THE EFFICACY OF METHANOL EXTRACT OF THE LEAVES OF *Anacardiumoccidentale*(Linn.-Holl.) ON EXPERIMENTAL *Salmonella* Gallinarum INFECTION IN SHIKABROWN PULLETS

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DECEMBER, 2017

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A THESIS SUBMITTED TO SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA NIGERIA IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN AVIAN MEDICINE

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DECEMBER, 2017

DECLARATION

I hereby declare that the work in this Dissertation entitled "The Efficacy of Methanol Extract of the Leaves of *Anacardium occidentale* (Linn.-Holl.) On Experimental*Salmonella* Gallinarum Infection in Shikabrown Pullets" has been carried out by me in the Department of Veterinary Medicine. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at any other institution.

RUFAI UMAR JIBRIN …….. ……

Name of Student Signature Date

CERTIFICATION

This Dissertation entitled "The Efficacy of Methanol Extract of the Leaves of *Anacardium occidentale* (Linn.-Holl.) On Experimental*Salmonella* Gallinarum Infection in Shikabrown Pullets" by Rufai Umar JIBRIN, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This MSc. Dissertation is dedicated to the Almighty ALLAH, the fashioner of the Heaven and the Earth, the Lord of an unfathomable majesty who in his infinite mercy made it possible for me to complete my work successfully throughout the period of my stay in the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria.Also, to my parents, wife and children.

ACKNOWLEDGEMENTS

I wish to express my appreciation and sincere gratitude to the Chairman Supervisory Committee, in person of Prof. Paul A. Abdu, for his financial contribution, the fatherly care and encouragement given to me in order to ensure I completed this study successfully. I want to also acknowledge the contributions of Prof. M. M. Suleiman, member of the Supervisory Committee particularly in the area of pharmacological aspect of my project. My appreciation goes to Prof. C. A. Kudi, who also contributed to see my project was successfully conducted. I will never forget to acknowledge the contribution of Dr. P. Mamman Department of Veterinary Microbiology ABU Zaria for improving my project especially the bacteriological aspect and Dr. M. Abdurrahman Department of Veterinary Surgery and Radiology ABU Zaria. Lastly, but not the least, I want to acknowledge the contribution of Messrs.A. S. Iliyaof Avian Clinic Veterinary Teaching Hospital, L. Musa Department of Veterinary Surgery and Radiology, A. G Hamiduof Department Public Health and Preventive Medicine and S. Isah casual staff, Ahmadu Bello University, Zaria respectively.

ABSTRACT

Fowl typhoid (FT) caused by *Salmonella* Gallinarum (*S*. Gallinarum) is an important bacterial disease of poultry. The aim of this study was to determine the efficacy of the crude methanol extract of *Anacardium occidentale* leaves against the bacterium in Shikabrown pullets. Phytochemical analyses,acute toxicity and *in vitro* study of methanol extract of *A. occidentale* leaves werecarried out. One hundred and thirty two pullets free of *S.* Gallinarum were purchased from hatchery in Kwara State and divided into 11 groups, A to K, with 12 birds per group. Each bird in group A to J was inoculated at 16 weeks of age with $3.3x10^8$colony forming unit (CFU/ml) of *S.* Gallinarum orally and group K served as negative control (NC). The pullets were observed for clinical signs, mortality and pathology for nine days post–inoculation (PI). Groups A, B, C and D were treated orally with methanol extract of *A. occidentale* leaves at 50, 100, 150 and 200 mg/kg body weight for 5days PI while group E was treated with Ceftazidime at 100 mg/kg. Groups F and G were administered *A. occidentale* leaves at 100 mg/kg and 200 mg/kg for 14days prior to inoculation of *S.* Gallinarum at fourteen weeks as prophylaxis (PX). Group J, positive control (PC), received normal saline. Result showed the methanol extract of *A. occidentale* leaves contained carbohydrates, alkaloids, tannins, saponins, steroids, triterpenes, flavonoids and cardiac glycosides and was non-toxic at 5,000 mg/kg. The extract of *A.occidentale* leaveshas *in vitro*antibacterial activity against *S*. Gallinarum at concentrations (C) of 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 mg/ml giving zones of inhibition (ZI) of 18.5, 15.9, 13.4, 8.3, 10.9 13.4 and 10.9 mm respectively. Pullet's chickens showed clinical signs six days PI in all the groups except group I. The clinical signs disappeared nine days PI and five days posttreatment in all the groups except group J, which it persisted. There was statistically significant difference ($P< 0.05$) in decrease in mean body weight (MBW) five

days post-treatment(PTX) between the treated groups (1.005±0.048 to1.046 ± 0.053kg) and NC (1.293±0.073kg). There was statistically significant diffirence (P< 0.05) in cloacal temperature (CT) between treated groups A, B, C and D (41.78, 41.85, 41.69 and 41.39)posttreatment compared to the PC group J (42.86). Changes in the weight of liver, spleen, kidneys, bursa of Fabricius and thymus showed group C (150 mg/kg) had the least percentage (%) increase in liver and spleen weight(23.18 and 56.65) among the treated groups compared to group J (36.7 and 19.55). Changes in kidney weight showed the second highest increase at 150 mg/kg dose (18.75) among the treated groups compared to J (3.05). Changes in weight of the bursa and thymus at 150 mg/kg showed the least reduction (38.9%) compared to PC group J (42.1%). *Salmonella*Gallinarum was only isolated in the liver of pullets in group J at day PI. The PCV in the treated groups was highest in group B (26.67±1.76 %) followed by group C (23.00±1.53 %) while in prophylactic groups, group G had the highest (24.33±0.67 %) than group F (21.67±1.67 %).The PCV of treated and prophylactic groups (A-G) was lower (P<0.05) compared to NC group K (34.67±1.45 %). Total protein **(**TP**)**values in treated, prophylactic and control groups were within normal (5.07±0.48 g/dl to3.07±0.07 g/dl) except in Ceftazidime treated group which showed statistically significant difference(P <0.01) lower (2.13±1.07 g/dl). Liver of inoculated and treated groups showed sinusoidal, hepatic and portal veins congestion, multifocal and perivascular infiltration with heterophils and mononuclear cells (MNC) with necrosis of hepatocytes. The spleen had circumscribed multifocal perivascular aggregates of heterophils and MNC predominantly lymphocytes and severe necrosis of lymphoid cells with congested splenic veins while the kidneys showed necrosis of tubular epithelium and congested blood vessels.In conclusion, methanol extract of *A. occidentale* leaves contained

flavonoids and tannins; its LD_{50} was ≥ 5,000 mg/kg and *in vitro* effect on *S.* Gallinarum was highest at 100 mg/ml with ZI of 18.5 mm. Treatment with ME of AOL at 150 mg/kg for five days reduced severity of clinical signs of *S.* Gallinarum infection in pullets andreduced PCV in treated groups while TP was high in all groups except group E. The methanol extract of *A. occidentale* leaves decreased MBW and CT in pullets inoculated with *S.* Gallinarum. It is recommended that, chronic toxicity and treatment of chronic infection of *S.* Gallinarum at150 mg/kg with methanol of *A. occidentale* leavesin pullets be carried out.

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
<	Less than
≥	Greater than or equal to
µg	Microgram
ºC	Degree Celsius
ABUCAUC	Ahmadu Bello University Committee on Animal Use and Care
AOL	*Anacardium occidentale*leaves
API	Analytical profile index
B2K	Gumboro vaccine
BeWo	Choriocarcinoma
BI	Before inoculation
C	Concentration
C/s	Clinical sign
CS	Cloacal swab
CFU	Colony forming unit
CNSL	Cashew nut shell liquid
CP	Crude protein
CRIN	Cocoa Research Institute of Nigeria
CT	Cloacal temperature
DC	Dead chick
DM	Dry matter
DNA	Deoxyribonucleic acid

DPPH	1,1-diphenyl-2-picrylhydrazyl
Dr	Doctor
DV	Daily value
DVM	Doctor of Veterinary Medicine
EAT	Erhlich ascites tumour
EDTA	Ethylene diamine tetraacetic acid
ENDC	Eastern Nigeria Development Corporation
FAO	Food and Agricultural Organisation
FDA	Federal Department of Agriculture
FLDPCS	Federal Livestock Department and Pest Control Services
FT	Fowl typhoid
Ft	Feet
FTV	Fowl typhoid vaccine
H&E	Haematoxyline and Eiosin
Hb	Haemoglobin
IC	Inhibitory concentration
Kg	Kilogram
L_3	Third larval stage
LD_{50}	Lethal dose fiftylethal concentration
Lv	Liver
M	Metre
MBW	Mean body weight
MC	Moribund chick

MCF-7	Michigan Cancer Foundation-7
ME	Methanol extract
Mg	Milligram
MIC	Minimum inhibitory concentration
Ml	Millilitre
Mm	Millimetre
MNC	Mononuclear cells
MSc	Master of Science
NC	Negative control
NDVL	Newcastle disease vaccine lasota
NE	Nasarawa Eggon
NFE	Nitrogen free extract
NS	Normal saline
NSP	Non starch polysaccharides
NVRI	National Veterinary Research Institute
OIE	Office of Internationale des Epizooties
P	Probability
Pr	Pre-inoculation
PC	Positive control
PCR	Polymerase chain reaction
PCV	Packed cell volume
PI	Post-inoculation
Prof	Professor

PTX	Post-treatment
PX	Prophylaxis
Pxd	Prophylactic
RBC	Red blood cells
RNA	Ribonucleic acid
SC	Simon citrate
SEM	Standard Error of Mean
SG	*Salmonella* Gallinarum
SGP	*Salmonella* Gallinarum-Pullorum
Sp	Spleen
SPI	*Salmonella* pathogenicity island
SSA	*SalmonellaShigella* Agar
SSD	Statistically significant difference
T_3SS	Type three secretory system
TAB	Typhoid-Paratyphoid A and B
TP	Total protein
TRBC	Total red blood cell
TSI	Triple sugar iron
TTSS	Type Three Secretory System
TWBC	Total white blood cell
TX	Treatment
Txd	Treated
UK	United Kingdom

USA	United States of America
USDA	United State Department of Agriculture
VEGF	Vascular endothelial growth factor
V-P	Voges-Proskauer
VTH	Veterinary Teaching Hospital
WNDC	Western Nigeria Development Corporation
ZI	Zone of inhibition
LC_{50}	Lethal concentration

TABLE OF CONTENTS

TITLE PAGE……ii

DECLARATION ..... **iii**

CERTIFICATION ..... **iv**

DEDICATION ..... **v**

ACKNOWLEDGEMENTS ..... **vi**

ABSTRACT ..... **vii**

CHAPTER ONE **1**

1.0 INTRODUCTION **1**

1.1 Cashew Tree **1**

1.2 Avian Salmonella Infection **3**

1.3 Statement of the Research Problem **4**

1.4 Justification of the Research **5**

1.5 Aim of the Study **6**

1.6 Objectives of the Study **6**

1.7 Research Questions **7**

CHAPTER TWO **8**

2.0 LITERATURE REVIEW **8**

2.1 History of *Anacardium occidentale* **8**

2.2 Origin of *Anacardium occidentale* **9**

2.3 Economic Importance of *Anacardium occidentale* **9**

2.4 Medicinal Uses of Cashew **10**

2.5 Medicinal Uses of Cashew Leaves **13**

2.6 Traditional Uses of *Anacardium occidentale*……14

2.7 Safety of *Anacardium occidentale*……15

2.8 History of Salmonellosis **15**

2.9 Avian Salmonellosis **16**

2.10 *Salmonella* Gallinarum **17**

2.11 Economic Importance of *Salmonella* Gallinarum **18**

2.12 Pathogenesis of Avian Salmonellosis **18**

2.13 Pathogenicity of *Salmonella* Gallinarum……19

2.14 Public Health Significance of *Salmonella* Gallinarum **20**

2.15 Diagnosis of Avian Salmonellosis **20**

2.16 Confirmatory Diagnostic Procedures for Avian Sallmonellosis **21**

2.17 Prevention and Control of Avian Salmonellosis **23**

2.18 Treatment of Fowl Typhoid Using Synthetic Drugs **23**

2.19 Treatment of Fowl Typhoid Using Medicinal Plants **24**

2.20 Significant of *Anacardium occidentale* Leaves Extract in the Management of Salmonellosis ... 24
CHAPTER THREE ... 26
3.0 MATERIALS AND METHODS ... 26
3.1 Plant Collection and Identification ... 26
3.3 Bacteria ... 26
3.4 Brilliiant Green and Mueller-Hinton Agars ... 27
3.5 Feeds ... 27
3.6 Medicines and Medication……27
3.7 Vaccines and Vaccination ... 28
3.8 Cleaning and Disinfection of Poultry House ... 28
3.9 Housing and Feeding of the pullets……..29
3.10 Approval for Animal Use ... 29
3.11 Experimental Design ... 29
3.12 Extraction of the Leaves of *Anacardium occidentale* ... 30
3.13 Phytochemical Analyses of the Methanol Extract of *Anacardium occidentale* Leaves ... 31
3.13.1 Test for carbohydrates (Molish test) ... 31
3.13.2 Test for alkaloids (Dragendoff test) ... 31
3.13.3 Test for tannins ... 31
3.13.4 Test for saponins (Frothing test) ... 32
3.13.5 Test for flavonoids ... 32
3.13.6 Test for cardiac glycosides (Keller-kiliani test) ... 32
3.13.7 Test for steroid and triterpenes (Libermann-Burchard test) ... 32
3.13.8 Test for free anthracene derivatives (Bentrager test) ... 33
3.14 Determination of Lethal Dose of Methanol Extract of *Anacardium occidentale* Leaves in Shikabrown Pullets ... 33
3.15 *In Vitro* Antibacterial Testing of Methanol Extract of *Anacardium occidentale* Leaves against *Salmonella* Gallinarum ... 34
3.16 Screening of Experimental Shikabrown Pullets for the Presence of *S.* Gallinarum ... 34
3.17 Preparation of *Salmonella* Gallinarum Inoculum ... 35S
3.18 Treatment with Extracts of *Anacardium occidentale* ... 35
3.19 Grading of clinical signs……..35
3.20 Blood Collection for Haematology ... 35
3.21 Gross weight of the liver, spleen, kidneys, thymus and bursa of Fabricius……..36
3.22 Histopathology of the Liver, Spleen and Kidneys……..36
3.23 Data Analyses ... 36

CHAPTER FOUR **38**

4.0 RESULTS **38**

4.1 Constituents present in *Anacardium occidentale* Leaves and Feeds **38**

4.2 Phytochemicals Present in Methanol Extract of *Anacardium occidentale* Leaves **38**

4.3 Lethal Dose of Methanol Extract of the Leaves of *Anacardium occidentale* in 16-week-old Shikabrown Pullets **38**

4.4 *In Vitro* Antibacterial Activity of Methanol Extract of *Anacardium occidentale* leaves against *Salmonella* Gallinarum **41**

4.5 Clinical Signs of Fowl typhoid Observed in Methanol Extract of *Anacardium occidentale*Leaves Treated, Prophylactic, Fowl typhoid Vaccinated and Control Groups…….....**41**

4.6 Live Body Weight of Shikabrown Pullets Pre-inoculation and Post-inoculation with *Salmonella* Gallinarum and Post-treatment with Methanol Extract of *Anacardium occidentale* Leaves …….**45**_Toc509402309

4.7 Effect of Methanol Extract of *Anacardium occidentale* Leaves on Slaughter Body Weight of Shikabrown Pullets **45**

4.8 Cloacal Temperature of Shikabrown Pullets Inoculated with Experimental *Salmonella* Gallinarum and Treated with Methanol Extract of *Anacardium occidentale* Leaves..... **48**

4.9 *Salmonella* Gallinarum from Cloacal Swabs and Liver of Shikabrown Pullets Pre-inoculation, Post-inoculation and Post-treatment with Methanol Extract of *Anacardium occidentale* Leaves **48**

4.10 Changes in the Weight of the Liver, Spleen, Kidneys, Bursa of Fabricius and Thymus of Shikabrown Pullets inoculated with *Salmonella* Gallinarum and treated with Methanol Extract of *Anacardium occidentale* Leaves…….....**48**

4.11 Changes in some Haematological Parameters of Shikabrown Pullets Inoculated with Experimental *Salmonella* Gallinarum and Treated with the Methanol Extract of *Anacardium occidentale* Leaves **57**

4.12 Histopathological Changes in the Liver, Spleen and Kidneys of Shikabrown Pullets Inoculated with Experimental *Salmonella* Gallinarum and Treated with Methanol Extract of *Anacardium occidentale* Leaves **57**

CHAPTER FIVE..... **63**

5.0 DISCUSSION **63**

CHAPTER SIX **70**

6.0 CONCLUSION AND RECOMMENDATIONS..... **70**

6.1 Conclusion **70**

6.2 Recommendations **71**

REFERENCES..... **73**

APPENDIXES…….....**86**

LIST OF FIGURES

Figure	Title	Page
4.1	Decrease in mean live body weight of Shikabrown pullets before and after inoculation with experimental *Salmonella* Gallinarum and treated with different concentrations of methanol extract of *Anacardium occidentale* leaves……..	46
4.2	Decrease in mean body weight after slaughter in Shikabrown pullets inoculated with experimental *Salmonella* Gallinarum and treated with methanol extract of *Anacardium occidentale* leaves……..	47
4.3	Changes in mean cloacal temperature of Shikabrown pullets post-inoculation with experimental *Salmonella* Gallinarum at sixteen weeks of age and treated with the methanol extract of *Anacardium occidentale* leaves for five days…….	49

LIST OF TABLES

Table	Title	Page
3.1	Experimental design to evaluate the efficacy of methanol extract of *Anacardium occidentale*leaves on *Salmonella* Gallinarum in experimentally inoculated Shikabrown pullets……..	37
4.1	Constituents (in percentages) of the feeds and *Anacardium occidentale*leaves obtained from Suleja, Niger State…….	39
4.2	Qualitative phytoconstituents present in the leaves of *Anacardium occidentale* obtained from Suleja, Niger state…….	40
4.3	Severity of clinical signs in Shikabrown pullets inoculated with *Salmonella* Gallinarum at sixteen weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* (**day six** post-inoculation and **day two** post-treatment)……..	43
4.4	Severity of clinical signs in Shikabrown pullets inoculated with *Salmonella* Gallinarum at sixteen weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale*(**day nine** post-inoculation and **day five** post-treatment)……...	44
4.5	Detection of *Salmonella* Gallinarum from the cloacal swabs, moribond and dead chicks of Shikabrown pullets on arrival, pre-inoculation, post inoculation and post-treatment with methanol extract of the leaves of *Anacardium occidentale*…..	50
4.6	Changes in average weight of the liver of Shikabrown pullets inoculated with *Salmonella*Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days…….	51
4.7	Changes in average weight of thespleen of Shikabrown pullets inoculated with *Salmonella*Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days…….	52
4.8	Changes in average weight of the kidney t of pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days…….....	53

4.9 Changes in average weight of thebursa of Shikabrown pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days…….. 54

4.10 Changes in average weight of thethymus of Shikabrown pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days……... 55

4.11 Some haematological parameters of Shikabrown pullets inoculated with experimental *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of *Anacardium occidentale* leaves for five days……..... 56

LIST OF PLATES

Plate	Title	Page
I	*In vitro* antibacterial activityindicating zones of inhibition of differentconcentrations of the methanol extract of *Anacardium occidentale* leaves……	42
II	Photomicrograph of the liver of Shikabrown pullets inoculated with *Salmonella*Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days……..	59
III	Photomicrograph of the spleen of Shikabrown pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days…….....	60
IV	Photomicrograph of the kidneys of Shikabrown pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days…….....	61

CHAPTER ONE

INTRODUCTION

1.1 Cashew Tree

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989; Eisner, 1990; Oyesomi and Ajao, 2011). The cashew tree *Anacardium occidentale* (*A. occidentale*) is a tropical evergreen tree that produces cashew seed and cashew fruit. It can grow as high as 14 metres (46 ft), but the dwarf cashew, growing up to 6 metres (20 ft), has proved more profitable, with earlier maturity and higher yields. The plant is a member of the family Anacardiaceae, originally native to northeastern Brazil. The tree is now widely cultivated in Vietnam, Nigeria and India as major producing countries (Togun, 1977; FAO, 2013; Sija *et al*., 2015).*Anacardium occidentale* is commonly known for its commercially valuable kernals and bioactive compounds like polyphenols which serve as natural antioxidant having an ability to put off cytotoxic free radicals that may prevent many diseases (Sija *et al*., 2015).

