TOXICITY STUDIES ON N-HEXANE AND METHANOL LEAF AND METHANOL SEED EXTRACTS OF *JATROPHA CURCAS* LINN. IN CHICKS AND MICE

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Declaration

I declare that the work in this thesis entitled "TOXICITY STUDIES ON N-HEXANE AND METHANOL LEAF AND METHANOL SEED EXTRACTS OF *JATROPHA CURCAS* LINN. IN CHICKS AND MICE" has been carried out by me in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria under the supervision of Prof. (Mrs.) H.O. Kwanashie and Dr. T.O. Olurishe. 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 university.

Omeiza Bashir ALIYU	_____	_____
Name of Student	Signature	Date

Certification

This thesis entitled "TOXICITY STUDIES ON N-HEXANE AND METHANOL LEAF AND METHANOL SEED EXTRACTS OF *JATROPHA CURCAS* LINN. IN CHICKS AND MICE" by Omeiza Bashir ALIYU meets the regulations governing the award of the degree of Master of Science in Pharmacology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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Dedication

This work is dedicated to the loving memory of my late father, Alhaji. Aliyu Jimoh Sallau. Daddy, you were such a loving dad and a wonderful friend. You will always be remembered. May your soul continue to rest in perfect peace.

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Abstract

Jatropha curcas is a toxic plant commonly used as food and remedy for several diseases. This study was designed to evaluate the protective and therapeutic effects of sodium nitrite, sodium thiosulphate, penicillamine, ethylene diamine tetra-acetic acids (EDTA) and atropine against acute and sub-acute n-hexane leaf, methanol leaf and seed extracts of *Jatropha curcas* intoxication in 7-14 day old chicks. The oral and intraperitoneal median lethal doses (LD_{50}) of the extracts were performed in mice, one and seven day old chicks. Effect of the antidotes on LD_{50} and signs of *J. curcas* extracts intoxication were determined in the acute toxicity study while the therapeutic effect of the antidotes co-administered with the extracts for 7 days were determined in sub-acute toxicity study. Phytochemical, anti-nutritional and elemental analysis of the extracts revealed the presence of secondary metabolites, anti-nutrients and heavy metals. The oral LD_{50} of hexane and methanol leaf extracts were above 5,000 mg/kg in chicks and mice while methanol seed extract was 1,100 mg/kg in 7-days old chicks. The intraperitoneal LD_{50} of hexane and methanol leaf extracts were 1,386 mg/kg and 775 mg/kg in mice, 894 mg/kg and 89 mg/kg in one day old chicks, 935 mg/kg and 74 mg/kg in seven days old chicks. The intraperitoneal LD_{50} of methanol seed extract in seven days old chicks was 22 mg/kg. The antidotes increased the intraperitoneal LD_{50} of the extracts in chicks and administration of extracts at 135% LD_{50} caused acute signs of intoxication such as pecking, ataxia, escape attempt, closing of eyes, gasping, crouching, sleeping and death. Treatment with antidotes significantly ($p<0.05$) decreased or had no effect on lethality and signs of intoxication due to *J. curcas* leaf and seed extracts. Seven days daily oral administration of methanol leaf and seed extracts at 10% LD_{50} for sub-acute intoxication caused diarrhoea, weakness, respiratory depression, weight loss and lethargy. Signs of intoxication were severe in

the extract treated with saline group (saline treated group). The methanol leaf extract significantly ($p<0.05$) increased the white blood cells (WBC). Sodium thiosulphate, penicillamine and atropine as antidotes against methanol leaf extract intoxication had no effect on the haematological parameters while sodium nitrite and EDTA significantly ($p<0.05$) decreased WBC and mean corpuscular haemoglobin concentration (MCHC) when compared to saline treated group. The methanol seed extract intoxicated chicks produced no significant ($p<0.05$) effect when compared to the control. The biochemical parameters (ALP, ALT, AST and Urea) of the methanol leaf extract treated with saline group showed significant ($p<0.05$) increase when compared to the control. Sodium thiosulphate, EDTA and atropine as antidotes for methanol leaf extract intoxication significantly ($p<0.05$) decreased the level of ALT while atropine significantly ($p<0.05$) decreased the level of urea when compared to the saline treated group. The methanol seed extract treated with saline group significantly ($p<0.05$) increased ALT and AST when compared to the control. Treatment with sodium thiosulphate significantly ($p<0.05$) decreased the level of ALT and AST. The study revealed the presence of anti-nutrients and heavy metals in *J. curcas*. Sodium thiosulphate and atropine were the most effective antidotes and may be used in the management of *J. curcas* intoxication.

