EVALUATION OF SOME CYTOKINES IN UNINJURED AND CUTANEOUSLY INJURED NIGERIAN INDIGENOUS DOGS AT THE VETERINARY TEACHING HOSPITAL, ZARIA

BY

DANIEL ONIMISI AVAZI

DEPARTMENT OF SURGERY AND RADIOLOGY,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

OCTOBER, 2017

EVALUATION OF SOME CYTOKINES IN UNINJURED AND CUTANEOUSLY INJURED NIGERIAN INDIGENOUS DOGS AT THE VETERINARY TEACHING HOSPITAL, ZARIA

BY

Daniel Onimisi AVAZI, DVM (ABU) 2013
P14VTSR8003

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY SOFT TISSUE SURGERY

DEPARTMENT OF SURGERY AND RADIOLOGY,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

OCTOBER, 2017

DECLARATION

I declare that the work reported in this dissertation, entitled '**Evaluation of some Cytokines in uninjuredand Cutaneously Injured Nigerian Indigenous Dogs at the Veterinary Teaching Hospital, Zaria.**' was carried out by me in the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria – Nigeria. The information derived from literature has been duly acknowledged in the text and in the list of references provided. No part of this dissertation has been presented for another degree or diploma at any Institution.

Daniel Onimisi AVAZI

Name of Student ....

Signature Date

CERTIFICATION

This dissertation, entitled '**EVALUATION OF SOME CYTOKINES IN UNINJURED AND CUTANEOUSLY INJURED NIGERIAN INDIGENOUS DOGS AT THE VETERINARY TEACHING HOSPITAL, ZARIA.**' carried out by Daniel Onimisi AVAZI meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. C. A. Awasum

Chairman, Supervisory Committee …….. ……

Signature Date

Prof. A. Z. Hassan

Member, Supervisory Committee …….. …….

Signature Date

Dr. S. T. Fadason

Head of Department …….. ……..…

Signature Date

Prof.Sadiq Zubair Abubakar

Dean,School of Postgraduate Studies ……...….. …..…….

Signature Date

DEDICATION

I dedicate this project to the Almighty God and my lovely mother; Mrs. E. O. Avazi.

ACKNOWLEDGMENTS

I thank the Almighty God for enabling me carry out this work to completion.

This research would not have been possible without the contributions of several people, to whom I am most grateful. I want to express my profound appreciation to members of my supervisory committee, Professor C. A. Awasum who provided mentorship, some financial support, guidance and vetting of the writeup, Professor A. Z. Hassan for the supervision and vetting. Their meticulous attention yielded these results.

I am very grateful to my mum and hero; Mrs. E.O. Avazi, whosponsored, encouragedand supported me throughout the period of my study. I lack words to express my profound appreciation. I appreciate my siblings; Mr.V. O. Avazi and Miss.B. O. Avazi for their support and prayers which enabled me go through all difficulties. I thank Miss. Dina Yerima for her tireless efforts, patience, support and encouragement throughout the period of my study.

With humility I thank Professor(s) J. O. Ayo, C. Kudi, Dr(s). E. O. Abidoye,T. Aluwong, S. A.Yila and M. H. Suleiman who provided some guidance and materials to enable me undertake the research work. I appreciate the support of my colleagues, Dr(s). S. T. Mohammad, A. Mohammed, M. N. Bappah, A. A. Bada, J. J. Kalang, C. Uchendu, A. Mohammad, E. T. Vandi,E. Ngbede, O. Bojuwoye, I. Aplakah, O. Orakpoghenor, O. N. Osonwa, J. A. Atata,A. A. Abimbola,A. Aliyu, M. A. Kilani, A. Obalowu, G. Abdullateef, A. Bello, I. O. Oyenekan, J. Sariki,F. O. Zakari, U. Felix, R. Lawal andR. Clement chukwuebuka,for assisting in the laboratory and operative phases of thiswork. I am very grateful to Mr. Adejoh and Mallam Sanni and Mr. I. Mohammed of the Haematology Laboratory, Ahmadu Bello University teaching Hospital andUniversity Health Services,Dr. Y. Yunusa and staff of the Ahmadu Bello University Center for Biothechnology Laboratory, members of staff of the Veterinary Surgery and Radiology Laboratory.Their efforts made it possible to timely complete the experiment. I appreciateMiss. F. J.Sanusi,Mr. I. Suleiman, B. Bimi, and Mrs.C.Daniel, for all the assistance rendered in the course of the research work. I am very grateful to every one who in one way or the other assisted me in the course of my study.

Abstract

Twelve apparently healthy Nigerian indigenous dogs (NIDs) (6 males and 6 females) within the age range of 9 - 13 months and weight range of 8 – 12 kilograms were assigned to experimental (injured dogs) and control (uninjured dogs) groups to determine the levels of some cytokines and blood cell counts in cutaneously injured and uninjuredNIDs at the Ahmadu Bello University Veterinary Teaching Hospital, Zaria. Both circulatory (serum) and wound fluid cytokines were studied in experimental group, while those in control group were used to study circulatory cytokines. Blood samples from each dog were collected via cephalic venopuncture at start (pre-operative), 12, 36, 60, 156 and 324 hours post operative and refrigerated in plain (for harvesting of serum) and anticoagulant-containing (for blood cell counts) sample bottles. Serum and wound fluid collected at 12, 36, 60 and 156 hours were stored at -20 ^{o}C for cytokine assay. Canine specific ELISA kits were used to assay for concentrations of cytokines [interleukin (IL) -6, -8, -10 and tumour necrosis factor (TNF) – α] as described in Abcam product protocol (ABCAM® UK, 2015). Results revealed peak concentrations of IL-6 in wound fluid (1.33. ± 0.33 ng/mL) and serum (1.13 ± 0.74 ng/mL) at 12 h, post-operation were higher ($P < 0.01$) than the control (0.30 ± 0.05 ng/mL). There was no significant ($P > 0.05$) difference in circulatory IL-8 levels between experimental and control group. Circulatory IL-10 concentration (1.67 ± 0.33 ng/mL) was significantly ($P < 0.001$) higher than those in wound fluid (0.87 ± 0.03 ng/mL) of experimental and control (0.94 ± 0.17 ng/mL) groups at 156 h. The concentrations of TNF-α in wound fluid (130.22 ± 35.15 pg/mL) was significantly ($P < 0.05$) higher than in serum (44.50 15.92 pg/mL) of the experimental group at 36 h, while the serum (116.17 ± 60.27 pg/mL) was higher than the control (29.79 ± 7.47 pg/mL) values at 60 h post-operation. Mean haematocrit of 0.354 ± 0.01 recorded at 12 hours post-operation in the

experimental group was lower than the corresponding values of 0.364 ± 0.00 recorded in the control group. The total Leucocyte counts remained within the normal limits with minimal changes in differential cellular activitiesof theexperimental and control group.Significant correlations of serum IL-6 with wound fluid IL-6 ($r = 0.827$; $P < 0.05$), wound fluid IL-6 with HCT ($r = - 0.893$; $P < 0.05$) and RBC ($r = - 0.923$; $P <0.01$), wound fluid IL-6 with monocytes ($r = 0.818$; $P < 0.05$), serum IL-10 with lymphocytes ($r = 0.846$; $P < 0.05$) and wound fluid TNF-α with monocytes ($r = 0.824$; $P = 0.05$) were observed in injured NIDs. Interleukin (IL) -6, -8, -10 and TNF-α of the experimental group were significantly ($P < 0.05$) higher than those of the control group of NIDs. Significant correlations were observed with IL -6, -10 and TNF-α and red blood cells, lymphocytes and monocytes of the NIDs within a range of $r = 0.818$ - 0.923. In conclusion, maximum mean concentrations of interleukin (IL) -6, -8, -10 and TNF – α detected at 450 nm from uninjured NIDs were 0.30 ± 0.05 ng/mL, 383.16 ± 157.57 pg/mL, 0.94 ± 0.17 ng/mL and 47.06 ± 14.34 pg/mL, respectively.

TABLE OF CONTENTS

Titles **Pages**

Title Page……....**i**

Declaration **……...ii**

Certification **.....iii**

Dedication……**iv**

Acknowledgments **..... v**

Abstract……**vi**

Table of Contents**.....viii**

List of Tables **.....xiii**

List of Figures**..... xv**

List of Plates **.....xvii**

List of Appendices**.....xviii**

List of abbreviations and Units **.....xxii**

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 Preamble..... 1

1.2 Statement of Research Problem 5

1.3 Justification for the Study..... 6

1.4 Aim and Objectives of the Study..... 7

1.4.1 Aim…… 7

1.4.2 Objectives of the study 8

1.5 Statement of Research Hypothesis

(H_0)……....8

1.6 Scope ……..……..9

CHAPTER TWO 10

2.0 LITERATURE REVIEW 10

2.1 Nigerian Indigenous Dogs 10

2.2 Wounds, Aetiology and Classifications..... 11

2.2.1 Wounds and wounds aetiology 11

2.2.2 Wounds classification 12

2.3 Wound Management Modalities 14

2.3.1 Disinfectants and wound antiseptics 15

2.3.2 Wound antibiotics 17

2.3.3 Wound examination and assessment 19

2.3.4 Cleansing of wounds..... 19

2.3.5 Debridement..... 20

2.3.6 Wound drainage 20

2.3.7 Surgical repair of underlying damaged tissues 21

2.3.8 Irrigation and lavage 21

2.3.9 Wound closure 22

2.3.10 Wound dressing 23

2.4 Alternative/Ethno-Medicine in Wound Care 24

2.5 Wound Management Advances 25

2.6 Cytokines 26

2.6.1 Properties/characteristics of cytokines 27

2.6.2 Cytokines production and classification 28

2.6.3 Interleukin-6 (IL-6) 33

2.6.4 Interleukin-8 (IL-8) 37

2.6.5 Interleukin-10 (IL-10) 38

2.6.6 Tumor necrosis factor (TNF-α) 40

2.7 Wound Healing……46

2.7.1 Types of wound healing 47

2.7.2 Stages and phases of wound healing 52

2.7.3 Factors influencing wound healing 61

2.8. Measurement of Cytokines 63

2.8.1 Bioassays 63

2.8.2 Immunoassays 64

2.8.3 ELISPOT (Enzyme-Linked Immuno Spot Assay) 65

2.8.4. Flow cytometry 66

2.9 Future of Cytokines in Wound Management 67

CHAPTER THREE 68

3.0 MATERIALS AND METHODS 68

3.1 Experimental Site 68

3.2 Ethical Clearance 68

3.3 Materials/Agents Used in the Study 68

3.4 Methodology 73

3.4.1 Study design 73

3.4.2 Research animals 74

3.4.3 Clinical procedures 74

3.4.4 Sample analyses 77

3.4.5 Statistical analysis 84

CHAPTER FOUR 87

4.0 RESULTS… 87

4.1 General Overview 87

4.2. Cytokines Analysed: Interleukin (IL) -6, -8, -10 and Tuour Necrosis Factor –alpha (TNF-α) 88

4.3 Haemogram 97

4.3.1 Erythrocyte parameters of both experimental and control groups (n = 12)…...97

4.3.2 White blood cells count of both experimental and control groups (n = 12)……....101

4.4 Relationship between Cytokines (IL-6, IL-8, IL-10 and TNF-α) Levels, Haematologic Parameters and Wound Contraction in the NIDs (n = 12)……108

CHAPTER FIVE 113

5.0 DISCUSSION 113

CHAPTER SIX 120

6.0 CONCLUSION AND RECOMMENDATIONS 120

6.1 Conclusions 120

6.2 Recommendations 122

REFERENCES 123

APPENDICES 154

LIST OF TABLES

Table: DESCRIPTION OF TABLES **Pages**

3.1: List of Consumables Used in the Study..... **69**

3.2: List of Some Surgical Instruments and Wears Used in the Study. **70**

3.3: List of Antiparasitics used in the Study **70**

3.4: List of Antimicrobials and Fluids Used in the Study **70**

3.5: List of Analgesics, Anaesthetics and Premedications Used in the Study..... **71**

3.6: List of Items Used to Feed and Water the Dogs in this Study..... **71**

3.7: Experimental Design Summary..... **85**

4.1: Mean Interleukin-6 (IL-6) Responses (ng/mL) of both Experimental and Control Groups of NIDs irrespective of sex (n = 12)……..……...89

4.2: Mean Interleukin-6 (IL-6) Values (ng/mL) of both Experimental and Control groups of NIDs (n = 12)..... **……90**

4.3: Mean Interleukin-8 (IL-8) Responses (pg/mL) of both Experimental and Control Groups of NIDs Irrespective of Sex (n = 12)……91

4.4: Mean Interleukin-8 (IL-8) Values (pg/mL) of both Experimental and Control Groups of NIDs (n = 12)..... **92**

4.5: Mean Interleukin-10 (IL-10) Responses (ng/mL) of both Experimental and Control Groups of NIDs Irrespective of Sex (n = 12)……...93

4.6: Mean Interleukin-10 (IL-10) Values (ng/mL) of both Experimental and Control groups of NIDs (n = 12)..... **…….94**

4.7: Mean Tumour Necrosis Factor Alpha (TNF-α) Responses (pg/mL) of both Experimental and Control Groups of NIDsIrrespective of Sex (n = 12)……..…..95

4.8: Mean Tumour Necrosis Factor Alpha (TNF-α) values (pg/mL) of both Experimental and Control Groups of NIDs (n = 12). **..... 96**

4.9: Mean Haematocrit (HCT) Values of Experimental and Control Groups of NIDs Obtained Pre- and Post-operative. **..... 98**

4.10: Mean Red blood cells count (10^{12}/L) Responses of Experimental and Control Groups of NIDs**Obtained Pre- and Post-operative** …. **..... 99**

4.11: Mean Haemoglobin (g/L) Responses of Experimental and Control Groups of NIDs Obtained Pre- and Post-operative. **..... 100**

4.12: Mean Total and Differential Leucocyte Counts of NIDs of Non-specific Sex Induced with Cutaneous Injuries..……102

LIST OF FIGURES

Figure: DESCRIPTION OF FIGURES **Pages**

2.1: Schematic illustration of cytokines production and mechanism of action. Modified from (Burtis *et al.*, 2006; Masih, 2011 and Owen *et al.*, 2013). **..... 30**

2.2: Time line of migration of immune cell populations correlated with the phases of wound healing (Witte and Barbul, 1997). **..... 48**

4.1: Total White Blood Cells Count of the Experimental and Control Groups with minimal fluctuations although all values are maintained within normal range limits for the NIDs. Mean (Means ± SEM), (n = 12). 103

4.2: Variations in Mean Neutrophils Count of the Experimental and Control Groups NIDs. Mean (Means ± SEM), (n = 12). 104

4.3: Variations in Mean Lymphocyte Count of the Experimental and Control Groups NIDs. Mean (Means ± SEM), (n = 12). 105

4.4: Variations in Mean Monocyte Count of the Experimental and Control Groups NIDs. Mean (Means ± SEM), (n = 12). 106

4.5: Variations in Eosinophil Count of the Experimental and Control Groups NIDs. Mean (Means ± SEM), (n = 12)**.....** 107

4.6: Relationship between the levels of IL-6, IL-8, IL-10 and TNF-α irrespective of sex: (A) Wound fluid with serum IL-6; (B) wound fluid IL-8 with IL-10; (C and D) wound fluid IL-6 with HCT and RBC; (E) serum IL-6 with RBC; (F) wound fluid IL-6 with monocytes; (G) Serum IL-10 with lymphocytes and (H) wound fluid TNF-α with monocytes. r = Pearson's correlation coefficient, $P < 0.05$ are considered significant……109

4.7: Relationship between the levels of IL-6, IL-8, IL-10 and TNF-α with each other. (A) Wound fluid and serum IL-6 in males; (B) Wound fluid and

serum IL-8 in males; (C) Wound fluid IL-8 and IL-10 in females; (D) Serum IL-6 and IL-10 in female NIDs. r = Pearson's correlation coefficient, P < 0.05 are considered significant..... 110

4.8: Relationship between levels of IL-6, IL-8, IL-10 and TNF-α and profiles of haematologic parameters. (A) HCT and wound fluid IL-6 in females; (B) MCHC and wound fluid IL-6 in males; (C) MCH and serum IL-8 in females; (D) HGB and serum IL-10 in female NIDs. HCT; haematocrit, HGB; haemoglobin concentration, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration; r = Pearson's correlation coefficient, P < 0.05 are considered significant 111

4.9: Relationship between levels of IL-6, IL-8, IL-10 and TNF-α and White Blood Cells profile (A) Monocyte and wound fluid IL-6; (B) Monocyte and serum IL-8; (C) Eosinophils and serum IL-6; (D) Lymphocytes and serum IL-10 and (E) Eosinophils and serum IL-10 levels in females (F) Lymphocytes and serum IL-10 in male NIDs. r = Pearson's correlation coefficient, P < 0.05 are considered significant 112

LIST OF PLATES

Plates: DESCRIPTION OF PLATES **Pages**

I: NID with moderate and predominantly brown hair coat, medium sized head (mesencephalic cranial index) and slender limbs with white patches at the ventral part of the neck, extremities of the limbs and tail... 72

II: Aseptically Drapped right forelimb with marked 2 x 2 cm^2 area for the Full thickness cutaneous excision at the mid lateral surface of the antebrachium (radius and Ulna). 76

III: Enzyme Linked Immunosorbent Assay Interleukin (IL)-6, -8, -10 and Tumour necrosis factor alpha (TNF-α) Kits acquired from Abcam®, Cambridge, United Kingdom. 79

IV: Displayed are all the reagents used in the study:Enzyme linked immunosorbent assay (ELISA) kits with Assay diluent A (A); Cytokine standards (B); 20X wash buffer (C); Assay diluent B (D); Biotinylated Canine cytokine antibody (E); Horseradish Perosidase (HRP)-streptoverdine (F); 3,3',5',5'-Tetramethylbenzidine (TMB) one-stop solution (G); Stop solution (H); Sealed antibody coated microtithre plate (I) 79

V: Thermo Multiskan Ascent Photometer for 96- and 384-well microplate format to measure absorbance at 450 nm. At the lower right corner is the 96 wells microplate with whitish rectangular base within a black frame (Thermo Scientific, USA) 83

LIST OF APPENDICES

Title DESCRIPTION OF APPENDICES **Pages**

I: Spectrophotometric Measurement Count of Interleukin (IL) -6 at Filter: 450nm……..... **154**

II: Graphical Output of Changes in IL-6 Standard Concentrations versus Optical Densities and Polynomial Calibration. IL-6 Trendline/Regression Graph (A), Standard Curve (B). **155**

III: Interleukin (IL) -6 Concentration (ng/mL) Extrapolated from Standard Curve using Trendline/Regression Formular. **156**

IV: Spectrophotometric Measurement Count of Interleukin (IL) -8 at Filter: 450nm……157

V: Graphical Output of Changes in IL-8 Standard Concentrations versus Optical Densities and Polynomial Calibration. IL -8 Trendline/Regression Graph (A), Standard Curve (B). **158**

VI: Interleukin (IL)-8 Concentration (pg/mL) Extrapolated from Standard Curve using Trendline/Regression Formular. **159**

VII: Spectrophotometric Measurement Count of Interleukin (IL) -10 at Filter: 450nm……. .. **160**

VIII: Graphical Output of Changes in IL-10 Standard Concentrations versus Optical Densities and Polynomial Calibration. IL -10 Trendline/Regression Graph (A), Standard Curve (B). **161**

IX: Interleukin (IL) -10 Concentration (ng/mL) Extrapolated from Standard Curve using Trendline/Regression Formular. **162**

X: Spectrophotometric Measurement Count of Tumour Necrosis Factor –alpha (TNF –α) at Filter: 450nm **..... 163**

XI: Graphical Output of Changes in TNF –α Standard Concentrations versus Optical Densities and Polynomial Calibration. TNF –α Trendline/Regression Graph (A), Standard Curve (B). **..... 164**

XII: Tumour Necrosis Factor –alpha (TNF –α) Concentration (pg/mL) Extrapolated from Standard Curve using Trendline/Regression Formular. **..... 165**

XIII: Mean (mean ± SEM) Age and Weight of Experimental and Control groups of the NIDs. **..... 166**

XIV: Some Mean Serum Biochemical Parameters of the Experimental (Pre- and Post-operative) and Control Groups……..167

XV: Some Mean Urinalysis Parameters of the Experimental (Pre- and Post-operative) and Control Groups……168

XVI: Mean Pre- and Post-operative vital Parameters of the Experimental Group of the NIDs. **..... 169**

XVII: Mean Pre- and Post-operative vital Parameters of the Control Group of the NIDs. **..... 171**

XVIII: Pre-operation Haemogram of the Experimental and Control Groups of NIDs. 173

XIX: Changes in the Surface area (cm2) of the Cutaneous Injuries on the Forelimb (Mid lateral aspect of the right Antebrachium) (n = 6)…….174

XX: Mean Morphometric Measurement (Percentage Wound Contraction) of the Wound Surface for 6 NIDsThat Underwent Study with Wound Healing Achieved in 42 Days Post Operation…….....175

XXI: Reference Haemogram for NIDs (Means ± SEM), (n = 12)……. 176

XXII: Haemogram of the Experimental and Control Groups of NIDs 12 hours Post-operation. 177

XXIII: Haemogramof the Experimental and Control Groups of NIDs36 hours Post-operation 178

XXIV: Haemogramof the Experimental and Control Groups of NIDs60 hours Post-operation 179

XXV: Haemogramof the Experimental and Control Groups of NIDs156 hours Post-operation 180

XXVI: Haemogramof the Experimental and Control Groups of NIDs324 hours Post-operation 181

XXVII:Relationship Between IL-6, IL-8, IL-10 and TNF-α Levels in Wound Fluids and Sera of Injured (Group B) NIDs 182

XXVIII:Relationship Between IL-6, IL-8, IL-10 and TNF-α Levels in Sera of Injured (Group B) NIDs..... 183

XXIX: Relationship Between IL-6, IL-8, IL-10 and TNF-α Levels in Sera of Uninjured (Control - Group A) NIDs. 184

XXX: Relationship Between IL-6, IL-8, IL-10 and TNF-α Levels in Wound Fluids with White Blood Cells Count of Injured (Group B) NIDs……..…….185

XXXI: Relationship Between IL-6, IL-8, IL-10 and TNF-α Levels in Sera with White Blood Cells count of Injured (Group B) NIDs……...……186

XXXII: Relationship Between IL-6, IL-8, IL-10 and TNF-α Levels in Sera with White Blood Cells Count of Uninjured (Control - Group A) NIDs……...187

XXXIII: Relationship Between IL-6, IL-8, IL-10 and TNF-α Levels in Wound Fluids with Erythrocyte Parameters of Injured (Group B) NIDs……...188

XXXIV: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Sera with Erythrocyte Parameters of Injured (Group B) NIDs..……...189

XXXV: Relationship Between IL-6, IL-8, IL-10 and TNF-α Levels in Sera with Erythrocyte Parameters of Uninjured (Control - Group A) NIDs……...190

XXXVI: Significant Correlations Between IL-6, IL-8, IL-10 and TNF-α Levels with Each Other and Blood Cell Counts in Injured (Group B) NIDs Irrespective of Sex……..……...……191

XXXVII: Relationship Between IL-6, IL-8, IL-10 and TNF-α Levels in Wound Fluids and Sera with Percentage Wound Contraction of Injured (Group B) NIDs……...192

LIST OF ABBREVIATIONS AND UNITS

NIDs	Nigerian indigenous dogs
IL	Interleukin
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
TNF-α	Tumour necrosis factor alpha
SIRS	Systemic inflammatory response syndrome
SOCS	Suppressors of cytokine signalling
EDTA	Ethylenediamintetraacetate
Exp	Experimental
ser	Serum
wf	Wound fluid (wound exudates collected by saline flush of the wound surface)
Ctrl	Control
h.	Hour
ng/mL	Nanogram per milliliter
pg/mL	Picogram per milliliter

mg/mL	Milligram per milliliter
mg/dL	Milligram/deciliter
mmol/L	Millimolar/litre
g/dL	Gram/decilitres
mL	Millilitres
L	Litre
kg	Killogram
^{0}C	Degree Celsius
Inch	Inches

CHAPTER ONE

INTRODUCTION

1.1 Preamble

Cytokines are low molecular-weight proteins (5-20 kDa) that are released by epithelial cells, endothelial cells, fibroblasts, activated lymphocytes, macrophages and monocytes and are important in cell signalling (Tracey *et al*., 1987; Dinarello, 1994; Wang and Tracey, 1999; Horst, 2013;Orzechowski, 2017). They mediate the metabolic and physiologic responses to injury and infection by binding to specific receptors, enabling immune cells communication, differentiation, proliferation and other activity which trigger inflammatory response to sites of injury and infection thereby enhancing wound healing (Haichao *et al.*, 2000; Horst, 2013; Orzechowski, 2017).

Cutaneous injuries are wounds which may be surgically induced or occur naturally. A wound is a traumatic separation/disruption of continuity of the skin, mucus membrane or an organ surface and it is one of the most frequent reasons for seeking medical attention (Hassan *et al.*, 2002; Kauvar and Wade, 2005; Ahmed *et al*., 2013; Borena *et al*., 2015). Wound is also defined as a loss or breakage of cellular and anatomic or functional continuity of living tissue following road traffic accidents, falls, cuts from sharp objects such as corrugated iron sheets and barbed wires, assault/acts of violence by individuals and surgery amongst others (Milner, 2008; Kokane *et al.*, 2009; Karen and Spencer 2012). Injury to the skin initiates series of events, which finally results to at least a partial reconstruction of the wounded tissue (Martin, 1997; Auf-dem-Keller *et al.*, 2006; Turkel *et al.*, 2014). Wounds, when left untreated may become complicated, leading to varying degrees of contamination, deformities and ultimately may result in

the death of the animal (Amber and Swaim, 1984; Hassan *et al.*, 2003; Namas *et al.*, 2009). When a wound is infected, it contains replicating micro-organisms which elicit a host response causing injury to the host. Etiologically, wounds are categorized into acute or chronic forms comprising surgical wounds, burns, lacerations and puncture wounds (Kokane *et al.*, 2009). In an acute wound, infection is met by a rapid inflammatory response initiated by complement fixation and an innate immune response followed by the release of cytokines and growth factors (Dow *et al*., 1999).

Cytokines are generally classified into Pro- and Anti-inflammatory cytokines, with the pro-inflammatory cytokines comprising of the tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) and anti-inflammatory cytokines which includes interleukin-10 (IL-10) amongst others. Others forms of cytokines include interferons and lymphokines (Blackwell and Christman,1996*;* McBride *et al.*, 1996; Lin *et al.*, 2000; Bown *et al.*, 2001; Chachkhiani *et al.*, 2005).

Wound healing is a complicated and very highly regulated pathway that varies in its processes longitudinally, temporally and spatially and involves a few phases (the main phases: Inflammation, Proliferation involving debridement and repair, and Maturation) which consumes vast resources (Hassan*et al.*, 2002; Grazul-Bilska *et al.*, 2003; Nguyen *et al.*, 2009; Guo and Dipiero, 2010). The inflammatory cascade produces vasodilation and a significant increase in blood flow to the injured site, thus increased cytokine response facilitating the breakdown of devitalised tissues while promoting the formation and preserving healthy granulation tissues with resultant restoration of tissue architecture and function (Robinson, 1993; Reddy *et al.*, 2008).

The various pro- and anti-inflammatory cytokine profile can be measured in serum and wound fluids by quantitative enzyme linked immunosorbent assay (ELISA) using the

cytokine canine ELISA kits with preset minimum detectable levels of the cytokines by the manufacturers (Zuhoor, 2012; John *et al.*, 2013). Pro-inflammatory cytokines such as tumour necrosis factor (TNF) and interleukin 1 (IL-1) are released by activated lymphocytes, macrophages and monocytes during infection, and acute inflammation following injury or wound (Tracey *et al.*, 1987; Dinarello, 1994; Wang and Tracey, 1999). Pro-inflammatory cytokines are pyrogenic and can stimulate thermogenesis by directly acting on the hypothalamic centers (Jones, 1993). These cytokines, especially TNF and IL-1 also influence the release of corticotropin, growth hormone (GH), pituitary hormones, prolactin, and macrophage migration inhibitory factor (MMIF), which function in the integration of neuro-endocrine and immune responses to various types of injuries and infections (Yamaguchi *et al.*, 1991; Nash *et al.*, 1992; Bucala, 1996; Fry *et al.*, 1998). Consequent to severe trauma, serum levels of TNF-α, IL-6, and IL-8 are significantly elevated. There appears to be a direct relationship between the extent of pro-inflammatory cytokine release and the severity of injury as well as hospital mortality (Hoch *et al.*, 1993; Carlstedt *et al*., 1997; Jiang *et al*., 1997). Anti-inflammatory cytokines such as IL-10, TNF-binding protein, IL-1 receptor antagonist (IL-1ra), and transforming growth factor beta (TGF-β) usually produced through a normal immune response, can inhibit the release of TNF- α and other pro-inflammatory cytokines by interfering with their binding to their respective receptors(Sakic *et al.*, 2009). Similarly, stress hormones such as epinephrine, glucocorticoids, norepinephrine, and α-melanocyte-stimulating hormone also inhibit cytokine production (Sakic *et al.*, 2009). There is a correlation between pro- and anti-inflammatory cytokines triggered by the Proinflammatory cytokines (McBride *et al.*, 1996). Pro-inflammatory cytokines such as TNF-α and IL-1 have short half-lives and system of membrane reservoirs (local control)(McBride *et al.*, 1996).The reservoirs include the compromised vascular

endothelium with its associated inflammatory and haemostatic activities which hold the cytokines close to the site of release and facilitate the modification of the host inflammatory response by the anti-inflammatory cytokines (McBride *et al.,* 1996).

Although cytokine activities have been stated by several researchers to be advantageous to wound healing, excessive cytokine activities are deleterious(McBride *et al.,* 1996). Some instances are as cited by McBride *et al.* (1996), where inappropriate synthesis and release of cytokines known as cytokine excess occurs with excessive and/or persistent activation of macrophages and neutrophils following some infectious or non-infectious conditions, burns, haemorrhagic shock, surgery, trauma and ischaemia-reperfusion injuries (Sakic *et al.,* 2009; Zura *et al.,* 2009). If this inflammatory response escapes local control, there will be widespread activation of inflammatory cascades with resultant systemic inflammatory response syndrome (SIRS) (McBride *et al.,* 1996). This SIRS evokes further release of inflammatory cytokines hence, a downward spiral of multi-system organ dysfunction/failure (MSOD/MSOF) and ultimately multiple-system organ dysfunction syndrome (MSODS) such as; kidney glomerular injury, tubular damage, left ventricular dysfunction and vasodilation, acute lungs injury and acute respiratory distress syndrome (ARDS), increased synthesis of hepatic acute phase proteins, inhibition of fibrinolysis with a role in the development of disseminated intravascular coagulation (DIC) and thrombosis (Pagani *et al.,* 1992*;* Esmon *et al*., 1994; Pawelec *et al.,* 1995; Kelly and Smith,1997;Oral *et al.,* 1997; Sheeran and Hall,1997; Bown *et al.,* 2001; Kudoh *et al.,* 2001; Shi-long *et al.,* 2016).

1.2 Statement of Research Problem

The Nigerian Indigenous Dog popularly referred to as mongrel are prone to injuries because they are well known for their hunting efficiency, as shepherd dogs, guard dogs at human inhabitants and company to farmers (Olayemi *et al.*, 2009; Olumayowa, 2011). For these and other uses, they are vulnerable to injuries especially cutaneous injuries(Hassan *et al.*, 2003). Often than not, their injuries heal but with others injuries may linger for longer than necessary either because of renewal of the already existing injuries or because of lack of care(Hassan *et al.*, 2003; Swai *et al.*, 2010). Even though it is known that injuries will heal whether aided or unaided, if not they die, the NIDs are exposed to these two sides of healing or healing defects. It is therefore important to have a clean understanding of factors that may assist healing of injuries in the NIDs. Healing of open injuries are further complicated or slowed down by numerous factors as described by Hassan *et al.* (2003); Benjamin and Christopher (2009); Velnar *et al*. (2009); Shashi *et al.* (2013) and Turkel *et al.* (2014).Effective wound healing process involves a delicate interplay of the various inflammatory cytokines and chemokines, destructive and constructive enzymes, growth factors, extracellular matrices and reactive oxygen species with the various cell types such as red blood cells, keratinocytes, endothelial cells, fibroblasts, platelets, neutrophils, monocytes, macrophages, lymphocytes and nerve cells(Dipietro, 1995; Cummings and Merriam, 2003; Grazul-Bilska, 2003). The continued modifications and changes of the various cytokines, cells and other contributory factors resulting in effective wound healing are thought to vary based on the origin and nature of insult as well as the duration of pathology (Dipietro, 1995; Cummings and Merriam, 2003; Grazul-Bilska *et al.*, 2003). These modifications have been influenced by various systemic and topical agents such as antimicrobials, wound healing ointments and creams though with useful but limited

outcomes (Benjamin and Christopher, 2009, Sheela *et al.*, 2012). Over the years, inappropriate responses to injury have been found to be more harmful than the original insult with resultant generalized systemic inflammatory response which damages distant tissues and causes more injury (Tracey, 2002; Van Westerloo *et al.*, 2005; Shi-long *et al.*, 2016). Increased Pro-inflammatory cytokine concentrations in plasma/serum have been demonstrated in humans after major surgeries (McBride *et al.*, 1996; Salo, 1996; Johnston and Webster, 2009) with the magnitude of the cytokine-mediated inflammatory response related to the extent of the surgical insults (McBride *et al.*, 1996). Littleefforts have been made to explore the modulating (upregulation and downregulation) effects of appropriate cytokines to modulate wound healing process and to use the levels of the cytokines expressed in serum to predict disease outcomes (Francisco *et al*., 1999; Carlstedt *et al*., 1997; Moseley *et* al., 2004; Ahmed *et al.*, 2013).

1.3 Justification for the Study

The various forms of traumatic injuries present major challenges to clinicians as they are usually acute in onset and may progress into chronic health problems especially when complicated by infections often resulting in relatively reduced quality of life affecting far beyond the individual victims (Kauvar and Wade, 2005; Namas *et al.*, 2009; Turkel *et al.*, 2014). However, following injuries, survival depends on the individuals' immune system's ability to respond and defend the body against further injuries and attack from invading pathogens. The extent of such a response is of critical importance as appropriate responses involving cytokines synthesis and release is a key component of the non-specific (innate) immune system's response (Tracey, 2002; Van Westerloo *et al.*, 2005). Site, severity of injury as well as nutrition and diet may impact these responses one of which is the inflammatory response. This inflammatory response

driven by cytokines and chemokines is partly propagated by damaged tissue-derived products (Damage-associated Molecular Patterns; DAMP's) which maintain inflammation through pro-inflammatory cytokines release or inhibition by anti-inflammatory cytokines (Namas *et al.*, 2009).

Various animal models with traumatic injuries and haemorrhagic shock in dog, mice, pigs, rats, and non-human primates have been utilised in an attempt to move from bench to bedside in understanding the biology of wound healing (Kauvar and Wade, 2005; Namas *et al.*, 2009; Kemin *et al.*, 2014). At the moment, knowledge derived from other studies has been useful in the understanding of cytokine dynamics in other animal species and breeds than injured NIDs (Namas *et al.*, 2009; Zuhoor, 2012; Anonymous[2], 2015; Bertha *et al.*, 2017). Thus, using the NID in this study, sufficient data when collected would serve as baseline, adding to the worth of knowledge and potential in the development of assessment/monitoring tools in diagnostic, therapeutic and prognostic evaluation and management of cutaneous injuries.

1.4 Aim and Objectives of the Study

1.4.1 Aim

This study aim at evaluating some cytokines in uninjured and cutaneously injured NIDs at the Veterinary Teaching Hospital (VTH), Zaria.

1.4.2 Objectives of the study

To:

i. Determine quantitatively the levels of interleukin (IL) -6, -8, -10 and tumour necrosis factor (TNF) -α in serum of uninjured NIDs.

ii. Determine the levels of interleukin (IL) -6, -8, -10 and tumour necrosis factor (TNF) -α expressed in the serum and wound fluid of injured NIDs

iii. Evaluate the blood cells (erythrocyte, total and differential leucocyte counts) in uninjured and injured NIDs.

iv. Correlate the circulatory levels of IL-6, IL-8, IL-10 and TNF-α in injuredNIDs with each other and with the blood cell counts.

1.5 Statement of Research Hypotheses (H_0)

a. There are no significant differences between the levels of circulatory cytokines of uninjured (control group) and injured (experimental group) NIDs.

b. There are no significant differences between the levels of circulatory cytokines and that in wound secretions of injured NIDs.

c. There are no significant correlations between the various studied cytokines, and the cytokines with the haematologic parameters in injured NIDs.

1.6 Scope

1. Only NIDswere used as study subjects.
2. Matured males and females not above the age of 2years were used.
3. Only Interleukin (IL) -6, -8, -10 and Tumour Necrosis Factor (TNF) –α were assayed
4. Blood samples were collected pre operative (start), 12 h., 36 h., 60 h., 156 h. and 324 h. post operative and sera harvested for evaluation of cytokines.
5. Wound fluids secreted at12 h., 36 h., 60 h., and 156 h. post operation were obtained for Enzyme Linked Immunosorbent assay by a surface flush into sterile containers using normal saline.
6. Basic haematology, urinalysis and serology (blood urea nitrogen, creatinine, glucose, total protein, albumen, sodium ion, potassium ion, hydrogen bicarbonate, chloride ion, calcium ion and phosphate ion) were evaluated to ascertain the healthiness of the NIDs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nigerian Indigenous Dog

Domestication of dogs from wolves can be dated back to some thousands of years or perhaps as early as 100 000 years ago based on recent genetic fossil and DNA evidences (Savolainen *et al*., 2002, Lindbald-Toh*et al.*, 2005; Morey, 2006). Evidence suggests East Asia, possibly China as the location where the first domestication of dogs took place and the first people to enter North America took dogs with them (Savolainen *et al*., 2002; Swai *et al.*, 2010). Dogs are the most common pet animals worldwide and perform a range of societal functions ranging from cultural and social to economic functions (Swai *et al.*, 2010). Some studies also suggest that keeping pets is associated with a higher level of self-esteem especially in children (Paul and Serpell, 1996; Knobel *et al*., 2008).

The Indigenous dogs to Nigeria known as the Nigerian indigenous dogs (NIDs) are mostly used for hunting in the wild by the Nigerian people with a recent increase in its use as guard dogs and pets in the urban areas (Olayemi *et al*., 2009; Olumayowa, 2011). NIDs are medium sized breed of dogs with unique features predominant of which are their average body size (adult weighing between 10 and 25 kg), slender limbs, moderate hair coats and mesencephalicor mesaticephalic or mesocranial (medium headed) cephalic indices orcranial index between 75 and 83 as against brachycephalic (short headed) and dolichocephalic (long headed) breads of dogs with cranian index of greater than 83 and less than 75 respectively (Arowolo, 1999; Olayemi *et al*., 2009; Oluwatoyin and Fayemi, 2011; Anonymous[3], 2017).

The cranial index (cephalic index) is used to categorize dogs, cats and other animals including humans. It is the ratio of the maximum width (biparietal diameter or side to side) of the head of an animal multiplied by 100 divided by its maximum length (occipitofrontal diameter or front to back) (Anonymous[3], 2017). There are about 4.5 million NIDs with recent upsurge in acquisition probably due to the cheaper cost of care and their considerable hardiness evident by elevated levels of resistance to diseases when compared with the improved or exotic breeds (Arowolo, 1999; Olayemi *et al*., 2009). The NIDs are now brought regularly to veterinary clinics for treatments.Although, little studies on the gross morphology, physiology, molecular biology, biochemistry and haematology of the NIDs and factors that affect them are available as tools for successful medical and surgical management of accidents and emergencies (Saror *et al.*, 1979; Awah and Nottidge, 1998; Arowolo, 1999; Ariyibi, *et al.,* 2002; Olayemi *et al.,* 2009).

2.2 Wounds, Aetiology and Classifications

2.2.1 Wounds and wounds aetiology

Wounds are inescapable events in lifefollowing physical, chemical, thermal, microbial or immunological insult to a body tissue, resulting in disruptions, breakage and loss of cellular and anatomic or functional continuity of living tissues or organs(Milne, 2008; Kokane *et al.,* 2009; Karen and Spencer 2012; Ahmed *et al*., 2013; Borena *et al*., 2015). They may extend into subcutaneous tissue with damage to tendons, muscles, vessels, nerves, parenchymal organs and even bones (Milne, 2008; Kokane*et al.*, 2009; Karen and Spencer 2012; Ahmed *et al*., 2013).

2.2.2 Wounds classification

Wounds are generally classified based on several factors among which are time frame of repair/healing, aetiology of such wounds, communication, degree of contamination and severity of the wounds.

2.2.2.1 Time frame

Based on time frame wounds can be classified into acute or chronic wounds which could get infected becoming complicated wounds (Lazurus *et al.*, 1994; Bischoff *et al.*, 1999; Robson *et al.*, 2001; Pirbalouti *et al*., 2012). Acute wounds are wounds that repair themselves in a timely (about 35 days) and orderly healing pathway with the end result being the normal or at least near normal anatomical and functional restoration of body part (Lazurus *et al.*, 1994; Robson *et al.*, 2001; Labler *et al.*, 2006). Excess levels of inflammatory cytokines, tissue hypoxia, necrosis, infection and exudation are factors delaying wound healing with resultant chronicity (Degreef, 1998; Robson *et al.*, 2001, Komarcevic, 2000; Vanwijck, 2001).

2.2.2.2 Aetiology

Classification based on aetiology takes into consideration the trigger factor such as physical, chemical and biologic causes.Physical causes includes falls, abrasions, crush wounds, cuts, lacerations, road traffic accidents, avulsions, stab wounds, shot wounds, burns and various sadistic expressions (Bischoff *et al.*, 1999; Lazurus *et al.*, 1994; Robson *et al.*, 2001; Broughton *et al.*, 2006a). Chemical causes occurs as a result of exposure to or contact with corrosive chemical and biologic causes includes bites, kicks and butting with resultant contusions and concussion in some cases (Komarcevic, 2000; Robson *et al.*, 2001).

2.2.2.3 Communication

Wounds may be presented as closed wounds; where the underlying tissue has been traumatised but not exposed to the exterior as seen in contusions and haematoma, or as opened wounds; where the skin layer has been damaged with the underlying tissue exposed as obtainable in lacerations, incisions, avulsions and puncture wounds (Degreef, 1998; Komarcevic, 2000; Attinger *et al.*, 2006). There could be morphological characteristics and communication with hollow or solid organs as seen in hernias such as seen in diaphragmatic hernia (Komarcevic, 2000; Robson *et al.,* 2001) or damages to muscles, tendons and neurovascular system (Bischoff *et al.*, 1999; Robson, 1997; Labler *et al.*, 2006).

2.2.2.4 Degree of contamination

Based on degree of contamination, we have aseptic or clean wounds, clean/contaminated wounds, contaminated wounds and septic or dirty wounds (Gezzer O. *et al*., 2012). Aseptic wounds are fresh uninfected and operative wounds in which minimal inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tracts are not entered. Clean/contaminated wounds involve controlled entery into the respiratory, alimentary and genital or urinary tracts without unusual contamination. Contaminated wounds include open fresh accidental wounds less than 4 hours of age, abdominal, lung, urinary tract, and respiratory tract operations with major breaks in sterile technique or gross spillage from the gastro-intestinal tract, and incisions in which acute, non-purulent inflammation is encountered (James, 2002; Broughton *et al.*, 2006a; Gezzer O. *et al*., 2012). Septic or dirty wounds include old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera as obtainable in bowel operations and lancing of abscesses

(Komarcevic, 2000; Vanwijck, 2001; James, 2002; Broughton *et al.*, 2006a; Gezzer O. *et al*., 2012).

2.2.2.5 Severity

Based on severity of wounds we have minor wounds, moderate wounds and major wounds (EPUAP, 2010). Minor wounds are characterized by discoloration of intact skin not affected by light finger pressure (non-blanching erythema), partial thickness skin loss or damage involving the epidermis and or dermis. It is superficial and presents clinically as abrasion, blister or shallow crater. Moderate wounds are full thickness skin loss involving damage of the subcutaneous tissue but not extending to the underlying fascia and major wounds are full thickness skin loss with extensive destruction and necrosis extending to underlying tissues/structures (James, 2002; Hassan *et al.*, 2003; EPUAP, 2010).

2.3 Wound Management Modalities

Adhering to the guidelines of the procedural ethics of wound examination and management effectively assist the healing processes impacting positively on the final clinical outcome (Diegelmann and Evans, 2004; Broughton, *et al*., 2006b; Strecker-McGraw *et al.*, 2007). Worthy of note are physiological, endocrine and nutritional supports which significantly influence repair without which wound healing often fails completely (Hunt, 1998; Attinger *et al.*, 2006). Disinfectants and antibiotics are vitals agents used in successful wound management.

2.3.1 Disinfectants and wound antiseptics

Disinfectants are agents with activity against virtually all disease-causing microorganisms, including spores; they are used primarily for sterilizing inanimate surfaces and may be toxic to tissues (Lineaweaver *et al*., 1985; Drosou *et al.*, 2003; Benjamin and Christopher, 2009). Most topical antimicrobials can be divided into antiseptics or antibiotics by virtue of its concentration (Drosou *et al.*, 2003; Benjamin and Christopher, 2009). Antiseptics like disinfectants are defined as chemicals with antimicrobial properties but differ in that antiseptics are suitable for application on living tissues such as intact skin, some skin wounds and burns to kill or inhibit microorganism, irrigate or lavage contaminated wounds for primary or secondary closure as well as post-operative sepsis control (Amber and Swaim, 1984; Lineaweaver *et al*., 1985; Drosou *et al.*, 2003). They often have broad antimicrobial spectrum and residual anti-infective activity which may become toxic to host tissues (eg, fibroblasts, keratinocytes and possibly leukocytes). Some compounds have antibacterial and desloughing actions but are generally safe when applied to intact skin (Lineaweaver *et al*., 1985; Drosou *et al.*, 2003). Most agents cause some toxicity to host cells *in vitro*, such as prolonging the acute inflammatory response or delaying the production of collagen, but these effects are not usually noted *in vivo* (Lineaweaver *et al*., 1985; Drosou *et al.*, 2003). Those routinely used include: some older agents such as sodium hypochlorite and hexacholorphene which are now infrequently used for infected wounds (Benjamin and Christopher, 2009). Commonly used antiseptics include: Acetic acid, hydrogen peroxide, iodophors, povidone iodine, chlorhexidine, alcohol, detergent, soap and newer formulations, such as cadexomer iodine and silver compounds (Castellano *et al.*, 2007; Cooper, 2007; Fonder *et al.*, 2008; Benjamin and Christopher, 2009).

2.3.1.1 Chlorhexidine gluconate

Chlorhexidine is cationic polybiguanide available in concentrations of 2% and 4% solutions, 2% and 4% liquids, 0.5% hand rinse, 0.5% wipes, 4% sponge/brush and 4% foam. It has long-acting activity against a wide range of both gram-positive bacteria (eg, *Staphylococcus aureus*) and gram-negative bacteria, including *Pseudomonas aeruginosa*(Cooper, 2007; Benjamin and Christopher, 2009). Persistent activity up to 6 h after application with few adverse effects comprising: Hypersensitivity, including anaphylaxis, generalized urticaria, bronchospasm, cough, dysponea, wheezing, and malaise; may cause serious injury to the eye and middle ear necessitating caution regarding its use on the face or head (Benjamin and Christopher, 2009). Some resistances have also been reported (Benjamin and Christopher, 2009). It is inexpensive and indicated as surgical hand scrub, hand wash, preoperative skin and skin wound cleanser (Cooper, 2007; Benjamin and Christopher, 2009). Other biguanides with similar characteristics include; polyhexanide and hexachlorophene which is available as 3% liquid, 0.23% foam with 56% alcohol biguanide. Hexachlorophene is very expensive and not recommended for routine us on wounds because of potential neurotoxicity with convulsions, dermatitis, photosensitivity reactions and death (Cooper, 2007; Benjamin and Christopher, 2009).

2.3.1.2 Silver compounds

Silver Nitrate is available as 0.5%, 10%, 25%, and 50% solution, 10% ointment and 25% - 50% swabs(Castellano *et al.*, 2007). Silver compounds (metallic, nanocrystalline, and ionic) have a broad bactericidal spectrum and have enjoyed a recent resurgence as topical antiseptics in various types of wound dressings. Silver ions kill bacteria by several mechanisms, including damaging their cell walls, cell membranes, respiratory enzymes, and ribonucleoproteins (Castellano *et al.*, 2007; Fonder *et al.*, 2008). Silver

dressing products act by slowly releasing silver ions providing sustained levels of active silver ions that has proven efficacy against several common wound pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and extended-spectrum b-lactamase producers (Patel *et al.*, 2008). Thus its usefullness in subclinically infected, highly colonized wounds or for wounds being prepared for skin grafting. These dressings do not stain tissues nor delay healing, rarely exhibit microbial resistance, its less painful and have fewer adverse effects than silver nitrate (Cooper, 2007; Benjamin and Christopher, 2009).

2.3.1.3 Super-oxidized water

Microcyn; Oculus is a brand of super-oxidized water available without prescription. This pH-neutral sterilant with reactive species of chlorine and oxygen in a stable formulation is rapidly bactericidal, has broad-spectrum coverage, does not appear to facilitate bacterial resistance or damage host tissues, and may be active in the presence of biofilm (Gonzalez-Espinosa *et al.*, 2007). It can be applied directly to wounds or be combined with dressings or other wound products and it is effective in treating infected ulcers (Zahumensky, 2006; Gonzalez-Espinosa *et al.*, 2007; Goretti *et al.*, 2007; Kaehn, 2007).

2.3.2 Wound antibiotics

Antibiotics are chemicals produced either naturally (by microorganisms) or synthetically that in dilute solution inhibit or kill other microorganisms (Lineaweaver *et al.*, 1985; Drosou *et al.*, 2003). They usually act on several classes of target by a wide spectrum of activity or one specific cell target as obtainable in topical antibiotics use, having narrower spectrum of activity (Lineaweaver *et al.*, 1985; Drosou *et al.*, 2003). Those with narrow spectrum of activity are relatively non-toxic and are more

susceptible to losing their effectiveness to bacterial resistance (Lineaweaver *et al*., 1985; Drosou *et al.*, 2003). Antibiotics are recommended for wounds in which the magnitude of tissue injury is extensive and contaminated or infected (Hassan *et al*., 2003; Neely *et al.*, 2009). Clinically infected wounds are usually treated with systemic antibiotic therapy such as sulphonamides, penicillins, streptomycins, cephazolins and ceftriaxones amongst others(O'Meara *et al.*, 2001; O'Meara *et al.*, 2008). Topical antibiotics are becoming more common in wound management as evidences from a collection of systematic review of controlled trials of antimicrobial agents in wound managements suggests that few systemic agents improved outcomes especially in chronic wounds(O'Meara *et al.*, 2008).Several topical substances applied on debrided wounds hastened healing, including silver-containing compounds (O'Meara *et al.*, 2001; O'Meara *et al.*, 2008).

Better results from topical antibiotics are probably due to the qualities such as; properly targeted antimicrobial spectrum for the particular type of infected wound and with rapid bactericidal activity, persistent or residual skin activity, allowing infrequent dosing, activity in the presence of body fluids and proteins in wound exudates, some local skin penetration but no systemic absorption, no associated toxic (to host tissue) or allergic reactions, acceptable cosmetic and aesthetic qualities and low cost (Neely *et al.*, 2009). Topical treatments may also prove helpful with the increasing problem of multidrug-resistant organisms that are untreatable with most systemic agents (Neely *et al.*, 2009). The first were derived from agents developed for systemic use (ie, sulfonamides in the mid-1930s), followed in the next decade by topical penicillins, bacitracin, gramicidin, aminoglycosides (including neomycin), polymixin, and tetracyclines (Benjamin and Christopher, 2009). Agents introduced later include fusidic acid, clindamycin, metronidazole, mupirocin and retapamulin (Paul and Pieper, 2008; Benjamin and

Christopher, 2009). Topical antibiotics come in forms of ointments, creams [These are semi-solids which are either oil in water emulsion (aqueous cream) or water in oil emulsion (oily cream) primarily used to apply solution or dispersions of medicaments to skin for therapeutic or prophylactic purposes (Hassan *et al*., 2003)]. Applying ointment to large wounds in azotemic patients can cause accumulation of polyethylene glycol; long-term use can lead to resistance among staphylococci (Paul and Pieper, 2008; Benjamin and Christopher, 2009).

2.3.3 Wound examination and assessment

Wound management process begins with the complete and detailed examination of the patient to assess, ascertain and document the wound's aetiology, environment and nature.These aids in optimization of the patient's medical condition particularly diagnosis of life threatening injuries, any underlying conditions (exposed delicate tissues such as nerves, vascular supply, tendons, visceral organs and bones) (Amber and Swaim, 1984; Dissemond and Goos, 2004) and the restoration of blood flow to the wound area which may be achieved by proper wound cleansing and debridement (Hunt *et al.*, 2000; Dissemond and Goos, 2004).

2.3.4 Cleansing of wounds

Cleansing of the wounds of excess wound exudates, dirt, debris, necrotic tissues and dressing residue is essential for optimum healing. It involves the use of appropriate solution such as water or antiseptics and an adequate mechanical force preventing unnecessary chemical and mechanical trauma to the wound surface (Diegelmann and Evans, 2004; Broughton, *et al*., 2006a; Strecker-McGraw *et al.*, 2007; Raut *et al.*, 2015).

2.3.5 Debridement

Wound debridement is the removal of devitalized (non-viable), infected and hyperkeratotic tissues and foreign materials from a wound. This forms the basis of non-delayed as well as delayed wound healing as avascularization of tissues prevents leukocytes, cytokines, growth factors and other humoral factors that would have otherwise helped in infection control and haemostasis from gaining access to the wound site, thus the need to debride thereby accelerating wound healing (Witte and Barbul, 1997; Hunt *et al.*, 2000; Hassan *et al.*, 2003). Debridement can be achieved surgically by excision of all non-viable or necrotic tissues and including margin of normal tissues(Mekkes *et al.*, 1998).Chemical debridement is by use of agents such as hydrogen peroxide and mechanical debridement involves the use of larval therapy such as allowing maggots of *Phaenicia sericata*to feed on dead wound tissues with minimal effect on healthy tissues of the host(Mekkes *et al.*, 1998). Also by means of autolysis/enzyme treatment such as trypsin, potent proteolytic enzymes isolated from the intestines of *Euphausia superb* (Antarctic krill), Pancreatic dornase (Dornavac®) extracted from cattle (Mekkes *et al.*, 1998).

2.3.6 Wound drainage

Drainage is undertaken to remove unwanted materials from tissue spaces, obliterate dead spaces as well as elimination of subcutaneous accumulation of fluid and gas. Different drain types exists and they include; flat drains which effects drainage by capillary action and gravity, e.g. Penrose drain and tube drains which are plastics or rubber drains usually adopted from infusion tubes (Hassan *et al.*, 2003; Katrin, 2015). Their stiff nature however, constitutes a disadvantage as it may be discomforting to the patient. Sump drains consists of an outer drainage tube with a smaller inner tube/sump

lumen which permits air entry displacing fluid into the outer drainage lumen. Active drains can remove fluid against gravity (Hassan *et al.,* 2003; Katrin, 2015). The introduction of Vacuum-Assisted Wound Closure Technique which entails placing open-cell foam dressing into the wound cavity and applying a sub atmospheric pressure thus, removing oedema and promoting healing (Hartnett, 1998; Hassan *et al.,* 2003).

2.3.7 Surgical repair of underlying damaged tissues

Surgical repair in wound management involves apposition of complementary underlying viable structures such as muscles and subcutaneous tissues with subsequent approximation of wound edges obliterating dead spaces.Thus, reducing contamination of site, preventing infection and enhancing wound healing processes with minimal scar tissue formation (Hassan, *et al.,* 2003; Dissemond and Goos, 2004). During the operation, non viable/vital special tissues such as tendons and fascia are not removed as they promote the healing process in the wound (Dissemond and Goos, 2004).

2.3.8 Irrigation and lavage

The next important step is the irrigation and lavage which aid in further decreasing tissue microbial counts and also dead tissue and foreign bodies. Commonly, an antibiotic solution is used such as Bacitracin solution. In contrast, large volumes of saline have been recommended for use in irrigation (Fildes *et al.*, 1991; Hassan *et al.,* 2003). Wound lavage refers to the high-pressure irrigation of wounds using lavage solution in 20 – 50 ml syringe with the needle places close to the wound as the content of the syringe is forcefully delivered onto the wound surface by application of maximum depressing force on the plunger of the syringe(Swaim, 1983). This is aimed at decreasing wound contamination, overcoming bacteria adhesions and physically

dislodging debris and dirt from wound surfaces (Swaim, 1983). Although high pressure irrigation reduces bacterial colonization of the wound and the frequency of infections, a high pressure jet can damage fine tissues and push dirt particles deeper into the wound or even into the bone, thus the need for extreme care (Fildes *et al.*, 1991).

2.3.9 Wound closure

This refers to the wound management modalities employed to hasten healing by apposing the wound edges. This is achieved by primary closure, delayed primary closure or secondary closure and second intention healing. Primary closure is used for clean and minimally contaminated wounds without infection. It can be cleaned, lavaged and sutured as need be (Amalsadvala and Swaim, 2006). For large wounds, primary closure may need flaps (partially freed skin moved to close defects while still attached at its base) of sophisticated designs such as rotation flaps, transposition flaps, single- or bi-pedicle advancement flaps and subcutaneous pedicle flaps or skin grafts (Amalsadvala and Swaim, 2006; Bohling and Swaim, 2012; Hunt, 2012; Wardlaw and Lanz, 2012). Delayed primary closure is based upon the fact that the healing of open wounds gradually gains sufficient resistance to infection to permit an uncomplicated closure often within a week of injury while, Secondary closure is undertaken after the wound has granulated and contracted. This should however be undertaken before granulation tissue becomes too rigid to permit closure (Amalsadvala and Swaim, 2006; Labler *et al.,* 2006; Rivera and Spencer, 2007). Second intention healing could be said to be an open wound healing in which wound contraction, granulation and epithelisation in a centripetal manner are the primary means of healing. This is particularly suitable for the management of severely damaged and infected wounds (Wong *et al*., 1998; Hassan *et al.*, 2003; Strecker-McGraw *et al.*, 2007; Rivera and Spencer, 2007).

2.3.10 Wound dressing

Wound dressing aims at protecting wounds, relieving tension on sutures, decreasing excessive granulation tissue and providing ideal environment for proper epithelisation (Swihart, 1996; Witte and Barbul, 1997; Hunt *et al.*, 2000). Wound dressing in form of bandaging is undertaken to decrease contamination, absorption of exudates and drainage creation; minimize edema, hemorrhage and swelling; obliteration of dead space; improve epithelisation and wound contraction; immobilization of osseous structures; maintainace of moist wound environment; protection from further trauma; vehicle for topical antimicrobials as well as pain reduction. Properly applied dressing should be comfortable, therefore application should be done with precaution and dressing should be changed once wet to avoid maceration of the skin. In all situations therefore dressing must be kept clean, dry and intact (Swihart, 1996).

Dressings that have been applied in wound management include the Primary contact layers (adherent and non-adherent primary dressings), secondary dressings and tertiary dressings (Hassan *et al.*, 2003; Linder, 1998). Adherent primary dressing, used in early wound dressing involves the use of materials such as gauze (Linder, 1998). While dry-dry adherent dressings are suitable for excessively discharging wounds, Wet-Dry forms are employed when there is need to dissolve the exudates to facilitate absorption and Wet-Wet dressing encourages wound maceration (Hassan *et al.*, 2003; Williams, 1998). The non-adherent primary dressing is less traumatic during change and has been classified into occlusive non-adherent dressing employed for minimally discharging wounds and the semi-occlusive or hydrocolloid non-adherent dressing better suited for moderately discharging wounds (Lavel *et al.*, 1997; Williams, 1998). Hydrocolloids are used where there is the desire to explore the warmth/heat and moisture in the wound environment and examples of such dressings are intra-site gel®, Op-site®, Derma-

Gel®and Kaltostat®. Other non-adherent dressings include; polyurethane foam dressings, hydrogel, vapour permeable film and perforated absorbent like melonin (Lavel *et al.,* 1997; Williams, 1998). The secondary (absorptive) layer usually made of cotton wool, pads or disposable nappies and is primarily useful for fluid and necrotic debris absorption as well as to secure the layer, obliterate dead space or prevent edema in the wound environment. The tertiary (protective) layer serves as the protective layer of wound dressing and is often made of gauze rolls, cotton bandage and elastic bandages which occasionally serve to immobilize skeletal instabilities by the incorporation of suitable rigid support within the bandage (Swaim and Amber, 1990).

2.4 Alternative/Ethno-Medicine in Wound Care

Ethnomedicinal use of plants is one of the most successful tools used in pharmaceutical industries' searching for new therapeutic agents for various fields of biomedicine (Cox and Balick, 2012). The skin provides the human body with protection and a major barrier to environmental assault. Caring for skin is sometimes an afterthought. In other words, if something isn't broken, don't fix it. However, in the case of the integument, nothing could be further from the truth. Intact skin is paramount to health and well-being (Cynthia and Mackenzie, 2012). Table salt, raw eggs, sugar and vinegar, various parts of oil palm (Chong *et al.*, 2012; Ibraheem *et al.*, 2012; Sasidharan *et al.*, 2012, Vijayarathna and Sasidharan, 2012; Phin *et al.* 2013; Rajoo *et al.*, 2013), shea butter (Suntar *et al*., 2012; Malachi, 2014) as well as *Aloe vera* and their extracts (Yadav *et al*., 2012; Pankaj *et al*., 2013; Ahmed *et al*., 2015; Seyyed *et al*., 2015), amongst others have been empirically used in skin care and wound management. The use of honey on wounds has also been extensively studied in recent times with its bioactive chemical

constituents identified (Jull *et al*., 2008; Al-Waili *et al*., 2011; Khiati *et al.,* 2013; Molan, 2011; Awasum *et al.,* 2015;Hananeh *et al*., 2015; Jull *et al*., 2015). Complementary wound management employs acupuncture, energy healing, guided imagery, hypnosis, prayer and relaxation techniques to enhance wound healing (Papantonio, 1998). Many of the current conventional wound healing drugs are not only expensive but also pose problems of toxic effects, allergies and drug resistance. This situations coupled with the economic situation in a developing country like ours where the per capital income is very low makes it difficult for most people to afford drugs aggravating the individual's condition, thus the proliferation of ethno-medicinal therapies.

2.5 Wound Management Advances

Various medical approaches and therapeutic interventions have been found to affect the different processes involved in the wound healing. Following wounding, the healing time may be shorter when there is less injured tissue, for example during minimally invasive surgery, which reduces the amount of soft tissue damage and post-operative morbidity. Novel techniques of topical and systemic cytokines measurement and modulation can optimize both the cellular and molecular environment, thus decreasing healing time by modifying inflammation and accelerating the proliferative phase (Chen *et al.*, 1995; Sosa *et al.*, 2008; Braund *et al.*, 2009). Other advances which may optimize the remodelling phase by promoting more efficient fibroblast recruitment and collagen deposition are the Electrical field stimulation (Goldman *and Pollack*, 1996;Lin *et al.*, 2008; Sosa *et al.*, 2008) and some prosthetic materials and gene therapy. Some of these are currently in pre-clinical development and it is hoped that in combinations with

cytokines modulations may be able to provide a way for selective healing (Hodde, 2006; Ramelet *et al.*, 2009).

2.6 Cytokines

As far back as the early ninety's, several investigators have demonstrated that certain low molecular-weight proteins (5-20 kDa) called cytokines are secreted by various immune cells and play vital roles in wound healing (Wahi *et al.*, 1989; Mckay and Leigh, 1991; Robson and Heggers, 1992; Horst, 2013). Most of the earlier studies were performed using *in vitro* techniques with a common difficulty in determining the actual secreted cytokines in the processes involved in wound healing, limiting the high expectations of dramatic therapeutic benefits in their clinical applications (Grayson *et al.*, 1993; Ono *et al*., 1994). Evidences suggest the modulatory effects of inflammatory cytokines on the healing processes as they were found important in cell signalling, regulating the biological processes including the metabolic and physiologic responses to injury and infection. They bind to specific receptors, enabling immune cells communication, differentiation, proliferation and other activity which trigger inflammatory response to sites of injury and infection thereby enhancing healing (Horst, 2013; Borena *et al.,* 2015; Orzechowski, 2017). More recently, *in vivo* techniques are being undertaken with remarkable results even though the exact role of each specific cytokine in the inflammatory response during certain organ injuries is still a subject of ongoing research (Borena *et al.,* 2015; Orzechowski, 2017). Although it seems that the roles of cytokines such as IL-6, IL-8, IL-10 and TNF-α have been established in some immune and inflammatory diseases, but little is known about the role of these cytokine in normal physiological processes of cutaneous wound healing.

2.6.1 Properties/characteristics of cytokines

2.6.1.1 Redundancy

Cytokines exhibit the properties of redundancy where different cytokines have similar or same effects. Example is the proliferation of B cells under the signalling of activated Th cells produced IL-2, IL-4 and IL-5 (Molloy *et al.*, 1993; Sheeran and Hall,1997; Owen *et al.*, 2013). Another example is the recruitment of phagocytes such as neutrophils and macrophages under the signalling of IL-6 and IL-8 (Witte and Barbul, 1997; Bacon *et al.* 2002).

2.6.1.2 Pleiotropic effect

This refers to the phenomenon where one cytokine has different effects depending on the target cell. Example is the IL-4 produced by activated Th cells regulating B cell activation, proliferation and differentiation, as well as thymocyte and mast cell proliferation. IL-8 produced by activated Th cells regulating Th2 polarization and chemotaxis. Also interferon gamma signalling the decreased $CD4^+$ Th2 cell proliferation and production of IL-4 and IL-5, increased macrophage expression of class II MHC molecules and microbicidal activities, dendritic cell expression of class II MHC molecules, NK cell cytotoxic activities and B celldifferentiation and antibody production (increased IgE and IgG) (Owen *et al.*, 2013). IL-6 stimulates production of neutrophils in bone marrow, antagonizes regulatory T-cells thus supports B-cells growth and synthesis of acute phase proteins such as hepatic creatinine phosphate production (Giuliani *et al.*, 1993; Gueret *et al.*, 2009; Modan-Moses *et al.*, 2009; Hudetz *et al.*, 2011).

2.6.1.3 Synergism

Synergism is the combined effect of two cytokines on a cellular activity producing a greater than the additive effects of the individual cytokines activities. Example is the

induction of a B cells class switch to IgE by the combined activities of IL-4 and IL-5 (Molloy *et al.*, 1993; Sheeran and Hall,1997; Owen *et al.*, 2013). Another is the inhibition of IL-1 by IL-6 activation of IL-1ra (receptor antagonist) and IL-10 (Pedersen, 2013).

2.6.1.4 Antagonism

Antagonism occurs when the effect of a cytokine inhibits that of another as seen in the blockade of the class switch to IgE induced by IL-4 upon release of IFN-gamma by the activated Th cells (Molloy *et al.*, 1993; Sheeran and Hall,1997; Owen *et al.*, 2013). Also the inhibition of self reactive T cells by IL-10 in protection against autoimmune disease antagonizes the production of other proinflammatory cytokines like IL-6, IL-8 and TNF-α (Sikka *et al.*, 2012; Pedoeem *et al.*, 2014).

2.6.1.5 Cascade effect

Cytokine effectssuch as IL-1 or IL-6 released from endothelial cells following injury with resultantstimulation of other proinflammatory cytokines production, influence cytokine receptors expression and regulation of haematopoiesis, innate immunity and acquire immunity (Molloy *et al.*, 1993; Sheeran and Hall,1997; Owen *et al.*, 2013).Also IL-8 induces the production of IL-10 and IL-6 via chemotactic factors involvement in acute phase response in target cells (Ono *et al.*, 2005).

2.6.2 Cytokines production and classification

2.6.2.1 Production

Cytokines are known to be released by endothelial cells, activated lymphocytes, macrophages and monocytes in acute inflammation following injury or wound and in infections. For example, tumour necrosis factor (TNF), interleukin-6 (IL-6) and interleukin-1 (IL-1) are produced and released by endothelial cells, epithelial cells and

other immune cells (Tracey *et al*., 1987; Dinarello, 1994; Wang and Tracey, 1999). The various pro- and anti-inflammatory cytokines profile can be measured in serum and wound fluids by quantitative enzyme linked immunosorbent assay (ELISA) using the canine cytokine ELISA kits with preset minimum detectable levels of the cytokines by the manufacturers (Zuhoor *et al.*, 2012). Cytokine production and mechanism of action is sumerised in Figure 2.1.

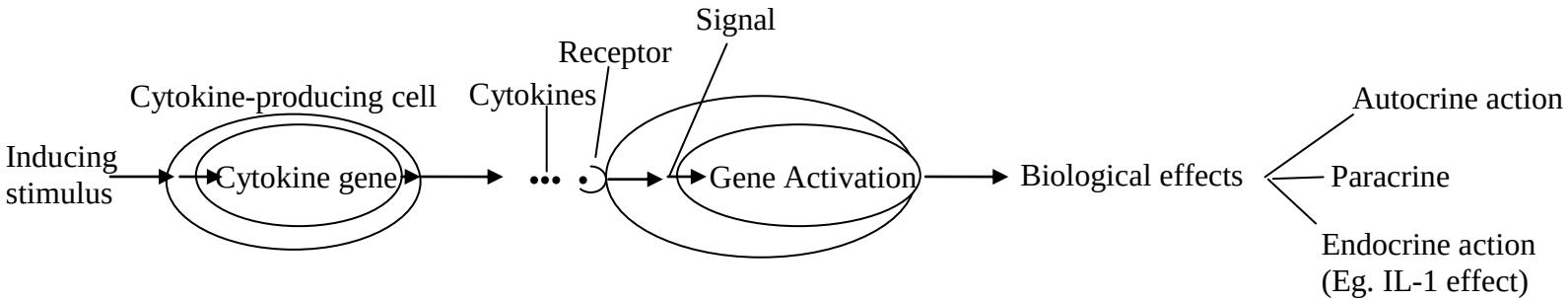


Figure 2.1: Schematic illustration of cytokines production and mechanism of action. Modified from (Burtis *et al.*, 2006, Masih, 2011 and Owen *et al.*, 2013).

2.6.2.2 Classification

Cytokines are generally classified based on their predominant function as pro-inflammatory and anti-inflammatory cytokines.

Pro-inflammatory cytokines:

Some of which include the tumour necrosis factor-alpha (TNF-α), interleukins such as interleukin-1 (IL-1β and IL-1α), interleukin-6 (IL-6), and interleukin-8 (IL-8), IL-17 and macrophage inflammatory proteins (MIP-1; CCL3, CCL4).

Anti-inflammatory cytokines:

Includes IL-4, interleukin-1 receptor antagonist (IL-1ra), interleukin-10 (IL-10), interleukin-13 (IL-13), transforming growth factor-β (TGF- β) and TNF-binding proteins 1 and 2 (TNF-BP-1 and TNF-BP-2).

Others cytokines form:

Include interferons and lymphokines (McBride *et al.*, 1996; Bown *et al.*, 2001; Quinton *et al.*, 2008; Bertesteanu *et al.*, 2014).

It's of importance to note that this classification is not rigid as many cytokines are capable of exerting both pro-inflammatory and anti-inflammatory effects depending on a variety of clinical and immunological factors. Although most investigators view cytokine responses in terms of pro-inflammatory and anti-inflammatory balance, there are suggestions that different patterns of cytokine response could best be described based on their concentrations as high, medium, and low, rather than their pro-inflammatory or anti-inflammatory activities (Kellum *et al*., 2007).

Another cytokines classification is based on receptor binding and seven cytokine receptors have been identified to be involves in the healing processes.

Immunoglobulin receptors (receptor-1):

include type I and II binding IL-1α and β respectively.

Haematopoietic receptor family (receptor-2):

This represents a large class displaying various combinations of cytokines receptor domain and binds IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, G-CSF and GM-CSF (Sheeran and Hall,1997).

Interferon receptor (IFNR) (receptor-3):

Includes IL-10 receptors and type I and II interferon receptors that binds to IFN-α and IFN-β, and INF-γ respectively.

Nerve growth factor receptor (NGFR) (receptor-4):

Comprises two tumour necrosis factor (TNF) receptors that specifically binds TNF-α and TNF-β.

G-protein-coupled seven transmembrane spanning family (receptor-5):

It's the α-helical receptor of complement 5a, PAF, IL-8 and many related chemokines.

Other receptor types:

They are classified from their intracellular catalytic domain; tyrosine kinase which phosphorylates an intracellular transcription protein upon activation with further passage of the incoming signal.

Soluble receptor and soluble binding proteins:

These represent the seventh receptor whose function is not clear but may act as an agonist (sIL-6R) and in other cases as an antagonist (sTNFR) and (IL-1ra) or transport

protein carrying cytokines to a distant target cell as seen with alpha-2-macroglobulin (Sheeran and Hall,1997).

2.6.3 Interleukin-6 (IL-6)

IL-6 is produced in wounds by epidermal keratinocytes, dermal fibroblasts, macrophages and many tissues including adipose tissue. Macrophages are responsible for an important part of the production of IL-6 (Weisberg *et al.*, 2003; Mateo *et al.*, 1994). Interleukin-6 has been shown to interact through activation of its cell-surface type cytokine receptor from a complex consisting of the ligand-binding with interleukin-6 receptor via the IL-6Rα signal chain (CD126) and the signal-transducing component glycoprotein 130 (gp-130, also called CD130) (Hibi *et al.*, 1990; Kallen*et al.*, 1997; Schwantner *et al.*, 2004). Cluster of differentiation 130 (CD130) is the common signal transducer for several cytokines as it is almost ubiquitously expressed in most tissues (Heinrich *et al.*, 1998). These complexes bring together the intracellular regions of gp-130 to initiate a signal transduction cascade through certain transcription factors, Janus kinases (JAKs) and Signal Transducers and Activators of Transcription (STATs) (Heinrich *et al.*, 1998; Owen *et al.*, 2013).

Interleukin-6 is a multifunctional cytokine which acts on many cells and tissues both as pro-inflammatory cytokine and anti-inflammatory myokine encoded by the IL-6 gene (Ferguson-Smith *et al.*, 1988; Sato *et al.*, 1999; Charlotte *et al.*, 2016). Generally, IL-6 secreted by T-cells and macrophages stimulate immune responses following trauma with resultant tissue damage, infections and in many diseases such as diabetes, atherosclerosis, depression, cancer and rheumatoid arthritis (van der Poll *et al.*, 1997; Smith *et al.*, 2001; Kristiansen and Mandrup-Poulsen, 2005; Nishimoto, 2006;

Dubiński and Zdrojewicz, 2007; Arjan *et al.*, 2017; James *et al.*, 2017). Following its production is the alteration of multiple pathologic and physiologic processes in favour of the healing process by the modulation of growth factors or their receptors such as the induction of keratinocytes growth factors (KGF) expression in fibroblasts as well as epidermal growth factors (EGF). When IL-6 is overexpressed in the skin, it induces transforming growth factor-alpha (TGFα); a potent keratinocyte mitogen, and inducer of IL-6 in keratinocyte *in vitro*, signifying a paracrine interaction (Navarro *et al.*, 1995; Sawamura *et al.*, 1998; Sato *et al.*, 1999). Interleukin (IL-6) are also secreted by macrophages in response to specific microbial molecules, referred to as pathogen-associated molecular patterns (PAMPs). These PAMPs binds to an important group of detection molecules of the innate immune system called pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). These are present on the cell surface and intracellular compartments and induce intracellular signaling cascades that give rise to inflammatory cytokine production (van der Poll *et al.*, 1997; Namas *et al.*, 2009). IL-6 is responsible for stimulating the production of neutrophils in the bone marrow and increases adhesion of neutrophils to dermal fibroblasts (Giuliani *et al.*, 1993). This is important to maintain the cellular homeostasis of neutrophils because neutrophils are viable in the circulation for 8–10 h before they succumb to cell death as they are terminally differentiated cells and have a very short lifespan (Jose *et al.*, 2013). IL-6 is also antagonistic to regulatory T-cells, supports the growth of B-cells and the synthesis of the acute phase protein such as the induction of hepatic creatinine phosphate production (Gueret *et al.*, 2009; Modan-Moses *et al.*, 2009; Hudetz *et al.*, 2011).

Interleukin-6 is considered a myokine; the first cytokine produced from muscle, which is elevated in response to muscle contraction especially due to exercise, preceding the appearance of other cytokines in the circulation(Petersen and Pedersen, 2005). It is thought to act in a hormone-like manner to mobilize extracellular substrates and/or augment substrate delivery during exercise (Petersen and Pedersen, 2005). The anti-inflammatory role of IL-6 is mediated through its inhibitory effects on IL-1 and TNF-alphaand activation of IL-1ra (interleukin-1 receptor antagonist) and IL-10 (Claus and Bente, 2010; Pedersen, 2013). IL-6 stimulates energy mobilization in muscle and fatty tissue, by crossing the blood-brain barrier initiating the synthesis of prostaglandin (PGE_2) in the hypothalamus, thereby changing the body's temperature set point that leads to increased body temperature (Banks *et al.*, 1994). It is now well known that IL-6 production by adipose tissue is enhanced in obesity (Fried*et al.*, 1998; Bastard *et al.*, 2002) and it is thought that 15 to 30 % of circulating IL-6 levels is derived from stroma vascular fraction of adipose tissues including pre-adipocytes, endothelial cells and monocytes-macrophages (Fain *et al.*, 2004).

It's been established that there is a strong interaction between cytokines (such as TNF-α and IL-6) and insulin signaling pathways that generally leads to impaired biological effect of insulin resulting in delayed wound healing(Kroder *et al.*, 1996; Mooney *et al.*, 2001). Although the exact mechanisms is yet to be clearly understood. Speculations have it that it could involve tyrosine phosphatase activation (Kroder *et al.*, 1996; Mooney *et al.*, 2001) or an interaction between suppressor of cytokine signalling (SOCS) proteins and the insulin receptor (Mooney *et al.*, 2001; Lagathu *et al.*, 2003; Rieusset *et al.*, 2004). Obesity is associated with a low-grade inflammation (with infiltration by macrophages) of white adipose tissue (WAT) which is the physiological

site of energy storage as lipids. Associated with this is the local production of some pro-inflammatory cytokines such as TNF-α and IL-6 which in excess may results in chronic activation of the innate immune system which can subsequently lead to insulin resistance, impaired glucose tolerance and even diabetes (Bastard *et al.*, 2006). Interestingly, weight loss is associated with a reduction in the macrophage infiltration of WAT and increased leptin, TNF-α, IL-6 and resistin levels but downregulated levels of adiponectin; an insulin-sensitising effector (Bruunsgaard, 2002; Bastard *et al.*, 2006; Arslan *et al.*, 2010; Salem *et al.*, 2015).

Exhibition of significantly delayed cutaneous wound healing characterized by minimal epithelial bridge formation, reduced inflammation and granulation tissue formation was observed in IL-6 - deficient transgenic mice (IL-6 Knockout (KO) when compared with the wild-counterpart serving as control animals which revealed IL-6 mRNA expression primarily in the epidermis at the leading edge of the wound when subjected to *In-situ* hybridization of wound tissue (Chen-Kiang *et al.*, 1993; Cressman *et al.*, 1995; Takeda *et al.*, 1998; Solis-Herruzo *et al.*, 1999). Inflammatory mediators such as TNF-α and IL-1 independent of IL-6 have been speculated as being responsible for the early transcriptional activation during wound healing with peak induction occurring 2 h after wounding (Kondo and Ohshima, 1996; Adcock, 1997). In contrast, IL-6 is induced more slowly after wounding in normal rats, reaching maximal induction within 12 and 16 h. before gradually returning to basal levels after 24 h. (Kondo and Ohshima, 1996; Gallucci *et al*., 2000). Worthy of note is the reversal of delayed wound healing in IL-6 KO mice with a single dose of recombinant murine IL-6 or intradermal injection of an expression plasmid containing the full-length murine IL-6 cDNA which also reestablished wound healing in glucocorticoid (dexamethasone) treated

immunosuppressed mice, indicating the potential of IL-6 as a therapeutic agent in delayed or impaired cutaneous wound resulting from varying events and circumstances (Gallucci *et al.,* 2000).

2.6.4 Interleukin-8 (IL-8)

Interleukin 8 (IL-8) is a member of the chemokine family also known as CXCL8 and are produced by macrophages, epithelial cells, airway smooth muscle cells, endothelial cells which store IL-8 in their storage vesicles, the Weibel-Palade bodies and other cell types (Wolff *et al.*¸1998; Utgaard *et al.,* 1998; Hedges *et al.*, 2000; Bacon *et al.*, 2002). In nature, IL-8 is encoded by the IL-8 gene which forms a cluster in a region mapped to chromosome 4q and is initially produced as a precursor peptide of 99 amino acids long which then undergoes cleavage to create several active IL-8 isoforms (Brat *et al.*, 2005). IL-8 is secreted through a chain of biochemical reactions and is an important mediator of immune reactions in the innate immune system's response. IL-8 can be secreted by cells with toll-like receptors such as macrophages which are the first cells to release IL-8 to recruit other cells (Brat *et al.*, 2005). Numerous receptors are capable of binding IL-8 on surface membranes with the most frequently studied types being the G protein-coupled serpentine receptors CXCR1 and CXCR2 whose expression and affinity for IL-8 differs with those of CXCR1 greater than CXCR2 (Brat *et al.*, 2005). IL-8 expression is regulated by a transcription factor; NF-κB(nuclear factor kappa-light-chain-enhancer of activated B cells) which represents a novel anti-IL-8 therapy for use in inflammatory diseases (Rottner *et al.*, 2009).

IL-8 frequently referred to as the neutrophil chemotactic factor is involved in acute phase response by the induction of chemotaxis in target cells, primarily neutrophils but

also other granulocytes, causing them to migrate toward the site of injury and/or infection. However, opposed to expectations, IL-1 which was believed to be the initiator of inflammatory process has been detected at low levels relative to the rather high IL-8 levels (Ono *et al.*, 1995). In target cells, IL-8 induces a series of physiological responses required for migration and phagocytosis, such as increases in intracellular Ca^{2+} and exocytosis (e.g. histamine release). They are a potent angiogenesis promoter with macrophages, mast cells and keratinocytes responding well to this chemokine (Köhidai and Csaba, 1998; Burton *et al.*, 2004). Interleukin-8 is often associated with inflammation as cited as a pro-inflammatory mediator in gingivitis and psoriasis (Bertha *et al.*, 2017). Its secretion is increased by oxidant stress causing the recruitment of inflammatory cells, making it a key parameter in localized inflammation (Sharabiani *et al.*, 2011). IL-8 is also suspected to be associated with obesity and its surrounding problems (Sharabiani, *et al.*, 2011; Bertha *et al.*, 2017).

2.6.5 Interleukin-10 (IL-10)

IL-10 also known ascytokine synthesis inhibitory factor (CSIF), is an anti-inflammatorycytokine encoded by the IL-10 gene. Monocytes are the primarily producers of IL-10 withlymphocytes, namely; type 2 T-helper cells (T_H2), mastocytes, $CD4^+CD25^+Foxp3^+$ regulatory T cells, and certain subset of activated T cells and B cells also producing IL-10 (Eskdale *et al.*, 1997; Said *et al.*, 2010). IL-10 belongs to a set of cytokine classified as class-2 comprising of IL-19, IL-20, IL-22, IL-24 (Mda-7), IL-26, interferons (IFN-alpha, -beta, -epsilon, -kappa, -omega, -delta, -tau, and -gamma) and interferon-like molecules (limitin, IL-28A, IL-28B, and IL-29) (Pestka *et al.*, 2004). The IL-10 protein is a homodimer with each subunit made-up of 178-amino-acid that signals through a functional receptor complex consisting of four IL-10 molecules (two

IL-10 receptor-1 and two IL-10 receptor 2 proteins) (Zdanov *et al.*, 1995; Mosser and Zhang, 2008). IL-10 binding induces STAT 3 signaling via the phosphorylation of the cytoplasmic tails of IL-10 receptor 1 + IL-10 receptor 2 by JAK1 and tyrosine kinase 2 (Tyk2) respectively (Mosser and Zhang, 2008; Jia *et al.*, 2015). IL-10 has been shown to interact with interleukin-10 receptor-alpha subunit with the receptor complex for IL-10 requiring the IL-10R2 chain to initiate signalling (Ho *et al.*, 1993; Tan *et al.*, 1995; Hoover *et al.*, 1999; Josephson *et al.*, 2001a; Josephson *et al.*, 2001b).

Programmed death (PD-1) pathway which is a co-receptor that is predominantly expressed on T cells binds to the ligands (PD-L1 or PD-L2) triggering monocytes to produce IL-10. The minimal expression of IL-10 in unstimulated tissues regulates the immune system by the inhibition of self rective T cells, which serve to protect against autoimmune diseases and thus extends life (Said *et al.*, 2010; Li *et al.*, 2012; Pedoeem *et al.*, 2014; Mehdi and Maryam, 2014; Dina *et al.*, 2016; Daniel *et al.*, 2017). Induction of IL-10 involves ERK1/2 (extracellular signal-regulated kinases 2), p38 (mitogen activated protein kinases) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signalling and its expression is tightly regulated at the transcriptional and post-transcriptional (involving control of mRNA stability via AU-rich elements (adenylate-uridylate-rich elements) and by microRNAs such as let-7 and miR-106) levels with extensive IL-10 locus remodelling observed in monocytes upon stimulation of its receptors (TLR or Fc receptor pathways) (Eskdale *et al.*, 1997; Powell *et al.*, 2000; Sharma *et al.*, 2009; Saraiva and O'Garra, 2010; Schulte *et al.*, 2011). IL-10 blockade of NF-κB activity is involved in the regulation of the JAK-STAT signalling pathway. Negative feed-back loop via autocrine stimulation of the IL-10 receptor and inhibition of the p38 signaling pathway makes possible the autoregulation of its expression (Hammer *et al.*, 2005). IL-10 is a cytokine with multiple, pleiotropic, effects

in immunoregulation and inflammation. It downregulates the expression of Th1 cytokines, MHC class II antigens, co-stimulatory molecules on macrophages, enhances B cell survival, proliferation, and antibody production. Knockout studies in mice supported the function of this cytokine as an immunoregulator demonstrating the counteracting effects of IL-10 for hyperactive immune response in the body (Braat *et al.*, 2006). Also study showed the mast cells production of IL-10 counteracts the inflammatory effect that these cells have at the site of an allergic reaction (Grimbaldeston, *et al.*, 2007).

IL-10 possess the capability of inhibiting macrophages and regulatory T-cells' synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, IL-6, IL-8, TNFα and GM-CSF. IL-10 checks Cyclo-oxygenase-2 (COX-2) as a lack of IL-10 has been shown to cause COX activation and resultant Thromboxane receptor activation causing vascular endothelial and cardiac dysfunctions in mice (Sikka *et al.*, 2013). It has also been linked to the myokines, as exercise provokes an increase in circulating levels of IL-1ra, IL-10, and sTNF-R, in response to the pro-inflammatory cytokines increase (Ostrowski *et al.*, 1999; Ostrowski *et al.*, 2000). IL-10 is actively involved in the regulation of TNF-α levels via TNF-α-converting enzyme regulation (Brennan *et al.*, 2008), enhancing release of soluble TNF receptor and inhibition of the expression of surface receptors/adhesion molecules (ICAM-1) thus, reduces neutrophil adhesion to activated endothelial cells (Willems *et al.*, 1994; Sheeran and Hall,1997).

2.6.6 Tumor necrosis factor (TNF-α)

In 1968 the physician; William B. Coley reported the anti-tumoral response of the immune system*in vivo* and recognized a cytotoxic factor produced by

lymphocytes which he later named lymphotoxin (LT) (Kolb and Granger, 1968; Ruddle and Waksman, 1968). Subsequently, report of another cytotoxic factor produced by macrophages was filed and named tumor necrosis factor (TNF) (Carswell *et al.*, 1975; Morteza *et al.*, 2017). Both factors were described based on their ability to kill mouse fibrosarcoma L-929 cells and these concepts were extended to systemic disease in working with pre-sequencing era data with inferences that the excessive production of TNF causes disease and endotoxin poisoning (Clark *et al.*, 1981; Clark, 1982; Morteza *et al.*, 2017). Further works revealed and confirmed the sequential and functional homology between the two factors with their cloned complementary DNA (cDNAs) encoding LT and TNF with the binding of TNF to its receptor and its displacement by LT which led to the renaming of TNF as TNFα and LT as TNFβ (Pennica, 1984). In 1985, came the discovery that cachectin; a hormone which induces cachexia, was actually TNF (Beutler *et al.*, 1985). Furthermore, the key mediator role of TNF in lethal septic shock and the therapeutic effects of monoclonal anti-TNF antibodies were identified (Tracey *et al.*, 1986; Tracey *et al.*, 1987). Research has also shown that TNF can prevent the death/apoptosis of neurons by a mechanism involving activation of the transcription factor NF-kappaB which induces the expression of factors such as Mn-SOD (Manganese-dependent superoxide dismutase) and Bcl-2 (B-cell lymphoma 2 – Apoptosis regulator). Recently, reserchers have argued that TNFα should simply be called TNF as TNFα has been shown to interact with TNFRSF1A (Bouwmeester *et al.*, 2004; Micheau and Tschopp, 2003; Clark, 2007; Olszewski *et al.*, 2007).

Tumor necrosis factor (TNF), also known as tumor necrosis factor alpha (TNF-α), cachexin, or cachectin is one of the cell signaling proteins, although produced by

many cell types such as CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, endothelial cells, cardiac monocytes, adipose tissues, fibroblast and neurons.It is chiefly produced by activated macrophages and make up the acute phase reaction and also involved in systemic inflammation. In the skin, mast cells appear to be the predominant source of pre-formed TNF, which can be released upon inflammatory stimulus (Walsh, 1991). TNF is produced as a type II transmembrane protein of 233-amino acid-long arranged in stable homotrimers (Kriegler *et al.*, 1988; Tang *et al.*, 1996). This membrane-integrated form of soluble homotrimeric cytokine (sTNF) is released via proteolytic cleavage by the TNF alpha converting enzyme metalloprotease (TACE, also called ADAM17) (Black *et al.*, 1997).

The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen like IL-6, is able to induce fever, apoptotic cell death, cachexia inflammation, inhibit tumorigenesis and viral replication and respond to sepsis via IL-1and IL-6 producing cells. TNF production dysregulation has been reported to play a role in a variety of diseases including cancer and inflammatory bowel disease (IBD) (Locksley *et al.*, 2001; Brynskov *et al.*, 2002; Mikocka *et al.*, 2007; Dowlati *et al.*, 2010; Swardfager *et al.*, 2010).

TNF can bind two receptors TNF receptor type 1 (TNFR1; CD120a; p55/60) which is 55-kDa and TNF receptor type 2 (TNFR2; CD120b; p75/80) which is 75kDa (Theiss *et al.*, 2005). While TNFR1 is expressed in most tissues and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF, TNFR2 is found only in cells of the immune system, with response directed to the membrane-bound form of the TNF homotrimer. As most information regarding TNF signaling is derived from TNFR1, the role of TNFR2 is likely underestimated (Theiss *et al.*, 2005). Upon contact with

their ligand, TNF receptors also form trimers, their tips fitting into the grooves formed between TNF monomers. This binding causes a conformational change to occur in the receptor, leading to the dissociation of the inhibitory protein; silencer of death domain (SODD) from the intracellular death domain. This dissociation enables the adaptor protein; TNF-1 associated death domain) TRADD to bind to the death domain, serving as a platform for subsequent protein binding. Following TRADD binding, three pathways can be initiated namely; activation of NF-κB (NF-κB is a heterodimeric transcription factor that translocates to the nucleus and mediates the transcription of a vast array of proteins involved in cell survival and proliferation, inflammatory response, and anti-apoptotic factors) (Chen and Goeddel, 2002 ;Wajant *et al.*, 2003), activation of the mitogen-activated protein kinases (MAPKs) in association with the c-Jun N-terminal kinases(JNK) pathway (The JNK pathway is involved in cell differentiation, proliferation, and generally pro-apoptosis) (Chen and Goeddel, 2002 ;Wajant *et al.*, 2003; Kant *et al.*, 2011) and induction of death signaling via TNFR1 superfamily. However, TNF-induced cell death plays only a minor role compared to its overwhelming functions in the inflammatory process. Its death-inducing capability is weak compared to other family members (such as Fas transmembrane protein) (Chen and Goeddel, 2002; Gaur and Aggarwal, 2003; Wajant *et al.*, 2003). Other factors, such as cell type, concurrent stimulation of other cytokines, or the amount of reactive oxygen species (ROS) can shift the balance in favor of one pathway or another. Such complicated signaling ensures that, whenever TNF is released, various cells with vastly diverse functions and conditions all respond appropriately to inflammation. TNF-α and IL-1b are transiently induced immediately after cutaneous injury, peaking at; 3 h, after which both cytokines decrease to near basal levels by 10 h (Kondo and Ohshima, 1996; Adcock, 1997).

It has a number of actions on various organ systems, generally together with IL-1 and Interleukin-6 (IL-6). On the hypothalamus they stimulate the hypothalamic-pituitary-adrenal axis by stimulating the release of corticotropin releasing hormone (CRH) with resultant suppression of appetite and fever. On the liver they stimulate the acute phase responses, leading to an increase in C-reactive protein and a number of other mediators. It also induces insulin resistance by promoting serine-phosphorylation of insulin receptor substrate-1 (IRS-1), which impairs insulin signaling. It is a potent chemoattractant for neutrophils, and promotes the expression of adhesion molecules on endothelial cells, helping neutrophils migrate. On macrophages it stimulates phagocytosis, and production of IL-1 oxidants and the inflammatory lipid prostaglandin E2 (PGE_2). A local increase in concentration of TNF will cause the cardinal signs of Inflammation to occur: heat, swelling, redness, pain and loss of function. Whereas high systemic concentrations of TNF induce shock-like symptoms, the prolonged exposure to abnormal concentrations of TNF can result in cachexia, a wasting syndrome as can be seen in cancer patients (Said *et al.*, 2010). TNFα causes an IL-10-dependent inhibition of CD4 T-cell expansion and function by up-regulating PD-1 levels on monocytes which leads to IL-10 production by monocytes after binding of PD-1 by PD-L (Said *et al.*, 2010; Pedoeem *et al.*, 2014). TNF-alpha has also been documented to induce endothelial dysfunction while IL-6 promotes pro-coagulant changes and both cytokines cause dyslipidaemia. TNF-alpha and IL-6 are also differently and independently of each other associated with mortality in elderly populations, indicating points of distinction in the biological effects of the two cytokines (Bruunsgaard, 2002). IL-10 regulates TNFα levels as IL-10 effectively regulates the TNF-α-converting enzyme and a decrease in IL-10 levels causes TNFα levels to rise resulting in inflammation of tissues and even neurons (Brennan *et al.*, 2008; Nakahara *et al.*, 2012).

Various cytokines are released at different levels over time by activated macrophages, monocytes, and lymphocytes and other cells during infection, injury, and acute inflammation (Wang and Tracey, 1999; Tracey *et al.*, 1987; Dinarello, 1994).Several classical risk factors are controlled by cytokines as seen with TNF-alpha and IL-6 overproduction in adipose tissue of several rodent models of obesity, local effects on white adipose tissue (WAT) physiology, pathophysiology of insulin resistance and systemic effects on other organs (Hotamisligil *et al.*, 1993; Bruunsgaard, 2002; Bastard *et al.*, 2006; Charlotte *et al.*, 2016; Caitlin *et al.*, 2017). It was recently demonstrated that neutropenic patients have local TNF-α production which may be of significance in determining the severity and possible reason for the poor prognosis in disease and injury (Allaouchiche *et al.*, 2004). Cytokines also provokes the acute phase responses with alteration in temperature set point in hypothalamus mediated through production of PGE_2 with the generation of a febrile response. There is also changes in plasma composition, increase in number of leucocytes especially granulocytes, stimulation of adrenocorticotrophic hormone (ACTH) secretion by modulators such as IL-6 and IL-1 with consequent cortisol and acute phase proteins (such as C-reactive protein (CRP)) production by various organs (Sheeran and Hall,1997; Burton *et al.*, 2004). Inferences drawn from results of various experimental reports suggests that IL-6, IL-8, IL-10 and TNF-α are important in wound inflammation with IL-6 and TNF-α actively involves in seroma formation after 24 and 48 hours as was obtained post-surgery. There appear to be a true compartmentalization with preferential cytokine secretion for specific locations as their levels in wound fluid as well as sputum were higher than serum for patients with lung injuries indicating their local production (Moret *et al*., 2011; Zuhoor *et al.*, 2012). Results from researches on cytokines (such as IL-1, IL-6, IL-8, and IL-10)

suggest that they act as acute-phase proteins with concentrations generally highest on admission and decline rapidly over the first few days (Ferna´ndez-Serrano, *et al.*, 2003; Kellum, *et al.*, 2007; Endeman, *et al.*, 2011). Analysis of serial samples demonstrated rapid increase in IL-6 and IL-8 concentrations within 12 h of injury. IL-6 and IL-8 remained increased for the next 24 h after injury and decreased markedly from their peak values during the next 24 h increased circulatory levels of these cytokines continues to be present for greater than 5 days in the severely injured patients. IL-6 and IL-8 concentrations were only minimally increased in patients 8 and 24 hrs after moderate injury (Hoch *et al.*, 1993; Bertha *et al.*, 2017). However, some authors suggests that cytokine levels might actually be more predictive of adverse outcomes when measured at the end of hospitalization, rather than on admission (Remick *et al.*, 2002; Ioanas *et al.*, 2004; Remick *et al.*, 2005; Wu *et al.*, 2007; Yende *et al.*, 2008; Martı´nez *et al.*, 2011; Mehdi and Maryam, 2014). Thus, understanding the different cytokine responses elicited in different conditions is vital in identification of early cytokine dysregulation which may lead to toxic inflammatory responses. This may serve in the development of potential therapeutic approaches targeting biomarkers enabling timely therapies to modulate inflammation (McConnell *et al.*, 2010; Mene´ndez *et al.*, 2012; Jose *et al.*, 2013; Ankit *et al.*, 2015; Charlotte *et al.*, 2016).

2.7 Wound Healing

Wound healing is initiated and proceeds from the moment an injury is sustained through the repair process and it involves the extracellular matrix, resident and migratory cell populations and the action of soluble mediators. The mechanisms underlying these processes involve inflammatory mediators; cytokines and growth factors which mediate cell to cell and cell to extracellular matrix interactions that govern cell proliferation,

migration and differentiation, events involved with angiogenesis, fibroplasias and epithelialization; wound contraction and remodelling (Lawrence, 1998; Robson *et al.*, 2001; Labler *et al.*, 2006; Broughton *et al.*, 2006a; Velnar *et al.*, 2009). However, there are differences between wound healing time in different tissues and locations as some may take up to a year or more to heal completely (Komarcevic, 2000; Flangan, 2000; Richardson, 2004). Disturbance in the finely balanced repair processes of wounds results in chronicity or non-healing wounds. A completely healed wound is defined as one that has been returned to a normal anatomical structure, function and appearance of the tissue within a reasonable period of time (Robson *et al.*, 2001; Strecker-McGraw *et al.*, 2007). It is worthy of note that separate parts of a wound may be at different stages of healing at any one time (Broughton *et al.*, 2006a).

2.7.1 Types of wound healing

Wounds heal either by regeneration as seen in fetal life or by repair that is seen in postnatal life. Successful wound healing depends on the timely and optimal functioning of the many and diverse processes involving different cell types, molecular mediators and structural elements of the diverse immunological and biological systems. These involves a cascade of carefully and precisely regulated steps and events that correlate with the appearance and disappearance of the various cytokines, growth factors and cell types in the wound bed (Glat and Longaker, 1997; Hunt, 2000; Broughton *et al.*, 2006a). Different cells dominate various phases of the repair, and cellular patterns which vary according to the different types of injury and the extent of tissue damage (Robson *et al.*, 2001; Izadi and Ganchi, 2005; Amalsadvala and Swaim, 2006).

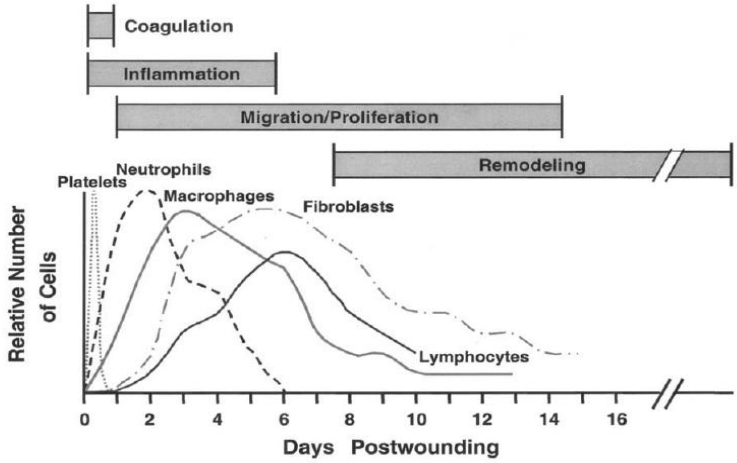


Figure 2.2: Time line of migration of immune cell populations correlated with the phases of wound healing (Witte and Barbul, 1997).

Healing types are largely dependent on the closure method/type in postnatal life. For better comprehension of the physiology of wound healing processes, it has been arbitrarily divided into two broad types; healing by primary intention and healing by secondary intention (Lawrence, 1998; Richardson, 2004; Toy, 2005).

2.7.1.1 Regenerative healing

The repair process as seen in fetal life which is also known as scarless healing is however not absolute but dependent on gestational age and wound size with the possibility of scarring in large wounds especially in late fetal life (Glat and Longaker, 1997; Simon and Ali, 2015).

2.7.1.2 Reparative healing

Although in postnatal life the almost permanent inevitable consequence of wounding beyond the epidermal basement membrane is scarring. Compromise between regeneration and repair at a clinical level is significantly patient related and subjective making scaring in wound healing inseparable at some level and over time (Nicholas *et al.*, 2012). Advances in regenerative medicine attempts to harness the same plasticity seen in lower vertebrate such as the regeneration of an amputated limb by an adult Salamander but what is generally achieved is only 'partial regeneration and not replacement of like with like owing partly to constraints posed by the risk of loss of control in the manipulation of adult/somatic stem cells resulting in carcinogenesis (Glat and Longaker, 1997; Simon and Ali, 2015). Most patients would perceive a wound as a scar from the time that the wound is no longer open with the absence of exudates and no requirement for dressing care. Most normotrophic young scars exhibit features of hypertrophy and then go through a plateau period of relatively little clinical change in the absence of treatment followed by the period of regression of inflammatory signs and

symptoms, and eventually settle to a mature state after many months or even years and sometimes because of treatment, become very thin, pale and atrophic (Nicholas *et al.*, 2012; Simon and Ali, 2015).

2.7.1.3 Epidermal wound healing

Epidermal wound healing in cell biology is relatively well understood with keratinocyte stem cells residing primarily in the bulge region of the hair follicle populating the interfollicular basement membrane by asymmetric division (Lavker, *et al.*, 2003; Bickenbach, 2005) providing the differentiating cells of the stratifying epidermis. All this takes place under the huge array of small wound healing and scar forming peptides (cytokines and growth factors) involving the coordinated response to epidermal wounding by autocrine, paracrine and juxtacrine signalling (Myers *et al.*, 2007). Regeneration then occurs from a population of follicle stem cells with the dermis serving the role of structural support to the epidermis and its related adnexae (Lavker, *et al.*, 2003).

2.7.1.4 Dermal wound healing

More attention has been concentrated on dermal wound studies than epidermal and particularly on macrophage production of interleukins (such as IL-1, IL-6, IL-8, IL-10, TNFα) and TGF-β isoforms. TGF-β; a multifunctional growth factor appears to play a key role in dermal healing and scarring (O'Kane and Ferguson, 1997; Rosengardten *et al.*, 2011). Although the TGF-β1 isoform promotes scarring, the TGF-β3 isoform appears to have the opposite effect (Occleston *et al.*, 2008) and was developed to improve the quality of normotrophic scarring (Renovo, 2011). The dermis is populated by fibroblasts and hosts vascular network (arterial, venous and lymphatic), neural network, and supports folliclar and other adnexal stem cell niches (Lim *et al.*, 2013).

There are many factors at play but the interleukins and platelet-derived growth factor (PDGF) has shown great promise when delivered (Steed, 2006; Lim *et al.*, 2013).

2.7.1.5 Healing by primary intention

The least complicated example of wound repair is the healing of clean wounds without tissue loss and the incisions are approximated by sutures. This is referred to as healing by primary intention (Greenhalgh, 1998; Attinger *et al.*, 2006; Labler *et al.*, 2006; Rosengardten *et al.*, 2011). Death of a limited number of epithelial and connective tissue cells may occur because of small disruptions in basement membrane continuity (Labler *et al.*, 2006; Strecker-McGraw *et al.*, 2007). It is a fast process achieved by primary closure, delayed primary closure or secondary closure. Primary closure is used for clean and minimally contaminated wounds without infection. It can be cleaned, lavaged and sutured as need be (Swaim, 1983; Attinger *et al.*, 2006). For large wounds, primary closure may need flaps (partially freed skin moved to close defects while still attached at its base) or sophisticated designs such as rotation flaps, transposition flaps, single- or bi-pedicle advancement flaps and subcutaneous pedicle flaps, skin grafts or surgical tape closure (Linder, 1988; Wong *et al.*, 1998; Dena and Jennifer, 2012). Delayed primary closure is based upon the fact that the healing of open wounds gradually gains sufficient resistance to infection to permit an uncomplicated closure often within a week of injury (Hassan *et al.*, 2003). Secondary closure is undertaken after the wound has granulated and contracted just before the granulation tissue becomes too rigid to permit closure (Eliya-Masamba and Banda, 2013).

2.7.1.6 Healing by secondary intention

Second intention healing is an open wound healing in which wound contraction, granulation and epithelisation occurs in a centripetal manner as the primary means of repair and this is particularly suitable for the management of severely damaged and

infected wounds (Wong *et al*., 1998; Hassan *et al.*, 2003; Rivera and Spencer, 2007). Here large tissue losses have to be filled making the reparative process more complicated than the healing in primary intention closures (Rice, 2000; Robson *et al*,, 2001; Rivera and Spencer, 2007; Dena and Jennifer, 2012). In comparison to healing by first intention, large amounts of granulation tissues is formed in order to fill the defect making this process longer (Rice, 2000; Robson *et al*,, 2001; Rivera and Spencer, 2007; Ward *et al.*, 2013).

2.7.2 Stages and phases of wound healing

Although acute and chronic wounds have similar phases of healing, the timing and interactions between the components taking part in the wound healing processes differ significantly (Glat and Longaker, 1997; Robson *et al.*, 2001; Diegelmann and Evans, 2004). The various processes of acute tissue repair, which are triggered by tissue injury, may be united into a sequence of four orderly interactions and time-dependent phases, namely; coagulation and haemostasis, inflammation, proliferation and wound remodelling (Hunt, 1998; Attinger, *et al.*, 2006; Broughton *et al.*, 2006a; Velnar *et al.*, 2009).

2.7.2.1 Coagulation and haemostasis phase

Noxious insult causes microvascular injury with extravasation of blood and immediate commencement of coagulation and haemostasis by activation of the clotting cascade, complement cascade and complement vasodilatation which principally prevents exsanguinations thereby protecting the vascular system (Simon and Ali, 2015). Saror *et al.*, (1979), Awah and Nottidge, (1998), Ariyibi *et al.*, 2002 and Olayemi *et al.*, (2009) reported on the various blood components and their ranges in apparently normal dogs. A dynamic balance between endothelial cells, thrombocytes (platelets), coagulation, and fibrinolysis regulates haemostasis and these determines the amount of fibrin deposited

at the wound site, thereby influencing the progress of the reparative processes (Lawrence, 1998). Temporary cessation of bleeding is achieved through the neuronal reflex mechanism involving several strong vascular smooth muscle cells' constrictions in the circular muscle layer of injured vessels (Lawrence, 1998). However, bleeding may resume as hypoxia and acidosis in the wound wall causes a change in muscle tone by passive relaxation but, the formation of an insoluble fibrin plug enhances the haemostatic mechanisms to be effective on the longer term (Robson *et al.*, 2001; Strecker-McGraw *et al.*, 2007). Synergistic with the haemostatic events is the coagulation cascade which is activated through extrinsic and intrinsic pathways involving platelet aggregation coming in contact with exposed collagen and other extracellular matrix components. This contact triggers the release of clotting factors from the platelets and the formation of a blood clot, composure of fibronectin, fibrin, vitronectin and thrombospondin, limiting blood loss and also serves as a provisional matrix for cell migration in the subsequent phases (Lawrence, 1998; Robson *et al.*, 2001; Velnar *et al.*, 2009; Simon and Ali, 2015). Platelets contain α-granules filled with growth factors and cytokines such as interleukins, platelet-derived growth factor (PDGF), epidermal growth factor, insulin-like growth factors and transforming growth factor beta (TGF-β) in their cytoplasms (Cho-Hee *et al.*, 2015). Mast cells release histamines and kinins as mediators upon degranulation, contributing to vasodilatation and increased vascular permeability. Increasing interest is also in platelet-rich plasma (PRP) clinically in regenerative medicine which is based on these early cascades (Lawrence, 1998; Simon and Ali, 2015). The molecules/mediators involved act by activating and attracting neutrophils, macrophages, endothelial cells and fibroblasts at different times with timely production of the appropriate cytokines such as the

interleukins-1(IL-1), IL-4, IL-6, IL-8, IL-10, TNF and growth factors which are promoters of the wound healing cascade (Lawrence, 1998; Broughton *et al.*, 2006a).

2.7.2.2 Inflammatory phase

The inflammatory phase aid in establishing an immune barrier against invading micro-organisms and it's been postulated that inflammatory response following cutaneous injury is a pre-requisite for healing with high possibilities of inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and Tumor necrosis factor-alpha (TNF-α) intimately involved in this process (Bluethmann *et al.*, 1994; Cressman *et al.*, 1996). Inflammation is a tightly balanced process between the pro-inflammatory and anti-inflammatory cytokines checking potential tissue damage from cytokines dysregulation. Therefore, in injury and infection, pro-inflammatory cytokine response is normally regulated by the anti-inflammatory cytokines (Antunes *et al.*, 2002; Ferna´ndez-Serrano *et al.*, 2003; Wattanathum *et al.*, 2005). Different inflammatory patterns are elicited by different forms of injuries and colonizing microorganisms and may provide a useful tool for both diagnosis and treatment (McConnell *et al.*, 2010; Mene´ndez *et al.*, 2012). When injuries occur, platelets releases vasoactive amines, such as serotonin, that are stored in their dense bodies and cause vasodilation and increased vascular permeability, leading to fluid extravasation in the tissue that result in oedema which, in turn, potentiates itself during the following inflammatory phase (Lawrence, 1998; Richardson, 2004). Eicosanoids and other products of arachidonic acid metabolism are released after injury to cell membranes and have potent biological functions in the immediate inflammatory response (Lawrence, 1998; Robson *et al.*, 2001; Toy, 2005; Strecker-McGraw *et al.*, 2007). Two separate division of this phase exists; an early inflammatory phase and a late inflammatory phase (Hart, 2002).

Early Inflammatory phase:

This is the time between injury and around 4 days post injury characterized by the classical signs of inflammation; redness, swelling, pain and loss of function accompanied by changes in temperature, pulse rates and respiratory rates (Slatter, 1985; Said *et al.*, 2010; Simon and Ali, 2015). This commences just shortly after the late phase of coagulation with early inflammatory response that has many functions including the activation of the complement cascade which initiates some molecular events, leading to the infiltration of wound site by immune cells such as phagocytes:macrophages followed by neutrophils and then lymphocytes (Broughton *et al.*, 2006a; Simon and Ali, 2015; Takeshi *et al.*, 2015). These immune cells are vital to the regulation of the wound-healing process through the secretion of signaling molecules, such as cytokines, lymphokines, and growth factors (Park and Barbul, 2004). Various chemoattractive agents, including cytokines such as IL-6, IL-8, TGF-β, complement components such as C3a and C5a, and formylmethionyl peptides produced by bacteria and platelet products ensures the continued neutrophils attraction within 24 – 36 h of injury (Robson *et al.*, 2001). This results in weak attachments of neutrophils to the endothelial cells due to alterations in the regulation of surface adhesion molecules mediated by selectin-dependent interactions. Migration of cells out of the venules by squeezing between the endothelial cells into the wound environment commences by a process known as diapedisis. Thus, chemokines (e.g. IL-8) secreted by endothelial cells rapidly activate a stronger adhesion system, which is mediated by integrins (Skover, 1991; Lawrence, 1998; Flangan, 2000; Hart, 2002). Neutrophils then phagocytose foreign material and bacteria, destroying them by releasing proteolytic enzymes and oxygen-derived free radical species (Skover, 1991; Flangan, 2000; Richardson, 2004; Broughton *et al.*, 2006b). Upon completing the task, redundant cells are disposed of by

extrusion to the wound surface as slough and by apoptosis, allowing elimination of the entire neutrophil population without tissue damage or potentiating the inflammatory response. The cell remnants and apoptotic bodies are then phagocytosed by macrophages (Skover, 1991; 2000; Hart, 2002; Diegelmann and Evans, 2004). Keratinocytes in the wound edge and follicle remnants migrate and proliferate, and fibroblasts are chemo-attracted, and become activated (Broughton *et al.*, 2006a).

Late inflammatory phase:

This commences 48 – 72 h after injury at which time more macrophages appear in the wound and continue the process of phagocytosis (Hunt, 1998; Hart, 2002; Ariyibi *et al.*, 2002). These cells are originally blood monocytes that undergo phenotypic changes on arrival into the wound to become tissue macrophages. Attracted to the wound site by a myriad of chemoattractive agents, including clotting factors, complement components, cytokines such as interleukines (IL-6, IL8, TNF-α), PDGF, TGF-β, leukotriene B4 and platelet factor IV, as well as elastin and collagen breakdown products, macrophages have a longer lifespan than neutrophils and continue to work at a lower pH (Witte and Barbul, 1997; Ramasastry, 2005). These cells are fundamental for the late stages of the inflammatory response, acting as key regulatory cells and providing an abundant reservoir of potent cytokines and tissue growth factors, particularly TGF-β, as well as other mediators (TGF-α, heparin binding epidermal growth factor, fibroblast growth factor [FGF], collagenase), activating keratinocytes, fibroblasts and endothelial cells (Diegelmann and Evans, 2004; Ramasastry, 2005). Clearly the depletion of monocytes and macrophages from the wound causes severe healing disturbances due to poor wound debridement, delayed fibroblast proliferation and maturation, as well as delayed angiogenesis, resulting in inadequate fibrosis and a more weakly repaired wound (Skover, 1991). The last cells to enter the wound site in the late inflammatory phase are

lymphocytes, attracted 72 h after injury by the action of interleukins such as IL-1 and IL-6, complement components and immunoglobulin G (IgG) breakdown products (Witte and Barbul, 1997; Hart, 2002; Broughton *et al.*, 2006b; Burtis *et al.*, 2006; Owen *et al.*, 2013). The IL-1 plays an important role in collagenase regulation, which is later needed for collagen remodelling, production of extracellular matrix components and their degradation (Hart, 2002; Hunt, 1998).

2.7.2.3 Proliferative phase

Following coagulation, haemostasis and successful setup of appropriate inflammatory and immune responses, the acute wound shifts toward tissue repair (Skover, 1991; Flangan, 2000; Hart, 2002; Richardson, 2004). The proliferative phase starts on the third day after wounding and lasts for about 2 weeks thereafter. It is characterized by fibroblast migration and deposition of newly synthesized extracellular matrix, acting as a replacement for the provisional network composed of fibrin and fibronectin. At the macroscopic level, this phase of wound healing can be seen as an abundant formation of granulation tissue. The diverse processes that take place in the proliferative phase are collagen synthesis, angiogenesis and granulation tissue formation, adhesion, traction and epithelialization (Hunt, 1998; Diegelmann and Evans, 2004).

Collagen synthesis:

Collagens are an important component in all phases of wound healing especially from day 4 and for 2 - 4 weeks when the wound bed becomes vascularized, and type III collagen is laid down by fibroblasts to replace any dermal loss. In the absence of epidermal cover, this appears clinically as granulation tissue (Simon and Ali, 2015). Synthesized by fibroblasts, they impart integrity and strength to all tissues and play a key role, especially in the proliferative and remodelling phases of repair (Greenhalgh, 1998; Baum and Arpey, 2005). Collagens act as a foundation for the intracellular matrix

formation within the wound. Unwounded dermis contains 80% type I and 25% type III collagen whereas wound granulation tissue expresses 40% type III collagen (Robson *et al.,* 2001).

Angiogenesis and granulation:

Establishment of new blood vessels (angiogenesis) is critical in wound healing and takes place concurrently during all phases of the reparative process (Oike, *et al.,* 2004; Simon and Ali, 2015). In addition to attracting neutrophils and macrophages, numerous angiogenic factors secreted during the haemostatic phase promote angiogenesis. Resident endothelial cells are responsive to a number of angiogenic factors, including Fibrobast growth factor (FGF), vascular endothelial growth factor (VEGF), PDGF, angiogenin, TGF-α and TGF-β. A fine balance is kept by the action of inhibitory factors, such as angiostatin, steroids and possibly IL-10 (Oike, *et al.,* 2004; English *et al.,* 1999). Inhibitory and stimulatory agents act on proliferating endothelial cells directly as well as indirectly, by activating mitosis, promoting locomotion and by stimulating the host cells to release endothelial growth factors. Under hypoxic conditions, molecules are secreted from the surrounding tissue, promoting proliferation and growth of endothelial cells. In response, a four-step process namely; production of proteases by endothelial cells for degradation of the basal lamina in the parent vessel in order to crawl through the extracellular matrix, chemotaxis, proliferation and remodelling/differentiation takes place (English *et al.,* 1999). Initially, there is no vascular supply in the wound centre, so viable tissue, which is limited to the wound margins, is perfused by uninjured vessels and by diffusion through undamaged interstitium (Philips, 2000; Robson *et al.,* 2001). Capillary sprouts from the surrounding edges invade the wound clot and, within a few days, a microvascular network composed of many new capillaries is formed (Hsu *et al.,* 2007). The contributing factors act as

mediators for neovascularization and vessel repair at the injury site are also important modulators of cell growth and differentiation, and they include endothelial cell growth factor, TGF-α, VEGF, angiopoietin-1, fibrin and lipid growth factors. Regulation is achieved by three mechanisms namely; chemotaxis (migration towards the concentration gradient of the chemoattractive substance), mechanotaxis (migration induced by mechanical forces) and haptotaxis (migration in response to a gradient of immobilized ligands) (Krizbai, *et al.,* 2000; Li *et al.,* 2005). Cellular motility requires three distinct actions; protrusion at the cell front, adhesion to attach the actin cytoskeleton to the substratum and finally traction propelling the trailing cytoplasm forward (Wolgemuth, 2005). A protruding structure forms, in the case of the endothelial cell these are known as filopodia, and they are filled with filamentous actin under the multiple signalling pathways and regulatory proteins control (Giannone *et al.*, 2004; Li *et al.*, 2005; Moon *et al.*, 2005).

Adhesion to a solid substratum is a particularly important step in cell migration (Lawrence, 1998; Giannone *et al.*, 2004). It is mediated by integrins, which act as the primary receptors for extracellular matrix proteins involved in signal transduction and required for cell motility consequently regulating and stimulating migration (Bokel and Brown, 2002). Adhesion with further attachment as the wound agesresults in reduced mobility. After attachment to the extracellular matrix, integrin signalling contacts with the extracellular matrix which governs the cells' change in morphology from oval or spindle-shape to an irregular, flattened shape (Bokel and Brown, 2002). The traction forces generated at the sites of contact during locomotion can be high enough to significantly rearrange the extracellular matrix and/or deform it (Augustin *et al.*, 1994; Chang *et al.*, 2004; Dowsett *et al.*, 2006; Hsu *et al.*, 2007). Acute granulation tissue constituents such as macrophages, proliferating fibroblasts and vascularized stroma

together with collagen matrix, fibrinogen, fibronectin and hyaluronic acid replaces the fibrin based provisional matrix (Baum and Arpey, 2005). With collagen accumulation, the density of the blood vessels diminishes and the granulation tissue gradually matures to produce a scar (English *et al.*, 1999; Moon *et al.*, 2005).

Epithelialization:

This involves the migration of epithelial cells which commences from the wound edges within a few hours of wound sustainance. A single layer of cells initially forms over the defect, accompanied by markedly increased epithelial cells mitotic activity around the wound edges with the migrating cells attaching to the provisional matrix below. Upon contact of the advancing epithelial cells, migration stops and the formation of basement membrane commences (Diegelmann and Evans, 2004; Baum and Arpey, 2005).

2.7.2.4 Remodelling phase

This is the final phase of wound healing and it is responsible for the development of new epithelium and final scar tissue formation. Granulation tissue development serves to initiate the synthesis of the extracellular matrix in the proliferative phases as well as the remodelling phase which may last a few weeks to 18 months or even more (Ramasastry, 2005; Simon and Ali, 2015). Regulatory mechanisms which aims at maintaining a tight balance between synthesis and degradation controls the remodelling of an acute wound leading to normal healing (Hunt, 1998; Baum and Arpey, 2005). About 3 weeks after injury is the steady state equilibration of the synthesis and degradation processes of collagen as well as extracellular matrix remodelling. The degradation of collagen is dependent on the tightly regulated and synchronized matrix metalloproteinase enzymes, produced by neutrophils, macrophages and fibroblasts in the wound. With the gradual increase in the activity of inhibitory cytokines and other tissue inhibitors of metalloproteinases is a culminating drop in activity of

metalloproteinase enzymes, thereby promoting new matrix accumulation (Mulder and Vande, 2002; Toy, 2005). Although the initial deposition of collagen bundles is highly disorganized, subsequent organization is achieved to a greater extent by the wound contraction (shrinkage in size of underlying connective tissue bringing the wound margins closer together as a result of fibroblast interactions with the extracellular matrix under the regulated control of factors like TNF-α, IL-10, PDGF, TGF-β and FGF) that has already begun in the proliferative phase. As the wound heals, the density of fibroblasts and macrophages is further reduced by apoptosisregulated by TNF-α.Blood flow to the area declines as the growth of capillaries stops, metabolic activity at the wound site decreases and the new collagen matrix becomes more oriented and cross-linked (Falanga, 1998; Baum and Arpey, 2005).

Summarily, as fibroblasts mature into myofibroblasts with wound contraction and type III collagen gradually replaced by type I collagen, disorganized collagen becomes lamellar (Simon and Ali, 2015). The end result is a fully matured scar with a decreased number of cells and blood vessels and a high tensile strength (Hart, 2002; O'Kane, 2002). Over time, the tensile strength of the wound increases progressively in parallel with collagen collection whose fibres may regain approximately 80% of its original strength depending on the localization of repair (Robson *et al.*, 2001; Baum and Arpey, 2005).

2.7.3 Factors influencing wound healing

Numerous physiologic, pathophysiologic and metabolic factors can influence wound healing locally, systemically or in combination and result in an enhanced, normal or poor outcome (Hunt, 1998; Rice, 2000). Tissue handling and surgical techniques are controllable local factors as proper surgical techniques and tissue handling accelerates

healing which finally results in proper or at least a partial reconstruction of the wounded tissue (Auf-dem-Keller *et al.*, 2006; Ramachandran *et al.*, 2013; Turkel *et al.*, 2014; Coutin *et al.*, 2015). This occurs especially in the presence of varying combinations of other beneficial factors such as good nutrition (balanced diet), good vascularity and proper topical and systemic wound mediators (antiseptic and antibiotic use, cytokines, growth factors and hormones such as growth hormones and insulin-like growth factor I) (Awah and Nottidge, 1998; Hassan *et al.*, 2002). Poor handling provides the necessary environment for adverse effects/results such as anastamotic thrombosis which may lead to varying degrees of contamination, deformities and ultimately may result in the death of the individual (Amber and Swaim, 1984; Hassan *et al.*, 2003; Namas *et al.*, 2009; Ramachandran *et al.*, 2013). Other adverse results include oedema, tissue hypoxia, infection, ischaemia, necrosis and cytokines imbalance, though imbalance in cytokines and growth factors could also be a local manifestation of a systemic factor which may be reflective of systemic comorbidities (Ogawa *et al.*, 2013; Hubenak *et al.*, 2014). Systemic factors may be congenital or acquired influencing the healing processes and in some instances the clinical quality of the tissue. A range of defects could make the skin vulnerable to defective healing such as deficiencies in collagen synthesis seen in Ehlers–Danlos syndrome (Whitaker *et al.*, 2009) and deficiency of lamina 5 in the junction of epidermal basement membrane as seen in epidermolysis bullosa (Fine, 2010). Delay in wound closure and a reduction in wound strength could be the result of a fairly global change in healing processes associated with aging (Rosengardten *et al.*, 2011). Acquired systemic factors such as stress, drugs like anti-inflammatory, steroidal (systemic glucocorticoids), non-steroidal anti-inflammatory drugs and cytotoxic (chemotherapeutic) drugs globally suppresses the cellular responses to wounding (Lemmens *et al.*, 2008). Diabetic effects are compounded by reduced tissue oxygen

delivery due to atherosclerosis, any neuropathy, and impaired cellular immunity, phagocytosis and bacterial killing (Sorensen, 2012; Tsourdi *et al.*, 2013). Acquired factors such aspoor nutritional status, metabolic disease, obesity, general perfusion disturbances or pre-existing illness also influence wound healing.Vitamin deficiencies such as deficiency of vitamin c which is an essential co-factor for collagen synthesis and can reverse steroid-induced collagenase activity and zinc which is also important to many enzyme systems which can be seen in large burn injuries (Albino *et al.*, 2009). These factors act by altering the wound repair environment and turning acute wounds into chronic wound. Chronic wounds are usually loaded with bacterial biofilm including anaerobic organisms not isolated by standard culture systems making it an area of particular current interest (Bertesteanu *et al.*, 2014). In such situations, all processes, from wound closure time, inflammatory cell influx and fibroblast migration, to collagen and extracellular matrix deposition are delayed (Hunt, 1998; Rice, 2000).

2.8. Measurement of Cytokines

Various techniques for cytokine measurement exist with the most common tests being bioassay and immunoassay techniques (Masih, 2011).

2.8.1 Bioassays

Many cytokines are measured by bioassays and recently by immunoassays techniques. Historically, bioassays of cytokines have been used to measure bioactivity in a particular biological model or cell line. Tests for chemotactic activity (Boyden chamber for IL-8), proliferation, or cytotoxicity tests (WEHI 164 or L929 for TNF), and inhibition of cytokine secretion (inhibitory activity of IFN-g on IL-10 production) are

among the earliest bioassays tests (Jacques, *et al.*, 2000; Burtis *et al.*, 2006). Good sensitivity and the capability of measuring biologically active molecules have been the advantages of bioassays while, their major drawback is their lack of specificity due to the fact that several cytokines can exert similar or synergistic effects (Masih, 2011). These methods are time-consuming (24–96 h) and their reproducibility is rather poor, with coefficients of variation (CV) ranging from 20 to 100% (Burtis *et al.*, 2006). The possibility of adding antibodies directed against an interfering cytokine improves the specificity of the assay, but augments its cost(Burtis *et al.*, 2006).

2.8.2 Immunoassays

Immunoassays currently are the method of choice for determination of cytokines. Enzyme-linked immunosorbent assay (ELISA) is the commonly used form of immunoassay. ELISA uses a primary antibody for the capture and a secondary antibody conjugated to an enzyme or radioisotope for the detection. The conventional ELISA kits, however, detect only one cytokine at a given time leading to the development and introduction of the novel technologies of multiplex analysis system. This allows for the simultaneous detection of upto 100 and more different biomolecules performed in a single microplate well (Burtis *et al.*, 2006). These cytokine antibody arrays can detect simultaneously multiple cytokine expressions in one assay at the protein levels in any body fluid, such as plasma, serum, cell lysates, cerebrospinal fluid, ascites, saliva or urine (Burtis *et al.*, 2006; Bozza *et al.*, 2007; Masih, 2011).

These arrays have high sensitivity and can detect cytokine at the pg/ml concentrations. Bozza *et al*, (2007) using multiplex cytokine technique, showed that various cytokines were upregulated with severe clinical manifestations when compared with mild disease forms. Cytokines, as modulators or effectors of inflammatory response, play an

important role in injuries, sepsis and multi-organ dysfunction. Cytokines such as IL-6, IL-8 and IL-10 have clinical utility in the prediction of outcomes in severe sepsis (Bozza *et al.*, 2007). The main advantages of immunoassays are their ability to be automated allowing the measurement of analytes (such as IL-1, IL6, IL-8, TNF- α and IL-2R) as emergency tests within a few hours as well as their excellent sensitivity of analytical performance. Major drawback is the fact that it often measure total cytokine concentration, which consists of both non-functional and functional molecules. Nonetheless, immunoassays have several advantages over traditional bioassays, such as higher specificity and precision, shorter assay times (only a few hours versus a few days for bioassays), and simpler calibration (Burtis *et al.*, 2006).

2.8.3 ELISPOT (Enzyme-Linked Immuno Spot Assay)

This represents one of the first attempts to monitor immune responses by the detection of cytokine producing cells amongt as well as the frequency of production in a heterogeneous population of cells (Czerkinsky *et al.*, 1983). In this technique, a stimulated cell suspension is laid on a microplate well coated with antibodies directed against the cytokine of interest. After incubation for 2 - 4 h at 37°C and washing, cytokine-producing cells are removed and the cytokine bound to the solid phase antibody is visualized by a second enzyme-labelled anti-cytokine antibody. A chromogenic substrate is then added and each cytokine-secreting cell can be visualized as a coloured spot (Masih, 2011; Navarrete, 2015). ELISPOT is 10- to 100-fold more sensitive than classical ELISAs allowing the detection of as few as three to five cytokine secreting cells per 100000. The detection of cells simultaneously producing two cytokines is possible by this method. Because of its exquisite sensitivity, ELISPOT can be used to detect circulating cytokine producing cells without stimulation *in vitro.*

Thus, the ELISPOT assay provides both qualitative and quantitative information (Czerkinsky *et al.*, 1983; Jacques, *et al.*, 2000; Navarrete, 2015).

2.8.4. Flow cytometry

Flow cytometry is a laser- or impedance-based, biophysicaltechnology used for cell counting, cell sorting and biomarker (intracellular cytokine) detection which can be performed for thousands in a short span of time, usually less than two hours. Flow cytometry can use blood or other body fluids such as cerebrospinal fluid or synovial fluid as the specimen of choice for cytokine analysis (Burtis *et al.*, 2006).Use of flow cytometry has the advantage of rapid analysis for cellular identification. The drawbacks of using flow cytometry, however, lie in the complexity of identifying and quantifying intracellular cytokines and related molecules, background induced autofluorescense that is seen in some body fluids, the automatic gating of cells, and the requirement for negative control for cutoff levels (Burtis *et al.*, 2006; Masih, 2011).

The introduction of cellular and tissue methods such as the detection of cell-associated cytokines as proteins after addition of specific particles and/or antibodies (immunohistochemistry), the detection of cytokine mRNA by *in situ* hybridization (ISH) or reverse transcriptase polymerase chain reaction (RT-PCR), Luminx assay, Magnetic bead cytokines tests are also newer methods of measurements are employed to overcome some of the drawbacks of the bioassay and immunoassay methods (Jacques, *et al.*, 2000; Saami *et al.*, 2015).

Regardless of the type of the technology chosen for cytokine testing, analysis of cytokines needs to be performed no more than five hours after sample collection to avoid cellular interaction except samples are stored at -20^{o}C or lower. Various factors

such as short half-lives, intra-individual variations, the presence of autoantibodies or soluble cytokines receptors/cytokine receptor antagonists or nonspecific inhibitors all can contribute to inaccurate cytokine measurements and inaccurate results (Burtis *et al.*, 2006).

2.9 Future of Cytokines in Wound Management

Wounds of diverse aetiology and classification occupy a remarkable place in everyday tissue pathology. Tissue injury triggers the host's response involving various processes of tissue healing that encompasses the four continuous phases including coagulation and haemostasis, inflammation, proliferation and wound remodelling with scar tissue deposition. Correct clinical management may positively influence the wound healing course and reduce potential complications (Velnar *et al.*, 2009). Much effort has been focused on wound care with an emphasis on new therapeutic approaches and the development of technologies for acute and chronic wound management. Wound healing involves multiple cell populations, the extracellular matrix and the action of soluble mediators such as growth factors and cytokines (Velnar *et al.*, 2009). Cytokines play important roles in growth modulation, angiogenesis, cytotoxicity, inflammation, and immune modulation in various disorders. Cytokine assay hold a great potential for diagnosing and imaging patients with acute and chronic inflammatory diseases. As research on cytokine testing continues, laboratory measurements of relevant cytokines profiles could therefore prove useful in the design of future biomarkers for diagnostics, prognostics and eventual routine testing in the clinical laboratories aiding proper and timely interventions in wound management with excellent repair and restoration (Velnar *et al.*, 2009; Glaudemans *et al.*, 2010; Masih, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site

The experiment was carried out in the Department of Veterinary Surgery and Radiology and Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, located in the Northern Guinea Savannah zone of Nigeria (11° 04/ N, 7° 42/ E) (Anonymous[1], 2013).

3.2 Ethical Clearance

Ethical clearance for the use of dogs in this study was sought and approval was gotten from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) Ahmadu Bello University, Zaria (Reference number: ABUCAUC/2016/026).

3.3 Materials/Agents Used in the Study

The materials used are summarised in Tables 3.1 to 3.6 including consumables (Tables 3.1), surgical instruements (Table 3.2) antiparasitics agents used to treat the dogs during acclimatization period (Table 3.3), antimicrobials and fluids (Table 3.4), analgesic agents, anaesthetic agents and premedications (Table 3.5) and the dogs and items used to feed and water them (Table 3.6) They were purchased from the Ahmadu Bello University Teaching Hospital Pharmacy, Shika and the Gospel Medical Equipment and Consumables shop,Zaria.

Table 3.1: List of Consumables Used in the Study

Description of item	Quantity
Adhesive tapes (3.5 inch)	8 rolls
Chlorhexidine gluconate BP (litres; 0.3 % w/v - Purit®)	1 litre
Absorbent cotton wool	2 pack
Gauze	2 roll
Liquid soap	1 litre
Sterile disposable syringes (5mls)	2 pack
Sterile disposable syringes (10mls)	2 pack
Surgical gloves	1 pack
Surgical blades (size 15)	20 pieces
Surgical caps and masks	1 pack
Razor blades (packets)	2 packs
Test tubes	9 packs
Nylon bags	1 pack
Microscopic glass slides	2packs
Microhaematocrit capillary tubes	1 pack
Distilled water	4 litre
*Enzyme Linked Immunosorbent Assay Kits	4 kits

* Interleukin (IL) -6 Canine ELISA kit (ab193686, Lot: GR252142-1);Interleukin (IL) -8 Canine ELISA kit (ab155465, Lot: GR252140-1); Interleukin (IL) -10 Canine ELISA kit (ab193685, Lot: GR252141-2) and Tumour Necrosis Factor (TNF) –α Canine ELISA kit (ab193687, Lot: GR252143-1),(Abcam®United Kingdom).

Table 3.2: List of Some Surgical Instruments and Wears Used in the Study.

Description of item	Quantity
Standard General Surgical park	2
Surgical gown	4
Sets of Surgical drapes	5

Table 3.3: List of Antiparasitics used in the Study

Description of item	Quantity
Ivermectin (20 mls, 1% w/v)	1 bottle
Praziquantel (600 mg tablet)	1 pack
Pyrantelpamoate (180mg tablet)	1 pack

Table 3.4: List of Antimicrobials and Fluids Used in the Study

Description of item	Quantity
Normal saline solution (1litre bag, Dana)	10 bags
Infusion set	10 pieces
Catheter and canula	10 pieces
Penicillin topical ointment	2 tubes

Table 3.5: List of Analgesics, Anaesthetics and Premedications Used in the Study

Description of item	Quantity
Pentazocine (2 mls ampoules)	3packs
Thiopental sodium (1g bottle)	2 bottles
Midazolam (3 mls ampoules)	1 pack
Atropine sulphate (2ml ampoules)	2 pack

Table 3.6: List of Items Used to Feed and Water the Dogs in this Study

Description of item	Quantity
Feeding bowls	12 pieces
Drinkers	4 pieces
Cooking stove and pot	1 each
Sweeping brooms	2 bundles
*****Dogs (6 males and 6 females)	12 dogs

(*): NIDs (Plate I).



Plate I: NIDswith moderate and predominantly brown hair coat, medium sized head (mesencephalic cranial index) and slender limbs with white patches at the ventral part of the neck, extremities of the limbs and tail.

3.4 Methodology

3.4.1 Study design

This study consisted of two groups; control and experimental groups to which 12 adult, apparently, healthy NIDs were randomly assigned to the groups consisting of 6 dogs per group. The control group consisted of Six (6)NIDs (3 males and 3 females) maintained throughout the research as uninjured dogs. Urine samples were collected from the urinary bladder of each dog via cystocentesis at start and finish of the experiment for urinalysis. Five mL of whole blood sampleswerecollected from each dog via cephalic venipuncture at start, 12 h., 36 h., 60 h., 156 h. and 324 hours for complete blood counts and serology. The Experimental group consisted of six (6) NIDs (3 males and 3 females) with 2 x 2 cm^2 cutaneous flap excision at the mid lateral aspect of the antebrachium (radius and ulna bones).Urine samples were collected from each dog at start and finish of the experiment for urinalysis. From all six dogs, 5 mL blood samples were collected via the cephalic vein pre-operative and continued post operatively at 12 h., 36 h., 60 h., 156 h. and 324 hours.Wound fluids (flushed exudates) were collected and processed following thewound wash/flush method,as described by Ambrosch *et al.* (2008). In this method, 3 mL ofnormal saline was obtained using sterile syringe and needle and used to flush the wound withthe effluents collected in plastic containers. The effluents were then centrifuged at 4, 500 g for 15 minutes; the supernatants were decanted and the sediment fluids collected and stored at -20 ^{o}C.This was done routinely at 12, 36, 60 and 156 hours post operation. Immediately after wounding, the wounds were occlusively dressed and only at times of wound fluids collection that the dressings were removed and wound flushed for further collection of exudates.

3.4.2 Research animals

From Zaria and environDog markets, 15 healthy looking NIDs were purchased and humanely transported by road in an open back conveyor van to the site of research. They were kept in transit in a section of the hospital kennels where they were immediately screened and maintained for a week for acclimatisation and conditioning. After screening and treatment with pyrantel pamoate (Vermisol® - GM Pharmaceutical INC, Arlington, Texas) at 5 mg/kg,oral, praziquantel (PRIZILCIDE® - Yanzhou Xier Kangtai Pharmaceutical co., Ltd, Shandong, China) at 10 mg/kg, oraland Ivermectin (IVERMEC SUPER® - Pharma Swede, Egypt) at 0.4 mg/kg SC., only the healthiest twelve (12) were moved to the cubicles which had been cleaned and disinfected prior to the arrival of the animals, where they were maintained throughout the period of research. All animals were adequately provided with food and water ad libitum.

3.4.3 Clinical procedures

3.4.3.1 General physical and laboratory examination

All dogs that underwent the study were thoroughly examined physically with vital parameters (rectal temperature, pulse rate and respiratory rate) obtained and recorded. Blood samples obtained via cephalic venipuncture, faeces and urine via cystocentesis were collected at start for screening at the various laboratories: Blood samples and sera were analysed at the Ahmadu Bello University Health service, Haematology and Chemical Pathology Laboratories for haemogram and serology, Urinalysis was performed at the Ahmadu Bello Teaching Hospital Chemical Pathology Laboratory, while blood and faeces were at protozoology and helminthology laboratories of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria.

3.4.3.2 Surgical operations

Pre-surgical considerations

In preparation for surgery, the lateral aspect of the antebrachium (radius and ulna) of each dog was shaved and scrubbed clean with plenty of water and soap and scrubbed with chlorhexidine gluconate (Purit® - Saro life care Ltd, Lagos Nigeria) at 2% solution. Food and water were withheald for 12 h. and 6 h. respectively.

Anaesthesia

The operations were done under general anaesthesia via cephalic venapunctures. The dogs were premedicated with atropine sulphate (Atropine® - Shanxi Shuguang Pharmaceutical Co., Ltd, Qixian, China) at 0.05 mg/kg, pentazocine (Pentalab® - Laborate Pharmaceuticals Ltd, India) at 1 mg/kg and midazolam (Roche Pharmaceuticals, Switzerland) at 0.25 mg/kg while the final anaesthetic agent was thiopental sodium 1g (Pauco Pharmaceuticals, Nigeria) at 12 mg/kg. Post-anaesthesia, the dogs were monitored throughout the operation until recovery from anaesthesia and they were carefully moved to their kennels and placed on beddings.

Techniques and approach of wound creation

In the operating theatre, the dogs were placed on left lateral recumbency on the surgical table with the right limb uppermost and draped with adequate exposure of the marked 2 x 2 cm^2surgical site (Plate II). The skin flap excision at the site; mid lateral aspect of the right antebrachium (Radius and Ulna)was achieved on all the dogs using size 15 surgical blades.

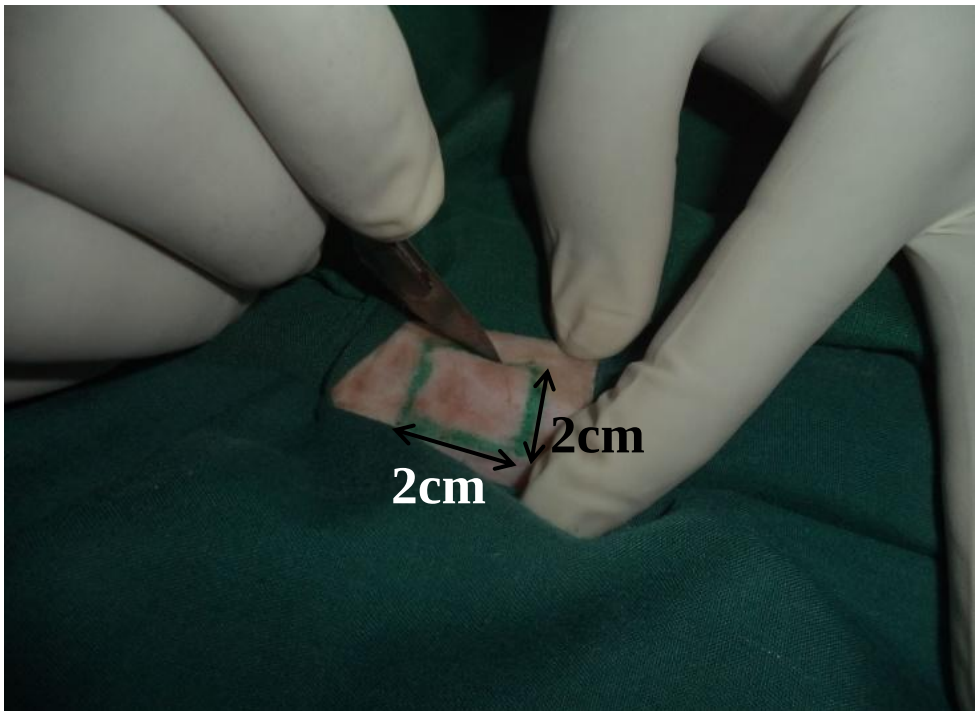


Plate II: **Aseptically Drapped right forelimb with marked 2 x 2 cm^2 area for the Full thickness cutaneous excision at the mid lateral surface of the antebrachium (radius and Ulna).**

3.4.4 Sample analyses

3.4.4.1 Haematology and serum biochemical tests

Haemogram of the anticoagulated (Sodium ethylenediamintetraacetate) 2 mL whole blood samples were obtained using the automated haematologic analyser (Mindray®BC-3600, Shenzhen, China). Serum was obtained from 3 mL stored whole blood without anticoagulant at room temperature for 1 h. and centrifuged at 4, 500 x g for 15 min. using automated centrifuge (BHG Hermle Z364, Gosheim, Germany). The sera from all blood samples were carefully extracted into sterile plastic tubes and stored at – 20^{o}C (Karr, 1989; Ramnath *et al.*, 2008; Zuhoor, 2012; Alessandra *et al.*, 2016; Najib *et al.*, 2016). The Serum samples were analysed for creatinine, urea, total protein, albumin, glucose, Na^{+}, K^{+}, $Cl^{-,}$ Ca^{2+}, P, $HCo_3^{-}$by enzyme immunoassay method and read using digital ultraviolet spectrophotometer (Digital System S. R. L, Italy) at 450 nm (Kerr, 1989).

3.4.4.2 Urinalysis

Urine samples obtained using sterile syringes and needles by cystocentesis were dispensed into sterile test tubes. Urinalysis test strips (Cybow health mate, Gyung-Nam, Korea. Lot. 150729) were immediately used to analyse the urine samples by dipping the strips into the urine and allowed for 60 seconds before reading the result by cross matchingthe colour change on the strips with the corresponding results colour change chart provided with the urinalysis strip(Kerr, 1989; Archer, 2005).

3.4.4.3 Cytokine assay in sera and wound fluids by ELISA

Reagents preparation:

The vials of detection Antibody and 200X Horseradish Perosidase concentrate were briefly spun, thereafter their contentswere pipetted up and down to mix gently. 5X Assay Diluent (5X Assay Diluent B)was diluted 5-fold with distilled water to yield 1X Assay Diluent (1X Assay Diluent B) before use and 20 mL of 20X wash Buffer was diluted by addition of 380 mL of distilled water to yield 400 mL of 1X Wash Buffer. 100 µL of 1X Assay diluentwas added into each vial of the detection Antibody (biotinylated anti-Canine cytokine antibody) concentrate, mixed gently and diluting it 80-fold by the addition of 8 mL of 1X Assay Diluent in a container. 1X Horseradish Perosidase (HRP)-Streptavidinfor IL- 6, 8, 10 or TNF-αwere prepared by addingtheir respective Horseradish Perosidase - Streptavidin concentrates(20 µL, 50 µL, 40 µL and 40 µL) to 1X Assay Diluent (12 mL, 10 mL, 14 mL and 14 mL) inseparate containers and mixed well giving 600-, 200-, 350- and 350-folds dilutions respectively.Other reagents present in the Enzyme Linked Immunosorbent Assay Kits for IL- 6, 8, 10 or TNF-α quantification are 3,3',5',5'-Tetramethylbenzidine (TMB) One-Step substrate reagent,Stop solution which comes ready for use and Assay Diluent A/D for use in preparation of Canine IL-6 and TNF-αStandards (Plate III and IV).



Plate III: Enzyme Linked Immunosorbent Assay Interleukin (IL)-6, -8, -10 and Tumour necrosis factor alpha (TNF-α) Kits acquired from Abcam®, Cambridge, United Kingdom.



Plate IV: Displayed are all the reagents used in the study:Enzyme linked immunosorbent assay (ELISA) kits with Assay diluent A (A); Cytokine standards (B); 20X wash buffer (C); Assay diluent B (D); Biotinylated Canine cytokine antibody (E); Horseradish Perosidase (HRP)-streptoverdine (F); 3,3',5',5'-Tetramethylbenzidine (TMB) one-stop solution (G); Stop solution (H); Sealed antibody coated microtithre plate (I).

Preparation of test tube standards from Stock standard:

Stock standard vials were spun briefly and used to prepare fresh test tube standards (recombinant proteins) asfollows;

Assay Diluent A (800 µL) was added to Canine IL-6 Standard vial preparing 25 ng/mL Standard #1 afterthorough but gently mixing. This was serially diluted by adding 300 µL Assay Diluent A into test tubes #2-8 and Standard #2 was prepared by a transfer of 200 µL of standard #1 into tube #2 which was then mixed thoroughly.Standard #3was also prepared by a transfer of 200 µL of standard #2into tube #3 withthorough mixing, and the serial dilution continued until the eighth dilution giving known concentrationsof 10, 4, 1.6, 0.64, 0.256, 0.102and 0 ng/mL respectively for tubes #2-8.

400 µL of Assay Diluent was added to Canine IL-8 Standard vialto prepare a 50 ng/mL Stock Standard. 15 µL Stock Standard was added into a test tube with 485 µL 1X Assay Diluent to prepare 1,500 pg/mL Standard #1 solutionwhich was mixed thoroughly but gently. This was serially diluted by adding 300 µL 1X Assay Diluent into test tubes #2-8 and Standard #2 was prepared by a transfer of 200 µL of standard #1 into tube #2 and mix thoroughly.Standard #3 was prepared by a transfer of 200 µL of standard #2 into tube #3 and mixed, and the serial dilution continued until the eighth dilution giving known concentrations of600.00, 240.00, 96.00, 38.40, 15.40, 6.14 and 0.00 pg/mL respectively for tubes #2-8.

500 µL of 1X Assay Diluent D was added to Canine IL-10 Standard vial to prepare 80 ng/mL Stock Standard and Standard #1 was prepared by adding 200 µL Stock Standard to 400 µL 1X Assay Diluent D which was mixed thoroughly but gently. It was serially diluting by adding 400 µL 1X Assay Diluent D into test tubes #2-8 and Standard #2 was

prepared by transfer of 200 µL of standard #1 into tube #2 and mixed thoroughly.Standard #3 was also prepared by a transfer of 200 µL of standard #2 into tube #3 and mixed thoroughly, and the serial dilution continued until the eighth dilutiongiving known concentrations of 26.670, 8.889, 2.963, 0.988, 0.329, 0.110 and 0.000 ng/mL respectively for tubes #2-8.

400 µLof Assay Diluent A was added to Canine TNF-α Standard vial to prepare 100 ng/mL Stock Standard and 700 pg/mL Standard #1 was prepared by adding 7 µL Stock Standard to 993 µLAssay Diluent Aand mixed thoroughly but gently. This was then serially diluted by adding 300 µLAssay Diluent A into test tubes #2-8. Standard #2 was prepared by a transfer of 200 µL of standard #1 into tube #2 and mixed thoroughly, standard #3 was prepared by transfer of200 µL of standard #2 into tube #3 and also mixed thoroughly, and the serial dilution continued until the eighth dilution giving known concentrations of 280.00, 112.00, 44.80, 17.92, 7.17, 2.87 and 0.00 pg/mL respectively for tubes #2-8.

Assay procedure:

We equilibrated all materials;serum and wound fluids samples, 96 well plates strips (pre-coated with specific Canine cytokine antibody for IL- 6, 8, 10 or TNF-α), prepared reagents and standards to room temperature (25 ^{0}C) prior to use.

The first 2 wells of each 96 well plates were left as blank and to the next 8 wells, 100 µL of the various serially diluted standards were deposited into separate wells.Into the remaining 86 wells were deposited separately 100 µL of each prepared serum or wound fluid sample using micropipettes with disposable cups. The 96 well platespre-coated with specific Canine cytokine antibody for IL- 6, 8, 10 and TNF-αcontaining the

standards and samples were then covered and incubated for two and half hours at room temperature to allow for the cytokine present in the samples to be bound by the immobilized antibody. The wells were washed four times with 1X wash buffer and then blot dried with paper towelsto wash away unbound samples and standards. 100 µL of prepared biotinylated anti-canine cytokine antibody was then added to each well and incubated for one hour at room temperature with gentle shaking thereafter washedwith 1X wash buffer (4 times as above). After washing away unbound biotinylated antibody, 100 µL of prepared 1X Horseradish Perosidase (HRP) - conjugated streptavidin was pipetted to the wells and incubated for 45 minutes at room temperature with gentle shaking before the solution was discarded and the wells were again washed (4 times and blot dried). 100 µL of 3,3',5',5'-Tetramethylbenzidine (TMB) One-Step substrate reagent was then added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking to allow colour development in proportion to the amount of cytokine bound. 50 µL of the Stop solution was dispensed into each well and this caused colour changes from blue to yellow which was observed visually and thecolour intensityread using spectrophotometry at a wavelength of 450 nm (Plate V).

The absorbencies in wavelengths obtained were used to extrapolate the concentrations of IL -6, -8, -10 and TNF-αfrom graphical standard curve plots using Trendline/Regression equation on Microsoft office excel-2010 as shown in Appendices I - III, IV - VI, VII - IXand X - XII respectively.

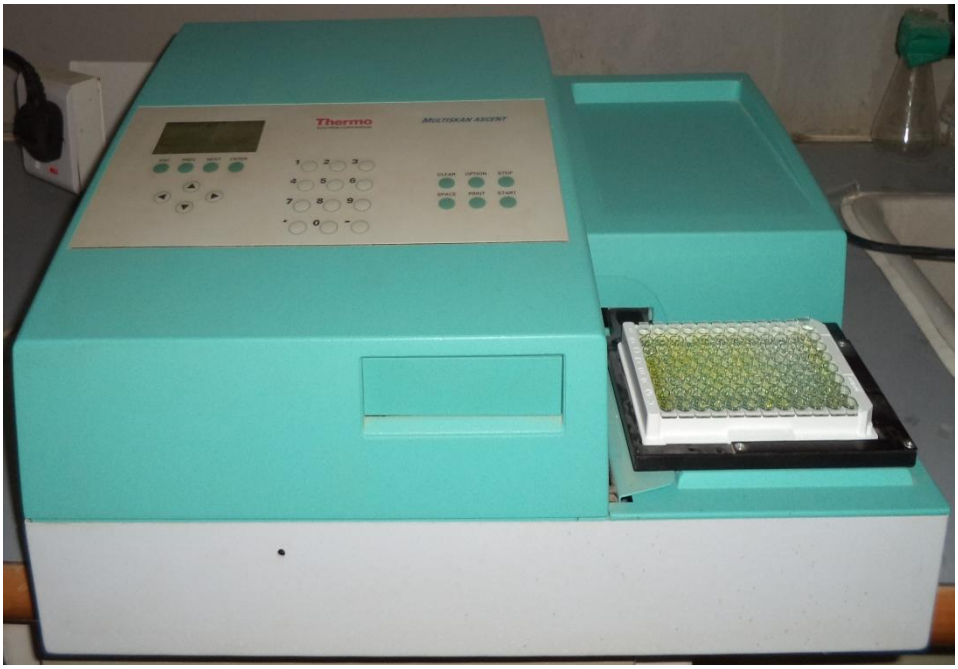


Plate V: Thermo Multiskan Ascent Photometer for 96- and 384-well microplate format to measure absorbance at 450 nm. At the lower right corner is the 96 wells microplate with whitish rectangular base within a blackframe (Thermo Scientific, USA).

3.4.5 Statistical analysis

The numerical data were analysed to determine the means and standard error of the means (means ± SEM). Data were subjected to analysis of variance (ANOVA) followed by Turkey's multiple comparison test and Bonferroni post hoc tests. Pearson correlation coefficients were also determined. Values of $P < 0.05$ were considered significant (Snedecor and Cochran, 1994; Das *et al.,* 2009), using GraphPad prism Version 5.03 (San Diego, California, USA) for windows.

Table 3.7: Experimental Design Summary

Headings	Descriptions
Animal selection:	Apparently healthy NIDs, aged eight month to two years within Zaria and environs. Randomly assigned into two groups of 6 dogs (3 males and 3 females) each; Control groupconsisted of uninjuredNIDs and Experimental group dogs had surgically induced cutaneous injuries.
Physical / Laboratory Examinations:	Rectal Temperatures (T^o), Pulserates (beats/min) and Respiratory rates (cycles/min). were obtained using clinical rectal thermometer, Lithmans Stethoscope and physical counts of the thoracic cage movements. Blood samples were obtained for haemogram, haemoparasites screening and serum biochemistry,urine samples for urinalysis andfecal samples for endoparasitesscreening. Only animals with values within normal range limits and indicating no health challenges were considered for the study of cytokines levels (Ihedioha *et al.*, 2012).
Samples/sampling:	Preoperative samplingswere undertaken to acertain healthiness and for baseline while, post operative were to be used to evaluate changes between pre- and post-operative.
Pre-operative	- Blood: Haemogram, Serology and Haemoparasite screening. - Urine: Urinalysis - Faeces: Endoparasite screening.
Post-operative	- Blood: Haemogram and Serology (including assay for cytokines) at 12, 36, 60, 156 and 324 hours. - Wound fluids: Exudates collected at 12, 36, 60 and 156 hours. - Urine: Urinalysis
General Anaesthesia:	Premedication: - Atropine sulphate (Atropine®- Shanxi Shuguang Pharmaceutical Co., Ltd, Qixian, China) at0.05 mg/kg. - Midazolam (Roche Pharmaceuticals, Switzerland)0.25 mg/kg. - Pentazocine(Laborate Pharmaceuticals Ltd.,India) at 1mg/kg

Headings	Descriptions
	Anaesthetic agent: - Thiopental sodium 1g, (Pauco Pharmaceuticals Industries Ltd., Nigeria) at 12 mg/kg.
Operative technique and approach	2 x 2cm(4 cm^2)full thickness cutaneous excision on the mid lateral aspect of the right antebrachium (radius and Ulna) and managed by second intention wound healing.
Observation/Monitoring and Care	At every change of dressings, wound evaluations were undertaken with changes in *wound size noted. Wound secretions/exudates were collected by flushing wound surface with normal saline. This was done at 12, 36, 60, 156 and 324 hours as stated above for samples and sampling.

*Percentage wound contraction = $\frac{\text{(Initial Wound Size – Current Wound Size)}}{\text{(Initial Wound Size)}}$

(Sussman and Bates, 2007).

CHAPTER FOUR

RESULTS

4.1 General Overview

A total of 12 apparently healthy (6 male and 6 female) NIDsrandomly assigned into 2 groups control (uninjured) and experimental (injured) were evaluated for cytokines: Interleukins (IL)-6, 8, 10, Tumour Necrosis Factor (TNF)-α, and blood cell counts.The basic demographic characteristics showed that the dogs had mean age range of 9 - 13 months and mean weight range of 8- 12 kilograms (appendix XIII).Serum biochemical test and urinalysis (appendix XIV and XV),pre-operative vital parameters andcomplete blood countsobtained following screening, acclimatization and conditioning of the dogs were used as to ascertain healthiness as well as served as baseline for the NIDs as shown in appendices XVI– XVIII.All the dogs that entered for the experiment were humanely handled as stipulated by theABUCAUC/2016/026and approved by the committee. All the wounds healed adequately within 45 days without major complications (Appendix XIX and XX) and the dogs have been fostered to families of staff members of the Ahmadu Bello University community.

4.2. Cytokines Analysed: Interleukin (IL)-6, -8, -10 and Tuour Necrosis Factor – alpha (TNF-α)

The cytokines;IL-6 IL-8, IL-10 and TNF-α were respectively detected at the ranges of (0.10 ± 0.01 - 0.44 ± 0.16 ng/mL, 213.20 ± 155.90 - 389.70 ± 142.90 pg/mL, 0.72 ± 0.03 - 1.16 ± 0.31 ng/mL and 16.02 ± 5.23 - 67.66 ± 5.23 pg/mL)in the sera of the dogs in the control group and in the sera (0.14 ± 0.04 - 1.01 ± 0.36 ng/mL, 330.40 ± 149.40 - 830.20 ± 320.5 pg/mL, 0.81 ± 0.00 - 1.73 ± 0.03 ng/mL and 23.06 ± 12.04 - 129.50 ± 111.60 pg/mL) and wound fluids (0.11 ± 0.01 - 1.90 ± 0.47 ng/mL, 111.80 ± 13.55 - 323.20 ± 209.40 pg/mL, 0.82 ± 0.02 - 1.05 ± 0.04ng/mL and 23.09 ± 7.56 - 179.00 ± 58.19 pg/mL) of those in the experimental group.The cytokine levels from sera in the experimental group were generally higher than those in the control group as shown in Tables 4.1 – 4.8.

Table 4.1: Mean Interleukin-6 (IL-6) Responses (ng/mL) ofboth Experimental and Control groups of NIDsirrespective of sex (n = 12).

Time(Hours)	Exp. WF. Response (ng/mL) (n = 6)	Exp. Serum response (ng/mL) (n = 6)	Cont. Serum response (ng/mL) (n = 6)
Pre-operative (Start)	-	0.29 ± 0.04 (0.21 - 0.49)	0.28 ± 0.05 (0.18 - 0.49)
Post-operative			
12	1.33. ± 0.33[a,b] (0.52 - 2.74)	1.13 ± 0.74[b,c] (0.17 - 4.84)	0.30 ± 0.05[c] (0.19 - 0.54)
36	0.51 ± 0.26 (0.14 - 1.77)	0.46 ± 0.04 (0.37 - 0.65)	0.29 ± 0.10 (0.10 - 0.70)
60	0.18 ± 0.02 (0.12 - 0.22)	0.47 ± 0.18 (0.23 - 1.38)	0.17 ± 0.05 (0.06 - 0.35)
156	0.14 ± 0.02 (0.10 - 0.20)	0.79 ± 0.32 (0.24 - 2.24)	0.16 ± 0.03 (0.10 - 0.26)
324	-	0.17 ± 0.03 (0.10 - 0.29)	0.21 ± 0.03 (0.10 - 0.28)
*Mean ± SEM	0.54 ± 0.28	0.55 ± 0.14	0.23 ± 0.02

[a,b,c]= Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly (P < 0.05) different. Sources of variation (Interaction: P = 0.151; Time: P < 0.001; Groups: P = 0.095). Values in parenthesis are the ranges (minimum – maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, ng/mL = nano-gram per millilitre, n = total number of individuals in group and (*) = Overrall values.

Table 4.2: Mean Interleukin-6 (IL-6) Responses (ng/mL)of both Experimental and Control groups of NIDs (n = 12).

(ng/mL)	Exp. WF. Response (ng/mL)		Exp. Serum response (ng/mL)		Cont. Serum response	
Time (Hours)	Males (n = 3)	Females (n = 3)	Males (n = 3)	Females (n = 3)	Males (n = 3)	Females (n = 3)
Pre-operative (Start)	-	-	0.22 ± 0.01 (0.21 - 0.25)	0.35 ± 0.08 (0.24 - 0.49)	0.33 ± 0.08 (0.22 - 0.49)	0.22 ± 0.02 (0.18 - 0.25)
Post-operative 12	1.66 ± 0.56[a] (0.52 - 1.71)	1.90 ± 0.47[a] (0.86 - 2.74)	1.01 ± 0.36[a] (0.41 - 4.84)	0.36 ± 0.09[b] (0.17 - 0.45)	0.36 ± 0.09[b] (0.25 - 0.54)	0.23 ± 0.04[b] (0.19 - 0.30)
36	0.19 ± 0.03 (0.14 - 0.24)	0.84 ± 0.49 (0.13 - 1.77)	0.44 ± 0.04 (0.37 - 0.49)	0.49 ± 0.08 (0.38 - 0.65)	0.13 ± 0.03 (0.10 - 0.18)	0.44 ± 0.16 (0.14 - 0.70)
60	0.21 ± 0.01 (0.18 - 0.22)	0.15 ± 0.02 (0.12 - 0.18)	0.64 ± 0.37 (0.23 - 1.38)	0.29 ± 0.02 (0.26 - 0.34)	0.10 ± 0.01 (0.06 - 0.09)	0.26 ± 0.06 (0.16 - 0.35)
156	0.11 ± 0.01 (0.10 - 0.13)	0.18 ± 0.02 (0.15 - 0.20)	0.96 ± 0.64 (0.24 - 2.24)	0.61 ± 0.23 (0.37 - 1.08)	0.10 ± 0.01 (0.10 - 0.12)	0.22 ± 0.02 (0.20 - 0.26)
324	-	-	0.14 ± 0.04 (0.10 - 0.21)	0.20 ± 0.05 (0.12 - 0.29)	0.19 ± 0.05 (0.10 - 0.28)	0.23 ± 0.03 (0.19 - 0.27)
*Mean ± SEM	0.54 ± 0.37	0.77 ± 0.41	0.57 ± 0.15	0.38 ± 0.06	0.20 ± 0.05	0.27 ± 0.04

[a,b] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. Sources of variation (Interaction: $P = 0.33$; Time: $P < 0.001$; Groups: $P = 0.11$). Values in parenthesis are the ranges (minimum – maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, ng/mL = nano-gram per millilitre, n = total number of individuals in group and (*) = Overrall values.

Table 4.3: Mean Interleukin-8 (IL-8) Responses (pg/mL) of both Experimental and Control groups of NIDsirrespective of sex (n = 12).

Time (Hours)	Exp. WF. Response (pg/mL) (n = 6)	Exp. Serum response (pg/mL) (n = 6)	Cont. Serum response (pg/mL) (n = 6)
Pre-operative (Start)		352.80 ± 101.87 (120.60 - 698.90)	353.28 ± 102.57 (120.60 - 714.60)
Post-operative			
12	206.95 ± 49.15[a] (62.18 - 360.30)	714.98 ± 205.31[b] (40.10 - 1413.0)	337.10 ± 87.14[a,b] (106.50 - 728.70)
36	126.04 ± 22.92 (57.76 - 225.8)	473.44 ± 165.33 (101.30 - 1059.0)	301.45 ± 102.46 (32.80 - 592.10)
60	217.51 ± 105.06[a] (89.30 - 740.90)	729.03 ± 240.59[b] (75.70 - 1452.0)	355.68 ± 138.74[a,b] (65.70 - 862.20)
156	248.78 ± 75. 27 (65.70 - 539.60)	458.17 ± 137.25 (124.50 - 1020.0)	383.16 ± 157.57 (51.10 - 918.30)
324		389.90 ± 52.04 (264.90 - 587.40)	221.65 ± 5.25 (205.10 - 230.80)
*Mean ± SEM	199.80 ± 26.15	519.7 ± 66.48	325.4 ± 23.45

[a,b] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. Sources of variation (Interaction: $P = 0.90$; Time: $P = 0.13$; Groups: $P < 0.0001$). Values in parenthesis are the ranges (minimum – maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, pg/mL = pico-gram per millilitre, n = total number of individuals in group and (*) = Overrall values.

Table 4.4: Mean Interleukin-8 (IL-8) Responses (pg/mL) of both Experimental and Control groups of NIDs (n = 12).

Time (Hours)	Exp. WF. Response (pg/mL) Males (n = 3)	Exp. WF. Response (pg/mL) Females (n = 3)	Exp. Serum response (pg/mL) Males (n = 3)	Exp. Serum response (pg/mL) Females (n = 3)	Cont. Serum response (pg/mL) Males (n = 3)	Cont. Serum response (pg/mL) Females (n = 3)
Pre-operative (Start)	-	-	330.40 ± 149.4 (130.70 - 622.70)	375.30 ± 170.5 (120.60 - 698.90)	380.50 ± 175.5 (120.60 - 714.60)	326.00 ± 145.20 (130.70 - 609.80)
Post-operative 12	167.20 ± 42.78 (81.89–216.00)	246.70 ± 93.1 (62.18 - 360.30)	767.90 ± 328.8 (334.70-1413.00)	662.00 ± 316.0 (40.10-1070.00)	364.70 ± 187.2 (106.50-728.70)	309.50 ± 46.3 (220.90-377.30)
36	118.70 ± 28.00 (99.65 - 141.60)	133.4 ± 49.22 (57.76 - 225.8)	515.00 ± 190.8 (199.40 - 858.60)	431.90 ± 313.9 (101.30 – 1059)	389.70 ± 142.9 (113.80 - 592.10)	213.20 ± 155.9 (32.80 - 523.60)
60	323.20 ± 209.4 (89.30 - 740.90)	111.80 ± 13.55[a] (89.30 - 136.10)	830.20 ± 320.5[b] (208.20-1275.00)	627.90 ± 420.1 (75.70-1452.00)	350.90 ± 180.7 (79.10 – 693.00)	360.40 ± 252.2 (65.70 - 862.20)
156	276.10 ± 132.4 (121.50-539.60)	221.50 ± 100.2 (65.70 - 408.60)	558.10 ± 258.8 (124.50-1020.00)	358.20 ± 131.2 (128.60 - 583.00)	380.30 ± 227.4 (145.10 - 835.10)	386.10 ± 269.1 (51.10 – 918.30)
324	-	-	418.50 ± 85.31 (312.70 - 587.40)	361.30 ± 73.77 (264.90 - 506.20)	221.60 ± 8.4 (205.10 - 230.80)	221.60 ± 8.3 (205.10 - 230.80)
*Mean±SEM	221.30 ± 47.29	178.30 ± 32.89	570.00 ±79.67	469.40 ± 56.72	348.0 ± 25.89	302.80 ± 29.13

[a,b] = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. Sources of variation (Interaction: $P = 0.10$; Time: $P = 0.21$; Groups: $P < 0.001$). Values in parenthesis are the ranges (minimum – maximum) from the values.Exp. = Experimental, WF. = wound fluids, Cont. = control, pg/mL = pico-gram per millilitre, n = total number of individuals in group and (*) = Overrall values.

Table 4.5: Mean Interleukin-10 (IL-10) Responses (ng/mL) of both Experimental and Control groups of NIDs irrespective of sex (n = 12).

Time (Hours)	Exp. WF. Response (ng/mL) (n = 6)	Exp. Serum response (ng/mL) (n = 6)	Cont. Serum response (ng/mL) (n = 6)
Pre-operative (Start)		0.85 ± 0.02 (0.81 - 0.93)	0.85 ± 0.02 (0.81 - 0.93)
Post-operative			
12	1.03 ± 0.11 (0.74 - 1.42)	0.90 ± 0.04 (0.82 - 1.07)	0.86 ± 0.02 (0.79 - 0.91)
36	0.88 ± 0.03 (0.81 - 0.99)	0.98 ± 0.09 (0.80 - 1.36)	0.81 ± 0.04 (0.62 - 0.88)
60	0.82 ± 0.02 (0.74 - 0.90)	0.91 ± 0.04 (0.81 - 1.10)	0.86 ± 0.02 (0.81 - 0.91)
156	0.87 ± 0.03[a] (0.82 - 0.98)	1.67 ± 0.33[b] (0.85 - 2.68)	0.94 ± 0.17[a] (0.68 - 1.78)
324		0.84 ± 0.03 (0.72 - 0.95)	0.87 ± 0.02 (0.81 - 0.91)
*Mean ± SEM	0.90 ± 0.05	1.02 ± 0.13	0.86 ± 0.02

a,b = Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. Sources of variation (Interaction: $P < 0.0001$; Time: $P < 0.0001$; Groups: $P < 0.0001$). Values in parenthesis are the ranges (minimum – maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, ng/mL = nano-gram per millilitre, n = total number of individuals in group and (*) = Overrall values.

Table 4.6: Mean Interleukin-10 (IL-10) Responses (ng/mL) of both Experimental and Control groups of NIDs(n = 12).

(ng/mL)	Exp. WF. Response (ng/mL)		Exp. Serum response (ng/mL)		Cont. Serum response	
Time (Hours)	Males (n = 3)	Females (n = 3)	Males (n = 3)	Females (n = 3)	Males (n = 3)	Females (n = 3)
Pre-operative (Start)	-	-	0.90 ± 0.02 (0.85 - 0.93)	0.81 ± 0.00 (0.81 - 0.81)	0.89 ± 0.02 (0.85 - 0.93)	0.81 ± 0.0 (0.81 - 0.81)
Post-operative 12	1.02 ± 0.16 (0.74 - 1.29)	1.05 ± 0.04 (0.82 - 1.42)	0.92 ± 0.08 (0.82 - 1.07)	0.88 ± 0.02 (0.85 - 0.91)	0.86 ± 0.03 (0.81 - 0.91)	0.86 ± 0.19 (0.79 - 0.91)
36	0.86 ± 0.02 (0.83 - 0.90)	0.91 ± 0.05 (0.81 - 0.99)	0.96 ± 0.08 (0.87 - 1.11)	1.01 ± 0.08 (0.80 - 1.36)	0.79 ± 0.18 (0.62 - 0.88)	0.82 ± 0.02 (0.78 - 0.87)
60	0.82 ± 0.02 (0.81 - 0.86)	0.82 ± 0.05 (0.74 - 0.90)	0.93 ± 0.08 (0.82 - 1.10)	0.88 ± 0.03 (0.81 - 0.91)	0.88 ± 0.02 (0.86 - 0.91)	0.84 ± 0.03 (0.81 - 0.90)
156	0.85 ± 0.53^{a,d} (0.82 - 0.91)	0.90 ± 0.50^{a,d} (0.85 - 0.98)	1.73 ± 0.03^{b} (0.85 - 2.68)	1.62 ± 0.04^{c} (0.86 - 2.55)	1.16 ± 0.31^{a} (0.83 - 1.78)	0.72 ± 0.03^{d} (0.68 - 0.77)
324	-	-	0.86 ± 0.05 (0.81 - 0.95)	0.82 ± 0.05 (0.72 - 0.89)	0.87 ± 0.02 (0.83 - 0.91)	0.86 ± 0.03 (0.81 - 0.90)
*Mean ± SEM	0.89 ± 0.04	0.92 ± 0.05	1.05 ± 0.14	1.00 ± 0.13	0.91 ± 0.05	0.82 ± 0.02

^{a,b,c,d}= Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. Sources of variation (Interaction: $P < 0.001$; Time: $P < 0.001$; Groups: $P < 0.001$). Values in parenthesis are the ranges (minimum – maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, ng/mL = nano-gram per millilitre, n = total number of individuals in group and (*) = Overrall values.

Table 4.7: Mean Tumour Necrosis Factor Alpha (TNF-α) Responses (pg/mL) of both Experimental and Control groups of NIDsirrespective of sex (n = 12).

Time (Hours)	Exp. WF. Response (pg/mL) (n = 6)	Exp. Serum response (pg/mL) (n = 6)	Cont. Serum response (pg/mL) (n = 6)
Pre-operative (Start)		27.03 ± 9.76 (6.56 - 65.56)	21.95 ± 4.57 (7.74 - 40.84)
Post-operative			
12	65.65 ± 8.72 (45.64 - 93.16)	47.21 ± 18.60 (9.87 - 128.20)	45.60 ± 12.29 (8.35 - 85.53)
36	130.22 ± 35.15[a] (41.96 - 277.30)	44.50 15.92[b] (10.53 - 98.49)	28.27 ± 5.24[b] (16.46 - 50.41)
60	54.74 ± 11.14[a,b] (13.74 - 81.08)	116.17 ± 60.27[a] (15.38 - 352.60)	29.79 ± 7.47[b] (9.87 - 56.89)
156	25.55 ± 4.94 (15.08 - 42.64)	66.85 ± 29.13 (12.59 - 160.60)	47.06 ± 14.34 (18.54 - 109.20)
324		48.00 ± 20.24 (13.89 - 145.70)	20.04 ± 1.78 (16.46 - 27.77)
*Mean ± SEM	69.04 ± 22.08	58.29 ± 12.67	32.12 ± 4.74

[a,b] = Mean (Mean ± Standard Error of Mean) with differentsuperscript letters are significantly (P < 0.05) different. Sources of variation (Interaction: P = 0.006; Time: P = 0.005; Groups: P = 0.198). Values in parenthesis are the ranges (minimum – maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, pg/mL = pico-gram per millilitre, n = total number of individuals in group and (*) = Overrall values.

Table 4.8: Mean Tumour Necrosis Factor Alpha (TNF-α) Responses (pg/mL) of both Experimental and Control groups of NIDs(n = 12).

(pg/mL)	Exp. WF. Response (pg/mL)		Exp. Serum response (pg/mL)		Cont. Serum response	
Time (Hours)	Males (n = 3)	Females (n = 3)	Males (n = 3)	Females (n = 3)	Males (n = 3)	Females (n = 3)
Pre-operative (Start)	-	-	23.06 ± 12.04 (7.74 - 46.81)	31.00 ± 17.77 (6.56 - 65.56)	27.88 ± 6.49 (20.88 - 40.84)	16.02 ± 5.23 (7.74 - 25.70)
Post-operative 12	68.12 ± 13.78 (45.64 - 93.16)	63.17 ± 13.58 (46.58 - 90.08)	52.72 ± 37.76 (12.73 - 128.20)	41.70 ± 16.56 (9.87 - 65.56)	23.54 ± 13.59 (8.35 - 50.65)	67.66 ± 9.15 (55.36 - 85.53)
36	179.00 ± 58.19[a] (75.86 - 277.30)	81.44 ± 20.31[b] (41.96 - 109.50)	46.48 ± 21.78[b,c] (18.87 - 89.47)	42.52 ± 28.08[b,c] (10.53 - 98.49)	21.93 ± 3.27[c] (16.46 - 27.77)	34.61 ± 9.30[b] (18.21 - 50.41)
60	44.66 ± 18.83 (13.74 - 78.74)	64.82 ± 12.82 (39.51 - 81.08)	102.90 ± 74.38 (20.20 - 251.30)	129.50 ± 111.60 (15.38 - 352.60)	30.13 ± 8.91 (12.45 - 40.84)	29.44 ± 14.13 (9.87 - 56.89)
156	23.09 ± 7.56 (15.08 - 38.20)	28.01 ± 7.69 (16.62 - 42.64)	69.17 ± 43.85 (24.78 - 156.90)	64.53 ± 48.1 (12.59 - 160.60)	43.38 ± 12.65 (18.54 - 59.98)	50.73 ± 29.24 (19.70 - 109.20)
324	-	-	62.84 ± 41.43 (20.03 - 145.70)	33.17 ± 10.59 (13.89 - 50.41)	21.93 ± 3.27 (16.46 - 27.77)	18.14 ± 1.26 (16.31 - 20.54)
*Mean±SEM	78.72 ± 34.67	59.36 ± 11.23	59.53 ± 10.85	57.07 ± 15.28	28.13 ± 3.34	36.10 ± 8.13

[a,b,c]= Mean (Mean ± Standard Error of Mean) with different superscript letters are significantly ($P < 0.05$) different. Sources of variation (Interaction: $P = 0.22$; Time: $P < 0.01$; Groups:$P=0.63$). Values in parenthesis are the ranges (minimum – maximum) from the values. Exp. = Experimental, WF. = wound fluids, Cont. = control, pg/mL = pico-gram per millilitre, n = total number of individuals in group and (*) = Overrall values.

4.3 Haemogram

4.3.1 Erythrocyte parameters of both experimental and control groups (n = 12).

The mean values as shown on tables 4.9 to 4.11 are for haematocrit (HCT), red blood cells count (RBC), haemoglobin concentration (HGB). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) obtained for both experimental and control groups are as shown on appendix XXI - XXVI. All values until termination of the experiment are as shown in Tables 4.9 to 4.11.

Table 4.9: Mean Haematocrit (HCT) Values of both Experimental and Control Groups of NIDsObtained Pre- and Post-operative.

TIME (Hours)	EXPERIMENTAL GROUP			CONTROL GROUP		
	Male + Female (n =6)	Males (n = 3)	Female (n = 3)	Male + Female (n =6)	Male (n = 3)	Female (n = 3)
Pre-operative (Start)	0.367 ± 0.003 (0.351 - 0.374)	0.371 ± 0.002 (0.367 - 0.374)	0.363 ± 0.006 (0.351 - 0.371)	0.368 ± 0.004 (0.357 - 0.39)	0.374 ± 0.004 (0.367 - 0.380)	0.362 ± 0.004 (0.357 - 0.369)
Post-operative						
12	0.354 ± 0.007 (0.326 - 0.370)	0.356 ± 0.010 (0.337 - 0.369)	0.353 ± 0.014 (0.326 - 0.370)	0.364 ± 0.003 (0.351 - 0.375)	0.369 ± 0.003 (0.365 - 0.375)	0.359 ± 0.004 (0.351 - 0.365)
36	0.358 ± 0.013 (0.309 - 0.3880)	0.363 ± 0.017 (0.329 - 0.385)	0.353 ± 0.023 `(0.309 - 0.388)	0.365 ± 0.003 (0.356 - 0.376)	0.366 ± 0.006 (0.356 - 0.376)	0.364 ± 0.003 (0.360 - 0.370)
60	0.368 ± 0.010 (0.326 - 0.392)	0.379 ± 0.006 (0.369 - 0.389)	0.356 ± 0.019 0.326 - 0.392)	0.365 ± 0.003 (0.356 - 0.376)	0.365 ± 0.060 (0.356 - 0.376)	0.366 ± 0.030 (0.360 - 0.370)
156	0.370 ± 0.005 (0.360 - 0.392)	0.376 ± 0.009 (0.361 - 0.392)	0.364 ± 0.003 (0.360 - 0.370)	0.365 ± 0.002 (0.360 - 0.373)	0.366 ± 0.003 (0.360 - 0.372)	0.365 ± 0.004 (0.361 - 0.373)
324	0.374 ± 0.007 (0.345 - 0.391)	0.378 ± 0.007 (0.368 - 0.391)	0.369 ± 0.013 (0.345 - 0.389)	0.370 ± 0.003 (0.361 - 0.380)	0.370 ± 0.002 (0.367 - 0.375)	0.369 ± 0.006 (0.361 - 0.380)

Values in parenthesis are the ranges (minimum – maximum) from the values.

Table 4.10: Mean Red blood cells count (10^{12}/L) Valuesof both Experimental and Control Groups of NIDs Obtained Pre- and Post-operative.

TIME (Hours)	EXPERIMENTAL GROUP			CONTROL GROUP		
	Male + Female (n = 6)	Males (n = 3)	Female (n = 3)	Male + Female (n = 6)	Male (n = 3)	Female (n = 3)
Pre-operative (Start)	6.19 ± 0.10 (5.87 - 6.48)	6.11 ± 0.13 (5.87 - 6.30)	6.27 ± 0.15 (5.97 - 6.48)	6.01 ± 0.14 (5.62 - 6.36)	6.11 ± 0.19 (5.72 - 6.36)	5.91 ± 0.22 (5.62 - 6.35)
Post-operative						
12	5.54 ± 0.37 (5.43 - 6.31)	5.65 ± 0.12 (5.43 - 5.83)	5.44 ± 0.81 (3.82 - 6.31)	6.20 ± 0.08 (5.97 - 6.46)	6.17 ± 0.10 (6.01 - 6.34)	6.23 ± 0.15 (5.96 - 6.46)
36	6.05 ± 0.28 (4.78 - 6.59)	6.32 ± 0.21 (5.91 - 6.59)	5.77 ± 0.53 (4.78 - 6.57)	6.29 ± 0.07 (6.13 - 6.58)	6.35 ± 0.13 (6.13 - 6.58)	6.22 ± 0.05 (6.16 - 6.31)
60	5.96 ± 0.26 (4.99 - 6.60)	6.47 ± 0.08 (6.33 - 6.60)	5.45 ± 0.28 (4.99 - 5.96)	6.19 ± 0.14 (5.49 - 6.43)	6.05 ± 0.29 (5.49 - 6.43)	6.32 ± 0.07 (6.19 - 6.41)
156	6.01 ± 0.13 (5.64 - 6.54)	6.24 ± 0.17 (5.97 - 6.54)	5.78 ± 0.08 (5.64 - 5.92)	6.53 ± 0.17 (6.01 - 7.10)	6.40 ± 0.28 (6.01 - 6.94)	6.66 ± 0.22 (6.42 - 7.10)
324	6.08 ± 0.30 (4.96 - 6.97)	6.59 ± 0.22 (6.22 - 6.97)	5.56 ± 0.36 (4.96 - 6.20)	6.04 ± 0.15 (5.62 - 6.54)	6.05 ± 0.22 (5.62 - 6.38)	6.02 ± 0.26 (5.70 - 6.54)

Values in parenthesis are the ranges (minimum – maximum) from the values.

Table 4.11: Mean Haemoglobin (g/L) Valuesof both Experimental and Control Groups of NIDs Obtained Pre- and Post-operative

TIME (Hours)	EXPERIMENTAL GROUP			CONTROL GROUP		
	Male + Female (n = 6)	Males (n = 3)	Female (n = 3)	Male + Female (n = 6)	Males (n = 3)	Female (n = 3)
Pre-operative (Start)	123.50 ± 8.35 (90.66 - 154.51)	124.80 ± 18.56 (90.66 - 154.51)	122.24 ± 1.41 120.03 - 124.86)	123.80 ± 1.98 (117.08 - 125.12)	127.04 ± 1.94 (124.76 - 130.90)	120.66 ± 2.36 (117.08 - 125.12)
Post-operative						
12	123.10 ± 1.28 (117.97 - 126.69)	121.87 ± 2.56 (117.97 - 126.69)	124.29 ± 0.39 (123.68 - 125.02)	119.30 ± 1.52 (115.45 - 124.59)	119.08 ± 2.80 (115.45 - 124.59)	119.53 ± 1.93 (117.16 - 123.35)
36	124.30 ± 3.98 (108.10 - 135.10)	126.93 ± 5.11 (117.54 - 135.10)	121.46 ± 6.76 (108.10 - 129.99)	132.40 ± 10.09 (117.52 - 182.60)	121.06 ± 1.88 (117.52 - 123.92)	143.81 ± 19.40 (123.82 - 182.60)
60	126.90 ± 4.14 (113.76 - 142.98)	128.43 ± 1.74 (126.59 - 131.91)	125.35 ± 8.96 (113.76 - 142.98)	124.20 ± 1.96 (115.37 - 128.20)	123.32 ± 3.98 (115.37 - 127.32)	124.99 ± 1.62 (122.37 - 128.20)
156	119.40 ± 2.62 (110.55 ± 128.27)	124.13 ± 2.60 (119.34 - 128.27)	114.71 ± 2.34 (110.55 - 118.65)	125.70 ± 2.88 (115.45 - 135.70)	123.35 ± 3.99 (115.45 - 128.32)	127.97 ± 4.50 (120.12 - 135.70)
324	123.80 ± 4.16 (104.88 - 131.70)	127.98 ± 3.39 (121.22 - 131.70)	119.64 ± 7.58 (104.88 - 130.00)	124.30 ± 1.70 (118.39 - 128.85	125.68 ± 1.70 (122.44 - 128.19)	122.89 ± 3.11 (118.39 - 128.85)

Values in parenthesis are the ranges (minimum – maximum) from the values.

4.3.2 White blood cell counts of both experimental and control groups (n = 12)

The mean white blood cell counts irrespective of sex are as shown on Table 4.12.Figures 4.1 – 4.5presents the white blood cell counts obtained both in male and female NIDs for the experimental and control groups. In the control group, the mean total white blood cell counts rangedbetween 10.23±0.32 and 11.12±0.75 x 10^9/L with the range values in both male and female NIDsas 10.53 ± 0.41 - 11.30 ± 1.49 x10^9/L and 9.63 ± 1.58 - 11.93 ± 0.33 x10^9/L, respectively. While those for the male and female NIDs in the experimental group, rose from the pre-operative ranges of 9.30 ± 1.27 x10^9/L and 8.33 ± 1.25 x10^9/L to post-operative ranges of10.23 ± 2.66 -13.43 ± 0.90 x10^9/L and 10.60 ± 0.51 - 12.50 ± 1.27 x10^9/L, respectively. The differential counts in the control group were maintained within normal range values while those for the male and female of the experimental group had higher values which were within the normal limits in the range of:Neutrophils (6.52 ± 0.84 - 9.21 ± 0.87 x10^9/L and 6.01 ± 0.85 - 9.61 ± 1.93 x10^9/L), lymphocytes (2.61 ± 0.38 - 4.15 ± 0.17 x10^9/L and 2.18 ± 0.37 - 3.78 ± 0.36 x10^9/L), Monocytes (0.03 ± 0.03 - 0.29 ± 0.05 x10^9/L and 0.08 ± 0.04 - 0.22 ± 0.11 x10^9/L) and Eosinophils (0.05 ± 0.05 - 0.15 ± 0.09 x10^9/L and 0.03 ± 0.03 - 0.05 ± 0.05 x10^9/L), respectively.

Table 4.12: Mean Total and Differential Leucocyte Counts of NIDs of Non-specific Sex Induced with Cutaneous Injuries.

Parameters/Groups	Pre-wounding	Hours Post-wounding				
	0	12	36	60	156	324
TOTAL WBC(6 – 17 x 10^9/L)						
Experimental (n = 6)	8.82±0.82 (6.30 - 11.20)	10.58±0.38 (9.50 - 11.80)	11.43±0.49 (9.60 - 12.90)	11.33±1.53 (6.90 - 15.80)	12.12±1.18 (9.10 - 16.30)	12.08±0.94 (9.00 - 14.50)
Control (n = 6)	10.32±1.10 (8.00 - 14.40)	11.12±0.75 (9.00 - 14.10)	10.23±0.32 (9.00 - 11.20)	10.10±0.48 (8.00 - 11.20)	11.08±0.73 (9.00 - 14.10)	10.32±1.10 (8.00 - 14.40)
NEUTROPHILS (3 – 12 x 10^9/L)						
Experimental (n = 6)	6.27±0.54 (4.59 - 7.84)	7.57±0.35 (6.37 - 8.93)	8.08±0.38 (6.91 - 9.29)	8.45±1.43 (4.55 - 13.11)	8.05±1.15 (5.19 - 11.74)	8.75±0.82 (5.67 - 11.02)
Control (n = 6)	6.94±0.76 (5.02 - 9.79)	7.66±0.46 (5.85 - 9.31)	6.93±0.36 (6.08 - 8.18)	7.25±0.38 (6.17 - 8.40)	7.66±0.46 (5.85 - 9.31)	6.94±0.76 (5.02 - 9.79)
LYMPHOCYTES (1 – 5 x 10^9/L)						
Experimental (n = 6)	2.40±0.26[a] (1.58 - 3.14)	2.71±0.16 (2.28 - 3.30)	3.05±0.23 (2.30 - 3.78)	2.41±0.20[a] (2.07 - 3.41)	3.96±0.20[b] (3.17 - 4.42)	3.23±0.14 (2.90 - 3.90)
Control (n = 6)	3.15±0.31 (2.24 - 4.18)	3.23±0.34 (2.42 - 4.51)	2.98±0.30 (1.84 - 3.70)	2.99±0.29 (1.88 - 3.70)	3.45±0.35 (2.41 - 4.51)	3.15±0.31 (2.24 - 4.18)
MONOCYTES (0 – 1.4 x 10^9/L)						
Experimental (n = 6)	0.07±0.03 (0.00 - 0.21)	0.25±0.06 (0.00 - 0.35)	0.22±0.06 (0.00 - 0.38)	0.18±0.05 (0.00 - 0.31)	0.06±0.03 (0.00 - 0.14)	0.08±0.05 (0.00 - 0.29)
Control (n = 6)	0.05±0.02 (0.00 - 0.14)	0.07±0.02 (0.00 - 0.12)	0.07±0.05 (0.00 - 0.32)	0.08±0.04 (0.00 - 0.21)	0.08±0.03 (0.00 - 0.14)	0.05±0.02 (0.00 - 0.14)
EOSINOPHIL (0.1 – 1.3 x 10^9/L)						
Experimental (n = 6)	0.09±0.03 (0.00 - 0.22)	0.05±0.02 (0.00 - 0.12)	0.08±0.03 (0.00 - 0.13)	0.09±0.05 (0.00 - 0.32)	0.05±0.03 (0.00 - 0.16)	0.04±0.03 (0.00 - 0.15)
Control (n = 6)	0.18±0.05 (0.00 - 0.30)	0.14±0.04 (0.00 - 0.28)	0.11±0.04 (0.00 - 0.22)	0.13±0.03 (0.00 - 0.22)	0.13±0.03 (0.00 - 0.22)	0.18±0.05 (0.00 - 0.30)

[a,b]= Mean (Mean ± Standard Error of Mean) with different superscript letters significantly ($P < 0.05$) different. Ranges are in parenthesis.

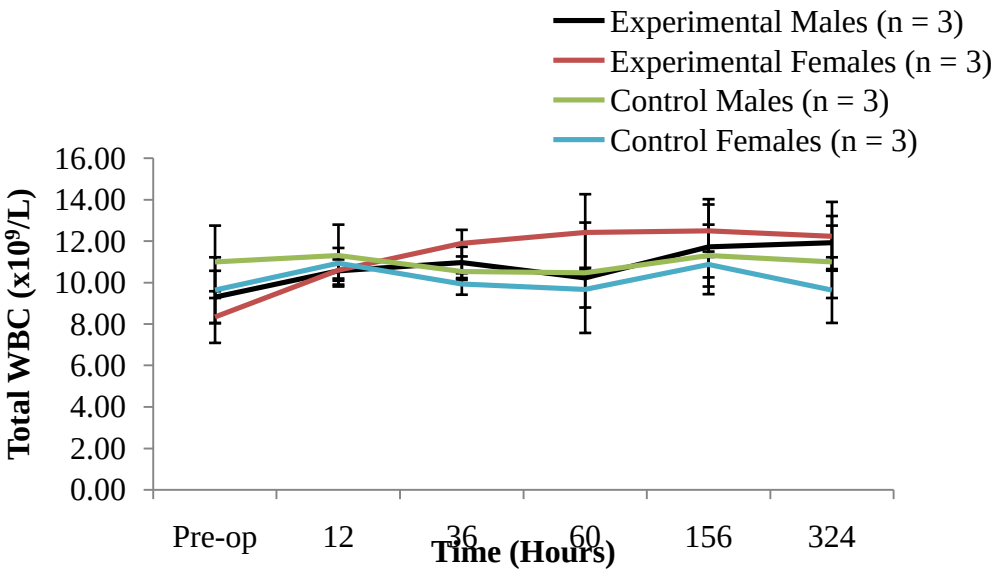


Figure 4.1: **Total White Blood Cells Count of the Experimental and Control Groups with minimal fluctuations although all values are maintained within normal range limits for the NIDs. Mean (Means ± SEM), (n = 12).**

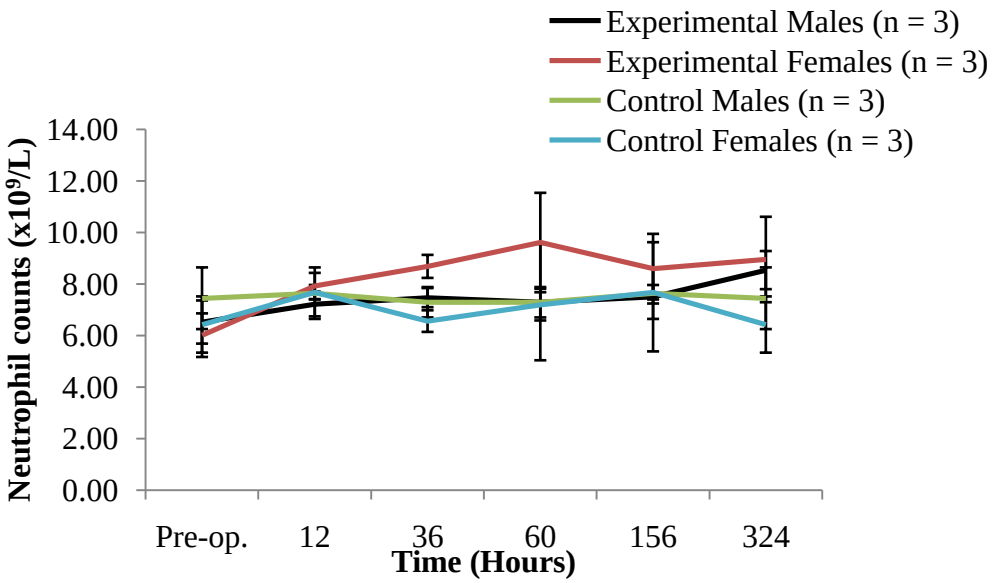


Figure 4.2: Variations in Mean Neutrophils Count of the Experimental and Control Groups NIDs. Mean (Means ± SEM), (n = 12).

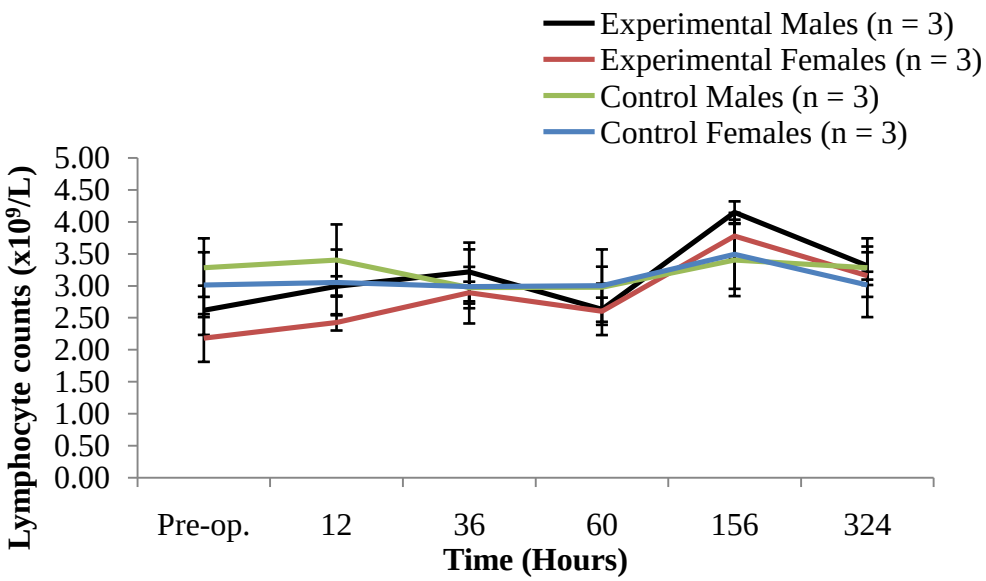


Figure 4.3: Variations in Mean Lymphocyte Count of the Experimental and Control Groups NIDs. Mean (Means ± SEM), (n = 12).

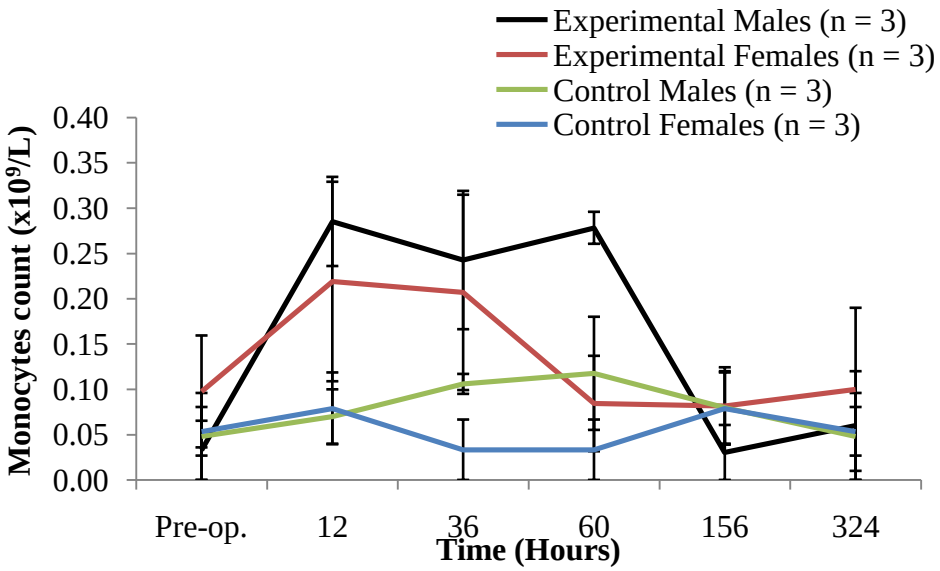


Figure 4.4: Variations in Mean Monocyte Count of the Experimental and Control Groups NIDs. Mean (Means ± SEM), (n = 12).

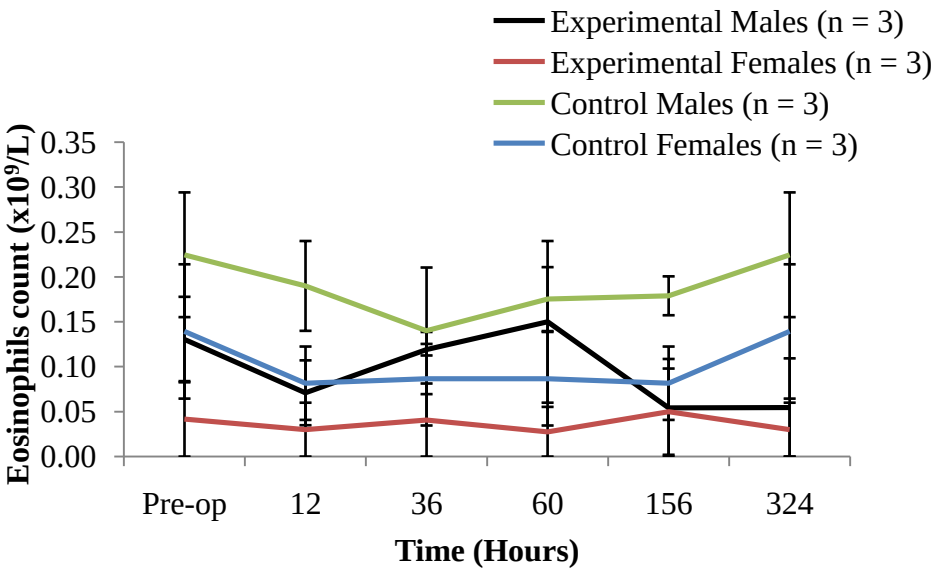


Figure 4.5: Variations in Eosinophil Count of the Experimental and Control Groups NIDs. Mean (Means ± SEM), (n = 12).

4.4. Relationship between Cytokines (IL-6, IL-8, IL-10 and TNF-α) Levels, Haematologic Parameters and Wound Contraction in injured NIDs (n = 12).

Appendix XXVII – XXXVII shows the associations (both significant and non significant) between the various studied cytokines (IL-6, IL-8, IL-10 and TNF-α)and the cytokines with the haematologic parameters and percentage wound contraction. The significant correlations were:

Wound fluid with serum IL-6, wound fluid IL-8 with IL-10, wound fluid IL-6 with HCT and RBC and monocytes, serum IL-6 with RBC, serum IL-10 with lymphocytes and wound fluid TNF-α with monocytes in NIDs irrespective of sex (Figure 4.6).

Correlations of the cytokines level between the sera and wound fluids IL-6, Sera and wound fluids IL-8 in males, wound fluids IL-8 and IL-10 and Sera IL-6 and IL-10 were significant ($P < 0.05$) (Figure 4.7).

Wound fluids IL-6 and haematocrit (HCT) in females, wound fluids IL-6 and mean corpuscular haemoglobin concentration (MCHC) in males, serum IL-8 and mean corpuscular haemoglobin (MCH) and serum IL-10 and Haemoglobin concentration (HGB) in females were significant ($P < 0.05$) (Figure 4.8).

Relationship between wound fluids IL-6 and monocytes, Serum IL-8 and monocytes, serum IL-6 and Eoisinophils, serum IL-10 with Lymphoctes in females and Eoisinophils in females and Lymphocytes in males were all significant ($P < 0.05$) as shown in figure 4.9.

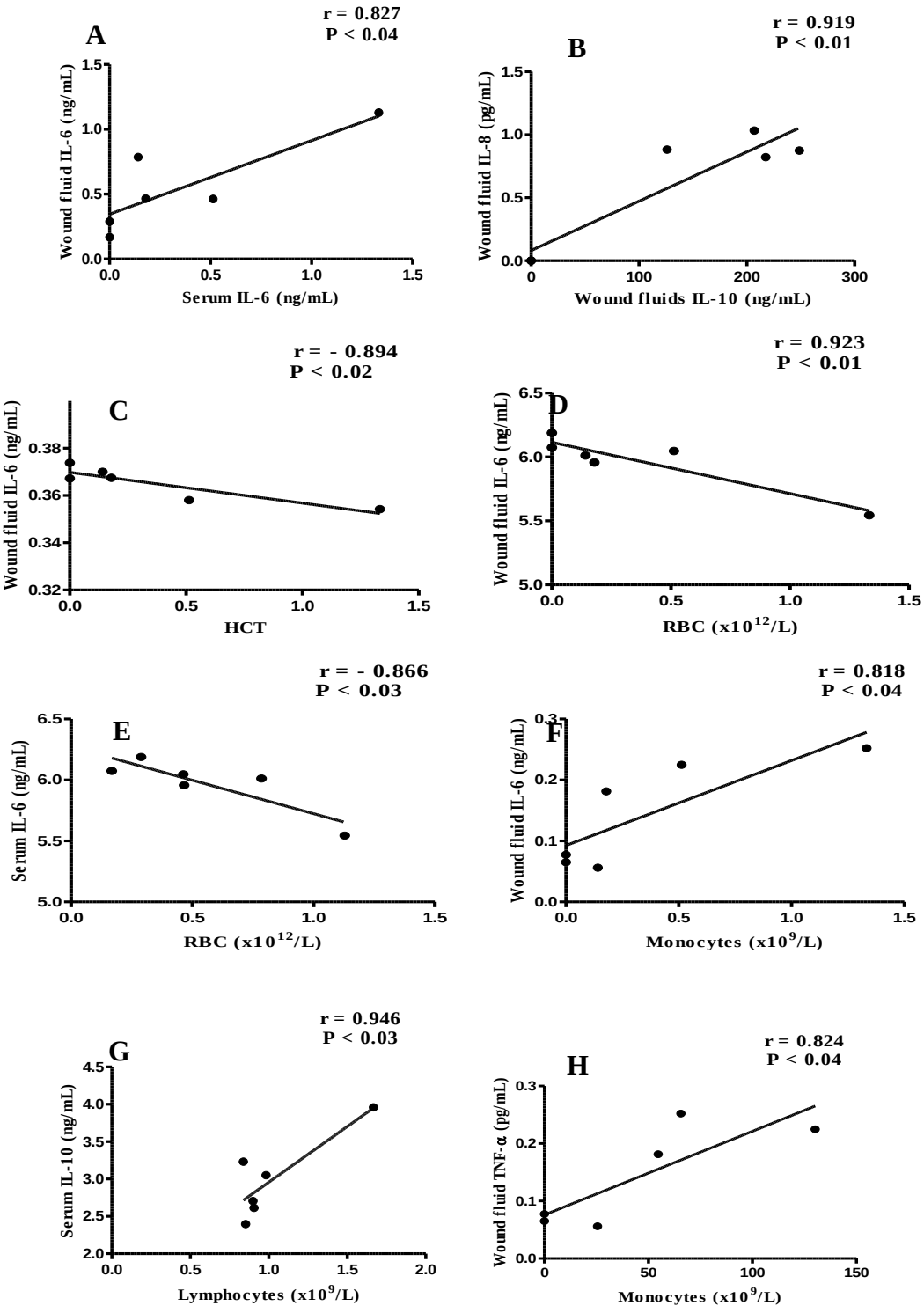


Figure 4.6: Relationship between the levels of IL-6, IL-8, IL-10 and TNF-α irrespective of sex: (A) Wound fluid with serum IL-6; (B) wound fluid IL-8 with IL-10; (C and D) wound fluid IL-6 with HCT and RBC; (E) serum IL-6 with RBC; (F) wound fluid IL-6 with monocytes; (G) Serum IL-10 with lymphocytes and (H) wound fluid TNF-α with monocytes. r = Pearson's correlation coefficient, P < 0.05 are considered significant.

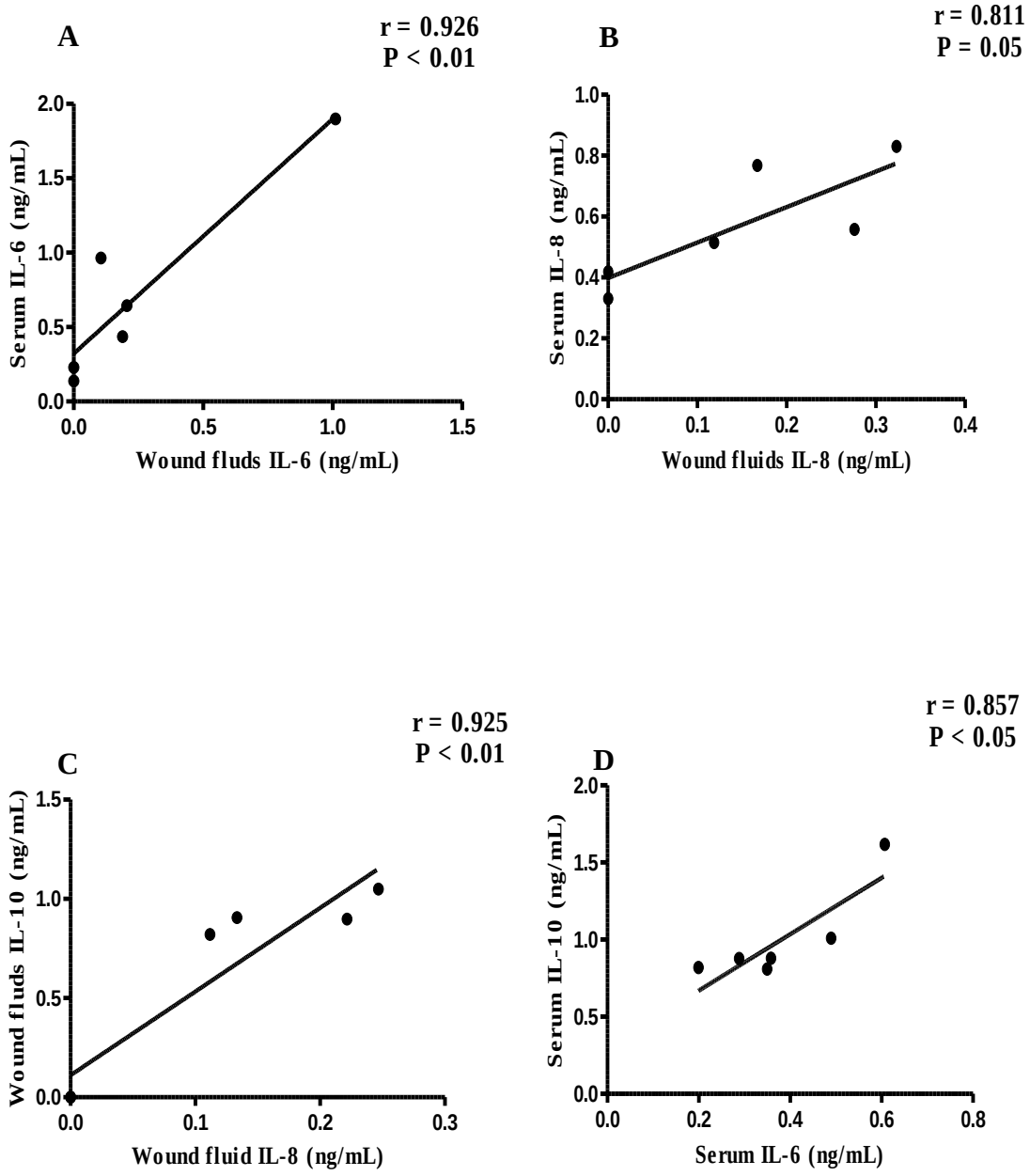


Figure 4.7: Relationship between the levels of IL-6, IL-8, IL-10 and TNF-α with each other. (A) Wound fluid and serum IL-6 in males; (B) Wound fluid and serum IL-8 in males; (C) Wound fluid IL-8 and IL-10 in female; (D) Serum IL-6 and IL-10 in female NIDs. r = Pearson's correlation coefficient, P < 0.05 are considered significant.

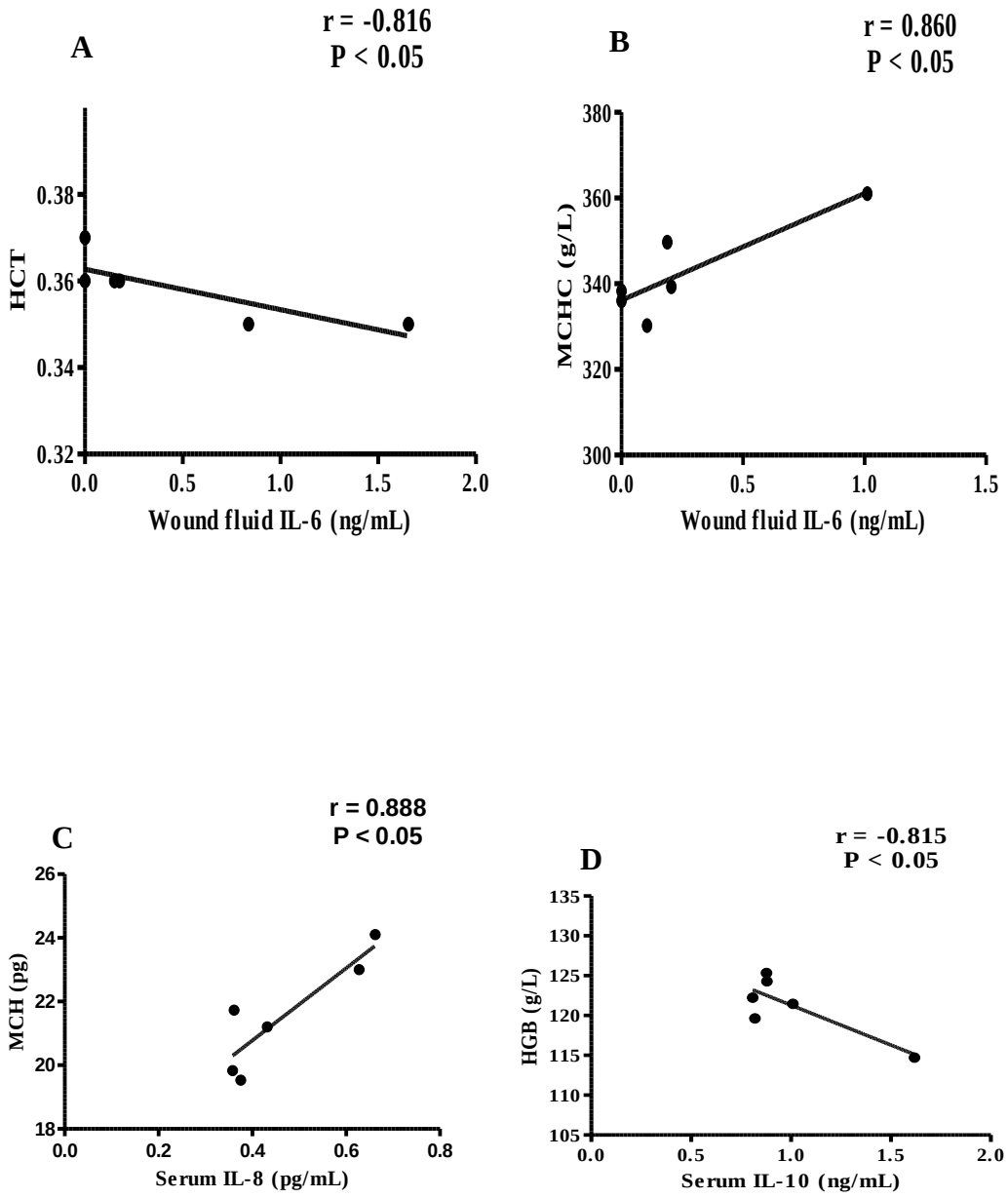


Figure 4.8: Relationship between levels of IL-6, IL-8, IL-10 and TNF-α and profiles of haematologic parameters. (A) HCT and wound fluid IL-6 in females; (B) MCHC and wound fluid IL-6 in males; (C) MCH and serum IL-8 in females; (D) HGB and serum IL-10 in female NIDs. HCT; haematocrit, HGB; haemoglobin concentration, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration; r = Pearson's correlation coefficient, P < 0.05 are considered significant.

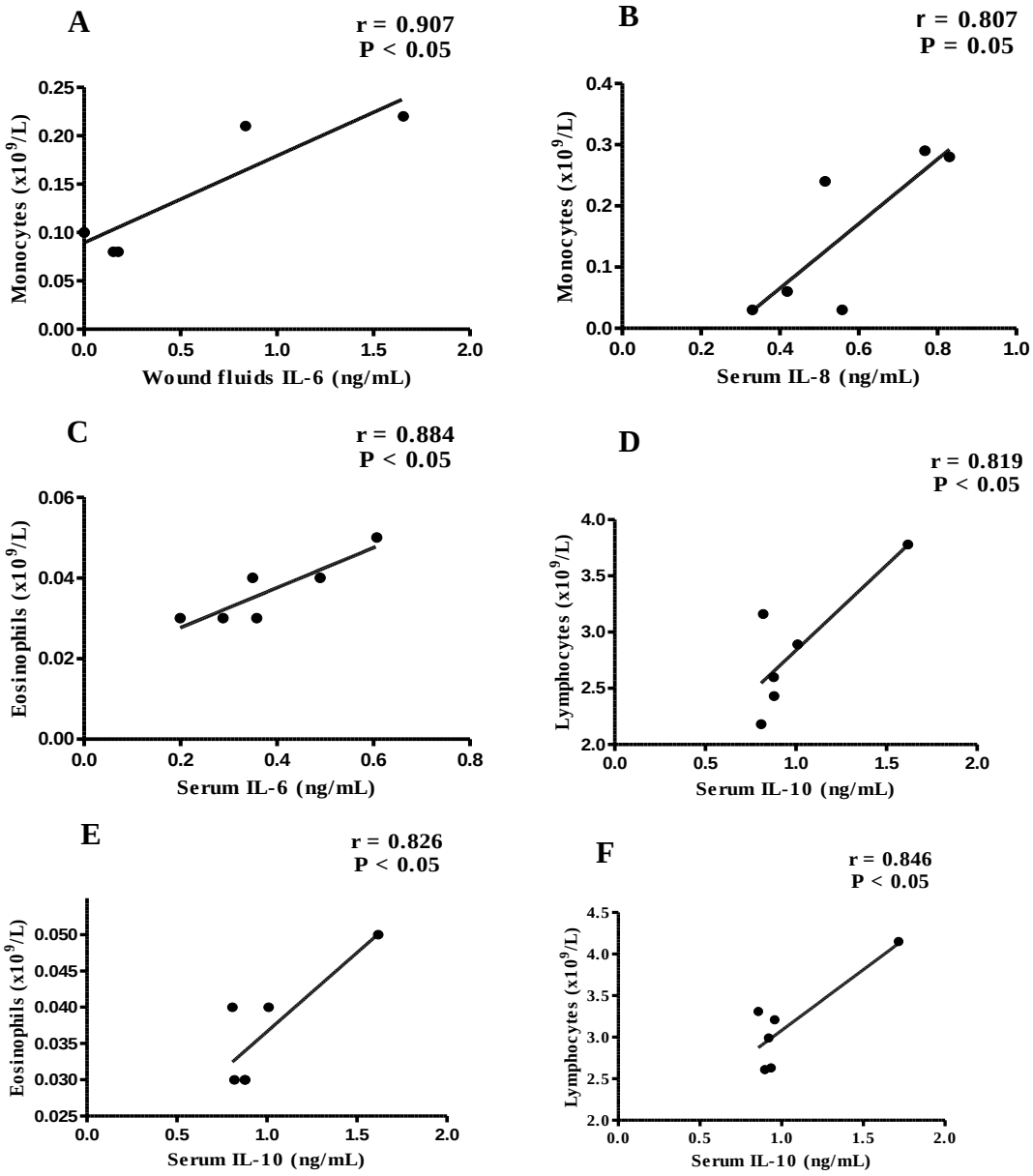


Figure 4.9: Relationship between levels of IL-6, IL-8, IL-10 and TNF-α and White Blood Cells profile (A) Monocyte and wound fluid IL-6; (B) Monocyte and serum IL-8; (C) Eosinophils and serum IL-6; (D) Lymphocytes and serum IL-10 and (E) Eosinophils and serum IL-10 levels in females (F) Lymphocytes and serum IL-10 in male NIDs. r = Pearson's correlation coefficient, P < 0.05 are considered significant.

CHAPTER FIVE

DISCUSSION

Nigerian Indigenous Dogs (NIDs) used in the study were adequate clinically and they demonstrated compliance and cooperation after successful screening, acclimatization and conditioning. There were no abnormal changes observed in terms of behavioural changes post acclimatization. As evidence of good preparation of accommodation spaces and study subject preparation, none of the selected animals studied descended with health complications in the course of the study.

This implied all strategies and medications used in the screening and treatment during the experimental phases were adequate and satisfactory. From the basic vital parameters studied, it is not unusual to have certain members appearing with high or low parameters but once the animals were treated by deworming and provided adequate nutrition and deticked, they were stable throughout the experiment as there were no other reasons for a follow up treatment.

The sharp increase in interleukin (IL-6) levels was observed 12 h post injury in wound fluids and serum ingroup B (injured), while group A (uninjured)serum levels remained unchanged. Thiscould be attributed to the local and systemic manifestation of the acute phase inflammatory responses following injury in group B NIDs. These responses involvedthe rapid release of IL-6 from epithelial cells with subsequent recruitment and activation of fibroblasts and phagocytic cells such as neutrophils, monocytes and macrophages (Warren et al., 2009; John *et al.*, 2013). This response is similar to reports

by Kondo and Ohshima, (1996) and Gallucci *et al.* (2000) in which case they observed the induction of IL-6 after wounding in normal rats, reaching maximal levels between 12 h and 16 h before gradually returning to basal levels after 24 h.The significantly ($P < 0.05$) higher Interleukin (IL) -6 levels in wound fluids compared with those in the serum within first 36 h could be ascribed to the dominating early local production similar to findings reported by Zuhoor *et al.* (2012)where higher proinflammatory cytokines were detected at operation site (seromas),compared with serum in the early hours following breast surgery in humans. In contrast, a second serum IL-6 production spike(at 156 h) of comparable level to the first observed (within first 36 h) in group B (injured NIDs) correlates with peak phagocytic, lymphocytic and fibroblastic activities at the interphase between the inflammatory and migratory/proliferative phases of wound healing (Witte and Barbul, 1997). Whilewound fluids IL-6 levels at 156 hwere maintained within control limits which is necessary for the regulation of normal biological processes such as metabolism, haematopoiesis, immune regulation and other physiologic functions (Sheeran and Hall,1997; Gueret *et al*., 2009; Owen *et al.*, 2013; Horst, 2013).

As anticipated, cutaneous injury triggered increased production of both serum and wound fluids IL-8whichpeaked at 12 h and 60 hpost injury before normalizing to control limits. These peaks encompasses the period of peak neutrophils and macrophages activities as well as the early stages of lymphocytic involvement in injuries which is usually between 12 h and 72 h (Witte and Barbul, 1997). This may serve as evidence of its chemotactic activities in target cells initiating the inflammatory processes required for migration and phagocytosis, such as increases in intracellular Ca^{2+} and exocytosis (e.g. histamine release) with consequent movement of neutrophils and other granulocytes toward the site of injury and/or infection (Utgaard *et al.,* 1998; Hedges *et al.,* 2000; Bacon *et al.,* 2002; Ono *et al.,* 1995). However, in this study, the

detected IL-8 in wound fluid of NIDs were below the controlcirculatory levels, although the trend was similar to those obtained in the sera of injured NIDs making its level a key parameter in localized injuries and inflammation (Sharabiani *et al.*, 2011).

Increased wound fluids and serum IL-10 concentrations respectively at 12 h and 36 h post injury similar to the IL-6 dynamics contrasted those of Risnes *et al*. (2003), where IL-10 levels tended to increase earlier than IL-6 levels. Also, the findings contrasted the report by Opal *et al.* (1998), where he stated that in injuries, IL-10 is synthesized later than other inflammatory cytokines in both T-cells and monocytes, suggesting its regulatory roles later in the healing process. The significantly ($P < 0.05$) and ($P < 0.001$) high peaks of IL-10 attained at 156 hfor males and females respectively is indicative of its regulatory (inhibitory) role in the later phases of the immune response to injury. These finding agree with those by Opal *et al.* (1998) and contrasted those by Risnes *et al*. (2003).The early IL-10 release occurring independently from systemic proinflammatory cytokine elevation remains unclear. It has been postulated that the programmed death (PD-1) pathway which is a co-receptor that is predominantly expressed on T-cells binds to the ligands PD-L1 or PD-L2 triggering monocytes to produce IL-10 with the minimal expression of IL-10 in unstimulated tissues, regulating the immune system by the inhibition of self reactive T-cells, which serve to protect against autoimmune diseases (Said *et al.*, 2010; Li *et al.*, 2012; Pedoeem *et al.*, 2014; Dina *et al.*, 2016; Daniel *et al.*, 2017). IL-10 possess the capability of inhibiting macrophages and regulatory T-cells' synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, IL-6, IL-8, TNFα and GM-CSF (Sikka *et al.*, 2012).

In response to injury, wound fluids TNF-α levels were significantly ($P < 0.05$) raised at 36 h as serum TNF-α levels also increased with an initial slight rise at 12 h and a later

higher rise at 60 h post injury. This finding was in contrast to reports by Kondo and Ohshima, (1996) and Adcock, (1997), which stated that TNF-α and IL-1b are transiently induced immediately after cutaneous injury, peaking at 3 h, after which both cytokines decrease to near basal levels by 10 h. These TNF-α levels serve important function as its primary role is in the regulation of immune cells via apoptotic and non apoptotic cell death pathways, inhibiting tumorigenesis and viral replication as regeneration and/or repair takes place (Brynskov *et al.*, 2002; Mikocka *et al.*, 2007; Dowlati *et al.*, 2010; Swardfager *et al.*, 2010; Morteza *et al.*, 2017).

In this study,higher levels of IL-6, IL-8, IL-10 and TNF-α in serum of healthy NIDs compared to reported levels in exotic breeds could be attributed to a stronger and more active immune system consequent to frequent exposure to environmental challenges as most NIDs are without proper shelter and allowed to scavenge for survival (Olayemi *et al.*, 2009; Mehdi and Maryam, 2014).

Increased vascularity of the injury location favours prompt and higher levels of inflammatory cytokines expression (Witte and Barbul, 1997; Park and Barbul, 2004). From the haemogram, the slight drop in haematocrit and haemoglobin concentration12 h post injury before normalising to values within control (uninjured NIDs) limits at 36 hcould be attributed to surgical stress with accompanying extravasation of blood and fluids due to increased vascular permeability resulting from the vasodilatory effects of released vasoactive amines by platelets (Lawrence, 1998; Richardson, 2004). These findings are similar to those bySimon and Ali, (2015) and Coutin *et al.* (2015),who reported that trauma associated with surgery creates a unique extravascular environment with increased capillary permeability in dogs. The mean erythrocyteparameters were largely higher in males than females but did not differ significantly ($P > 0.05$) both

between groups and sexes and within each sex with time. In this study, the values for these parameters obtained were consistent with reportby Olayemi *et al.* (2009) for apparently healthy NIDs, but slightly lower than values earlier reported for clinically healthy tropical dogs (Saror *et al.*, 1979; Awah and Nottidge, 1998; Ariyibi *et al.*, 2002).

Total WBC count were non-significantly ($P > 0.05$) elevated following injury probably due to sufficient regulation of the immune cells under the signalling of the cytokines produced by the circulatory leucocytes as the wounds were not extensive enough to trigger more immune cells recruitments (Park and Barbul, 2004). The higher values observed in female than in male with less fluctuations in the males than females for both injured and uninjured NIDs was probably due to hormonal effects. These were similar to observations by Ariyibi *et al.* (2002) in apparently healthy dogs and Takeshi *et al.*, (2015) in dogs that underwent cardiopulmonary bypass.

The sharp spike in neutrophils countin femalesat 60 hwas similar to reports by Robson *et al.* (2001) which stated that neutrophils counts rise in injury due to increased production of IL-6, IL-8, TGF-β, complement components such as C3a and C5a, and formylmethionyl peptides produced by bacteria and platelet products. Increased need of wound debridement and phagocytosis which constitute a part of neutrophils' primary function may also account for the spike(Canturk *et al.*, 2001).Lymphocytes count rose gradually in injured NIDs with time factor as the source of significant ($P < 0.01$) variation especially at its peak value at 156 h for males and females, while the control values remained within the reference range (Hassan and Hassan, 2003; Ihedioha *et al.*, 2013). This finding is in agreement with the reports by Park and Barbul, (2004) thatT-lymphocytes migrate into the wound after inflammatorycells and macrophages on the

fifth day following injuryduring the proliferative phase, and peak at day 7.Significant ($P < 0.05$) increase in monocytes count 12 h post injury and maintained through to 36 h and 60 h before decline to values within control ranges at 60 h and 156 h respectively for females and males. This finding collaborates report byWitte and Barbul, (1997), that macrophages migrate into the wound 48 to 96 hours after injury and become the predominant cell population toparticipate in the inflammatory and debridement processes. The major contribution of these monocytes to wound healing as they differentiate to macrophages is the secretion of cytokines and growth factors which directs the healing process (Broughton *et al.*, 2006a; Soehnlein*et al.*, 2009; Owen *et al.*, 2013; Simon and Ali, 2015). Eosinophils count remained within reference ranges with minimal fluctuations during the period of experiment likely due to cytokines balancing activites in cell recruitment.

In this study, the initial increase in wound size observed on day 1 in both male and female could be ascribed toinflammatory oedema and/or as a result of necrotic tissue debridement as earlier reported by Janice, (2005) and Richardson, (2004).The subsequent reduction in wound size could be attributed to decreased contamination as healing via the processes of contraction and epithelialization proceededwith complete closure of the entire wounds leaving no raw wound surfaces by day 42(Hunt, 1998; Janice, 2005; Diegelmann and Evans, 2004; Turkel *et al.*, 2014).

The significant ($P < 0.05$) positive correlations obtained between wound fluid and serum IL-6, wound fluid and serum IL-8 and wound fluids IL-8 with IL-10 suggests the existence of both their local and systemic production.However, the significant association between serum IL-10 and TNF-α emphasizes the balancing role of IL-10 (an anti-inflammatory cytokines) as an effective anti-inflammatory response is a protective

factor especially in individuals showing unbalanced pro-inflammatory patterns (McBride *et al.*,1996; Tasaka *et al.*, 2010).

A negative non-significant association of HCT and haemoglobin predominated upon correlation with wound fluids IL-6, IL-8, IL-10 and TNF-α in both males and females. The significant ($P < 0.05$) association between wound fluids IL-6 and HCT, IL6 and MCHC, serum IL-8 with MCH, MCHC; IL-10 with HGB and MCH could be ascribed to response due to extravasation of blood following surgical trauma and microvascular injuries (Strecker-McGraw *et al.*, 2007; Simon and Ali, 2015; Coutin *et al.*, 2015).

Significant ($P < 0.05$) positive correlations obtained between monocytes count and wound fluids levels of IL-6 in females, monocyte counts and serum IL-8 in males and lymphocytes and eosinophils counts with IL-10 in serum of females points to some of the important relationship between the various leucocytes and the different cytokines in wound healing. This is vital as the modulating (upregulation and downregulation) effects of these cytokines on the wound healing processes are thought to be a prospective predictive tool of outcomes and could further influence decisions regarding adoption of specific management modalities as healing progresses (Francisco *et al*., 1999; Carlstedt *et al*., 1997; Moseley *et* al., 2004; Masih, 2011; Ahmed *et al.*, 2013).

The significant ($P < 0.05$) negative association between wound contraction and IL-10 in males suggests improved wound healing due to sufficient circulatory levels of appropriate proinflammatory cytokines (Masih, 2011; Mehdi and Maryam, 2014).

The serum biochemical parameters and urinalysis were normal through out the study period, indicative of healthiness of the animals(Awah and Nottidge, 1998; Prause and Grauer, 1998; Archer, 2005; Ihedioha, *et al.*, 2013).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

It was concluded that:

i. Maximum mean concentrations of interleukin (IL) -6, -8, -10 and TNF – α detected at 450 nm from uninjured NIDs were 0.30 ± 0.05 ng/mL, 383.16 ± 157.57 pg/mL, 0.94 ± 0.17 ng/mL and 47.06 ± 14.34 pg/mL, respectively.

ii. Interleukin (IL) 6, 8, 10 and TNF-αattained maximum values in cutaneously injured NIDs as follows:

   a. In sera

      - IL-6: 1.13± 0.74 ng/mL
      - IL-8: 729.03 ± 240.59 pg/mL
      - IL-10: 1.67 ± 0.33 ng/mL
      - TNF-α: 116.17 ± 60.27 pg/mL

   b. In wound fluids

      - IL-6: 1.33 ± 0.33 ng/mL
      - IL-8: 248.78 ± 75.27 pg/mL
      - IL-10: 1.03 ± 0.11 ng/mL
      - TNF-α: 130.22 ± 35.15 pg/mL

iii. Interleukin (IL) -6, -8, -10 and TNF-α of the injured NIDs were significantly ($P < 0.05$) higher than those of the uninjured NIDs.

iv. Generally,changes in the haemogram ofthe injured dog were:

a. Minimal HCT decrease was observed as follows:

- Pre operative: 0.367 ± 0.003
- Post operative (12 h.): 0.354 ± 0.01
- Control (12 h.): 0.364 ± 0.03

b. The total Leucocyte counts remained within the normal limits with minimal changes in differential cellular activities of the experimental and control group with peak valuesof $12.12 \pm 1.18 \times 10^9$/L and $11.12 \pm 0.75 \times 10^9$/L, respectively.

v. Significant correlations were observed with IL -6,-10 and TNF-α and red blood cells, lymphocytes and monocytes of the NIDs within a range of $r = 0.818 - 0.923$.

6.2 Recommendations

It is recommended that:

1) The detected cytokines (interleukin (IL) -6, 8, 10 and tumour necrosis factor (TNF)-α)levels of uninjuredNIDs should be used clinically as baseline references.
2) Interleukin (IL) -6, 8, 10 and tumour necrosis factor (TNF) –α levels from uninjured and injured NIDs should be conducted in clinical evaluation of cases.
3) Further studies should be conducted for other cytokines in order to build up a holistic data for the cytokine biology of uninjured and/or injured NIDs.
4) Investigations into the use of exogenous cytokines in NIDs may reveal fascinating results in ameliorating healing in injured patients.

REFERENCES

Adcock, I. M. (1997). Transcription factors as activators of gene transcription: AP-1 and NF- kappa B. *Monaldi archives for chest disease, 52*(2),178-186.

Ahmed, A. M., Abdel, H. and Mona, F. M. (2015). Soliman Effect of topical aloe vera on the process of healing of full-thickness skin burn: a histological and immunohistochemical study. *Journal of Histology and Histopathology, 2*(3), 1-9.

Ahmed, H. R., Mohamed, E. and Adel, F. A. (2013). Tissue extract fluid cytokine levels as markers for wound vitality: An experimental comparative study. *Journal of American Science,9*(1), 188-193.

Albino, F. P., Koltz, P. F. and Gusenoff, J. A. (2009). A comparative analysis and systematic review of the wound-healing milieu: implications for body contouring after massive weight loss. *Plastic and Reconstructive Surgery, 124*(5), 1675-1682. dio: 10.1097/PRS.0b013e3181b98bb4

Alessandra, A., Comelia, D. G., Nadia, D., Davide, B., Sabina, S., Vittorio, K. and Giuseppe, M. (2016). Anticoagulants used in plasma collection affects adipokine multiplexed measurement (IL6, TNF-α). *Cytokine,85*, 5 - 10. dio.org/10.1016/j.cyto.2016.03.001

Allaouchiche, B., Coronel, B., Gagnieu, M. C., Chassard, D. and Mercatello, A. (2004). Cytokine levels in bronchoalveolar lavage fluid and blood of neutropenic patients with pneumonia. *Bull Cancer,91*(5), E77-80.

Al-Waili, N. S., Salom, K. and Al-Ghamdi, A. A. (2011). Honey for wound healing, ulcers and burns; data supporting its use in clinical practice. *The Scientific Would Journal, 5*(11), 766-787. dio:10.1100/tsw.2011.78

Amalsadvala, T. and Swaim, S. F. (2006). Management of hard-to-heal wounds. *Veterinary Clinics of North America: Small Animal Practice,36*(4),693-711.

Amber, E. I. and Swaim, S. F. (1984). An update on common wound antiseptics. *Australian Veterinary Practice, 14*(1), 29-33.

Ambrosch, A., Lobmann, R., Pott, A. and Preissler, J. (2008). Interleukin-6 concentrations in wound fluids rather than serological markers are useful in assessing bacterial triggers of ulcer inflammation. *International Wound Journal,5*(1), 99-106.

Ankit, S., Sam, K., Sanjeev, N., Dirya, M., Thomas, D. and Abdel Rahim, A. H. (2015). Interleukin-10 paradox. A potent immunoregulatory cytokine that has been difficult to harness for immunotheraphy. *Cytokine, 74*, 27 - 34. dio.org/10.1016/j.cyto.2014.10.031

Anonymous[1] (2013). *Location of Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.* Retrieved March 14, 2013 @ 18:20:05 GMT from http://Zaria,en. Wikipedia.Org/Wiki/Zaria.

Anonymous[2] (2015). Abcam®, specific Canine cytokine antibody Enzyme Linked Immunosorbent assay -Quantitative. Retrieved on 10th May, 2015 @ 13:40 GMT from www.abcam.com.

Anonymous[3] (2017). *Cephalic Index.* Retrieved on 18th April 2017 @ 20:52:25 GMT from https://en.wikipedia..org/wiki/cephalicindex.

Antunes, G., Evans, S. A., Lordan, J. L. and Frew, A. J. (2002). Systemic cytokine levels in community- acquired pneumonia and their association with disease severity. *European Respiratory Journal,20*(4), 990-995.

Archer, J. (2005). Urine analysis. Chapter 10. In: Villiers, E., Blackwood, L., editors. Manual of Canine and Feline Clinical Pathology. 2nd edition. Gloucester, UK: *British Small Animal Veterinay Association*, 149-168.

Ariyibi, A. A., Oyeyemi, M. O. and Ajadi, R. A. (2002). Comparative study of some haematological and biochemical parameters of clinically healthy Alsatian and local dogs. *African Journal of Biomedical Research, 5*(3), 145-147.

Arjan, m., Harry, T., Nienke, J. W., Joep, K., Marinus, A. B. and Charlette, E. T. (2017). Comparison of multiplex platforms of cytokines assesments and their potential use for biomarker profiling in multiple sclerosis. *Cytokine*, *91*, 145 - 152. dio.org/10.1016/j.cyto.2016.12.021

Arowolo, R. O. A. (1999). *Protecting Our Livestock Resources.*1995/96 Inaugural lecture. University Press, U.I. Ventures, Ltd., Ibadan, pp 5.

Arslan, N., Erdur, B. and Aydin, A. (2010). Hormones and cytokines in childhood obesity. *Indian Pediatrics,47*(10), 829-839.

Attinger, C. E., Janis, J. E., Steinberg, J., Schwartz, J. Al-Attar, A. and Couch, K. (2006). Clinical approach to wounds: debridement and wound bed preparation including the use of dressings and wound-healing adjuvants. *Plastic and Reconstructive Surgery,117*(7 suppl),72S-109S.

Auf-dem-Keller, U., Kümin, A., Braun, S. and Werner, S. (2006). Reactive oxygen species and their detoxification in healing skin wounds. *Journal of Investigative Dermatology Symposium Proceedings*, *11*(1), 106-111.

Augustin, H. G., Kozian, D. H. and Johnson, R. C. (1994). Differentiation of endothelial cells: analysis of the constitutive and activated endothelial cell phenotypes. *Bioassays,16*(12),901-906.

Awah, J. N. and Nottidge, H. O. (1998). Serum biomedical parameters in clinically healthy dogs in Ibadan. *Tropical Veterinarian16,* 123-129.

Awasum, C. A., Fotzo, S. L. M., Ndukum, J. A., Genesis, C. W. D. and Zoli, A. (2015). Gas Chromatography-Mass Spectroscopy Analysis and Chemical Composition

of Ngaoundere, Cameroon Honey. *American Journal of Bioscience and Bioengineering, 3*(5), 33-36.

Bacon, K., Baggiolini, M., Broxmeyer, H., Horuk, R., Lindley, I., Mantovani, A., … and IUIS/World Health Organisation Subcommittee on chemokine nomenclature (2002). "Chemokine/chemokine receptor nomenclature". *Journal of Interferon and Cytokine Research, 22*(10), 1067-1068. doi:10.1089/107999002760624305

Banks, W. A., Kastin, A. J. and Gutierrez, E. G. (1994). "Penetration of interleukin-6 across the murine blood-brain barrier". *Neuroscience Letters. 179*(1-2), 53-56. doi:10.1016/0304-3940(94)90933-4

Bastard, J. P., Maachi, M., Lagathu, C., Kim, M. J., Caron, M., Vidal, H., … and Feve, B. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. Obesity, inflammation, and insulin resistance, *European Cytokine Network, 17*(1), 4-12.

Bastard, J. P., Maachi, M., Tran Van Nhieu, J., Jardel, C., Bruckert, E., Grimaldi, A., … and Hainque, B. (2002). Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. *The Journal of Clinical Endocrinoly and Metabolism,87*(5), 2084-2089.

Baum, C. L. and Arpey, C. J. (2005). Normal cutaneous wound healing: clinical correlation with cellular and molecular events. *Dermatologic Surgery,31*(6),674-686.

Benjamin, A. L. and Christopher, H. (2009). Topical antimicrobial therapy for treating chronic wounds. *Clinical Practice,49*(10), 1541-1549.

Bertesteanu, S., Triaridis, S., Stankovic, M., Lazar, V., Chifiriuc, M. C., Vlad, M. and Grigore, R. (2014). Polymicrobial wound infections: Pathophysiology and current therapeutic approaches. *International Journal of Pharmaceutics, 463*(2), 119-126.

Bertha, A. B., Oscar, R. C., Sandra, L. M., Gibran, P. V., Viridiana, Y. G., Teresa, J., … and Armanda, L. (2017). Relationship of inflammatory profile of elderly patients serum and senescence associated secretory phenotype with human breast cancer cells proliferation: Role of IL6/IL8 ratio. *Cytokine, 91*, 13 - 29. dio.org/10.1016/j.cyto.2016.12.001

Beutler, B., Greenwald, D., Hulmes, J. D., Chang, M., Pan, Y. C., Mathison, J., … and Cerami, A. (1985). "Identity of tumour necrosis factor and the macrophage-secreted factor cachectin". *Nature, 316*(6028), 552-554. doi:10.1038/316552a0

Bickenbach, J. R. (2005). Isolation, characterization, and culture of epithelial stem cells. *Methods in Molecular Biology, 289*, 97-102.

Bischoff, M., Kinzl, L. and Schmelz, A. (1999). The complicated wound. *Unfallchirurg,102*(10),797-804.

Black, R. A., Rauch, C. T., Kozlosky, C. J., Peschon, J. J., Slack, J. L., Wolfson, M. F., … and Cerretti, D. P. (1997). "A metalloproteinase disintegrin that releases

tumour-necrosis factor-alpha from cells". *Nature, 385*(6618), 729-733.doi:10.1038/385729a0

Blackwell, T. S. and Christman, J. W. (1996). Sepsis and cytokines: current status. *British Journal of Anaesthesia,77*(1),110-177.

Bluethmann, H., Rothe, J., Schultze, N., Tkachuk, M., and Koebel, P. (1994). Establishment of the role of IL-6 and TNF receptor 1 using gene knockout mice. *Journal of Leukocyte Biology,56*(5),565-570.

Bohling, M. W. and Swaim, S. F. (2012). Skin grafts. In Tobias, K. M. and Johnston, S. A. (Eds.). *Veterinary Surgery: Small Animal* (pp. 1271-1290). St. Louis: Elsevier.

Bokel, C. and Brown, N. H. (2002). Integrins in development: moving on, responding to, and sticking to the extracellular matrix. *Developmental Cell,3*(3),311-321.

Borena, B. M., Martens, A., Broeckx, S. Y., Meyer, E., Chiers, K., Duchateau, L. and Spaas, J. H. (2015). Regenerative skin wound healing in mammals: State-of-the-art on growth factor and stem cell based treatments. *Journal of Cellular Physiology and Biochemistry,36*(1), 1-23.

Bouwmeester, T., Bauch, A., Ruffner, H., Angrand, P. O., Bergamini, G., Croughton, K., … and Superti-Furga, G. (2004). "A physical and functional map of the human TNF alpha/NF-kappa B signal transduction pathway". *Nature Cell Biology, 6*(2), 97-105. doi:10.1038/ncb1086

Bown, M. J., Nicholson, M. L., Bell, P. R. F. and Sayers, R. D. (2001). Cytokines and inflammatory pathways in the pathogenesis of multiple organ failure following abdominal aortic aneurysm repair. *European Journal of Vascular and Endovascular Surgery,22*(6),485-495.

Bozza, F. A, Salluh, J. I., Japiassu, A. M., Soares, M., Assis, E. F., Gomes, R. N., … and Bozza P. T. (2007). Cytokine profiles as markers of disease severity in sepsis; A multiplex analysis. *Critical Care*, *11*(2), R49.

Braat, H., Rottiers, P., Hommes, D. W., Huyghebaert, N., Remaut, E., Remon, J. P., … and Steidler, L. (2006). "A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease". *Clinical Gastroenterology and Hepatology, 4*(6), 754-759. doi:10.1016/j.cgh.2006.03.028

Brat, D. J., Bellail, A. C. and Van Meir, E. G. (2005). The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. *Neuro-oncology,7*(2), 122-133.

Braund, R., Hook, S. M., Greenhill, N. and Medlicott, N. J. (2009). Distribution of fibroblast growth factor-2 (FGF- 2) within model excisional wounds following topical application. *Journal of Pharmacy and Pharmacology,61*(2),193-200. doi.10.1211/jpp/61.02.0008

Brennan, F. M., Green, P., Amjadi, P., Robertshaw, H. J., Alvarez-Iglesias, M. and Takata, M. (2008). "Interleukin-10 regulates TNF-alpha-converting enzyme

(TACE/ADAM-17) involving a TIMP-3 dependent and independent mechanism". *European Journal of Immunology, 38*(4), 1106-1117. doi:10.1002/eji.200737821

Broughton, G., Janis, J. E. and Attinger C. E. (2006a). The basic science of wound healing. *Plastic and Reconstructive Surgery, 117*(7 suppl),12S-34S.

Broughton, G., Janis, J. E. and Attinger, C. E. (2006b). Wound healing: an overview. *Plast Reconstr Surgery,117*(7 suppl),1e-S-32e-S.

Bruunsgaard, H. (2002). Effects of tumor necrosis factor-alpha and interleukin-6 in elderly populations. *European Cytokine Network, 13*(4), 389-391.

Brynskov, J., Foegh, P., Pedersen, G., Ellervik, C., Kirkegaard, T., Bingham, A. and Saermark, T. (2002). Tumour necrosis factor alpha converting enzyme (TACE) activity in the colonic mucosa of patients with inflammatory bowel disease. *Gut, 51*(1), 37-43. doi:10.1136/gut.51.1.37

Bucala, R. (1996). Macrophage migratory inhibitory factor (MIF) rediscovered: cytokine, pituitary hormone, and glucocorticoid-induced regulator of the immune response. *Federation of American Society for Experimental Biology Journal, 10*(14), 1607-1613.

Burtis, C. A., Ashwood, E. R. and Bruns, D. E. (2006). *TIETZ textbook of clinical chemistry and molecular diagnostics* fourth edition (pp. 2448). St. Louis, MO: Elsevier Saunders. doi:10.1373/clinchem.2005.062638

Burton, D., Nicholson, G. and Hall, G. (2004). Endocrine and metabolic response to surgery. Continuing Education in Anaesthesia, *Critical Care and Pain*, *4*(5), 144-147.

Caitlin, N. C., Kristin, A. B., Hannah , E. R. and Dastin, T. Y. (2017). Acute exposure of primary rat soleus muscle to zilpaterol HCL (β_2 adrenergic agonist), TNF-α, or IL-6 in culture increases glucose oxidation rates independent of the impact on insulin signaling or glucose uptake. *Cytokine,*96, 107 -113. dio.org/10.1016/j.cyto.2017.03.014

Canturk, N. Z., Esen, N., Vural B, Canturk, Z., Kirkali, G., Oktay, G. and Solakoglu, S. (2001). The relationship between neutrophils and incisional wound healing. *Skin Pharmacolology and Applied Skin Physiology, 14*(2),108–116.

Carlstedt, F., Lind, L. and Lindahl, B. (1997). Pro-inflammatory cytokines, measured in a mixed population on arrival in the emergency department, are related to mortality and severity of disease. *Journal of Internal Medicine,242*(5),361-365.

Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. and Williamson, B. (1975). "An endotoxin-induced serum factor that causes necrosis of tumors". *Proceedings of the National Academy of Science, U.S.A., 72*(9), 3666–3670. doi:10.1073/pnas.72.9.3666

Castellano, J. J., Shafii, S. M., Ko, F., Donate, G., Wright, E. T., Mannari, R. J., … and Robson, M. C. (2007). Comparative evaluation of silver containing antimicrobial dressings and drugs. *International Wound Journal, 4*(2), 114-22.

Chachkhiani, I., Gurlich, R., Maruna, P., Fraskor, and Lindner, J. (2005). The post-operative stress response and its reflection in cytokine network and leptin plasma levels. *Physiological Research,54*(3),279-285.

Chang, R. R., Mehrara, B. J., Hu, Q. Y., Disa, J. J. and Cordeiro, P. G. (2004). Reconstruction of complex oncologic chest wall defects: a 10-year experience. *Annals of Plastic Surgery,52*(5),471-479; discussion 479.

Charlotte, T., Ruth, D. and Ernest, C. (2016). Anti cytokine therapy in chronic inflammatory artheritis. *Cytokine,* 86, 92-99. dio.org/10.1016/j.cyto.2016.17.015

Chen, G. and Goeddel, D. V. (2002). "TNF-R1 signaling: a beautiful pathway". *Science 296*(5573), 1634-1635. doi:10.1126/science.1071924

Chen, J. D., Lapiere, J. C., Sauder, D. N., Peavey, C. and Woodley, D. T. (1995). Interleukin-1 alpha stimulates keratinocytes migration through an epidermal growth factor/transforming growth factor-alphaindependent pathway. *Journal of Investigative Dermatology,104*(5),729-733.

Chen-Kiang, S., Hsu, W., Natkunam, Y., and Zhang, X. (1993). Nuclear signaling by interleukin-6. *Current Opinion in Immunology 5*(1),124-128.

Cho-Hee, J., Na-Young, E., Hyo-Mi, J., Hae-Won, J., Eul-Soo, C., Jin-Hee, W., … and Dong-In, J. (2015). Effect of autologous platelet-rich plasma application on cutaneous wound healing in dogs. *Journal of Veterinary Science,* 1-22.

Chong, K. P., Atong, M. and Rossall, S. (2012). The roles of syringic, caffeic and 4-hydroxybenzoic acids in *Ganoderma* - oil palm interaction. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences,14*(2), 157-166.

Clark, I. A. (1982). "Suggested importance of monokines in pathophysiology of endotoxin shock and malaria". *Klin Wochenschr, 60*(14), 756-758. doi:10.1007/BF01716573

Clark, I. A. (2007). "How TNF was recognized as a key mechanism of disease". *Cytokine Growth Factor Reviews, 18*(3-4), 335-343. doi:10.1016/j.cytogfr.2007.04.002

Clark, I. A., Virelizier, J. L., Carswell, E. A. and Wood, P. R. (1981). "Possible importance of macrophage-derived mediators in acute malaria". *Infection and Immunity, 32*(3), 1058–1066.

Claus, B. and Bente, K. P. (2010). The Role of Exercise-InducedMyokines in Muscle Homeostasis and the Defense against Chronic Diseases. *Journal of Biomedicine and Biotechnology, 2010,* 1-6. doi:10.1155/2010/520258

Cooper, R. A. (2007). Iodine revisited. *International Wound Journal, 4*(2), 124–37.

Coutin, J. V., Otto, I. L., Geraldine, C. M., Marion, F. E., Emily, I. M., Stephen, R. W., and Thomas, O. R. (2015). Cefazolin Concentration in Surgically Created Wounds Treated With Negative Pressure Wound Therapy Compared to Surgically Created Wounds Treated With Nonadherent Wound Dressings. *Veterinary Surgery, 44*(2015), 10. doi:10.1111/j.1532-950X.2014.12218.x

Cox, P. A. and Balick, M. J. (2012). The ethnobotanical approach to drug discovery. *Scientific American, 270*(6), 82–87.

Cressman, D. E., Diamond, R. H. and Taub, R. (1995). Rapid activation of the Stat3 transcription complex in liver regeneration. *Hepatology, 21*(15),1443-1449.

Cressman, D. E., Greenbaum, L. E., DeAngelis, R. A., Ciliberto, G., Furth, E. E., Poli, V. and Taub, R. (1996). Liver failure and defective hepatocyte regeneration in interleukin-6- deficient mice. *Science, 274*(5291),1379–1383.

Cummings, D. and Merriam G. (2003). Growth Hormone Therapy in Adults. *Annual Review of Medicine*, *54*(1), 513-533.

Cynthia, A. F. and Mackenzie, N. (2012). Advanced Skin Care – A Novel Ingredient. *Journal of the American College of Clinical Wound Specialists*, *4*, 92-94.

Czerkinsky, C., Nilsson, L., Nygren, H., Ouchterlony, O. and Tarkowski, A. (1983). A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specific antibody-secreting cells. Journal of Immunological Methods, *65*(1-2), 109-121. doi: 10.1016/0022-1759(83)90308-3

Daniel, W. T., Lisa, A. L. amd Anthony, J. D. (2017). Selected life-extending interventions reduce arterial CXCL 10 and macrophage colony-stimulating factor in aged mouse artery. *Cytokine,96*, 102 -106. dio.org/10.1016/j.cyto.2017.03.008

Das, S. K., Varadham, S., Gupta, G., Mukherjee, S., Dhanya, L., Rao, D. N. and Vasudevan, D. M. (2009). Time-dependent effect of ethanol in blood oxidative stress parameters and cytokines. *Indian Journal of Biochemistry and Biophysics. 46*(2009), 116-121.

Degreef, H. (1998). How to heal a wound fast. *Dermatology Clinics,16*(2),365-375.

Dena, L. L. and Jennifer, L. W. (2012). Use of Random SubdeRmal Flaps to Repair Extensive Skin Defects. *Today's Veterinary Practice,* pp 21-25.

Diegelmann, R. F. and Evans, M. C. (2004). Wound healing: an overview of acute, fibrotic and delayed healing. *Frontiers Bioscience,1*(9),283-289.

Dina, R., Dina, S., Dalia, S. and Aisha, Y. (2016). Vitamine D status and its modulatory effect on interferon gamma and interleukin 10 production by peripheral blood mononuclear cells in culture. *Cytokine, 85*, 5 - 10. dio.org/10.1016/j.cyto.2016.05.024

Dinarello, C. A. (1994). The interleukin-1 family: 10 years of discovery. *Federation of American Society for Experimental Biology Journal,8*(15), 1314-1325.

Dipietro, L. A. (1995). Wound healing: the role of the macrophages and other immune cells. *Shock,4*(4), 233-240.

Dissemond, J. and Goos, M. (2004). Options for debridement in the therapy of chronic wounds. *Journal der Deutschen Dermatologischen Gesellschaft,2*(9),743-751.

Dow, G., Browne, A. and Sibbald, R. G. (1999). Infection in chronic wounds: controversies in diagnosis and treatment. *Ostomy Wound Managagement,45*(8),23-40.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K. and Lanctôt, K. L. (2010). "A meta-analysis of cytokines in major depression". *Biology and Psychiatry*, *67*(5), 446-457. doi:10.1016/j.biopsych.2009.09.033

Dowsett, C. and Claxton, K. (2006). Reviewing the evidence for wound bed preparation. *Journal ofWound Care, 15*(10),439-442.

Drosou, A., Falabella, A. and Kirsner, R. (2003). Antiseptics on wounds: an area of controversy. *Wounds,15*(5). Retrieved October 7, 2015 from http://www.medscape .com/viewarticle/456300_5.

Dubiński, A. and Zdrojewicz, Z. (2007). The role of interleukin-6 in development and progression of atherosclerosis. *Polski Merkuriusz Lekarski* (in Polish), *22*(130), 291-294.

Eliya-Masamba, M. C. and Banda, G. W. (2013). Primary closure versus delayed closure for non bite traumatic wounds within 24 hours post injury. *Cochrane Database of Systematic Review*, 10:CD008574. doi:10.1002/14651858.CD008574

Endeman, H., Meijvis, S. C., Rijkers, G. T., van Velzen-Blad, H., van Moorsel, C. H., Grutters, J. C. and Biesma, D. H. (2011). Systemic cytokine response in patients with community-acquired pneumonia. *European Respiratory Journal,37*(6), 1431-1438. doi:10.1183/09031936.00074410

English, D., Kovala, A. T., Welch, Z., Harvey, K. A., Siddiqui, R. A., Brindley, D. N. and Garcia, J. G. (1999). Induction of endothelial cell chemotaxis by sphinosine 1- phosphate and stabilization of endothelial monolayer barrier function by lysophosphatidic acid, potential mediators of hematopoietic angiogenesis. *Journal of Hematotherapy and Stem Cell Research,8*(6),627-634.

Eskdale, J., Kube, D., Tesch, H. and Gallagher, G. (1997). Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. *Immunogenetics, 46*(2), 120-128. doi: 10.1007/s002510050250

Esmon, C. T. (1994). Possible involvement of cytokines in diffuse intravascular coagulation and thrombosis. *Bailliere's Clinical Haematology, 7*(3), 453-468.

European Pressure Ulcer Advisory Panel (2010). Pressure ulcer Classification-differentiation between pressure ulcer and moisture lesions. European Pressure

Ulcer Advisory Panel. Retrieved July 5, 2016 from http://www.judy-waterlow.co.uk.

Fain, J. N., Madan, A. K., Hiler, M. L., Cheema, P. and Bahouth, S. W. (2004). Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology, 145*(5), 2273-2282.

Falanga, V. (1998). Wound healing and chronic wounds. *Journal of Cutaneous Medicine and Surgery,3*(suppl 1),1-5.

Ferguson-Smith, A. C., Chen, Y. F., Newman, M. S., May, L. T., Sehgal, P. B. and Ruddle, F. H. (1988). "Regional localization of the interferon-beta 2/B-cell stimulatory factor 2/hepatocyte stimulating factor gene to human chromosome 7 (pp. 15-21)". *Genomics, 2*(3), 203–8. doi: 10.1016/0888-7543(88)90003-1

Ferna´ndez-Serrano, S., Dorca, J., Coromines, M., Carratala`, J., Gudiol, F. and Manresa, F. (2003). Molecular inflammatory responses measured in blood of patients with severe community-acquired pneumonia. *Clinical Diagnostic Laboratory Immunology Journal,10*(5), 813–820.

Fildes, J., Bannon, M. P. and Barrett, J. (1991). Soft tissue infection after trauma. *Surgical Clinics of North America,71*(2)**,**371-384.

Fine, J. D. (2010). Inherited epidermolysis bullosa: past, present, and future. *Annals of the New York Academy of Sciences, 194*(2), 213–222. doi: 10.1111/j.1749-6632.2010.05463.x

Flangan, M. (2000). The physiology of wound healing. *Journal of Wound Care,9*(6),299-300.

Fonder, M. A., Lazarus, G. S., Cowan, D. A., Aronson-Cook, B., Kohli, A. R. and Mamelak, A. J. (2008). Treating the chronic wound: a practical approach to the care of nonhealing wounds and wound care dressings. *Journal of the American Academy of Dermatology*, *58*(2), 185-206.

Francisco, A., Julia, L., Rosa, C., Manuel, J., Rosario, M. and Carmen, M. (1999). Relationship of Plasma Leptin to Plasma Cytokines and Human Survival: In Sepsis and Septic Shock. *The Journal of Infectious Diseases*, *180*(3), 908-911.

Fried, S. K., Bunkin, D. A. and Greenberg, A. S. (1998). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot differenceand regulation by glucocorticoid. *Journal of Clinical Endocrinology and Metabolism, 83*(3), 847-850.

Fry, C., Gunter, D. R., McMahon, C. D., Steele, B. and Sartin, J. L. (1998). Cytokine-mediated growth hormone release from cultured ovine pituitary cells. *Neuroendocrinology, 68*(3), 192-200.

Gallucci, R. M., Simeonova, P. P., Matheson, J. M., Kommineni, C., Guriel, J. L., Sugawara, T. and Luster, M. I. (2000). Impaired cutaneous wound healing in

interleukin-6–deficient and immunosuppressed mice. *Federation of American Societies for Experimental Biology Journal, 14*(15), 2525–2531.

Gallucci, R. M., Sugawara, T., Yucesoy, B., Berryann, K., Simeonova, P. P., Matheson, J. M. and Luster, M. I. (2001). Interleukin-6 treatment augments cutaneous wound healing in immunosuppressed mice. *Journal of Interferon and Cytokine Research, 21*(18), 603–609.

Gaur, U. and Aggarwal, B. B. (2003). Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. *Biochemical Pharmacology, 66*(8), 1403-8. doi: 10.1016/S0006-2952(03)00490-8

Gezzer O., Daniel S. R., Dominic J. P., Jessica Y., Andrew M. I., Andrew D. S., … and Fizan A. (2012). An Evaluation of Surgical Site Infections by Wound Classification System Using the American College of Surgeons National Surgical Quality Improvement Program dataset between 2005 and 2008 In: Association for Academic Surgery. *Journal of Surgical Research, 174*(1), 33–38.

Giannone, G., Dubin-Thaler, B. J., Dobereiner, H. G., Kieffer, N., Bresnick, A. R. and Sheetz, M. P. (2004). Periodic lamellipodial contractions correlate with rearward actin waves. *Cell, 116*(3),431-443.

Giuliani, A. L., Spisani, S., Cavalletti, T., Reali, E., Melchiorri, L., Ferrari, L., … and Traniello, S. (1993). Fibroblasts increase adhesion to neutrophils after stimulation with phorbol ester and cytokines. *Cellular Immunology Journal. 149*(1),208-222.

Glat, P. M. and Longaker, M. T. (1997). *Wound healing*. In: *Grabb and Smith's Plastic Surgery*, 5th edition. Aston S. J., Beasley, R. W. and Thorne, C. H., (Eds), (pp 3-12). Philadelphia: Lippincott-Raven.

Glaudemans, A. W., Dierckx, R. A., Kallenberg, C. G. and Fuentes, K. L. (2010). The role of radiolabelled anti- TNF-α monoclonal antibodies for diagnostic purposes and therapy evaluation. *Journal of Nuclear Medicine and Molecular Imaging, 54*(6), 639 – 653.

Goldman, R. and Pollack, S. (1996). Electric fields and proliferation in a chronic wound model. *Bioelectromagnetics,17*(6),450-457.

Gonzalez-Espinosa, D., Perez-Romano, L., Guzman-Soriano, B., Ariasm, E., Bongiovanni, C. M. and Gutierrez, A. A. (2007). Effects of pH-neutral, super-oxidised solution on human dermal fibroblasts in vitro. *International Wound Journal*, *4*(3), 241-250.

Goretti, C., Mazzurco, S., Nobili, L. A., Macchiarini, S., Tedeschi, A., Palumbo, F., … and Piaggesi, A. (2007). Clinical outcomes of wide postsurgical lesions in the infected diabetic foot managed with 2 different local treatment regimens compared using a quasi-experimental study design: a preliminary communication. *International Journal of Lower ExtremityWounds, 6*(1), 22-27.

Grayson, L. S., Hansbrough, J. F., Zapta-Sirvent, R. L., Dore, C. A., Morgan, J. L. and Nicolson, M. A. (1993). Quantitation of cytokine levels in skin graft donor site wound fluid. *Burns, 19*(5), 401-405.

Grazul-Bilska, A. T., Johnson, M. L., Bilski, J. J., Redmer, D. A., Reynolds, L. P., Abdullah, A. and Abdullah, K. M. (2003). Wound healing: The role of growth factors. *Drugs Today, 39*(10), 787-800.

Greenhalgh, D. G. (1998). The role of apoptosis in wound healing. *International Journal of Biochemistry and Cell Biology, 30*(9),1019-1030.

Grimbaldeston, M. A., Nakae, S., Kalesnikoff, J., Tsai, M. and Galli, S. J. (2007). Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nature Immunology, 8*(10), 1095-1104. Doi:10.1038/ni1503.

Gueret, G., Lion, F., Guriec, N., Arvieux, J., Dovergne, A., Guennegan, C., … and Arvieux, C. C. (2009). Acute renal dysfunction after cardiac surgery with cardiopulmonary bypass is associated with plasmatic IL6 increase. *Cytokine, 45*(2), 92–98. doi: 10.1016/j.cyto.2008.11.001

Guo, S. and Dipietro, L. A. (2010). Factors affecting wound healing. *Journal of Dental Research, 89*(3), 219-229.

Haichao, W., Jaideep, M. V., Ona, B., Minghuang, Z., Michael, O., Andrew, S. *and* Kevin, J. T. (1999). Pro-inflammatory cytokines (tumour necrosis factor and interleukin-1) stimulate release of high mobility group protein-1 by pituicytes. FACS, *Manhasset, NewYork.*

Hammer, M., Mages, J., Dietrich, H., Schmitz, F., Striebel, F., Murray, P. J., Wagner, H. and Lang, R. (2005). Control of dual-specificity phosphatase-1 expression in activated macrophages by IL-10. *European Journal of Immunology, 35*(10), 2991-3001. doi: 10.1002/eji.200526192

Hananeh, W. M., Ismail, Z. B., Alshehabat, M. A., Abeeleh, M. A. and Ali, J. H. (2015).Effects of Sidr honey on second-intention healing of contaminated full-thickness skin wounds in healthy dogs. *Bulletin of Veterinary Institute in Pulawy, 59*(3), 433-439. doi: 10.1515/bvip-2015-0063

Hart, J. (2002). Inflammation. 1: its role in the healing of acute wounds. *Journal of Wound Care, 11*(6),205-209.

Hartnett, J. M. (1998). Use of vacuum-assisted wound closure in three chronic wounds. Journal of Wound *Ostomyology Continence Nursing,25*(6), 281-290.

Hassan, A. Z. and Hassan, F. B. (2003). *An introduction to Veterinary Practice.* Ahmadu Bello University Press Limited, Zaria Nigeria. Pp: 47 – 65.

Hassan, A. Z., Amber, E. I., Awasum, C. A., Remi-Adewumi, B. D., Yila, A. S., Hassan, F. B. and Jahun, B. M. (2002). A review of wound healing. *Nigerian Veterinary Journal,23*(2), 1-14.

Hassan, A. Z., Remi-Adewumi, B. D., Adawa, D.A.Y., Hassan, F.B. and Adeiza, A. A. (2003). Wound Management-A Review. *Sahel Journal of Veterinary Science, 2*, 1-9.

Hedges, J. C., Singer, C. A. and Gerthoffer, W. T. (2000). Mitogen-activated protein kinases regulate cytokine gene expression in human airway myocytes. *American Journal of Respiratory Cell and Molecular Biology. 23*(1), 86-94. Doi: 10.1165/ajrcmb.23.1.4014.

Heinrich, P. C., Behrmann, I., Müller-Newen, G., Schaper, F. and Graeve, L. (1998). Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochemical Journal,334*(Pt 2), 297-314.

Hibi, M., Murukami, M., Saito, M., Hirano, T., Taga, T. and Kishimoto, T. (1990). Molecular cloning and expression of an IL-6 signal tranducer, gp 130. *Cell ,63*(6), 1149-1157.

Ho, A. S., Liu, Y., Khan, T. A., Hsu, D. H., Bazan, J. F. and Moore, K. W. (1993). A receptor for interleukin 10 is related to interferon receptors. *Proceedings of the National Academy of Sciences of the United States of America, 90*(23), 11267–11271. Doi: 10.1073/pnas.90.23.11267.

Hoch, R. C., Rodriguez, R., Manning, T., Bishop, M., Mead, P., Shoemaker, W. and Abraham, E. (1993). Effects of accidental trauma on cytokine and endotoxin production. *CriticalCare Med, 21*(6), 839-845.

Hodde, J. (2006). Extracellular matrix as a bioactive material for soft tissue reconstruction. *ANZ Journal of Surgery,76*(12),1096-1100.

Hoover, D. M., Schalk-Hihi, C., Chou, C. C., Menon, S., Wlodawer, A. and Zdanov, A. (1999). "Purification of receptor complexes of interleukin-10 stoichiometry and the importance of deglycosylation in their crystallization". *European Journal of Biochemistry, 262*(1), 134-141. doi: 10.1046/j.1432-1327.1999.00363.x

Horst, I. (2013). Cytokines and Cells. Online Pathfinder Encyclopaedia Version 31.4 (Spring/Summer 2013 Edition). Retrieved October 12, 2015 from http://www.cell-talk.com. pp 1-5.

Hotamisligil, G. S., Shargill, N. S. and Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science,259*(5091), 87-91.

Hsu, S., Thakar, R. and Li, S. (2007). Haptotaxis of endothelial cell migration under flow. *Methods in Molecular Medicine,139*,237-250.

Hubenak, J. R., Zhang, Q., Branch, C. D. and Kronowitz, S. J. (2014). Mechanisms of injury to normal tissue after radiotherapy: a review. *Plastic and Reconstructive Surgery, 133*(1), 49e–56e.

Hudetz, J. A., Gandhi, S. D., Iqbal, Z., Patterson, K. M. and Pagel, P. S. (2011). Elevated postoperative inflammatory biomarkers are associated with short- and

medium-term cognitive dysfunction after coronary artery surgery. *Journal of Anesthesia, 25*, 1-9. doi: 10.1007/s00540-010-1042-y

Hunt, G. B. (2012). Local or subdermal plexus flaps. In Tobias, K. M. and Johnston, S. A. (Eds): *Veterinary Surgery: Small Animal* (pp. 1243-1255). St. Louis: Elsevier.

Hunt, T. K. (1988). The physiology of wound healing. *Annals of Emergency Medicine, 17*(12),1265-1273.

Hunt, T. K., Hopf, H. and Hussain, Z. (2000). Physiology of wound healing. *Advances in Skin and Wound Care,13*(2 Suppl),6-11.

Ibraheem, Z. O., Sattar, M. A., Abdullah, N. A., Hassan, R. and Johns, E. J. (2012). Toxicity, phytochemical content and antioxidant activity assessment studies for standardized ethanolic fraction of palm oil leaf extract. *Pharmacognosy Communications, 2*(1), 21-30.

Ihedioha, J. I., Ugwuja, J. I., Noel-Uneke, O. A., Udeani, I. J. and Daniel-Igwe, G. (2012). Reference values for the haematology profile of conventional grade outbred albino mice (Mus musculus) in Nsukka, Eastern Nigeria. *Animal Research International, 9*(2), 1601 – 1612.

Ihedioha, J. J., Anosa, V. O. ad Esievo, K. A. N. (2013). Prevelence of and clinicopathologic findings associated with ascites in dogs in Enugu state, Nigeria. *Comparative clinical pathology, 22*(2), 185-193.

Ioanas, M., Ferrer, M., Cavalcanti, M., Ferrer, R., Ewig, S., Filella, X., … and Torres, A. (2004). Causes and predictors of non-response to treatment of intensive care unit-acquired pneumonia. *Critical Care Medicine, 32*(4), 938-945.

Izadi, K. and Ganchi, P. (2005). Chronic wounds. *Clinics in Plastic Surgery,32*(2),209-222.

Jacques, B., Guillaume, M., Nicole, F. and Jean-Pierre, R. (2000). The clinical usefulnesss of the measurement of cytokines. *Clinical Chemistry and Laboratory Medicine.* Abstract. dio: 10.1515/CCLM.2000.040

James S. A., Laura T., Catherine L. H., David I. D., Edward H. Y and Patricia P. K. (2017). Serum biomarkers of inflammation and muscle strength among women with systemic lupus erythrematosus. *Cytokines, 90*, 109-112. dio.org/10.1016/j.cyto.2016.11.003

James, S. (2002). *The Domestic Dog: Its Evolution, Behaviour and Interaction with People* (pp. 219). Cambridge University Press.

Janice, L., Schroeder, R. N., Elaine, E. and Steinke, R. N. (2005). Necrotising fasciitis – The importance of early debridement. *Association of perioperative Registered Nurses Journal,82*(6), 1031-1037. dio: 10.1016/S0001-2092(06)60255-X

Jia Z., Jingjing L. J., Yong W., Bainian F and Fengfeng P. (2016). Interleukin 10 protects primary melanocytes by activation of stat-3 and P13K/Akt/NF-kB signaling pathway. *Cytokine, 83*, 275-281. dio.org/10.1016/j.cyto.2016.05.013

Jiang, J. X., Tian, K. L., Chen, H. S., Zhu, P. F. and Wang Z. G. (1997). Plasma cytokines and endotoxin levels in patients with severe injury and their relationship with organ damage. *Injury, 28*(8),509-513.

John, T., Petter, S., Joel, E., Victoria, T. and Allan, H. B. W. (2013). Reference range and short-and-long-term biological variation of IL-6, IL-17A and tissue necrosis factor-alpha using high sensitivity assays. *Cytokine, 64*(3), 660-665. dio.org/10.1016/j.cyto.2013.09.018

Johnston, G. R. and Webster, N. R. (2009). Cytokines and the immunomodulatory function of the vagus nerve. *British Journal of Anaesthesia, 102*(4),453-462.

Jones, T. H. and Kennedy, R. L. (1993). Cytokines and hypothalamic-pituitary function. *Cytokine, 5*(6), 531-538.

Jose, B., Stefano, A., Rafael, F-B., Silvia, M. U., Madhavi, J. R., Padmaraj, D., … and Julio, A. R. (2013). Understanding the roles of cytokines and neutrophil activity and neutrophil apoptosis in the protective versus deleterious inflammatory response in pneumonia. *International Journal of Infectious Diseases,17*(2), e76-e83.

Josephson, K., Logsdon, N. J. and Walter, M. R. (2001a). Crystal structure of the IL-10/IL-10R1 complex reveals a shared receptor binding site. *Immunity, 15*(1), 35-46. doi: 10.1016/S1074-7613(01)00169-8

Josephson, K., McPherson, D. T. and Walter, M. R. (2001b). Purification, crystallization and preliminary X-ray diffraction of a complex between IL-10 and soluble IL-10R1. *Acta Crystallographica. Section D, Biological Crystallography 57*(Pt 12), 1908-1911. doi: 10.1107/S0907444901016249

Jull, A. B., Cullum, N., Dumville, J. C., Westby, M. J., Deshpande, S. and Walker, N. (2015). Honey as a topical treatment for wounds. Cochrane Database *Systematic Reviews, 3,* CD005083. doi: 10.1002/14651858.CD005083

Jull, A. B., Rodgers, A. and Walker, N. (2008). Honey as a topical treatment for wounds. Cochrane Database *Systematic Reviews, 4,* CD005083. doi: 10.1002/14651858.CD005083

Kaehn, K. (2007). Dermacyn on the infected foot ulcers of 10 patients. *Journal of Wound Care,16*(2), 60-61.

Kallen, K. J., zum Büschenfelde, K. H. and Rose-John, S. (1997). The therapeutic potential of interleukin-6 hyperagonists and antagonists. *Expert Opinion on Investigational Drugs, 6*(3), 237–266. doi: 10.1517/13543784.6.3.237

Kant, S., Swat, W., Zhang, S., Zhang, Z. Y., Neel, B. G., Flavell, R. A. and Davis, R. J. (2011). TNF-stimulated MAP kinase activation mediated by a Rho family

GTPase signaling pathway. *Genes and Development, 25*(19), 2069-2078. doi: 10.1101/gad.17224711

Karen M. T. and Spencer A. J. (2012). Veterinary Surgery: Small animal surgery. *Elsevier, 1*(2), 1601.

Katrin S. (2015). Drains: Proper Use and Management. Louisiana State University Baton Rouge, Los Angeles. (pp. 1-4). Retrieved August 20, 2015 from http://www.google.com.

Kauvar, D. S. and Wade, C. E. (2005). The epidemiology and modern management of traumatic haemorrhage: US and international perspectives. *Critical Care, 9*(5), S1-S9.

Kellum, J. A., Kong, L., Fink, M. P., Weissfeld, L. A., Yealy, D. M., Pinsky, M. R.,…, and Angus, D. C. (2007). Understanding the inflammatory cytokine response in pneumonia and sepsis, *Archives of Internal Medicine, 167*(15), 1655-1663.

Kelly, R. A. and Smith, T. W. (1997). Cytokines and cardiac contractile function. *Circulation, 95*(4),778-781.

Kemin, L., Fei, W., Zhigang, C., Sihai, L. and Xinzuo, H. (2014). Inflammatory cytokine expression in the quadricepts of rats with posttraumatic knee stiffness: A prelilinary study. dio: 10.1016/j.cyto.2014.12.018

Kerr, M. G. (1989). Veterinary Laboratory Medicine, Clinical Biochemistry and Haematology. Oxford, London, Edinburgh, Boston, Melbourne, Blackwell Scientific Publications, Pp 1 - 386.

Khiati, B., Bacha, S., Ahmed, M., Aissat, S., Meslem, A. and Djebli, N. (2013). Wound Care with *Euphorbia* Honey after Nucleation: A Case Report. *Clinical Microbiology*, *2*, 129. doi: 10.4172/2327-5073.1000129

Knobel, D. L., Laurenson, M. K., Kazwala, R. R., Boden, L. A. and Cleaveland, S. (2008). A crosssectional study of factors associated with dog ownership in Tanzania. *BMC Veterinary. Research, 4,* 5. doi: 10.1186/1746-6148-4-5

Köhidai, L. and Csaba, G. (1998). Chemotaxis and chemotactic selection induced with cytokines (IL-8, RANTES and TNF-alpha) in the unicellular Tetrahymena pyriformis.*Cytokine, 10*(7), 481-486. doi:10.1006/cyto.1997.0328

Kokane, D. D., More, R. Y., Kale, M. B., Nehete, M. N., Mehendale, P. C. and Gadgoli, C. H. (2009). Evaluation of wound healing activity of root of *Mimosa pudica. Journal of Ethnopharmacology, 124*(2), 311-315. doi: 10.1016/j.jep.2009.04.038

Kolb, W. P. and Granger, G. A. (1968). Lymphocyte in vitro cytotoxicity: characterization of human lymphotoxin. *Proceedings of the National Academy of Sciences United States of America, 61*(4), 1250-1255. doi: 10.1073/pnas.61.4.1250

Komarcevic, A. (2000). The modern approach to wound treatment. *Medicinski Pregled Journal,* 2000; *53*(7-8),363-368.

Kondo, T. and Ohshima, T. (1996). The dynamics of inflammatory cytokines in the healing process of mouse skin wound: a preliminary study for possible wound age determination. *International Journal of Legal Medine, 108*(5),231-236.

Kriegler, M., Perez, C., DeFay, K., Albert, I. and Lu, S. D. (1988). A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell, 53*(1), 45-53. doi:10.1016/0092-8674(88)90486-2

Kristiansen, O. P. and Mandrup-Poulsen, T. (2005). Interleukin-6 and diabetes: the good, the bad, or the indifferent?". *Diabetes*, *54*(Suppl 2), S114-124, doi: 10.2337/diabetes.54.suppl_2.S114

Krizbai, I. A., Bauer, H., Amberger, A., Hennig, B., Szabo, H., Fuchs, R. and Bauer, H. C. (2000). Growth factor-induced morphological, physiological and molecular characteristics in cerebral endothelial cells. *European Journal of Cell Biology,79*(9),594-600.

Kroder, G., Bossenmaier, B., Kellerer, M., Capp, E., Stoyanov, B., Mühlhöfer, A., … and Häring, H. (1996). Tumor necrosis factor-alpha- and hyperglycemia-induced insulin resistance. Evidence for different mechanisms and different effects on insulin signalling. *The Journal of Clinical Investigation, 97*(6), 1471-1477, doi: 10.1172/JCI118569

Kudoh, A., Katagai, H., Takazawa, T. and Matsuki, A. (2001). Plasma proinflammatory cytokine response to surgical stress in elderly patients. *Cytokine*, *15*(5),270-273.

Labler, L., Mica, L., Härter, L., Trentz, O. and Keel, M. (2006). Influence of V.A.C.-therapy on cytokines and growth factors in traumatic wounds. *Zentralblatt fur Chirurgie,131*(suppl 1),S62-S67.

Lagathu, C., Bastard, J. P., Auclair, M., Maachi, M., Capeau, J. and Caron, M. (2003). Chronic Interleukin-6 (IL-6) treatment increased IL-6 secretion and induced insulin resistance in adipocyte: Prevention by Rosiglitazone. *Biochemical and Biophysical Research Communications,311*(2), 372-379.

Lavel, B., Barthes-Biesel, D., Regnier, C. and Chauve, T. (1997). Swelling of hydrocolloid dressing. *Biorheology, 34*(2), 139-153.

Lavker, R. M., Sun, T. T., Oshima H, Barrandon, Y., Akiyama, M., Ferraris, C., … and Christiano, A. M. (2003). Hair follicle stem cells. *Journal of Investigative Dermatology Symposium Proceedings,8*(1), 28-38.

Lawrence, W. T. (1998). Physiology of the acute wound. *Clinics in Plastic Surgery,25*(3),321-340.

Lazurus, G. S., Cooper, D. M., Knighton, D. R., Margolis, D. J., Pecoraro, R. E., Rodeheaver, G. and Robson, M. C. (1994). Definitions and guidelines for assessment of wounds and evaluation of healing. *Archives of Dermatology,130*(4),489-493.

Lemmens, L., Claes, V. and Uzzell, M. (2008). Managing patients with metastatic colorectal cancer on bevacizumab. *British Journal of Nursing, 17*(15), 944-949.

Li, S., Huang, N. F. and Hsu, S. (2005). Mechanotransduction in endothelial cell migration. *Journal of Cellular Biochemistry,96*(6),1110-1126.

Li, X., Mai, J., Virtue, A., Yin, Y., Gong, R., Sha, X., … and Yang, X. F. (2012). IL-35 is a novel responsive anti-inflammatory cytokine-A new system of categorizing anti-inflammatory cytokines. *PloS One, 7*(3), e33628. doi: 10.1371/journal.pone.0033628

Lim, E. H., Sardinha, J. P., Myers, S. and Stevens, M. (2013). Latent transforming growth factor-beta1 functionalised electrospun scaffolds promote human cartilage differentiation: Towards an engineered cartilage construct. *Archives of Plastic Surgery, 40*(6), 676-686. doi: 10.5999/aps.2013.40.6.676

Lin, E., Calvano, S. E. and Lowry, S. F. (2000). Inflammatory cytokines and cell response in surgery. *Surgery, 127*(2),117–126.

Lin, F., Baldessari, F., Gyenge, C. C., Sato, T., Chambers, R. D., Santiago, J. G. and Butcher, E. C. (2008). Lymphocyte electrotaxis in vitro and in vivo. *Journal of Immunology,181*(4),2465-2471.

Lindblad-Toh, K., Wade, C. M., Mikkelsen, T. S., Karlsson, E. K., Jaffe, D. B., Kamal, M., … and Lander, E. S. (2005). Genome sequence, comparative analysis and halotype structure of the domestic dogs. *Nature,438*(7069), 803-819. doi: 10.1038/nature04338

Linder, R. (1998). Effect of dressing materials on wound healing. *Langenbecks-Arch-Chir-Suppl-Kongressbd,115,* 694-697.

Lineaweaver, W., Howard, R., Soucy, D., McMorris, S., Freeman, J., Crain, C., … and Rumley, T. (1985). Topical antimicrobial toxicity. *Archive of Surgery,120*(3), 267-270.

Locksley, R. M., Killeen, N. and Lenardo, M. J. (2001). The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell, 104*(4), 487-501. doi:10.1016/S0092-8674(01)00237-9

Malachi, O. I. (2014). Effects of Topical and Dietary Use of Shea Butter on Animals. *American Journal of Life Sciences*. *2*(5), 303-307. doi: 10.11648/j.ajls.20140205.18

Martı´nez, R., Mene´ndez, R., Reyes, S., Polverino, E., Cillo´ niz, C., … and Torres, A. (2011). Factors associated with inflammatory cytokine patterns in community-acquired pneumonia. *European Respiratory Journal, 37*(2), 393-399. doi: 10.1183/09031936.00040710

Martin, P. (1997). Wound Healing - Aiming for Perfect Skin Regeneration. *Science, 276*(5309), 75-81.

Masih, S. (2011). Cytokines: Utility and loboratory measurement. *Medical Laboratory Observer, 2011*, pp1 – 3.

Mateo, R. B., Reichner, J. S. and Albina, J. E. (1994). Interleukin- 6 activity in wounds. *American Journal of Physiology, 266*(6 Pt),R1840-1844.

McBride, W. T., Armstrong, M. A. and McBride, S. J. (1996). Immunomodulation: an important concept in modern anaesthesia. *Anaesthesia, 51*(5), 465-473.

McConnell, K. W., McDunn, J. E., Clark, A. T., Dunne, W. M., Dixon, D. J., Turnbull, I. R., … and Coopersmith, C. M (2010). Streptococcus pneumoniae and Pseudomonas aeruginosa pneumonia induce distinct host response. *Critical Care Medicine, 38*(1), 223-41. doi: 10.1097/CCM.0b013e3181b4a76b

Mckay, I. A. and Leigh, I. M. (1991). Epidermal cytokines and their roles in cutaneous wound healing. *British Jounal of Dermatology, 124*(6), 513-518.

Mehdi, R. and Maryam, N. (2014). Evaluation of serum levels of interleukin 10 with trauma intensity and patients prognosis. *International Journal of Current Research and Academic Review. 2*(9), 214 -219.

Mekkes, J. R., Le-Poole, I. C., Das, P. K., Bos, J. D. and Westerhof, W. (1998). Efficient debridement of necrotic wounds using proteolytic enzymes derived from Antarctic Krill: a double-blind, placebo controlled study in a standardized animal wound model. *Wound Repair and Regeneration,6*(1), 50-57.

Mene´ndez, R., Sahuquillo-Arce, J. M., Reyes, S., Martı´nez, R., Polverino, E., Cillo´niz, C., … and Torres, A. (2012). Cytokine activation patterns and biomarkers are influenced by microorganisms in community-acquired pneumonia. *Chest,141*(6), 1537-1545. doi: 10.1378/chest.11-1446

Micheau, O. and Tschopp, J. (2003). Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell, 114*(2), 181-90. doi:10.1016/S0092-8674(03)00521-X

Mikocka-Walus, A. A., Turnbull, D. A., Moulding, N. T., Wilson, I. G., Andrews, J. M. and Holtmann, G. J. (2007). Controversies surrounding the comorbidity of depression and anxiety in inflammatory bowel disease patients: a literature review. *Inflammatory Bowel Diseases, 13*(2), 225-234. doi:10.1002/ibd.20062

Milner, C. T., (2008). Wound and skin care centre, American Association of Nurses. Retrieved February 26, 2016 from http://www.peacehealth.org/klbase/cam/hm.

Modan-Moses, D., Prince, A., Kanety, H., Pariente, C., Dagan, O., Roller, M., … and Paret, G. (2009). Patterns and prognostic value of troponin, interleukin-6, and leptin after pediatric openheart surgery. *Journal of Critical Care, 24*(3), 419-425. doi: 10.1016/j.jcrc.2009.02.011

Molan, P. C. (2011). The evidence and the rationale for the use of honey as a wound dressing. *Wound Practice and Research, 19*(4), 204-221.

Molloy, R. G., Mannick, J. A. and Rodrick, M. L. (1993). Cytokines, sepsis and immunomodulation. *British Journal of Surgery, 80*(3), 289-297.

Moon, J. J., Matsumoto, M., Patel, S., Lee, L., Guan, J. L. and Li, S. (2005). Role of cell surface heparin sulphate proteoglycans in endothelial cell migration and mechanotransduction. *Journal of Cell Physiology,203*(1),166-176.

Mooney, R. A., Senn, J., Cameron, S., Inamdar, N., Boivin, L. M., Shang, Y. and Furlanetto, R. W. (2001). Suppressors of cytokine signaling-1 and -6 associate with and inhibit the insulin receptor. A potential mechanism for cytokine-mediated insulin resistance. *The Journal of Biological Chemistry,276*, 25889-25893. doi: 10.1074/jbc.M010579200

Moret, J., Lorenzo, M. J., Sarria, B., Cases, E., Morcillo, E., Perpin˜a´, M., … and Menendez, R. (2011). Increased lung neutrophil apoptosis and inflammation resolution in nonresponding pneumonia. *European Respiratory Journal, 38*(5), 1158-1164. doi: 10.1183/09031936.00190410

Morey, D. (2006). Burying key evidence: The social bond between dogs and people. *Journal of Archaeological science*, *33*(2), 158-175. doi: 10.1016/j.jas.2005.07.009

Morteza, G., Javad, B., Khalil, A., Melika, E. G. and Fatemeh, M. (2017). TNF-α exerts cytotoxic effects on multidrug resistant breast cancer MCF-7/MX cells via non-apoptotic death pathway. *Cytokine, 97*, 167-174. dio.org/10.1016/j.cyto.2017.06.014

Moseley, R., Stewart, J. E., Stephens, P., Waddington, R. J. and Thomas, D. W. (2004). Extracellular matrix metabolites as potential biomarkers of disease activity in wound fluid: lessons learned from other inflammatory diseases? *British Journal of Dermatology,150*(3), 401-413.

Mosser, D. M. and Zhang, X. (2008). Interleukin-10: new perspectives on an old cytokine. *Immunological Reviews*, *226*(1), 205-218. doi:10.1111/j.1600-065X.2008.00706.x

Mulder, G. D. and Vande, B. J. S. (2002). Cellular senescence and matrix metalloproteinase activity in chronic wounds. Relevance to debridement and new technologies. *Journal of the American Podiatric Medical Association,92*(1),34-37.

Myers, S. R., Leigh, I. M. and Navsaria, H. (2007). Epidermal repair results from activation of follicular and epidermal progenitor keratinocytes mediated by a growth factor cascade. *Wound Repair and Regeneration, 15*(5), 693-701.

Najib, A., Roger, D., Joshua, J. Q., Qian, L., David, G. and Anthony, W. B. (2016). Stability of cytokines and soluble activation markers in unprocessed blood stored under different conditions. *Cytokine, 84*, 17 - 24. dio.org/10.1016/j.cyto.2016.05.010

Nakahara, J., Maeda, M., Aiso, S. and Suzuki, N. (2012). Current concepts in multiple sclerosis: autoimmunity versus oligodendrogliopathy. *Clinical Reviews in Allergy and Immunology 42*(1), 26–34. doi:10.1007/s12016-011-8287-6

Namas, R., Ghuma, A., Hermus, L., Zamora, R., Okonkwo, D., Billiar, T. R. and Vodovotz, Y. (2009). The acute inflammatory response in trauma / hemorrhage and traumatic brain injury: current state and emerging prospects.Insights into trauma-induced inflammation. *Libyian Journal of Medicine, 4*(3), 97-103. doi: 10.4176/090325

Nash, A. D., Brandon, M. R. and Bello, P. A. (1992). Effects of tumor necrosis factor-alpha on growth hormone and interleukin 6 mRNA in ovine pituitary cells. *Molecular Cell Endocrinology,84*(1-2), R31-37.

Navarrete, M. A. (2015). ELISpot and DC-ELISpot assay to measure frequency of antigen-specific IFNγ-secreting cells. *Methods in Molecular Biology*, *1318*, 79-86. dio: 10.1007/978-1-4939-2742-5_8

Navarro, J. M., Casatorres, J. and Jorcano, J. L. (1995). Elements controlling the expression and induction of the skin hyperproliferation- associated keratin K6. *Journal of Biological Chemistry,270*(36),21362-21367.

Neely, A. N., Gardner, J., Durkee, P., Warden, G. D., Greenhalgh, D. G., Gallagher, J. J., … and Kagan, R. J. (2009). Are topical antimicrobials effective against bacteria that are highly resistant to systemic antibiotics? *Journal of Burn and Care Research,30*(1), 19-29.

Nguyen, D. T., Orgill, D. P. and Murphy, G. F. (2009). The pathophysiologic basis for wound healing and cutaneous regeneration. Biomaterials for treating skin loss (pp. 25-57). CRC press (US) and Woodhead Publishing (UK/Europe), Boca Raton/Cambridge.

Nicholas, R. S., Falvey, H., Lemonas, P., Damodaran, G., Ghanem, A. M., Selim, F., … and Myers, S. (2012). Patient-related keloid scar assessment and outcome measures. *Plastic Reconstructive Surgery, 129*(5), 648-656. doi: 10.1097/PRS.0b013e3182402c51

Nishimoto, N. (2006). Interleukin-6 in rheumatoid arthritis. *Current Opinion in Rheumatology, 18*(3), 277-281. doi:10.1097/01.bor.0000218949.19860.d1

O'Kane, S (2002). Wound remodelling and scarring. *Journal of Wound Care, 11*(8),296-299.

O'Kane, S. and Ferguson, M. W. (1997). Transforming growth factor beta s and wound healing. *International Journal of Biochemistry and Cell Biology, 29*(1), 63-78.

O'Meara, S. M., Cullum, N. A., Majid, M. and Sheldon, T. A. (2001). Systematic review of antimicrobial agents used for chronic wounds. *British Journal of Surgery,88*(1), 4-21.

O'Meara, S., Al-Kurdi, D. and Ovington, L. G. (2008). Antibiotics and antiseptics for venous leg ulcers. *Cochrane Database Systematic Review*, 1, CD003557. doi: 10.1002/14651858.CD003557

Occleston, N. L., Laverty, H. G., O'Kane, S. and Ferguson, M. W. (2008). Prevention and reduction of scarring in the skin by transforming growth factor beta 3 (TGFbeta3): from laboratory discovery to clinical pharmaceutical. *Journal of Biomaterials Science. Polymer Edition, 19*(8), 1047-1063. doi: 10.1163/1568562084909345

Ogawa, R., Huang, C., Akaishi, S., Dohi, T., Sugimoto, A., Kuribayasi, S., … and Hyakusoku, H. (2013). Analysis of surgical treatments for earlobe keloids: analysis of 174 lesions in 145 patients. *Plastic and Reconstructive Surgery, 132*(5), 818e-825e. doi: 10.1097/PRS.0b013e3182a4c35e

Oike, Y., Ito, Y., and Maekawa, H.(2004). Angiopoietin related growth factor (AGF) promotes angiogenesis. *Blood, 103*,3760-3765. doi: 10.1182/blood-2003-04-1272

Olayemi, F. O., Azeez, I. O., Ogunyemi, A. and Ighagbon, F. O. (2009). Study on erythrocyte values on the Nigerian indigenous dog. *Folia Veterinaria,53*(2), 65-67.

Olszewski, M. B., Groot, A. J., Dastych, J. and Knol, E. F. (2007). TNF Trafficking to Human Mast Cell Granules: Mature Chain-Dependent Endocytosis. *The Journal of Immunology,178*(9), 5701-5709. doi:10.4049/jimmunol.178.9.5701

Olumayowa, O. I. (2011). Gross morphometric study of the eyeball and tongue of the Nigerian local dog. *Italian Journal of Anatomy and Embryology, 116*(1), 104-110.

Oluwatoyin A. O. and Fayemi, O. E. (2011). A retrospective study of reproductive conditions and requested procedures in Dogs in South Western Nigeria: 1999-2008. *Journal of Animal and Veterinary Advances, 10*(19), 2612-2617.

Ono, I., Gunji, H., Suda, K., Iwatsuki, K. and Kaneko, F. (1994). Evaluation of cytokines in donor-site wound fluids. *Scandinavian Journal of Plastic and reconstructive Hand Surgery, 28*(4), 269-273. dio: 10.3109/02844319409022010

Ono, I., Gunji, H., Zhang, J-Z., Maruyama, K. and Kaneko, F. (1995). A study of cytokines in burn blister fluid related to wound healing. *Bums,21*(5), 352-355.

Opal, S. M., Wherry, J. C. and Grint, P. (1998). Interleukin-10: potential benefits and possible risks in clinical infectious diseases. *Clinical Infectious Disease, 27*, 1497-1507.

Oral, H., Dorn, G. W. and Mann, D. L. (1997). Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-a in the adult mammalian cardiac myocyte. *Journal of Biological Chemistry, 272*(8),4836-4842.

Orzechowski, A. (2017). Cytokines in skeletal muscle growth and decay. In: Sakuma, L. (eds). The plasticity of skeletal muscle. Springer, Singapore, Pp 113 – 139. dio: 10.1007/978-981-10-3292-9_5

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P. and Pedersen, B. K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *The Journal of Physiology*. *515*(Pt 1), 287-291. doi:10.1111/j.1469-7793.1999.287ad.x

Ostrowski, K., Schjerling, P. and Pedersen, B. K. (2000). Physical activity and plasma interleukin-6 in humans--effect of intensity of exercise. *European Journal of Applied Physiology*, *83*(6), 512-515. doi:10.1007/s004210000312

Owen, J. A., Punt, J., Stranford, S. A. and Jones, P. P (2013). Receptors and signalling: Cytokines and chemokine. *In Kuby Immunology* (pp. 105-140). W. H. Freeman and Company.

oxidised solution on human dermal fibroblasts in vitro. *International Wound Journal*, *4*(3), 241-250.

Pagani, F. D., Baker, L. S., His, C., Knox, M., Fink, M. P. and Visner, M. S. (1992). Left ventricular systolic and diastolic dysfunction after infusion of tumor necrosis factor-a in conscious dogs. *Journal of Clinical Investigation*,*90*(2),389-398.

Pankaj, K. S., Deen, D. G., Ritu S., Priyanka, P., Sharmistha, G., Atul, K. S., … and Kapil, D. P. (2013). Therapeutic and Medicinal Uses of *Aloe vera*: A Review. *Pharmacology and Pharmacy, 4*, 599-610.doi.org/10.4236/pp.2013.48086

Papantonio, C. (1998). Alternative Medicine and Wound Healing. *Ostomy Wound Management*,*44*(4), 44-50.

Park, J. E. and Barbul, A. (2004).Understanding the role of immune regulation in wound healing. *The American Journal of Surgery*, *187*(Suppl to May 2004), 11S–16S.

Patel, P. P., Vasquez, S. A., Granick, M. S. and Rhee, S. T. (2008). Topical antimicrobials in pediatric burn wound management. *Journal of Craniofacial Surgery*, *19*(4), 913-922. doi: 10.1097/SCS.0b013e318175b516

Paul, E. and Serpell, J. (1996). Obtaining a new pet dog: Effects on middle childhood children and their families. *Applied Animal Behaviour Science*., *47*, 17-29.

Paul, J. C. and Pieper, B. A. (2008). Topical metronidazole for the treatment of wound odor: A review of the literature. *Ostomy Wound Management*,*54*(3), 18–27.

Pause, L. C. and Grauer, G. F. (1998). Association of gastrointestinal haemorrhage with increased blood urea nitrogen and BUN/Creatinie ratio in dogs: Literature review and retrospective study. *Veterinary Clinical Pathology*, *27*(4), 107 – 111.

Pawelec, G., Adibzadeh, M., Pohla, H. and Schaudt, K. (1995). Immunosenescence: ageing of the immune system. *Immunology Today, 16*(9),420-422.

Pedersen, B. K. (2013). Muscle as a secretory organ. *Comprehensive Physiology*, *3*(3), 1337-1362. doi:10.1002/cphy.c120033

Pedoeem, A., Azoulay-Alfaguter, I., Strazza, M., Silverman, G. J. and Mor, A. (2014). Programmed death in cancer and autoimmunity. *Clinical Immunology*, *153*(1), 145-152. dio: 10.1016/j.clim.2014.04.010

Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., … and Goeddel, D. V. (1984). Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature*, *312*(5996), 724-729. doi:10.1038/312724a0

Pestka, S., Krause, C. D., Sarkar, D., Walter, M. R., Shi, Y. and Fisher, P. B. (2004). Interleukin-10 and related cytokines and receptors. *Annual Review of Immunology*, *22*(1), 929-79. doi:10.1146/annurev.immunol.22.012703.104622

Petersen, A. M. and Pedersen, B. K. (2005). "The anti-inflammatory effect of exercise". *Journal of Applied Physiology*, *98*(4), 1154-1162. doi: 10.1152/japplphysiol.00164.2004

Phillips, S. J. (2000). Physiology of wound healing and surgical wound care. *American Society for Artificial Internal Organs Journal*,*46*(6),S2-S5.

Phin, C. K., Yin, N. S. and Abdullah, S. (2013). Phytochemical Constituents from Leaves of E*laeis guineensis* and Their Antioxidant and Antimicrobial Activities. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(suppl 4). Issn- 0975-1491.

Pirbalouti, G. A., Azizi, S. and Koohpayeh, A. (2012). Healing potential of Iranian traditional medicinal plants on burn wounds in alloxan-induced diabetic rats. *Revista Brasileira de Farmacognosia*, *22*(2), 397-403. doi: 10.1590/S0102-695X2011005000183

Powell, M. J., Thompson, S. A., Tone, Y., Waldmann, H. and Tone, M. (2000). Posttranscriptional regulation of IL-10 gene expression through sequences in the 3'-untranslated region. *Journal of Immunology*, *165*(1), 292-296. doi:10.4049/jimmunol.165.1.292

Quinton, L. J., Jones, M. R., Robson, B. E., Simms, B. T., Whitsett, J. A. and Mizgerd, J. P. (2008). Alveolar epithelial STAT3, IL-6 family cytokines, and host defense during Escherichia coli pneumonia. *American Journal of Respiratory Cell and Molecular Biology*,*38*(6), 699-706. doi: 10.1165/rcmb.2007-0365OC

Rajoo, A., Sasidharan, S., Jothy, S. L., Ramanathan, S. and Mansor, S. M. (2013). Ultrastructural Study of Elaeis guineensis (Oil Palm) Leaf and Antimicrobial Activity of its Methanol Extract against Staphylococcus Aureus.*Tropical Journal of Pharmaceutical Research, 12*(3), 419-423. doi: 10.4314/tjpr.v12i3.22

Ramachandran S., Ghanem, A. M. and Myers, S. R. (2013). Assessment of microsurgery competency-where are we now? *Microsurgery*, *33*(5), 406–415. doi: 10.1002/micr.22111

Ramasastry, S. S. (2005). Acute wounds. *Clinics in Plastic Surgery, 32*(2),195-208.

Ramelet, A. A., Hirt-Burri, N., Raffoul, W., Scaletta, C., Pioletti, D. P., Offord, E., … and Applegate, L. A. (2009). Chronic wound healing by fetal cell therapy may be explained by differential gene profiling observed in fetal versus old skin cells. *Experimental Gerontology,44*(3),208-218. doi: 10.1016/j.exger.2008.11.004

Ramnath, V., Rekha, P. S. and Sujatha, K. S. (2008). Amelioration of heat stress-induced disturbances of antioxidant defence system in chickens by *Brahma rasyanaEvidence-Based Complementary and Alternative Medicine*, *5*(1), 77-84.

Raut G., Vanmali H., Pimpliskar M. and Rahul J. (2015). Efficacy of Some Antiseptics and Disinfectants: A Review. In.Human journals.*4*(4),182-197.

Reddy, B. S., Reddy, R. K. K., Naidu, V. G. M., Madhusudhana, K., Agwane, S. B., Ramakrishna, S. and Diwan, P. V. (2008). Evaluation of antimicrobial, antioxidant and wound healing potentials of *Holoptelea integrifolia. Journal of Ethanopharmacology*, *115*(2), 249-256.

Remick, D. G., Bolgos, G. R., Siddiqui, J., Shin, J. and Nemzek, J. A. (2002). Six at six: interleukin 6 measured 6 h after the initiation of sepsis predicts mortality over 3 days. *Shock*, *17*(6): 463-467.

Remick, D. G., Bolgos, G., Copeland, S. and Siddiqui, J. (2005). Role of interleukin 6 in mortality and physiologic response to sepsis. *Infection and Immunity*, *73*(5): 2751-2757.

Renovo. *Juvista EU Phase 3 trial results*. (2011). Retrieved June, 23 2015 from http://www.renovo. com/en/news/juvista-eu-phase-3-trial-results.

Rice, J. (2000). Secondary intention wound healing pathophysiology and management. *Collegian*, *7*(3),40-41.

Richardson, M. (2004). Acute wounds: an overview of the physiological healing process. *Nursing Times,100*(4),50-53.

Rieusset, J., Bouzakri, K., Chevillotte, E., Ricard, N., Jacquet, D., Bastard, J. P., … and Vidal, H. (2004). SOCS (Suppressor of Cytokine Signaling)-3 Expression and Insulin Resistance in Skeletal Muscle of Obese and Type 2 Diabetic Patients. *Diabetes,53*(9), 2232-2241.

Risnes, I., Ueland, T., Lundblad, R., Mollnes, T. E., Baksaas, S. T., Aukrust, P. and Svennevig, J. L. (2003). Changes in the cytokine network and complement parameters during open heart surgery. *Interactive Cardiovascular and Thoracic Surgery*, *2*(1), 19 - 24.

Rivera, A. E. and Spencer, J. M. (2007). Clinical aspects of fullthickness wound healing. *Clinics in Dermatology, 25*(1),39-48.

Robinson, C. J. (1993). Growth factor: therapeutic advances in wound healing. *Annals of Medicine, 25*(6), 535-538.

Robson, M. C. (1997). Wound infection: a failure of wound healing caused by an imbalance of bacteria. *Surgical Clinics of North America,77*(3),637-650. doi: 10.1016/S0039-6109(05)70572-7

Robson, M. C. and Heggers, I. P. (1992). Eicosinoid, cytokines and free radicals. In: Cohen, I. K., Diegelmann, R. F. and Lindbiad, W. I. (Eds). *Wound healing*, pp. 292-304. Philadelphia, WB Saunders.

Robson, M. C., Steed, D. L. and Franz, M. G. (2001). Wound healing: biologic features and approaches to maximize healing trajectories. *Current Problems in Surgery,38*(2),72-140.

Rosengardten, Y., McKenna, T., Grochová, D. and Eriksson, M. (2011). Stem cell depletion in Hutchinson-Gilford progeria syndrome. *Aging Cell, 10*(6), 1011-1020. doi: 10.1111/j.1474-9726.2011.00743.x

Rottner, M., Freyssinet, J. M. and Martínez, M. C. (2009). Mechanisms of the noxious inflammatory cycle in cystic fibrosis. *Respiratory Research, 10*(1), 23. doi: 10.1186/1465-9921-10-23

Ruddle, N. H. and Waksman, B. H. (1968). Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. 3. Analysis of mechanism. *Journal of Experimental Medicine, 128*(6), 1267-1279. doi: 10.1084/jem.128.6.1267

Saami, K., Giorgio, R. and Gerald, B. (2015). The use of luminex assays to measure cytokines. *Journal of Investigative Dermatology, 135*(4), 1 – 5. dio: 10.1038/jid.2015.36

Said, E. A., Dupuy, F. P., Trautmann, L., Zhang, Y., Shi, Y., El-Far, M., … and Sekaly, R. P. (2010). "Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection". *Nature Medicine, 16*(4), 452-459. doi: 10.1038/nm.2106

Sakic, K., Zura, M., Sakic, L., Vrbanovic, V. and Bagatin, D. (2009). Neuroimmunomodulation by regional and general anaesthesia. *Periodicum Biologorum, 111*(2),209-214.

Salem, A. H., Entsar, A. S., Ashraf, A. E., and Zeinab, R. A. (2015). Pro-inflammatory adipocytokines, oxidative stress, insulin, Zn and Cu: Interrelations with obesity in Egyptian non-diabetic obese children and adolescents. *Advances in Medical Sciences, 60*(2), 179-185. doi: 10.1016/j.advms.2015.02.002

Salo, M. (1996). Cytokines and attenuation of responses to surgery. *Acta anaesthesiologica Scandinavica, 40*(2), 141-142.

Saraiva, M. and O'Garra, A. (2010). "The regulation of IL-10 production by immune cells". *Nature Reviews Immunology, 10*(3), 170-81. doi: 10.1038/nri2711

Saror, D. I., Schillhorn Van Veen, T. W. and Adeyanju, J. B. (1979). The haemogram of dogs with intestinal parasites in Zaria, Nigeria. *Journal of Small Animal Practice, 20*(4), 243-247.

Sasidharan, S., Logeswaran, S. and Latha, L. Y. (2012). Wound healing activity of *Elaeis guineensis* leaf extract ointment. *International Journal of Molecular Science,13*(1), 336-347. doi: 10.3390/ijms13010336

Sato, M., Sawamura, D., Ina, S., Yaguchi, T., Hanada, K. and Hashimoto, I. (1999). In vivo introduction of the interleukin 6 gene into human keratinocytes: induction of epidermal proliferation by the fully spliced form of interleukin 6, but not by the alternatively spliced form. *Archives of Dermatological Research, 291*(7-8),400-404.

Savolainen, P., Y. Zang, J. Luo, J. Lundeberg and T. Leitner, (2002). Genetic evidence for an East Asian origin of domestic dogs. *Science*, *298*(5598), 1610-1613.

Sawamura, D., Meng, X., Ina, S., Sato, M., Tamai, K., Hanada, K. and Hashimoto, I. (1998). Induction of keratinocyte proliferation and lymphocytic infiltration by in vivo introduction of the IL-6 gene into keratinocytes and possibility of keratinocyte gene therapy for inflammatory skin diseases using IL-6 mutant genes. *Journal of Immunology, 161*(10),5633-5639.

Schulte, L. N., Eulalio, A., Mollenkopf, H. J., Reinhardt, R. and Vogel, J. (2011). Analysis of the host microRNA response to Salmonella uncovers the control of major cytokines by the let-7 family. *The European Molecular Biology Organization Journal, 30*(10), 1977-1989. doi: 10.1038/emboj.2011.94

Schwantner, A., Dingley, A. J., Ozbek, S., Rose-John, S. and Grötzinger, J. (2004). Direct determination of the interleukin-6 binding epitope of the interleukin-6 receptor by NMR spectroscopy. *Journal of Biological Chemistry, 279*(1), 571-576. doi:10.1074/jbc.M311019200

Seyyed, A. H., Seyyed, A. M. and Saied, A. (2015).The Review on Properties of Aloe Vera in Healing of Cutaneous Wounds (pp. 1-6). *BioMedical Research International,* Article ID 714216. doi: org/10.1155/2015/714216

Sharabiani, M. T., Vermeulen, R., Scoccianti, C., Hosnijeh, F. S., Minelli, L., Sacerdote, C., … Vineis, P. (2011). "Immunologic profile of excessive body weight". *Biomarkers 16*(3), 243-51. doi: 10.3109/1354750X.2010.547948

Sharma, A., Kumar, M., Aich, J., Hariharan, M., Brahmachari, S. K., Agrawal, A. and Ghosh, B. (2009). "Posttranscriptional regulation of interleukin-10 expression by hsa-miR-106a".*Proceedings of the National Academy of Sciences of the United States of America, 106*(14), 5761-5766. doi: 10.1073/pnas.0808743106

Shashi, P. M., Shivanshu, S. and Sanjeev, K. G. (2013). Necrotising soft tissue infection: Surgeon's prospective. *International journal of inflammation, 7*. dio: 10.115/2013/609628

Sheela, D. J., Jeeva, S., Shamila, I. M. R., Lekshmi, N. C. J. P. and Brindha, J. R. (2012). Antimicrobial activity and phytochemical analysis of *Sansevieria roxburghiana* leaf. *Asian Journal of Plant Science and Research*, *2*(1), 4-44.

Sheeran, P. and Hall, G. M. (1997). Cytokines in anaesthesia. *British Journal of Anaesthesia*, *78*(2),201-202.

Shi-long, Y., Xiao-Jun, X., Yong-min, T., Hua, S., Wei-Qun, X., Fen-Ying, Z. and Ying, S. (2016). Association between inflammatory cytokines and organ damage in pediatric patients with hemophagocytic lymphohistocytosis. *Cytokine, 85,* 14-17. dio.org/10.1016/j.cyto.2016.05.022

Sikka, G., Miller, K. L., Steppan, J., Pandey, D., Jung, S. M., Fraser, C. D., … and Barouch, L. A. (2013). " Interleukin 10 knockout frail mice develop cardiac and vascular dysfunction with increased age". *Experimental Gerontology, 48*(2), 128-135.doi:10.1016/j.exger.2012.11.001

Simon R. M. and Ali M. G. (2015). Wound healing and scar formation. *Plastic and Reconstructive Surgery: Approaches and Techniques*, First Edition. Ross, D. F., Neil, W. B. and Sabrina, C. (Eds). John Wiley & Sons, Ltd.

Skover, G. R. (1991). Cellular and biochemical dynamics of wound repair. Wound environment in collagen regeneration. *Clinics in Podiatric Medicine and Surgery,8*(4),723-756.

Slatter, D. H. (1985). External Skeleton fixation. In *Textbook of Samll Animal Surgery, 2,* 1972-1988. Philadelphia: W. B. Saunders Company.

Smith, P. C., Hobisch, A., Lin, D. L., Culig, Z. and Keller, E. T. (2001). "Interleukin-6 and prostate cancer progression". *Cytokine Growth Factor Review,12*(1), 33-40. doi: 10.1016/S1359-6101(00)00021-6

Snedecor, G. W. and Cochran, W. G. (1994). *Statistical Methods* (pp. 509). Oxford and IBH Publishing Corperation, Calcutta, India. Pp 509.

Soehnlein, O., Weber, C. and Lindbom, L. (2009). Neutrophil granule proteins tune monocytic cell function. *Trends Immunology, 30,* 538 - 546.

Solis-Herruzo, J. A., Rippe, R. A., Schrum, L. W., de La Torre, P., Garcia, I., Jeffrey, J. J., … and Brenner, D. A. (1999). Interleukin-6 increases rat metalloproteinase-13 gene expression through stimulation of activator protein 1 transcription factor in cultured fibroblasts. *Journal of Biological Chemistry, 274*(43),30919-30926.

Sorensen, L. T. (2012). Wound healing and infection in surgery: the pathophysiological impact of smoking, smoking cessation, and nicotine replacement therapy: a systematic review. *Annals of Surgery, 255*(6), 1069-1079. doi: 10.1097/SLA.0bO13e31824f632d

Sosa, I. J., Reyes, O. and Kuffler, D. P. (2008). Elimination of a pressure ulcer with electrical stimulation - A case study. *Puerto Rico Health Sciences Journal,27*(2),175-179.

Steed, D. L. (2006). Clinical evaluation of recombinant human plateletderived growth factor for the treatment of lower extremity ulcers. *Plastic and Reconstructive Surgery, 117*(7 Suppl.), 143S-149S; discussion 150S-151S.

Strecker-McGraw, M. K., Jones, T. R. and Baer, D. G. (2007). Soft tissue wounds and principles of healing. *Emergency Medicine Clinics of North America,25*(1),1-22.

Suntar, I., Kupeli, A. E., Nahar, L., Satyajit, D. and Sarker, S. D. (2012). Wound healing and antioxidant properties: do they coexist in plants? *Free Radicals and Antioxidants*, *2*(2), 1-7.

Sussman, C. and Bates-Jensen, B. (2007). *Wound assessment*: Wound care: A collaborative practice manual for health professionals. USA:Lippincott Williams & Wilkins.

Swai, E. S., Kaaya, E. J., Mshanga D. A. and Mbise, E. W. (2010). A Survey on Gastro-Intestinal Parasites of Non-Descript Dogs in and Around Arusha Municipality, Tanzania. *Journal of Animal and Veterinary Advances*, *3*(2), 63-67, 2010.

Swaim, S. F. (1983). Management and bandaging of soft tissue injuries of the dog and cat feet. *Journal of the American Animal Hospital Association*, *21*, 329-340.

Swaim, S. F. and Amber, E. I. (1990). Bandages and topical agents. *Veterinary Clinics of North Amerian Small Animal Practice*, *20*, 47-64.

Swardfager, W., Lanctôt, K., Rothenburg, L., Wong, A., Cappell, J. and Herrmann, N. (2010). "A meta-analysis of cytokines in Alzheimer's disease". *Biological Psychiatry, 68*(10), 930-941. doi: 10.1016/j.biopsych.2010.06.012

Swihart, E. V. (1996). New Options in Wound Healing. *Veterinary Forums*, (May) 64-49.

Takeda, K., Kaisho, T., Yoshida, N., Takeda, J., Kishimoto, T. and Akira, S. (1998). Stat3 activation is responsible for IL-6- dependent T cell proliferation through preventing apoptosis: generation and characterization of T cell-specific Stat3-deficient mice. *Journal of Immunology, 161*(9),4652-4660.

Takeshi, M., Hiroshi, K., Masashi, M., Takahiro, M., Asako, S., Kayoko, H., … and Masami, U., (2015). Plasma cytokine levels in dogs undergoing cardiopulmonary bypass. *Research in Veterinary Science,101*, 99-105.

Tan, J. C., Braun, S., Rong, H., DiGiacomo, R., Dolphin, E., Baldwin, S., Narula, S. K., Zavodny, P. J. and Chou, C. C. (1995). Characterization of recombinant extracellular domain of human interleukin-10 receptor. *The Journal of Biological Chemistry, 270*(21), 12906-11911. doi: 10.1074/jbc.270.21.12906

Tang, P., Hung, M-C. and Klostergaard, J. (1996). Human pro-tumor necrosis factor is a homotrimer.*Biochemistry, 35*(25), 8216-8225. doi: 10.1021/bi952182t

Tasaka, S., Kobayashi, S., Kamata, H., Kimizuka, Y., Fujiwara, H., Funatsu, Y., … and Hasegawa, N. (2010). Cytokine profiles of bronchoalveolar lavage fluid in patients with Pneumocystis pneumonia. Microbiol Immunol, *54*(7), 425–433. dio: 10.1111/J.1348-0421.2010.00229.x

Theiss, A. L., Simmons, J. G., Jobin, C. and Lund, P. K. (2005). Tumor necrosis factor (TNF) alpha increases collagen accuulation and proliferation in intestinal myofibrobasts via TNF Recptor 2. *The Journal of Biological Chemistry, 280*(43), 36099-36109.

Toy, L. W. (2005). Matrix metalloproteinases: their function in tissue repair. *Journal of Wound Care,14*(1),20-22.

Tracey, K. J. (2002). The inflammatory reflex. *Nature,420*(6917),853-859.

Tracey, K. J., Beutler, B., Lowry, S. F., Merryweather, J., Wolpe, S., Milsark, I. W., … and Albert, J. D. (1986). Shock and tissue injury induced by recombinant human cachectin. *Science, 234*(4775), 470-474. doi: 10.1126/science.3764421

Tracey, K. J., Fong, Y., Hesse, D. G., Manoque, K. R., Lee, A. T., Kuo, G. C., … and Cerami, A. (1987). Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature, 330*(6149), 662-664. doi: 10.1038/330662a0

Tsourdi, E., Barthel, A., Rietzsch, H., Reichel, A. and Bornstein, S. R. (2013). Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. *BioMedical Research International,* 385641. doi: 10.1155/2013/385641

Turkel, Y., Turker, H., Demir, I. A., Bayrak, A. O. and Onar, M. K. (2014). Validation of self report version of the Leeds Assessment of Neuropathic symptoms and signs score for identification of neuropathic pain in patients from northern Turkey. *Advanced Clinical Experimental Medicine, 23*(4), 599-603.

Utgaard, J. O., Jahnsen, F. L., Bakka, A., Brandtzaeg, P. and Haraldsen, G. (1998). "Rapid secretion of prestored interleukin 8 from Weibel-Palade bodies of microvascular endothelial cells". *Journal of Experimental Medicine, 188*(9), 1751-1756. doi: 10.1084/jem.188.9.1751

Van der Poll, T., Keogh, C. V., Guirao, X., Buurman, W. A., Kopf, M. and Lowry, S. F. (1997). "Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia". *Journal of Infectious Diseases, 176*(2), 439-444. doi: 10.1086/514062

Van Westerloo, D. J., Giebelen, I. A., Florquin, S., Daalhuisen, J., Bruno, M. J., de Vos, A. F., … and van der Poll, T. (2005). The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *Journal of Infectious Diseases, 191*(12),2138-2148.

Vanwijck, R. (2001). Surgical biology of wound healing. *Bulletin et Memoires de'l Academie Royale de Medecine Belgique, 156*(2-4),175-184; discussion 185.

Velnar, T. Bailey, T. and Smrkolj, V. (2009). The Wound Healing Process: an Overview of the Cellular and Molecular Mechanisms. *The Journal of International Medical Research, 37*(5), 1528-1542.

Vijayarathna S. and Sasidharan S. (2012). Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and Vero cell lines. *Asian Pacific Journal of Tropical Biomedicine,2*(10), 826-829. doi: 10.1016/S2221-1691(12)60237-60238

Wahi, S. M., Wong, H. and McCartney-Francis, N. (1989). Role of growth factors in inflammation and repair. *Journal of cell Biochemistry,40*(2), 193-199.

Wajant, H., Pfizenmaier, K. and Scheurich, P. (2003). Tumor necrosis factor signaling. *Cell Death and Differentiation, 10*(1), 45-65. doi: 10.1038/sj.cdd.4401189

Walsh, L. J., Trinchieri, G., Waldorf, H. A., Whitaker, D. and Murphy, G. F. (1991). Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1. *Proceedings of the National Academy of Science of United State of America, 88*(10), 4220-4224. doi: 10.1073/pnas.88.10.4220

Wang, H. and Tracey, K. J. (1999). Tumor necrosis factor, interleukin-6, macrophage migration inhibitory factor, and macrophage inflammatory protein-1 in inflammation. In: Gallin, J. I., Snyderman, R., editors. Inflammation: basic principles and clinical correlates (pp. 471-486). Philadelphia, USA: Lippincott Williams and Wilkins. Pp 471 - 486.

Ward, P. D., London, N. and Collar, R. (2013). Role of secondary intention healing. *Facial Plastic Surgery, 29*, 346-350. doi: 10.1055/s-0033-1353374

Wardlaw, J. L. and Lanz, O. I. (2012). Axial pattern and myocutaneous flaps. In Tobias, K. M. and Johnston, S. A. (Eds), *Veterinary Surgery: Small Animal* (pp. 1256-1270). St. Louis: Elsevier.

Warren, O. J., Smith, A. J., Alexiou, C., Rogers, P. L., Jawad, N., Vincent, C., et al., (2009). The inflammatory response to cardiopulmonary bypass: part 1–mechanisms of pathogenesis. *Journal of Cardiothoracic and Vascular Anesthesia*, *23*, 223-231.

Wattanathum, A., Manocha, S., Groshaus, H., Russell, J. A. and Walley, K. R. (2005). Interleukin-10 haplotype associated with increased mortality in critically ill patients with sepsis from pneumonia but not in patients with extrapulmonary sepsis. *Chest, 128*(3), 1690-1698.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L. and Ferrante Jr. A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation,112*(12), 1796-1808.

Whitaker, I. S., Rozen, W. M., Cairns, S. A., Howes, J., Pope, F. M. and Hamish, L. J. (2009). Molecular genetics and clinical review of Ehlers-Danlos Type VIIA: implications for management by the plastic surgeon in a multidisciplinary setting. *Journal of Plastic, Reconstructive & Aesthetic Surgery, 62*(5), 589-594. doi: 10.1016/j.bjps.2008.11.119

Willems, F., Marchant, A., Delville, J. P., Gerard, C., Delvaux, A., Velu, T., de Boer, M. and Goldman, M. (1994). Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. *European Journal of Immunology, 24*(4), 1007-1009.

William, C. (1998). Hydrocoll: A ‘New Breed’ of Hydrocolloid wound dressing. *British Journal of Nursing.7*(21), 1337-1340.

Witte, M. B. and Barbul, A. (1997). General principles of wound healing. *Surgery Clinics of North America,* 77(3): 509–528. *In:* Park, J. E. and Barbul, A. (2004).Understanding the role of immune regulation in wound healing. *The American Journal of Surgery, 187*(Suppl to May 2004), 11S–16S.

Wolff, B., Burns, A. R., Middleton, J. and Rot, A. (1998). Endothelial cell "memory" of inflammatory stimulation: human venular endothelial cells store interleukin 8 in Weibel-Palade bodies. *Journal of Experimental Medicine, 188*(9), 1757-62. doi:10.1084/jem.188.9.1757

Wolgemuth, C. W. (2005). Lamellipodial contractions during crawling and spreading. *Biophysical Journal,89*(3), 1643-1649. doi: 10.1529/biophysj.105.066720

Wong, T. W., Shen, H. M., Lee, J. Y. and Chao, S. C. (1998). Use of tissue meshing technique to facilitate side to side closure of large defects. *Dermatologic Surgery,24*(12), 1338-1341.

Wu, Q., Martin, R. J., Rino, J. G., Breed, R., Torres, R. M. and Chu, H. W. (2007). IL-23-dependent IL-17 production is essential in neutrophil recruitment and activity in mouse lung defense against respiratory Mycoplasma pneumoniae infection. *Microbes and Infection, 9*(1), 78–86.

Yadav. K. C. H., Kumar, J. R., Basha, S. I., Deshmukh, G. R., Ravi, G. and Santhamma, B. (2012). Wound healing activity of topical application of aloe vera gel In experimental animal models. *International Journal of Pharmacology and Bio Sciences, 3*(2), 63-72.

Yamaguchi, M., Koike, K., Yoshimoto, Y., Ikegami, H., Miyake, A. and Tanizawa, O. (1991). Effect of TNF alpha on prolactin secretion from rat anterior pituitary and dopamine release from the hypothalamus: comparison with the effect of interleukin-1 beta. *Endocrinologia Japonica.38*(4), 357-361.

Yende, S., D'Angelo, G., Kellum, J. A., Weissfeld, L., Fine, J., Welch, R. D., Kong, L., Carter, M., Angus and D. C. (2008). Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. *American Journal of Respiratory and Critical Care Medicine, 177*(11), 1242-1247. doi: 10.1164/rccm.200712-1777OC

Zahumensky, E. (2006). Infections and diabetic foot syndrome infield practice. *Vnitrni Lekarstvi,52*(5), 411-446.

Zdanov, A., Schalk-Hihi, C., Gustchina, A., Tsang, M., Weatherbee, J. and Wlodawer, A. (1995). Crystal structure of interleukin-10 reveals the functional dimer with an unexpected topological similarity to interferon gamma. *Structure, 3*(6), 591-601. doi: 10.1016/S0969-2126(01)00193-9

Zuhoor, *K. A.* (2012).Proinflammatory cytokine dynamics at operation site and serum after breast surgery.*Archives of Clinical and Experimental Surgery,2*(3), 161-169.

Zura, M., Sakic, K., Malenica, B. and Vrbanovic, V. (2009). Immune response to surgical stress in spinal anaesthesia. *Periodicum Biologorum, 111*(2),193-19.

APPENDICES

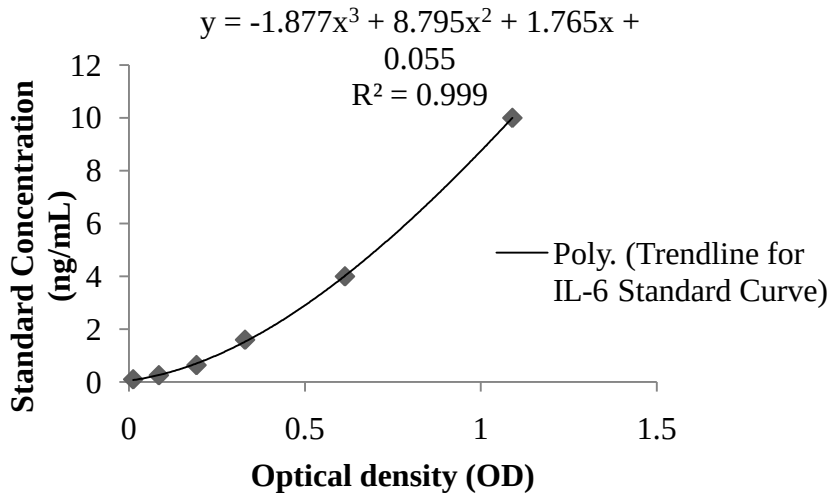
Appendix I: Spectrophotometric Measurement Count of Interleukin (IL) -6 at Filter: 450nm.

ID No.	Standards		Optical Density (OD) of Samples									
	Conc. (ng/mL)	Absorbance (OD)	1	2	3	4	5	6	7	8	9	10
TIME:			PRE-	12 h wf	12 h ser	36 h wf	36 h ser	60 h wf	60 h ser	156 h wf	156 h ser	324 h ser
1	25.000	1.201	0.073	0.211	0.134	0.059	0.134	0.055	0.101	0.037	0.077	0.023
2	10.000	1.090	0.066	0.355	0.690	0.040	0.146	0.070	0.308	0.015	0.125	0.067
3	4.000	0.614	0.078	0.153	0.125	0.075	0.114	0.068	0.073	0.023	0.424	0.022
4	1.600	0.330	0.146	0.306	0.136	0.034	0.132	0.055	0.105	0.062	0.260	0.030
5	0.640	0.192	0.098	0.222	0.052	0.364	0.181	0.045	0.081	0.042	0.116	0.093
6	0.256	0.085	0.077	0.482	0.135	0.173	0.117	0.033	0.087	0.058	0.114	0.058
7	0.102	0.012	0.146		0.078		0.040		0.014		0.020	0.021
8			0.089		0.157		0.012		0.003		0.016	0.059
9			0.070		0.093		0.057		0.018		0.032	0.090
10			0.073		0.062		0.190		0.110		0.083	0.087
11			0.056		0.059		0.038		0.047		0.064	0.058
12			0.078		0.095		0.143		0.089		0.065	0.074

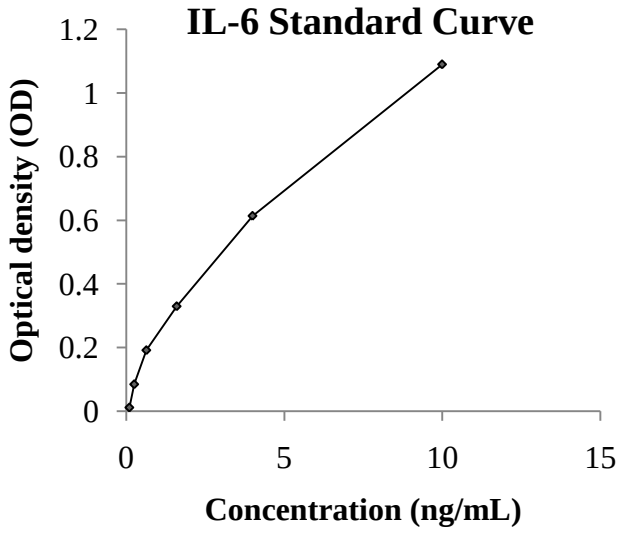
ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, Conc.; concentration, PRE-; pre-experimental serum values, wf; wound fluid, ser; serum, h; hours.

Appendix II: Graphical Output of Changes in IL-6 Standard Concentrations versus Optical Densities and Polynomial Calibration. IL-6 Trendline/Regression Graph (A), Standard Curve (B).

A



B



Appendix III: Interleukin (IL) -6 Concentration (ng/mL) Extrapolated from Standard Curve using Trendline/Regression Formular.

	Concentations (ng/mL) of Samples									
ID No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
TIME:	**PRE-**	**12 h wf**	**12 h ser**	**36 h wf**	**36 h ser**	**60 h wf**	**60 h ser**	**156 h wf**	**156 h ser**	**324 h ser**
1	0.23	0.80	0.45	0.19	0.45	0.18	0.32	0.13	0.24	0.10
2	0.21	1.71	4.84	0.14	0.49	0.22	1.38	0.08	0.41	0.21
3	0.25	0.52	0.41	0.24	0.37	0.22	0.23	0.10	2.24	0.10
4	0.49	1.37	0.45	0.13	0.44	0.18	0.34	0.20	1.08	0.12
5	0.31	0.86	0.17	1.77	0.65	0.15	0.26	0.15	0.38	0.29
6	0.24	2.74	0.45	0.61	0.38	0.12	0.27	0.19	0.37	0.19
7	0.49		0.25		0.14		0.08		0.09	0.10
8	0.28		0.54		0.08		0.06		0.09	0.19
9	0.22		0.29		0.18		0.09		0.12	0.28
10	0.23		0.20		0.70		0.35		0.26	0.27
11	0.18		0.19		0.14		0.16		0.20	0.19
12	0.25		0.30		0.48		0.28		0.21	0.23

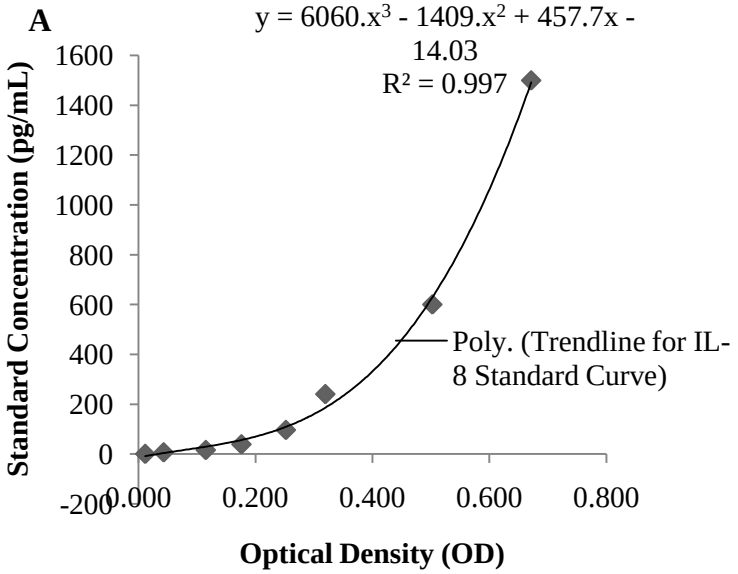
ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, PRE-; pre-experimental serum values, wf; wound fluid, ser; serum, h; hours.

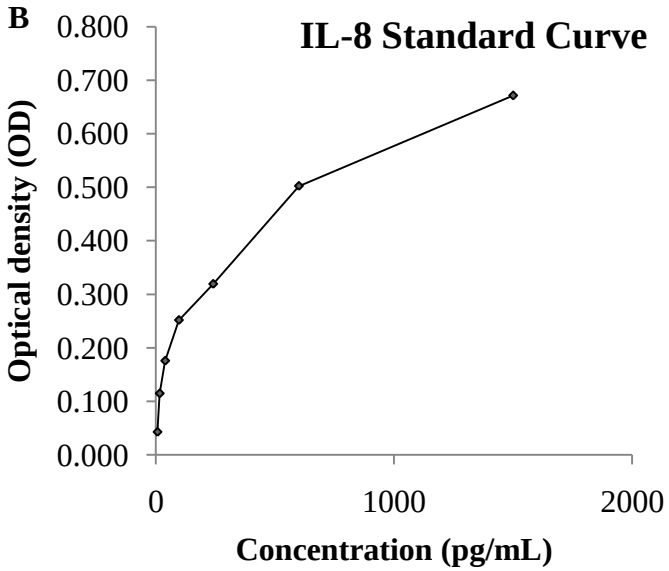
Appendix IV: Spectrophotometric Measurement Count of Interleukin (IL) -8 at Filter: 450nm

	Standards		Optical Density (OD) of Samples									
ID No.	Conc. (ng/mL)	Absorbance (OD)	1	2	3	4	5	6	7	8	9	10
TIME:			PRE-	12 h wf	12 h ser	36 h wf	36 h ser	60 h wf	60 h ser	156 h wf	156 h ser	324 h ser
1	1500.000	0.672	0.353	0.332	0.660	0.259	0.622	0.532	0.335	0.477	0.269	0.392
2	600.000	0.503	0.275	0.219	0.482	0.242	0.289	0.283	0.590	0.306	0.525	0.411
3	240.000	0.320	0.508	0.340	0.402	0.285	0.422	0.229	0.638	0.266	0.474	0.494
4	96.000	0.252	0.389	0.405	0.832	0.180	0.600	0.280	0.210	0.194	0.458	0.392
5	38.400	0.176	0.525	0.413	0.143	0.261	0.244	0.254	0.666	0.323	0.273	0.467
6	15.400	0.115	0.265	0.188	0.602	0.346	0.279	0.229	0.411	0.455	0.490	0.369
7	6.140	0.043	0.389		0.365		0.452		0.215		0.301	0.333
8	0.000	0.011	0.525		0.529		0.487		0.520		0.288	0.348
9			0.265		0.250		0.258		0.392		0.554	0.349
10			0.353		0.343		0.125		0.560		0.167	0.333
11			0.275		0.400		0.472		0.194		0.322	0.348
12			0.475		0.420		0.221		0.295		0.572	0.349

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, Conc.; concentration, PRE-; pre-experimental serum values, wf; wound fluid, ser; serum, h; hours.

Appendix V: Graphical Output of Changes in IL-8 Standard Concentrations versus Optical Densities and Polynomial Calibration. IL -8 Trendline/Regression Graph (A), Standard Curve (B).





Appendix VI: Interleukin (IL)-8 Concentration (pg/mL) Extrapolated from Standard Curve using Trendline/Regression Formular.

	Concentations (pg/mL) of Samples									
ID No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Time:	**PRE-**	**12 h wf**	**12 h ser**	**36 h wf**	**36 h ser**	**60 h wf**	**60 h ser**	**156 h wf**	**156 h ser**	**324 h ser**
1	237.59	203.55	1413.02	114.77	858.63	740.92	208.16	539.65	124.54	312.74
2	130.74	81.89	556.04	99.65	199.42	139.40	1007.38	167.02	1019.77	355.51
3	622.73	216.02	334.72	141.64	486.81	89.25	1274.95	121.54	529.99	587.35
4	306.37	317.79	2875.87	57.76	1059.45	136.09	75.71	65.66	363.01	312.74
5	698.85	360.27	40.10	116.67	101.33	110.13	1452.44	190.23	128.64	506.19
6	120.55	62.18	1070.09	225.77	135.01	89.25	355.51	408.59	583.04	264.88
7	306.37		258.99		463.03		79.10		160.66	205.08
8	714.65		728.71		592.12		692.96		145.05	229.10
9	120.55		106.54		113.83		280.64		835.07	230.77
10	237.59		220.85		32.80		862.18		51.07	205.08
11	130.74		330.24		523.62		65.66		188.80	229.10
12	609.76		377.28		83.32		153.29		918.32	230.77

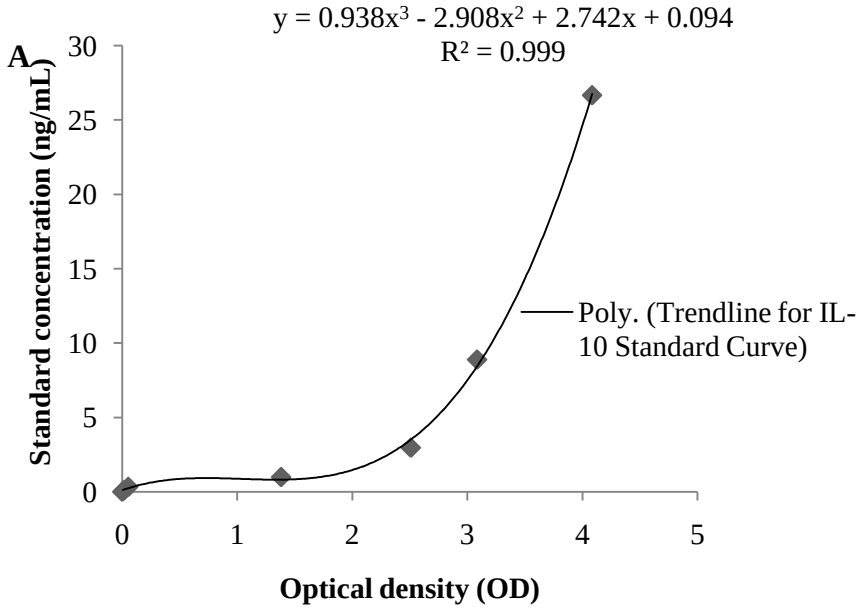
ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, PRE-; pre-experimental serum valueS, wf; wound fluid, ser; serum, h; hours.

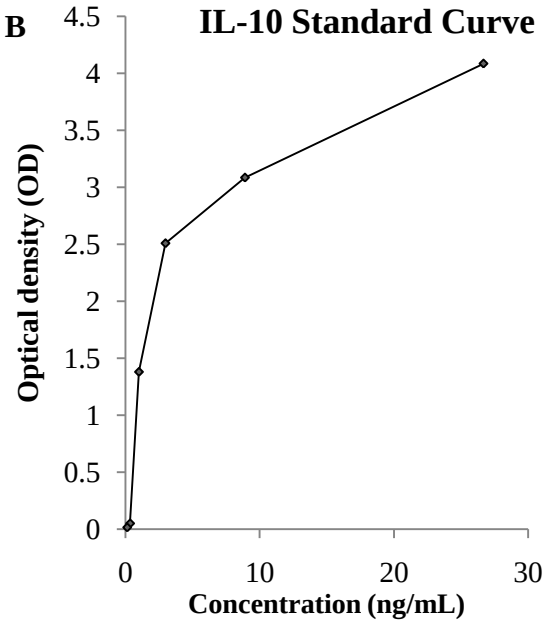
Appendix VII: Spectrophotometric Measurement Count of Interleukin (IL) -10 at Filter: 450nm.

ID No.	Standards		Optical Density (OD) of Samples									
	Conc. (ng/mL)	Absorbance (OD)	1	2	3	4	5	6	7	8	9	10
TIME:			PRE-	12 h wf	12 h ser	36 h wf	36 h ser	60 h wf	60 h ser	156 h wf	156 h ser	324 h ser
1	80.000	4.240	1.661	1.923	0.536	1.104	0.998	1.045	1.813	1.178	1.056	1.408
2	26.670	4.085	0.761	0.719	1.223	0.628	1.817	1.366	1.188	0.689	2.063	1.409
3	8.889	3.085	0.743	1.421	1.856	1.154	1.621	1.370	0.944	0.439	2.354	1.688
4	2.963	2.509	1.425	1.986	0.493	1.421	0.987	0.352	0.792	1.718	1.565	1.264
5	0.988	1.381	1.368	1.215	0.711	1.729	0.414	1.203	0.655	3.575	1.994	0.899
6	0.329	0.052	0.424	0.793	0.968	0.696	1.957	0.827	1.060	1.076	2.326	1.063
7	0.110	0.017	1.660		1.431		0.258		0.715		1.483	0.725
8	0.000	0.005	1.076		0.762		0.547		0.505		1.043	0.451
9			0.643		1.074		0.964		1.577		2.121	0.977
10			1.421		0.714		1.424		0.839		0.296	0.839
11			1.368		0.400		0.518		1.459		0.383	1.419
12			0.424		0.907		0.395		0.431		0.315	0.531

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, Conc.; concentration, PRE-; pre-experimental serum value, wf; wound fluid, ser; serum, h; hours.

Appendix VIII: Graphical Output of Changes in IL-10 Standard Concentrations versus Optical Densities and Polynomial Calibration. IL -10 Trendline/Regression Graph (A), Standard Curve (B).





Appendix IX: Interleukin (IL) -10 Concentration (ng/mL) Extrapolated from Standard Curve using Trendline/Regression Formular.

	Concentations (ng/mL) of Samples									
ID No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Time:	**PRE-**	**12 h wf**	**12 h ser**	**36 h wf**	**36 h ser**	**60 h wf**	**60 h ser**	**156 h wf**	**156 h ser**	**324 h ser**
1	0.93	1.29	0.87	0.84	0.87	0.86	1.10	0.82	0.85	0.81
2	0.91	0.74	0.82	0.90	1.11	0.81	0.82	0.91	1.62	0.81
3	0.85	13.02	1.07	0.83	0.90	0.81	0.88	0.82	2.68	0.95
4	0.81	1.42	0.85	0.81	0.87	0.74	0.91	0.98	0.86	0.89
5	0.81	0.82	0.91	0.99	0.80	0.82	0.91	0.87	1.44	0.72
6	0.81	0.91	0.88	0.91	1.36	0.90	0.81	0.85	2.55	0.85
7	0.93		0.81		0.62		0.91		0.83	0.91
8	0.85		0.91		0.88		0.86		0.86	0.83
9	0.90		0.85		0.88		0.87		1.78	0.87
10	0.81		0.91		0.81		0.90		0.68	0.90
11	0.81		0.79		0.87		0.82		0.77	0.81
12	0.81		0.89		0.78		0.81		0.70	0.87

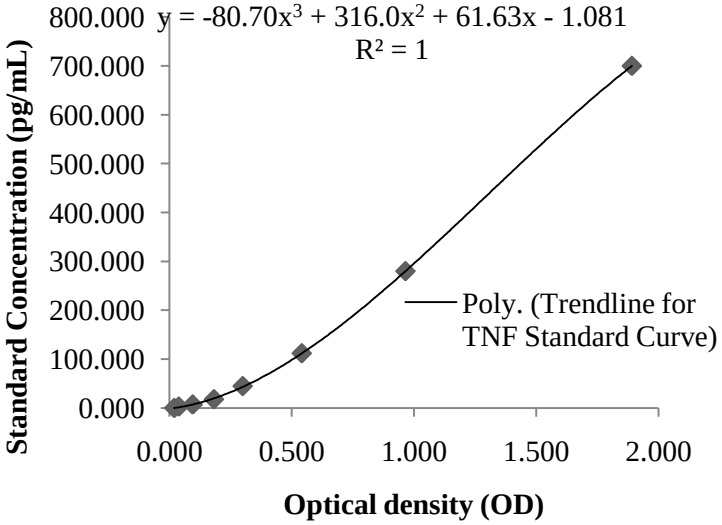
ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, PRE-; pre-experimental serum values, wf; wound fluid, ser; serum, h; hours.

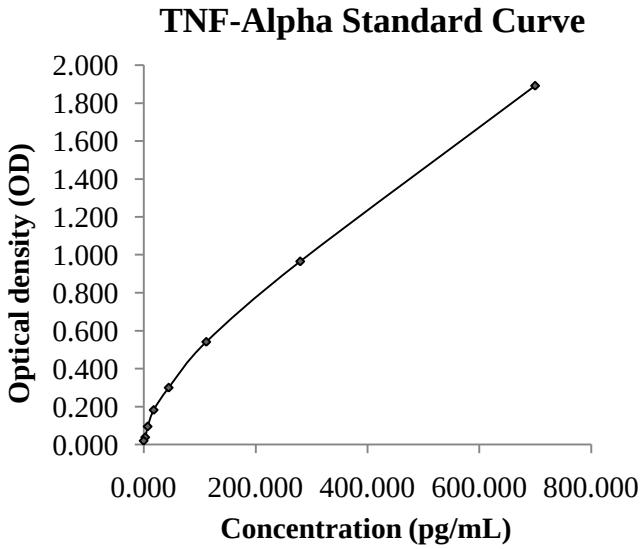
Appendix X: Spectrophotometric Measurement Count of Tumour Necrosis Factor –alpha (TNF –α) at Filter: 450nm

ID No.	Standards		Optical Density (OD) of Samples									
	Conc. (ng/mL)	Absorbance (OD)	1	2	3	4	5	6	7	8	9	10
TIME:			PRE-	12 h wf	12 h ser	36 h wf	36 h ser	60 h wf	60 h ser	156 h wf	156 h ser	324 h ser
1	700.000	1.892	0.097	0.309	0.135	0.959	0.175	0.142	0.183	0.276	0.215	0.182
2	280.000	0.965	0.148	0.483	0.165	0.738	0.471	0.435	0.900	0.151	0.668	0.659
3	112.000	0.541	0.314	0.388	0.589	0.425	0.242	0.291	0.271	0.157	0.209	0.198
4	44.800	0.299	0.388	0.473	0.388	0.534	0.119	0.443	1.124	0.296	0.134	0.143
5	17.920	0.181	0.187	0.339	0.114	0.482	0.173	0.282	0.153	0.161	0.184	0.262
6	7.170	0.094	0.087	0.313	0.326	0.293	0.500	0.418	0.184	0.209	0.678	0.329
7	2.870	0.037	0.288		0.102		0.225		0.288		0.334	0.225
8	0.000	0.019	0.187		0.127		0.160		0.271		0.173	0.160
9			0.193		0.330		0.191		0.133		0.367	0.191
10			0.097		0.349		0.171		0.355		0.180	0.185
11			0.148		0.458		0.262		0.114		0.201	0.159
12			0.214		0.375		0.329		0.191		0.533	0.167

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, Conc.; concentration, PRE-; pre-experimental serum values, wf; wound fluid, ser; serum, h; hours.

Appendix XI: Graphical Output of Changes in TNF –α Standard Concentrations versus Optical Densities and Polynomial Calibration. TNF –α Trendline/Regression Graph (A), Standard Curve (B).





Appendix XII: Tumour Necrosis Factor –alpha (TNF –α) Concentration (pg/mL) Extrapolated from Standard Curve using Trendline/Regression Formular.

		Concentations (pg/mL) of Samples									
ID No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	Time:	**PRE-**	**12 h wf**	**12 h ser**	**36 h wf**	**36 h ser**	**60 h wf**	**60 h ser**	**156 h wf**	**156 h ser**	**324 h ser**
1		7.74	45.64	12.73	277.27	18.87	13.74	20.20	38.20	25.88	20.03
2		14.63	93.16	17.25	183.89	89.47	78.74	251.32	15.08	156.86	145.69
3		46.81	65.56	128.19	75.86	31.10	41.51	37.12	16.00	24.78	22.80
4		65.56	90.08	65.56	109.49	10.53	81.08	352.62	42.64	12.59	13.89
5		20.88	52.86	9.87	92.85	18.54	39.51	15.38	16.62	20.37	35.20
6		6.56	46.58	49.68	41.96	98.49	73.86	20.37	24.78	160.64	50.41
7		40.84		8.35		27.77		40.84		51.63	27.77
8		20.88		11.61		16.46		37.12		18.54	16.46
9		21.92		50.65		21.57		12.45		59.98	21.57
10		7.74		55.36		18.21		56.89		19.70	20.54
11		14.63		85.53		35.20		9.87		23.33	16.31
12		25.70		62.08		50.41		21.57		109.16	17.57

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, PRE-; pre-experimental serum values, wf; wound fluid, ser; serum, h; hours.

Appendix XIII: Mean (mean ± SEM) Age and Weight of Experimental and Control Groups of the NIDs.

PARAMETERS	EXPERIMENTAL		CONTROL
Age (Months)	11.50 ± 0.56		11.00 ± 0.37
	(9.00 - 13.00)		(10.00 - 12.00)
P-value		0.473	
Weight (Kg)	9.00 ± 0.45		10.17 ± 0.54
	(8.00 - 11.00)		(8.00 - 12.00)
P- value		0.128	

n = total number of animals within group.

Appendix XIV: Some Mean Serum Biochemical Parameters of the Experimental (Pre- and Post-operative) and Control Groups of NIDs.

PARAMETERS	PRE-OPERATIVE (n = 6)	POST-OPERATIVE (n = 6)	CONTROL (n = 6)	REFERENCE RANGE
Urea (mg/dL)	13.82±1.75	15.53±2.48	13.73±0.78	6 – 25
Creatinine (mg/dL)	1.27±0.122	1.17±0.07	1.26±0.11	0.5 – 1.6
Glucose (mg/dL)	78.67±1.98[a,c]	95.67±4.26[b]	78.83±1.91[c]	70 – 138
Total protein (g/dL)	7.32±0.26	7.50±0.38	7.37±0.30	5.0 – 7.4
Albumin (g/L)	2.57±0.08	2.60±0.09	2.55±0.09	2.3 – 3.1
Na^+(mmol/L)	140.50±1.59	137.00±3.08	144.20±2.15	139 – 154
K^+(mmol/L)	3.62±0.13	3.92±0.25	3.60±0.13	3.6 – 5.5
HCO_3^-(mmol/L)	19.67±0.42	20.33±1.43	19.67±0.84	18 – 25
Cl^- (mmol/L)	112.30±2.93	106.30±3.52	113.50±3.21	102 – 120
Ca^{2+} (mmol/L)	2.40±0.07	2.45±0.04	2.40±0.15	2.2 – 3.0
PO_4^{3-} (mmol/L)	1.35±0.15	1.48±0.15	1.57±0.19	0.81 – 2.42

Values are Mean ± SEM, Mean values of parameters in rows for males and females separately did not differ significantly ($p \geq 0.05$),
Values were observed to be within normal limits with a slight upward shift.
Na^+; Sodium ion,K^+; Potassium ion, HCO_3^-; Hydrogen bicarbonate, Cl^-; Chloride ion and Ca^{2+}; Calcium ion, PO_4^{3-}; Phosphate. Reference range (Awah and Nottidge, 1998; Prause and Grauer, 1998; Ihedioha, *et al.*, 2013).

Apprndix XV: Some Mean Urinalysis Parameters of the Experimental (Pre- and Post-operative) and Control Groups of NIDs.

PARAMETERS	PRE-OPERATIVE	POST-OPERATIVE	CONTROL
Specific Gravity	1.02±0.00	1.02±0.00	1.02±0.00
	(1.015 - 1.030)	(1.015 - 1.030)	(1.015 - 1.030)
PH	6.50±0.43	6.50±0.22	6.67±0.33
	(5.00 - 8.00)	(6.00 - 7.00)	(6.00 - 8.00)
Ascobic acid mg/dL(mmol/L)	Neg	Neg	Neg
Leukocytes (WBC/uL)	Neg	Neg	Neg
Nitrite mg/dL(g/L)	Neg	Neg	Neg
Protein mg/dL(g/L)	Neg	Neg	Neg
Blood (RBC/uL)	Neg	Neg	Neg
Ketones mg/dL(mmol/L)	Neg	Neg	Neg
Bilirubin mg/dL(mmol/L)	Neg	Neg	Neg
Glucose mg/dL(mmol/L)	Neg	Neg	Neg
Urobilinogenmg/dL(mmol/L)	Neg	Neg	Neg

Values are Mean ± SEM, Values in parenthesis are the ranges (minimum – maximum) values which were observed to be within normal limits.
Neg: Negative, Reference ranges for Specific gravity: 1.015 – 1.050; PH: 5.5 - 9.0 (Archer, 2005)

Appendix XVI: Mean Pre- and Post-operative Vital Parameters of the Experimental Group of the NIDs.

Parameters	Temperature (^{0}C) *(38.5 - 39.4)		Pulse rate (Beats/min) *(65 - 90)		Respiratory rate (Cycles/min) *(15 - 30)	
Days	Male	Female	Male	Female	Male	Female
Pre-op (0)	38.17±0.12	37.83±0.12	74.00±2.31	68.00±0.00	24.00±1.16	22.00±1.16
Post-op (1)	38.00±0.21	38.10±0.21	70.00±1.16	72.00±3.06	22.00±1.16	22.67±0.67
2	38.00±0.26	37.97±0.09	70.00±3.06	69.33±0.67	22.00±1.16	23.33±0.67
3	37.80±0.12	38.13±0.13	72.00±1.16	69.33±1.33	24.00±0.00	22.00±1.16
4	38.13±0.15	38.03±0.15	68.67±0.67	69.33±3.53	22.67±1.33	23.33±0.67
5	37.97±0.27	37.87±0.09	70.00±1.16	70.67±2.67	22.67±0.67	24.00±1.15
6	38.17±0.15	38.07±0.17	71.33±1.76	71.33±2.40	23.33±0.67	26.00±1.16
7	38.43±0.23	38.33±0.15	72.00±1.16	73.33±1.76	24.00±0.00	23.33±0.67
8	38.33±0.07	38.07±0.03	70.00±1.16	74.00±1.16	22.67±0.67	22.67±1.33
9	38.00±0.10	37.97±0.09	71.33±0.67	71.33±2.40	23.33±0.67	23.33±0.67
10	37.93±0.07	38.07±0.09	71.33±1.76	72.00±3.06	23.33±0.67	24.67±0.67
11	38.03±0.19	38.270.27±	72.00±2.31	73.33±4.37	22.67±0.67	22.67±1.33
12	38.23±0.28	38.37±0.42	74.67±4.67	75.33±5.46	22.00±1.16	24.67±1.76
13	38.37±0.37	38.43±0.48	75.33±4.37	76.00±6.00	24.00±1.16	26.00±2.00
14	38.27±0.37	38.37±0.37	74.67±4.81	76.00±6.00	24.00±1.16	24.67±1.76
15	38.37±0.32	38.23±0.23	75.33±3.33	76.67±5.70	24.00±1.15	24.00±0.00
16	38.37±0.27	38.17±0.32	75.33±3.33	75.33±4.37	23.33±0.67	23.33±0.67
17	38.23±0.19	38.07±0.16	75.33±3.33	74.00±3.06	24.00±0.00	24.00±1.16
18	38.10±0.10	38.00±0.06	75.33±3.33	73.33±3.33	23.33±0.67	24.00±0.00
19	38.10±0.10	37.90±0.06	75.33±2.40	72.67±2.67	22.67±0.66	24.00±0.00
20	38.10±0.06	38.00±0.00	74.67±1.76	72.00±2.00	23.33±0.66	24.00±0.00
21	38.10±0.05	38.03±0.03	74.00±2.30	71.33±1.33	23.33±0.67	24.00±0.00
22	38.00±0.06	37.97±0.03	72.67±1.76	71.33±1.33	24.00±0.00	22.67±0.67
23	37.90±0.06	38.00±0.06	71.33±1.33	68.67±0.67	23.33±0.66	23.33±0.67
24	37.90±0.06	37.93±0.09	72.00±1.16	70.00±0.00	24.67±0.67	24.00±0.00
25	37.97±0.03	38.10±0.06	74.00±0.00	70.67±1.67	22.00±1.16	22.67±0.67
26	37.97±0.03	37.93±0.06	72.67±1.33	70.00±1.16	23.33±0.67	24.00±0.00
27	38.00±0.03	38.00±0.07	71.33±1.33	71.33±1.33	23.33±0.67	24.00±0.00

Parameters	Temperature (^{0}C) *(38.5 - 39.4)		Pulse rate (Beats/min) *(65 - 90)		Respiratory rate (Cycles/min) *(15 - 30)	
Days	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
28	37.80±0.06	38.07±0.03	68.00±0.00	73.33±1.67	22.67±0.67	24.00±0.01
29	37.93±0.03	37.90±0.06	70.00±0.00	72.67±1.67	22.67±0.67	23.33±0.67
30	38.07±0.03	38.00±0.06	72.67±0.67	72.67±1.33	21.33±0.67	24.00±0.02
31	37.97±0.09	37.90±0.06	72.00±1.16	72.00±1.16	22.00±1.15	22.67±0.67
32	37.90±0.06	37.97±0.03	72.67±1.33	73.33±1.66	22.67±1.33	22.67±0.67
33	38.07±0.03	37.90±0.00	71.33±1.33	72.67±1.67	23.33±0.67	22.00±0.15
34	37.93±0.07	37.97±0.03	72.00±1.16	73.33±1.67	24.00±0.00	21.33±0.67
35	38.07±0.03	37.87±0.07	71.33±1.76	72.00±1.16	23.33±0.67	21.33±1.33
36	37.93±0.03	37.87±0.09	72.67±1.33	71.33±1.67	23.33±0.67	23.33±0.67
37	38.00±0.06	37.97±0.03	71.33±1.33	71.33±1.76	21.33±0.66	22.00±1.16
38	37.87±0.15	37.970.15±	68.67±0.67	70.00±1.16	20.67±1.33	23.33±0.67
39	37.93±0.12	37.93±0.09	70.00±1.16	70.67±0.67	23.33±0.66	24.00±0.00
40	37.87±0.09	38.07±0.18	70.00±1.16	70.00±1.16	23.33±0.67	23.33±0.67
41	38.00±0.05	37.90±0.06	71.33±1.76	72.00±1.15	23.33±0.66	22.00±1.16

* Reference values (Hassan and Hassan, 2003).

Appendix XVII: Mean Pre- and Post-operative period Vital Parameters of the Control Group of the NIDs.

Parameters	Temperature (^{0}C) *(38.5 - 39.4)		Pulse rate (Beats/min) *(65 - 90)		Respiratory rate (Cycles/min) *(15 - 30)	
Days	Male	Female	Male	Female	Male	Female
Pre-op (0)	38.03±0.15	37.90±0.12	68.00±2.31	78.00±2.30	20.67±1.76	23.33±0.67
Post-op (1)	38.10±0.00	37.73±0.09	73.33±3.33	74.00±2.00	24.00±1.55	24.00±0.00
2	37.93±0.03	38.37±0.27	72.00±2.00	73.33±0.67	24.00±0.00	22.00±1.16
3	38.07±0.23	37.97±0.03	78.67±5.21	70.00±1.16	23.33±0.67	22.67±1.76
4	37.97±0.12	38.13±0.15	69.33±0.67	72.67±1.76	22.67±0.67	22.00±1.16
5	38.00±0.06	38.13±0.12	72.00±2.00	71.33±2.40	21.33±0.67	20.67±1.76
6	38.10±0.06	38.27±0.19	70.67±1.76	72.00±2.31	20.67±0.67	22.00±1.16
7	37.83±0.12	38.17±0.12	71.33±1.76	71.33±2.67	23.33±0.67	22.67±0.67
8	38.10±0.21	38.00±0.21	73.33±3.3	70.67±1.33	21.33±1.33	23.33±0.67
9	37.97±0.09	38.00±0.26	71.33±2.40	71.33±3.71	22.00±1.56	23.33±0.67
10	38.13±0.13	37.80±0.12	70.00±1.16	72.67±3.33	23.33±0.67	22.00±0.00
11	38.03±0.15	38.13±0.15	72.00±2.00	72.00±4.00	22.67±1.33	22.00±1.16
12	37.83±0.12	38.17±0.12	72.00±1.15	75.33±3.33	22.67±0.67	23.33±0.67
13	38.10±0.21	38.00±0.21	73.33±2.41	70.67±1.76	22.00±1.16	21.33±1.33
14	38.03±0.15	37.90±0.12	68.00±2.31	78.00±2.31	20.67±1.76	23.33±0.67
15	38.10±0.00	37.73±0.09	73.33±3.33	74.00±2.00	23.33±0.67	22.00±1.16
16	37.93±0.03	38.37±0.27	72.00±2.00	73.33±0.67	24.67±0.67	22.67±1.33
17	38.07±0.23	37.97±0.03	78.67±5.21	70.00±1.15	23.33±0.67	23.33±1.33
18	37.97±0.12	38.13±0.15	69.33±0.67	72.67±1.76	22.67±0.67	22.00±1.16
19	38.00±0.06	38.13±0.12	72.00±2.00	71.33±2.40	21.33±0.67	20.67±1.76
20	38.10±0.06	38.27±0.19	70.67±1.76	72.00±2.31	20.67±0.67	22.00±1.16
21	38.10±0.21	38.00±0.21	73.33±0.67	71.33±3.33	22.00±0.00	22.67±0.67
22	38.00±0.06	38.07±0.03	72.67±0.67	71.33±1.33	23.33±0.67	22.00±1.16
23	38.03±0.15	37.90±0.12	72.67±1.33	72.67±0.67	24.00±0.00	22.00±1.16
24	38.07±0.03	37.80±0.06	72.00±1.16	72.00±1.16	22.00±1.16	23.33±0.67
25	37.90±0.06	37.93±0.03	73.33±1.67	72.67±1.33	24.00±0.00	22.67±0.67
26	38.10±0.00	37.73±0.09	72.67±1.67	71.33±1.33	24.00±0.00	22.67±1.33

Parameters	Temperature (^{0}C) *(38.5 - 39.4)		Pulse rate (Beats/min) *(65 - 90)		Respiratory rate (Cycles/min) *(15 - 30)	
Days	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
27	38.03±0.07	37.80±0.06	71.33±1.67	74.00±1.16	22.00±2.00	21.33±0.67
28	37.90±0.06	37.93±0.03	68.00±2.31	78.00±2.31	22.67±1.33	23.33±0.67
29	38.10±0.00	37.73±0.09	73.33±3.33	74.00±.00	24.00±1.16	24.00±0.00
30	37.93±0.03	38.37±0.27	72.00±2.00	73.33±0.67	23.33±0.67	21.33±0.67
31	38.07±0.23	37.97±0.03	78.67±5.21	70.00±1.16	23.33±0.67	21.33±0.67
32	37.97±0.12	38.13±0.15	69.33±0.67	72.67±1.76	22.00±0.00	22.67±1.76
33	38.00±0.06	38.13±0.12	72.00±2.00	71.33±2.40	21.33±0.67	21.33±1.33
34	38.10±0.06	38.27±0.19	70.67±1.76	72.00±2.30	20.00±0.00	21.33±1.76
35	37.90±0.06	38.00±0.06	71.33±1.76	72.67±1.33	22.67±0.67	20.67±0.67
36	38.00±0.06	38.07±0.03	72.00±1.16	71.33±1.76	24.00±0.00	24.00±0.00
37	37.90±0.05	38.03±0.03	72.00±1.15	71.33±0.67	22.67±1.33	23.33±0.67
38	37.97±0.03	37.93±0.09	70.00±1.16	72.67±1.33	22.67±1.33	23.33±0.67
39	37.90±0.00	38.07±0.03	71.33±1.33	69.33±0.67	23.33±0.67	23.33±0.67
40	37.97±0.03	37.97±0.03	70.67±1.76	70.00±0.00	21.33±1.33	21.33±1.76
41	37.87±0.07	38.07±0.03	68.67±0.67	68.67±0.67	22.00±0.00	20.67±0.67

* Reference values (Hassan and Hassan, 2003).

Appendix XVIII: Pre-operation Haemogram of the Experimental and Control Groups of NIDs.

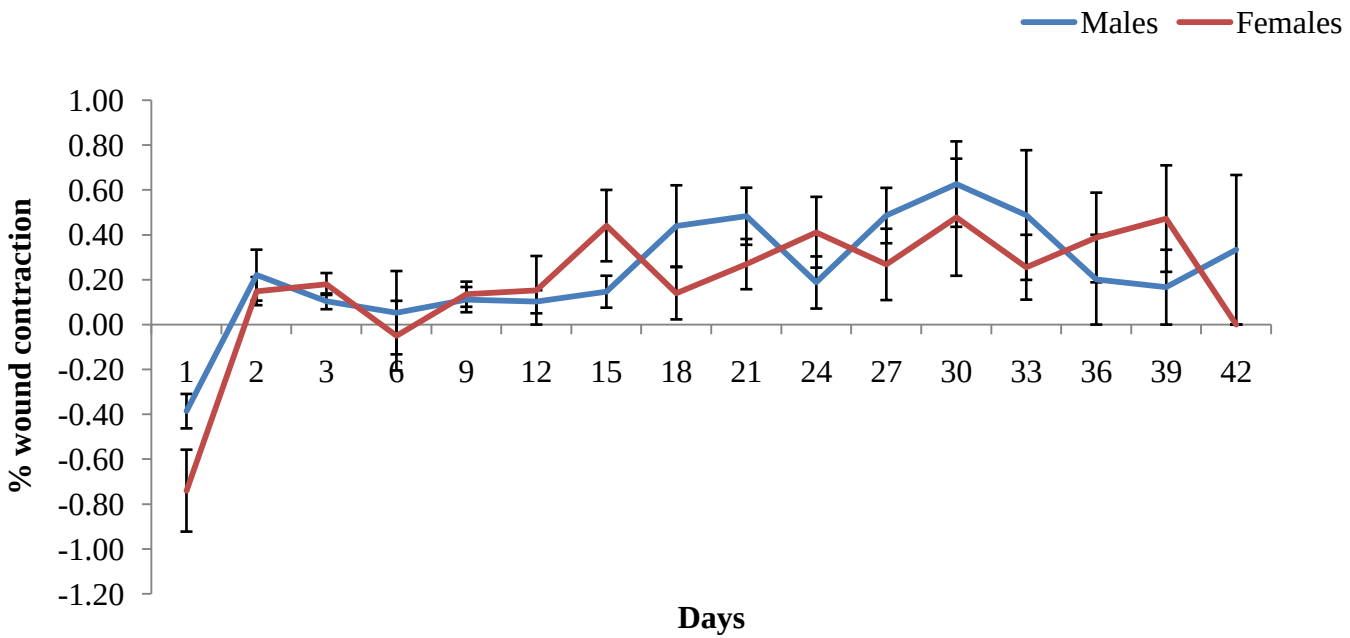
ID No.	HCT	HGB (g/L)	RBC (10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC (10^9/L)	Neut (10^9/L)	Lymph (10^9/L)	Mono (10^9/L)	Eosin (10^9/L)
1	0.374	129.22	5.693	65.70	22.70	345.51	6.90	4.97	1.86	0.00	0.07
2	0.367	90.66	5.429	67.60	16.70	247.04	9.80	6.76	2.84	0.10	0.10
3	0.372	154.51	5.831	63.80	26.50	415.36	11.20	7.84	3.14	0.00	0.22
4	0.368	121.82	6.345	58.00	19.20	331.03	8.10	5.91	2.11	0.08	0.00
5	0.351	120.03	6.488	54.10	18.50	341.96	6.30	4.60	1.58	0.00	0.13
6	0.371	124.86	5.974	62.10	20.90	336.55	10.60	7.53	2.86	0.21	0.00
7	0.376	124.76	5.723	65.70	21.80	331.81	8.60	5.85	2.67	0.00	0.09
8	0.367	125.45	6.241	58.80	20.10	341.84	14.40	9.79	4.18	0.14	0.29
9	0.380	130.90	6.355	59.80	20.60	344.48	10.00	6.70	3.00	0.00	0.30
10	0.369	125.12	6.351	58.10	19.70	339.07	12.80	8.58	3.97	0.00	0.26
11	0.357	117.08	5.767	61.90	20.30	327.95	8.10	5.02	2.84	0.08	0.16
12	0.361	119.77	5.623	64.20	21.30	331.78	8.00	5.68	2.24	0.08	0.00

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, HCT; haematocrit, HGB; haemoglobin concentration, RBC; red blood cells count, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, WBC; white blood cells count, Neut; neutrophils count, lymph; lymphocytes count, mono; monocytes count and eosin; eosinophils count.

Appendix XIX: Changes in the Surface area (cm2) of the Cutaneous Injuries on the Forelimb (Mid lateral aspect of the right Antebrachium) of the NIDs (n = 6).

DAYS	MALES			FEMALES		
	A	B	B	A	B	C
1	4.00	4.00	4.00	4.00	4.00	4.00
2	4.96	6.00	5.67	6.09	8.41	6.38
3	4.76	3.42	4.59	5.94	6.75	4.94
4	4.56	3.06	3.84	5.40	5.00	3.99
7	3.40	2.38	5.06	4.56	4.75	5.40
10	2.88	1.95	5.06	4.32	3.60	4.80
13	2.40	1.68	5.06	4.32	1.95	1.95
16	2.21	1.56	3.60	1.87	0.72	0.88
19	1.35	0.35	3.06	1.17	0.72	0.48
22	0.60	0.12	2.34	0.77	0.42	3.80
25	0.36	0.12	1.96	0.30	0.20	3.42
28	0.12	0.09	0.90	0.30	0.09	2.55
31	0.06	0.00	0.56	0.24	0.00	1.95
34	0.00	0.00	0.30	0.12	0.00	1.43
37	0.00	0.00	0.12	0.06	0.00	0.48
40	0.00	0.00	0.06	0.02	0.00	0.12
43	0.00	0.00	0.00	0.00	0.00	0.00

A, B, C; Individual male and female animals.



Appendix XX: Mean Morphometric Measurement (Percentage Wound Contraction) of the Wound Surface for 6 NIDs that Underwent Study with Wound Healing Achieved in 42 Days Post Operation.

Appendix XXI: Reference Haemogram for NIDs (Means ± SEM), (n = 12).

PARAMETERS	UNITS	NORMAL VALUES
HCT		0.36 - 0.55
HGB	g/L	120 – 180
Total protein	g/dL	6 - 8.5
RBC	10^{12}/L	5.5 - 8.5
WBC	10^{9}/L	6 – 17
Neutrophils	10^{9}/L	3 – 12
Lymphocytes	10^{9}/L	1 – 5
Monocytes	10^{9}/L	0 - 1.4
Eosinophils	10^{9}/L	0.1 - 1.3
Basophils	10^{9}/L	0 - 0.14
MCV	fL	58 -78
MCH	Pg	19 – 28
MCHC	g/L	330 – 380

HCT; haematocrit, HGB; haemoglobin, RBC; red blood cells count, WBC; white blood cells count, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin and MCHC; mean corpuscular haemoglobin concentration. (Hassan and Hassan, 2003; Olayemi, *et al.*, 2009; Ihedioha, *et al.*, 2013).

Appendix XXII: Haemogram of the Experimental and Control Groups of NIDs 12 hours Post-operation.

ID No.	HCT	HGB (g/L)	RBC (10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC (10^9/L)	Neut (10^9/L)	Lymph (10^9/L)	Mono (10^9/L)	Eosin (10^9/L)
1	0.361	126.69	6.15	58.70	20.60	369.00	9.50	6.37	2.85	0.19	0.10
2	0.337	117.97	5.87	57.42	20.10	371.00	10.40	7.28	2.81	0.31	0.00
3	0.369	120.94	6.30	58.58	19.20	343.00	11.80	8.02	3.30	0.35	0.12
4	0.362	124.17	6.18	58.60	20.10	343.00	11.60	8.93	2.32	0.35	0.00
5	0.326	123.68	3.82	85.40	32.40	379.00	9.90	7.52	2.28	0.00	0.10
6	0.370	125.02	6.31	58.60	19.80	338.00	10.30	7.31	2.68	0.31	0.00
7	0.365	115.45	6.01	60.70	19.20	317.00	9.00	5.85	2.88	0.09	0.18
8	0.367	124.59	6.17	59.50	20.20	339.00	14.10	9.31	4.51	0.00	0.28
9	0.375	117.21	6.34	59.19	18.50	357.00	10.80	7.78	2.81	0.11	0.11
10	0.365	123.35	6.46	56.52	19.10	363.00	12.40	7.94	4.22	0.12	0.12
11	0.351	118.09	5.96	58.85	19.80	355.00	10.10	7.99	3.99	0.00	0.12
12	0.361	117.16	6.27	57.62	18.70	333.00	10.30	7.12	4.07	0.11	0.00

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, HCT; haematocrit, HGB; haemoglobin concentration, RBC; red blood cells count, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, WBCC; white blood cells count, Neut; neutrophils count, lymph; lymphocytes count, mono; monocytes count and eosin; eosinophils count.

Appendix XXIII: Haemogramof the Experimental and Control Groups of NIDs36 hours Post-operation.

ID No.	HCT	HGB (g/L)	RBC (10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC (10^9/L)	Neut (10^9/L)	Lymph (10^9/L)	Mono (10^9/L)	Eosin (10^9/L)
1	0.385	135.10	6.59	58.42	20.50	350.91	12.200	8.174	3.782	0.122	0.122
2	0.329	117.54	5.91	55.70	19.90	357.27	9.600	6.912	2.304	0.384	0.000
3	0.376	128.14	6.47	58.10	19.80	340.79	11.100	7.326	3.552	0.222	0.000
4	0.388	129.99	6.57	59.10	19.80	335.03	12.900	9.288	3.225	0.258	0.129
5	0.309	108.10	4.78	64.60	22.60	349.85	10.700	7.811	2.782	0.000	0.107
6	0.361	126.29	5.96	60.60	21.20	349.83	12.100	8.954	2.662	0.363	0.121
7	0.356	121.74	6.58	54.10	18.50	341.96	10.60	7.526	3.120	0.318	0.000
8	0.365	123.92	6.13	59.50	20.20	339.50	9.80	6.174	3.430	0.000	0.196
9	0.376	117.52	6.35	59.19	18.50	312.55	11.20	8.176	2.352	0.000	0.224
10	0.360	182.60	6.19	58.16	29.50	507.22	9.00	7.400	3.420	0.000	0.180
11	0.361	123.82	6.16	58.60	20.10	343.00	10.80	6.080	1.840	0.000	0.080
12	0.370	125.02	6.31	58.60	19.80	337.88	10.00	6.200	3.700	0.100	0.000

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, HCT; haematocrit, HGB; haemoglobin concentration, RBC; red blood cells count, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, WBC; white blood cells count, Neut; neutrophils count, lymph; lymphocytes count, mono; monocytes count and eosin; eosinophils count.

Appendix XXIV: Haemogramof the Experimental and Control Groups of NIDs60 hours Post-operation.

ID No.	HCT	HGB (g/L)	RBC (10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC (10^9/L)	Neut (10^9/L)	Lymph (10^9/L)	Mono (10^9/L)	Eosin (10^9/L)
1	0.378	131.91	6.47	58.46	20.40	348.96	15.50	11.78	3.41	0.31	0.00
2	0.369	126.59	6.33	58.30	20.00	343.05	8.30	5.56	2.41	0.25	0.08
3	0.389	126.80	6.60	58.90	19.20	325.98	6.90	4.55	2.07	0.28	0.00
4	0.392	142.98	5.96	65.80	24.00	364.74	12.00	9.24	2.64	0.00	0.12
5	0.326	119.32	4.99	65.30	23.90	366.00	15.80	13.11	2.21	0.16	0.32
6	0.351	113.76	5.39	65.10	21.10	324.12	9.50	6.46	2.95	0.10	0.00
7	0.362	127.32	6.24	58.00	20.40	351.72	10.60	7.53	3.17	0.53	0.11
8	0.356	115.37	5.49	64.80	21.00	324.07	9.80	6.17	3.43	0.00	0.20
9	0.376	127.28	6.43	58.49	19.80	338.52	11.20	8.18	2.35	0.00	0.22
10	0.370	128.20	6.35	58.30	20.20	346.48	11.00	8.40	3.42	0.00	0.18
11	0.367	123.78	6.19	59.30	20.00	337.27	8.00	6.80	1.84	0.00	0.08
12	0.360	122.99	6.41	56.20	19.20	341.64	10.00	6.40	3.70	0.10	0.00

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, HCT; haematocrit, HGB; haemoglobin concentration, RBC; red blood cells count, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, WBC; white blood cells count, Neut; neutrophils count, lymph; lymphocytes count, mono; monocytes count and eosin; eosinophils count.

Appendix XXV: Haemogramof the Experimental and Control Groups of NIDs156 hours Post-operation.

ID No.	HCT	HGB (g/L)	RBC (10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC (10^9/L)	Neut (10^9/L)	Lymph (10^9/L)	Mono (10^9/L)	Eosin (10^9/L)
1	0.375	124.79	6.21	60.40	20.10	332.78	9.80	5.59	4.21	0.00	0.00
2	0.361	119.34	5.97	60.50	20.00	330.58	16.30	11.74	4.40	0.00	0.16
3	0.392	128.27	6.54	59.90	19.60	327.21	9.10	5.19	3.82	0.09	0.00
4	0.370	110.55	5.64	65.60	19.60	298.78	13.00	8.58	4.42	0.00	0.00
5	0.360	118.65	5.79	62.20	20.50	329.58	14.40	10.94	3.17	0.14	0.14
6	0.362	114.94	5.92	61.10	19.40	317.51	10.10	6.26	3.74	0.10	0.00
7	0.365	115.45	6.01	60.70	19.20	316.31	9.00	5.85	2.88	0.09	0.18
8	0.372	126.29	6.25	59.50	20.20	339.50	14.10	9.31	4.51	0.14	0.14
9	0.360	128.32	6.94	51.90	18.50	356.45	10.80	7.78	2.81	0.00	0.22
10	0.373	135.70	7.10	52.50	19.10	363.81	10.20	7.94	4.22	0.12	0.12
11	0.361	128.10	6.47	55.80	19.80	354.84	12.10	7.99	3.99	0.00	0.12
12	0.361	120.12	6.42	56.20	18.70	332.74	10.30	7.12	4.07	0.11	0.00

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, HCT; haematocrit, HGB; haemoglobin concentration, RBC; red blood cells count, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, WBC; white blood cells count, Neut; neutrophils count, lymph; lymphocytes count, mono; monocytes count and eosin; eosinophils count.

Appendix XXVI: Haemogramof the Experimental and Control Groups of NIDs324 hours Post-operation.

ID No.	HCT	HGB (g/L)	RBC (10^{12}/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC (10^9/L)	Neut (10^9/L)	Lymph (10^9/L)	Mono (10^9/L)	Eosin (10^9/L)
1	0.376	131.70	6.58	57.10	20.00	350.26	9.40	8.21	3.13	0.17	0.00
2	0.368	121.22	6.22	59.20	19.50	329.39	12.90	9.95	3.90	0.00	0.15
3	0.391	131.03	6.97	56.10	18.80	335.12	13.50	7.47	2.90	0.00	0.00
4	0.389	130.00	4.96	78.40	26.20	334.18	9.00	5.67	3.24	0.00	0.09
5	0.374	124.05	6.20	60.30	20.00	331.67	13.20	10.16	3.04	0.00	0.00
6	0.345	104.88	5.52	62.50	19.00	304.00	14.50	11.02	3.19	0.29	0.00
7	0.369	122.44	5.62	65.70	21.80	331.81	8.60	5.85	2.67	0.00	0.09
8	0.375	128.19	6.38	58.80	20.10	341.84	14.40	9.79	4.18	0.14	0.29
9	0.367	126.42	6.14	59.80	20.60	344.48	10.00	6.70	3.00	0.00	0.30
10	0.380	128.85	6.54	58.10	19.70	339.07	12.80	8.58	3.97	0.00	0.26
11	0.361	118.39	5.83	61.90	20.30	327.95	8.10	5.02	2.84	0.08	0.16
12	0.366	121.43	5.70	64.20	21.30	331.78	8.00	5.68	2.24	0.08	0.00

ID Nos: 1 – 3; Experimental males, 4 – 6; Experimental females, 7 – 9; Control males, 10 – 12; Control females, HCT; haematocrit, HGB; haemoglobin concentration, RBC; red blood cells count, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, WBC; white blood cells count, Neut; neutrophils count, lymph; lymphocytes count, mono; monocytes count and eosin; eosinophils count.

Appendix XXVII: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Wound Fluids and Sera of Injured (Group B) NIDs.

wf IL-6		IL-8 wf (m)	IL-8 wf f	IL-10 wf m	IL-10 wf f	TNF-αwf m	TNF wf f	IL 6 ser m	IL 6 ser f
	Pearson r	0.247	0.695	0.628	0.665	0.272	0.654	0.926	0.159
	R square	0.061	0.482	0.394	0.442	0.074	0.428	0.857	0.025
	P value	0.637	0.126	0.182	0.150	0.602	0.159	0.008	0.764
	Summary	ns	Ns	ns	ns	ns	ns	**	Ns
wf IL-8		IL 10 wf m	IL 10 wf f	TNF wf m	TNF wf f	IL 8 ser m	IL 8 ser f		
	Pearson r	0.793	0.925	0.153	0.629	0.811	0.487		
	R square	0.629	0.855	0.023	0.396	0.657	0.237		
	P value	0.060	0.008	0.772	0.181	0.050	0.328		
	Summary	ns	**	ns	Ns	*	ns		
wf IL 10		TNF wf m	TNF wf f	IL 10 ser m	IL 10 ser f				
	Pearson r	0.596	0.863	0.354	0.442				
	R square	0.355	0.745	0.125	0.195				
	P value	0.212	0.027	0.491	0.380				
	Summary	Ns	*	ns	ns				
wf TNF		TNF ser m	TNF ser f						
	Pearson r	-0.071	0.417						
	R square	0.005	0.174						
	P-value	0.893	0.411						
	Summary	ns	ns						

WF = Wound fluid; Ser = Serum; (m) = Male; (f) = Female; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = P < 0.05, ** = P < 0.01 and *** = P < 0.001), ns = not significant.

Appendix XXVIII: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Sera of Injured (Group B) NIDs.

Ser IL6		IL 8 ser m	IL 8 ser f	IL 10 ser m	IL 10 ser f	TNF ser m	TNF ser f
	Pearson r	0.681	-0.254	0.217	0.857	0.124	-0.040
	R square	0.463	0.065	0.047	0.735	0.015	0.002
	P value	0.137	0.627	0.680	0.029	0.815	0.940
	Summary	ns	ns	ns	*	ns	ns
Ser IL 8		IL 10 ser m	IL 10 ser f	TNF ser m	TNF ser f		
	Pearson r	0.023	-0.345	0.724	0.511		
	R square	0.001	0.119	0.524	0.261		
	P value	0.966	0.503	0.104	0.301		
	Summary	ns	ns	ns	ns		
Ser IL 10		TNF ser m	TNF ser f				
	Pearson r	0.193	0.116				
	R square	0.037	0.013				
	P value	0.714	0.828				
	Summary	ns	ns				

Ser = Serum; (m) = Male; (f) = Female; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = P < 0.05, ** = P < 0.01 and *** = P < 0.001), ns = non significant.

Appendix XXIX: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Sera of Uninjured (Control - Group A) NIDs.

Ctrl IL 6		IL 8 Ctrl m	IL 8 Ctrl f	IL 10 Ctrl m	IL 10 Ctrl f	TNF Ctrl m	TNF Ctrl f
	Pearson r	0.050	-0.580	-0.306	0.081	-0.419	-0.053
	R square	0.002	0.336	0.094	0.007	0.175	0.003
	P value	0.925	0.228	0.555	0.878	0.409	0.920
	Summary	ns	ns	ns	ns	ns	ns
Ctrl IL 8		IL 10 Ctrl m	IL 10 Ctrl f	TNF ctrl m	TNF ctrl f		
	Pearson r	0.164	-0.542	0.362	0.306		
	R square	0.027	0.294	0.131	0.094		
	P value	0.756	0.267	0.481	0.556		
	Summary	ns	ns	ns	ns		
Ctrl IL 10		TNF ctrl m	TNF ctrl f				
	Pearson r	0.950	-0.159				
	R square	0.902	0.025				
	P value	0.004	0.764				
	Summary	**	ns				

Ser = Serum; (m) = Male; (f) = Female; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = P < 0.05, ** = P < 0.01 and *** = P < 0.001), ns = non significant.

Appendix XXX: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Wound Fluids with White Blood Cells Count of Injured (Group B) NIDs.

A: Wound fluid of experimental dogs

Wf		Twbc (m)	Twbc (f)	Neut (m)	Neut (f)	Lymph (m)	Lymph (f)	Mono (m)	Mono (f)	Eosin (m)	Eosin (f)
IL-6	Pearson r	-0.213	-0.068	-0.225	-0.011	-0.139	-0.268	0.677	0.907	-0.168	-0.256
	R square	0.045	0.005	0.051	0.000	0.019	0.072	0.458	0.823	0.028	0.066
	P value	0.685	0.899	0.668	0.984	0.792	0.608	0.140	0.013	0.751	0.624
		ns	ns	ns	ns	ns	ns	ns	*	ns	ns
IL-8	Pearson r	-0.163	0.323	-0.243	0.264	0.229	0.288	0.445	0.438	0.140	0.235
	R square	0.026	0.105	0.059	0.069	0.052	0.083	0.198	0.191	0.019	0.055
	P-value	0.759	0.532	0.643	0.614	0.662	0.581	0.376	0.386	0.792	0.655
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
IL-10	Pearson r	-0.186	0.435	-0.292	0.437	0.237	0.191	0.700	0.477	0.030	0.129
	R square	0.035	0.189	0.085	0.191	0.056	0.036	0.490	0.228	0.001	0.017
	P-value	0.724	0.389	0.574	0.386	0.652	0.718	0.122	0.339	0.956	0.807
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
TNF-α	Pearson r	-0.134	0.363	-0.149	0.462	-0.002	-0.109	0.631	0.623	0.285	-0.145
	R square	0.018	0.131	0.022	0.214	0.000	0.012	0.398	0.388	0.081	0.021
	P-value	0.800	0.480	0.778	0.356	0.998	0.837	0.179	0.187	0.585	0.784
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

WF = Wound fluid; Ser = Serum; (m) = Male; (f) = Female; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = P < 0.05, ** = P < 0.01 and *** = P < 0.001). WBCC; white blood cells count, Neut; neutrophils count, lymph; lymphocytes count, mono; monocytes count and eosin; eosinophils count, ns = non significant.

Appendix XXXI: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Sera with White Blood Cells Count of Injured (Group B) NIDs.

Ser		Twbc (m)	Twbc (f)	Neut (m)	Neut (f)	Lynph (m)	Lymph (f)	Mono (m)	Mono (f)	Eosin (m)	Eosin (f)
IL-6	Pearson r	-0.182	0.145	-0.282	-0.056	0.136	0.507	0.510	0.134	-0.301	0.884
	R square	0.033	0.021	0.080	0.003	0.018	0.257	0.260	0.018	0.090	0.781
	P-value	0.730	0.784	0.588	0.916	0.798	0.305	0.301	0.801	0.563	0.020
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
IL-8	Pearson r	-0.183	0.060	-0.2822	0.269	-0.139	-0.491	0.51	0.398	-0.3008	-0.624
	R square	0.033	0.004	0.07962	0.072	0.019	0.241	0.2601	0.158	0.09046	0.390
	P-value	0.729	0.911	0.588	0.607	0.793	0.323	0.3013	0.435	0.5625	0.185
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
IL-10	Pearson r	0.182	0.433	-0.079	0.203	0.846	0.819	-0.408	-0.235	-0.446	0.826
	R square	0.033	0.188	0.006	0.041	0.717	0.670	0.167	0.055	0.199	0.682
	P-value	0.730	0.391	0.882	0.700	0.034	0.046	0.422	0.655	0.376	0.043
	Summary	ns	ns	ns	ns	*	*	ns	ns	ns	*
TNF-α	Pearson r	0.249	0.486	0.295	0.607	0.101	0.044	0.380	-0.413	0.064	-0.168
	R square	0.062	0.236	0.087	0.369	0.010	0.002	0.144	0.171	0.004	0.028
	P-value	0.634	0.328	0.571	0.201	0.849	0.934	0.458	0.416	0.905	0.750
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Ser = Serum; (m) = Male; (f) = Female; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = P < 0.05, ** = P < 0.01 and *** = P < 0.001). WBCC; white blood cells count, Neut; neutrophils count, lymph; lymphocytes count, mono; monocytes count and eosin; eosinophils count, ns = non significant.

Appendix XXXII: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Sera with White Blood Cells Count of Uninjured (Control - Group A) NIDs.

Ser		Twbc (m)	Twbc (f)	Neut (m)	Neut (f)	Lymph (m)	Lymph (f)	Mono (m)	Mono (f)	Eosin (m)	Eosin (f)
IL-6	Pearson r	0.5092	-0.5009	0.4422	-0.5531	0.577	-0.3841	-0.6798	-0.6058	0.5088	-0.2799
	R square	0.2593	0.2509	0.1955	0.3059	0.333	0.1475	0.4621	0.367	0.2589	0.07834
	P-value	0.3022	0.3115	0.3799	0.255	0.2305	0.4522	0.1374	0.2024	0.3026	0.5911
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
IL-8	Pearson r	-0.0571	0.4615	0.04382	0.4459	-0.1972	0.4871	0.4271	0.4159	-0.5535	-0.3704
	R square	0.00326	0.213	0.00192	0.1988	0.03888	0.2373	0.1824	0.1729	0.3063	0.1372
	P-value	0.9145	0.3569	0.9343	0.3755	0.7081	0.3271	0.3983	0.4122	0.2546	0.4698
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
IL-10	Pearson r	0.5789	-0.4053	0.6117	-0.3933	0.5014	-0.4183	-0.134	-0.3753	0.1046	0.2809
	R square	0.3351	0.1643	0.3742	0.1547	0.2514	0.175	0.01796	0.1408	0.01094	0.07888
	P-value	0.2286	0.4254	0.1969	0.4404	0.3109	0.4091	0.8002	0.4635	0.8437	0.5898
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
TNF-α	Pearson r	0.3643	0.8109	0.4208	0.7609	0.2647	0.893	0.1099	0.6911	-0.0457	-0.8258
	R square	0.1327	0.6576	0.1771	0.579	0.07004	0.7974	0.01209	0.4776	0.00209	0.682
	P-value	0.4777	0.0502	0.406	0.0789	0.6123	0.0166	0.8357	0.1284	0.9315	0.0429
	Summary	ns	ns	ns	ns	ns	*	ns	ns	ns	*

Ser = Serum; (m) = Male; (f) = Female; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = P < 0.05, ** = P < 0.01 and *** = P < 0.001). WBCC; white blood cells count, Neut; neutrophils count, lymph; lymphocytes count, mono; monocytes count and eosin; eosinophils count, ns = non significant.

Appendix XXXIII: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Wound Fluids with Erythrocyte Parameters of Injured (Group B) NIDs.

Wf		HCT (m)	HCT (F)	HGB (m)	HGB (F)	RBC (m)	RBC (F)	MCV (m)	MCV (F)	MCH (m)	MCH (F)	MCHC (m)	MCHC (F)
IL-6	Pearson r	-0.6069	-0.8159	-0.667	0.3657	-0.0855	-0.4176	-0.3228	0.3702	-0.2102	0.6493	0.8597	0.6003
	R square	0.3683	0.6657	0.4449	0.1337	0.0073	0.1744	0.1042	0.1371	0.04418	0.4216	0.7391	0.3604
	P value	0.2014	0.0477	0.1479	0.4759	0.8721	0.4100	0.5326	0.4700	0.6894	0.1629	0.0281	0.2077
	Summary	ns	*	ns	ns	ns	ns	ns	ns	ns	Ns	*	ns
IL-8	Pearson r	0.2486	-0.6593	-0.0213	-0.1172	0.3163	-0.4475	-0.3019	0.3331	-0.4036	0.3772	-0.0543	0.1909
	R square	0.06178	0.4347	0.0006	0.0137	0.1000	0.2003	0.09115	0.1110	0.1629	0.1423	0.0030	0.03646
	P-value	0.6349	0.1543	0.968	0.8249	0.5414	0.3735	0.5609	0.5188	0.4275	0.4611	0.9187	0.7171
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns	ns	ns
IL-10	Pearson r	-0.348	-0.7428	-0.3228	0.0823	0.2105	-0.5158	-0.4895	0.2902	-0.4243	0.4566	0.4745	0.4027
	R square	0.1211	0.5518	0.1042	0.0068	0.0443	0.2661	0.2396	0.0842	0.18	0.2084	0.2251	0.1621
	P-value	0.4991	0.0907	0.5326	0.8768	0.6889	0.2949	0.3244	0.5769	0.4018	0.3627	0.3417	0.4286
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns	ns	ns
TNF-α	Pearson r	-0.7002	-0.7917	0.0564	0.4236	0.1697	-0.5002	-0.4868	0.2121	-0.2031	0.5581	0.5749	0.6951
	R square	0.4903	0.6268	0.0032	0.1795	0.0288	0.2502	0.237	0.0450	0.04124	0.3114	0.3306	0.4831
	P-value	0.1214	0.0606	0.9156	0.4025	0.748	0.3123	0.3274	0.6866	0.6996	0.2498	0.2326	0.1253
	P value summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

WF = Wound fluid; Ser = Serum; (m) = Male; (f) = Female; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = P < 0.05, ** = P < 0.01 and *** = P < 0.001). HCT; haematocrit, HGB; haemoglobin concentration, RBC; redblood cells count, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, count, ns = non significant.

Appendix XXXIV: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Sera with Erythrocyte Parameters of Injured (Group B) NIDs.

Ser		HCT (m)	HCT (F)	HGB (m)	HGB (F)	RBC (m)	RBC (F)	MCV (m)	MCV (F)	MCH (m)	MCH (F)	MCHC (m)	MCHC (F)
IL-6	Pearson r	-0.4089	-0.4864	-0.7695	-0.5928	-0.1002	0.2749	-0.2435	-0.4127	-0.2418	-0.4553	0.6273	-0.2898
	R square	0.1672	0.2366	0.5921	0.3514	0.01005	0.0756	0.05931	0.1703	0.05848	0.2073	0.3936	0.08397
	P-value	0.4208	0.3279	0.0736	0.2149	0.8502	0.598	0.6419	0.4161	0.6443	0.3643	0.1824	0.5775
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
IL-8	Pearson r	-0.4089	-0.5032	-0.7695	0.7698	-0.1002	0.6457	-0.2435	0.5293	-0.2418	0.8875	0.6273	0.8458
	R square	0.1672	0.2532	0.5921	0.5926	0.01005	0.417	0.05931	0.2802	0.05848	0.7877	0.3936	0.7154
	P-value	0.4208	0.3089	0.0736	0.0734	0.8502	0.166	0.6419	0.2802	0.6443	0.0183	0.1824	0.0338
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*
IL-10	Pearson r	0.3606	-0.0573	-0.3064	-0.8152	0.0101	0.0581	0.08204	-0.1189	-0.1643	-0.4345	-0.4977	-0.6058
	R square	0.13	0.0033	0.0939	0.6646	0.0001	0.0034	0.00673	0.01413	0.027	0.1888	0.2477	0.367
	P-value	0.4826	0.9142	0.5547	0.0481	0.9849	0.9129	0.8772	0.8225	0.7557	0.3893	0.3151	0.2025
	Summary	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
TNF-α	Pearson r	0.5831	0.0428	0.4563	0.2964	0.7398	-0.447	-0.5491	0.2321	-0.6823	0.3201	-0.1724	0.3005
	R square	0.34	0.0018	0.2082	0.08785	0.5473	0.1998	0.3016	0.05385	0.4656	0.1025	0.02972	0.09032
	P-value	0.2245	0.9359	0.363	0.5684	0.0927	0.3742	0.2591	0.6582	0.1353	0.5362	0.744	0.5628
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Ser = Serum; (m) = Male; (f) = Female; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = P < 0.05, ** = P < 0.01 and *** = P < 0.001). HCT; haematocrit, HGB; haemoglobin concentration, RBC; red blood cells count,

MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, ns = non significant.

Appendix XXXV: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Sera with Erythrocyte Parameters of Uninjured (Control - Group A) NIDs.

Ctrl		HCT (m)	HCT (F)	HGB (m)	HGB (F)	RBC (m)	RBC (F)	MCV (m)	MCV (F)	MCH (m)	MCH (F)	MCHC (m)	MCHC (F)
IL-6	Pearson r	-0.5497	-0.3594	0.2556	0.9403	-0.2920	0.0206	0.4669	-0.0643	0.1437	0.9068	0.3345	-0.2709
	R square	0.3022	0.1292	0.0653	0.8841	0.0852	0.0004	0.218	0.0041	0.0207	0.8223	0.1119	0.07337
	P-value	0.2585	0.4841	0.625	0.0052	0.5745	0.9691	0.3505	0.9037	0.7859	0.0126	0.5169	0.6036
	Summary	ns	ns	ns	**	ns	ns	ns	ns	ns	*	ns	ns
IL-8	Pearson r	-0.4798	0.3055	-0.338	-0.4403	0.5767	0.5542	-0.5605	-0.5712	-0.5758	-0.7455	-0.4546	0.624
	R square	0.2302	0.0933	0.1142	0.1939	0.3326	0.3071	0.3142	0.3263	0.3315	0.5558	0.2067	0.3894
	P-value	0.3356	0.556	0.5123	0.3822	0.2309	0.2538	0.2473	0.2364	0.2318	0.0889	0.3651	0.1855
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
IL-10	Pearson r	0.3606	-0.2274	0.4474	-0.2312	0.4704	-0.6651	-0.3969	0.6301	-0.167	0.149	0.2944	-0.3662
	R square	0.13	0.0517	0.2002	0.0535	0.2212	0.4423	0.1576	0.3971	0.0279	0.0222	0.08667	0.1341
	P-value	0.4826	0.6648	0.3737	0.6594	0.3465	0.1495	0.4359	0.1799	0.7519	0.7781	0.5712	0.4753
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
TNF-α	Pearson r	0.5431	-0.1832	0.3718	-0.0361	0.4678	0.613	-0.4415	-0.7264	-0.1861	-0.3891	0.202	0.9139
	R square	0.2949	0.0336	0.1383	0.0013	0.2188	0.3758	0.1949	0.5277	0.0346	0.1514	0.0408	0.8352
	P-value	0.2655	0.7283	0.4679	0.9459	0.3495	0.1957	0.3808	0.102	0.7241	0.4458	0.7012	0.0108
	Summary	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*

Ser = Serum; (m) = Male; (f) = Female; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = P < 0.05, ** = P < 0.01 and *** = P < 0.001). HCT; haematocrit, HGB; haemoglobin concentration, RBC; red blood cells count, MCV; mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, ns = non significant.

Appendix XXXVI: Significant correlations between IL-6, IL-8, IL-10 and TNF-α Levels with Each Other and Blood Cell Counts In injured (Group B) NIDs Irrespective of Sex.

IL-6		ser IL-6 / wf IL-6	Ser IL-6 / RBC	wf IL-6 / HCT	wf IL-6 / RBC	wf IL-6 / mono
	Pearson r	0.827	0.866	- 0.894	- 0.923	0.818
	R square	0.684	0.750	0.799	0.851	0.669
	P value	0.042	0.026	0.016	0.009	0.047
	Summary	*	*	*	**	*
IL-8		wf IL-8 / wf IL-10				
	Pearson r	0.919				
	R square	0.845				
	P value	0.010				
	Summary	**				
IL 10		ser IL-10 / lymph				
	Pearson r	0.846				
	R square	0.716				
	P value	0.034				
	Summary	*				
TNF		wf TNF-α / mono				
	Pearson r	0.824				
	R square	0.679				
	P-value	0.044				
	Summary	*				

wf = wound fluid; ser = serum; RBC = red blood cell counts; HCT = hematocrit; mono = monocyte count; lymph = lymphocyte counts; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinant; P values are levels of significance where (* = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$), ns = not significant.

Appendix XXXVII: Relationship between IL-6, IL-8, IL-10 and TNF-α Levels in Wound Fluids and Sera with Percentage Wound Contraction of Injured (Group B) NIDs.

		Males	Females	Males	Females
IL 6		WF IL-6/Contraction	WF IL-6/Contraction	Ser IL-6/Contraction	Ser IL-6/Contraction
	Pearson r	0.4918	0.4034	0.5568	0.1857
	R square	0.2418	0.1627	0.31	0.03449
	P value	0.3218	0.4278	0.2512	0.7246
	Summary	ns	ns	ns	ns
IL 8		WF IL-8/Contraction	WF IL-8/Contraction	Ser IL-8/Contraction	Ser IL-8/Contraction
	Pearson r	0.4622	0.5654	0.613	0.2091
	R square	0.2137	0.3197	0.3758	0.04373
	P value	0.356	0.2423	0.1956	0.6909
	Summary	ns	ns	ns	ns
IL 10		WF IL-10/Contraction	WF IL-10/Contraction	Ser IL-10/Contraction	Ser IL-10/Contraction
	Pearson r	-0.8507	0.5919	0.1999	0.3442
	R square	0.7237	0.3504	0.03998	0.1185
	P value	0.0318	0.2158	0.7041	0.504
	Summary	*	ns	ns	ns
TNF		WF TNF-α/Contraction	WF TNF-α/Contraction	Ser TNF-α/Contraction	Ser TNF-α/Contraction
	Pearson r	0.3841	0.4993	0.5267	0.1224
	R square	0.1475	0.2493	0.2774	0.01497
	P value	0.4522	0.3133	0.283	0.8174
	Summary	ns	ns	ns	ns

Ser = Serum; WF – wound fluids; r = Pearson's correlation coefficient; R^2 = Coefficient of Determinantn P values are levels of significance where (* = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$), ns = not significant.