The ethanol extract of cashew nuts has been shown to contain various phytochemical compounds such as triterpenoids, phenolic, flavonoids and xantoprotein which are responsible for its antimicrobial effects (Rajesh *et al*., 2009). The liquid obtained from the shell of cashew nut (CNSL) has wide commercial applications and medicinal properties (Murthy and Sivasamban, 1985). The presence of anacardic acid in cashew nut shell liquid, cashew nut and cashew fruit was also reported (Kubo *et al*., 2006; Trevisan *et al*., 2006; Praveen *et al*., 2013; Sija *et al*., 2015).Anacardic acid is a highly valuable natural polyphenolic compound used in medicine and industry. Medicinally, it has been

successfully used to treat warts, ringworms and elephantiasis and also used in beauty therapy to remove the skin of the face in order to grow a new skin (Sija *et al*., 2015).

Cashew is a multipurpose tree whose leaves, stems and bark extracts and shell oil are used extensively for the treatment of different diseases worldwide (Agedah *et al*., 2010; Arekemase *et al*., 2011). All the parts of the plant (apple, bark, leaves, gum, nut and roots) have different ethnomedicinal and ethnoveterinary uses for both man and animal alike (Ramiakajato *et al*., 2001). The antimicrobial activity of the cashew leaf extract has been reported by several workers (Omojasola and Awe, 2004; Agedah*et al*., 2010; Ifesan*et al*., 2013; Chabi*et al*., 2014). Anacardic acid and other phytoconstituents (flavonoids and tannins) found in cashew have been shown to have antimicrobial effects through the inactivation of microbial adhesions enzymes and cells envelop transport cells present in the bacterial organism. Flavonoids cause inhibition of DNA gyrase enzyme in bacteria thereby inhibiting DNA synthesis (Aderiye *et al*., 2015).

*Anacardium occidentale*has antifungal activity against *Cryptococcus neoformans* due to its phenolic lipids, flavonoid and tannins component (Nopamart, 2011). Ademola and Eloff (2011) reported the *in vitro* antihelminthic effect of the plant against the ova and larvae of *Haemonchus contortus*. It has also been reported to possess antidiabetic (Ukwenya *et al*., 2012), anti-inflammatory and anti-ulcerogenic properties (Akinpelu, 2001; Bacchi and Konan, 2007). Kamtchouing *et al*. (1998) also reported the antidiabetic and anti-inflammatory properties of the leaf and bark extracts of cashew plant.

Extracts of the leaves of *A. occidentale*is used in Nigeria to lower blood pressure and sugar (Esimone *et al*., 2001). The ethanol and water extracts of fresh bark of *A. occidentale*

possess activity against malaria parasite (Sha'a *et al*., 2014). The aqueous extracts of leaves, bark and nutshell of *A. occidentale*also showed activity against larvae of *Anopheles gambiae* (Nnamani *et al*., 2011). The ethanol extract of the leaves of *A. occidentale*inhibited cell proliferation of different tumour cells such as Ehrlich ascites tumour cells (EAT), choriocarcinoma (BeWo) and breast cancer (MCF-7) *in vitro* in a dose-dependent manner and reduced the vascular endothelial growth factor (VEGF) level in the ascites of treated mice (Salimath *et al*., 2008). The ethanol extract of the leaves of *A. occidentale*did not produce any changes in the brain and kidneys when given to Wistar albino rats to investigate neurotoxic and nephrotoxic effects at a dose of 2,000 mg/kg by oral route (Edet *et al*., 2013).

1.2 Avian Salmonella Infection

Avian *Salmonella* infections are important as both a cause of clinical disease in poultry and source of food-borne disease in humans (Kabir, 2010). *Salmonella* belongs to the family *Enterobacteriaceae* and genus *Salmonella*. It is a facultative intracellular pathogen causing localized or systemic infections; as well as a chronic asymptomatic carrier state (Shivaprashad, 1997; Kabir, 2010). Abbas *et al*. (2015) reported an overall incidence of fowl typhoid (FT) in broilers and in layers to be 6.58% and 6.97%, respectively in Nigeria. Similarly, Ukashatu *et al*. (2012) reported 7.14% prevalence rate of FT among exotic breeds of poultry in Katsina metropolis, Katsina State, Nigeria. Reports put the prevalence of FT in sampled flocks in some parts of northern Nigeria at 18.4% (Mbuko *et al*., 2009; Ogie *et al*., 2013).

1.3 Statement of the Research Problem

In Nigeria, the incidence of *Salmonella* Gallinarum (*S*. Gallinarum) was first reported in 1930 National Veterinary Research Institute, Vom, Nigeria,as cited by Salihu *et al*.(2014). Since then *S.* Gallinarum has been reported in different parts of the country according to report on the notification of the absence or presence of all diseases in Nigeria (FLDPCS, 2010). Moreover, according to data on the diagnosis of animal diseases obtained from the Central Diagnostic Division of National Veterinary Research Institute, Vom, Nigeria, FT ranks higher among the bacterial diseases of poultry, causing huge economic losses to commercial poultry farmers (Salihu *et al*., 2014).

Fowl typhoid has affected both government and private farms and*S.* Gallinarum have been found in birds in chicken markets (Nwobu *et al*., 1990; Salihu*et al*., 2014). Prevention of Salmonella infection is important for the profitable expansion of the poultry industry in Nigeria. This is necessitated by the fact that, with the continuous expansion of poultry farming in Nigeria, avian salmonellosis caused by *S.* Gallinarum has also gained ground as a major disease of poultry (Agbaje *et al*., 2010), resulting in heavy economic loss through mortality and reduced egg production (Salihu *et al*., 2014). However, even though FT has been successfully eradicated in developed countries like USA, UK, Canada and Japan (OIE, 2004); the disease is still endemic in developing countries like Nigeria (Sa'idu *et al*., 1994; Okwori *et al*., 2007).

Salmonella Gallinarum is an avian adapted bacterial pathogen and the causative agent of FT (Abdu, 2007; OIE, 2008); a severe systemic disease which affects primarily chickens and turkeys (Lee *et al*., 2005) with geographical distribution in Mexico, Central and South America, Africa, Asia and Indian subcontinent (OIE, 2005). The birds that survive from

clinical disease when infected at a young stage may show few signs of infection but become carriers (Berchieri *et al*., 2001). Both exotic and indigenous breeds of chickens are susceptible to *S.* Gallinarum (Alvarez *et al*., 2003; Ogie *et al*., 2013).

1.4 Justification of the Research

The World Health Organization's Commission on Intellectual Property and Innovation in Public Health has continually recognized the role of natural ethnomedicinal and ethnoveterinaryproducts,as an aid in treating human and animal diseases in developing and developed countries (Hoareau and DaSilva, 1991; Nopamart, 2011). The re-emerging interest in ethnopharmacological research on natural products, particularly medicinal plants, as evidenced by numerous publications in recent decades has additionally signified the clinical potential of medicinal plants beyond traditional medical practices (Nopamart, 2011). It has been anticipated that drug discovery and development may not necessarily be confined to new molecule entities, but the rationally designed, carefully standardized, synergistic traditional herbal formulations (Nopamart, 2011).

More so, with the development of resistance to drugs of pathogens like*S.* Gallinarum, ethnoveterinary medicine might be an alternative since herbs tend to be broad spectrum (Mwale *et al*., 2005). However, other molecular research findings revealed that, resistance to antibiotics by *S.* Gallinarum might be due to its ability to evade host immune responses through the use of syringe and needle-like apparatus called type three secretory systems (TTSS, T3SS or injectisome) to inject sectretory protein known as Sic or Sip (Salmond and Reeves, 1993; Kimbrough and Miller, 2000).Therefore, *A. occidentale*leaves extract can be used to treat bacterial infection in poultry because of its antimicrobial activity against many

diseases as reported by different researchers (Kudi *et al*., 1999; Omojasola and Awe, 2004; Agedah *et al*., 2010; Ifesan *et al*., 2013; Chabi *et al*., 2014). Moreover, the research attention given to *A. occidentale*is far below its usefulness (Country Report, 2008). Despite its medicinal importance, to the best of my knowledge there was no record of scientific research reported to ascertain the safety and efficacy of *A. occidentale*leaves in treating FT in chickens in Nigeria.

1.5 Aim of the Study

The aim of the study was to determine the efficacy of methanol extract of the leaves of *Anacardium occidentale*on experimental *Salmonella* Gallinarum infection in Shikabrown pullets.

1.6 Objectives of the Study

The objectives of the study were to determine the:

1. phytochemical constituents of methanol extract of the leaves of*A. occidentale*.
2. median lethal dose (LD_{50}) of methanol extract of the leaves of *A. occidentale*in Shikabrown pullets.
3. *in vitro*and *in vivo*effect of methanol extract of the leaves of *A. occidentale*against *S.* Gallinarum.
4. effect of methanol extract of the leaves of*A. occidentale*against *S.* Gallinarum infection on clinical and pathological changes in Shikabrown pullets.

1.7 Research Questions

1. What are the phytoconstituents present in the methanol extract of *A. occidentale* leaves?
2. What dose of methanol extract of the leaves of *A. occidentale*is lethal to Shikabrown pullets?
3. What is the effect of methanol extract of the leaves of *A. occidentale*on *S.* Gallinarum *in vitro*and *in vivo*?
4. What are the possible clinico-pathological changes in Shikabrown pullets infected with *S.* Gallinarum *in vivo* and treated with methanol extractof the leavesof *A. occidentale?*

CHAPTER TWO

LITERATURE REVIEW

2.1 History of *Anacardium occidentale*

*Anacardium occidentale*is a tree in the family of the flowering plant Anacardiaceae with 73 genera and about 600 species of which eight were native to tropical America (Morton, 1987; Dare *et al*., 2011). It is a multipurpose economically important tree of the Amazon that grows up to 15m high witha thick and tortuous trunk andbranches so winding that they frequently reach the ground (Morton, 1987; Dare *et al*., 2011). The history of cashew in Nigeria dated back to 15th century, where it was mainly used in afforestation schemes for the control of erosion in the former Eastern Nigeria (Ohler, 1967; Venkataramah, 1976; Togun, 1977; Asogwa *et al*., 2008). However, the commercial cashew plantations started in Nigeria in the early 1950s with the establishment of the first commercial plantations at Oghe, Oji and Mbala by the defunct Eastern Nigeria Development Corporation (ENDC) and Iwo, Eruwa and Upper Ogun by the defunct Western Nigeria Development Corporation (WNDC). From these locations, the planting of the *A. occidentale*spread to other parts of Nigeria particularly the Central and Northern States of Nigeria (Ohler, 1967; Venkataramah, 1976; Togun, 1977; Asogwa *et al*., 2008). Research on cashew production and its uses started at the Cocoa Research Institute of Nigeria (CRIN), Ibadan in 1971.The introduction of Brazilian cashew biotype with improved and desirable nut and kernel quality characteristics by CRIN further increased the crops spread and popularity in Nigeria (Hammed *et al*., 2007; Asogwa *et al*., 2008).

2.2 Origin of *Anacardium occidentale*

*Anacardium occidentale*is a native of Tropical Central and South America, notably North Eastern Brazil, from where the Portuguese explorers introduced it into Nigeria in the 15th/16th century (Ohler, 1967; 1979; Venkataramah, 1976; Asogwa*et al*., 2008).The tree is now widely cultivated in Vietnam, Nigeria, India,Côte d'Ivoire and Benin as major production countries (FAO, 2013).

2.3 Economic Importance of *Anacardium occidentale*

The *A. occidentale*seed is widely consumed, used in recipes or processed into cheese or butter, while *A. occidentale*oil is dark yellow andobtained by pressing the nutsfor use in cooking or dressing salad (Anon, 2015). *Anacardium occidentale*nutshell liquid (CNSL) or *A. occidentale*shell oil (CAS registry number 8007-24-7) is a natural resin with a yellowish sheen found in the honeycomb structure of the *A. occidentale*nutshell, and is a by-product of processing cashew nuts with multiple uses in developing drugs, antioxidants, fungicides and anti-termite treatment of timber (Jason, 2015). *Anacardium occidentale* apples contained valuable substances with cosmetic and antimicrobial applications as reported by Gonçalves and Gobbo (2012). The shell of the cashew seed yields derivatives that can be used in many applications from lubricants to paints and other parts of the tree have traditionally been used for snake-bites and other folk remedies (Wikipedia, 2016).The world total production of cashew nuts (in shells) was 4.4 million metric tons with Vietnam as the world's largest individual producer in 2013 with 1.1 million tons (FAO, 2013). As at year 2014, rapid growth of cashew cultivation in Côte d'Ivoire made this country the top African exporter(Bavier, 2014).In a 100 gram serving, raw cashews provide 553 calories, 67% of the Daily Value (DV) in total fats, 36% DV of protein, 13% DV of dietary fibre and

11% DV of carbohydrates (USDA, 2015). Cashews are rich sources (> 19% DV) of dietary minerals, including particularly copper, manganese, phosphorus and magnesium (79-110% DV), and of thiamin, vitamin B6 and vitamin K (32-37% DV) (USDA, 2015). Iron, potassium, zinc and selenium are present in significant content (14-61% DV). Cashews (100 grams, raw) contain 113 mg of beta-sitosterol (USDA, 2015).

2.4 Medicinal Usesof Cashew

The bark, leaves and shell oil of *A. occidentale*were used medicinally to treat different ailments (Agedah *et al*., 2010; Aderiye *et al*., 2015). All the parts of the plant (apple, bark, leaves, gum, nut and roots) have different ethnomedicinal uses for both man and animal alike (Ramiakajato *et al*., 2001; Aderiye *et al*., 2015). Plants and their derivatives play key role in world health and have long been known to possess biological activity with 30% of all modern drugs derived from plants (Riaz *et al*., 2010; Omoboyowa *et al*., 2013). According to the WHO, about 80% of the world's population relies essentially on plants for primary health care (Mckay and Blumberg, 2007;Omoboyowa *et al*., 2013).Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Oyesomi and Ajao, 2011).

The phytocontituents present in cashew extracts such as anacardic acid, flavonoids and tannins are responsible for the anti-microbial activities of the extract through the inactivation of microbial adhesions enzymes and cell envelop transport cells. Flavonoids, which are responsible for the inhibition of DNA gyrase thereby inhibiting DNA synthesis; phenolic compounds like anacardic acids, cardols, triterpenoids, methylcardols, xantoprotein and cardanols that are responsible for bacteria cell wall inhibition, by

inhibiting the cell wall synthesis in addition to a growing cell and blocking the DNA, RNA and protein pathways (Aderiye*et al*., 2015).The cashew apple contains tannins, vitamin C, sugars, carotenoids, organic acids, proteins, fibres and water (Queiroz *et al*., 2011; Gonçalves and Gobbo, 2012). Tannins are widely used in the leather industry for transforming the skins of animals into finished leather and they have important gustatory components responsible for their astringency like many fruits and vegetables.

Antimicrobial effect of ethanol and aqueous extracts of*A. occidentale*bark and leaf on some bacteria that can cause gastrointestinal infections has been reported (Kudi *et al*., 1999; Arekemase *et al*., 2011). *Anacardium occidentale*stem extract was found to be effective against spirochete bacterium (*Treponema pallidum*) a causative agent of syphilis (Akinpelu, 2001; Bassey *et al*., 2012; Aderiye *et al.*, 2015). It was reported that, flavonoids and tannins are responsible for the antimicrobial activity of cashew stem against syphilis (Ajibesin*et al*., 2011; Aderiye, *et al.*, 2015). Moreover, *A. occidentale*has beenreported to be effective against *Cryptococcus neoformans* due its phenolic lipids, flavonoids and tannins contents. Over one hundred thousand plants extracts that had been assayed for their pharmacological properties, based onthe current available data and the recently proposed IC50 < 100 ug/ml or MIC < 500 ug/ml as a cut-off value if the extracts would possess any promising antifungal effect, *A. occidentale* is a potential candidate (Algiannis *et al*., 2001; Cos *et al*., 2006;Nopamart, 2011).

Anacardium occidentale leaves and bark had been reported for their amaebicidal, antioxidant and astringent properties, in which the extractshad been used extensively in the treatment of malaria(Razalia *et al*., 2008; Orwa *et al*., 2009; Obembe *et al*., 2012; Kunle *et al*., 2013; Aderiye *et al*., 2015). The tannin that is present in both the leaf and stem extracts

of cashew was said to be responsible for the antimicrobial activity against *Plasmodium* spp. which is the causative organism of malaria, however, the mechanism of its action isnot yet known (Obembe *et al*., 2012). Fresh bark ethanol and aqueousextracts possess a moderate antimalarial activity*in vitro* against *Plasmodium falciparum*, but the ethanol extract of *A. occidentale*exhibited higher antimalarial activity than water extract as reported by Sha'a *et al*. (2014).Aqueous extracts of leaves, bark and nutshell of *A. occidentale*has potential bioactive compound against the larvae of*Anopheles gambiae*,therefore,it could be used in stagnant water bodies, which are known to be breeding ground for mosquitoes (Nnamani *et al*., 2011).

Anacardium occidentale extracts is effective against nematode of sheep, predominantly, *Haemonchus contortusin vitro* in which they decreased the hatchability of eggs and survival of L3 larvae in a concentration dependent manner, the LC_{50} values of acetone extract was 0.311 and 1.72 mg/ml for egg hatch and larval viability test (Ademola and Eloff, 2011). The false fruit extract of *A. occidentale*caused paralysis anddeath of *Ascaridia galli*(Aderiye *et al*., 2015).

Field observation revealed that *A. occidentale*was among 52 plant species belonging to 29 families that were identified as being used for the treatment of ruminant animals' pests and diseases in Ekiti State, Nigeria (Olanipekun and Tedela, 2013).*Anacardium occidentale* extract possessed hypoglycemic and antioxidant effect in alloxan-induced diabetic rats by decreasing the concentration of total cholesterol, low density lipoprotein and triglyceride to an extent and increased the concentration of high density lipoprotein (Shafe*et al*., 2014). The compounds present in the extract of cashew bark and stem which are suspected to be flavonoids probably inhibit the cyclo-oxygenase and lipoxygenase pathways of

arachidonate metabolism, blocking the leukotriene mechanism, which is important to chemotaxis, and also blocking prostaglandin mechanism, which is important to the genesis of the oedema and pain (Morrow and Roberts II, 2001; Aderiye*et al.*, 2015).

2.5 Medicinal Uses of Cashew Leaves

The fats and oils in cashew nuts contain 54% monounsaturated (oleic acid), 18% polyunsaturated (linoleic acid) and 16% saturated fatty acids (palmitic and stearic acids). Phenols, sitosterin, tannins and resorcinol derivatives are also found in the plant (Orwa *et al*., 2009; Atawodi *etal*., 2014).*Anacardium occidentale* leaves extract possess antiulcerogenic effect at doses higher than 100 mg/kg body weight in which it was found to be more effective than 30 mg/kg of lansoprazol in inhibiting gastric lesions in mice (Bacchi and Konan, 2007). More specifically, antibacterial activity of ethanol extract of*A. occidentale*leaves have all been demonstrated scientifically *in vitro*against *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Shigella dysentery, Salmonella typhimerium* at the concentration of 200mg/ml of the extract (Omojasola and Awe, 2004).