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List of Abbreviations

A.B.U.	Ahmadu Bello University
ABC	ATP Binding Cassette
AChE	Acetylcholinesterase
ADP	Adenosine Di-Phosphate
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
AST	Aspartate Transaminase
ATP	Adenosine Triphosphate
ATPase	Adenine Triphosphatase
CNS	Central Nervous System
CYP	Cytochrome P_{450} Oxidase
DAG	Diacyl Glycerol
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
Ext	Extract
FAO	Food and Agricultural Organization
GABA	Gamma Amino Butyric Acid
GIT	Gastro-intestinal Tract
Hb	Haemoglobin
HCN	Hydrogen Cyanide
HCT	Hematocrit

HLE	Hexane Leaf Extract
LD_{50}	Median Lethal Dose
MAO	Monoamine Oxidase
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MDR	Multiple Drug Resistance
MLE	Methanol Leaf Extract
MSE	Methanol Seed Extract
NAPRI	National Animal Production Research Institute
PI	Protective Index
PKC	Protein Kinase C
PLT	Platelet
PNA	Penicillamine
PPM	Parts Per Million
PS	Phosphatidyl Serine
RBC	Red Blood Cell
RIP	Ribosome Inactivating Protein
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SEM	Standard Error of Mean
SN	Sodium Nitrite
STS	Sodium Thiosulphate
TI	Trypsin Inhibitor
WBC	White Blood Cell
W.H.O	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The use of plants in the treatment of ailments dates back to antiquity (Sofowora, 1993). Increasing number of patients and consumers are using herbs and herbal products as complementary therapy in the treatment and management of chronic ailments while neglecting their harmful effect to the body. Among the genus of herbal plants reported to be poisonous to humans and livestock include *Euphorbiaceae, Excoecaria, Hyaenanche, Manihot* and *Sapium.* These groups of poisonous plants produce a variety of toxins such as alkaloids, terpenoids, tannins, cyanogenic glycosides, saponins and toxic amino acids. For example, over 10,000 different alkaloids and 25,000 different terpenes have been identified and generally, these toxins are called secondary metabolites (Da Rocha *et al*., 2001; Bent and Ko, 2004; Devappa *et al*., 2010).

The toxins present in medicinal herbs may modulate or modify the effects of their "active principles". Therefore, toxic plants with medicinal value are unsafe but can be made useful by isolation of the active principle or detoxification before usage (Devappa *et al*., 2010).

Certain diterpenoids possess therapeutic potential as anti-hypertensive, anti-retroviral, anti-inflammatory, analgesic and anti-bacterial drugs. In addition to these properties, these compounds may function as anti-oxidants, hallucinogens, and sweeteners; and may stimulate contraction of the uterus (Kumari *et al*., 2003). Ingestion of some

diterpene esters produced symptoms of severe toxicity in livestock and humans (Breitmaier, 2006). Therefore, some of these metabolites present in plants are toxic while others are of therapeutic values.

The toxicity of plants may depend on strength of toxin, quantity consumed, time of exposure, individual body chemistry, climate, soil and genetic differences within the species. Toxicity problems of most herbal formulations develop because their potency are unknown resulting to overdose in most cases. In humans and animals, toxins produce irreparable damage or exert transient effects which may be overcomed with treatment using antidotes, supportive measures or simply ware off with time (Hoy *et al*., 1998; Min, 2005). Unfortunately, there are few antidotal therapies for plant poisonings. The most available approach for treating plant poisoning often involves routine decontamination procedures such as induction of emesis (in appropriate species), administration of activated charcoal and a cathartic to hasten elimination of the plant from the gastrointestinal tract. In addition, symptomatic and supportive care needs to be provided and continued exposure to the suspected plant should be stopped (Cheeke, 1998).

1.2 Statement of Research Problem

The root, leaves and seeds of *Jatropha curcas* have been widely used in traditional folk medicine in many parts of West Africa, Central and South America. Previous studies have shown that *J. curcas* exhibits bioactive activities for the management of several diseases such as fever, mouth infections, jaundice, and guinea worm sores (Oliver-Bever, 1986; Balaji *et al*., 2009; Igbinosa *et al*., 2009 and 2011).

Despite these beneficial effects of *J. curcas*, some studies have also demonstrated that *J. curcas* exhibited toxicity in human and animals. