EFFECTS OF DETOXIFICATION ON SOME PHARMACOLOGICAL PROPERTIES OF *JATROPHA CURCAS* LINN. (EUPHORBIACEAE) SEED OIL IN LABORATORY ANIMALS

By

Shola Paul EMIADE, B.Sc Biochemistry (ABU) 2011
MSc/Pharm Sci/38610/2012-2013

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A
MASTER DEGREE IN PHARMACOLOGY.

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
FACULTY OF PHARMACEUTICAL SCIENCES
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

MARCH, 2016

Declaration

I declare that the work in this dissertation entitled "EFFECTS OF DETOXIFICATION ON SOME PHARMACOLOGICAL PROPERTIES OF *JATROPHA CURCAS* LINN. (EUPHORBIACEAE) SEED OIL IN LABORATORY ANIMALS" has been carried out by me in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria under the supervisions of Prof. (Mrs.) H.O. Kwanashie, Prof. B.A. Chindo and Dr. J.I. Ejiofor. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project was previously presented for another degree or diploma at this or any other Institution.

Shola Paul EMIADE	_____	_____
Name of Student	Signature	Date

Certification

This dissertation entitled "EFFECTS OF DETOXIFICATION ON SOME PHARMACOLOGICAL PROPERTIES OF *JATROPHA CURCAS* LINN. (EUPHORBIACEAE) SEED OIL IN LABORATORY ANIMALS" by Shola Paul EMIADE meets the regulations governing the award of the degree of Master of Science in Pharmacology of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Prof. (Mrs.) H.O. Kwanashie		
Chairman, Supervisory committee	Signature	Date
Prof. B.A. Chindo		
Member Supervisory committee	Signature	Date
Dr. J.I. Ejiofor		
Member supervisory committee	Signature	Date
Dr. N.M. Danjuma		
Head, Department of Pharmacology and Therapeautics	Signature	Date
Prof. K. Bala		
Dean, School of Postgraduate Studies	Signature	Date

Dedication

This work is dedicated to the glory of God and my lovely family for their support during the course of this programme.

Acknowledgement

I wish to sincerely express my profound gratitude to Almighty God, for His infinite mercy, grace, favour, and divine provision through this programme.

I am greatly indebted to my Supervisors, Prof. (Mrs.) H.O. Kwanashie, Prof. B.A. Chindo and Dr. J.I. Ejiofor for their parental guidance and determination to get this study done against all odds. I will always cherish your encouragement and support.

I thank the Head of Pharmacology and Therapeutics Department, Dr N.M. Danjuma for his support.

I wish to also thank all academic and non-academic staff of the department, all of whom have been of help to me in one way or the other.

My appreciation also goes to the entire technologists of the department, especially Mallam Mohammed, Mr. John, Mallam Salihu, Mallam Aliyu, Mallam Hassan and Alhaji Yau as well as Mallam Kabir and Mallam Bala Riti of Department of Pharmacognosy and Pharmaceutical and Medicinal Chemistry respectively, for their immense assistance during the experimental stage of this work.

I am thankful to my lovely parents Mr. and Mrs. S.A. Emiade, my siblings, Sanmi and Ruth for their prayers and encouragements.

I also appreciate my colleagues and friends, Isaac Agyigra, Mohammed Highab, Dr. Iwuozo, Ahmed Danbala, James Fatika, Oyeronke Oyeleke, Fatima Bello and Inekwe Victory. Thanks for being there.

Abstract

Jatropha curcas seed oil is a known energy source in biodiesel which but for its toxicity, also has potential of being used as food and in the pharmaceutical / cosmetic industries. This study was designed to detoxify solvent and mechanically extracted *J. curcas* oils and to ascertain the effects of detoxification on acute and sub-acute toxicities as well as on analgesic and laxative activities in laboratory animals. Following determination of the physicochemical properties of the extracted oils, detoxification of the oils was attempted by both the usual synthetic method of orthophosphoric acid degumming, sodium hydroxide deacidification, tonsil decolourisation and vacuum-heat deodorization; and by the use of natural materials in place of the synthetic agents such as distilled water for orthophosphoric acid, plantain peel ash extract for sodium hydroxide and bentonite for tonsil. Phytoconstituents, elemental and phorbol esters compositions of the oils were also determined. Acute toxicity studies for LD_{50} determination and sub-acute (14 days) studies for toxicity symptoms of the mechanically extracted oil in male rats were carried out. Weight changes, mortality, haematological liver / kidney assessment as well as histopathological analyses of liver, kidney and intestines were checked. Wherever appropriate olive oil was used as vehicular control. Statistical analyses were by ANOVA with appropriate post hoc tests. The extraction yields of the oils were 49.1% (petroleum ether solvent) and 14.9% (hand operated hydraulic screw press). The physicochemical properties of the extracted oils were similar. Alkaloids were the only phytoconstituent present in both the undetoxified and detoxified oils. Detoxification resulted in oil losses upward of 60%, but with reasonable amounts of formed soaps (fatty-acid precipitate). Elemental analyses showed higher toxic elements (lead, cadmium and copper) and nutritional elements (iron, calcium and zinc) in the undetoxified oils compared to detoxified. The level of phorbol ester rich fraction of solvent extracted oil detoxified with synthetic materials was significantly ($p<0.05$) reduced, but such reduction was not significant ($p>0.05$) for mechanically extracted oils detoxified either with synthetic or more natural materials. The oral LD_{50} of the detoxified oils of both extraction processes in both 3-day old cockerels and adult male mice were above 5,000 mg/kg. Sub-acute oral treatment with 125, 250 and 500 mg/kg of mechanically extracted oil resulted in dose-dependent toxic symptoms of ruffled fur, hyperpnoea, restlessness and decreased mobility mostly of the

undetoxified (moderate to severe) as opposed to the detoxified oil (mostly mild). The rats that received the undetoxified oil suffered weight loss and dose-dependent mortality, but there was neither weight loss nor mortality in rats treated with the detoxified oil. Administration of the undetoxified oil caused significant ($p<0.05$) dose-dependent increased levels of WBC and platelets and also of serum Na^+. Both the undetoxified and detoxified oils produced significantly ($p<0.05$) increased levels of kidney function (urea and creatinine) as well as liver function indices (serum albumin and enzymes, ALT, ALP, AST), but the increase in AST was more for the undetoxified oil. The undetoxified oil at 500 mg/kg, resulted in reduced liver and kidney to body weight ratios. Both the undetoxified and detoxifed oils at 250 and 500 mg/kg showed significant ($P<0.05$) central and peripheral analgesic activity, which was dose-dependent only for the undetoxified oil. The laxative activity in mice was not significant ($p>0.05$) for any of the oils. Histopathological examinations of the liver, kidney and intestines indicated a dose-dependent moderate to severe necrosis and degeneration of tissues from the undetoxified oil**,** while detoxified oil produced mild degeneration of tissues. But for the high oil losses in the process of detoxification, the detoxified oil, based on the various indices studied, ameliorated the toxic effects of *J. curcas* oil and revealed its potential for use medicinally.

TABLE OF CONTENT

Title Page---------- i

Declaration---------- ii

Certification---------- iii

Dedication---------- iv

Acknowledgement---------- v

Abstract---------- vi

Table of Contents---------- viii

List of Figures---------- xiii

List of Tables---------- xiv

List of Plates---------- xv

List of Appendices---------- xvii

List of Abbreviations---------- xviii

1.0 INTRODUCTION----------1

1.1 Background of the Study----------1

1.2 Statement of Research Problem----------3

1.3 Justification of the Study----------5

1.4 Aim and Objectives of the Study----------5

1.4.1 Aim---------- 5

1.4.2 Specific objectives---------- 6

2.0 LITERATURE REVIEW---------- 7

2.1 Overview of Plant Toxicity---------- 7

2.2 Common Plant Toxins---------- 8

2.2.1 Alkaloids---------- 9

2.2.2 Cyanogenic glycosides---------- 9

2.2.3. Lectins---------- 11

2.2.4 Saponin---------- 11

2.2.5 Tannins---------- 11

2.2.6 Phytates---------- 11

2.2.7 Trypsin inhibitors---------- 12

2.2.8 Phorbol esters---------- 12

2.2.9 Curcin---------- 13

2.3 Factors Influencing Plant Toxicity---------- 13

2.4 Classification of Poisonous Plants ---------- 13

2.4.1 Plants that affect the digestive system---------- 13

2.4.2 Plants that affect the blood---------- 14

2.4.3 Cardiotoxic plants---------- 15

2. 4.4 Hepatotoxic plants---------- 15

2 4.5 Plants that affect the urinary system---------- 16

2.4.6 Plants that affect the nervous system----------16

2.4.7 Plants that affect the skin---------- 17

2.5 Prevention and Defenses Against Plant Poisoning---------- 17

2.6 The Genus *Jatropha*---------- 18

2.6.1 *Jatropha curcas*---------- 19

2.6.2 Origin and distribution of *Jatropha curcas*----------19

2.6.3 Names of *Jatropha curcas*----------19

2.6.4 Taxonomic hierarchy----------20

2.6.5 Description of the plant----------20

2.7 Products of *Jatropha curcas*----------21

2.7.1 *Jatropha curcas* seed cake----------21

2.7.2 *Jatropha curcas* seed shell----------21

2.7.3 *Jatropha curcas* seed oil----------23

2.8 Uses of *Jatropha curcas* Oil----------23

2.9 Methods of Extraction of *Jatropha curcas* Seed Oil----------24

2.9.1 Solvent extraction----------24

2.9.2 Aqueous enzymatic oil extraction----------24

2.9.3 Mechanical extraction----------25

2.10 Toxic Components in *Jatropha curcas* Seed Oil----------25

2.10.1 Phorbol esters----------25

2.10.2 Curcin----------26

2.11 Methods of Detoxification of *Jatropha curcas* Seed Oil----------26

2.11.1 Physical method----------26

2.11.2 Conventional edible oil refining methods----------27

3.0 MATERIALS AND METHODS----------29

3.1 Materials----------29

3.1.1 Collection of plant materials----------29

3.1.2 Experimental animals----------29

3.1.3 Ethics statement---------- 30

3.1.4 Chemicals, solvents and drugs---------- 30

3.2 Methods----------30

3.2.1 Extraction of seed oils and determination of yield---------- 31

3.2.2 Determination of physicochemical properties---------- 31

3.2.3 Detoxification of *Jatropha curcas*---------- 33

3.2.4 Phytochemical screening---------- 36

3.2.5 Acid digestion of oil samples and determination of elements---------- 38

3.2.6 Isolation and estimation of phorbol esters from *Jatropha curcas* oils---------- 38

3.2.7 Acute toxicity studies of *Jatropha curcas* seed oils---------- 39

3.2.8 Sub-acute toxicity of *Jatropha curcas* oils---------- 40

3.2.9 Pharmacological studies---------- 42

3.2.10 Statistical analysis---------- 43

4.0 RESULTS

4.1 Oil Yields from Both Extraction Methods----------44

4.2 Physicochemical Properties of *Jatropha curcas* Oils----------45

4.3 Detoxification of *Jatropha curcas* Oils----------46

4.4 Qualitative Phytochemical Analysis---------- 47

4.5 Mineral Element Concentration of *Jatropha curcas* Oils----------48

4.6 Phorbol Ester Rich Fraction of *Jatropha curcas* Oils----------49

4.7 Median Lethal Dose (LD_{50}) Values of Acute Toxicity Studies---------- 50

4.8 Physical Toxicity Symptoms of Undetoxified and Detoxified *Jatropha curcas* Oils in Male Wistar Rats----------51

4.9 Body Weight Changes and Mortality in Male Wistar Rats that Received Undetoxified and Detoxified *J. curcas* Oils ---------- 52

4.10 Haematological Parameters in Male Wistar Rats That Received Undetoxified and Detoxified *J. curcas* Oils ---------- 53

4.11 Serum Electrolytes and Kidney Function Indices in Male Wistar Rats that Received Undetoxified and **Detoxified *J. curcas* Oils---------- 55**

4.12 Liver Function Enzymes and Serum Albumin in Male Wistar Rats that Received Undetoxified and Detoxified *J. curcas* Oils---------- 56

4.13 Liver and Kidney to Body Weight Ratios in Male Wistar Rats that Received Undetoxified and Detoxified *J. curcas* Oils ---------- 57

4.14 Effects of Undetoxified and Detoxified *J. curcas* Oils on Formalin-Induced Paw Licking in Mice---------- 59

4.15 Effects of Undetoxified and Detoxified *J. curcas* Oils on Hot Plate Latency in Mice---------- 60

4.16 Effects of Undetoxified and Detoxified *J. curcas* Oils on Hot Plate Induced Pain Inhibition---------- 61

4.17 Laxative Effects of Undetoxified and Detoxified *J. curcas* Oils---------- 62

4.18 Effects of Undetoxified and Detoxified *Jatropha curcas* Oils on Liver, Kidney and Intestinal Tissues---------- 64

4.18.1 Effects of undetoxified and detoxified *Jatropha curcas* oils on liver tissues---------- 63

4.19.1 Effects of undetoxified and detoxified *Jatropha curcas* oils on kidney tissues---------- 66

4.20.3 Effects of undetoxified and detoxified *Jatropha curcas* oils on GIT tissues---------- 69

5.0 DISCUSSION---------- 72

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS-------- 83

REFERENCES---------- 86

APPENDICES---------- 101

List of Figures

Figure Page

2.1: The fruit, seeds, oil and defatted seed cake of *Jatropha curcas*----------22

4.1: Effects of synthetic and natural detoxification processes on levels of phorbol ester rich fractions in solvent and mechanically extracted *Jatropha curcas* oils-----49

4.2: Liver to body weight ratio in male Wistar rats that received undetoxified and detoxified *Jatropha curcas* oils---------- 57

4.3: Kidney to body weight ratio in male Wistar rats that received undetoxified and detoxified *Jatropha curcas* oils---------- 58

4.4: Effects of undetoxified and detoxified *Jatropha curcas* oils on formalin induced paw licking in mice.----------59

4.5: Effects of undetoxified and detoxified *Jatropha curcas* oils on hot plate induced pain inhibition----------61

List of Tables

Table Page

4.1 Percentage yield of *Jatropha curcas* seed oils extracted with solvent and mechanically---------- 44

4.2: Physicochemical properties of solvent and mechanically extracted *Jatropha curcas* oils---------- 45

4.3: Oil recoveries, losses and soaps formed during the various stages of detoxification of *Jatropha curcas* oils---------- 46

4.4: Phytochemical constituents of undetoxified and detoxified solvent and mechanically extracted *Jatropha curcas* oils---------- 47

4.5: Mineral element concentrations of undetoxified and detoxified *Jatropha curcas* oils---------- 48

4.6: LD_{50} of undetoxified and detoxified solvent and mechanically extracted *Jatropha curcas* oils---------- 50

4.7: Physical toxicity symptoms of undetoxified and detoxified *Jatropha curcas* oils in male Wistar rats---------- 51

4.8: Body weight changes and mortality in male Wistar rats that received undetoxified and detoxified *Jatropha curcas* oils---------- 52

4.9: Haematological parameters in male Wistar rats that received undetoxified and detoxified *Jatropha curcas* oils ---------- 54

4.10: Serum electrolyte and kidney function indices in male Wistar rats that received undetoxified and detoxified *Jatropha curcas* oils---------- 55

4.11: Liver function enzymes and serum albumin in male Wistar rats that received undetoxified and detoxified *Jatropha curcas* oils---------- 56

4.12: Effects of undetoxified and detoxified *Jatropha curcas* oils on hot plate latency in mice---------- 60

4.13: Laxative effects of undetoxified and detoxified *Jatropha curcas* oils------- 62

List of Plates

Plate Page

Plate I: Normal liver tissues (control)---------- 63

Plate II: Effects of undetoxified *J. curcas* oil on liver tissues (125 mg/kg)-------- 63

Plate III: Effects of detoxified *J. curcas* oil on liver tissues (125 mg/kg)---------- 63

Plate IV: Normal liver tissues (control)---------- 64

Plate V: Effects of undetoxified *J. curcas* oil on liver tissues (250 mg/kg)------- 64

Plate VI: Effects of detoxified *J. curcas* oil on liver tissues (250 mg/kg)--------- 64

Plate VII: Normal liver tissues (control)---------- 65

Plate VIII: Effects of undetoxified *J. curcas* oil on liver tissues (500 mg/kg)--- 65

Plate IX: Effects of detoxified *J. curcas* oil on liver tissues (500 mg/kg)--------- 65

Plate X: Normal kidney tissues (control)---------- 66

Plate XI: Effects of undetoxified *J. curcas* oil on kidney tissues (125 mg/kg)-- 66

Plate XII: Effects of detoxified *J. curcas* oil on kidney tissues (125 mg/kg)---- 66

Plate XIII: Normal kidney tissues (control)---------- 67

Plate XIV: Effects of undetoxified *J. curcas* oil on kidney tissues (250 mg/kg)- 67

Plate XV: Effects of detoxified *J. curcas* oil on kidney tissues (250 mg/kg)------ 67

Plate XVI: Normal kidney tissues (control)---------- 68

Plate XVII: Effects of undetoxified *J. curcas* oil on kidney tissues (500 mg/kg)- 68

Plate XVIII: Effects of detoxified *J. curcas* oil on kidney tissues (500 mg/kg)--- 68

Plate XIX: Normal GIT tissues (control)---------- 69

Plate XX: Effects of undetoxified *J. curcas* oil on GIT tissues (125 mg/kg)------- 69

Plate XXI: Effects of detoxified *J. curcas* oil on GIT tissues (125 mg/kg)--------- 69

Plate XXII: Normal GIT tissues (control)---------- 70

Plate XXIII: Effects of undetoxified *J. curcas* oil on GIT tissues (250 mg/kg)---- 70

Plate XXIV: Effects of detoxified *J. curcas* oil on GIT tissues (250 mg/kg)------- 70

Plate XXV: Normal GIT tissues (control)---------- 71

Plate XXVI: Effects of undetoxified *J. curcas* oil on GIT tissues (500 mg/kg)---- 71

Plate XXVII: Effects of detoxified *J. curcas* oil on GIT tissues (500 mg/kg)------ 71

LIST OF APPENDICES

Appendix Page

Appendix 1: Table of doses for first and second phases of LD_{50} determination by Lorke's method----------101

LIST OF ABBREVIATIONS

A.B.U.	Ahmadu Bello University
ABC	ATP Binding Cassette
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
ATP	Adenosine Triphosphate
CNS	Central Nervous System
CV	Central Vein
CYP	Cytochrome P_{450} Oxidase
DAG	Diacylglycerol
EMEA	Europeans Medicines Agency
FD	Fat Droplets
FFA	Free Fatty Acids
GIT	Gastro-Intestinal Tract
H	Hepatocytes
HGB	Haemoglobin
HPLC	High Performance Liquid Chromatography
ILAR	Institute for Laboratory Animal Research
K	Kupffer Cells
LD_{50}	Median Lethal Dose

MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MDR	Multiple Drug Resistance
NAPRI	National Animal Production Research Institute
PAs	Pyrolizidine Alkaloids
PE	Phorbol Esters
PKC	Protein Kinase C
PLT	Platelet
PO	Per Oral
PPM	Parts Per Million
RBC	Red Blood Cell
RIP	Ribosome Inactivating Protein
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
S	Sinusoids
SD	Standard Deviation
SDME	Synthetically Detoxified Mechanically Extracted
SDSE	Synthetically Detoxified Solvent Extracted
SEM	Standard Error of Mean
SPSS	Statistical Package for Social Sciences
TI	Trypsin Inhibitor
UME	Undetoxified Mechanically Extracted
UNIFEM	United Nations Development Fund for Women
USE	Undetoxified Solvent Extracted

UV	Ultraviolet
W.H.O.	World Health Organization
WBC	White Blood Cell

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Plants are a rich source of many natural products most of which have been extensively used for human welfare including treatment of various diseases (Meskin, 2002; Haubrich, 2003). Medicinal plants have been used as a source of medicine to treat illness since time immemorial (Prasad *et al*., 2012; Eja *et al*., 2007). Plants have provided a source of old and modern drugs, as plant-derived medicines have made large contributions to humans (Prasad *et al*., 2012; Tapsell *et al*., 2006). Their role is twofold in the development of new drugs: they may become the base for development of a medicine and a natural blueprint for the development of new drugs to be used for the treatment of diseases (Iwu, 1993). Medicinal plants provide bountiful resource of active compounds for the pharmaceutical, cosmetics and food industries, and also in agriculture for pest control (Rice, 1995). For example, *Arnica montana* is used for treatment of osteoarthritis (Widrig *et al*., 2007) and *Azadirachta indica* is used in treatment of malaria and rheumatism (Ganguli, 2002). Herbal medicinal plants are preferred to synthetic chemical agents because of higher cultural acceptability and lesser side effects (Prasad *et al*., 2012). The chemical compounds present in herbal products are believed to have better compatibility with the human body (Khanna *et al*., 1986).

Many varieties of seed are cultivated for their oil, but more commonly, the residue left after removal of the oil is used for feeding of animals (Trabi *et al*., 1997). The protein content of the residue is usually high and the carbohydrate content low (Makkar and

Becker, 1999). The fat content depends on the method used to remove the oil. More than 90% of oil producing plants are found in the tropics and sub tropics, a far higher percentage than other group of food plants (Rehm and Espig, 1991). The cultivation of oil plants is considered therefore to play a major role in their political and economic development (Rehm and Espig, 1991). Oil seeds have an advantage over other seeds in that, the oil with a higher nutritional and economic value can be extracted first, while protein rich fraction left after extraction of the oil can be exploited as a protein supplement for animal diets, thus reducing cost as protein is the most expensive component of animal diet (Makkar and Becker, 1999).

Seeds usually contain large amount of stored materials such as starch, oil and minerals required for seed growth and early plant growth. These stored materials make plants highly susceptible to attack by microorganisms, insects, birds and animals so that protection against predators is necessary. Plants have evolved to rely upon elaborate chemical defenses towards unwanted predators (Ames *et al*., 1990). For this reason, plants produce a wide variety of chemical compounds in form of toxins and antinutritional factors which are toxic to species, ranging from bacteria to humans (Keddy, 2007). Many plants are therefore not edible and are unsafe for use as medicines simply because they produce toxins. Plants produce a variety of secondary metabolites, and chemists have subdivided them into various classes based upon their molecular structure. The classes of secondary metabolites include alkaloids, terpenoids, tannins, cyanogenic glycosides and saponins and so forth. The isolation and detoxification of these toxic secondary metabolites and antinutritional factors in conventional oil and non-oilseeds have been attempted (Huisman *et al*., 1989; Liener, 1994; D'Mello, 1995a,b; Hass and Mittelbach, 2000).

Jatropha curcas is a plant found in nearly all parts of the world and is not edible. This is due to its toxicity which is primarily attributed to the presence of a diterpene (phorbol esters) and other antinutrients such as saponins, phytic acids, trypsin inhibitors and lectins (Jongschaap *et al*., 2007). Despite its toxicity, oil from *Jatropha curcas* seed has been used in folkloric medicine for the treatment of syphilis, soothing of pain, treatment of paralysis, dropsy, constipation (due to its strong purgative property) as well as skin diseases which include eczema and rashes, in Africa and various parts of the world (Mujumdar *et al.*, 2000).

The various potential uses of *J. curcas* oil especially for industrial purposes (biodiesel and soap making) have led to the use of various methods of extraction of the seed oil to obtain a maximum oil yield. These processes include use of solvents such as n-hexane, isopropanol, methanol and petroleum ether among which petroleum ether has been reported to give the highest oil yield (Nzikou *et al*., 2009). Other extraction processes include mechanical method as well as enzymatic-solvent extraction (Sharma *et al*., 2002).

1.2 Statement of Research Problem

J. curcas is a plant with many potential uses, all parts of which have been used in traditional medicine and for veterinary purposes for a long time (Dalziel, 1955; Duke, 1985). The oil from its seed has been found particularly useful for medicinal and veterinary purposes (Gubitz *et al*., 1999). It is also used as an insecticide, for soap production and as a fuel substitute (Gubitz *et al*., 1999). The seed contains about 20-40% oil with a fatty acid composition similar to that of oils used for human nutrition

(Gubitz *et al*., 1998; Augustus *et al*., 2002). However, antinutrients like trypsin inhibitor, lectin and phytate (Martinez-Herrera *et al*., 2006), and two major toxic components - curcin and phorbol esters (PEs), are present in Jatropha seeds (Makkar and Becker, 1997a), though only phorbol esters and curcin (a toxalbumin) have been reported as the toxic components present in the oil (Makkar and Becker, 1997a; Kpoviessi *et al*., 2004), making it unsuitable for consumption and ordinarily unsafe for medicinal uses as this may cause both acute and chronic toxicity effects following long term use hence the need for detoxification.

Various methods such as the use of alcohols to extract phorbol esters from seeds (Gross *et al*., 1997), as well as physical methods which include the use of heat and ultraviolet light rays have been employed for its detoxification. Most of these methods have their limitations; both money and time consuming (Aregheore *et al*., 2003) and are only directed towards the industrial potentials of the oil and not its undeniable uses in folkloric medicine.

Conventional edible oil refining methods as described by Bokisch (1993) and O'Brian (1998), have been reported to cause a reduction in the level of phorbol esters in the oil in an *in vitro* study (Haas and Mittelbach, 2000), a process whose effects have not been confirmed on its toxicity and pharmacological activities in animals.

Solvent extraction process yields more oil relative to other methods (Sharma *et al*., 2002). However, trace quantities of the extraction solvents might be retained in the oils after extraction; a factor which may increase the toxicity and alter the physicochemical

properties of the oil. Such may pose more harm especially when the oil is envisaged for medicinal and consumption purposes.

1.3 Justification of the Study

J. curcas oil has been reported to have good medicinal and nutritional potentials but for its toxicity (Gubitz *et al*., 1999). Though the oil has been mostly exploited for its industrial potentials, it is also widely used in folkloric medicine in many countries in Africa and other parts of the world, despite its toxicity (Jongschaap *et al*., 2007); a factor which raises concern for the need for detoxification through simple conventional edible oil refining methods which may not destroy the medicinal activity of the oil in the process so as to make the oil safer and still medicinally available for both folkloric, modern medicinal as well as industrial uses.

The variation in the level of toxins in the plant among geographical locations (Ahmed and Salimon, 2009), gives the possibility of total or near-total detoxification in regions with low toxicity. This study therefore seeks to determine the extraction method that yields possibly less toxic starting oil for detoxification (by comparing the toxicity of the solvent extracted oil against the mechanically extracted oil through their phorbol ester levels), as well as compare the use of naturally available and cheaper materials with synthetic ones in the detoxification process of the mechanically extracted oil.

1.4 Aim and Objectives

1.4.1 Aim

The aim of this study was to investigate the effect of detoxification on the safety and some pharmacological properties of *J. curcas* seed oil in laboratory animals.

1.4.2 Specific Objectives

1. To determine the phytoconstituents and elemental compositions of undetoxified and detoxified *J. curcas* seed oils as well as the amount of the major toxic constituent (phorbol esters) present in the variously extracted oils, both before and after detoxification.
2. To evaluate the acute toxicity of the variously extracted and detoxified *J. curcas* oils in chicks and mice, as well as the sub-acute toxicity in rats.
3. To evaluate the effects of the detoxification on some pharmacological properties of the oils.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Plant Toxicity

Plants cannot move to escape their predators, so they must have other means of protecting themselves from herbivorous animals. Some plants have physical defenses such as thorns, but by far the most common protection is chemical (Keddy, 2007). Plants have evolved to rely upon elaborate chemical defenses towards unwanted predators (Ames *et al*., 1990). For this reason, plants produce a wide variety of chemical compounds, which are toxic to species, from bacteria to humans. Many of the plants, spread across the globe, are not edible simply because they produce toxins. Natural plant toxins may be present inherently in plants such as fruits and vegetables which are common food sources. They are usually metabolites produced by plants for the purpose of self defense against threats from other organisms (Wink, 1988). Natural toxins may also be present in food plants as a result of natural selection and new breeding methods that enhance these protective mechanisms. Toxic food components of plant origin may be low-molecular-weight endogenous toxins or products of secondary metabolism. Products of secondary metabolism are species-specific and give the plant its particular characteristics. They include plant pigments, flavours and compounds that serve to protect the plants. Some of these secondary metabolic products impart toxicity to the individual when taken orally. These substances may be growth inhibitors, neurotoxins, carcinogens and teratogens (Omaye, 2004).

Different types of natural toxins may be found in different crop plants and in different parts of a plant. The parts of a plant which may contain toxins and may also be used as

food sources include the foliage, buds, stems, roots, fruits and tubers. Toxicological effects following ingestion of plant toxins may range from acute effects of gastroenteritis to more severe toxicities in the central nervous system leading to death, as are seen in cases of poisoning due to cyanide or certain alkaloids. In addition to acute toxicities, some plant toxins such as pyrrolizidine alkaloids may also cause chronic systemic effects, organ toxicities or teratogenicity (Hong Kong Center for food safety, 2007).

2.2 Common Plant Toxins

Common classes of plant toxins include: alkaloids, cyanogenic glycosides, lectins, saponins and other antinutrients.

2.2.1 Alkaloids

They are the bitter components of plants found widely in nature and frequently have pharmacological properties. Mostly acting as secondary plant metabolites, alkaloids are often basic nitrogen-containing compounds able to form salts with acid. Alkaloids have been isolated from the roots, seeds, leaves or bark of some members of at least 40% of plant families. Families being particularly rich in alkaloids include Amaryllidaceae, Papaveraceae and Solanaceae (Petterson, 1991). Some of the alkaloids found in the plant kingdom include: pyrrolizidine alkaloids and glycoalkaloids.

2.2.1.1 *Pyrrolizidine alkaloids*

They are tumorigenic plant toxins which are widely distributed in the plant kingdom as they are found in about 3% of flowering plants (Australia New Zealand Food Authority, 2001). About half of them exhibit hepatotoxicity (Radominska-Pandya, 2010). Ingestion

of plants containing pyrrolizidine alkaloids are usually through contaminated crops or via intake of herbal foods and preparations. Disease associated with consumption of pyrrolizidines is known as pyrrolizidine alkaloidosis (Fu *et al*., 2002).

2.2.1.2 *Glycoalkaloids*

They are found in common food plants acting like a natural pesticide against common pests. Common examples include tomatine which is found in tomatoes (McMillan and Thompson, 1979) and solanine which is present in small amounts in potatoes. Its level is increased in greened or blighted potatoes, and can reach very high levels in the sprouts. Cooked potatoes that contain high level of solanine have a bitter taste and cause a burning sensation in the throat.

Glycoalkaloids produce toxic effects at high doses. There have been many reported cases of human poisonings (sometimes fatal) due to the ingestion of greened, damaged or sprouted potatoes as a consequence of high levels of glycoalkaloids (Maga, 1980). Acute toxicity syndromes in humans have been observed at glycoalkaloid levels of more than 2.8 mg/kg body weight while onset of symptoms has ranged from minutes to 2 days after ingestion of toxic potatoes, with longer incubation periods generally associated with the more severe cases (WHO, 1988).

2.2.2 Cyanogenic glycosides

Cyanogenic glycosides occur in at least 2,000 plant species. Important staple foods for some parts of the world (such as cassava and sorghum) contain cyanogenic glycosides. Toxicity of cyanogenic glycosides-containing plant is due to the cyanide produced on ingestion. The plant species that produce cyanogenic glycosides usually also have a

corresponding hydrolytic enzyme (β-glucosidase). In the presence of water, the non-toxic cyanogenic glycosides are hydrolysed by the enzyme producing cyanohydrins which quickly decompose to the toxic hydrogen cyanide.

In humans, the clinical signs of acute cyanide intoxication may include: rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pains, vomiting, diarrhoea, mental confusion, stupor, cyanosis with twitching and convulsions followed by terminal coma (WHO, 1993). The acute lethal dose of hydrogen cyanide for humans is reported to be 0.5-3.5 mg/kg body weight (WHO, 1993).

Chronic toxicity effects usually accompany individuals who have underlying dietary deficiency such as inadequate protein and/or iodine intake. Cyanide is detoxified in the body, by the enzyme rhodanese in the presence of sulphur-containing amino acids, to produce thiocyanate while food processing procedures such as soaking, fermentation, drying, grating and storage also reduce the levels of hydrogen cyanide before consumption (Kendirim *et al.*, 1995; Obilie *et al.*, 2004; Onabolu *et al.*, 2002).

2.2.3 Lectins

Lectins are proteins or glycoproteins of non-immune origin which have multiple highly specific carbohydrate binding sites (Els *et al.*, 1998). They are particularly concentrated in legume seeds majorly; green beans, red kidney beans and white kidney beans and have been shown to cause immune (allergic) reactions, nutritional deficiencies and other noxious effects such as gastroenteritis, nausea and diarrhoea which are due to gastrointestinal distress through interaction of the lectins with the epithelial cells of the gut. A recent *in vitro* study has suggested that the mechanism of lectin damage may

occur by interfering with the repair of already damaged epithelial cells (Miyake *et al*., 2007). Other symptoms of acute toxicity of lectin are local haemorrhages, kidney, liver and heart damage and agglutination of red blood cells. Food processing methods such as soaking, boiling and cooking with moist heat can reduce the toxicity of lectins (Hong Kong Center for food safety, 2007).

2.2.4 Saponins

They are secondary metabolites widely distributed in higher plants. They have ability to lyse erythrocytes or to foam even at low concentrations (Bruneton, 1995; Rao and Gurfinkel 2000; Francis *et al*., 2002). Haemolysis of red blood cells seems to result from their ability to form complexes with cell membrane cholesterol leading in consequence to pore formation and cell permeabilization (Abe *et al*., 1981; Melzig *et al*., 2001; Gauthier *et al*., 2009).

2.2.5 Tannins

Tannins are phenolic substances associated with toxic and antinutritional effects including reduced food/feed intake, growth retardation and impaired nutrient absorption (Butler *et al*., 1986). Tannins possess multiple phenolic hydroxyl groups leading to formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids and polysaccharides.

2.2.6 Phytates

Phytate is the principal storage form of phosphorus in most plant seeds. Phosphorus in phytate form is, in general, not bioavailable to nonruminant animals because these animals lack the digestive enzyme phytase, which is required to separate phosphorus

from the phytate molecule. When these molecules are consumed along with their diet, the phytates chelate with di and/or trivalent mineral ions such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{3+}, and Fe^{3+}, resulting in these ions becoming unavailable to consumers (Duffus and Duffus, 1991).

2.2.7 Trypsin inhibitors

They are protease inhibitors which are widespread antinutrient substances present in many plant derived nutritional ingredients (Norton, 1991) and potency is dependent upon origin and target enzyme. Trypsin inhibitors are known to decrease protein digestibility.

2.2.8 Phorbol esters in toxic plants

They are amphiphilic molecules which are regarded as co-carcinogens and have a tendency to bind phospholipid membrane receptors. During the normal signal transduction process the enzyme is activated by diacyl glycerol (DAG) which is then rapidly hydrolyzed. DAG is responsible for activating protein kinase C (PKC) function by increasing its affinity for phosphatidylserine containing membranes. Upon activation, PKC enzymes are translocated to the plasma membrane by membrane-bound receptor protein to initiate various signal transduction pathways. Phorbol esters act as an analogue for DAG, a potent PKC activator, by enhancing PKC and triggering cell proliferation, thus amplifying the efficacy of carcinogens. Phorbol esters themselves do not induce tumors but promote tumor growth following exposure to sub-carcinogenic doses of carcinogen (Goel *et al*., 2007).

2.2.9 Curcin in toxic plants

Curcin is a toxalbumin belonging to a group of proteins called ribosome-inactivating proteins which inhibit prokaryotic and eukaryotic ribosome by specific modification of the larger rRNA. Thus curcin inhibits protein synthesis (Endo *et al*., 1987; Endo and Tsurugi, 1987). Curcin has protein translation inhibitory activity or N-glycosidase activity (Lin *et al*., 2003; Weike *et al*., 2006; King *et al*., 2009).

2.3 Factors Influencing Plant Toxicity

Toxin concentrations in plant species vary based on several factors which include: environmental stresses on the plant, age of the plant, individual susceptibility, different parts of the plant (root, stem, leaves, and seeds), and season of the year. The level of toxicity of plants to humans or animals may depend on nature of toxin, quantity consumed, time of exposure, individual body chemistry, climate, soil and genetic differences within species. Some of the toxins produce irreparable damage to predators, while others exert transient effects (Hong Kong Center for food safety, 2007).

2.4 Classification of Poisonous Plants

2.4.1 Plants that affect the digestive system

2.4.1.1 *Plants that cause irritation of the oral cavity*

Plants belonging to the Araceae family contain needle-shaped calcium oxalate crystals in their leaves. These crystals when chewed in the plant leaves are expelled, causing an immediate burning sensation in the oral cavity tissues. Plants that accumulate calcium oxalates such as *Dieffenbachia picta* (Cao, 2003) can cause severe inflammation and necrosis of the epithelium of the tongue and oral cavity and may even cause death. Besides calcium oxalates, *D. picta* contains proteolytic enzymes that induce histamine

release causing a severe inflammatory response that may lead to asphyxia and death (Loretti *et al*., 2003).

2.4.1.2 *Plants that affect the gastrointestinal tract*

Ricinus communis contain lectins which cause necrosis of the cells lining the gastrointestinal tract. Ricin is comprised of two subunits: Unit B (for binding) is the actual lectin that binds to galactosyl residues in cellular membranes, whereas unit A (for activity) is an enzyme capable of inactivating ribosomes in eukaryotic cells (Barbieri *et al*., 1993). All animal species are sensitive to the effects of ricin. Clinical signs include weakness, salivation, profuse aqueous diarrhoea, dehydration, mydriasis, teeth grinding, hypothermia and recumbence; the major postmortem finding is severe gastroenteritis (Aslani *et al*., 2007).

2.4.2 Plants that affect the blood

2.4.2.1 *Plants causing haemolytic anaemia*

Allium cepa, which includes all types of onions, is capable of causing toxicosis in both large and small animals due to its content of organic sulphoxides, especially alkyl or alkenyl cysteinyl sulphoxides (Rae, 1999; Parton, 2000). After ingestion, the organosulphoxides are transformed into a complex mixture of organic sulphur compounds, some of which are capable of causing intravascular haemolysis in animals.

2.4.2.2 *Plants causing methemoglobinemia*

The nitrite ion, which is formed by bacteria in the rumen from plant nitrate, is the major cause of methaemoglobinemia in ruminants. *Amaranthus* species have been associated with nitrate intoxication. *Chenopodium album*, a plant recently reported in Colombia

(Fernández-Alonso and Hernández-Schmidt, 2007), which can cause lethal intoxication in ruminants because of its high nitrate levels.

2.4.2.3 *Plants that affect the coagulation of blood*

Plants such as *Anthoxanthum odoratum* (Poaceae) contain coumaric glycosides which have anticoagulant property on the blood. When hay from these plants becomes mouldy, the coumarinic glycosides can produce dicumarol, an anticoagulant that causes depletion of active vitamin K in the liver resulting in reduced clotting factors being released into the blood (Hallak and Wedlund, 1991).

2.4.3 Cardiotoxic plants

Cardiac glycosides are a specific type of toxic glycosides that affect the cardiac muscle, sometimes causing fatal toxicosis. Cardiac glycosides increase the contraction force of the heart by inhibiting the myocardial Na-K ATP-ase, which can lead to cardiac arrest (Poindexter *et al*., 2007). *Digitalis purpurea* contains cardiac glycosides in all parts of the plant but the concentration is higher in the leaves (Diaz, 2011).

2.4.4 Hepatotoxic plants

Hepatotoxic plants affect the liver by causing either hepatocellular necrosis or intrahepatic cholestasis. Pyrrolizidine alkaloids (PAs) are a large group of hepatotoxins capable of causing hepatocellular necrosis. Extensive literature reviews on the chemistry, mechanism of action and effects of PAs in animals and humans have been published (Mattocks, 1986; Diaz, 2001; Fu *et al*., 2004; Rietjens *et al*., 2005). In general, PAs induce hepatocyte necrosis that progresses to the destruction of the

parenchymal cells of the organ and eventually to liver failure. PAs are also potent carcinogens at levels below those causing hepatic necrosis.

2.4.5 Plants that affect the urinary system

Urinary bladder tumors in cattle have been associated with the intake of *Pteridium aquilinum* (Smith, 1997). The toxicosis results from the chronic intake of ptaquiloside and its major sign is haematuria caused by the development of multiple bleeding tumors in the bladder mucosa (Pedraza *et al*., 1983; Smith, 1997). The glycoside can be excreted in the milk (Alonso-Amelot, 1997).

2.4.6 Plants that affect the nervous system

Plants that affect the nervous system are divided into 2 major groups namely: plants that affect the neuromuscular junction and those that affect the central nervous system.

2.4.6.1 *Plants that block the neuromuscular junction*

Plants such as *Conium maculatum* cause paralysis of the musculature due to the blockade of the neuro-muscular junction. The clinical signs of *Conium maculatum* poisoning in domestic animals and humans include muscle weakness, tremors, incoordination and mydriasis, followed by bradycardia, depression, coma and death from respiratory failure (Panter *et al.*, 1988; Vetter, 2004).

2.4.6.2 *Plants that affect the central nervous system (CNS)*

Plants such as *Ipomoea carnea* has been reported to affect the central nervous system of animals and humans (Antoniassi *et al*., 2007). The toxic compound of this plant was found to be the swainsonine which acts to inhibit lysosomal hydroxylases, particularly

the enzyme α-mannosidase. Swainsonine causes a cellular alteration known as lysosomal storage disease, characterized by excessive carbohydrate accumulation within the lysosomes (Jolly and Walkley, 1997).

2.4.7 Plants that affect the skin

Toxic plant-induced primary and secondary photosensitization is a common cause of skin lesions. The flowers and seeds of *Fagopyrum esculentum* contain a conjugated photo-reactive quinone known as fagopyrin (Hagels *et al*., 1995), which has been known to induce primary photosensitization. Primary photosensitization is caused by the reaction of the photoreactive compound in non-pigmented skin when it is exposed to solar radiation in the ultraviolet range. The photoactive compounds absorb solar energy, forming reactive molecules (free radicals) that react with nearby macromolecules, causing inflammation, erythema, oedema, serous exudation, scar formation and skin necrosis. Several plants of the Euphorbiaceae family are potentially toxic due to their content of phorbol esters, which are highly irritating to the skin and mucosa, and some are tumor promoters (Goel *et al*., 2007). The phorbol esters from *J. curcas* are also highly irritating to the skin, and its seeds contain caustic oil (Pérez-Arbeláez, 1931; Diaz, 2011).

2.5 Prevention and Defenses Against Plant Poisoning

In human, adequate phytochemical screening of plants used as remedies is required to ascertain their level of safety. Also, public awareness on toxic plants is required to prevent accidental poisoning especially among children due to their vulnerability. Plants which are toxic are not, or less eaten by herbivores (Wink, 2007, 2008). The liver of herbivores and some omnivores have active set of detoxification enzymes. Among them

are cytochrome P_{450} oxidases (CYP), which can add hydroxyl groups to mostly lipophilic xenobiotics. These hydroxylated metabolites are then conjugated with hydrophilic molecules, such as glucuronic acid, sulfate or amino acids and excreted via the kidneys in the urine (Alberts *et al.*, 2008; Mutschler *et al*., 2008).

Other lines of defense are ABC transporters (ATP Binding Cassette) such as multiple drug resistance proteins (MDR), which are membrane proteins that can pump lipophilic xenobiotics, that have entered intestinal cells by free diffusion, back to the gut lumen (Wink, 2007; Alberts *et al*., 2008; Mutschler *et al*., 2008).

Some herbivores have microorganisms in their intestine or rumen, which can help to degrade nutritional toxins (Aguiar and Wink, 2005). A few toxin eaters (e.g. parrots) are known to ingest clay which can bind most toxins, in a manner similar to charcoal (Aufreiter *et al*., 2001).

Many plants such as *J. curcas* used ethnobotanically for treatment of disease in humans and animals have therapeutic efficacy at lower doses, where overdosing can induce poisoning. However, poisonous plants may contain active compounds with useful biological activities (McGraw and Eloff, 2005).

2.6 The Genus *Jatropha*

The genus *Jatropha* that belongs to Euphorbiaceae family contains approximately 170 known species. The name *Jatropha* is derived from the Greek word ‘‘jatros’’ (doctor) and ‘‘trophe’’ (food), which implies its medicinal uses (Kumar and Sharma, 2008). *Jatropha* is a large genus of diverse growth forms and are attractive monoecious or

dioecious plants. These species are woody trees, shrubs and sub shrubs of disjunct distribution in the seasonally dry tropics of the Old and the New World.

2.6.1 *Jatropha curcas*

Jatropha curcas is the most widely available species; all parts of *J. curcas* have been used in traditional human medicine and for veterinary purposes for a long time (Duke, 1985). During the past two decades, *Jatropha* plant has gained interest in particular for its oil, which can be used as a feedstock for biodiesel production. Among the many Jatropha species, *J. curcas* is the most studied as the seeds are rich in oil and protein. The oil is used for biodiesel production, and attempts were made to use cake or kernel meal left after extraction of oil as livestock feed following detoxification.

2.6.2 Origin and distribution of *Jatropha curcas*

Jatropha curcas (physic nut), named by Carrolus Linnaeus originated in the Central America and has become naturalized in many tropical and sub-tropical areas including India, Africa and Asia. Today, it is cultivated in almost all tropical and sub-tropical countries (Heller, 1996).

2.6.3 Names of *Jatropha curcas*

Scientific/Botanical Name (species)**:** *Jatropha curcas* Linn.

Common names: The plant is known by various names in different languages viz:

English: Barbados nut, Chinese castor oil, fig nut, physic nut, pig nut, purging nut, wild oil nut and so forth.

Nigeria: Hausa- *binidazugu*

Igbo- *wuluidu*

Yoruba- *lapa lapa*.

2.6.4 Taxonomic hierarchy

According to Cronquist (1981), the taxonomic hierarchy of *Jatropha curcas* is as follows:

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Rosidae

Order: Euphorbiales

Family: Euphorbiaceae – Spurge family

Genus: *Jatropha* L. – Nettlespurge P

Species: *Jatropha curcas* Linnaeus – Barbados nut.

2.6.5 Description of the plant

Jatropha curcas has thick branchlets. The tree has a straight trunk and grey or reddish bark, masked by large white patches. It has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The leaves are arranged alternately. The branches contain whitish latex, which causes brown stains, which are very difficult to remove. Normally, five roots are formed from seeds: one tap root and others are lateral roots. Plants from cuttings develop only lateral roots. Inflorescences are formed terminally on branches. The plant is monoecious and flowers are unisexual. Pollination is by insects. After pollination, a trilocular ellipsoidal fruit is formed. The exocarp remains fleshy

until the seeds are mature. The seeds are black and in the average, 18 mm long and 10 mm wide in ripe *Jatropha* fruits. The seed weight (per 1000) is about 727g (Raju and Ezradanam, 2002; Kochhar *et al*., 2008). Figure 2.1 shows the fruits, seeds, oil and defatted seed cake of *J. curcas.*

2.7 Products of *Jatropha curcas* Seeds

2.7.1 *Jatropha curcas* seed cake

The extraction of oil from *Jatropha* seeds is associated with generation of substantial amount of seed cake waste at an average rate of 500 g cake per kg of seeds used (Zanzi *et al*., 2008). Inspite of its high protein content along with presence of all essential amino acids, except lysine (Makkar and Becker, 1997b), it cannot be used in feed formulation due to the presence of potential anti-nutritional components like phorbol esters (PE), lectins and trypsin inhibitors (Makkar *et al*., 1997). The PEs, have been identified as main toxicants in *Jatropha curcas* seed cake.

2.7.2 *Jatropha curcas* seed shell

Jatropha curcas shell is composed of mainly of fibre (>83% neutral detergent fibre). The high acid detergent lignin (>45%) and very low protein (<5%) contents in the shell indicate its poor nutritional value. However, the shell can be a good source of combustible fuel for cooking due to its high gross energy content. It could also be used as fertilizer (Gubitz *et al*., 1999).



***Jatropha curcas* fruits**



***Jatropha curcas* defatted seedcake**



***Jatropha curcas* oil**



***Jatropha curcas* seeds**

Figure 2.1: *Jatropha curcas*. Retrieved from https://www.google.com.ng

2.7.3 *Jatropha curcas* seed oil

Jatropha curcas seeds have been reported to contain around 20-40% viscous oil (Augustus *et al*., 2002). Its oil fraction consists of both saturated (14.1% palmitic acid and 6.7% stearic acid) and unsaturated fatty acids (47% oleic acid and 31.6% of linoleic acid). Recently, it has been reported that the major fatty acids found in the oil samples were oleic (41.5-48.8%), linoleic (34.6-44.4%), palmitic (10.5- 13.0%), and stearic (2.3-2.8%) acids (Martinez-Herrera *et al*., 2006). The about 36% linoleic acid (C18:2) content in *J. curcas* seed oil is of possible interest for skin care aside its other reported uses (Kumar and Sharma, 2008).

2.8 Uses of *Jatropha curcas* Oil

Jatropha oil has been found useful for manufacture of candles and soap, in cosmetic industry and also for medicinal purposes (Gubitz *et al*., 1999; Akbar *et al*., 2009) as it has been applied to treat eczema and skin diseases and to sooth pain; such as rheumatic pain (Heller, 1996). The oil is also used externally for the treatment of sciatica, dropsy and paralysis (Mujumdar *et al*., 2000). It has very high saponification value and being extensively used for making soap in India and other countries. In India, *J. curcas* oil is being imported to meet the demand of cosmetic industry. In China, a varnish is prepared by boiling the oil with iron oxide. In villages, it is used as an illuminant as it burns like candles as in case of castor oil. It is used for wool spinning in England (Gubitz *et al*., 1999; Mahanta *et al*., 2008). The oil may also be converted to biodiesel without refining (Becker and Makkar, 2009).

2.9 Methods of Extraction of *Jatropha curcas* Oil

The conventional methods employed in the extraction of oil from *Jatropha curcas* seeds include: solvent extraction, enzyme and mechanical extraction methods (Sharma *et al*., 2002).

2.9.1 Solvent extraction

Solvent extraction is the most widely used technique, owing to their high efficiency in oil recovery (90 to 98%). It involves the use of solvents such as petroleum ether, hexane, liquid carbon dioxide and isopropanol for oil extraction from pulverized seed either by centrifugation, cold maceration or Soxhlet apparatus. But the major disadvantage in using Solvent extraction technique is its high energy input and toxicity of solvent. This has lead to the development of enzyme-based techniques (Sharma *et al*., 2002).

2.9.2 Aqueous enzymatic oil extraction

This is a promising technique for extraction of oil from *Jatropha* seeds. The presence of certain enzymes such as protease and cellulase during extraction enhances oil recovery by breaking cell walls and oil bodies (Rhee *et al*., 1972). Plant cell walls have complex structure; different enzymatic preparation is required to break up the cell wall. This process is eco-friendly and does not produce volatile organic compounds as atmospheric pollutants. The major disadvantage associated with the technique is the long process and time necessary for the enzymes to liberate oil bodies. Another factor is the use of enzymes which are not commercially available (Gupta *et al*., 2005).

2.9.3 Mechanical extraction

Mechanical method involves the application of pressure to already pre-treated oil-bearing products. It employs the use of devices like screw and hydraulic presses as a means of applying the pressure (Gunstone and Norris, 1983). Other mechanical devices include oil expellers and improved ghanis which are used for seeds and nuts because of the high pressure required to express the oil (UNIFEM, 1993). Whichever method is employed, researchers (Norris, 1964; Ward, 1976; Khan and Hanna, 1983; Adekola, 1991) reported that the yields and quality of the oil extracted depend on the content adjustment, heating time, pressure application, operating temperature and so forth.

2.10 Toxic Components in *Jatropha curcas* Seed Oil

Due to their toxicity, *J. curcas* seed and oil are not edible however; the oil is traditionally used for manufacturing soap and medicinal applications (Jongschaap *et al.*, 2007). The toxic components present in the seeds of *J. curcas* are: phorbol esters (diterpenes), tannins, phytates, saponins, curcin, trypsin inhibitors and lectins. It is therefore noteworthy that only two of these toxic components in the seed have been reported in the oil; phorbol ester and curcin (King *et al*, 2009).

2.10.1 Phorbol esters in *Jatropha cursas* seed oil

Phorbol esters present in *Jatropha* seed have been identified as the main toxic agent responsible for *Jatropha* toxicity (Adolf *et al*., 1984; Makkar *et al*., 1997). The concentration of phorbol esters varies from 2 to 3 mg/g kernel meal and from 2 to 4 mg/g oil in different provenances of *J. curcas* (Makkar *et al*., 1997; Devappa *et al*., 2010).

2.10.2 Curcin in *Jatropha cursas* seed oil

Curcin is a toxalbumin belonging to a group of proteins called ribosome-inactivating proteins (RIP), which inhibit prokaryotic and eukaryotic ribosome by specific modification of the larger rRNA. It is heat labile, and can be positively identified by precipitation reactions with sera containing known antibodies (Kingsbury, 1964). Curcin is said to be highly irritant and is contained in *J. curcas* oil.

2.11 Methods of Detoxification of *Jatropha curcas* Oil

Various methods aimed at totally destroying or reducing antinutrients in the treatment of *J. curcas* oil have been employed namely: physical and conventional edible oil refining methods.

2.11.1 Physical method

This is a method for detoxifying phorbol esters in plant products or materials containing phorbol ester which comprises treating the plant products or materials with short wavelength U.V. light irradiation sufficient to detoxify or degrade the phorbol esters in the plant products or material. *J. curcas* oil was exposed to about 6,000 W-sec/cm^2 to 7,500 W-sec/cm^2 irradiation level of U.V. light at 254 nm wavelength for 3 min after which the level of phorbol esters was found to have been reduced by about 98% of its level in the undetoxified oil (Hong *et al*., 2012).

2.11.2 Conventional edible oil refining methods

This comprises of four processes namely: degumming, deacidification, bleaching or decolourization and deodorization (Bokisch, 1993 and O'Brian, 1998). It has been reported that the use of this method yielded about 50% reduction in the level of phorbol esters in *J. curcas* oil treated by this method in Austria (Haas and Mittelbach, 2000).

2.11.2.1 *Degumming*

The degumming processes convert the phosphatides to hydrated gums, which are insoluble in oil and readily separated as sludge by settling, filtering, or centrifugal action. Hydratable phosphatides can be precipitated by adding water to the oil, nonhydratable ones must be destroyed by adding acids. This process was reported to have no reducing effect on the level of phorbol esters in *J. curcas* oil (Haas and Mittelbach, 2000; Ahmed and Salimon, 2009).

2.11.2.2 *Deacidification*

Free fatty acids (FFA) are removed by neutralization with alkali hydroxides leading to soaps which can be removed. The addition of an alkali solution to a crude oil brings about a number of chemical and physical reactions. The alkali combines with the FFA present to form soaps; the phosphatides and gums absorb alkali and are coagulated through hydration or degradation. This process of deacidification of oil has been reported to have brought about a reduction in the level of phorbol esters in *J. curcas* oil (Haas and Mittelbach, 2000; Ahmed and Salimon, 2009).

2.11.2.3 *Bleaching*

Undesirable coloured impurities are removed by bleaching with an adsorptive reagent; the undesirable compounds are adsorbed and can be removed together with the adsorbent by filtration (Haas and Mittelbach, 2000; Ahmed and Salimon, 2009).

2.11.2.4 *Deodorization*

Undesirable volatile and odoriferous materials are removed by steam distillation at reduced pressure and high temperature for about 2 h (Haas and Mittelbach, 2000; Ahmed and Salimon, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection of plant materials

Jatropha curcas seeds were collected from the Institute for Agricultural Research (IAR), Ahmadu Bello University (A.B.U.), Zaria in September 2014 during the rainy season. The seeds were authenticated by Mr. Namadi Sanusi at the herbarium section, Department of Biological Sciences, A.B.U., Zaria and the voucher specimen number 22873 was obtained.

3.1.2 Experimental animals

Three day-old chicks weighing 25-35 g (Ranger cockerels, also known as Shika Brown) were obtained from National Animal Production Research Institute (NAPRI) A.B.U., Shika, Zaria. They were housed in a room at the animal house, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The chicks were placed on vital starter mash chick feed (Vital feeds, Jos, Nigeria) and water *ad libitum* for three days before the commencement of the experiment.

Male mice weighing approximately 20-30 g and rats weighing approximately 140-220 g for the study were obtained from the animal house, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were maintained in a well ventilated room, fed on laboratory animal feeds (Vital feeds, Jos, Nigeria) and granted access to water *ad libitum*. They were kept in clean aluminum cages and allowed to

acclimatize to laboratory conditions for one week before the commencement of the experiment.

3.1.3 Ethics statement

The care and use of animals in this study was carried out according to the international ethical standards (Institute of Laboratory Animal Research, 1996). Protocols were presented in a seminar to academic staff and postgraduate students and approved by the Ethics Committee in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

3.1.4 Chemicals, solvents and drugs

Pentazocine (Ranbaxy, India), petroleum ether (Guandong Guanghua, China), sodium hydroxide (NaOH), sodium thiosulphate ($Na_2S_2O_3$), sulphuric acid (H_2SO_4), hydrochloric acid (HCl), potassium hydroxide (KOH), potassium iodide (KI) (BDH chemicals, England), chloroform, glacial acetic acid, methanol, orthophosphoric acid (Guandong Guanghua, China), pharmaceutical grade bentonite (Sigma Aldrich, St. Loius USA), Olive oil (Andralucia, Spain), tonsil (W.R. Grace, USA).

3.2 Methods

3.2.1 Extraction of seed oils and determination of yield

Seeds of *J. curcas* were collected, washed, air-dried under shade and pulverized into coarse powder using clean mortar and pestle. The coarse powder was weighed and divided into two equal portions of 500 g each, after which the oil content of a portion of the weighed powdered seeds was extracted with petroleum ether (60-80^{o}C) for 24 h using a Soxhlet apparatus. Residual solvent in the extracted oil was allowed to

evaporate. The oil content of the second portion of the weighed powdered seeds was extracted mechanically using a hand operated hydraulic screw press.

Portions of the oils were used for qualitative phytochemical screening, test for the presence or absence of heavy metals, phorbol esters and analgesic activity. Solutions of the seed oils were obtained by dissolving appropriate quantities of the oil in olive oil to obtain stock solutions of the oils for the individual experiments.

3.2.2 Determination of physicochemical properties

3.2.2.1 *Determination of density*

The density of each oil sample was determined using a density bottle. The density bottle was weighed empty with an electronic weighing balance. The bottle was then filled with the oil of known volume after which the weight of both the bottle and the oil were determined (AOAC, 1990).

$$\text{Density (g/ml):} = \frac{W_2 - W_1}{V}$$

Where W_2= weight of oil + weight of density bottle (g)

W_1= weight of empty density bottle (g)

V = Volume of oil (ml).

3.2.2.2 *Determination of specific gravity*

The specific gravity of each oil sample was determined using the specific gravity bottle at 25^{o}C. The specific gravity bottle was weighed empty (W_1) after which the oil was carefully poured into the bottle which was then re-weighed (W_2). The same step was repeated with distilled water (AOAC, 1990).

Calculations: Weight = $W_2 - W_1$

Where W_2= weight of oil sample + weight of specific gravity bottle

W_1= weight of empty specific gravity bottle

Specific gravity of oil = $\frac{\text{Weight of oil held in specific gravity bottle}}{\text{Weight of water held in same specific gravity bottle.}}$

3.2.2.3 *Determination of refractive index*

The refractive indexes of the oils were determined using an Abbe refractometer with temperature control set at 25^{o}C (AOAC, 1990).

3.2.2.4 *Determination of free fatty acids*

One (1) g of each oil sample was placed in a 250 ml conical flask and warmed. Twenty five (25) ml of methanol was added with thorough stirring followed by addition of 2 drops of phenolphthalein indicator and a drop of 0.14M sodium hydroxide. The content was titrated with 0.14M sodium hydroxide until a light pink colour which persisted for 1 min was seen. The titre was recorded and used to calculate the free fatty acids (FFA).

FFA (as oleic) = $\frac{\text{Titre x M x 28.2}}{\text{Weight of oil sample.}}$

And M is the molarity of the base.

3.2.2.5 *Determination of acid value*

The acid value was calculated from the FFA as follows:

Acid Value (mg/g) = %FFA (as oleic) x 1.99.

3.2.2.6 *Determination of peroxide value*

Four (4) g of each oil sample was weighed into a 250 ml conical flask to which 30 ml of glacial acetic acid / chloroform (3:2) was added. The contents were shaken until they dissolved. One (1) ml of 5% KI solution was then added followed by addition of 0.5ml of 1% starch indicator. This was titrated with 0.1M $Na_2S_2O_3$ until the dark colour just disappeared. Blank determinations were also carried out (British Standard Institute 684). The peroxide value was calculated thus:

$$\text{Peroxide value (mEq/kg)} = \frac{(S - B) \times 10{,}000 \times M}{W}$$

Where B = blank titre, S = sample titre, M = molarity of $Na_2S_2O_3$, and W = weight of oil.

3.2.2.7 *Determination of saponification value*

Two (2) g of each oil sample was weighed into a conical flask and 25 ml of 0.5M ethanol KOH solution was added. A reflux condenser was fitted to each of the conical flasks and the mixture was refluxed for 30 min, while swirling until it simmered. The mixture was titrated with 0.5M HCl using phenolphthalein as indicator. Blank determinations were carried out under the same conditions (British Standard Institute 684). The saponification value was calculated thus.

$$\text{Saponification value (mg/g)} = \frac{(B - S) \times 12}{W}$$

Where B = blank titre, S = sample titre, and W = weight of oil.

3.2.3 Detoxification of *Jatropha curcas* oil

The detoxification processes used for *J. curcas* seed oil comprised of the four major conventional edible oil treatment methods described by Bokisch (1993) with modifications, namely: Degumming, Deacidification, Bleaching (decolourization) and Deodorization in a stepwise sequence. Three detoxifications were carried out: solvent extracted oil synthetically detoxified (SDSE), mechanically extracted oil synthetically detoxified (SDME) and mechanically extracted oil detoxified with more natural materials (NDME).

3.2.3.1 *Degumming of the oils*

Four hundred and twenty five (425) ml solvent extracted and 325 ml mechanically extracted *J. curcas* seed oils were separately heated to 80°C on a hot plate under constant stirring with magnetic stirrer at 100 rpm in a beaker. To each oil sample, distilled water which was first heated to approximately 90°C was added at 3% of the volume of the oil before 0.2% of *ortho*-phosphoric acid (90%, p.a.) was added. The mixture was further stirred for 1 h. After cooling, the formed milky-white precipitate (for the solvent extracted) and brown precipitate (for the mechanically extracted) were separated by centrifugation for 0.5 h at 3,500 rpm. The degummed oils were dried at 100°C for 0.5 h under reduced pressure with the use of a rotary evaporator. For degumming with more natural materials, the same process was repeated, but starting with 28.2 ml of the mechanically extracted oil and replacement of the *ortho*-phosphoric acid with distilled water.

3.2.3.2 *Deacidification (neutralisation) of the degummed oils*

Free fatty acids were determined as described above, prior to this deacidification or neutralization stage. Known weights of the oils were heated to 70°C under constant stirring at 100 rpm in a beaker. These were respectively, 360 ml, 275 ml and 26 ml for SDSE, SDME and NDME. Then 2.5M aqueous NaOH was added to each of the oils to neutralize the free fatty acids. The appropriate amount of NaOH solution to neutralize the free fatty acids was calculated by the following equation:

$$L = \frac{d \times FFA \times 10{,}000}{M \times N}$$

Where *L*= appropriate volume of N-molar aqueous NaOH solution

d= density of the oil

FFA= free fatty acids in the oil

M = average molecular weight of the fatty acids (278)

N = concentration of the aqueous NaOH solution (mol/dm^3).

The mixture was stirred for 60 min and allowed to cool, then centrifuged at 3,500 rpm to separate the formed precipitate. Alternatively, unripe plantain peel ash extract which was obtained by ashing unripe plantain peel and extracting it with distilled water for 48 h, was used as alkali in place of NaOH (Onyegbado *et al.*, 2002 and Olabanji *et al.*, 2012). The soaps derived from this stage were harvested, examined and weighed.

3.2.3.3 *Decolourisation (bleaching) of the oils*

Known volumes of the neutralized oils (225 ml of SDSE and 210 ml of SDME) were stirred at 100 rpm in a beaker at 80°C. After adding 2% of the bleaching reagent the mixture was stirred for 0.5 h and allowed to cool, whereupon, the bleaching agent was

separated by suction filtration. The bleaching reagent used was Tonsil standard, a Trisyl-Type bleaching substance based on silica gel (Grace Worms, Germany). For the NDME (16 ml), 2% pharmaceutical grade bentonite was used as the bleaching reagent (Haas and Mittelbach, 2000; Ahmed and Salimon, 2009).

3.2.3.4 *Deodorization of the oils*

Known volumes of the bleached oils (140 ml of SDSE, 138 ml of SDME and 14 ml of NDME) were heated to75°C and left under steam using a steam distillation setup under vacuum for 2 h, to give the final detoxified *J. curcas* oils (Haas and Mittelbach, 2000; Ahmed and Salimon, 2009).

3.2.4 Phytochemical screening

The oils were subjected to duplicate qualitative phytochemical tests for plant secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, phenols, saponins and tannins using standard procedures described by Trease and Evans (1989; 1996).

3.2.4.1 *Test for alkaloids*

a). *Mayer's test*: Few drops of Mayer's reagent were added to samples of the oils in test tubes, and a cream precipitate indicated the presence of alkaloid.

b). *Dragendorff's test*: Few drops of Dragendorff's reagent were added to the oils and a red precipitate indicated the presence of alkaloid.

3.2.4.2 *Test for cardiac glycosides*

Keller-killiani test: Oils were dissolved in glacial acetic acid containing traces of ferric chloride in a test tube held at an angle of 45^{o}. One (1) ml of concentrated sulphuric acid

was added down the side of the tube. A purple ring colour at the interface should indicate cardiac glycosides.

3.2.4.3 *Test for flavonoids*

Sodium hydroxide test: About 0.5 ml of each oil was dissolved in about 5 ml of ethanol. Few drops of aqueous NaOH were added to 5 ml of the solution. A yellow colouration should show the presence of flavonoid.

3.2.4.4 *Test for phenols*

One (1) ml of each oil was dissolved in 0.5 ml of water-alcohol mixture and 2 drops of 1% aqueous iron (III) chloride solution was added. A red, blue, green, or purple colouration should show the presence of phenol.

3.2.4.5 *Test for saponins*

a). *Frothing test*: About of 0.5 ml of each oil was shaken with 2 ml of distilled water in a test tube. Frothing, which should persist on warming should show the presence of saponins.

b). *Lieberman-Burchards test*: Equal volume of acetic anhydride was added to 0.5 ml of each oil in a tube, after which 1 ml of concentrated sulphuric acid was added down the side each tube. Red, pink or purple colour should indicate the presence of triterpenes while blue or blue-green should indicate the presence of steroids.

3.2.4.6 *Test for tannins*

Ferric chloride test: About 0.5 ml of each oil was stirred in 5 ml of ethanol. Few drops of ferric chloride were added to the solution. The appearance of a blue-black precipitate

should indicate hydrolysable tannins while a green precipitate should indicate the presence of condensed tannin.

3.2.5 Acid digestion of oil samples and determination of elements

One (1) g of the *J. curcas* seed oil samples were weighed into separate beakers for various mineral analyses. To each beaker, 2 ml of hydrogen peroxide and 7.5 ml of concentrated nitric acid (HNO_3) were added to form a solution. The sample solutions were then heated at a temperature of 170^{o}C in a fume cupboard until it became clear. After cooling to room temperature, 20 ml of deionized water was added to the digested samples and filtered using filter paper to remove particles. The filtrates were made up to 50 ml with deionized water and analyzed using atomic absorption spectrophotometer (Polarized Zeeman Hitachi 2000) for lead, cadmium, copper, iron, calcium and zinc. This procedure used was as described in ETHOS D User Manual (2001).

3.2.6 Isolation and estimation of phorbol esters from *J. curcas* oils

The isolation of phorbol esters from *J. curcas* oils (both undetoxified and detoxified), were carried out according to the method described by Gaudani *et al*. (2009). Briefly, two hundred and fifty (250) ml of n-hexane was added to 10 g of the oil to increase the volume and surface area of the oil. The mixture was homogenized by gentle mixing. The mixture was transferred into a separating funnel and 200 ml of methanol was added dropwise. The mixture was allowed to settle in two layers for 2 h. The lower layer of methanol was collected dropwise into a clean beaker without disturbing the upper layer of n-hexane. The collected methanol extract was kept on a water bath at 70^{o}C in order to remove the methanol to give the phorbol esters rich fraction. The phorbol ester rich fractions were weighed to estimate the amounts of phorbol esters present in each of the

J. curcas oil samples. The amount of phorbol esters present in the oil was estimated by relating the weight of the phorbol esters rich fraction to the starting oil weight, as a percentage, thus:

$$\% \text{ Phorbol esters in the oil} = \frac{W_2 - W_1}{W_3} \times 100$$

Where;

W_1 = weight of empty beaker to hold the methanol extract

W_2 = weight of beaker and the isolated phorbol esters

W_3 = weight of *J. curcas* oil at the start (typically, 10 g).

Attempts to determine the exact amounts of phorbol esters present in the oils by HPLC method such as that described by Wink *et al.* (1997) was foiled by the unavailability of the instrument and its consumables.

3.2.7 Acute toxicity studies of *J. curcas* seed oils

The median lethal dose (LD_{50}) determination was used as an index to define the acute toxicity of undetoxified and detoxified *J. curcas* seed oils. The oral LD_{50} of the seed oil was determined in chicks and mice using the method of Lorke (1983) which comprised of two phases. The chicks or mice were deprived of food for 3 to 4 h prior to administration of the oils respectively. In phase one, chicks or mice were assigned into 3 groups of 3 animals each. Groups 1, 2 and 3 were administered orally by gastric lavage, with 10 mg/kg, 100 mg/kg and 1,000 mg/kg of each seed oil. The chicks or mice were observed for clinical signs of toxicity for 2 h and mortality for 24 h. In the second phase, 4 groups of 1 chick or mouse each, were given *J. curcas* seed oil orally in geometrically increasing doses based on results from phase one (see Appendix I). Since

none of the animals died in the first phase, doses used in the second phase were: 1,600 mg/kg, 2,900 mg/kg and 5,000 mg/kg. The animals were observed for clinical signs of toxicity for 2 h and mortality for 24 h. The LD_{50} values for the extracts were calculated as the geometric mean of the highest non-lethal dose multiplied by the lowest lethal dose.

3.2.8 Sub-acute toxicity of *J. curcas* oils

The sub-acute toxic effects of undetoxified and detoxified *J. curcas* oils in rodents were determined using male albino rats. Thirty five (35) rats were divided into 7 groups of 5 rats each. Six (6) groups of the rats received oral daily doses of 125 mg/kg, 250 mg/kg and 500 mg/kg of the oils, with the groups 1-3 receiving the undetoxified mechanically extracted oil while groups 4-6 received the detoxified oil at the same doses, all for 14 days. Rats in group 7 served as the control and received equivalent volumes of olive oil which was used as the vehicle for the test oils for the 14 days. Weight changes, other physical toxicity symptoms and mortality among the various animal groups were observed (daily for 2 h post treatment) and recorded. The animals were sacrificed on the 15^{th} day of the experiment and blood samples were collected for hematological and biochemical analyses. Some organs (liver, kidney and intestine) were also harvested and weighed before histological analyses.

3.2.8.1 *Haematological parameters*

Following 14 days treatment, blood samples were collected from rats by cardiac puncture into EDTA tubes for analysis. Standard operating procedures as described by Afia and Momoh (2006) using the BC-3200 Auto-Haematology Analyzer was used to conduct assays for the haematological parameters. White blood cells (WBC), red blood

cells (RBC), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), platelet (PLT) and haemoglobin (HGB) were then calculated.

3.2.8.2 *Liver and kidney parameters*

The blood samples from the rats were collected by cardiac puncture into non-heparinized tubes for analysis of liver and kidney parameters. Standard operating procedures (Afia and Momoh, 2006) were used to conduct assays for the following: Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), creatinine and urea were then calculated.

3.2.8.3 *Histopathological examination of organs*

Each tissue (liver, kidney and intestine) was fixed in 10% phosphate buffered formalin, thereafter they were dehydrated with graded doses of xylene, embedded in molten paraffin wax and sectioned at 5 µm with a microtome. The sectioned tissues were placed on glass slides and stained with Haematoxylin and Eosin (H and E) stain. The tissues were observed under light microscope for pathological changes in structure and pictures were taken with digital camera attached to the eye piece of the light microscope (Ganter and Jolles, 1970; Lucia *et al*., 2008).

3.2.9 Pharmacological studies

3.2.9.1 *Analgesic Activity: Hot Plate Method*

Male albino mice (20-30 g) were randomly distributed into 8 groups of 5 mice each. Mice in groups 1-3 received doses of 125 mg/kg, 250 mg/kg and 500 mg/kg of undetoxified mechanically extracted *J. curcas* oil while groups 4-6 received the equivalent detoxified oil. Positive control (group 7) and negative control (group 8) were

treated with pentazocine (10 mg/kg) and equivalent volumes of vehicular olive oil respectively. Each mouse was placed on hot plate kept at a temperature of 55±0.5^{o}C, at 0, 30, 60, 90 and 120 min after oral administration of the oils, reaction time was recorded when animals licked their fore or hind paw or jumped off the hot plate (Eddy and Leimback, 1953; Kulkarni, 1999; Toma *et al*., 2003). A cut-off period of 15 s was observed in order to avoid damage to the paw.

3.2.9.2 *Analgesic activity: Formalin induced pain method*

Eight (8) groups of 5 male albino mice were used. Three (3) groups were pretreated with undetoxified *J. curcas* oil at 125, 250 and 500 mg/kg, per oral while the other 3 groups were pretreated with detoxified *J. curcas* oil through the same route and at same doses (as the undetoxified), 1 h before subcutaneous injection of 0.025 ml of 0.5% formalin into the dorsal surface of the right hind paw. Animals in positive control group were pretreated with pentazocine (10 mg/kg, intraperitoneally), 30 min before the subcutaneous formalin injection. The vehicle group (negative control) was pretreated with olive oil (10 ml/kg, per oral) at an interval of 1 h before the formalin injection. All animals were observed from 0 to 5 min (first phase) and from 15 to 30 min (second phase) and each was scored according to a pain scale of 0-3; where zero (0) indicated that mice walked or stood firmly on injected paw, one (1) indicated that the injected paw was partially raised or favoured, two (2) indicated that the injected paw was fully raised or favoured and three (3) indicated that the injected paw was licked. Pain responses were indicated by elevation or favoring of the paw or excessive licking and biting of the injected paw (Shibata *et al.*, 1989).

3.2.9.3 *Laxative studies*

A total of 30 male mice were randomly distributed into six groups of five animals each. The mice which were fasted for 12 h prior to the time of the experiment were placed in individual cages lined with adsorbent paper. Group 1 which served as negative control received normal saline (10 ml/kg), group 2 received 0.5 ml castor oil, group 3 and 4 received 0.5 and 1 ml of undetoxified *J. curcas* oil respectively while group 5 and 6 received 0.5 and 1 ml of detoxified *J. curcas* oil respectively, each administration was done orally by gavage. Each mouse was observed for the frequency of diarrhoea droppings on the adsorbent paper placed in their individual cages for 4 h (Izzo *et al*., 1992; Mukherjee *et al*., 1995; Karim *et al*., 2010).

3.2.10 Statistical analyses

The statistical analyses of the data was done using SPSS version 20 software. Data for the body weight, haematological and biochemical parameters as well as analgesic activity data were analysed using one way analysis of variance (ANOVA) followed by Dunnet and Bonferroni tests as post hoc. Data were analyzed by comparing various test groups with the normal saline/olive oil (negative control) and standard drug (positive control) groups. Values of $P \leq 0.05$ were considered statistically significant. The research results were calculated as mean ± standard error of the mean (SEM) and percentages. Data were presented as tables or charts, as appropriate.

CHAPTER FOUR

RESULTS

4.1 Oil Yield from Both Extraction Methods

The solvent extracted oil was light yellow while that from the mechanical extraction process was dark brown. Yields of 49.1% and 14.9% w/w were obtained for the solvent and mechanically extracted oils respectively (Table 4.1).

Table 4.1: Percentage Yield of *Jatropha curcas* Seed Oils Extracted with Solvent and Hand Operated Mechanical Hydraulic Screw Press

Extraction method	Weight of powdered material (g)	Weight of oil obtained (g)	Yield (%)
Solvent (petroleum ether)	500	245.7	49.1
Mechanical (hydraulic screw press)	500	74.5	14.9

4.2 Physicochemical Properties of *J. curcas* Oils

The physicochemical properties determined for both solvent extracted and mechanically extracted *J. curcas* oils were density, specific gravity, refractive index, free fatty acids, acid value, peroxide value and saponification value. Both oils showed similar values for all the determined physicochemical properties with no significant differences (Table 4.2).

Table 4.2: Physicochemical Properties of Solvent and Mechanically Extracted *Jatropha curcas* Oils

Physicochemical properties	Solvent extracted oil	Mechanically extracted oil
Density (g/ml)	0.91 ± 0.00	0.91 ± 0.00
Specific gravity	0.91 ± 0.00	0.91 ± 0.00
Refractive index	1.46 ± 0.00	1.47 ± 0.00
Free fatty acids (mg/g)	16.47 ± 2.67	15.07 ± 0.03
Acid value (mg/g)	32.80 ± 5.30	29.95 ± 0.05
Peroxide value (Meq/kg)	4.91 ± 0.01	4.75 ± 0.04
Saponification value (mg/g)	272.90 ± 2.00	280.40 ± 1.35

Data are expressed as Mean ± S.E.M.

4.3 Detoxification of *J. curcas* Oils

Oil recoveries and losses following the detoxification processes employing synthetic and more natural materials for both solvent and mechanically extracted *J. curcas* oils, are shown in Table 4.3. Total losses ranged from 63.69% to 71.76%, and occurred mostly during the deacidification / neutralization stage while the least losses occurred during the degumming stage. The table also shows the amount of soaps produced per 100 ml of starting oil after the deacidification stage, which was 14.18-16.92 g/100 ml for synthetic detoxification and 35.71 g/100 ml for the more natural detoxification.

Table 4.3: Oil Recoveries, Losses and Soaps Formed During the Various Stages of Detoxification of *Jatropha curcas* Oils

Detoxification stage ↓	Solvent extracted oil	Mechanically extracted oil	
Detoxification materials →	Synthetic	Synthetic	More Natural
Degumming	425, 370, 12.94	325, 285, 12.31	28, 26, 7.14
Deacidification	360, 300, 16.67	275, 220, 20.00	26, 16, 38.46
Decolourisation	225, 190, 15.56	210, 188, 10.48	16, 14, 12.50
Deodorization	140, 120, 14.29	138, 118, 14.49	14, 10, 28.57
Total oil loss (%)	425, 120, **71.76**	325, 118, **63.69**	28, 10, **64.28**
Soaps formed: total (g), (g/100 ml oil)	60, 14.18 g/100 ml	55, 16.92 g/100 ml	10, 35.71 g/100 ml

For the detoxification stages, data are: starting oil volumes (ml), ending oil volumes (ml), losses (%)

4.4 Qualitative Phytochemical Analysis

Preliminary phytochemical analyses revealed the presence of only alkaloids while cardiac glycosides, flavonoids, phenols, saponins and tannins were absent in both the undetoxified and detoxified, solvent and mechanically extracted *J. curcas* oil (Table 4.4).

Table 4.4: Phytochemical Constituents of Undetoxified and Detoxified Solvent and Mechanically Extracted *Jatropha curcas* Oils

Phytoconstituents ↓	Solvent extracted oil		Mechanically extracted oil	
	Undetoxified	Synthetically detoxified	Undetoxified	Synthetically detoxified
Alkaloids	+	+	+	+
Cardiac glycosides	-	-	-	-
Flavonoids	-	-	-	-
Phenols	-	-	-	-
Saponins	-	-	-	-
Tannins	-	-	-	-

Key: + = Present; - = Absent

4.5 Mineral Element Concentration of *J. curcas* Oils

In general, Table 4.5 shows that solvent and mechanically extracted undetoxified oils showed higher concentrations of mineral elements compared to the synthetically detoxified oils. However, naturally detoxified mechanically extracted oil showed the highest concentration of most of the detected mineral elements. This was true for toxic elements (Lead, cadmium and copper) as well as nutritional elements (iron and calcium).

Table 4.5: Mineral Element Concentrations of Undetoxified and Detoxified *Jatropha curcas* Oils

Oil sample ↓	Toxic elements (ppm)			Nutritional elements (ppm)		
	Lead	Cadmium	Copper	Iron	Calcium	Zinc
USE	0.044	0.000	0.000	0.361	1.380	0.022
SDSE	0.003	0.000	0.000	0.302	1.374	0.022
UME	0.151	0.000	0.000	0.354	0.617	0.870
SDME	0.015	0.000	0.000	0.111	0.480	0.028
NDME	0.000	0.000	0.008	0.506	4.798	0.332

USE = Undetoxified Solvent Extracted

SDSE = Synthetically Detoxified Solvent Extracted

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

NDME = Naturally Detoxified Mechanically Extracted

4.6 Phorbol Ester Rich Fractions of *J. curcas* Oils

The phorbol ester rich fractions in undetoxified *J. curcas* oils were used as references (100%) for the phorbol ester rich fractions in both synthetically and naturally detoxified oils. The synthetically detoxified solvent extracted (SDSE) oil contained 72% phorbol ester rich fraction compared to the undetoxified solvent extracted (USE) oil, showing 28% reduction in the levels of phorbol esters in the detoxified oil. The synthetically and naturally detoxified mechanically extracted (SDME) and (NDME) oils showed 91% and 97% phorbol ester rich fractions respectively, when compared to the undetoxified mechanically extracted (UME) oil which was used as reference with 100% phorbol ester rich fraction (Figure 4.1).

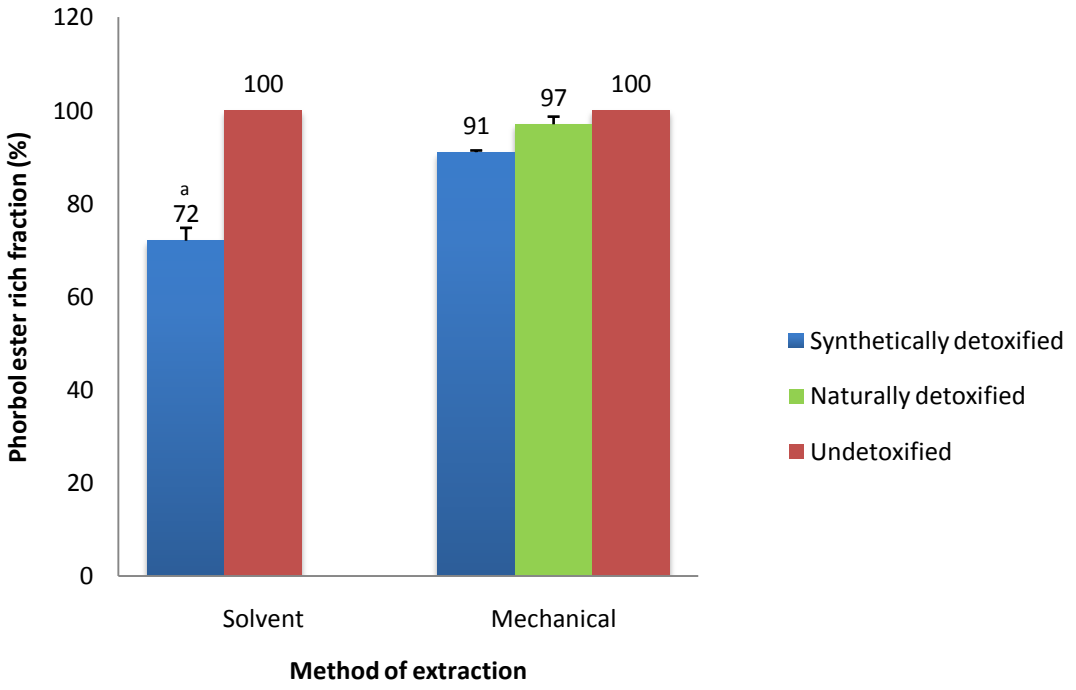


Figure 4.1. Effects of Synthetic and Natural Detoxification Processes on Levels of Phorbol Ester Rich Fractions in Solvent and Mechanically Extracted *Jatropha curcas* Oils

Values are mean ± S.E.M. of percentages

Data analyzed using one way ANOVA and Dunnet's post hoc

[a] significantly different from the reference (100%) at $p<0.05$

4.7 Median Lethal Dose (LD_{50}) Values of the Oils

The oral LD_{50} values for both undetoxified and detoxified solvent and mechanically extracted *J. curcas* oil was found to be above 5,000 mg/kg in both cockerels and mice as no mortality was observed at this dose (Table 4.6).

Table 4.6: Median Lethal Dose (LD_{50}) of Undetoxified and Detoxified Solvent and Mechanically Extracted *Jatropha curcas* Oils

Treatment	Animal	Age	LD_{50} [p.o.] (mg/kg)
USE	Cockerel	3-day	>5,000
	Mice	Adult	>5,000
SDSE	Cockerel	3-day	>5,000
	Mice	Adult	>5,000
UME	Cockerel	3-day	>5,000
	Mice	Adult	>5,000
SDME	Cockerel	3-day	>5,000
	Mice	Adult	>5,000

USE = Undetoxified Solvent Extracted

SDSE = Synthetically Detoxified Solvent Extracted

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

4.8 Signs of Toxicity of the Undetoxified and Detoxified *J. curcas* Oils in Male Wistar Rats

The undetoxified mechanically extracted (UME) *J. curcas* oil at 500 mg/kg dose produced severe toxicity symptoms in animals. The undetoxified oil at 250 mg/kg produced moderate symptoms of toxicity while at 500 mg/kg, the synthetically detoxified mechanically extracted (SDME) oil produced mild to moderate symptoms. However, both undetoxified and detoxified oils at 125 mg/kg dose produced no or undetected toxicity symptoms (Table 4.7).

Table 4.7: Physical Toxicity Symptoms of Undetoxified and Detoxified *Jatropha curcas* Oils in Male Wistar Rats

Treatment	Dose (mg/kg)	Ruffled fur	Hyperpnoea	Restlessness	Decreased mobility
Olive oil	10*	-	-	-	-
UME	125	-	±	-	-
	250	+	+	+	+
	500	++	++	++	++
SDME	125	-	±	-	-
	250	±	±	±	±
	500	+	±	±	+

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

* = ml/kg; n = 5 (number of rats); Drug administration; p.o. daily for 14 days

- = Absent/not detected; ± = mild; + = moderate; ++ = severe

4.9 Body Weight Changes and Mortality in Male Wistar Rats that Received Undetoxified and Detoxified *J. curcas* Oils

Undetoxified mechanically extracted oil at 500 mg/kg resulted in significant ($p<0.05$) weight changes with decrease (21.00±0.00 g) in their body weight and corresponding 80% mortality in rats and 250 mg/kg of the undetoxified oil resulted in 7.33±2.40 g decrease in body weight and 20% mortality. However, the undetoxified oil at 125 mg/kg dose and all doses of the detoxified oil (SDME) produced significant ($p<0.05$) increases in body weight of rats and no mortality (Table 4.8).

Table 4.8: Body Weight Changes and Mortality in Male Wistar Rats that Received Undetoxified and Detoxified *Jatropha curcas* Oils

Treatment	Dose (mg/kg)	Weight change ** (g)	Mortality		
			Day 10	Day 11	Total (%)
Olive oil	10*	+25.50±2.06[b]	0	0	0
UME	125	+21.60±8.51[b]	0	0	0
	250	-7.33±2.40[a]	1	0	20
	500	-21±0.00[a]	1	3	80
SDME	125	+25.75±3.27[b]	0	0	0
	250	+8.50±1.50	0	0	0
	500	+17.00±3.00[b]	0	0	0

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

* = ml/kg; n = 5 (number of rats); Drug administration; p.o. daily for 14 days

** = Day 15 compared to day 1; - = Weight loss; + = Weight gain

Values are mean ± S.E.M.; Data analysed using one way ANOVA and Dunnett's post hoc

[a] = Significant weight loss at $p<0.05$; [b] = Significant weight gain at $p<0.05$

4.10 Haematological Parameters in Male Wistar Rats that Received Undetoxified and Detoxified *J. curcas* Oils

The Red blood cell (RBC), White blood cell (WBC), Platelet (PLT), Haemoglobin (HGB), Mean cell haemoglobin concentration (MCHC) and Mean cell volume (MCV) levels in the control group were: 8.6±0.18, 5.6±1.80, 308.00±9.00, 11.57±2.43, 39.80±0.52, and 65.40±0.95 respectively. Levels of WBC was significantly ($p<0.05$) increased at 500 mg/kg dose of the undetoxified *J. curcas* oil. PLT values of 549.25±12.60, 573.00±0.00 and 549.50±12.08 were obtained at 250, 500 mg/kg of undetoxified and 500 mg/kg dose of detoxified oil respectively, showing significantly increased levels of PLT compared to the control. However, the undetoxified oil showed higher levels of WBC and PLT compared to the detoxified (Table 4.9).

Table 4.9: Haematological Parameters in Male Wistar Rats that Received Undetoxified and Detoxified *Jatropha curcas* Oils

Treatment	Dose (mg/kg)	Haematological parameters					
		RBC (10^{12}/L)	WBC (10^{9}/L)	PLT (10^{9}/L)	HGB (g/dL)	MCHC (g/dL)	MCV (fL)
Olive oil	10*	8.63±0.18	5.60±1.80	341.00±9.00	11.57±2.43	39.80±0.52	65.40±0.95
UME	125	8.76±0.35	5.70±1.40	471.25±11.50	9.67±3.68	40.20±0.41	67.97±1.48
	250	7.14±1.72	6.22±1.87	549.66±12.60[a]	12.62±2.14	28.72±0.88[a]	62.52±5.43
	500	7.10±0.00	10.00±0.00[ab]	573.00±0.00[a]	13.30±0.00	27.70±0.00[a]	67.70±0.00
SDME	125	9.38±0.50[a]	5.70±1.30	345.33±7.20	11.50±1.61	35.30±2.40	66.85±0.28
	250	6.85±1.50	5.57±1.47	515.25±15.00	11.72±2.74	27.42±0.89[a]	64.62±4.36
	500	7.77±1.40	5.50±2.10	549.00±12.08[a]	12.80±0.77	27.5±0.40[a]	63.92±3.21

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

* = ml/kg; n = 5 (number of rats); Drug administration; p.o. daily for 14 days

Values are mean ± S.E.M.; Data analyzed using one way ANOVA and Bonferonni's post hoc

[a] significantly different from the control group at $p<0.05$

[b] significantly different from other groups at $p<0.05$

RBC = Red Blood Cell; WBC = White Blood Cell; PLT = Platelet

HGB = Haemoglobin; MCHC = Mean Cell Haemoglobin Concentration

MCV = Mean Cell Volume

4.11 Serum Electrolytes and Kidney Function Indices in Male Wistar Rats that Received Undetoxified and Detoxified *J. curcas* Oils

The sodium (Na^+), potassium (K^+), bicarbonate (HCO_3^-), urea and creatinine levels of rats in the control group were 140.50 ± 0.50, 5.22 ± 0.07, 28.75 ± 0.25, 5.07 ± 0.08, 127.25 ± 1.10 respectively. Administration of 500 mg/kg of undetoxified mechanically extracted *J. curcas* oil caused significant ($p<0.05$) increase in the levels of Na^+ compared to the control and all other groups. All groups showed similar range of values for K^+, HCO_3^-, urea and creatinine, though some groups were significantly ($p<0.05$) different from the control (Table 4.10).

Table 4.10: Serum Electrolyte and Kidney Function Indices in Male Wistar Rats that Received Undetoxified and Detoxified *Jatropha curcas* Oils

Treatment	Dose (mg/kg)	Serum electrolyte and kidney function indices				
		Na^+ (mmol/L)	K^+ (mmol/L)	HCO_3^- (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Olive oil	10*	140.25±1.00	5.22±0.15	28.75±0.50	5.07±0.17	127.25±2.21
UME	125	143.00±0.81	5.20±0.18	24.50±2.08[a]	5.60±0.31[a]	132.25±2.62[a]
	250	142.00±2.16	5.30±0.81	27.50±1.29	5.10±0.18	125.00±0.81
	500	148.00±0.00[ab]	5.20±0.00	24.00±0.00[a]	5.80±0.00[a]	133.00±0.00[a]
SDME	125	141.00±0.81	5.20±0.21	25.50±1.91	5.47±0.35	129.00±0.81
	250	144.25±0.95[a]	5.17±0.25	26.25±0.95	5.37±0.05	128.00±0.81
	500	143.00±0.81	5.20±0.18	22.60±0.95[a]	5.93±0.14[a]	135.25±0.95[a]

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

* = ml/kg; n = 5 (number of rats); Drug administration; p.o. daily for 14 days

Values are mean ± S.E.M.; Data analyzed using one way ANOVA and Bonferonni's post hoc

[a] significantly different from the control group at $p<0.05$

[b] significantly different from other groups at $p<0.05$

Na^+ = sodium; K^+ = potassium and HCO_3^- = bicarbonate

4.12 Liver Function Enzymes and Serum Albumin in Male Wistar Rats that Received Undetoxified and Detoxified *J. curcas* Oils

The levels of liver test enzymes; Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) and Albumin (ALB) in the control group were 14.5±1.73, 23.7±4.34, 3.70±3.36 and 32.50±4.35 respectively. The effect of 125 and 250 mg/kg on the serum liver enzyme and albumin in both undetoxified and detoxified oils were not as pronounced as those of 500 mg/kg. Both 500 mg/kg of undetoxified and detoxified *J. curcas* oil produced significantly ($p<0.05$) increased levels of AST, ALT, ALP enzymes and ALB compared to the control. However, the undetoxified produced higher levels of AST enzyme compared to the detoxified oil (Table 4.11).

Table 4.11: Liver Function Enzymes and Serum Albumin in Male Wistar Rats that Received Undetoxified and Detoxified *Jatropha curcas* Oils

Treatment	Dose (mg/kg)	Liver function enzymes			
		AST (IU/L)	ALT (IU/L)	ALP (IU/L)	ALB (g/dL)
Olive oil	10*	14.50±1.73	23.75±4.34	32.50±4.35	3.70±3.36
UME	125	17.00±1.15	24.75±0.95	37.00±1.63	3.80±1.82
	250	19.50±0.57[a]	25.00±1.15	31.00±0.11	3.75±0.57
	500	35.00±0.00[ab]	28.00±0.00	45.00±0.00[a]	4.30±0.00[a]
SDME	125	17.25±1.89	27.75±2.21	36.50±2.64	4.05±2.88

250	22.00±2.16[a]	25.00±0.81	36.00±2.58	3.90±1.41
500	30.50±1.73[ab]	31.25±4.27[a]	46.50±3.10[a]	4.42±0.95[a]

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

[*] = ml/kg; n = 5 (number of rats); Drug administration; p.o. daily for 14 days

Values are mean ± S.E.M.; Data analyzed using one way ANOVA and Bonferonni's post hoc

[a] significantly different from the control group at $p<0.05$

[b] significantly different from other groups at $p<0.05$

AST = Aspartate aminotransferase; ALT = Alanine transaminase

ALP = Alkaline phosphatase and ALB = Albumin

4.13 Liver and Kidney to Body Weight ratios in Male Wistar Rats that Received Undetoxified and Detoxified *J. curcas* Oils

Undetoxified and detoxified *J. curcas* oils resulted in similar liver to body weight ratios in control, 125, as well as 250 mg/kg in rats. However, undetoxified *J. curcas* oil at 500 mg/kg resulted in decreased liver to body weight ratio (0.024) compared to the same dose of detoxified oil (0.035). The kidney to body weight ratio obtained for the control group was 0.0063 Kidney to body weight ratio of 0.0056 and 0.0055 were obtained for 125 mg/kg undetoxified and detoxified *J. curcas* oils respectively, showing no difference between each of the two groups and the control. Undetoxified and detoxified *J. curcas* oils at 250 mg/kg showed similar values. However, 500 mg/kg of the undetoxified *J. curcas* oil resulted in a decreased kidney to body weight ratio (0.005) compared to the detoxified *J. curcas* oil (0.0067), (Figure 4.2, 4.3).

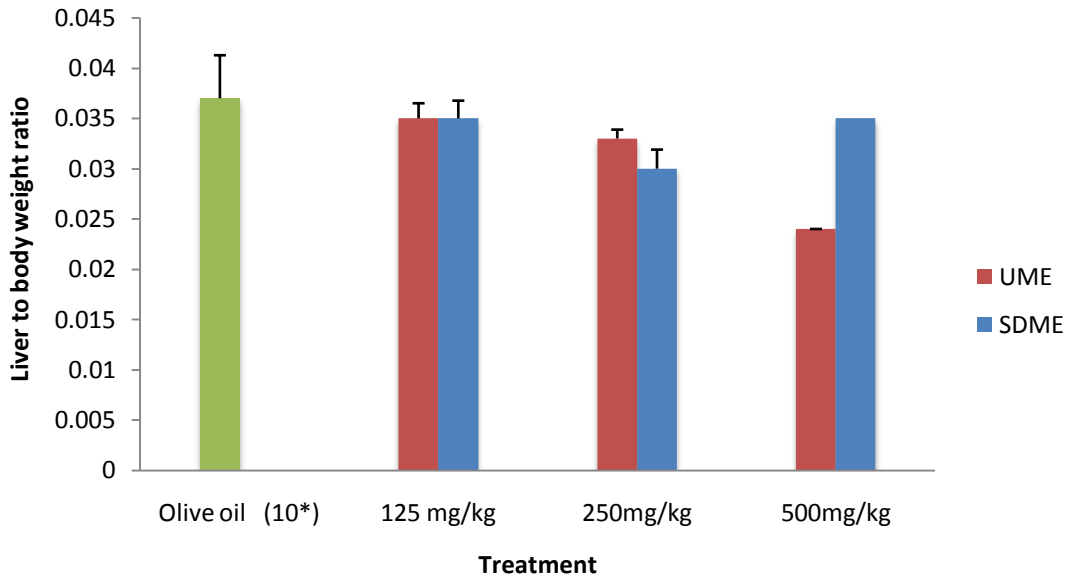


Figure 4.2. Liver to Body Weight Ratio in Male Wistar Rats that Received Undetoxified and Detoxified *Jatropha curcas* Oils

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

* = ml/kg; n = 5 (number of rats); Drug administration; p.o. daily for 14 days

Values are mean ± S.E.M.; Data analyzed using one way ANOVA and Bonferonni's post hoc

No significant difference between groups and control and among groups at $p<0.05$

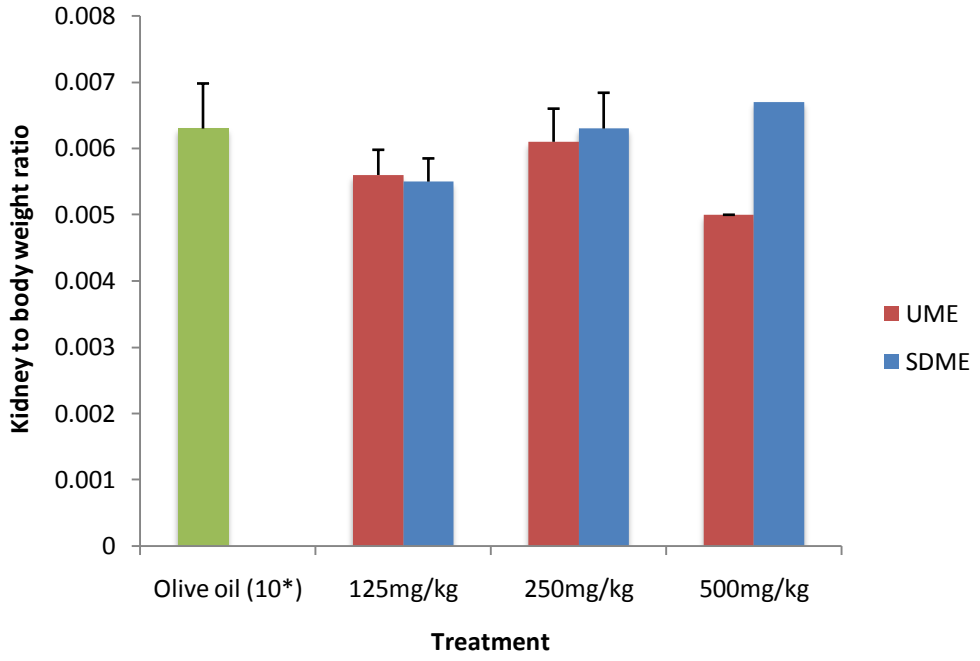


Figure 4.3. Kidney to Body Weight Ratio in Male Wistar Rats that Received Undetoxified and Detoxified *Jatropha curcas* Oils

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

* = ml/kg; n = 5 (number of rats); Drug administration; p.o. daily for 14 days

Values are mean ± S.E.M.; Data analyzed using one way ANOVA and Bonferonni's post hoc

No significant difference between groups and control and among groups at $p<0.05$

4.14 Effects of Undetoxified and Detoxified *J. curcas* Oils on Formalin-Induced Pain in Mice

In the first phase, only the standard drug (pentazocine, 10 mg/kg) and undetoxified 500 mg/kg groups produced significantly ($p<0.05$) reduced paw licking scores compared to the control. The standard drug group produced the lowest paw licking score (1.4±0.24) followed by undetoxified 500 mg/kg (1.6±0.40). In the second phase, there were significant ($p<0.05$) reduction in paw licking scores in the standard drug, 250 mg/kg undetoxified and detoxified oils as well as 500 mg/kg undetoxified and detoxified oils groups compared to the control. The standard drug produced the lowest paw licking score (1.0±0.00), followed by undetoxified at 500 mg/kg (1.2±0.24) and detoxified at 250 mg/kg (1.4±0.5).

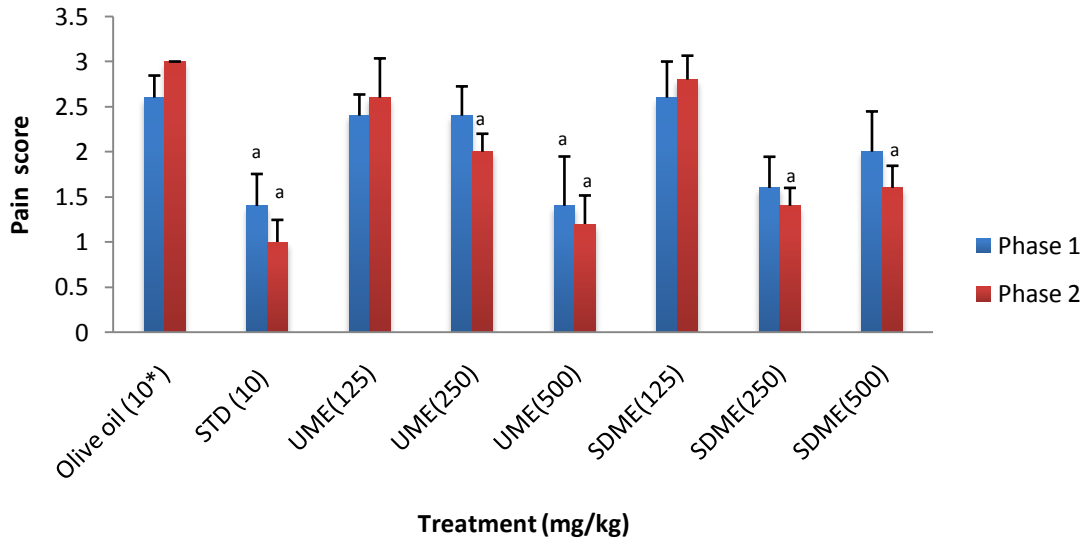


Figure 4.4. Effects of Undetoxified and Detoxified *Jatropha curcas* Oils on Formalin-Induced Pain in Mice.

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

STD = Standard drug (pentazocine)

* = ml/kg; n = 5 (number of mice); Drug administration; p.o.

Values are mean ± S.E.M.; Data analyzed using one way ANOVA and Dunnett's post hoc

[a] significantly different from the control group at $p<0.05$

4.15 Effects of Undetoxified and Detoxified *J. curcas* Oils on Hot Plate Latency in Mice

Pretreatment with pentazocine (10 mg/kg) produced the highest significant ($p<0.05$) increase in pain reaction time both at the early and late stages having its highest analgesic activity at 60 min time interval (10.56±1.10). The undetoxified *J. curcas* oil at 125 mg/kg (5.20±0.23) and 500 mg/kg (10.80±0.03) produced greater significant ($p<0.05$) increases in pain reaction time at 120 min time interval where they produced their highest analgesic activity compared to the detoxified *J. curcas* oil at 125 mg/kg (6.51±0.66) and 500 mg/kg (6.84±0.31) at the same time (Table 4.12).

Table 4.12: Effects of Undetoxified and Detoxified *Jatropha curcas* Oils on Hot Plate Latency in Mice

Treatment	Dose (mg/kg)	Response time (Sec)				
		0 min	30 min	60 min	90 min	120 min
Olive oil	10*	3.17±0.14	3.46±0.06	4.05±1.67	3.29±0.77	4.90±0.32
STD	10	2.77±0.92	5.95±0.39[a]	10.56±1.10[a]	7.85±0.25[a]	10.33±0.33[a]
UME	125	3.58±0.36	6.61±0.96[a]	4.80±0.57	5.20±0.23[a]	6.96±0.34[a]
	250	3.29±0.10	6.27±0.79[a]	5.26±0.19	5.35±0.03[a]	6.73±0.94[a]
	500	3.85±0.66	6.54±0.42[a]	6.97±0.87[a]	10.21±0.64[a]	10.80±1.62[a]
SDME	125	3.90±0.28	5.27±0.61[a]	4.79±0.56	5.20±0.23[a]	6.51±0.66
	250	2.57±0.20	4.60±0.31	4.14±0.21	5.17±0.64[a]	6.84±0.56[a]
	500	3.24±0.05	5.01±0.56[a]	4.64±0.04	5.16±0.15[a]	6.96±0.31[a]

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

STD = Standard drug (pentazocine)

[*] = ml/kg; n = 5 (number of mice); Drug administration; p.o.

Values are mean ± S.E.M; Data analyzed using one way ANOVA and Dunnett's post hoc

[a] significantly different from the control group at $p<0.05$

4.16 Effects of Undetoxified and Detoxified *J. curcas* Oils on Hot Plate Induced Pain Inhibition

Pretreatment with pentazocine (10 mg/kg) produced the highest significant ($p<0.05$) increase in pain reaction time both at the early and late stages having its highest analgesic activity at 60 min (73.7%). The undetoxified *J. curcas* oil at 125 mg/kg (48.5%) and 500 mg/kg (64.3%) produced greater significant ($p<0.05$) increases in pain reaction time at 120 min time interval where they produced their highest analgesic activity compared to the detoxified *J. curcas* oil at 125 mg/kg (40.1%) and 500 mg/kg (53.4%) at the same time. However, detoxified oil produced greater significant ($p<0.05$) increase in pain reaction time at 250 mg/kg (62.4%) and 120 min time interval compared to the undetoxified (51.1%) at the same dose and time.

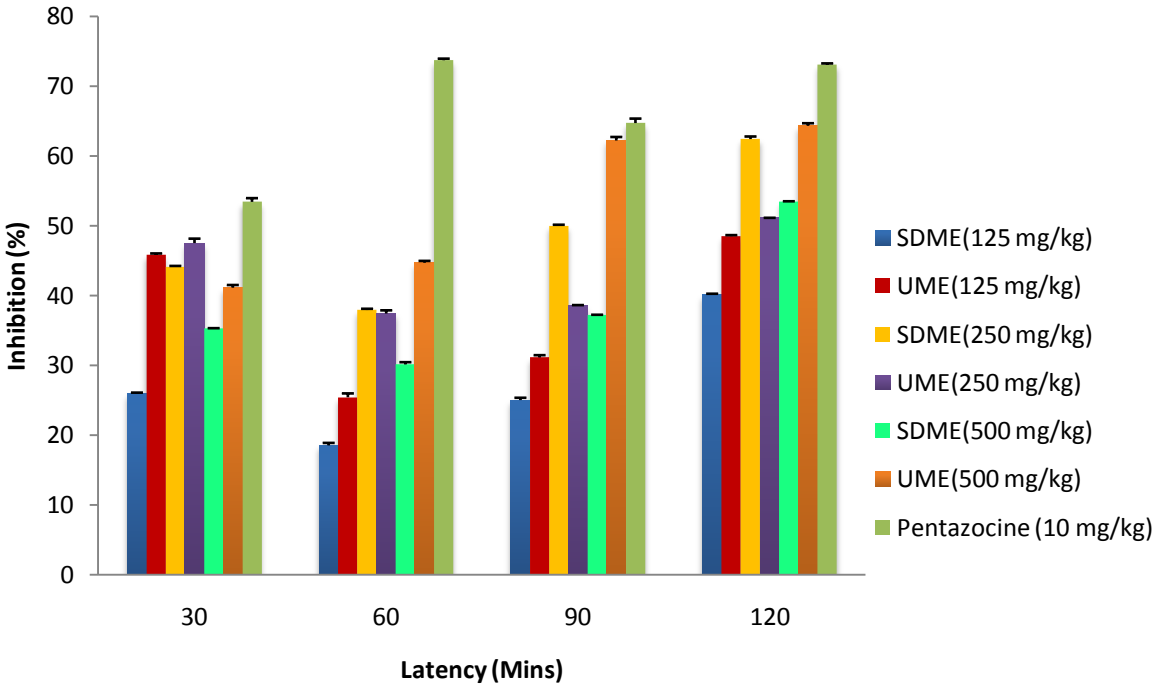


Figure 4.5. Effects of Undetoxified and Detoxified *Jatropha curcas* Oils on Hot Plate Induced Pain Inhibition

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

n = 5 (number of mice); Drug administration p.o.

Values are mean ± S.E.M. and percentages

4.17 Laxative Effects of Undetoxified and Detoxified *J. curcas* Oils

Castor oil showed significant ($p<0.05$) laxative activity in albino mice when compared with normal saline. Both undetoxified and detoxified *J. curcas* oils at 1 ml administration did not show any laxative activity. However, both undetoxified and detoxified *J. curcas* oils at 0.5 ml administration showed some laxative activity, though not significant ($p>0.05$) (Table 4.13).

Table 4.13: Laxative Effects of Undetoxified and Detoxified *Jatropha curcas* Oils

Treatment	Dose (ml)	Number of diarrhoea faeces
Normal saline	10*	0
Castor oil	0.5	1.83±0.47[a]
UME	0.5	0.60±0.60
	1	0
SDME	0.5	0.40±0.40
	1	0

UME = Undetoxified Mechanically Extracted

SDME = Synthetically Detoxified Mechanically Extracted

* = ml/kg; n = 5 (number of mice); Drug administration; p.o.

Values are mean ± S.E.M.; Data analyzed using one way ANOVA and Dunnett's post hoc

[a] significantly different from the control group at $p<0.05$

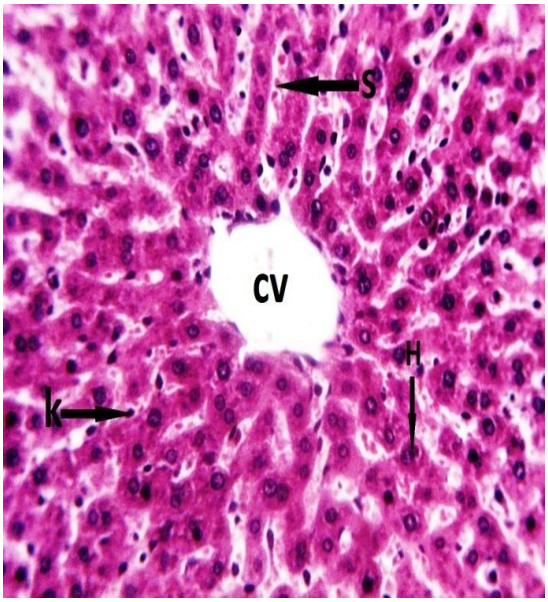


Plate I: Liver Control x 250



Plate II: Liver (125 mg/kg UME) x250



Plate III: Liver (125 mg/kg SDME) x250

4.18.1 Effects of undetoxified and detoxified *Jatropha curcas* oils on liver tissues (H and E stain)

Plate I: Section of the liver tissue from the control (olive oil 10 ml/kg) group with normal hepatocytes (H), sinusoids (S), central vein (CV) and kupffer cells (K).

Plate II: Section of the liver treated with 125 mg/kg of undetoxified (UME) *J. curcas* oil showing normal cytoarchitecture of the liver with cellular debris and slight infiltration of atypical lymphocyte in the central vein (CV) and the sinusoid slightly degenetrated.

Plate III: Section of the liver treated with 125 mg/kg detoxified (SDME) *J. curcas* oil showing apparently normal histological cytoarchitecture of the hepatocyte (H), kupffer cells (K), sinusoids (S) and central vein (CV).

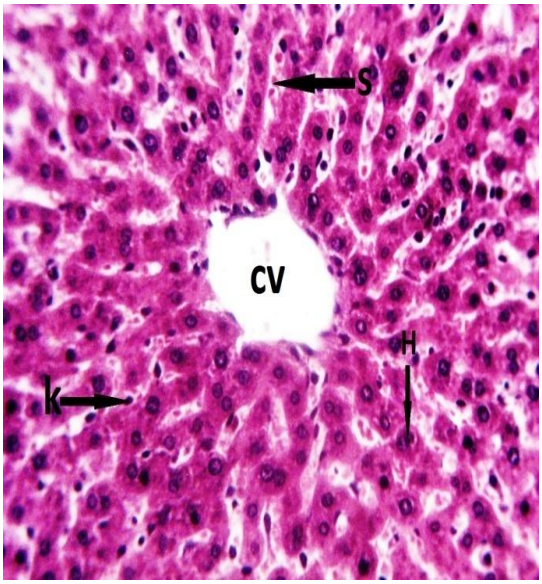


Plate IV: Liver (Control) x250

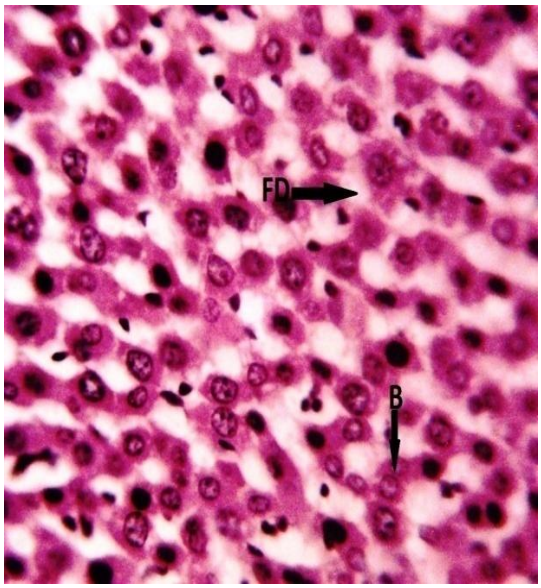


Plate V: Liver (250 mg/kg UME) x400

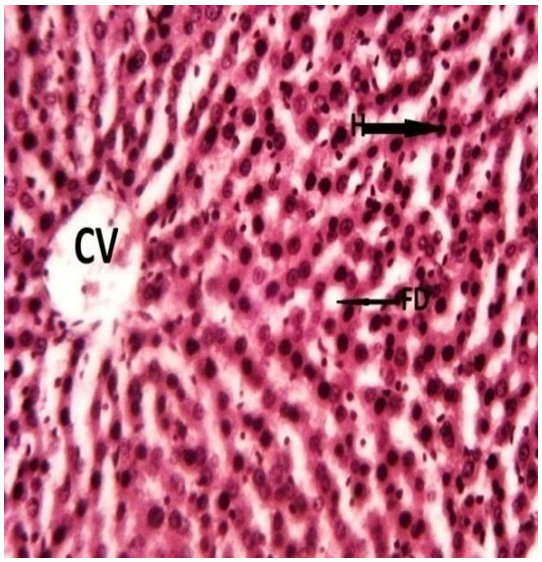


Plate VI: Liver (250 mg/kg SDME) x250

4.18.2 Effects of undetoxified and detoxified *Jatropha curcas* oils on liver tissues (H and E stain)

Plate IV: Section of the liver tissue from the control (olive oil 10 ml/kg) group with normal hepatocytes (H), sinusoids (S), central vein (CV) and kupffer cells (K).

Plate V: Section of the liver treated with 250 mg/kg of undetoxified *J. curcas* oil showing a cytoarchitecture of the liver with microvasculating fat droplets (FD) and binucleated hepatocytes (B).

Plate VI: Section of the liver treated with 250 mg/kg detoxified *J. curcas* oil showing apparently normal cytoarchitecture of the liver with few microvascular fat droplets (FD) and some binucleated hepatocytes (B).

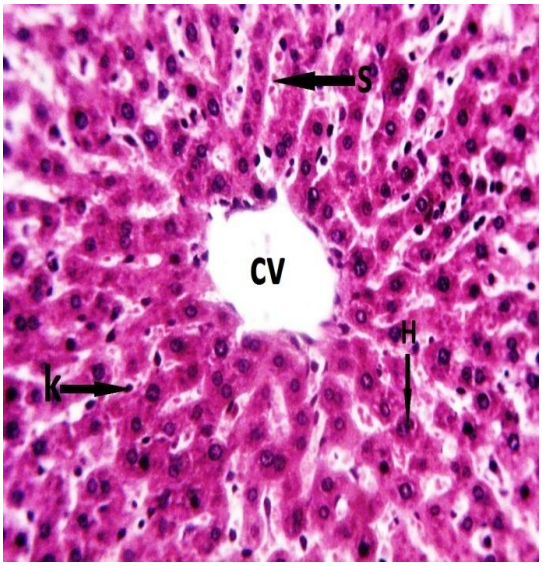


Plate VII: Liver (Control) x250

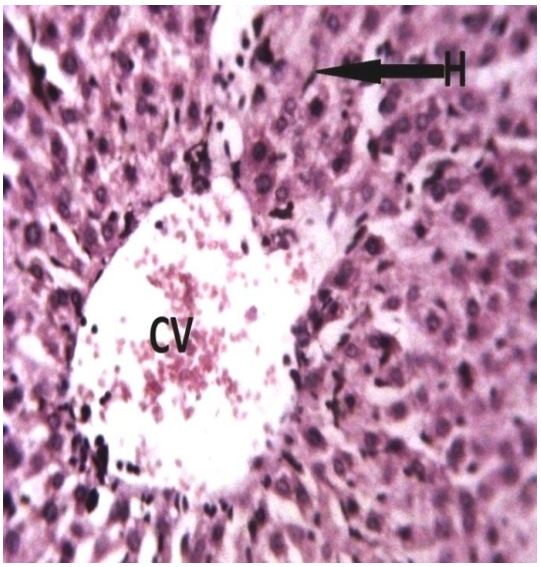


Plate VIII: Liver (500 mg/kg UME) x400

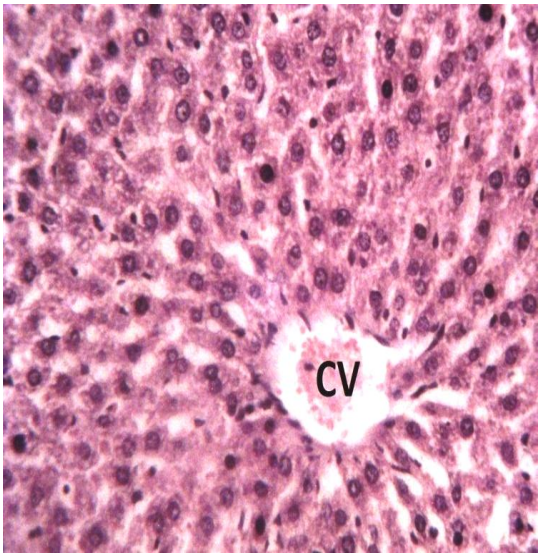


Plate IX: Liver (500 mg/kg SDME) x250

4.18.3 Effects of undetoxified and detoxified *Jatropha curcas* oils on liver tissues (H and E stain)

Plate VII: Section of the liver tissue from the control (olive oil 10 ml/kg) group with normal hepatocytes (H), sinusoids (S), central vein (CV) and kupffer cells (K).

Plate VIII: Section of liver treated with 500 mg/kg undetoxified *J. curcas* oil showing the cytoarchitecture of the liver with cellular debris and atypical lymphocyte in the central vein (CV), necrotic hepatolysis (H) and degenerated sinusoids (S).

Plate IX: Section of the liver treated with 500 mg/kg detoxified *J. curcas* oil showing apparently normal architecture of the liver with cellular debris in the central vein (CV) and slightly degenerated sinusoid.

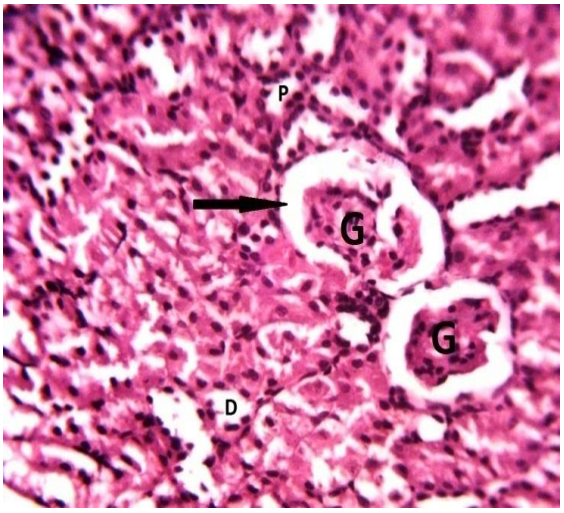


Plate X: Kidney Control x250

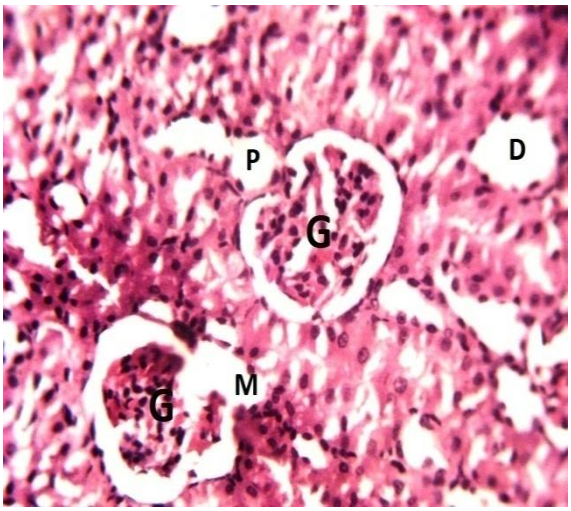


Plate XI: Kidney (125 mg/kg UME) x250

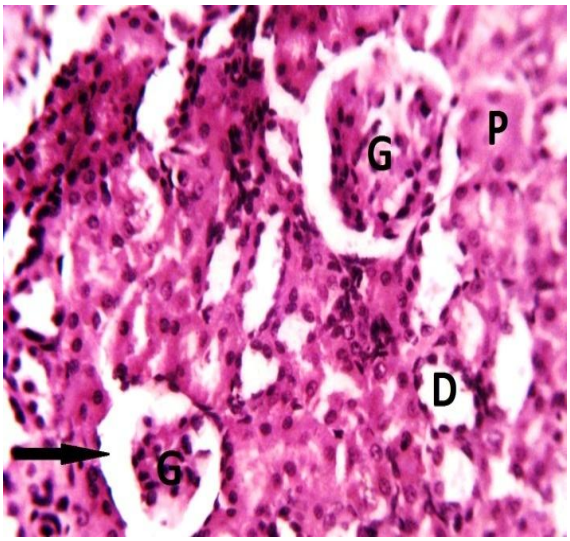


Plate XII: Kidney (125 mg/kg SDME) x250

4.19.1 Effects of undetoxified and detoxified *Jatropha curcas* oils on kidney tissues (H and E stain)

Plate X: Section of the renal cortex of the control (olive oil 10 ml/kg) group showing normal malphigian renal corpuscle containing glomerulus (G) surrounded by Bowman's space (arrow head), proximal and distal convoluted tubules.

Plate XI: Section of renal cortex of kidney treated with 125 mg/kg undetoxified (UME) *J. curcas* oil showing malphigian renal corpuscle and mildly shrunken glomerulus (G). Lining tubular epithelial cells show cytoplasmic vacuolation (V) and necrotic macula densa (M).

Plate XII: Section of renal cortex of kidney treated with 125 mg/kg detoxified (SDME) *J. curcas* oil showing two malphigian renal corpuscle with mildly shrunken glomeruli (G) and widened bowman's space (arrow head). Normal proximal (P) and distal convoluted tubule (D) were observed.

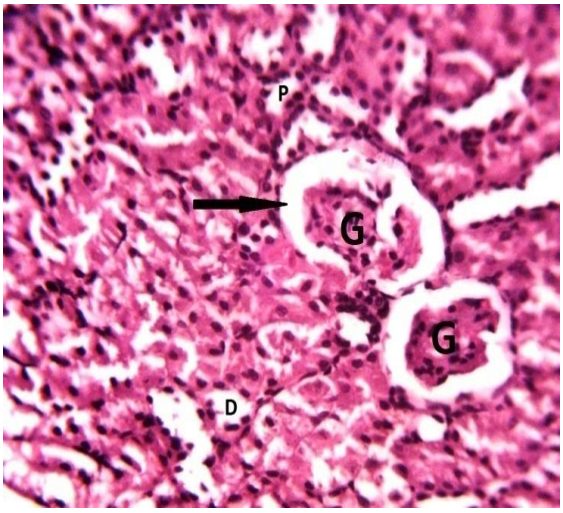


Plate XIII: Kidney (Control) x250

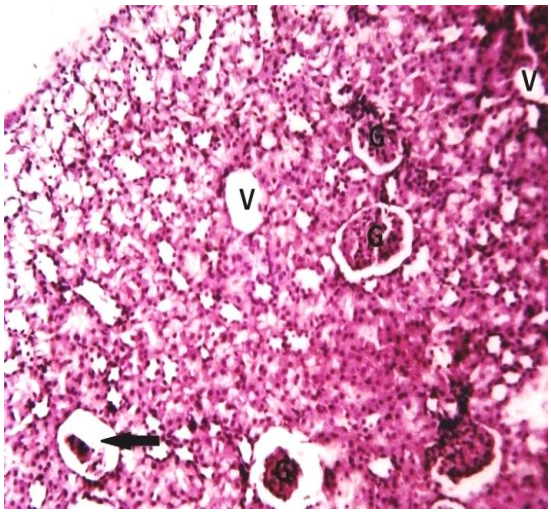


Plate XIV: Kidney (250 mg/kg UME) x100

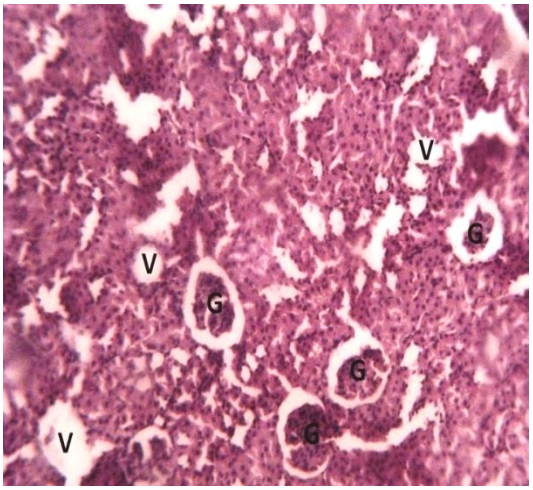


Plate XV: Kidney (250 mg/kg SDME) x100

4.19.2 Effects of undetoxified and detoxified *Jatropha curcas* oils on kidney tissues (H and E stain)

Plate XIII: Section of the renal cortex of the control (olive oil 10 ml/kg) group showing normal malphigian renal corpuscle containing glomerulus (G) surrounded by Bowman's space (arrow head), proximal and distal convoluted tubules.

Plate XIV: Section of renal cortex of kidney treated with 250 mg/kg undetoxified *J. curcas* oil showing vacuolated and shrunken renal corpuscle and widened bowman's space (arrow). Some of the lining tubular epithelial cells show cytoplasmic vacuolation (V). Cellular debris was observed in the lumina of some tubules.

Plate XV: Section of renal cortex of kidney treated with 250 mg/kg detoxified *J. curcas* oil showing mildly shrunken renal corpuscle (G) and widened bowman's capsules. Some of the lining tubular epithelial cells show mild cytoplasmic vacuolation (V). Metaplasia tubules were observed.

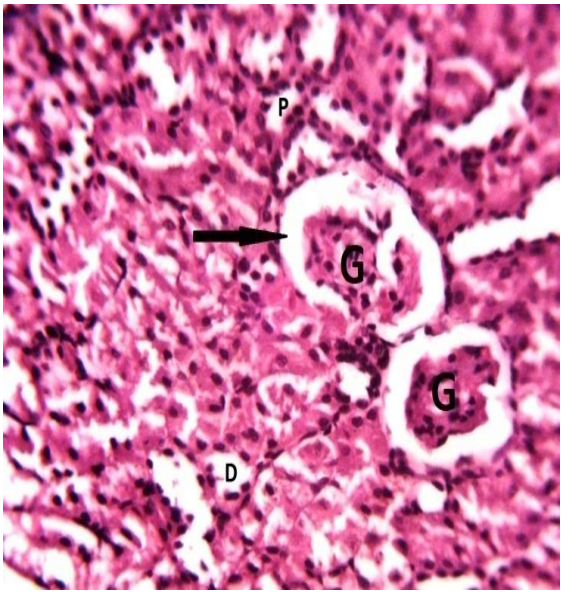


Plate XVI: Kidney (Control) x250

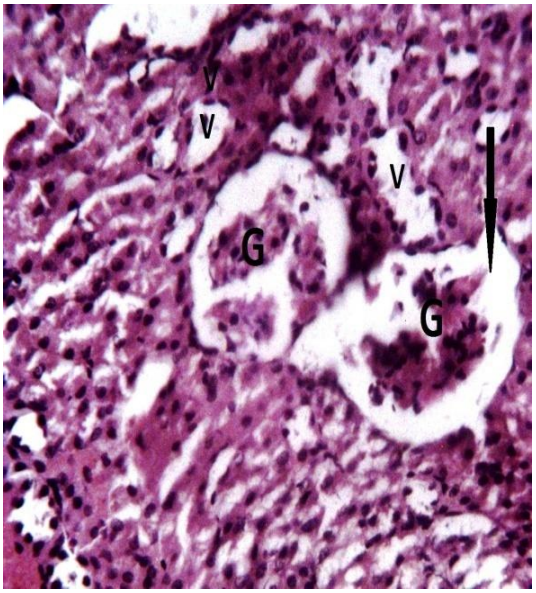


Plate XVII: Kidney (500 mg/kg UME) x100

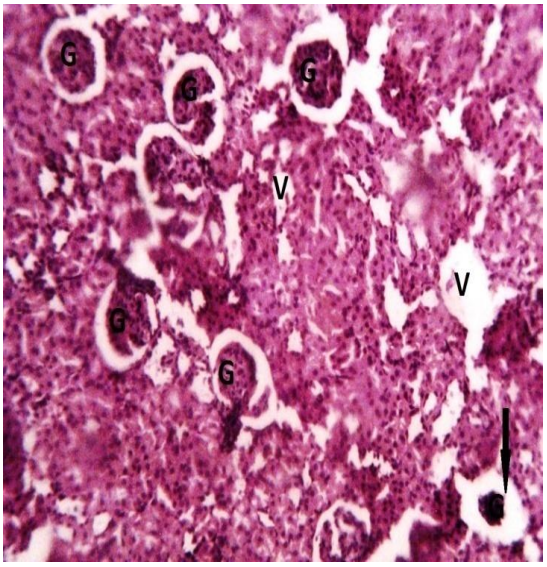


Plate XVIII: Kidney (500 mg/kg SDME) x100

4.19.3 Effects of undetoxified and detoxified *Jatropha curcas* oils on kidney tissues (H and E stain)

Plate XVI: Section of the renal cortex of the control (olive oil 10 ml/kg) group showing normal malphigian renal corpuscle containing glomerulus (G) surrounded by Bowman's space (arrow head), proximal and distal convoluted tubules.

Plate XVII: Section of the renal cortex of kidney treated with 500 mg/kg undetoxified *J. curcas* oil showing chronic shrunken renal corpuscle (G) and widened bowman's capsule (arrow head). Large amount of metaplasic renal tubules (V) with cellular debris were observed (arrow).

Plate XVIII: Section of the renal cortex of kidney treated with 500 mg/kg detoxified *J. curcas* oil showing shrunken renal corpuscles (G) and widened bowman's capsule (arrow) with metaplastic renal tubules.

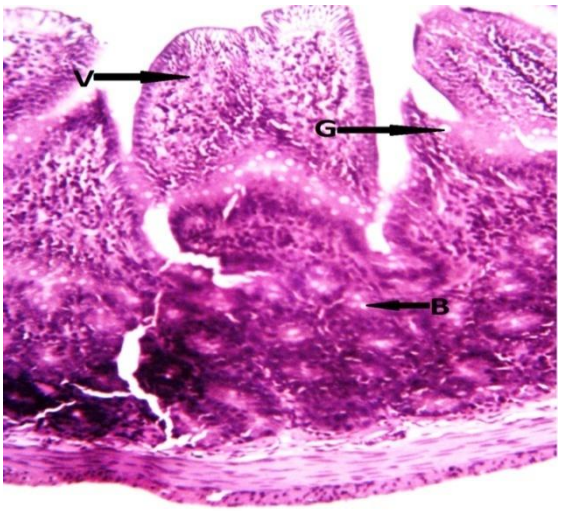


Plate XIX: GIT (Control) x250

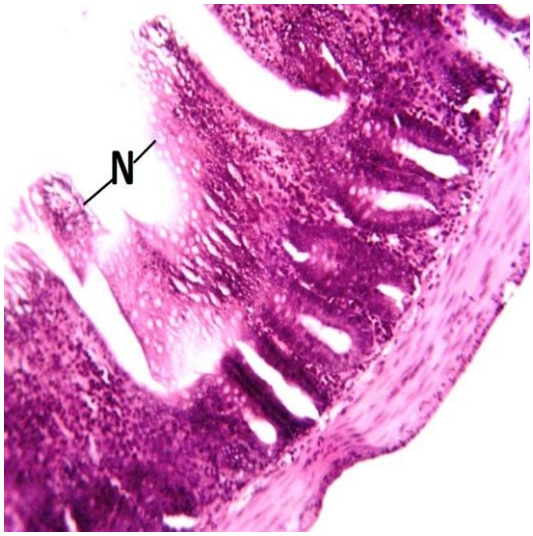


Plate XX: GIT (125 mg/kg UME) x250

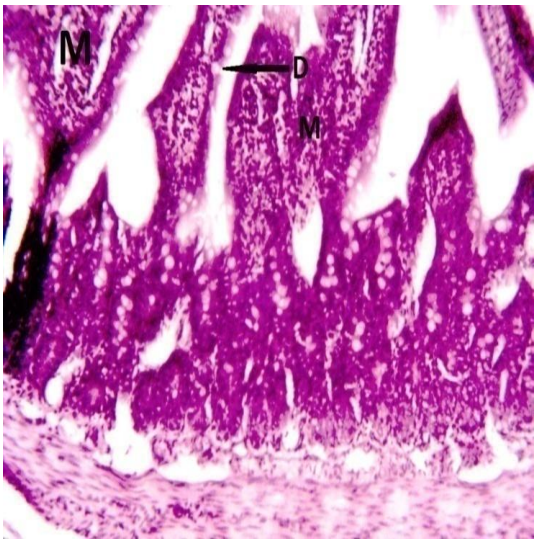


Plate XXI: GIT (125 mg/kg SDME) x250

4.20.1 Effects of undetoxified and detoxified *Jatropha curcas* oils on GIT (duodenal) tissues (H and E stain)

Plate XIX: Section of the wall of the small intestine of control (olive oil 10 ml/kg) group with normal sub mucosa with Brunner's gland (B) and tall cylindrical villi (V) with goblet cells (G).

Plate XX: Section of the small intestine treated with 125 mg/kg undetoxified (UME) *J. curcas* oil showing desquamation and necrosis of the tips of the villi (N) infiltrated with lymphocytes.

Plate XXI: Section of the small intestine treated with 125 mg/kg detoxified (SDME) *J. curcas* oil showing normal cytoarchitecture with mild desquamation (D), metaplasia (M) and necrosis at the tip of the villi epithelium.

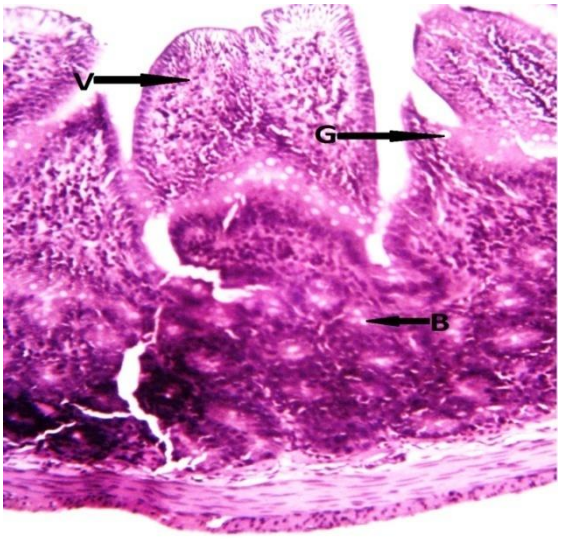


Plate XXII: GIT (Control) x250

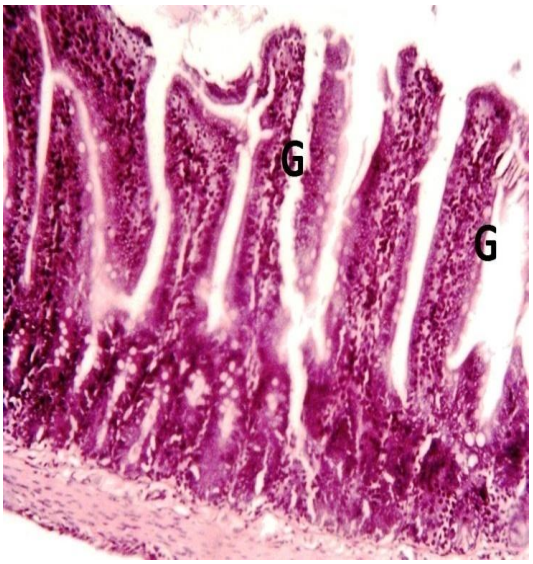


Plate XXIII: GIT (250 mg/kg UME) x250

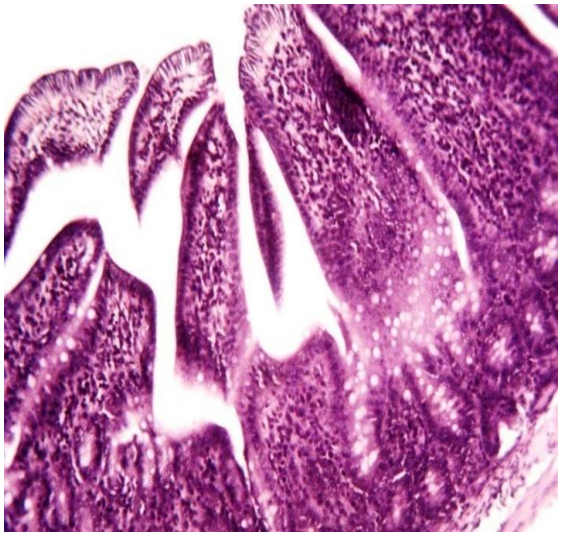


Plate XXIV: GIT (250 mg/kg SDME) x250

4.20.2 Effects of undetoxified and detoxified *Jatropha curcas* oils on GIT (duodenal) tissues (H and E stain)

Plate XXII: Section of the wall of the small intestine of control (olive oil 10 ml/kg) group with normal sub mucosa with Brunner's gland (B) and tall cylindrical villi (V) with goblet cells (G).

Plate XXIII: Section of the small intestine (SI) treated with 250 mg/kg undetoxified *J. curcas* oil showing chronic desquamation and necrosis of the tips of the villi epithelium. Hyperplasia and metaplasia (G) were also observed.

Plate XXIV: Section of the small intestine (SI) treated with 250 mg/kg detoxified *J. curcas* oil showing mild desquamation of the tips of the villi epithelium. Hyperplasia and metaplasia were also observed.

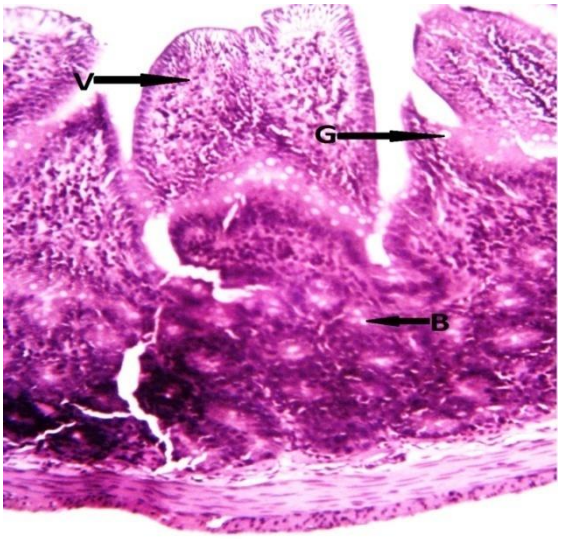


Plate XXV: GIT (Control) x250

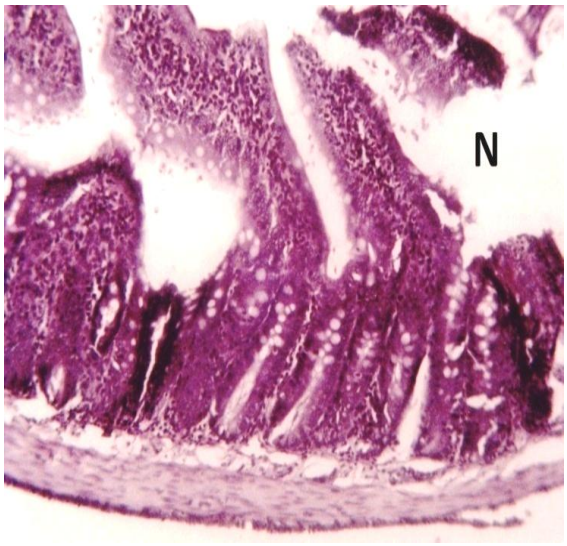


Plate XXVI: GIT (500 mg/kg UME) x100

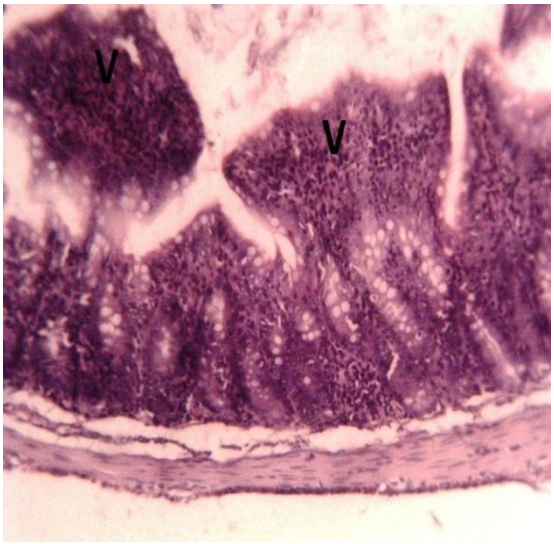


Plate XXVII: GIT (500 mg/kg SDME) x100

4.20.3 Effects of undetoxified and detoxified *Jatropha curcas* oils on GIT (duodenal) tissues (H and E stain)

Plate XXV: Section of the wall of the small intestine of control (olive oil 10 ml/kg) group with normal sub mucosa with Brunner's gland (B) and tall cylindrical villi (V) with goblet cells (G).

Plate XXVI: Section of the small intestine (SI) treated with 500 mg/kg undetoxified *J. curcas* oil showing chronic necrosis (N) and desquamation of the villi epithelium. Hyperplasia, metaplasia and chronic infiltration of lymphocytes were observed.

Plate XXVII: Section of the wall of the small intestine (SI) treated with 500 mg/kg detoxified *J. curcas* oil showing desquamation and necrosis of the villi (V). Metaplasia infiltrated with lymphocytes were observed.

CHAPTER FIVE

DISCUSSION

Defatting *J. curcas* seeds through solvent extraction yielded 49.1% w/w oil which was 3.3 fold higher than the yield obtained with mechanical extraction using hand-operated hydraulic screw press which was only 14.9% w/w oil. The 49.1% oil yield obtained from solvent extraction falls within the 44.2-49.7% range of values reported by Derkyi *et al*. (2014) and close to the 50% yield reported by Nzikou *et al*. (2009). However, Becker and Makkar (2009) reported 22-40% range of values which is lower than that obtained in this study. The 14.9% oil yield obtained from the mechanical oil extraction method was also lower than the 30.0-33.7% range of values obtained by Ahmed and Salimon (2009) possibly because of the hydraulic screw press used. While hand operated hydraulic screw press was used in the present study, that used by Ahmed and Salimon (2009) was not stated, but could have been motor driven. The higher percentage of oil yield obtained from the solvent extraction as compared to that obtained from mechanical extraction was because solvent extraction through the use of Soxhlet apparatus is exhaustive (Luque de Castro and Garcia-Ayuso, 2000). The repeated flow of the solvent through the powdered seed of increased surface area resulted in the recovery of more oil in contrast to mechanical extraction through hand operated screw press which only allowed for partial extraction.

The high oil content (49.1% in the present study) was such that *J. curcas* seed oil can be used as a biodiesel precussor (Gubitz *et al*., 1999; Becker and Makkar, 2009), in the cosmetic industry (Akbar *et al*., 2009) and as a pharmaceutical agent (Heller, 1996), and

for nutritional purposes (Makkar *et al*., 1997). Other than for biodiesel, other potential uses require that the oil be devoid of toxic constituents.

The density, specific gravity and refractive indices for both the solvent and mechanically extracted *J. curcas* oil were practically the same. Suggesting that these physical properties of the oil were not affected by the various methods of extraction, an observation that is supported by the findings of previous researchers in their comparisons of the physichochemical properties of solvent and mechanically extracted *J. curcas* oils (Belewu *et al*., 2010). The range of values obtained in this study for density, specific gravity and refractive index also agree with those obtained by previous studies (Akbar *et al*., 2009; Maricela *et al*., 2010). Specific gravity and density of oil give information about the concentraction of the oil while refractive index that measures the effect of the oil on rays of light passing through it, gives insight into its degree of purity (Inekwe *et al*., 2012). The free fatty acids, acid, peroxide and saponification values obtained for both the solvent and mechanically extracted oil were comparable, and ranged between 15.07-16.47%, 29.95-32.80 mg/g, 4.75-4.91 Meq/kg and 272.90-280.40 mg/g respectively. The values obtained for the free fatty acids is comparable to the 18% reported by Umaru and Aberuagba (2012) but slightly higher than 11.94% reported by Belewu *et al*. (2010). Acid value is an indicator of the degree of edibility of oil (Esuoso and Odetokun, 1995). Umaru and Aberuagba (2012) reported an acid value of 36.2 mg/g, a value similar to that obtained in this study. The peroxide and saponification values were also higher than those reported in previous studies (Akbar *et al*., 2009; Belewu *et al*., 2010; Umaru and Aberuagba, 2012). Peroxide value is an indicator of the deterioration of lipids due to oxidation of the double bond of an unsaturated fatty acid which causes rancidity. The higher saponification value obtained

in this study as compared to those reported previously by other researchers indicates the richness of the oil in triacylglycerols which are useful in the production of soap (Akbar *et al*., 2009).

Oil losses are common to oil refining processes due to factors such as amount of free fatty acids, phospholipids and the efficiency of the refining conditions (International Conference on Palms and Palm Products, 1989). The high amount of oil losses in this study was probably due to the laboratory-scale oil refining processes employed as compared to the industrial setting where losses are more likely to be minimised (Hamm *et al*., 2013a). The highest oil losses in this study occurred during the deacidification (neutralisation) process, possibly due to the high level of free fatty acids and saponification values of *J. curcas* oil. This may also be a reason for the amount of soaps formed (Hamm *et al*., 2013b). Oil loss during the decolourization process may have been due to the unavailability of high power pressure pumps in the laboratory, such as are used in the oil refining industries for more efficient oil recovery from bleaching reagents (Kellens, 1997; Hamm *et al*., 2013b).

Although cardiac glycosides, flavonoids, phenols, saponins and tannins were all absent, preliminary phytochemical screening showed the presence of alkaloids in the solvent and mechanically extracted oils, undetoxified and detoxified. Plant derived alkaloids have been reported to have among other pharmacological activities, analgesic activity (Grover *et al*., 2002; Sonavane *et al*., 2001) Common plant derived alkaloids with analgesic activity in clinical use include codeine and morphine (Doughari, 2012). The presence of alkaloids in the oil may be responsible for the analgesic activity observed in animal studies. Essential oils such as that of *Cinnamon osmophloeum* twigs have been

reported to have excellent antiinflamatory activity (Jakhetia *et al*., 2010). Falodun *et al*. (2013) also observed the presence of alkaloids in the preliminary phytochemical screening of the root bark of *J. multifida* on evaluation of its analgesic activity.

The presence of heavy metals and some mineral elements have been reported in *J. curcas* seed, small percentage of which have also been reported in the seed oil (Harder *et al*., 2013). The concentration of lead, cadmium, copper, iron, calcium and zinc obtained in this study for the undetoxified oil were found to be below European Medicines Agency (2007) maximum acceptable limits of metal residues in drug substances and excipients of 1.0, 0.1, 250, 1300, 2500 and 1300 ppm respectively. While cadmium and copper were undetected in both the undetoxified and synthetically detoxified oils, levels of all detected mineral elements were found to be reduced in the synthetically detoxified oils, a finding that is consistent with reported decrease in levels of copper and iron in groundnut oil after refining by Aluyor *et al*. (2009). This reduction may have resulted from the effect of the adsorbent property of the bleaching reagent used in the detoxification process that caused it to bind metals and colour pigments in oils (O'Brien, 2009). The reduction in levels of heavy metals in the synthetically detoxified oil was necessary because copper and iron have been reported to be involved in catalyzing oil oxidation (International Conference on Palm and Palm materials products, 1989) and also to prevent accumulation of heavy metals above the tolerable limits due to continuous usage of the oil.

The increased levels of both calcium and iron in the naturally detoxified oil could have resulted from the high concentration of these elements in the plantain peel ash extract used in place of caustic soda for the deacidification of the oil since Onyegbado *et al*.

(2002) and Olabanji *et al*. (2012) had reported high levels of these elements in plantain peel ash extracts. Calcium is essential nutritionally as bone and teeth mineral content (Weaver, 2001). Similarly, iron is an essential trace element that has important metabolic functions, including oxygen transport and storage and many redox reactions. Insufficient intake results in deficiency conditions such as anaemia, adverse outcomes of pregnancy, impaired psychomotor development and cognitive performance and reduced immune function. The decreased levels of the toxic elements due to detoxification as well as increased levels of the naturally detoxified *J. curcas* oils was therefore an advantageous outcome of the detoxification processes.

The main toxins present in *J. curcas* oil are phorbol esters, which prevent their utilization as feed ingredients (Makkar *et al.*, 1997). Detoxification of the oil both synthetically and naturally produced reduced levels of phorbol esters in the detoxified oil compared to the undetoxified. The percentage reduction in levels of phorbol esters obtained in this study was less than those reported in previous studies (Haas and Mittelbach, 2000; Ahmed and Salimon, 2009; Norhani *et al*., 2014). The variation in the percentage reduction in phorbol esters of this study from previous ones could be because of the direct weighing of phorbol ester rich fractions in the estimation of the levels of phorbol esters in the oils in place of the high performance liquid chromatography (HPLC) method due to unavailability; a limitation in this study. Phorbol esters act as analogues for diacylglycerol and are strong protein kinase activators; with some of the acute toxicity symptoms including irritation and inflammation of the gastrointestinal walls, diarrhoea and haemorrhagic eyes (Goel *et al*., 2007).

The variation in the percentage reduction in the levels of phorbol esters in the solvent and mechanically extracted oil may have been due to the difference in viscosity of both oils. Viscosity is described as the characteristic thickness of a fluid that causes it to resist deformation under shear stress. It is also defined as the resistance of a fluid to pouring or its internal resistance to flow (Nzikou *et al*., 2009). Mechanically extracted *J. curcas* oil has been reported to be about twice as viscous as the solvent extracted in previous studies (Belewu *et al*., 2010) although this feature was not ascertained in the present study. The higher viscosity of the mechanically extracted oil could have prevented the free flow of alkali solution and bleaching reagent used in the deacidification and bleaching steps respectively, of the detoxification process, resulting in reduced adsorption of phorbol esters from the oil. The bleaching and deacidification steps have been reported by other researchers to be the most effective steps in the detoxification of *J. curcas* oil by other researchers (Haas and Mittelbach, 2000 and Ahmed and Salimon, 2009). Other antinutrinitional factors such as trypsin inhibitors and lectins, also reported to be present in the oil, may have been reduced to non or less toxic levels during the detoxification process as they are heat labile and may have therefore been destroyed by heat treatment of the oil during the detoxification processes (Aderibigbe *et al*., 1997).

Both undetoxified and detoxified *J. curcas* oils showed LD_{50} values greater than 5,000 mg/kg when administered orally to chicks and mice. These high LD_{50} values showed that the oil is practically safe on acute exposure. The high LD_{50} values obtained in this study are similar to the 6 ml/kg (6,000 mg/kg) reported in previous studies by Gandhi *et al*. (1995).

The major toxicity symptoms observed in Wistar rats on administration of undetoxified and detoxified *J. curcas* oils were ruffled fur, hyperpnoea, restlessness followed by decreased mobility. Restlessness is a central nervous system symptom which may have resulted from electrolyte changes in the rats (Castilla-Guerra *et al*., 2006). These occurred mostly in the undetoxified oil (moderate to severe) as opposed to the detoxified oil (mostly mild). The degree of severity of the observed toxicity symptoms were also dose dependent, which is consistent with the previous research findings (Li *et al*., 2010). Similarly, the highest degree of body weight loss occurred in animals at higher and middle doses of the undetoxified oil while lower doses of the undetoxified oil as well as all doses of detoxified oil caused significant increases in the animal body weight.

The undetoxified oil at 500 mg/kg dose produced increased levels of WBC and PLT, though the detoxified oil at the same dose also produced milder increases. This shows the ameliorative effect of the detoxification of the oil. WBC increase in the blood has been implicated in pathological conditions such as inflammation which is one of the major toxic effects of *J. curcas* oil due to its chemical irritant effect (Farhangi *et al*., 2013) as observed in the histopathological examination of the organs in this study. This is also consistent with the reports of other researchers who have reported inflammation as one of the effects of *J. curcas* toxicity (Gandhi *et al*., 1995).

Electrolyte change due to the toxic effect of undetoxified *J. curcas* oil occurred in the group that received 500 mg/kg dose of the undetoxified oil, though moderate changes were observed at other doses of both the undetoxified and detoxified oil. The increased level of Na^+ (hypernatremia) may be due to distortions and pathological changes in the

structure and function of the kidney caused by the toxicity of the oil which also resulted in increased levels of serum creatinine and urea, an indication of impaired kidney function (Saidu *et al*., 2010). This effect may also account for the decrease in kidney to body weight ratio of rats administered the highest dose of the undetoxified oil. Hypernatremia has been implicated in body dehydration due to severe loss of water that accompanies the increased excretion of Na^+ from the blood (Laing, 2002). This observation is consistent with the findings of previous researches which have reported dehydration as one of the symptoms of *J. curcas* toxicity (Setty *et al*., 2013).

The increased level of liver enzymes observed in Wistar rats due to the administration of 500 mg/kg of the undetoxified oil indicates pathological effects of the oil on the liver function and may therefore account for the reduced liver to body weight ratio on administration of undetoxified *J. curcas* oil at this dose (Setty *et al*., 2013). The detoxified oil also produced pathological effects on the liver suggesting that the detoxification only provided mild or no protection to the liver. The significant increase in the level of AST is suggestive of necrosis of hepatic parenchymal tissues and therefore may be indicative of acute liver disease (Adedapo *et al*., 2007)

The formalin test is a biphasic analgesic test that consists of two distinctive phases; early and late phase. Drugs that act primarily on the central system inhibit both phases while peripherally acting drugs inhibit the late phase (Shibata *et al*., 1989). The early phase is probably a direct result of stimulation of nociceptors in the paw and reflects centrally mediated pain while the late phase is due to inflammation with a release of serotonin, histamine, bradykinin and prostaglandin (Tjolsen *et al*., 1992). The undetoxified oil at higher doses significantly inhibited both phases of the formalin-

induced pain with a more pronounced effect on the second than the first phase, suggesting the presence of both central and peripheral analgesic effects of the oil. Detoxified oil only significantly inhibited the second phase of formalin induced pain, suggesting the presence of peripheral analgesic activity. The hot-plate test is employed for the evaluation of centrally acting analgesics because centrally acting analgesic drugs elevate the pain threshold of rodents towards heat (Kiron *et al*., 2012). The significant activity observed in this test supports the central analgesic effects of the oils. Both undetoxified and detoxified oils showed the presence of analgesic activity in mice as widely reported (Duke, 1994; Edeoga *et al*., 2005; Agbogidi and Eruotor, 2012). This may have been due to the presence of phytochemicals such as alkaloids as detected in the phytochemical analysis and as also reported by (Aiyelaagbe *et al*., 2007). Alkaloids, which are one of the largest groups of phytochemicals in plants, have amazing effects on humans and this has led to the development of powerful painkiller medications (Kam and Liew, 2002). However, the undetoxified oil showed greater analgesic effect compared to the detoxified. This reduction in the observed analgesic effect of the detoxified may have been affected by the heat treatment during the detoxification process which may have led to the denaturation of the phytochemicals responsible for the analgesic activity of the oil (Rungapamestry *et al*., 2007; Volden *et al*., 2009). The decreased analgesic activity on administration of 500 mg/kg of the detoxified oil compared to the analgesic activity on administration of 250 mg/kg of the same oil suggests that the peak analgesic activity for the detoxified oil had been reached at 250 mg/kg. From this study, the analgesic activity of the undetoxified oil was dose dependent. The seed, latex and root of *J. curcas* have also been reported to have anti-inflammatory activity (De Feo, 1989; Mujumdar *et al*., 2000 and Mujumdar and Visar 2004).

The ability of *Jatropha curcas* oil to induce diarrhoea in animals has been reported (Heller, 1996) and is attributed to the presence of ricinoleic acid (Hosamami and Katagi, 2008); the active metabolite in castor oil which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa and diarrhoea induction. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin (Pierce *et al*., 1971). Both the undetoxified and detoxified oils elicited slight laxative activity in mice on administration of 0.5 ml of each oil though both were not statistically significant. Both undetoxified and detoxified oils did not induce diarrhoea on administration of 1 ml of each. The slight laxative activity observed in mice on administration 0.5 ml of *J. curcas* as well as lack of laxative activity on administration of 1 ml of the oil suggest the possibility of obtaining a higher laxative activity on administration of lower doses of the oil.

Histopathological examinations of the liver, kidney and intestine of the animals to which the undetoxified oil was administered all showed severe pathological impairment in the structures of these organs as compared to the mild degree observed in the animals to which the detoxified was administered, suggesting that the pre-treatment of the oil before administration to rats ameliorated the severe toxicity symptoms. This observed impairment is consistent with reports in previous studies (Gandhi *et al*., 1995; Setty *et al*., 2013).

Histopathologic examination suggests inflammation and hemorrhage in the liver which could have caused the presence of lymphocytes in the central vein and degeneration of sinusoids in rats administered the undetoxified oil. However, binucleated hepatocytes

were observed in rats at lower doses and due to administration of the detoxified oil, suggesting the ability of the hepatocytes to regenerate and recover from the toxic effect of the oil (Nabil *et al.*, 2011).

The widening of the Bowman's capsule and shrinkage of the glomerulus suggest renal impairment effect of the oil which may have resulted in the elevated levels of urea, creatinine and electrolyte imbalance. These observed effects are indicative of the acute renal toxic effect of the undetoxified oil which became mild due to detoxification of the oil (Ojediran and Emiola, 2014).

The villi are the part of the small intestine involved in the absorption of digested nutrients in the digestive system. The severe desquamation and necrosis of the villi epithelium observed at the higher doses of the undetoxified oil may have been responsible for the impairment of the ability of the villi to absorb nutrients from the gastrointestinal tract (GIT) resulting in its unavailability for use by the body cells thereby leading to the significant loss in body weight observed in the animals to which they were administered. The observed hyperplasia and infiltration of the intestinal cells suggest inflammatory responses by the immune system to the inflammation of the small intestine (duodenum) which may have been caused by the irritant effect of the oil. Detoxification of the oil may have been responsible for the reduction of these toxic symptoms to a milder degree at which the organ could easily recover from, enabling its ability to function in the absorption of nutrients and probably account for the significant weight gain resulting from administration of the detoxified oil (Ojediran and Emiola, 2014).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

J. curcas seeds yielded more oil when extracted through solvent extraction as compared to the mechanical method of extraction by hand operated screw press. Solvent and mechanically extracted oils contained the same levels of phorbol esters before ddetoxification. Heavy metals and other mineral elements were also present at low levels which may accumulate to toxic levels on constant ingestion of the oil. The toxic effects of the oil were not detected both in chicks and mice in acute toxicity studies, but were detected on further sub-acute toxicity studies which lasted for 14 days. These toxic effects were mostly those of inflammation and necrosis of various organs such as the liver, kidney and intestine and overall impairment of their functions primarily due to the irritating effect of the oil on the organs; and these effects were dose dependent. Signs of toxicity such as ruffled fur, hyperpnoea, restlessness followed by decreased mobility and weight loss were also observed. Detoxification of the oil by edible oil treatment method involving the use of synthetic and more naturally available materials separately, resulted in reduction in the level of phorbol esters and other toxic principles more in the solvent extracted oil than in the mechanically extracted. The effect of the detoxification resulted in the amelioration of the toxic symptoms to milder levels in rats. The oils showed analgesic activity in animals, though the detoxified showed reduced activity compared to the undetoxified. Laxative activities of undetoxified and detoxified oils in albino mice were not found to be statistically significant at all administered doses, though mild laxative activities were observed on administration of 0.5 ml of undetoxified and detoxified oils.

Contribution of the Current Research to the Body of Knowledge

1. *J. curcas* oil from seeds located in Zaria, North-Western part of Nigeria is toxic as it contains phorbol esters. It also contains heavy metals and mineral elements in minute quantities but may accumulate to toxic levels on constant exposure to the oil.

2. Detoxification of *J. curcas* oil through edible oil refining methods using synthetic materials brought about greater reduction in the level of phorbol esters in the detoxified oil as compared to the use of more naturally available materials for detoxification. Reduction in levels of phorbol esters was also more in the solvent extracted oil than in the mechanically extracted.

3. *J. curcas* oil detoxification through edible oil refining method by the use of more naturally available materials brought about increased levels of iron, calcium and copper in the detoxified oil while the use of synthetic materials brought about a decrease in the levels of heavy metals and mineral elements.

4. The effect of detoxification of *J. curcas* oil in reduction of phorbol esters and other toxic antinutrient in the oil translated to the amelioration of the toxic effects of the oil to mild levels in rats.

5. Analgesic activity of the detoxified *J. curcas* oil was reduced to a lower level after detoxification of the oil.

6.2 Conclusion

The study revealed that *J. curcas* oil is toxic in rats. The toxicity is attributable to the presence of phorbol esters and heavy metals. Detoxification by edible oil treatment method reduced the level of phorbol ester more in solvent extracted than mechanically extracted oil and ameliorated the toxic effects of the oil in animals. The analgesic activity of the oil was significant and was retained but to a lower degree after detoxification.

6.3 Recommendations

The recommendations for further research on this study include:

i) Studies on other pharmacological activities of detoxified *J. curcas* oil.

ii) Chronic toxicity studies of detoxified *J. curcas* oil.

iii) Determination of detoxification methods that will result in minimal oil losses.

REFERENCES

Abe, H., Konishi, H., Komiya H. and Arichi, S. (1981). Effects of saikosaponins on biological membranes. *Planta Medica*, 42: 356-363.

Adedapo, A. A., Abatan, M. O. and Olorunsogo, O. O. (2007). Effects of some plants of the spurge family on haematological and biochemical parameters in rats. *Veterinarski Arhiv* 77 (1): 29-38.

Adekola, K. A. (1991). Process optimization of oil expression from coconut (cocosuncifera L). An M.Sc. Thesis submitted to Department of Agric. Engg, University of Ibadan, Nigeria. NSAE Proc. 16: 102-11.

Aderibigbe, A.O., Johnson, C.O.L.E, Makkar, H.P.S, Becker, K. and Foidl, N. (1997). Chemical composition and effect of heat on organic matter and nitrogen degradability and some antinutritional components of *Jatropha* meal. *Animal Feed Science and Technology*, 67: 347-354.

Adolf, W., Opferkuch, H.J. and Hecker, E. (1984). Irritant phorbol derivatives from four Jatropha species. *Phytochemistry*, 23: 129-132.

Afia, E.S. and Momoh, J.A. (2006). Standard operating procedure. Unpublished guideline for the chemical pathology laboratory, National Hospital, Abuja.

Agbogidi, O.M., Erutor, P.G. (2012). Morphological Changes Due to Spent Engine Oil and its Heavy metal Components of *Jatropha curcas.* In: Baby, S. and Sandhu, P.S. (Ed) *Proceedings of The International Conference On Bioscience, Biotechnology and Health Sciences organized by Plenary Science Centre Research*, Singapore, pp. 88-93.

Aguiar, R. and Wink, M. (2005). How do slugs cope with toxic alkaloids. *Chemoecology*, 15: 167-177.

Ahmed, W.A. and Salimon, J. (2009). Phorbol ester as toxic constituents of tropical *Jatropha curcas* seed oil. *European Journal of Scientific Research*, 31(3): 429-436.

Aiyelaagbe, O.O., Adeyeni, B.A., Fatunsin, O.F. and Arimah, B.D. (2007). *In vitro* antimicrobial activity and phytochemical analysis of *Jatropha curcas* roots. *International Journal of Pharmacology*, 3(1): 106-110.

Akbar, E., Yaakob, Z., Kamarudin, S.K., Ismail, M. and Salimon, J. (2009). Characteristic and composition of *Jatropha curcas* oil seed from Malaysia and its potential as biodiesel feedstock, *European Journal of Scientific Research*, 29(3): 396-403.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. (2008). *Molecular Biology of the Cell*. Retrieved from http://www.pdfcampus.net/molecular-biology-of-the-cell-5th-edition/

Alonso-Amelot, M.E. (1997). The link between bracken fern and stomach cancer: milk. *Nutrition*, 13: 694-696.

Aluyor, E. O., Aluyor, P. and Ozigagu, C. E. (2009). Effect of refining on the quality and composition of groundnut oil. *African Journal of Food Science*, 3(8): 201-205.

Ames, B. N., Profet, M. and Gold, L. S. (1990). *Nature's chemicals and synthetic chemicals: Comparative toxicology*. Proceedings of the National Academy of Science, USA, 87: 7782-7786.

Antoniassi, N.A.B., Ferreira, E.V., Dos Santos, C.E.P., Arruda, L.P., Campos, J.L.E., Nakazato, L. and Codel, E.M. (2007). Intoxicação espontânea por *Ipomoea carnea* subsp. *fistulosa* (Convolvulaceae) em bovinos no Pantanal Matogrossense. *Pesquisa Veterinaria Brasileira*, 27: 415-418.

Association of Official Analytical Chemists (AOAC). (1990). *Official methods of analysis of the association of official analytical chemist* (17th ed.). Arlington, Virginia: Association of official analytical chemist.

Aregheore, M., Becker, K. and Makkar, H.P.S. (2003). Detoxification of a toxic variety of *Jatropha curcas* using heat and chemical treatments, and preliminary nutritional evaluation with rats. *The South Pacific Journal of Natural Sciences*, 21: 50-56.

Aslani, M.R., Maleki, M., Mohri, M., Afshari, A., Sharifi, K. and Najarnezhad, V. (2007). Castor bean (*Ricinus communis*) toxicosis in a sheep flock. *Toxicon,* 49: 400-406.

Aufreiter, S., Mahaney, W.C., Milner, M.W., Hancock, R.G.V., Ketch, L., Malloch, D., ...Sodhi, R. (2001). Mineralogical and chemical interactions of soils eaten by chimpanzees of the Mahale mountains and Gombe stream National parks, Tanzania. *Journal of Chemical Ecology,* 27: 285-311.

Augustus, G.D.P.S., Javabeans, M. and Seiler, G.J. (2002). Evaluation and bioinduction of energy components of *Jatropha curcas*. *Biomass Bioenergy*, 23: 161-164.

Australia New Zealand Food Authority. (2001). *Pyrrolizidine Alkaloids in Food. A toxicological review and risk assessment*. Technical Report Series No.2. Retrieved from: www.foodstandards.gov.au/_srcfiles/TR2.pdf.

Barbieri, L., Battelli, M.G. and Stirpe, F. (1993). Ribosome-inactivating proteins from plants. *Biochimica et Biophysica Acta*, 1154: 237-282.

Becker, K. and Makkar, H.P.S. (2009). *Jatropha curcas*: A biodiesel source for tomorrow's oil and biodiesel. *European Journal of Lipid Science and Technology,* 20: 104-7.

Belewu, M.A., Adekola, F.A., Adebayo, G.B., Ameen, O.M., Muhammed, N.O., Olaniyan, A.M. and Adekola, O.F. (2010). Physicochemical characteristics of

oil and biodiesel from Nigerian and Indian *Jatropha curcas* seeds. *International Journal of Biological and Chemical Sciences*, 4(2): 524-529.

Bokisch, M. (1993). Handbuch der Lebensmitteltechnologie- Nahrungsfette und -o¨ le. Ulmer, Stuttgart, pp. 346.

British Standard Institutions (1936). *Standard Methods for the Analysis of Fats*. Victoria, London, pp. 684.

Bruneton, J. (1995). *Pharmacognosie, Phytochimie, Plantes Médicinales; Éditions Technique & Documentation*. Paris, France, pp. 1120.

Butler, L., Rogler, J., Mehansho, H. and Carlson, D. (1986). Dietary Effects of Tannins. In: Plant Flavonoids in Biology and Medicine; Biochemical, Pharmacological, and Structure Activity Relationships, New York, USA, pp. 141-157.

Castilla-Guerra, L., Fernandez-Moreno, M.C., Lopez-Chozas, J.M. and Fernandez-Bolanos, R. (2006). Electrolyte disturbances and seizures. *Epilepsia*, 47(12): 1990-1998.

Cao, H. (2003). The distribution of calcium oxalate crystals in genus *Dieffenbachia* Schott and the relationship between environmental factors and crystal quantity and quality. (University of Florida, Master's thesis).

Cronquist, A. (1981). *Integrated System of Classification of Flowering Plants*. Columbia University Press, New York, U.S.A, pp. 1262.

D'Mello, J.P.F. (1995a). Antinutritional Substances in Legume Seeds. In: D'Mello, J.P.F. and Devendra, C. (Ed.) *Tropical Legumes in Animal Nutrition*. CAB International, U.K., pp. 135-172.

D'Mello, J.P.F. (1995b). Underutilized Legume Grains in Non-Ruminant Nutrition. In: D'Mello, J.P.F. and Devendra, C. (Ed.) *Tropical Legumes in Animal Nutrition.* CAB International, U.K., pp. 289-329.

Dalziel, J.M. (1955). *The Useful Plants of West-Tropical Africa*. Crown Agents for Oversea Governments and Administration, London, pp. 147.

De Feo, V. (1989). Use of plant action anti-inflammatoria upper Ucayall, eastern Peru. *Fitoterapia*, 62: 481-494.

Derkyi, Agyemang, N.S., Sekyere, Daniel, Oduro and Antwi, K. (2014). Variations in oil content and biodiesel yield of *Jatropha curcas* from different agro-ecological zones of Ghana. *International Journal of Renewable and Sustainable Energy*. Vol. 3(4): 76-81.

Devappa, R.K., Makkar, H.P.S. and Becker, K. (2010). *Jatropha* toxicity – A review. *Journal of Toxicology and Environmental Health*, 13: 476-507.

Diaz, G.J. (2001). Naturally Occurring Toxins Relevant to Poultry Nutrition. In: Leeson, S. and Summers, J.D. (Ed) *Scott´s Nutrition of the Chicken*, 4th ed., University Books, Guelph, Ontario, Canada. pp. 544-591.

Diaz, G.J. (2011). Toxic plants of veterinary and agricultural interest in columbia. *International Journal of Poisonous Plant Research*, 1(1): 5.

Doughari, J.H. (2012). *Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health*. doi: 10.5772/26052

Duffus, C. M. and Duffus, J. H. (1991). Introduction and Overview. In: D‟‟Mello, F. J.P., Duffus, Duffus, C. M. and Duffus, J. H. *Toxic substances in crop plants* (eds). Royal Society of Chemistry, Cambridge, pp. 1-21.

Duke, J. A. (1985). Medicinal plants. *Science*, 229: 1036.

Duke, J.A. (1988). CRC Hand book of Medicinal Herbs. CRC Press, Boca Raton, USA, pp. 253-254.

Duke, J.A. (1994). Biologically Active Compounds in Important Species. In: Charalambous, E. (Ed) *Species, Herbs in Edible Fungi*. Elsevier science, Oxford, pp. 225-250.

Eddy, N.B. and Leimback, D. (1953). Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *Journal of Pharmacology and Experimental Therapeautics*, 107: 385-393.

Edeogba, H.O., Okwu, D.E. and Mbaebre, B.O. (2005). Phytochemical constituents of some Nigerian plants. *African Journal of Biotechnology*, 44(7): 685-688.

Eja, M.E., Asikong, B.E., Abriba, C., Arikpo, G.E., Anwan, E.E. and Enyi-Idoh, K.H. (2007). A comparative assessment of the antimicrobial effects of garlic (*Allium sativum*) and antibiotics on diarrheagenic organisms. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 38(2): 343-8.

Els, J. M., Damme, V., Peumans, W. J., Pusztai, A. and Bardocz, S. (1998). *Handbook of Plant Lectins: Properties and Biomedical Applications*. John Wiley and Sons, pp. 78.

Endo, Y. and Tsurugi, K. (1987). RNA N-glycosidase activity of ricin A-chain: Mechanism of action of the toxic lectin ricin on eukaryotic ribosomes. *Journal of Biological Chemistry*, 262: 8128-8130.

Endo, Y., Mitsui, K., Motizuki, M. and Tsurugi, K. (1987). The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes: The site and the characteristics of the modification in 28S ribosomal RNA caused by the toxin. *Journal of Biological Chemistry*, 262: 5908-5912.

Esuoso, K.O. and Odetokun S.M. (1995). Proximate chemical composition and possible industrial utilization of *Biphia sapida* seed oils. *La Rivista Italiana Delle Sostanze Grasse*, 72:7.

ETHOS User Manual Rev 01/2001. Retrieved from, acid digestion guidelines http//www.yumpu.com/en/documents/view/37608950/mpr

European Medicines Agency (2007). *Pre-authorisation Evaluation of Medicines for Human Use: Guideline on the Specification Limits for Residues of Metal Catalysts*. London, UK.

Falodun, A., Igbe, I., Erharuyi, O. and Agbanyim, O.J. (2013). Chemical characterization, anti-inflamatory and analgesic properties of *J. multifida* root bark. *Journal* of *Applied Science* and *Environmental Management*, 17 (3): 357-362.

Farhangi, M.A., Keshavarz, S.A., Eshraghian, M., Ostadrahimi, A. and Saboor-Yaraghi, A.A. (2013). White blood cell count in women: Relation to inflammatory biomarkers, haematological profiles, visceral adiposity, and other cardiovascular risk factors. *Journal of Health Population and Nutrition*, 31(1): 58-64.

Fernández-Alonso, J.L., and Hernández-Schmidt, M. (2007). Catálogo de la flora vascular de la cuenca alta del río Subachoque (Cundinamarca, Colombia). *Caldasia*, 29: 73-104.

Food Standard Australia New Zealand (2004). Final assessment report proposal: Advice on the preparation of cassava and bamboo shoots. Report Number 2-04. Canberra: FSANZ.

Francis, G., Kerem, Z., Makkar H.P.S. and Becker, K. (2002). The biological action of saponins in animal systems: A review. *British Journal of Nutrition*, 88:587-605.

Fu, P.P., Xia, Q., Lin, G. and Chou, M.W. (2004). Pyrrolizidine alkaloids-Genotoxicity,

metabolism enzymes, metabolic activation and mechanisms. *Drug Metabolism Reviews*, 36: 1-55.

Fu, P.P., Yang, Y.C., Xia, Q., Chou, M.C., Cui, Y.Y. and Lin G. (2002). Pyrrolizidine alkaloids-tumorigenic components in Chinese herbal medicines and dietary supplements. *Journal of Food and Drug Analysis*, 10(4): 198-211.

Gandhi, V. M., Cherian, K. M. and Mulky, M. J. (1995). Toxicological studies on Ratanjyot oil. *Food and Chemical Toxicology*. 33: 39-42.

Ganguli, S. (2002). Neem: A therapeautic for all seasons. *Current Science*, 82: 11.

Ganter, P. and Jolles, G. (1970). In tissus conjonctifs in histichimie normales et pathologique, Gauthier-Villars Paris, 2: 1045-1126.

Gaudani, H., Gupta, M., Guptaa, N., Trivedi, S., Patil, P., Gupta, G. and Vamshi Krishna, K. (2009). Isolation and characterization of phorbol esters (toxin) from *Jatropha curcas* L. *International Journal of Microbiology Research*, 1(1): 1-7.

Gauthier, C., Legault, J., Girard-Lalancette, K., Mshvildadze, V. Pichette, A. (2009). Haemolytic activity, cytotoxicity and membrane cell permeabilization of semi-synthetic and natural lupane and oleanane-type saponins. *Bioorganic and Medicinal Chemistry*, 17: 2002-2008.

Goel, G., Makkar, H.P.S., Francis, G. and Becker, K. (2007). Phorbol esters: structure, biological activity, and toxicity in animals. *International Journal of Toxicology*, 26: 279-288.

Gross, H., Foidl, G. and Foidl, N. (1997). Detoxification of *J. curcas* Press Cake and Oil and Feeding Experiments on Fish and Mice. In: Gubitz, G.M., Mittelbach, M., Trabi, M. (Ed) *Biofuels and Industrial Products from Jatropha curcas*. Dubrovnik Graz, Austria, pp. 179-182.

Grover, J.K., Khandkar, S., Vats, V., Dhunnoo, Y. and Das, D. (2002). Pharmacological studies on *Myristica fragrans* – antidiarrhoeal, hypnotic, analgesic and hemodynamic (blood pressure) parameters. *Experimental and clinical pharmacology*, 24(10): 675-680.

Gubitz, G.M., Mittelbach, M. and Trabi, M. (1998). Exploitation of the tropical oil seed plant *Jatropha curcas* L. *Bioresource Technology*, 67: 73-82.

Gunstone, F. D. and Norris, F. A. (1983). Lipids in food; Chemistry, Biochemistry and Technology, Pergamon Press, Oxford, U.K. pp. 95-100.

Gupta, M.N., Sharma, A. and Shah, S. (2005). Extraction of *Jatropha curcas* L. oil from kernel by combination of ultrasonic and aqueous enzymatic oil extraction. *Bioresource Technology*, 96: 121-123.

Haas, W. and Mittelbach, M. (2000). Detoxification experiments with the seed oil from *Jatropha curcas* L. *Industrial Crops and Products*, 12: 111-118.

Hagels, H., Wagenbreth, D. and Schilcher, H. (1995). Phenolic Compounds of Buckwheat Herb and Influence of Plant and Agricultural Factors (*Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Gärtner). In: Matano, T. and Ujihara, A. (Ed) *Current Advances in Buckwheat Research*: *Proceedings of the 6th International Symposium on Buckwheat*, Shinshu University Press, Shinshu, China. pp. 801-809.

Hallak, H.O. and Wedlund, P.J. (1991). Vitamin K epoxide reductase activity and its inhibition by warfarin in young and old rats. *Drug Metabolism and Disposition*, 19: 278-279.

Hamm, W., Hamilton, R.J. and Gijs, C. (2013a). *Edible Oil Processing*. John Willey and Sons, U.K., pp. 129.

Hamm, W., Hamilton, R.J. and Gijs, C. (2013b). *Edible Oil Processing*. John Willey and Sons, U.K., pp. 170-194.

Harder, M.N.C., Duarte, E.C.M., Barros, L.L.S., Maciel, P.B., Abreu Jnr. C.H., Villanueva, F.C.A. and Arthur, V. (2013). *Jatrofa* Seeds; Oil and Biodiesel Quality: Nutrients and Potentially Toxic Elements Determined by Mass Spectroscopy Inductively Coupled Plasma. Materials and Processes for Energy: Communicating Current Research and Technology Developments.

Haubrich, W.S. (2003). "officina". *Medical Meanings: a Glossary of Word Origins*. (2nd ed.). Philadelphia, American College of Physicians, USA, pp. 201.

Heller, J. (1996). Physic nut, (*Jatropha curcas* L.) promoting the preservation and utilization of underutilized and neglected crops. Institute of plant genetics and crop plant research, Garrtersleben, Germany. *Industrial Crops and Products*, 28: 1-10.

Hosamami, K.M. and Katagi, K.S. (2008). Characterization and structure elucidation of 12-hydroxyoctadec-cis-9-enoic acid in *Jatropha gossypiifolia* and *Havea brasiliensis* seed oils: a rich source of hydroxyl fatty acid. *Chemistry and Physics of Lipids*, 152: 9-2.

Hong, Y., B.U., Y. and LO, L.C. (2013). Methods and systems for detoxifying phorbol esters in plant products. *Patentscope*, 13 (78): 22.

Hong Kong Center for Food Safety, Food and Environmental Hygiene Department (2007). Natural toxins in food plants. *Risk Assesment Studies*, (27): 4-39.

Huisman, J., Van der poel, A.F.B. and Liener, I.E. (1989). Recent advances of research in antinutritional factors in legume seeds. PUDOC, Wageningen.

Inekwe, U. V., Odey, O., Gauje, B., Dakare, A. M., Ugwumma, C. D. and Adegbe E. S. (2012). Fatty acid composition and physicochemical properties of *Jatropha Curcas* oils from Edo and Kaduna states of Nigeria and India. *Annals of Biological Research*, 3(10): 4860-4864.

Institute of Laboratory Animal Research. (1996). *Guide for the care and use of laboratory animals.* Retrieved from: www.nap.edu.

International Conference on Palms and Palm Products. (1989). *The Impart of Research and Development on the Production, Processing and Utilisation of Palms and Palm Products* 2: 707-708.

Iwu, M. (1993). *Handbook of African medicinal plants*. CRC Press, Boca Raton, Florida.

Izzo, A.A., Nicoletti, M. Giannattasio, B. and Capasso, F. (1992). Antidiarrhoeal Activity of *Terminalia sericea Burch* ex. DC Extract. In: Capasso, F. and N. Mascolo (Eds.), Natural Drugs and the Digestive Tract. EMSI, Rome, pp: 223-230.

Jakhetia, V., Patel, R., Khatri, P., Pahuja, N., Garg, S., Pandey, A. and Sharma, S.A. (2010). Cinnamon: A pharmacological review. *Journal of Advanced Scientific Research*, 1(2): 19-23.

Jolly, R.D. and Walkley, S.U. (1997). Lysosomal storage diseases of animals, an essay in comparative pathology. *Veterinary Pathology*, 34: 527-548.

Jongschaap R.E.E., Corre W.J., Bindraban, P.S. and Brandenburg W.A. (2007). *Claims and Facts on Jatropha curcas L.: Global Jatropha curcas evaluation, breeding and propagation Programme*. B.V. Wageningen, The Netherlands. pp. 1-12.

Kam, P.C.A. and Liew, S. (2002). Traditional Chinese herbal medicine and anaesthesia. *Anaesthesia*, 57: 1083-1089.

Karim, A., Makhfi, H., Ziyyat, A., Legssyer, A. and Bnouham, M. (2010). Antidiahoeal activity of crude aqueous extract of *Rubia Tinctorum l*. Roots in rodent. *Journal of Smooth Muscle Research,* 46(2): 119-123.

Keddy, P.A. (2007). *Plants and Vegetation: Origins, Processes, Consequences*. Cambridge University Press, Cambridge, UK, pp. 666.

Kendirim, O.C., Chukwu, O.A. and Achinewhu S.C. (1995). Effect of traditional processing of cassava on the cyanide content of garri and cassava flour. *Plant Foods for Human Nutrition*, 48(4): 335-339.

Khan, L. M. and Hanna, M. A. (1983). Expression of oil from oil-seeds: A review. *Journal of Agricultural Engineering Research*, 28: 495-503.

Khanna, S., Gupta, S.R. and Grover, J.K. (1986). Effect of long term feeding of tulsi on reproductive performance of adult albino rats. *Indian Journal of Experimental Biology*, 24: 302-304.

Kellens, M. (1997). Current developments in oil refining technology. Antwerp Belgium: Technical report De Smet-Belgium. pp. 31-48.

King, A.J., He, W., Cuevas, J.A., Ramiaramanana, D. and Graham, I.A. (2009). Potential of *Jatropha curcas* as a source of renewable oil and animal feed: Review paper. *Journal of Experimental Botany*, 10: 1093.

Kingsbury, J. M. (1964). *Poisonous Plants of the United States and Canada.* Englewood Cliffs, Prentice Hall Inc., Englewood Cliffs, USA, pp. 626.

Kiron, S.S., Nizar, K., Rajagopal, P.L., Saritha, M. and Narayaswamy, V.B. (2012). Analgesic activity study of *Polygonum glabrum* willd in rodents. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 3(3): 1158.

Kochhar, S., Singh, S.P. and Kochhar, V.K. (2008). Effect of auxins and associated biochemical changes during clonal propagation of the biofuel plant – *Jatropha curcas*. *Biomass and Bioenergy*, 32: 1136-1143.

Kpoviessi, D.S.S., Accrombessi, G.C., Kossouoh, C., Soumanou, M.M. and Moudachirou, M. (2004). Propriétés physico-chimiques et composition de l'huile non conventionnelle de pourghère (*Jatropha curcas*) de différentes régions du Bénin. *Comptes Rendus Chimie*, 7: 1007-1012.

Kulkarni, S.K. (1999). *Hand Book of Experimental Pharmacology*. (3rd ed), Vallabh Prakashan, New Delhi, India, pp. 115-133.

Kumar, A. and Sharma, S. (2008). *An Evaluation of Multipurpose Oil Seed Crop Jatropha curcas for Industrial Uses*. Druck- Beratungs- und VerlagsgesmbH, Geidorfgürtel, Austria, pp. 160-166.

Laing, I.A. (2002). Hypernatremic dehydration in newborn infants. *Acta Pharmaceutica Sinica B*, 23: 48-51.

Li, C.Y., Devappa, R.K., Liu, J.X., Lv, J.M., Makkar, H.P.S. and Becker, K. (2010). Toxicity of *Jatropha curcas* phorbol esters in mice. *Food and Chemical Toxicology*, 48: 620-625.

Liener, I.E. (1994). Implication of antinutritional components in soyabeans foods. *Food Science and Nutrition*, 34: 31-64.

Lin, J., Yan, F., Tang, L. and Chen, F. (2003). Antitumor effects of curcin from seeds of *Jatropha curcas*. *Acta Pharmacologica Sinica*, 24: 241-246.

Loretti, A.P., Da Silva, I.M.R. and Ribeiro, R.E. (2003). Accidental fatal poisoning of a dog by *Dieffenbachia picta* (dumb cane). *Veterinary and Human Toxicology*, 45: 233-239.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54(4): 275-87.

Lucia, B., Nibia, B. and Guillermo, D. (2008). Annual male reproductive cycle of a Hantavirus reservoir, the long-tailed mouse *Oligoryzomys flavescens* (Rodentia,

Crcentigae, Sigmodontinae) from Uruguay. *Mastozoologia Neotropica Mendoza*, 15(1): 135-140.

Luque de Castro, M.D. and Garcia-Ayuso, L.E. (2000). *Environmental Application*/*Soxhlet Extraction*. Academic press, Cordoba, Spain, pp. 2701.

Maga J.A. (1980). Potato alkaloids. *Critical Reviews in Toxicology Journal*, 12: 371-405.

Mahanta, N., Gupta, A. and Khare, S.K. (2008). Production of protease and lipase by solvent tolerance *Pseudomonas aeruginosa* PseA in solid state fermentation using *Jatropha curcas* seed cake as substrate. *Bioresource Technology*, 99: 1729-1735.

Makkar, H. P. S. and Becker, K. (1997a). Potential of *Jatropha curcas* seed meal as a protein supplement to livestock feed, constraints to its utilization and possible strategies to overcome constraints to its utilization. In Proceedings *Jatropha* 1997: International symposium on Biofuels and Industrial Products from *Jatropha curcas* and other tropical oil seed plants, February 23-27, Managua, Mexico.

Makkar, H.P.S. and Becker, K. (1997b). Potential of *J. curcas* seed meal as a protein supplement to livestock feed, constraints to its utilisation and possible strategies to overcome constraints. In: Gu¨ bitz, G.M., Mittelbach, M., Trabi, M. (Eds.), Biofuels and Industrial Products from *Jatropha curcas*. Dubrovnik Graz, Austria, pp. 190-205.

Makkar, H. P. S., Becker, K., Sporer, F. and Wink, M. (1997). Studies on nutritive potential and toxic constituents of different provenanaces of *Jatropha curcas*. *Journal of Agriculture and Food Chemistry*, 45: 3152-3157.

Makkar, H. P. S. and Becker, K. (1999). Plant toxins and detoxification methods to improve feed quality of tropical seeds. *Asian-Australian Journal of Animal Science*, 12(3): 467-480.

Maricela, R., Acosta, J.S., Ramirez, R.Z. and Diaz, (2010). Extraction and characterization of oil from three Mexican *Jatropha curcas* seed oil from Malaysia and its potential as biodiesel feed stock. *European Journal of Scientific Research*, 29(3): 89-91.

Martinez-Herrera, J., Siddhuraju, P., Francis, G., Davila-Ortiz, G. and Becker, K. (2006). Chemical composition, toxic/antimetabolic constituents and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. *Journal of Agricultural and Food Chemistry*, 96: 80-89.

Mattocks, A.R. (1986). *Chemistry and Toxicology of Pyrrolizidine Alkaloids.* Academic Press, London, UK, pp. 393.

McGraw, L.J. and Eloff, J.N. (2005). Screening of 16 poisonous plants for antibacterial, anthelmintic and cytotoxic activity in vitro. *South African Journal of Botany*, 71: 302-306.

McMillan, M. and Thompson, J.C. (1979). An outbreak of suspected solanine poisoning in school boys: Examination of criteria of solanine poisoning. *Quarterly Journal of Medicine*, 48: 227-443.

Melzig, M.F., Bader, G. and Loose, R. (2001). Investigations of the mechanism of membrane activity of selected triterpenoid saponins. *Planta medica*, 67: 43-48.

Meskin M.S. (2002). *Phytochemicals in Nutrition and Health*. CRC Press, Boca Raton, USA, pp. 123.

Miyake, K., Tanaka, T. and McNeil, P.L. (2007). Lectin based food poisoning: A new mechanism of protein toxicity. doi:10.1371/journal.pone.0000687.

Mujumdar, A.M., Upadyhe, A.S. and Misar, A.V. (2000). Studies on antidiarrhoeal activity of *Jatropha curcas* root extract in albino mice. *Journal of Ethnopharmacology*, 70: 183-187.

Mujumdar, A.M. and Visar, A.V. (2004). Anti-inflammatory activity of *Jatropha curcas* roots in mice and rats. *Journal of Ethnopharmacology*, 90: 11-15.

Mukherjee, P.K., Das, J., Balasubramanian, R., Saha, K., Pal, M. and Saha, B.P. (1995). Antidiarrhoeal evaluation of *Nelumbo nucifera* rhizome extract. *Indian Journal Pharmacology*, 27: 262-264.

Mutschler, E., Geisslinger, H., Kroemer, H.K., Ruth, P. and Schäfer-Korting, M. (2008). *Mutschler-Arzneimittelwirkungen*. Lehrbuch der Pharmakologie und Toxikologie, Stuttgart. Retrieved from http://www.amazon.de/Mutschle-Arzneimittel wirkungen-Pharmakologie-Klinische-Toxikologie/dp/3804728987.

Nabil, A.E.A., Neveen, A. E., Eman, E. E. and Eman, A.E. (2011). Chemical and Pathological evaluation of *Jatropha curcas* seed meal toxicity with or without heat and chemical treatment. *Australian Journal* of *Basic* and *Applied Sciences*, 5(12): 49-59.

Norhani, A., Azhar, N., Wan, Z.S., Syahida, A., Ehsan, O., Farida, A. and Youssuf, G. (2014). Detoxification of toxic phorbol esters from *Jatropha curcas* Linn. by *Tricoderma* species and endophytic fungi. *International Journal of Molecular Sciences*, 15: 2274-2288.

Norris, F. A. (1964). *Extraction of Fats and Oils. Baily's Industrial Oil and Fat Products*. John Wiley and Sons, NY, pp. 40.

Norton, G. (1991). Proteinase inhibitors. In: D"Mello, F.J.P., Duffus, C.M. and Duffus, J.H., *Toxic Substances in Crop Plants*. Royal Society of Chemistry, Cambridge. pp. 68-106.

Nzikou, J.M., Matos, L., Mbemba, F., Ndangui, C.B., Pambou-Tobi, N.P.G., Kimbonguila, A., Silou, T.H., Linder, M. and Desobry, S. (2009). Characteristics and composition of *Jatropha curcas* oils variety, Congo-

Brazzaville, *Research Journal of Applied Sciences, Engineering and Technology*, 1(3): 154-159.

O'Brian, R.D. (1998). *Fats and Oils:Formulating and Processing for Applications*. Technomic, Lancaster, England. Pp. 78-113.

O'Brien, R. D. (2009). *Fat and Oils: Formulating and Processing for Applications* (3rd ed). CRC press, Boca Raton, USA, pp. 83-109.

Obilie, E.M., Tano-Debrah, K. and Amoa-Awua, W.K. (2004). Souring and breakdown of cyanogenic glucosides during the processing of cassava into akyeke. *International Journal of Food Microbiology*, 93: 115-121.

Olabanji, I.O., Oluyemi, E.A. and Ajayi, O.S. (2012). Metal analyses of ash derived alkalis from banana and plantain peels (*Musa* spp.) in soap making. *African Journal of Biotechnology*, 11(99): 16512-16518.

Omaye S.T. (2004). *Animal Toxins and Plant Toxicants: Food and Nutritional Toxicology*. CRC press, Boca Raton, USA, pp. 205-213.

Onabolu, A.O., Oluwale, O.S. and Bokanga, M. (2002). Loss of residual cyanogens in a cassava food during short-term storage. *International Journal of Food Science and Nutrition*, 53(4): 343-349.

Onyegbado, C.O., Iyagba, E.T. and Offor, O.J. (2002). Solid soap production using plantain peel ash as source of alkali. *Journal of Applied Science and Environmental Management*, 6(1): 73-74.

Ojediran, T.K. and Emiola, I.A. (2014). Toxicological impact of processed *J. curcas* kernel meals on the gut morphology and kidney of broiler chicks. *Journal* of *Scientific Advances*, 4(11): 1122-1131.

Panter, K.E., Keeler, R.F. and Baker, D.C. (1988). Toxicoses in livestock from the hemlocks (*Conium* and *Cicuta* spp.). *Journal of Animal Science*, 66: 2407-2413.

Parton, K. (2000). Onion toxicity in farmed animals. *New Zealand Veterinary Journal*, 48: 89.

Pedraza, C., Villafañe, F. and Torrenegra, R.D. (1983). Hematuria vesical bovina y su relación con algunas especies vegetales. *Revista Acovez*, 7: 11-19.

Pérez-Arbeláez, E. (1931). Plantas venenosas de Colombia. *Revista de Medicina Veterinaria*, 3: 189-198.

Petterson, D.S., Harris, D.J. and Allen D.G. (1991). Alkaloids. In: D'Mello J.P.F., Duffus C.M. and Duffus J.H. (Ed) *Toxic Substances in Crop Plants*. The Royal Society of Chemistry, Cambridge, pp.148-149.

Pierce, N.F., Carpentor, C.C.J., Ellior, H. and Greenough, W.B. (1971). Effect of prostaglandin, theophyllin and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. *Gastroenterology*, 60: 22-32.

Poindexter, B. W., Feng, A., Dasgupta and Bick, R. (2007). Oleandrin produces changes in intracellular calcium levels in isolated cardiomyocytes, a real-time fluorescence imaging study comparing adult to neonatal cardiomyocytes. *Journal of Toxicology and Environmental Health*, 70: 568-574.

Prasad, R. D. M., Izam, A. and Khan, M.D. (2012). *Jatropha curcas*: Plant of medical benefits. *Journal of Medicinal Plant Research*, 6(14): 2691-2699.

Radominska-Pandya, A. (2010). Invited speakers. *Drug Metabolism Reviews*, 42(1): 1-2.

Rae, H.A. (1999). Onion toxicosis in a herd of beef cows. *Canadian Veterinary Journal*, 40: 55-57.

Raju, A.J.S. and Ezradanam, V. (2002). Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (Euphorbiaceae). *Current Science*, 83, 1395-1398.

Rao, A.V. and Gurfinkel, D.M. (2000). The bioactivity of saponins: triterpenoid and steroid glycosides. *Drug Metabolism and Drug Interaction*, 17: 211-235.

Rehm, S. and Espig, G. (1991). The Cultivated Plants of the Tropics and Subtropics. Cultivation, Economic Value, Utilization, Margraf Scientific books, CTA, pp. 522-528.

Rhee, K.C., Carter, C.M. and Matill, K.F. (1972). Simultaneous recovery of protein and oil from raw peanuts for separation of major constituents. *Journal of American Chemical Society*, 37: 90-93.

Rice, E.L. (1995). *Biological Control of Weeds and Plant Diseases; Advances in Applied Allelopathy*. University of Oklahoma Press, Norman, USA, pp. 439.

Rietjens, I.M., Martena, M.J., Boersma, M.G., Spiegelenberg, W. and Alink, G.M. (2005). Molecular mechanisms of toxicity of important food borne phytotoxins. *Molecular Nutrition and Food Research*, 49: 131-158.

Rungapamestry, V., Duncan, A.J., Fuller, Z. and Ratcliffe, B. (2007). Effect of cooking Brassica vegetables on the subsequent hydrolysis and metabolic fate of glucosinolates. *Nutrition Society*, 66 (1): 69-81.

Saidu, Y. Nwachukwu, F.C. Bilbis, L.S. Faruk, U.Z. and Abbas, A.Y. (2010). Toxicity studies of the crude aqueous root extract of *Albizzia chevalieri* harms in albino rats. *Nigerian Journal of Basic and Applied Science*, 18(2): 308-314.

Setty, M.M., Laxane, S.N., Swarnkar, S., Mruthunjaya, K. and Zanwar, S. (2013). *Jatropha curcas*: A systemic review on pharmacological, phytochemical, toxicological profiles and commercial applications. *Research Journal of Pharmaceutical, Biological and Chemical Research*, 4(1): 989.

Sharma, A., Khare, S.K. and Gupta, M.N. (2002). Enzyme assisted aqueous extraction of Peanut oil. *Journal of American Chemical Society*. 79: 215-218.

Sharma, O.P. and Sharma, S. (2007). A review of the hepatotoxic plant *Lantana camara*. *Critical Reviews in Toxicology*, 37: 313-352.

Shibata, M., Ohkubo, T., Takahashi, H. and Inoki, R. (1989). Modified formalin test; characteristic biphasic pain response. *Pain*, 38: 347-352.

Smith, B.L. (1997). The Toxicity of Bracken Fern (Genus *Pteridium*) to Animals and Its Relevance to Man. In: D'Mello, J.P.F. (Ed) *Plant and Fungal Toxicants*, CRC Press, New York, USA, pp. 63-76.

Sonavane, G., Sarveiya, V., Kasture, V. and Kasture, S.B. (2001). Behavioural actions of *Myristica fragrans* seeds. *Indian Journal of Pharmacology*, 33(6): 417-424.

Tapsell, L.C., Hemphill, I., Cobiac, L., Patch, C.S., Sullivan, D.R., Fenech, M., and Inge, K.E. (2006). Health benefits of herbs and spices: The past, the present, the future. *Medical Journal of Australia*, 185(4): 4-24.

Toma W., Graciosa J.S., Hiruma-Lima C.A., Andrade F.D.P., Vilegas W., and Souza-Brita A.R.M., (2003). Evaluation of the analgesic and antimutagenic activities of *Quassia amara* bark extract. *Journal* of *Ethnopharmacology*, 95: 19-23.

Trabi, M., Gubitz, G.M., Steiner, W. and Foidi, N. (1997). Toxicity of *Jatropha curcas* seeds. Biofuel and Industrial products from *Jatropha curcas*. Proceeding of a symposium held in Managua, Nicaragua, Technical University of Graz, Uhlandgasse, Graz, Austria.

Trease GE, Evans WC (1996). *Pharmacognosy*. Alden Press, Oxford, England, pp. 213 -232.

Trease, G. E. and Evans W. C. (1989). *Pharmacognosy*, 13th. Braillière Tindall, London, UK, pp. 290.

Tjolsen, A., Berger, O.G., Hunskaar, S., Rosland, J.H. and Hole, K. (1992). The formalin test: An evaluation of the method. *Pain*, 51: 5-17.

Umaru, M. and Aberuagba, F. (2012). Characteristics of a typical Nigerian *Jatropha curcas* oil Seeds for biodiesel production. *Research Journal of Chemical Sciences*, 2(10): 7-12.

UNIFEM, (1993). United Nations Development Fund for Women: Oil Processing, Food Cycle, Technology Source Books. *International Technological Proceedings*, London.

Vetter, J. (2004). Poison hemlock (*Conium maculatum* L.) – Review. *Food and Chemical Toxicology*, 42: 1373-1382.

Volden, J., Borge, G.I.A., Hansen, M., Wicklund, T. and Bengtsson, G.B. (2009). Processing (blanching, boiling, steaming) effects on the content of glucosinolates and antioxidant-related parameters in cauliflower (Brassica oleracea L. ssp. botrytis). *Food Science and Technology*, 42(753): 63-73.

Ward, J. A. (1976). Processing high oil content seeds in continuous screw presses. *American Journal of Oil and Chemical Society*, 53 (1): 261-264.

Weaver, C.M. (2001). Calcium. In: Bowman, B.A. and Russell R.M. (Ed.) *Present Knowledge in Nutrition* (8th ed). ILSI Press, Washington, DC, pp. 273-280.

Weike, C., Qi, Z., Xingchun, C., Kexiu, W., Ying, X., Lin, T., …Fang, C. (2006). Chemical modification of *Jatropha curcas* RIPs (curcin) and effect of the modification on relative activity of curcin. *Chinese Journal of Applied Environmental Biology*, 12: 329-333.

Widrig, R., Suter, A., Saller, R. and Melzer, J. (2007). Choosing between NSAID and arnica for topical treatment of hand osteoarthritis in a randomised, double-blind study. *Rheumatology International*, 27(6): 585-91.

Wink, M. (1988). Plant breeding: Importance of secondary metabolites for protection against pathogens and herbivores. *Theoretical and Applied Genetics*, 75: 225-233.

Wink, M. (2007). Importance of Plant Secondary Metabolites for Protection Against Insects and Microbial Infections. In: Carpinella, C., Rai, M., *Naturally Occurring Bioactive Compounds: A New and Safe Alternative for Control of Pests and Diseases*. pp. 251-268.

Wink, M. (2008). Evolutionary advantage and molecular modes of action of multicomponent mixtures in phytomedicine. *Current Drug Metabolism*, 9: 996-1009.

Wink, M., Koschmieder, C., Sauerwein, M. and Sporer, F. (1997). Phorbol Esters of *J. curcas*- Biological Activities and Potential Applications. In: Gubitz, G.M., Mittelbach, M. and Trabi, M. (Ed) *Biofuels and Industrial Products from Biofuels and Industrial Products from Jatropha curcas.* DBV Graz, pp. 160-166.

World Health Organization (1993). *Cyanogenic Glycosides. Toxicological additives Series 30* Geneva, Retrieved from: http://www.inchem.org/documents/jecfa/jecmono/v30je18htm

World Health Organization. (1988). *Pyrrolizidine Alkaloids, Environmental Health Criteria* 80. Geneva, Retrieved from: http://www.inchem.org/documents/ehc/ehc/ehc080.htm

Zanzi, R., Perez, J.A.S. and Soler P.B. (2008). Production of Biodiesel from *Jatropha curcas* in the Region of Guantanamo in Cuba. In: Proceedings of the 3rd International congress University-Industry cooperation, Ubatuba, Brazil.

APPENDIX 1

Range of doses for first and second phases of LD_{50} determination (Lorke 1983)

Doses in mg/kg and results of initial test			Doses chosen for the second test			
10	100	1,000				
0/3	0/3	0/3		1,600	2,900	5,000
0/3	0/3	1/3	600	1,000	1,600	2,900
0/3	0/3	2/3	200	400	800	1,600
0/3	0/3	3/3	140	225	370	600
0/3	1/3	3/3	50	100	200	400
0/3	2/3	3/3	20	40	80	160
0/3	3/3	3/3	15	25	40	60
1/3	3/3	3/3	5	10	20	40
2/3	3/3	3/3	2	4	8	16
3/3	3/3	3/3	1	2	4	8