The leaf of *A. occidentale*was also successfully used in the treatment of wounds due to its tannin content (Agedah *et al.,* 2010; Aderiye *et al*., 2015). Tannins not only heal burns and stop bleeding but they also stop infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more (Ashok and Upadhyaya, 2012; Aderiye *et al*., 2015).In addition, the leaf of *A. occidentale*can be used in treatment of dyspepsia (Franca *et al*., 1996; Aderiye *et al*., 2015). The ability of the cashew leaf had also been attributed to the presence of flavonoids found in the leaf of cashew (Jellin *et al*., 2002;Aderiye *et al*.,

2015).The leaf extract of *A. occidentale*can also be used in treating nausea due to its anacardic acid and tannins contents (Li *et al*., 1999; Aderiye *et al*., 2015). Tannins cause contraction of the stomach surface with which they come in contact and reduce permeability. When consumed, they reduce the permeability of the intestinal lining of the intestine, and thereby relieve the discomfort (Oyesomi and Ajao, 2011; Aderiye *et al*., 2015).

The *A. occidentale* leaves extract possess anti-inflammatory and analgesic activity. Pawar and Pal (2002) reported the anti-inflammatory effect of cashew leaf extract, as shown in carrageenan induced rat paw oedema. The acetone soluble part of ethanol extract of *A. occidentale* leaves showed better analgesic and anti-inflammatory activity. While the analgesic activity of *A. occidentale* leaves wascompared by tail flick test. The pentazocine showed 67.47% of analgesia, while petroleum ether, chloroform and acetone soluble part of methanol extracts of *A. occidentale* leaves showed 46.48, 33.17 and 53.80% of analgesia respectively ($P< 0.05$). Acetone soluble part of petroleum ether extract of *A. occidentale*exhibited significant analgesic activity. In the control group, the tail flick latency was 2.79 ± 0.23 s, while in pentazocine, petroleum ether, chloroform and acetone soluble part of methanol extract of *A. occidentale*showed tail flick latency increased to 10.5 ± 0.32, 8.5 ± 0.25, 8.0 ± 0.25 and 11.25 ± 0.43s, respectively ($P< 0.05$). This result indicated that the extract of cashew can be used as analgesic (Pawar and Pal, 2002; Aderiye *et al*., 2015).

2.6 Traditional Uses of *Anacardium occidentale*

*Anacardium occidentale*leaves decoction was among some medicinal plants used for the management of hypertension in the Igala-speaking area of Kogi State, Nigeria. The anti-hypertensive properties of *A. occidentale*may be due to some of these phytochemicals (Atawodi *et al*., 2014).

2.7 Safety of *Anacardium occidentale*

The ethanol extract of *A. occidentale*leaves was found to be non-toxic to brain and kidney via the oral route in rats, at 2,000 mg/kg body weight (Edet *et al*., 2013). It has been indicated that chronic administration of sitosterol (lipids isolated from *A. occidentale* leaves extract) subcutaneously to rats for 60 days was well tolerated and there was no clear evidence of any lesions either in the liver or in the kidneys (Malini and Vanithakumari, 1990; Edet *et al*., 2013). The leaves extract of *A. occidentale*was also reported to be non-teratogenic on Pregnant Wistar Rats (Dare *et al*., 2011).

2.8 History of Salmonellosis

Salmonella was first visualized in 1880 by Karl Eberth in the Peyer's patches and spleens of typhoid patients (Eberth, 1880). Four years later in 1884, Georg Theodor Gaffky, was able to successfully grow the pathogen in pure culture (Hardy, 1999). A year after that, medical research scientist Theobald Smith discovered what would be later known as *Salmonella* enterica (var. choleraesuis). At the time, Smith was working as a research laboratory assistant in the Veterinary Division of the United States Department of Agriculture. The department was under the administration of Daniel Elmer Salmon, a veterinary pathologist (FDA, 2008). Initially, *Salmonellacholeraesuis* was thought to be the causative agent of hog cholera, so Salmon and Smith named it "Hog-cholerabacillus". The name *Salmonella*

was not useduntil 1900, when Joseph Leon Lignières proposed that the pathogen discovered by Daniel Salmon's group be called Salmonella in his honor (Heymann *et al*., 2006).

2.9 Avian Salmonellosis

World-wide, birds were kept for meat or egg production, companionship, sports, scientific or educational purposes. In 2007, an estimated 17.9 billion chickens, 1.1 billion ducks, 447 million turkeys and 343 million geese and guinea fowls were farmed world-wide (Tomley and Shirley, 2009; Hoelzer *et al*., 2011). But, the major constraints identified in the development of the poultry industry in countries like Nigeria, thus causing huge economic losses to farmers were diseases (Abdu *et al*., 1992).

The aetiological agent of FT and pullorum disease is *Salmonella* enterica subsp. enterica serovar Gallinarum, which is divided into two distinct biovars under the serogroup D1, Gallinarum and Pullorum, which are denoted as *S.* Gallinarum and *S.* Pullorum, respectively (Minor, 1984; Shivaprashad, 1997; Kabir, 2010). In addition to *S.* Gallinarum-Pullorum, other salmonellae such as *S.* Enteritidis, *S.* Panama and *S.* Dublin also belong to the serogroup D1 (Minor, 1984; Kabir, 2010). The various motile and non-host adapted highly invasive serotypes such as *Salmonella enteritidis* and *Salmonella typhimurium* are commonly referred to as Paratyphoid Salmonellae (Gast, 1997 and Kabir, 2010). Age wise prevalence of avian salmonellosis showed highest infection rate in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) chickens (Rahman *et al*., 2004; Kabir, 2010).

2.10 *Salmonella* Gallinarum

*Salmonella*Gallinarumis an avian adapted bacterial pathogen and the causative agent of FT (Abdu, 2007; OIE, 2008; Salihu *et al*., 2014).It was formerly known as *Shigella gallinarum* when first isolated in 1989 by Klein in England (Shivaprasad, 2000). Fowl typhoid is common in both backyard and commercial poultry (Fricker, 1987; Kabir, 2010).Outbreaks of FT in Korea have been reported since 1992 and causes serious economic losses in brown layers (Kim *et al*., 1995; Yong-Ju Lee *et al*., 2001). In Pakistan, FT prevalence of 6.58% was reported by Abbas *et al*. (2015).In Bangladesh the prevalence of FT was found to be 62.5% (Hossain *et al*., 2006).

A serological survey for antibodies to *S.* Gallinarum among chickens under two different management systems around Jos, Plateau State, Nigeria.In the free range system 19.3% of the flocks sampled were seropositive for *S.* Gallinarum antibodies while semi intensive, 18.6% of the flock tested positive (Okwori *et al*., 2007).In Gombe State, Nigeria a prevalence of 12.02% was reported(Bukar-Kolo *et al*., 2004).Based on the survey conducted in Katsina State, Nigeria a prevalence of (7.14%) FT has been reported (Ukashatu*et al*., 2012). A recent report puts the prevalence of FT in sampled flocks in part of northern Nigeria at 18.4% (Mbuko *et al*., 2009; Ogie *et al*., 2013). Although, SG infection is frequently considered as a problem of adult and grower chickens, chicks are also affected (Wales and Davies, 2011; Ogie *et al*., 2013). Avian salmonellosis and colibacillosis are considered to be the major bacterial disease problems in the poultry industry worldwide because they constitute a major public burden and represent a significant cost in manydeveloping countries including Nigeria(Berchieri *et al*.,2001; Wigley *et al*., 2001; Balami *et al*., 2014).

2.11 Economic Importance of *Salmonella* Gallinarum

High prevalence of FT caused by *S.* Gallinarum was among the major factors limiting high productivity of indigenous chickens in the tropics(Yongolo, 1996; Ogie *et al*., 2013). Fowl typhoid is recognized worldwide as a septicaemic disease of social and economic significance which affects primarilychickens and turkeys, although natural infections in ducks, pheasants, guinea fowls, ostriches, wood pigeons, swans, sparrows, peacocks and quails have been reported (Yongolo, 1996; Ogie *et al*., 2013).

2.12 Pathogenesis of Avian Salmonellosis

Salmonella spp. genetics allows these bacteria to adapt too many environments, fomites and both mammal and non-mammal hosts (Sanchez-Vargas *et al*., 2011; Cardoso *et al*., 2013). The consequences of Salmonella infections depend on inherent pathogen factors, such as virulence, and on the host's capacity of building an adequate immune response to fight the pathogenic agent (Lahiri *et al*., 2010; Cardoso *et al*., 2013).

The main site of multiplication of *S.* Gallinarum is the digestive tract, which may result in widespread contamination of the environment due to bacterial excretion through faeces. Following invasion through the intestinal mucosa, caecal tonsils and Peyer's patches, the organisms are engulfed by macrophages, and through the blood stream and/or lymphatic systems, they spread to organs rich in reticuloendothelial tissues (RES), such as the liver and spleen, which are the main sites of multiplication (Barrow *et al*., 1994; Kabir, 2010). In the case of inadequate body defense mechanism, they may lead to second invasion and be localized in other organs, particularly ovary, oviduct, myocardium, pericardium, gizzard, yolk sac and/or lungs (Barrow, 1993; Kabir, 2010). In the bird challenge, *S. typhimurium*

rapidly caused inflammation of the intestinal mucosa, but *S.* Pullorum preferentially targeted the bursa of Fabricius (BF) prior to eliciting intestinal inflammation (Henderson *et al*., 1999; Kabir, 2010). Pullorum disease manifests itself predominantly as an enteric disease of chickens, while FT shows signs of septicemic disease (Shivaprashad, 1997; Kabir, 2010). Both biovars can cause septicemic infections, which may be acute or chronic, but unlike *S.* Pullorum, *S.* Gallinarum is capable of producing per acute infection and haemolytic anemia in both young and adults (Christensen, 1996; Kabir, 2010). *Salmonella* Gallinarum is extremely pathogenic to young broiler chicks (Lowry *et al*., 1999; Kabir, 2010).

Fowl typhoid is indistinguishable from pullorum disease unless the aetiological agent is isolated and identified (Fricker, 1987; Kabir, 2010). Clinical signs in chicks and poults include anorexia, diarrhoea, dehydration, weakness and high mortality (Shivaprasad, 2000; Kabir, 2010). In mature fowls, FT and pullorum disease are manifested by anorexia; drop in egg production, increased mortality, reduced fertility and hatchability (Shivaprasad, 2000; Kabir, 2010). *S.* Pullorum infected adult birds may or may not exhibit any clinical signs, or they cannot be detected by their physical appearance (Shivaprashad, 1997; Kabir, 2010).

2.13 Pathogenicity of *Salmonella* Gallinarum

The pathogenicity of Salmonella depends on its ability to survive and multiply within the cells, specifically macrophages (Humbert and Salvat, 1997; Kabir, 2010). The virulence of SG requires functional mutations in the type III secretion systems (TTSS) encoded by *Salmonella* pathogenicity island 1 (SPI-1) and Salmonellapathogenicity island 2 (SPI-2),

Salmonella serovar Gallinarum virulence function requires SPI-2 primarily through promoting survival within macrophages allowing multiplication within the reticuloendothelial system, but this does notpreclude the involvement of SPI-2 in uptake from the gut to the spleen and liver (Jones *et al*., 2001).

2.14 Public Health Significance of *Salmonella* Gallinarum

Only motile serovars for which poultry are known to be reservoirs are zoonotic(Gast, 2007). The most frequently reported serovars in the United States are *S. typhimurium*, *S.* Enteritidis, *S. newport, S. heidelberg* and S. enterica subsp. enterica, although persistency andprevalence of different serovars vary from place to place (Gast, 2007). However, two cases of *S.*Gallinarum infection were reported in twenty six year old Pakistani and twenty four year old Indian housemaid both residing in Saudi Arabia, manifested with high grade remittent fever of up to 40°C and 41°C with anorexia vomiting watery diarrhoea and abdominal pain after receiving TAB vaccination for enteric fever (Yousuf *et al*., 2001). Another case of S. Gallinarum emphyema in twenty four years old man was reported in Iran by Sherifi-Mood *et al*. (2006).However, *S.* Gallinarum is highly host adapted, and it is not considered to be a serious public health concern because in one survey, only eight out of more than 450,000 isolations of *Salmonella* from humans were *S.*Gallinarum (OIE, 2009).

2.15 Diagnosis of Avian Salmonellosis

Avian Salmonellosis can be diagnosed by isolation and identificationusing specific biochemical tests like carbohydrate fermentation test, methyl red test, Voges-Proskauer (V-P) test, Indole test, and motility test (OIE,1996).

2.16 Confirmatory Diagnostic Procedures for Avian Sallmonellosis

According to terrestrial manual (OIE, 2012) typical *S.*Gallinarum colonies on non-selective media are round, translucent, glistening, domed, smooth and 1–2 mm in diameter after 24–48 h incubation. *Salmonella* Pullorum colonies are slightly smaller andtranslucent. On selective media like Brilliant Green Agar, Triple Iron Sugar and Salmonella Shigella Agar, their appearance varies with the medium, but suspect colonies can beinvestigated serologically by testing for 'O'9 somatic antigens, and observing for motility and testing biochemically. After incubation for 20–24 h, the plates should be examined carefully for the presence of typical *S*. Pullorumand *S*. Gallinarum colonies. If growth is slight after 24 h incubation, the plates should be re-incubated for a further 24 h and examined again. For biochemical and serological confirmation from each plate, five typical or suspect colonies should be chosen for further examination. If there are fewer than five typical or suspect colonies, all of them should be taken for further examination. Selected colonies should be streaked on to the surface of nutrient agar, in a manner that allows the growth of separate colonies. For biochemical confirmation, only pure cultures taken from non-selective media should be used. The following media should be streaked using an inoculating loop: triple sugar iron (TSI) agar; lysine iron agar (or l-lysine decarboxylation medium); urea agar according to Christensen*et al*. (1996); tryptone/tryptophan medium for indole reaction; glucose with an inverted Durham tube for acid and gas production; dulcitol, maltose, ornithine decarboxylation medium and semisolid agar, for motility.

Identification kits are commercially available, for example Analytical Profile Index (API) system for Enterobacteriaceae. However, care must be taken when using API because *S*. Pullorum may be mis-identified as *Hafnia* spp. Molecular tests using ribotyping techniques

and PCR have been developed in research laboratories (Kang *et al*., 2011), and can be used for confirmation and differentiation between*S*. Gallinarum and *S*. Pullorum. For serological confirmation to serogroup level, colonies from non-selective media (nutrient or blood agar) are used. The first stage is elimination of autoagglutinable strains. For this, material taken from a single colony of pureculture is transferred to a glass slide and mixed with a drop of sterile saline. The slide is rocked gently or the dropstirred with a loop for 30–60 sec and observed for agglutination against a dark background, preferably with the aid of a magnifying glass or dissecting microscope. If the bacteria have clumped into more or less distinctive units, the strain is considered to be autoagglutinable and must not be submitted to the following tests. If the bacterial sample is recognised as non-autoagglutinable, it is tested with a polyvalent 'O' (A–G) antiserum. For this purpose, the material from a single colony is dispersed in the drop of polyvalent 'O' antiserum on the glass slide to obtain a homogenous and turbid suspension. After gently rocking for 30–60 sec, the reaction is observed against a dark background for agglutination.

Alternatively the slide agglutination test may be carried out with smaller volumes of suspension under a dissecting microscope. In this case a portion of the colony to be checked is added to a loopful of saline on the microscope slide to produce a light suspension to check for autoagglutination ('rough strains'). If no agglutination takes place, one or two loops of antisera are added, the drop stirred with a loop and observed for agglutination. *Salmonella* Pullorum and *S*. Gallinarum should agglutinate with polyvalent 'O' antisera but not with polyvalent flagella (poly 'H' phase 1 and phase 2) antisera. If the reaction is positive, the single colony is tested further in the same manner using group-specific sera for *S*. Pullorum and *S*. Gallinarum serotypes ('O'9 antiserum). After serogrouping, isolates may

be sent to a reference laboratory for serotyping.It is also possible to confirm and differentiate *S.*Gallinarum by specific PCR (Shah *et al*., 2005).

2.17 Prevention and Control of Avian Salmonellosis

The most effective means of control of avian Salmonellosis is a combination of stringent biosecurity procedures and eradication (Snoeyenbos, 1991). Selection for disease resistance in poultry strains can be an effective strategy for prevention and control of several important infectious diseases (Smith, 1956; Bumstead and Barrow, 1993; Lindell *et al*., 1994). As reported by Alshawabkeh and Tabbaa(2001) when three commercial strains bred between US (Hubbard), Holland (Lohman), Canada (Shavar), Jordan (Balady) are infected with 10^5CFU of *S. Gallinarum* per chick, it was found that, Lohman breed was the most resistance over the other two commercial strains, while Hubbard had the highest susceptibility. The Balady chicks were the most resistant. However, they may serve as a possible reservoir of *S.* Gallinarum.

2.18 Treatment of Fowl Typhoid Using Synthetic Drugs

Doxifin 50 PS®is an antimicrobial drug with a wide spectrum based on doxycycline, semisynthetic molecule belonging to the group of tetracycline'sis effective against FT and pullorosis in poultry. Each 100 g contain (Doxycycline (Hyclate)- 50.000 g Excipient q.s - 100.000 g Equivalent to Doxycycline Hyclate- 62.500 g). In addition field experience revealed that, Neoceryl-plus®, Conflux 20%, Neomycin Sulphate, and Flornicol were all effective in reducing the severity of clinical signs in avian salmonellosis.

2.19 Treatment of Fowl Typhoid Using Medicinal Plants

Natural antimicrobials from plant products, such as bioactive compounds from berry juice by productsblackberry and blueberry pomace extracts inhibited *S.* Gallinarum growth *in vitro* as reported by Biswas *et al*.(2015). Another example is the use of *Aloe secundiflora* which was used by locals in Tanzania for the treatment of FT (FAO/IAEA, 2001). Treatment with *A. secundiflora* resulted in delayed clinical signs and a reduced severity of the symptoms. The mortality decreased from 43% in the infected and non-treated to 14% in the treated group. Hexane extracts of the leaf, stem and root of *Ageratum conyzoides* had 100% activity against *S.*Gallinarum *in vitro* (Okwori *et al*., 2006). Moreover, field experience revealed that phytogenic feed additives Digestarom® (a mixture of essential oils, herbs, spices, extract with 1317 peppermint flavour) manufactured by Micro-plus Konzentrate GmbH of Germany was effective in treating avian salmonellosis *in vivo*. Also, *Persea americana* could be used to treat salmonellosis in poultry as reported by Rebecca,(2010). Supplementation of *Withania somnifera* (Ashwagandha) root powder at 0.5% in feed may reduce the severity of *S.*Gallinaruminfection in broiler chicken as reported by Deepika *et al*. (2015).Incorporation of plantain nonstarch polysaccharide (NSP) derived from the plantain (*Musa paradisiaca*) into the poultry feed reduced *S.*Gallinarum levels in the spleen and liver of chicks following experimental infection, although their numbers in the caeca increased (Parsons *et al*., 2015).

2.20 Significant of *Anacardium occidentale*Leaves Extract in the Management of Salmonellosis

The most important aspect of ethnoveterinary medicine is that the knowledge base resides in the local population and has been acquired over many years based on their experience in

the treatment of livestock diseases using different plant products found in the regions in which they live (FAO/IAEA, 2001). In order to qualify a standardize, scientific approach to the use of medicinal plants, it is necessary to carry out a comprehensive analysis of phytotherapeutic potentials of various plant extracts that are used by local people to treat various animal diseases (FAO/IAEA, 2001). Among the plants with ethnomedicinal and ethnoveterinary importance is *A. occidentale*,proven by many researchers to treat viral, bacterial, and parasitic diseases in humans and animals.Even though, few researchers reported that, therapeutic treatment of Salmonellosis has limitation and cannot be recognized as an effective means to clean up *S. Gallinarum* contaminated flocks because carries may remain (Smith and Tucker, 1975; Alshawabkeh, 1987). But current research showed that, ethnoveterinary therapeutic approaches are promising more especially with plants extracts as in the case of phytogenic feed additive Digestarom®. Therefore, *A. occidentale*can be a good candidate in the production of phytobiotic against *Salmonella*Gallinarum.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Collection and Identification

Fresh leaves of *A. occidentale*were collected in December, 2015 from Suleja Local Government Area of Niger State, Nigeria and identified by Namadi Sunusi at the Herbarium Unit, Department of Biological Sciences Ahmadu Bello University, Zaria. A voucher specimen number 991 was deposited for reference purpose.

3.2 Experimental Chickens

Two hundred day-old Shikabrown pullet chicks were purchased from a reputable hatchery in Kwara State, Nigeria. Immediately after arrival the chicks were screened for the presence of *S.*Gallinarum.

3.3 Bacteria

Pure seed culture of *S.*Gallinarum (NE/03/2007)was obtained from National Veterinary Research Institute Vom, Plateau State. The culture was transported to A.B.U Zaria in a plastic cooler packed with ice; on arrival it was stored on nutrient agar slants at 4°C in the laboratory of Department of Veterinary Microbiology, A.B.U Zaria. Overnight broth culture was used for testing the antibacterial activity *in vitro*as described by Rajesh *et al*. (2015).

3.4 Brilliiant Green and Mueller-Hinton Agars

Brilliiant Green and Mueller-Hinton agars manufactured by Oxoid-USA were purchased for culturing *S.* Gallinarum and *in vitro* antibacterial testing of methanol extract of the leaves of *A. occidentale*. The Brilliiant Green Agar was prepared by dissolving 10 g in 200 ml of distilled water,while Mueller-Hinton Agar was prepared by dissolving 7.6 g in 200 ml of distilled water. Each agar was heated with frequent agitation and boiled for one minute in order to dissolve the medium completely after which it was sterilized by autoclaving at 121 ^{0}C for 15 minutes. After cooling at room temperature it was poured into sterile petri dishes on a required level, horizontal to the surface to give uniform depth and allow cooling at room temperature.

3.5 Feeds

Chick mash and grower mash were purchased from commercial feed mill company, Hybrid feed Kaduna, Nigeria.

3.6 Medicines and Medication

The drugs used include, intracox oral® containing 25 mg toltrazuril per ml and administered at 1ml/litre of water as anticoccidial; aminogrow® multivite liquid administered at 0.25ml/litre of waterin which each one ml contains 9.6 mg alanine, 12 mg arginine, 25.2 mg aspartic acid, 0.4 mg cysteine, 39.6 mg glutamic acid, 8.4 mg glycine, 4.8 mg histadine, 6 mg isoleucine, 13.2 mg leucine, 10.8 mg lycine, 2.4 mg methionine, 8.4 mg phenylalanine, 10.8 mg proline, 9.6 mg serine, 6 mg threonine, 0.5 mg tryptophan, 4.8 mg tyrosine, 6 mg valine and 1ml solvents ad.Intracox oral® and aminogrow® were manufactured by Inter Chemie Holland. Ceftazidime® antibiotic (containing 1 g ceftazidime as pentahydrate for

injection) manufactured byGlaxoSmithKline S.p.A. via A. Fleming, 2 Verona, Italy was used as a control drug at (0.2 ml/bird) orally sixteen weeks of age.

3.7 Vaccines and Vaccination

Newcastle disease vaccine La sota Strain live, B.P (Vet.) (INDOVAX Private Limited Siswala, P.O Rewalwas Khurd, Hisar 125001(Haryana) India). Bursa® B2K Strain infectious bursal disease vaccine, Inversive Intermediate Strain Live, B.P. (Vet) (INDOVAX Private Limited Siswala, P.O Rewalwas Khurd, Hisar 125001(Haryana) India) and Bio-Vac SGP 695® (FATRO S.p. A.-Ozzano Emilia (BO) Italy).The birds were vaccinated against Newcastle Disease using (NDVL) Indovac® (day 7 and 21of age) intraocularly and orally, Infectious Bursal Disease using Bursa B2K® at day 14 and day 28 of age orally. They were also administered with oral *Salmonella* Gallinarum-Pullorum vaccine at day 49.

3.8 Cleaning and Disinfection of Poultry House

Broom, shovel, plastic parker, plastic bucket and detergent were used for cleaning. Virkon®S disinfectant (containing Potassium peroxomonosulphate…50 % m/m) (DuPont Nemours International Societe Anonyme,DuPont Animal Health Solutions Co, Halfway House, South Africa) and CID 20®containing (Quaternary Ammonium compounds; Alkyldimethyl benzylammonium chloride-61.5 g/l., Aldehydes; Glyoxal-19.8 g/l, Formaldehyde-84.4 g/l, Glutaraldehyde-58 g/l, isopropanol-37.6 g/l., Terpentine derivatives; Pine oil-20 g/l) (CID LINE Innovative hygiene solutions, Waterpoortstraat 2, 8900 leper, Belgium) were used to disinfect the research pen at the Animal House Pen

House of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria.

3.9 Housing and Feeding of the pullets

The birds were brood and rose from day old up to 16 weeks of age under deep litter management system, with floor spacing of 0.093 square metre per bird. They were fed with chick mash at 1-8 weeks and grower mash at 9-16 weeks produced by Hybrid feed and a portable drinking water was provided,*ad libitum*.

3.10 Approval for Animal Use

Approval was obtained from the Committee on Animal Use and Care (ABUCAUC), Directorate of Academic Planning and Monitoring, Ahmadu Bello University, Zaria, with an approval number ABUCAUC/2016/0018.

3.11 Experimental Design

At 16 weeks of age, the pullets were randomly selected and divided into eleven groupscomprising: A, B, C, D, E, F, G, H, I, J and K with 12 birds in each. All the groups were administered 0.2 ml of *S.*Gallinarum inoculum orally except group K which served as negative control as described by Garcia *et al*. (2010). All the inoculated groups were monitored daily for five days until clinical signs of FT appeared and cloacal swab samples were taken for bacteriology. All the groups were assigned with identification number using a permanent marker, after which individual weights and cloacal temperature were taken.

Group A, B, C and D were treated with the methanol extract of *A. occidentale* leaves at 50, 100, 150, and 200 mg/kg body weight orally with the help of sterile syringe without needle for five days post inoculation. Group E was treated with ceftazidime (standard antibiotic) for five days. Group F and G were given the methanol extract of *A. occidentale* leaves at 100 mg/kg and 200 mg/kg body weights 14 days pre-inoculation as prophylaxis for 5 days. Group H and I were vaccinated with foreign oral *Salmonella* Gallinarum vaccine and indigenous fowl typhoid vaccine pre-inoculationrespectively. Pullets in groups J and K that served as positive and negative control were given 0.2 ml of normal saline orally. After treatment with the methanol extract of *A. occidentale* leaves, three birds from each group were sacrificed and liver, kidney, spleen, bursa of fabricius and thymus were harvested and weight. Liver swabs were taken for bacterial culture in order to re-isolate the inoculated *S.* Gallinarum and samples of the liver, kidney and spleen were collected and fixed in 10% phosphate buffered formalin for histopathology (Table 3.1).

3.12 Extraction of the Leaves of *Anacardium occidentale*

The mature leaves of *A. occidentale* were washed with clean water and air dried under shed until a constant weight was obtained by weighing at interval using digital electronic scale. The dried leaves were grinded into fine powder using electric blender. Maceration method of extraction was used in which 2,000g of the powdered plant material was soaked in 5,000ml of 99 % methanol and allow standing for three days. The mixture of the soaked *A. occidentale* leaves with methanol was subsequently filtered using clean cotton wool. The maceration was repeated three times to extract most of the phytoconstituent in the plant (Ncube *et al*., 2008; Banu and Cathrine, 2015). All the filtrate was pooled together and the extract obtained was placed on a water bath at 100 °C to dry yielding 108.78g (5.4%). The

dried powder of the extract obtained was kept in a clean bottle at room temperature until required to treat the Shikabrown pullets post-inoculation as described by Arekemase *et al*. (2011) and Edet *et al*. (2013).

3.13 Phytochemical Analyses of the Methanol Extract of *Anacardium occidentale* Leaves

3.13.1 Test for carbohydrates (Molish test)

To a small portion of the methanol extract of the leaves of *A. occidentale*in a test tube, few drops of concentrated sulphuric acid was added down the side of the test tube to form a layer, a reddish coloured ring at the interphase indicates presence of carbohydrates (Evans and Trease, 2009).

3.13.2Test for alkaloids (Dragendoff test)

To a portion ofthe methanol extract of the leaves of *A. occidentale,* few drops of Mayer's reagent was added. A cream precipitates indicated the presence of alkaloids (Evans and Trease, 2009).

3.13.3Test for tannins

To a small portion ofthe methanol extract of the leaves of *A. occidentale*, 3-5 drops of lead sub-acetate solution was added. A coloured precipitate indicated the presence of tannins (Evans and Trease, 2009).

3.13.4Test for saponins (Frothing test)

To a small amount ofthe methanol extract of the leaves of *A. occidentale*, 10ml of distilled water was added in a tube and shaked vigorously for 30 sec. The tube was allowed to stand in a vertical position and observed for 30 min. A honeycomb froth that persisted for 10-15 min indicated the presence of saponins (Evans and Trease, 2009).

3.13.5Test for flavonoids

Few drops of 10% sodium hydroxide (NaOH) were added to the methanol extract of the leaves of *A. occidentale* in a tube. Yellow colouration indicatedthe presence of flavonoids (Evans and Trease, 2009).

3.13.6Test for cardiac glycosides (Keller-kiliani test)

A portion of the methanol extract of the leaves of *A. occidentale*was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. A purple-brown ring at the interphase indicates the presence of desoxy sugars and a pale green colour in the upper acetic acid layer indicated the presence of cardiac glycosides (Evansand Trease, 2009).

3.13.7Test for steroid and triterpenes (Libermann-Burchard test)

To a portion of the methanol extract of the leaves of *A. occidentale*, equal volume of acetic acid anhydride was added and mixed gently. One ml concentrated sulphuric acid was added down the side of the tube to form a lower layer. Color changes were observed immediately and over a period of one hour. Light green colour in the upper acetic layer indicated the

presence of triterpene and a reddish, pink or purple color indicated the presence of steroids (Evans and Trease, 2009).

3.13.8Test for free anthracene derivatives (Bentrager test)

To a small portion of the methanol extract of the leaves of *A. occidentale*in a dry test tube, 5ml of chloroform was added and was shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of 10% ammonia solution, bright pink colour in the aqueous upper layer indicated the presence of free anthracene (Evansand Trease,2009).

3.14 Determination of Lethal Dose of Methanol Extract of *Anacardium occidentale*Leaves in Shikabrown Pullets

The method used to determine lethal dose (LD_{50}) was described by Lorke, (1983). The crude methanol extract of the leaves of *A. occidentale*was dissolved in distilled water before being administered to the pullets. The study was conducted in two phases. In the first phase, nine pullets were randomly allocated into three groups of three pullets each. The pullets were deprived of food for 20 h and water for 8 h. Group 1, 2 and 3 were treated with the extract at respective oral doses of 350, 500 and 1,000 mg per kg body weight. The pullets were observed for signs of toxicity and possible deaths for 48 h. After 48 h there was no mortality in all the groups in the first phase. Therefore, the second phase of the study was conducted. In the second part of the study, three pullets were assigned into three groups of 1 pullet each. Pullets in the three groups were treated with the extract at graded doses of 1,600, 2,900 and 5,000 mg per kg body weight. All treatmentswere given orally and all pullets were observed as earlier described. The LD_{50} was calculated as the geometric mean of doses that produce 0% and 100% mortality (Appendix 2).

3.15 *In Vitro* Antibacterial Testing of Methanol Extractof *Anacardium occidentale*Leaves against *Salmonella* Gallinarum

Bacterial suspension containing (0.2 ml) 3.3×10^{8} CFU of *S.*Gallinarum from nutrient broth were taken and mixed in a sterile universal bottle with 25ml of the Mueller-Hinton agar. The mixture was poured into labeled plates and allowed to settle and 6mm diameter wells were made on the solidified plates using a sterilized steel cork borer, after which the wells were labeled. A stock solution of the extract was made by dissolving 100 mg of the extract in 1 ml of water. Thereafter, six fold serial dilutions werecarried out on the extract to obtain concentrations of 50, 25, 12.5, 6.25, 3.13, and 1.56 mg/ml. Then, 100 µl of the extract was poured into the wells and the plate was initially incubated at 4^{0}C in refrigerator for 1 h and before incubated at 37^{0}C for 24 h. The diameter of each zone of inhibition was measured with a graduated ruler as described by Rajesh *et al*. (2015). Control drug, Ceftazidime antibiotic (H) at 100 mg/ml was set up along with the experiment as shown in plate I.

3.16 Screening ofExperimental Shikabrown Pullets for the Presence of *S.*Gallinarum

On arrival cloacal swabs and liver swabs were taken from apparently healthy, dead and moribond chicks for isolation of*S.*Gallinarum, in order to ensure that the birds were free the bacterium, which may compromise the experiment as reported by Zancan *et al*.(2000) and Garcia *et al*.(2013). The swabs were appliedon Salmonella-Shigella Agar (SSA) and incubated at 37 °C for 24 h. The screening for *S.* Gallinarum was also done pre-inoculation and post-inoculation using cloacal swabs from live pullets and liver swabs from the sacrificed pullets for screening posttreatment. The swabs were pre-enriched before applied on brilliant green agar (BGA) and incubated at 37 °C for 24 h.

3.17 Preparation of *Salmonella* Gallinarum Inoculum

The turbidity of *S.* Gallinarum suspension used for susceptibility testing was adjusted to 0.5 McFarland standard (10^8 CFU/mL) using sterile normal saline, 0.9% sodium chloride (McFarland, 1907).

3.18 Treatment with Extracts of *Anacardium occidentale*

Dried methanol extract of *A. occidentale* leaves was used to treat the Shikabrown pullets at sixteen weeks of age for five days post-inoculated with *S.*Gallinarum*in vivo*and for *in vitro*antibacterial testing. The Methanol extract of *A. occidentale* leaves was dissolved in sterile distilled water before administration to obtain a concentration of 500 mg/ml; this is achieved by dissolving 6 g of the extract into 12 ml of distilled water. The amount administered to each pullet orally was determined using the following formula = dose (mg/kg) × body weight (kg) / concentration (mg/ml). However, for *in vitro* study 100 mg of the extract was dissolved in 1 ml of distilled water.

3.19 Grading of clinical signs

The clinical signs observed were graded as: absence (0), slightly severe (1), moderately severe (2), severe (3), highly severe (4) and strongly severe (5) which was a modification of the grading used by Christensen *et al*. (1996).

3.20 Blood Collection for Haematology

Blood (2.5 ml/bird) was collected usingsterile syringe 5 ml size with 23 gauge from the wing vein into a sample bottle containing ethylene diamine tetraacetate (EDTA) as anticoagulant for the determination of haematological valueswhich includes total red blood

cell (TRBC), total white blood cell (TWBC), packed cell volume (PCV), total protein (TP), haemoglobin concentration (Hb) and heterophils as described by (Coles, 1974: Geidam *et al*., 2013).

3.21 Gross weight of the liver, spleen, kidneys, thymus and bursa of Fabricius

The liver, spleen, kidneys, thymus and bursa of Fabricius, were weighed using digital electronic scale (Appendix 7) to observed any gross changes in weight.

3.22 Histopathology of the Liver, Spleen and Kidneys

Samples of tissues from liver, spleen and kidneys were obtained and prepared for histopathological examination using the method described by Behmer*et al*. (1976) and Garcia*et al*. (2013). The tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin and graded in absolute ethanol and ethyl acetate, then, the tissues were cut at 5μm microtome. The sections were stained with Haematoxylin and Eosin and examined under light microscope at different magnifications (×10, ×20, ×30 and ×40) and the lesions observed were recorded. Photomicrographs of stained tissues were taken using Nikon digital camera (COOLPIX L29), 16.1 megapixels, China.

3.23 Data Analyses

Data were presented in tables and bar charts. The mean body weights, clinical signs and cloacal temperature were expressed as mean ± SEM. Statistical analyses were carried out by Two Way ANOVA and One Way ANOVA followed by Bonferroni posttest using GraphPad InStat version 3 USA. $P<0.05$, $P<0.01$, $P<0.001$ and $P>0.05$ were regarded as statistically significant, highly significant, strongly significant and not significant respectively.

Table 3.1: Experimental design to evaluate the efficacy of methanol extract of *Anacardium occidentale* leaves on *Salmonella* Gallinarum in experimentally inoculated Shikabrown pullets

Group	Number of birds	Sample pre-inoculation	Age of inoculation (weeks)	Quantity of inoculum (ml)	Sample of blood (ml) post-infection	Dose of extract (mg/kg)	Type of treatment	Number of birds sacrificed	Organs collected for histopathology
A	12	CS	16	0.2	2.5	50	RX	3	Lv, Kd, Sp
B	12	CS	16	0.2	2.5	100	RX	3	Lv, Kd, Sp
C	12	CS	16	0.2	2.5	150	RX	3	Lv, Kd, Sp
D	12	CS	16	0.2	2.5	200	RX	3	Lv, Kd, Sp
E	12	CS	16	0.2	2.5	Ceftazidime	TX	3	Lv, Kd, Sp
F	12	CS	16	0.2	2.5	100	PX	3	Lv, Kd, Sp
G	12	CS	16	0.2	2.5	200	PX	3	Lv, Kd, Sp
H	12	CS	16	0.2	2.5	SGPV	VX	3	Lv, Kd, Sp
I	12	CS	16	0.2	2.5	FTV	VX	3	Lv, Kd, Sp
J(positive)	12	CS	16	0.2	2.5	0.2 (NS)	N	3	Lv, Kd, Sp
K(negative)	12	CS	16	0.2 (NS)	2.5	0.2 (NS)	N	3	Lv, Kd, Sp
Total	**132**							**33**	

Key: CS = cloacal swab, +ve C = positive control, -ve C = negative control, RX = phytotherapy, TX = chemotherapy, PX = prophylacxis NS = normal saline, ml = milliliter, mg = milligram, kg = kilogram, Lv = liver, Kd = kidneys, Sp = spleen, SGPV = oral *S.* Gallinarum-Pullorum vaccine, FTV = fowl typhoid vaccine, VX = vaccination and N = none, 0.2 = 3.3×10^8 CFU of SG.

CHAPTER FOUR

RESULTS

4.1 Constituents present in *Anacardium occidentale*Leaves and Feeds

The constituents present in the feeds (chick mash andgrower mash) and dried powder leaves of *A. occidentale* leaves are shown in Table 4.1. The crude protein (% CP) of the chick mash was 15.99 higher than that of grower's mash which was15.06, while the dried powder of *A. occidentale*leaves had the least 5.59.

4.2 Phytochemicals Present in Methanol Extract of *Anacardium occidentale*Leaves

Screening of methanol extract *A. occidentale*leaves revealed the presence of carbohydrates, alkaloids, tannins, saponins, flavanoids, cardiac glycosides, steroids and triterpenes. Free anthracene derivatives were absent (Table 4.2).

4.3 Lethal Dose of Methanol Extract of the Leaves of *Anacardium occidentale* in 16-week-old Shikabrown Pullets

The methanol extract of *A. occidentale* leaves in pullets did not produce any observable site effect or mortality at a lowest to the highest dosesof 350 to 5,000 mg/kg. Therefore, it was considered as relatively safe and the LD_{50} was assumed to be > 5,000 mg/kg (Appendix 2).

Table 4.1:Constituents (in percentages) of the feeds and of *A. occidentale* leaves obtained from Suleja, Niger State

Sample	Moisture	Crude protein	Crude fibre	Fat	Ash	Nitrogen Free Extract	Calcium	Phosphorus	Dry matter
Chick mash	9.10	15.99	8.71	4.42	10.33	55.85	2.87	0.33	9.10
Grower mash	8.70	15.06	12.58	4.86	11.41	51.75	4.00	0.29	8.70
A. occidentale leaves	8.10	5.59	19.29	1.21	3.29	66.56	0.35	0.04	8.10

Table 4.2: Qualitative phytochemicals present in *A. occidentale* leaves of obtained from Suleja, Niger State

Phytoconstituent	Result
Carbohydrate	+
Alkaloid	+
Tannins	+
Saponin	+
Flavanoids	+
Cardiac glycosides	+
Steroid	+
Triterpenes	+
Free anthracene derivatives	-

+ = detected, - = not detected

4.4 *InVitro* Antibacterial Activity of Methanol Extract of *Anacardium occidentale* leaves against *Salmonella* Gallinarum

In vitro antibacterial activity of methanol extract A. occidentale leaves showed activity on *S.*Gallinarum at concentrations (C) of 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 mg/ml with zones of inhibition (ZI) of 18.5, 15.9, 13.4, 8.3, 10.9, 13.4 and 10.9 mm, respectively. The ZI of ceftazidime was found to be 46.4 mm at 100 mg/ml (Plate I).

4.5 Clinical Signs of Fowl typhoid Observed in Methanol Extract of *Anacardium occidentale* Leaves Treated, Prophylactic, Fowl typhoid Vaccinated and Control Groups

The clinical signs manifested by pullets post-inoculation with experimental *Salmonella* Gallinarumin the treated, prophylactic, and vaccinated and control groups are shown in table 4.3, 4.4 and appendix 3, 4, 5, and 6, respectively.Full manifestation of clinical signs appeared at day 6 PI (table 4.3). Treated pullets in group A showed presence of weakness, moderately severe depression, diarrhoea and severe anorexia while B showed weakness, moderately severe inactivity, with severe depression, diarrhoea and anorexia. Group C manifested weakness, moderately severe inactivity with depression and severe diarrhoea with anorexia. Group D showed weakness, moderately severe inactivity, sneezing and coughing with severe depression, diarrhoea and highly severe anorexia. The NVRI vaccine-vaccinated groups showed absence of clinical signs while the foreign vaccine showed presence of diarrhea.

At day 9 PI and day 5 PTX, there was absence of depression, in all treated, prophylactic, vaccinated and negative control groups. Also, among the treated groups only group A and F

showed moderately severe diarrhoea while positive control group showed severe depression and diarrhoea with one mortality recorded (table 4.4).

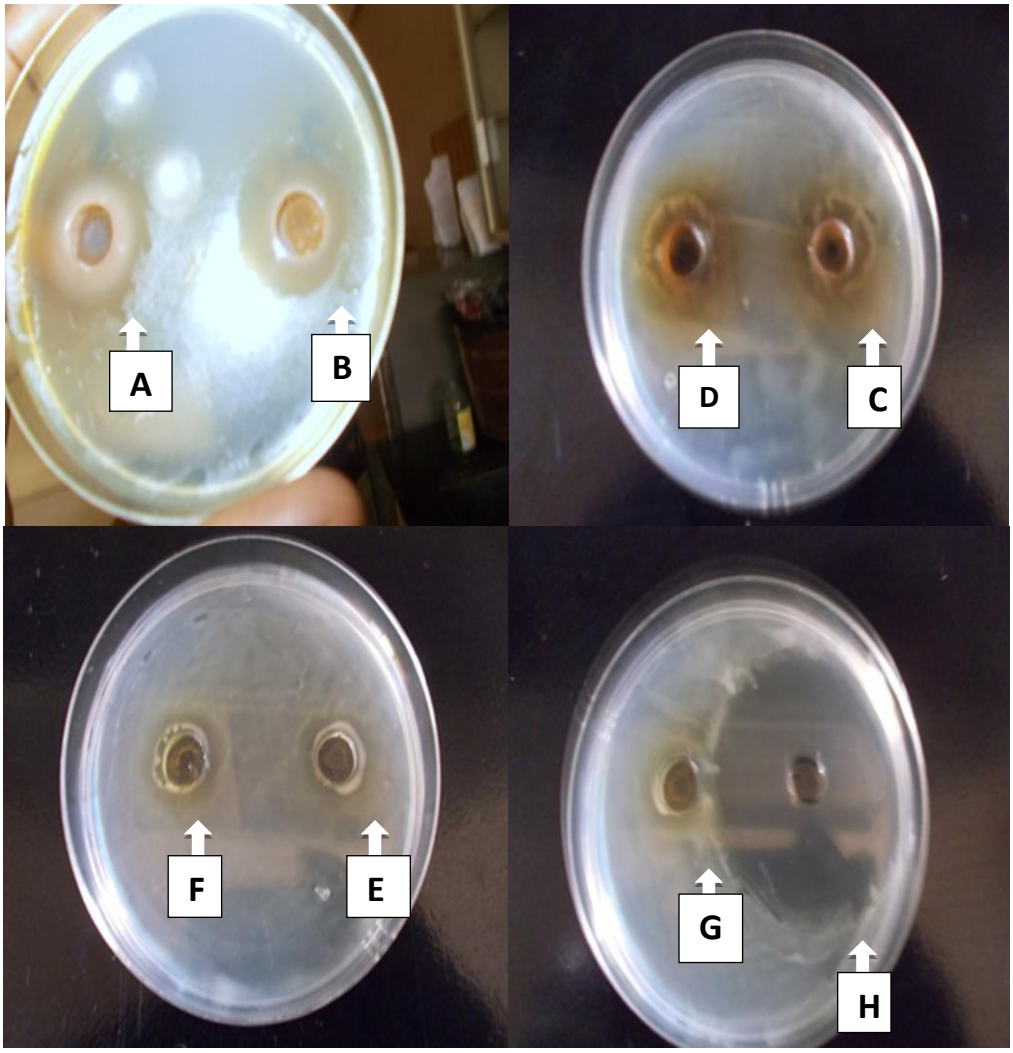


Plate I: *In vitro* antibacterial activity of methanol extract of *Anacardium occidentale* leaves and ceftazidime drug on *Salmonella* Gallinarumat at different concentrations of A=100, B=50, C=25, D=12.5, E=6.25, F=3.13, G=1.56 and H=100 mg/ml with zones of inhibition of 18.5, 15.9, 13.4, 8.3, 10.9, 13.4, 10.9 and 46.4 mm respectively.

Table 4.3: Severity of clinical signs in Shikabrown pullets inoculated with *Salmonella* Gallinarum at sixteen weeks of age and treated with methanol extract of *Anacardium occidentale* leaves (day six post-inoculation and day two post-treatment)

Group	Depression	Sneezing	Coughing	Diarrhoea	Anorexia	Inactivity	Weakness	Mean±SEM
A	2	0	0	2	3	2	1	1.4±0.4
B	3	0	0	3	3	2	1	1.7±0.5
C	2	0	0	3	3	2	1	1.6±0.5
D	3	2	2	3	4	2	1	2.4±0.4
E	3	0	0	4	2	0	1	1.4±0.6
F	3	0	0	1	2	0	1	1.0±0.4
G	1	0	0	1	0	0	0	0.3±0.2
H	0	0	0	1	0	0	0	0.1±0.1
I	0	0	0	0	0	0	0	0.0±0.0
J	3	0	0	3	5	2	3	**2.3±0.7**
K	0	0	0	0	0	0	0	0.0±0.0
Mean±SEM	1.8±0.4	0.2±0.2	0.2±0.2	1.9±0.4	2.0±0.5	0.9±0.3	0.8±0.3	

0 = absence, 1 = slightly severe, 2 = moderately severe, 3 = severe, 4 = highly severe, 5 = strongly severe

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control

Table 4.4: Severity of clinical signs in Shikabrown pullets inoculated with *Salmonella* Gallinarum at sixteen weeks of age and treated with methanol extract of *Anacardium occidentale* leaves (day nine post-inoculation and day five post-treatment)

Group	Depression	Sneezing	Coughing	Diarrhoea	Anorexia	Inactivity	Weakness	Mean±SEM
A	0	0	0	2	0	0	0	0.3±0.3
B	0	0	0	0	0	0	0	0.0±0.0
C	0	0	0	0	0	0	0	0.0±0.0
D	0	0	0	0	0	0	0	0.0±0.0
E	0	0	0	0	0	0	0	0.0±0.0
F	0	0	0	2	0	0	0	0.3±0.3
G	0	0	0	0	0	0	0	0.0±0.0
H	0	0	0	0	0	0	0	0.0±0.0
I	0	0	0	0	0	0	0	0.0±0.0
J	2	0	0	3	2	0	0	**1.0 ± 0.5**
K	0	0	0	0	0	0	0	0.0±0.0
Mean±SEM	0.2±0.2	0.0±0.0	0.0±0.0	0.6±0.3	0.2±0.2	0.0±0.0	0.0±0.0	

0 = absent, 1 = slightly severe, 2 = moderately severe, 3 = severe, 4 = highly severe, 5 = strongly severe.

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control

4.6 Live Body Weight of Shikabrown Pullets Pre-inoculation and Post-inoculation with *Salmonella* Gallinarum and Post-treatment with Methanol Extract of *Anacardium occidentale* Leaves

There was no statistically significant difference in mean decrease of live body weight between treated group A(0.953 ± 0.036 kg), B (0.946 ± 0.039 kg) and C (0.957 ± 0.039 kg) ($P > 0.05$) pre-inoculation and also treated group A (0.973 ± 0.042 kg), B (1.031 ± 0.041 kg) and C (1.012 ± 0.042 kg) post-inoculation compared to negative control K (1.084 ± 0.037 kg) pre-inoculation and (1.151 ± 0.049 kg) post-inoculation. There was significant difference in decrease in mean live body weight between treated group D (0.925 ± 0.033 kg) ($P < 0.05$) pre-inoculation and (0.995 ± 0.039 kg) post-inoculation compared to negative control K (1.084 ± 0.037 kg) pre-inoculation and (1.151 ± 0.049 kg) post-inoculation (Appendix 1). There was significant difference in decrease in mean live body weight at day 5 PTX ($P < 0.001$) between treated group A (1.046 ± 0.053 kg), C (1.055 ± 0.039 kg), and D (1.005 ± 0.048 kg) compared to negative control K (1.293 ± 0.073 kg). There was no significant difference in decrease in mean live body weight at day 5 PTX between treated group B (1.109 ± 0.060 kg) compared to negative control K (1.293 ± 0.073 kg) (figure 4.1).

4.7 Effect of Methanol Extract of *Anacardium occidentale* Leaves on Slaughter Body Weight of Shikabrown Pullets

The negative control group K hadsignificantly higher ($P < 0.05$) mean slaughter body weight (1,093±35.59g)followed by group I (1,030±61.9g), group E (971.66±107.4g), group H (964.33±49.27g)and group C (963.33±104.2g) (Figure 4.2).

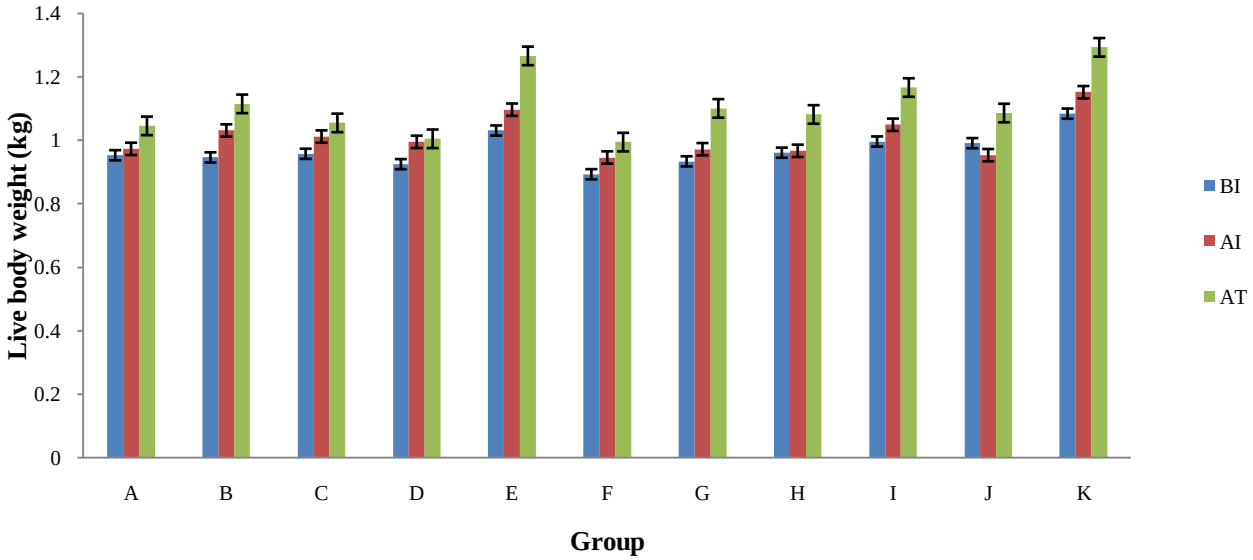


Figure 4.1: Decrease in mean live body weight of Shikabrown pullets before and after inoculation with experimental *Salmonella* Gallinarum and treated with different concentrations of methanol extract of *Anacardium occidentale*leaves.

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control, BI = pre-inoculation at sixteen week, AI = post-inoculation at seventeen week, AT = post-treatment at eighteen weeks of age.

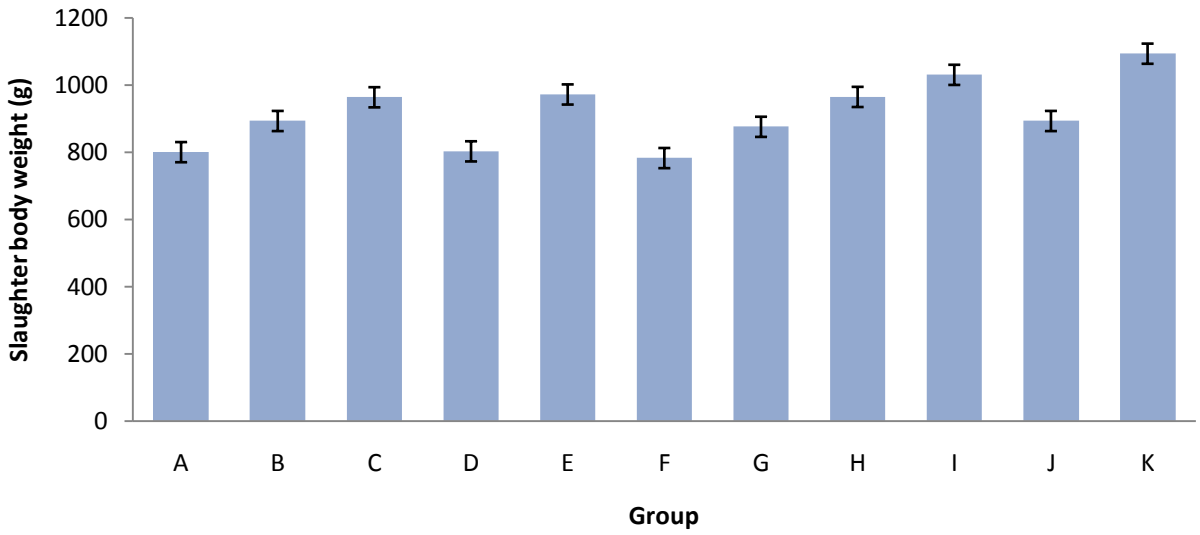


Figure 4.2: Decrease in mean body weight after slaughter in Shikabrown pullets inoculated with experimental *Salmonella* Gallinarum and treated with methanol extract of *Anacardium occidentale*leaves.

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control.

4.8 CloacalTemperature of Shikabrown Pullets Inoculated with Experimental *Salmonella* Gallinarum and Treated with Methanol Extract of *Anacardium occidentale* Leaves

Mean cloacal temperature (^{0}C) for treated groups A, B, and C ($P<0.05$) was 42.06, 41.96, and 41.52 but for treated group D ($P>0.05$) it was 42.60 post-inoculation when compared with positive control group J (43.23). Also, the mean cloacal temperature post treatment of groups A and C ($P < 0.05$), and D ($P<0.01$) were 41.78, 41.69 and 41.39 respectively. For treated group B ($P>0.05$) it was 41.85 compared to positive control J (42.86) (Figure 4.3).

4.9 *Salmonella* Gallinarum from Cloacal Swabs and Liver of Shikabrown Pullets Pre-inoculation, Post-inoculation and Post-treatment with Methanol Extract of *Anacardium occidentale*Leaves

After five days of treatment with methanol extract of *A. occidentale* Leaves, liver swabs of all the samples from all groups were negative of *S.*Gallinarum except those of positive control group J as shown (Table 4.5).

4.10 Changes in the Weight of the Liver, Spleen, Kidneys, Bursa of Fabricius and Thymus of Shikabrown Pullets inoculated with *Salmonella* Gallinarum and treated with Methanol Extract of *Anacardium occidentale* Leaves

Among the treated groups, group C showed increase in the weights of the kidneys (18.75%) compared to the control positive group J (3.05%) (Table 4.8). Changes observed in the weight of the thymus showed reduction, group I (NVRI vaccine) had 10.12 %, (foreign vaccine) had 7.03%, and C had 6.04 % and group B 4.70 %.Group A and D showed increase in thymic weight of 41.35% and 35.46%, respectively compared to the positive control (group J) which had 15.05% (Table 4.10). More so, the specific changes observed in liver, spleen and kidney was on an increase in their sizes (table 4.6 to 4.8) while the bursa of Fabricius and thymus showed reduction in size (table 4.9 and table 4.10).

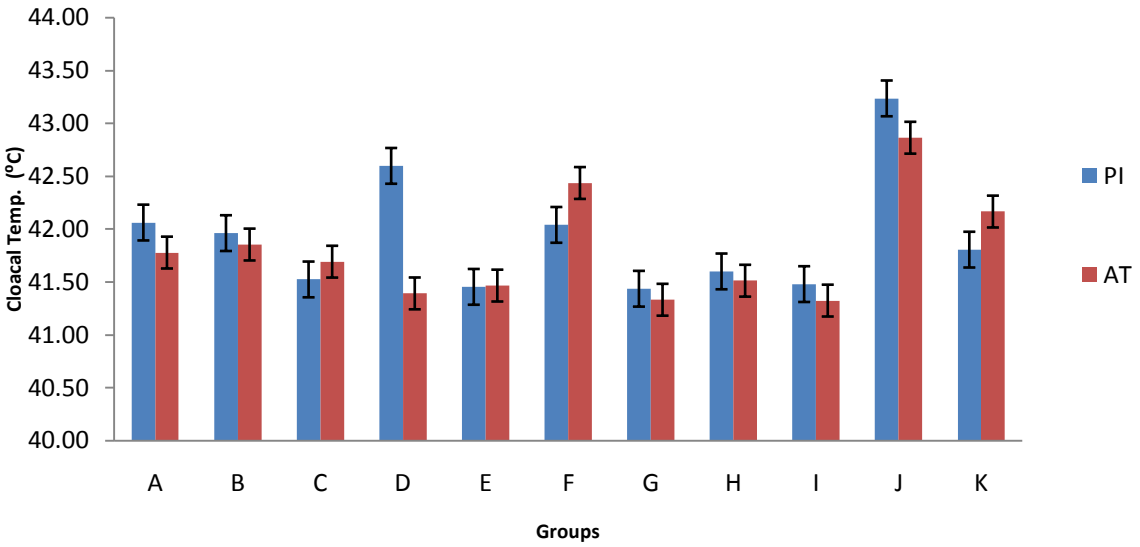


Figure 4.3: Changes in mean cloacal temperature of Shikabrown pullets post-inoculation with experimental *Salmonella* Gallinarum at sixteen weeks of age and treated with the methanol extract of *Anacardium occidentale*leaves for five days.

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control, PI = postinoculation, AT = treatment.

Table 4.5: Detection of *Salmonella* Gallinarum from the cloacal swabs, moribond and dead chicks of Shikabrown pullets on arrival, pre-inoculation, post inoculation and post treatment with methanol extract of the leaves of *Anacardium occidentale*.

Group	Birds on arrival (at day old)		Pre-inoculation (16 weeks of age)		Post inoculation (17 weeks of age)		Post treatment (18 weeks of age)	
	Sample	Result	Sample	Result	Sample	Result	Sample	Result
A	CS, MC	-	CS	-	CS	-	LV	-
B	CS, DC	-	CS	-	CS	-	LV	-
C	CS, DC	-	CS	-	CS	+	LV	-
D	CS, MC	-	CS	-	CS	-	LV	-
E	CS, DC	-	CS	-	CS	+	LV	-
F	CS, DC	-	CS	-	CS	-	LV	-
G	CS, DC	-	CS	-	CS	-	LV	-
H	CS, MC	-	CS	-	CS	+	LV	-
I	CS, DC	-	CS	-	CS	-	LV	-
J	CS, DC	-	CS	-	CS	-	LV	+
K	CS, DC	-	CS	-	CS	-	LV	-

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control, CS = cloacal swab, MC = moribond chick, DC = death chick, LV = liver, - = negative and + = positive.

Table 4.6: Changes in liver weight of Shikabrown pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days

Group	Type of treatment	Dose	%change	Type of change
I	Vaccination	0.5 ml	12.58	Increase
H	Vaccination	0.1 ml	13.14	Increase
E	Chemotherapy	100 mg/kg	13.79	Increase
C	Phytotherapy	150 mg/kg	23.18	Increase
B	Phytptherapy	100 mg/kg	28.45	Increase
G	Prophylacxis	200 mg/kg	32.26	Increase
J	Positive control	None	36.71	Increase
D	Phytotherapy	200 mg/kg	58.46	Increase
F	Prophylaxis	100 mg/kg	71.55	Increase
A	Phytothyrapy	50 mg/kg	87.59	Increase
K	Negative control	None	100.00	Normal

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control, mg = milligram, kg = kilogram, % = percentage, 0.5 = dose of NVRI vaccine administered by injection per pullet, 0.1 = dose of foreign vaccine administered orally per pullet.

Table 4.7: Changes in spleen weight of Shikabrown pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days

Group	Type of treatment	Dose	% change	Type of change
E	Chemotherapy	100 mg/kg	3.64	Increase
J	Positive control	None	19.55	Increase
I	Vaccination	0.5 ml	28.92	Increase
H	Vaccination	0.1 ml	44.99	Increase
C	Phytotherapy	150 mg/kg	56.65	Increase
B	Phytotherapy	100 mg/kg	62.49	Increase
G	Prohylacxis	200 mg/kg	73.11	Increase
F	Prophylaxis	100 mg/kg	129.00	Increase
D	Phytotherapy	200 mg/kg	131.00	Increase
A	Phytotherapy	50 mg/kg	174.00	Increase
K	Negative control	None	100.00	normal

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control,mg = milligram, kg = kilogram, % = percentage, 0.5 = dose of NVRI vaccine administered by injection per pullet, 0.1 = dose of foreign vaccine administered orally per pullet.

Table 4.8: Changes in kidney weight of pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days

Group	Type of treatment	Dose	% change	Type of change
F	Prophylaxis	100 mg/kg	0.90	Increase
J	Positive control	None	3.05	Increase
G	Prophylacxis	200 mg/kg	3.83	Increase
A	Phytotherapy	50 mg/kg	6.83	Increase
D	Phytotherapy	200 mg/kg	7.03	Increase
H	Vaccination	0.1 ml	7.93	Increase
E	Chemotherapy	100 mg/kg	12.48	Increase
B	Phytotherapy	100 mg/kg	14.71	Increase
C	Phytotherapy	150 mg/kg	18.75	Increase
I	Vaccination	0.5 ml	25.95	Increase
K	Negative control	None	100.00	Normal

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control, mg = milligram, kg = kilogram, % = percentage, 0.5 = dose of NVRI vaccine administered by injection per pullet, 0.1 = dose of foreign vaccine administered orally per pullet.

Table 4.9: Changes in bursa weight of Shikabrown pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days

Group	Type of treatment	Dose	% change	Type of change
B	Phytotherapy	100 mg/kg	76.17	Reduction
G	Prophylaxis	200 mg/kg	71.45	Reduction
D	Phytotherapy	200 mg/kg	65.15	Reduction
F	Prophylaxis	100 mg/kg	62.49	Reduction
E	Chemotherapy	100 mg/kg	62.37	Reduction
A	Phytotherapy	50 mg/kg	57.74	Reduction
J	Positive control	None	42.10	Reduction
H	Vaccination	0.1 ml	39.53	Reduction
C	Phytotherapy	150 mg/kg	38.90	Reduction
I	Vaccination	0.5 ml	16.86	Reduction
K	Negative control	None	100.00	Normal

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control,mg = milligram, kg = kilogram, % = percentage, 0.5 = dose of NVRI vaccine administered by injection per pullet, 0.1 = dose of foreign vaccine administered orally per pullet.

Table 4.10: Changes in thymus weight of Shikabrown pullets inoculated with *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of the leaves of *Anacardium occidentale* for five days

Group	Type of treatment	Dose	% change	Type of change
A	Phytotherapy	50 mg/kg	41.35	Reduction
D	Phytotherapy	200 mg/kg	35.46	Reduction
F	Prophylaxis	100 mg/kg	20.35	Reduction
J	Positive control	None	15.05	Reduction
I	Vaccination	0.5 ml	10.12	Reduction
H	Vaccination	0.1 ml	7.03	Reduction
C	Phytotherapy	150 mg/kg	6.04	Reduction
B	Phytotherapy	100 mg/kg	4.70	Reduction
E	Chemotherapy	100 mg/kg	3.89	Reduction
G	Prophylaxis	200 mg/kg	3.42	Reduction
K	Negative control	None	100.00	Normal

Key: A = RX 50 mg/kg, B = RX 100 mg/kg, C = RX 150 mg/kg, D = RX 200 mg/kg, E = TX Control drug 100 mg/kg, F = PX 100 mg/kg, G = PX 200 mg/kg, H = foreign vaccine, I = NVRI vaccine, J = positive control and K = negative control,mg = milligram, kg = kilogram, % = percentage, 0.5 = dose of NVRI vaccine administered by injection per pullet, 0.1 = dose of foreign vaccine administered orally per pullet.

Table 4.11: Some heamatological parameters of shikabrown pullets inoculated with experimental *Salmonella* Gallinarum at 16 weeks of age and treated with methanol extract of *Anacardium occidentale* leaves for five days

Group (mg/kg)/CD	Dose of extract	Haematological Parameters (MEAN±SEM)							
		PCV (%) RV(35-50)	HGB(g/dl) (2.5-4.5)	TRBC(cu mm) (2.5 and 5.5)	TP (g/dl)	TWBC(%)	Lymphocytes (%)	HETERO (%)	H/L RATIO
A	50	15.00 ± 4.04[a]	4.97±1.33[c]	2.63±0.63[c]	5.07±0.48[b]	11.60 ± 1.53[c]	82.00 ± 7.57[c]	17.00±7.37[c]	0.23±0.12
B	100	26.67 ± 1.76[a]	8.87±0.59[c]	4.43±0.30[c]	3.67±0.27[b]	8.97±1.03[c]	83.67 ± 0.88[c]	13.00±2.52[c]	0.15 ± 0.03
C	150	23.00 ± 1.53[a]	7.63±0.52[c]	3.93±0.29[c]	4.40±0.40[b]	11.03±1.52[c]	79.33 ± 5.21[c]	18.67±5.21[c]	0.25±0.08
D	200	17.00 ± 2.52[a]	5.63±0.82[c]	2.80±0.35[c]	4.40±0.35[b]	11.73±3.79[c]	87.67 ± 3.38[c]	10.33±4.48[c]	0.12±0.06
E	100 mg/ml	17.33 ± 8.97[a]	5.77±2.99[c]	2.97±1.51[c]	2.13±1.07[b]	6.93±4.04[c]	77.00 ± 0.58[c]	15.00±7.55[c]	0.29±0.01
F	100	21.67 ± 1.67[a]	7.17±0.57[c]	3.53±0.24[c]	3.93±0.07[b]	10.67±2.60[c]	74.67±5.7[c]	21.67±4.91[c]	0.30±0.08
G	200	24.33 ± 0.67[a]	8.07±0.23[c]	4.13±0.07[c]	4.00±0.00[b]	15.07±3.84[c]	84.00±2.00[c]	13.00± 1.00[c]	0.16±0.01
H	0.1ml	24.33 ± 0.67[a]	8.07±0.23[c]	4.07±0.03[c]	3.33±0.33[b]	10.63±1.81[c]	82.33±4.81[c]	15.67±4.70[c]	0.20±0.07
I	0.5ml	28.33 ± 4.37[a]	9.40±1.46[c]	4.77±0.72[c]	3.13±0.13[b]	10.07±0.84[c]	81.67±4.81[c]	17.33±4.18[c]	0.22±0.06
J	Saline	24.33 ± 1.20[a]	8.07±0.39[c]	4.07±0.19[c]	3.87±0.13[b]	9.53±0.79[c]	74.33±3.81[c]	20.67±1.76[c]	0.28±0.02
K	Saline	34.67 ± 1.45[a]	8.40±0.92[c]	4.30±0.51[c]	3.07±0.07[b]	8.93±0.66[c]	73.00±10.69[c]	26.67±10.71[c]	0.43±0.22

n=3 Data with superscript[a] in the same column are statistically significant (P<0.05), while data with superscript[b] in the same column are highly significant (P<0.01) and data with superscript[c] in the same column are not significant.

Key:CD= control drug, -ve= negative, +ve= positive,RV= reference value, PCV = packed cell volume, HGB = haemoglobin, TP = total protein, TRBC = total red blood cells, TWBC = total white blood cells, % = percentage, g/dl = gram per deciliter, cu mm = cubic millimeter, I = fowl typhoid vaccine, H = salmonella pullorum-gallinarum vaccine, K = negative control, J = positive control, E = standard antibiotic (ceftazidime), H/L = heterophils lymphocytes ratio.

4.11 Changes in some Haematological Parameters of Shikabrown Pullets Inoculated with Experimental *Salmonella* Gallinarum and Treated with the Methanol Extract of *Anacardium occidentale* Leaves

The PCV of the extract treated groups ranges between 15.00±4.04% to 26.67±1.76%, prophylactic group F and G had PCV of 21.67±1.67 % and 24.33±0.67%, vaccinated groups H and I had PCV of 24.33±0.67% and 28.33±4.37%, while positive and negative controls had PCV of 24.33±1.20% and 34.67±1.45%. The total protein value for all the groups ranged from 3.07 ± 0.07 to 5.07 ± 0.48 g/dl while it was 2.13±1.07 g/dl in Ceftazidime treated group E (Table 4.11).

4.12 Histopathological Changes in the Liver, Spleen and Kidneys of Shikabrown Pullets Inoculated with Experimental *Salmonella* Gallinarum and Treated with Methanol Extract of *Anacardium occidentale*Leaves

Microscopic findings in the liver of treated groups A, B, C and D showed sinusoidal congestion, congested hepatic and portal veins, multifocal and perivascular infiltration with heterophils and mononuclear cells. There was also dissociative necrosis of the hepatocytes as shown in Plate II. There was no lesion observed in the liver of Ceftazidime treated group E. The liver of prophylactic groups F and G also showed mild to moderate diffused and perivascular infiltration with heterophils and mononuclear cells, mild sinusoidal congestion and necrosis of the hepatocytes.

The liver of the vaccinated groups H and I showed mild congestion of the hepatic and portal veins (Plate IV). There was on lesion observed in the liver of the negative control K (Plate I). The liver of the positive control J showed marked congestion of hepatic vein, portal vein and sinusoids with severe necrosis of the hepatocytes (Plate II).

The spleen of the treated groups A, B, C and D showed numerous severe circumscribed multifocal perivascular aggregates of heterophils and mononuclear cells predominantly lymphocytes. There was also congestion of splenic veins with severe necrosis of lymphoid cells. Moderate necrosis of the lymphoid cells with multifocal aggregates of heterophils and mononuclear cells was observed in Ceftazidime treated group E. The spleen of the prophylactic groups F and G showed mild areas of necrosis with few multifocal and perivascular aggregates of heterophils and mononuclear cells. The vaccinated groups H and I showed mild areas of necrosis, few multifocal and perivascular aggregates of heterophils and Mononuclear cells predominantly lymphocytes.

The kidneys of the extract treated groups A, B, C and D showed congestion of blood vessels, Glomerular and tubular necrosis. Ceftazidime treated group E showed congested blood vessels with renal glomerular and tubular necrosis while prophylactic groups F and G showed infiltration with heterophils and mononuclear cells predominantly lymphocytes with glomerular and tubular necrosis. The vaccinated groups H and I showed tubular necrosis and heterophilic and Mononuclear cells infiltration while Positive control group J showed congested blood vessels and necrosis of the tubular epithelium.

Table 4.11: Some heamatological parameters of Shikabrown pullets inoculated with experimental *Salmonella* Gallinarum at 16 weeks and treated with methanol extract of *Anacardium occidentale* leaves for five days.

Groups	Dose of extract (mg/kg)/CD	Haematological Parameters (MEAN±SEM)					
		PCV (%) RV(35-50)	HGB(g/dl)	TP (g/dl) (2.5 and 5.5)	HETERO (%) (2.5-4.5)	TRBC(cu mm)	TWBC(%)
A	50	15.00±4.04^{a}	4.97±1.33^{c}	5.07±0.48^{b}	17.00±7.37^{c}	2.63±0.63^{c}	11.60±1.53^{c}
B	100	26.67±1.76^{a}	8.87±0.59^{c}	3.67±0.27^{b}	13.00±2.52^{c}	4.43±0.30^{c}	8.97±1.03^{c}
C	150	23.00±1.53^{a}	7.63±0.52^{c}	4.40±0.40^{b}	18.67±5.21^{c}	3.93±0.29^{c}	11.03±1.52^{c}
D	200	17.00±2.52^{a}	5.63±0.82^{c}	4.40±0.35^{b}	10.33±4.48^{c}	2.80±0.35^{c}	11.73±3.79^{c}
E (Ceftazidime)	100 mg/ml	17.33±8.97^{a}	5.77±2.99^{c}	2.13±1.07^{b}	15.00±7.55^{c}	2.97±1.51^{c}	6.93±4.04^{c}
F	100	21.67±1.67^{a}	7.17±0.57^{c}	3.93±0.07^{b}	21.67±4.91^{c}	3.53±0.24^{c}	10.67±2.60^{c}
G	200	24.33±0.67^{a}	8.07±0.23^{c}	4.00±0.00^{b}	13.00± 1.00^{c}	4.13±0.07^{c}	15.07±3.84^{c}
H (SPGV)	0.1ml	24.33±0.67^{a}	8.07±0.23^{c}	3.33±0.33^{b}	15.67±4.70^{c}	4.07±0.03^{c}	10.63±1.81^{c}
I (FTV)	0.5ml	28.33±4.37^{a}	9.40±1.46^{c}	3.13±0.13^{b}	17.33±4.18^{c}	4.77±0.72^{c}	10.07±0.84^{c}
J (+ve Control)	Saline	24.33±1.20^{a}	8.07±0.39^{c}	3.87±0.13^{b}	20.67±1.76^{c}	4.07±0.19^{c}	9.53±0.79^{c}
K (-ve Control)	Saline	34.67±1.45^{a}	8.40±0.92^{c}	3.07±0.07^{b}	26.67±10.71^{c}	4.30±0.51^{c}	8.93±0.66^{c}

n=3 Data with superscript^{a} in the same column are statistically significant ($P<0.05$), while data with superscript^{b} in the same column are highly significant ($P<0.01$) and data with superscript^{c} in the same column are not significant.

Key: CD= control drug, -ve= negative, +ve= positive,RV= reference value, PCV = packed cell volume, HGB = haemoglobin, TP = total protein, TRBC = total red blood cells, TWBC = total white blood cells, % = percentage, g/dl = gram per deciliter, cu mm = cubic millimeter, FTV = fowl typhoid vaccine, SPGV = salmonella pullorum-gallinarum vaccine.

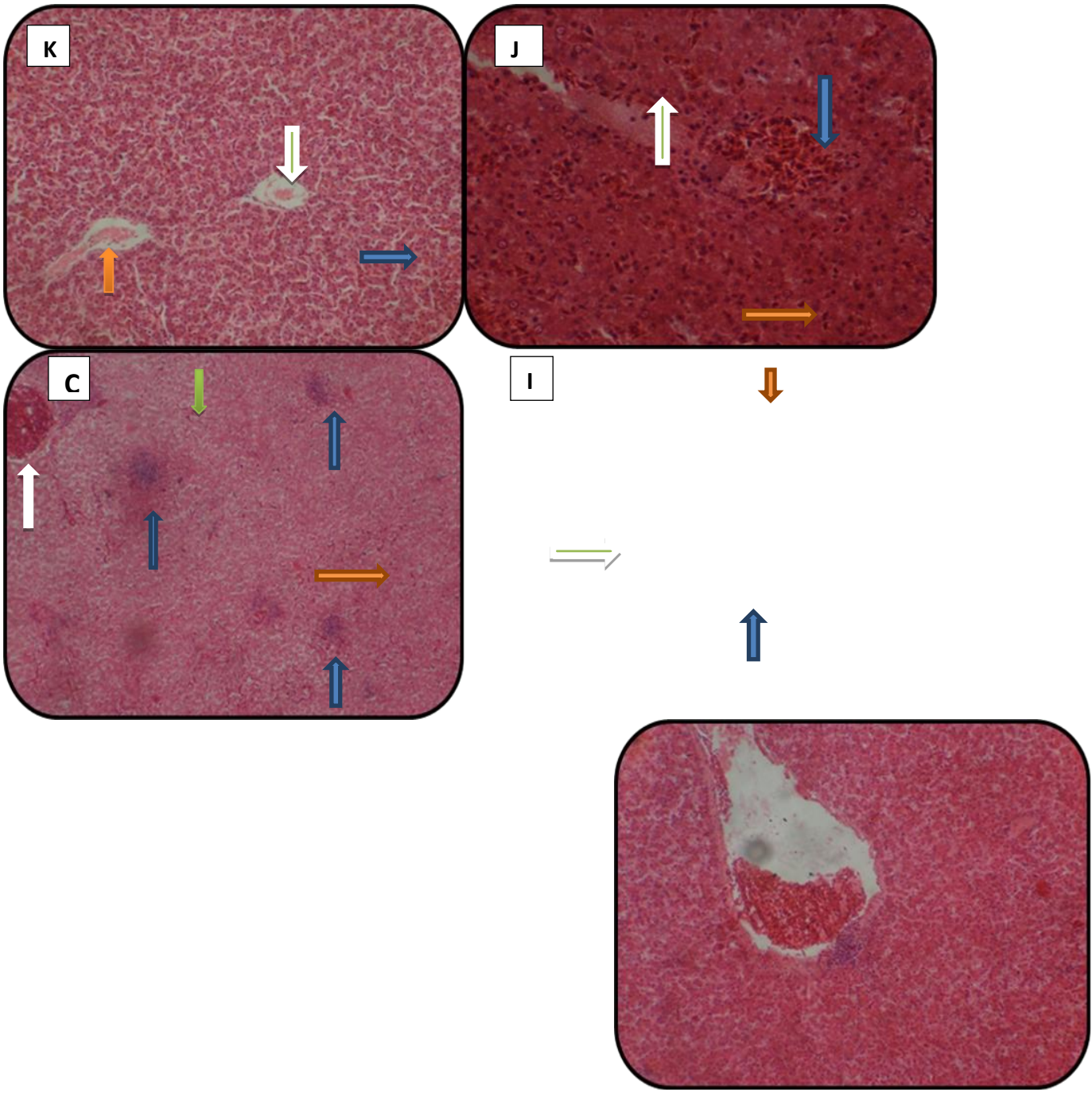


Plate II: Photomicrograph of liver of Shikabrown pullet (H&E X40) **K**: negative control with white arrow indicate uncongested hepatic vein, blue arrow indicate clean uncongested sinusoid, and yellow arrow indicate uncongested portalvein. **J:** positive control with blue arrow indicating marked congestion of portal vein,white arrow indicate marked sinusoidal congestion and yellow arrow indicating severe necrosis of hepatocytes. **C:** 150 mg/kg TX group with blue arrows indicating multifocal and perivascular infiltration of heterophils and mononuclear cells,white arrow indicate congested hepatic vein, green arrow indicating sinusoidal congestion and yellow arrow indicating dissociation and necrosis of hepatocytes, **I:** National Veterinary Research Institute vaccine vaccinated group with white arrow Indicating mild congestion of portal vein, blue arrow indicate mild perivascular aggregates of heterophils and mononuclear cells, yellow arrow indicating uncongested sinusoids.

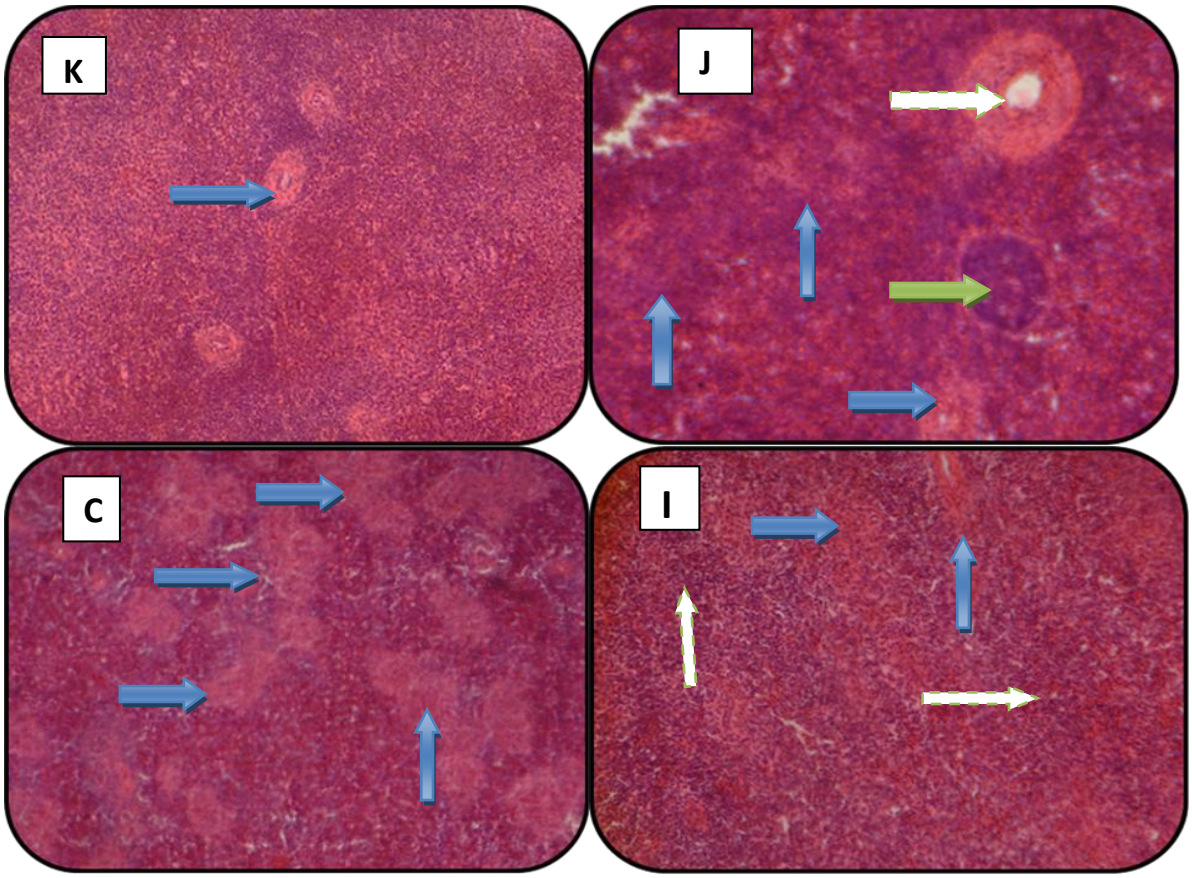
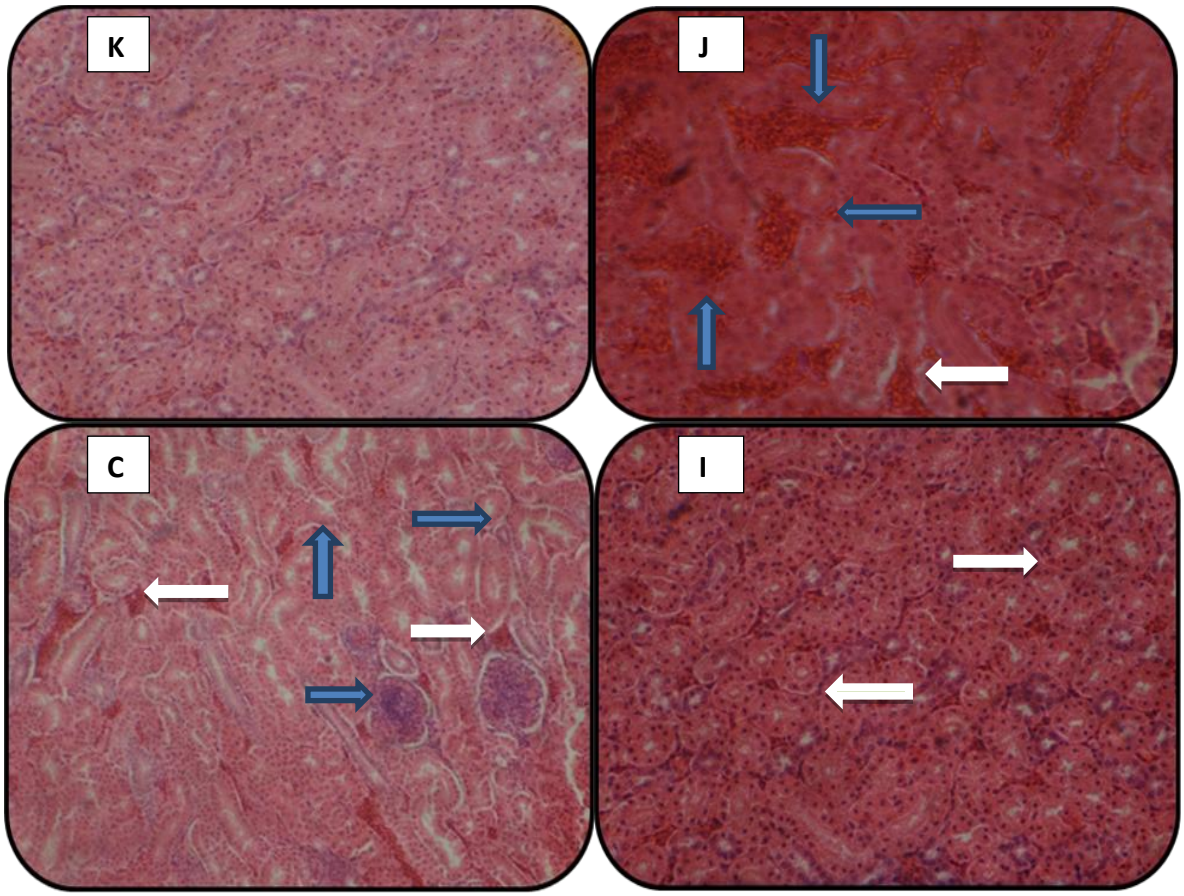


Plate III: Photomicrograph of the spleen of Shikabrown pullets (H&E X40) **K:** negative control with blue arrow indicatingsplenic artery, **J**: positive control with white arrow indicating splenic artery, green arrow indicating congested splenic vein, blue arrows indicating numerous severe necrotic areas, **C:** 150 mg/kgmethanol extract of *Anacardium occidentale* leaves treated group with blue arrows indicating numerous severe circumscribed necrotic areas, **J**: National Veterinary Research Institutevaccine vaccinated group with blue arrow indicating mild areas of necrotic and white arrows indicating multifocal aggregates of heterophils and mononuclear cell



PlateIV: Photomicrograph of the kidneys of pullets (H&E X40) **K:** negative control group **J:** positive control With white arrow indicating tubular necrosis, blue arrows indicating heterophils infiltration and MNC predominantly lymphocytes, **C:** 150 mg/kg TX group with blue arrows indicating tubular and glomerular Necrosis and white arrows indicating heterophils and MNC infiltration predominantly lymphocytes. **I:** NVRIvaccine vaccinated group with blue arrow indicatingtubular necrosis and white arrows indicating heterophils and MNC infiltration predominantly lymphocyte.

CHAPTER FIVE

DISCUSSION

The qualitative phytochemical screening of methanol extract of *A. occidentale*leaves revealed the following phytoconstituents; carbohydrates, alkaloids, tannins, saponins flavonoids, cardiac glycosides, steroids and triterpenes.Aiswarya *et al*. (2011) also reported the presence of steroids, glycosides, tannins, phenolicsand triterpenoids.However, alkaloids and carbohydrates were absence in the ethanol extract false fruit of *A. occidentale*.The absence of alkaloidsand carbohydrates from ethanol extract of the false fruit of *A. occidentale* might be attributed to the type of solvent used for extraction, the plant parts and location. Findings from this research werealso not consistent with findings of Desai*et al.* (2017)whoreportedthe absence of flavonoid from methanol extract of *A. occidentale* leaves and bark.

The methanol extract of *A. occidentale* leaves in pullets did not produce any toxic effect or mortality at a dose of 5,000 mg/kg. Therefore, it was considered as relatively safe and the $LD_{50}$of the methanol extract of *A. occidentale* leaves was assumed to be > 5,000 mg/kg.This finding agreed with Omoboyowa *et al*. (2013) who reported the LD_{50} of chloroform and ethanol extracts of AOkernel to be greater than 5000mg/kg in mice. However, many researchers used rat model to determine the LD_{50} of different medicinal plants,it is important to note that; this is the first time to determined LD_{50}using methanol extract of *A. occidentale* leaves in pullets.

Findings from this research revealed that, methanol extract of *A. occidentale* leaves had antibacterial *in vitro*activity against *S.*Gallinarum. This is consistent with the findings of Jothi *et al*. (2013)who reported the antimicrobial activity of methanol extract of *A. occidentale* leaves against *Porphyromonas gingivalis* and*Prevotella intermedia.*Findings from this work also agreed with that of Ayepola and Ishola (2009), who also foundmethanol extract of *A. occidentale* leaves to be highly effective against *Bacillus subtilis, Klebsiella pneumonia*and *E. coli*. Aderiye *et al*. (2015) reported that, anacardic acid, flavonoids and tannins found in *A. occidentale*were responsible to its antimicrobial effects through inactivation of microbial adhesions enzymes and cells envelop transport cells present in the bacterial organism. But flavonoids in particular caused inhibition of deoxyribonucleic acid gyrase enzyme in bacteria thereby inhibiting DNA synthesis (Aderiye *et al*., 2015). In addition,other molecular findings as reported by Cushnie and Lamb, (2006)on antibacterial mechanisms of action of selected flavonoids like quercetinhad been attributed to inhibition of deoxyribonucleic acid gyrase while sophoraflavone G and (-)-epigallocatechin gallate inhibit cytoplasmic membrane function and licochalcones A and C inhibit energy metabolism of the bacteria.Ajileye *et al*. (2015)reported the isolation of agathisflavone, a mixture of quercetin 3-O-rutinoside and quercetin 3-O-rhamnoside by bioassay-guided fractionation using DPPH autobiographic analysis ofthe ethyl acetate soluble fraction of methanol extract of *A. occidentale* leaves crude extract.

Tannins are polyphenols that can bind and precipitate proteins as reported by Chung *et al*. (1998) and Jothi *et al*. (2013).The different mechanisms proposed so far to explain tannin antimicrobial activity include inhibition of extracellular microbial enzymes, deprivation of

the substrates required for microbial growth or direct action on microbial metabolism through inhibition of oxidative phosphorylation (Augustin, 1991).But in this research we did not go into molecular level to look for the phytoconstituents responsible for the efficacy of methanol extract of *A. occidentale* leaves against *S.*Gallinarum. Therefore, the antibacterial activity against *S.*Gallinarum might be attributed to the presence of flavonoids and tannins detected from phytochemical screening ofmethanol extract of *A. occidentale* leaves.

In vitro antibacterial activity of methanol extract of *A. occidentale* leaves against *S.* Gallinarum from this research using agar well diffusion method showed zone of inhibition (ZI) of the standard drug Ceftazidime to be 46.4 mm which was higher than that of methanol extract of *A. occidentale* leaves, 18.5 mm at 100 mg/ml. This is consistent with finding of Sija *et al*. (2015) who also reported antibacterial activity of the standard streptomycin discs at10µg/ml against S. *aureus* and *E. coli*to be higher thanmethanol extract of young leaves of *A. occidentale* at 1 g/ml.

The highest ZI of18.5 mmproduced by methanol extract of *A. occidentale* leaves against *S.* Gallinarum at 100 mg/ml obtained from this research was higher than 15 mm reported by Sija *et al*. (2015)against*S. aureus* using methanol extract of young *A. occidentale* leaves at 1 g/ml. But it was lower than 26mm and 28mm reported by Aiswarya *et al*. (2011)against *B. cereus* and *K. pneumoniae* using alcohol extract of AOL. It was also lower than 18mm, 15mm, 14mm and 12mm reported by Rajesh *et al*. (2015) against *E. coli, Serratia marcescens, S. typhi and S. aureus*. Lower ZI of 4.0 mm and 7.0 mm had been reported by Ajileye *et al*. (2015) against *E. coli*and *B. subtilis*at 10.0 and 20.0 mg/ml using crude extract of *A. occidentale* leaves.

The clinical signs recorded in this research includes depression, anorexia, diarrhoea, weakness and inactivity and this is in agreement with reports of Shivaprasad, (2000) and Freitas *et al*. (2007) who reported that, clinical signs of fowltyphoid inyoung and adult birds includes anorexia, diarrhoea, dehydration, weakness. The methanol extract of *A. occidentale* leaves in this study eliminates the severity of clinical signs five days post-treatment in Shikabrown pullets orally inoculated with *Salmonella* Gallinarum.

The virulence of *Salmonella* Gallinarum(NE/03/2007) from this study was not consistent with thefindings of other researchersparticularly relative to the time mortality was recorded, at day 9 PI. Like Shivaprasad, (2000) reported that birds infected with *S.*Gallinarum may die within four days after exposure, but they usually started dying five days later and mortality commonly persists for 10 days. While Cardoso *et al*. (2013) observed mortality in quails due to *S.*Gallinarum started from 5 to11 days PI. Chadfield *et al*. (2003) also found that, mortality started 5 days PI in young broilers orally inoculated with *S.*Gallinarum.

There was statistically significant decrease in the mean body live weight ($P<0.01$) between treated group A, Cand D at 9 days PIand 5 days PTXwhen compare with the negative control, Kas shown in (figure 4.1). This finding agrees with those of Balicka-Ramisz *et al*. (2007) who reported slightly decreased weight gainin broilers infected with *S.*Gallinarumand treated with 3% chitosan in feed for seven days. The weight of the infected and treated groups was about 2.5% lower than negative control group. The lowest mean body live weight gain obtained from this study post-treatment with methanol extract of *A. occidentale* leaves is 1.005 ± 0.048 kg.

The negative control group K had the highest mean body weight of after slaughter, this might be attributed to the fact that, the group was uninoculated, untreated and

unvaccinated. It was followed by NVRI vaccine, ceftazidime antibiotic, foreign vaccine and TX 150 mg/kg. Among the treated groups, C had the highest mean weight and this is an indication that, 150mg/kg treatment dose is effective in reducing the severity of the clinical signsof FT in pullets with minimal effect on body weight.Meanwhile, among the prophylactic groups, G had the highest mean body weight, and between the vaccinated groups, NVRI vaccine had the highest mean body weight than the foreign vaccine. It is important to note that, there was no statistically significant difference in mean body weight in the treated, prophylactic and vaccinatedgroups after slaughter ($P>0.05$). But numerically there were differences in the mean body weight; this might be attributed to the fact that, *S.* Gallinarum caused an acute infection.

Normal PCV value ranges from 35-50 % in birds and is lowerin younger birds as reported by Jeannine-Miesle(2011). Birds with PCV values below 37% are considered anemic; and those with values below 15% may need blood transfusion (Jeannine-Miesle**,** 2011). A higher PCV level indicates dehydration or *polycythemia* (abnormally high number of erythrocytes). Polycythemia is an indication of bone marrow dysplasia and anemia-related disease like salmonellosis (Jeannine-Miesle**,** 2011). In this study, the PCV in treated groups was higher in group B followed by C. While between the prophylactic groups, G has the highest than F Also, NVRI vaccine had the highest PCV than foreign vaccine with. The PCV values of the treated, prophylactic and vaccinated groups were specifically lower when compared with negative control K. This agrees with the findings of Garcia*et al*. (2013) who reported that, in most cases of salmonellosis anaemia might occur in the acute phase of infection in inoculated groups while negative control would show no alteration in PCV value. It is important to note that the PCV (17.33±8.97 %) of control drug

(Ceftazidime) group E was lower than that of the negative control but higher than the treated groups A and D. The manufacturer reported that, ceftazidime rarely cause haemolytic anemia in humans.In pullets there was anaemia in group E treated with ceftazidime, possibly as a result of the acuteinfection with*S.* Gallinarum.

The TP level is an indicator of the health status of a patient and the normal value in birds is between 2.5 and 5.5 g/dl (Jeannine-Miesle**,** 2011). A low total protein value indicates chronic disease, especially of the liver and kidneys. The TP values in treated, prophylactic, vaccinated and control groups were within the normal ranges for birds except in ceftazidime treated group which was lower (2.13 ± 1.07 g/dl), ($P < 0.01$). This might be attributed to the fact that, *S.* Gallinarum infection was acute and TP values were normally lower in chronic infection ((Jeannine-Miesle**,** 2011).

Gross changes observed from the liver of pullet that died from the positive control group J includes bronze discolouration, white necrotic foci and hepatomegaly (Appendix 17). This agreed with the findings of Nazir *et al*. (2012) who reported that, bronze discoloration of liver was a prominent lesion in FT because *S.* Gallinarum has a predilection for the bile canaliculi which causes bile stasis in the liver. Bronze disclouration was also reported by others (Basnet *et al*., 2008;Nazir *et al*., 2012) in adult chickens infected with*S.* Gallinarum. Bronze discolouration of liver, mottling, necrotic and haemorrhagic foci in liver were also reported by Chishti *et al*. (1985) and Nazir *et al*.(2012). Results from this research found that, 150mg/kg treated dose in group C caused the least increase in liver weight and NVRI vaccine had the least increase in liver weight followed by foreign vaccine and control drug compared to the positive control J. This agreed with the findings of Shivaprasad (2000) and Saha *et al*. (2012) who reported that, FT is always associated with white necrotic foci and

enlargement of the liver. The least increase in the liver weight might be attributed to the fact that, methanol extract treatment dose of 150mg/kg, NVRI vaccine, foreign vaccine and ceftazidime were effective against*S.* Gallinarum*in vivo*.

Splenomegaly is a common finding of FT (Shivasprasd, 2000;Freitas *et al*., 2007; Saha *et al*., 2012). Treatment with methanol extract of *A. occidentale* leaves at a dose of 150 mg/kg had the least increase in spleen weight when compared with 50mg/kg, 200mg/kg and 100mg/kg. The result showed that, the extract might have effect on the spleen compared with non-treated positive control.

Among themethanol extract of *A. occidentale* leaves treated groups, 150mg/kg treated group C had the least reduction in bursa weight, while among the vaccinated groups, the NVRI vaccine had the least reduction when compared with positive control J. This agreed with the findings of Nazir *et al*. (2012) who reported mild to moderate atrophy in bursa of broilers infected with fowl typhoid.

Microscopic findings of the liver of treated groups A, B, C and D revealed sinusoidal congestion, congested hepatic and portal veins, multifocal and perivascular infiltration with heterophils and mononuclear cells. These findings agrees with Shivaprasad, (2000) and Nazir *et al*. (2012) who reported hepatocellular necrosis along with congestion and diffused perivascular mononuclear cell infiltration in liver of broilers infected with *S.*Gallinarum.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the findings of this study it was concluded that the methanol extract of *A. occidentale* leaves:

1. Contained carbohydrates, alkaloids, tannins, saponins, flavanoids, cardiac glycosides, steroids and triterpenes.
2. Was not toxic at a dose of 5,000 mg/kg, and therefore, considered as relatively safe.
3. Had antibacterial effect *in vitro*against *S.* Gallinarum (NE/03/2007) with zone of inhibition of 18.5 mm at 100 mg/ml.
4. Had phytobioticeffect on *S.*Gallinarum*in vivo*because inoculated bacterium was not detected in the liver of Shikabrown pullets five days post treatment.
5. Eliminated the severity of clinical signs from 1.6±0.5 at day 6 PI to 0.0±0.0 at day 5 PTX at the dose of 150mg/kgin Shikabrown pullets inoculated with *S.* Gallinarum as compared to positive control with severity of 2.3±0.7 at day 6 PI to 1.0±0.5 at day 11 PI.
6. Had phytoprophylactic effect because it prevented severity of clinical signs due to *S.* Gallinarum (0.0±0.0) 4DPI, (0.0±0.0) 5DPI, (0.3±0.2) 6DPI, (0.0±0.0) 7DPI, (0.0±0.0) 8DPI and (0.0±0.0) 9DPI a t a dose of 200 mg/kg when administered 14DBI with *S.* Gallinarum.

6.2 Recommendations

Based on the findings of this research it is recommended that;

1. Chronic toxicity study of methanol extract of *A. occidentale* leaves in pulletsbe carried out.
2. Quantitative phytochemical analyses and gas chromatography-mass spectrometry of methanol extract of *A. occidentale* leaves be conducted to ascertain the phytoconstituents responsible foritsefficacy against *S.*Gallinarum.
3. Indigenous fowl typhoid vaccine (FTV) and foreign oral *Salmonella* Gallinarum vaccine be administered by farmers to prevent FT.
4. On farm trial to treat FT using methanol extract of *A. occidentale* leaves at 150 mg/kg be carried out.
5. *In vitro* antibacterial study of ME of AOLagainst SG be carried out using Salmonella pathogenicity inland 1(SPI -1) and Salmonella pathogenicity Inland 2 (SPI-2).
6. *In vitro* study to determine minimum inhibitory concentration and minimum bacteriacidal concentration against *S.* Gallinarum should be carried out.

6.3 Limitations of the Research

1. The doses used in this study were limited to treatment of acute FT in pullets rather than chronic FT.
2. It was not possible to determine if the methanol extract of *A. occidentale* leaves has chronic toxic effects on pullets.

3. Liver enzymes and stress biomarkers were not assayed.
4. Microscopic lesions in tissues were not scored.
5. It was not possible to determine if phytoprophylactic doses of methanol extract of *A. occidentale* leaves were eliminated from body of the pullets.
6. As the histopathology of the bursa of Fabricius and thymus was not conducted it was not possible to determine the effects of treatment with methanol extract of *A. occidentale* leaves against *S.* Gallinarum on the organs.

6.4 Contributions of the Research to Knowledge

The present study contributed to scientific knowledge particularly in ethnoveterinary medicine, because it has provided base line data/ information for phytotherapeutic and phytoprophylactic potential of *A. occidentale* leaves in treating and preventing FT in poultry. The study also found that;

1. methanol extract of *A. occidentale* leaves eliminatedthe severity of clinical signs of FT in pullets at the treatment dose of 150 mg/kg.
2. had prophylactic effect against FT in pullets at a dose of 200 mg/kg, it was not toxic to pullets at a dose of 5,000 mg/kg.
3. indigenous and foreign fowl typhoid vaccines could be used by farmers to prevent FT.

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APPENDIXES

Appendix 1:Bonferroni posttests to compare the body weight of the pullets pre-inoculation, Post-inoculation and post-treatment

Groups	P- VALUES		
	PreinoculationPostinoculationPostreatment		
A vs B	P>0.05	P>0.05	P>0.05
A vs C	P>0.05	P>0.05	P>0.05
A vs D	P>0.05	P>0.05	P>0.05
A vs E	P>0.05	P>0.05	P<0.01 **
A vs F	P>0.05	P>0.05	P>0.05
A vs G	P>0.05	P>0.05	P>0.05
A vs H	P>0.05	P>0.05	P>0.05
A vs I	P>0.05	P>0.05	P>0.05
A vs J	P>0.05	P>0.05	P>0.05
A vs K	P>0.05	P<0.05*	P<0.001***
B vs C	P>0.05	P>0.05	P>0.05
B vs D	P>0.05	P>0.05	P>0.05
B vs E	P>0.05	P>0.05	P<0.05 *
B vs F	P>0.05	P>0.05	P>0.05
B vs G	P>0.05	P>0.05	P>0.05
B vs H	P>0.05	P>0.05	P>0.05
B vs I	P>0.05	P>0.05	P>0.05
B vs J	P>0.05	P>0.05	P>0.05
B vs K	P>0.05	P>0.05	P>0.05
C vs D	P>0.05	P>0.05	P>0.05
C vs E	P>0.05	P>0.05	P<0.01**
C vs F	P>0.05	P>0.05	P>0.05
C vs G	P>0.05	P>0.05	P>0.05
C vs H	P>0.05	P>0.05	P>0.05
C vs I	P>0.05	P>0.05	P>0.05
C vs J	P>0.05	P>0.05	P>0.05
C vs K	P>0.05	P>0.05	P<0.001***
D vs E	P>0.05	P>0.05	P<0.001***
D vs F	P>0.05	P>0.05	P>0.05
D vs G	P>0.05	P>0.05	P>0.05
D vs H	P>0.05	P>0.05	P>0.05
D vs I	P>0.05	P>0.05	P<0.05*
D vs J	P>0.05	P>0.05	P>0.05
D vs K	P<0.05*	P<0.05*	P<0.001***
E vs F	P>0.05	P>0.05	P<0.001***
E vs G	P>0.05	P>0.05	P<0.05*
E vs H	P>0.05	P>0.05	P<0.05*
E vs I	P>0.05	P>0.05	P>0.05
E vs J	P>0.05	P>0.05	P<0.05*
E vs K	P>0.05	P>0.05	P>0.05
F vs G	P>0.05	P>0.05	P>0.05
F vs H	P>0.05	P>0.05	P>0.05
F vs I	P>0.05	P>0.05	P<0.05*
F vs J	P>0.05	P>0.05	P>0.05
F vs K	P<0.01**	P<0.01**	P<0.001***
G vs H	P>0.05	P>0.05	P>0.05
G vs I	P>0.05	P>0.05	P>0.05
G vs J	P>0.05	P>0.05	P>0.05
G vs K	P>0.05	P<0.05*	P<0.01**
H vs I	P>0.05	P>0.05	P>0.05
H vs J	P>0.05	P>0.05	P>0.05
H vs K	P>0.05	P<0.05*	P<0.01**
I vs J	P>0.05	P>0.05	P>0.05
I vs K	P>0.05	P>0.05	P>0.05
J vs K	P>0.05	P<0.01**	P<0.01**

*= significant, **= highly significant and ***= strongly significant

Appendix 2: Acute toxicity level of methanol extract of *Anacardium occidentale* leaves on pullets

Groups	**Methanol extract**		
	Dose level mg/kg	**n**	**N**
1	350	3	0
2	500	3	0
3	1000	3	0
4	1600	1	0
5	2900	1	0
6	5000	1	0

n =number of experimental birds, **N** =number of dead birds

The volumes of the extract doses administered to the pullets were calculated using the following

Formula: Volume (ml) = body weight (kg) x dose levels (mg/kg)/stock drug concentration

(mg/ml). The extract was administered orally.

The LD_{50} was calculated as the geometric mean of doses that produce 0% and 100% mortality using the formula below.

$LD_{50} = \sqrt{(D_0 \times D_{100})}$

D_0= Highest dose that gave no mortality,

D_{100}= Lowest dose that produced mortality.

Appendix 3: Severity of clinical signs in Shikabrown pullets inoculated with Salmonella Gallinarum at sixteen weeks and treated with methanol extract of the leaves of *Anacardium occidentale* (day four post inoculation)

Group	Depression	Sneezing	Coughing	Diarrhoea	Anorexia	Inactivity	Weakness	Mean±SEM
A	1	0	0	0	2	1	0	0.6 ± 0.3
B	1	0	0	0	2	0	1	0.6 ± 0.3
C	2	0	0	0	2	0	0	0.6 ± 0.4
D	2	4	4	0	2	0	0	1.7 ± 0.7
E	1	0	0	0	2	0	1	0.6 ± 0.3
F	1	0	0	0	0	0	0	0.1 ± 0.1
G	0	0	0	0	0	0	0	0.0 ± 0.0
H	0	0	0	0	0	0	0	0.0 ± 0.0
I	0	0	0	0	0	0	0	0.0 ± 0.0
J	2	0	0	0	2	0	0	0.6 ± 0.4
K	0	0	0	0	0	0	0	0.0 ± 0.0
Mean±SEM	0.9 ± 0.3	0.4 ± 0.4	0.4 ± 0.4	0.0 ± 0.0	1.1 ± 0.3	0.1 ± 0.1	0.2 ± 0.1	

0 = absence, 1 = slightly severe, 2 = moderately severe, 3 = severe, 4 =highly severe, 5 = strongly severe

Appendix 4: **Severity of clinical signs in Shikabrown pullets inoculated with Salmonella Gallinarum at sixteen weeks and treated with methanol extract of the leaves of *Anacardium occidentale* (day five post inoculation and day one post treatment)**

Group	Depression	Sneezing	Coughing	Diarrhea	Anorexia	Inactivity	Weakness	Mean±SEM
A	2	0	0	1	2	1	1	1.0 ± 0.3
B	3	0	0	2	3	1	1	1.4 ± 0.5
C	2	0	0	3	2	1	1	1.3 ± 0.4
D	3	4	4	3	4	1	1	2.9 ± 0.5
E	3	0	0	4	2	0	1	1.4 ± 0.6
F	3	0	0	0	0	0	0	0.4 ± 0.4
G	0	0	0	0	0	0	0	0.0 ± 0.0
H	0	0	0	0	0	0	0	0.0 ± 0.0
I	0	0	0	0	0	0	0	0.0 ± 0.0
J	2	0	0	0	3	3	0	1.1 ± 0.6
K	0	0	0	0	0	0	0	0.0 ± 0.0
Mean±SEM	1.6±0.4	0.4±0.4	0.4±0.4	1.2±0.5	1.5±0.6	0.6±0.3	0.5±0.2	

0 = absence, 1= slightly severe, 2 = moderately severe, 3 = severe, 4 = highly severe, 5 = strongly severe

Appendix 5: Severity of clinical signs in Shikabrown pullets inoculated with Salmonella Gallinarum at sixteen weeks and treated with methanol extract of the leaves of *Anacardium occidentale* (day seven post inoculation and day three post treatment)

Groups	Depression	Sneezing	Coughing	Diarrhea	Anorexia	Inactivity	Weakness	Mean±SEM
A	0	0	0	1	0	0	0	0.1 ± 0.1
B	0	0	0	1	0	0	0	0.1 ± 0.1
C	0	0	0	1	0	0	0	0.1 ± 0.1
D	0	2	0	1	0	0	0	0.4 ± 0.3
E	0	0	0	1	0	0	0	0.1 ± 0.1
F	1	0	0	1	0	0	1	0.4 ± 0.2
G	0	0	0	0	0	0	0	0.0 ± 0.0
H	0	0	0	1	0	0	0	0.1 ± 0.1
I	0	0	0	0	0	0	0	0.0 ± 0.0
J	5	0	0	4	5	3	4	3.0 ± 0.8
K	0	0	0	0	0	0	0	0.0 ± 0.0
Mean±SEM	0.6±0.5	0.2±0.2	0.0±0.0	1.0±0.3	0.5±0.5	0.3±0.3	0.5±0.4	

0 = absence, 1= slightly severe, 2 = moderately severe, 3 = severe, 4 = highly severe, 5 = strongly severe

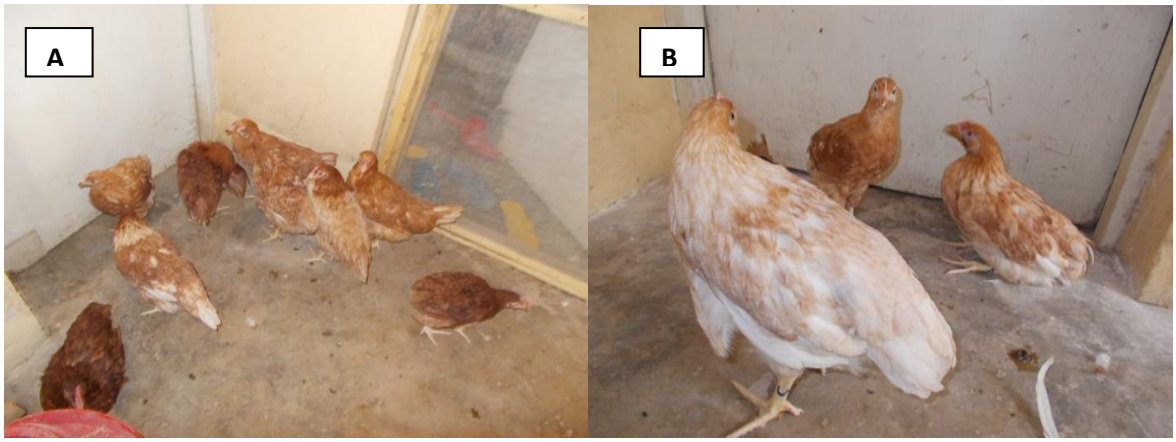
Appendix 6: Severity of clinical signs in Shika brown pullets inoculated with Salmonella Gallinarumat sixteen weeks and treated with methanol extract of the leaves of *Anacardium occidentale*(day eight post inoculation and day four post treatment)

Groups	Depression	Sneezing	Coughing	Diarrhea	Anorexia	Inactivity	Weakness	Mean±SEM
A	2	0	0	4	0	0	0	0.9 ± 0.6
B	1	0	0	0	0	0	0	0.1 ± 0.1
C	1	0	0	1	0	0	0	0.3 ± 0.2
D	0	2	0	0	0	0	0	0.3 ± 0.3
E	4	0	0	1	0	0	0	0.7 ± 0.6
F	3	0	0	3	0	0	0	0.9 ± 0.6
G	0	0	0	0	0	0	0	0.0 ± 0.0
H	0	0	0	2	0	0	0	0.3 ± 0.3
I	1	0	0	0	0	0	0	0.1 ± 0.1
J	3	0	0	4	0	0	0	1.0 ± 0.7
K	0	0	0	0	0	0	0	0.0 ± 0.0
Mean±SEM	1.4±0.4	0.2±0.2	0.0±0.0	1.4±0.5	0.0±0.0	0.0±0.0	0.0±0.0	

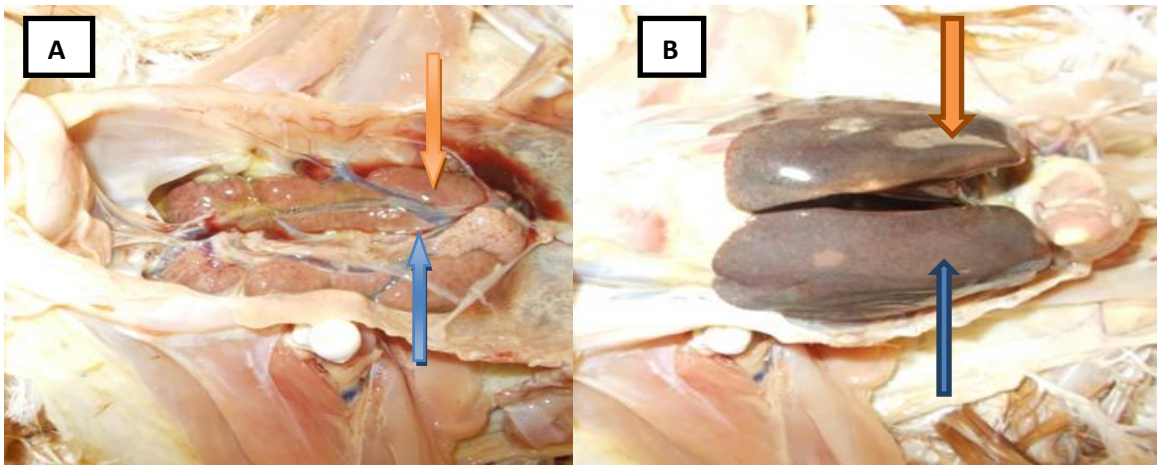
0 = absence, 1= slightly severe, 2 = moderately severe, 3 = severe, 4 = highly severe, 5 = strongly severe



Appendix 7: Digital electronic scale model: EK5350, ISO 9001 certified by SGS Made in China used to weigh the individual pullets and Methanol Extract of *Anacardium occidentale* leaves.



Appendix 8: Shikabrown pullets after administration of methanol extract of the leaves of *Anacardium occidentale* in first phase and second phase of LD_{50}determination.



Appendix 9: Gross pathology of the liver (B) of pullet that died from positive control group with yellow arrow indicating hepatomegaly and blue arrow indicating white necrotic foci,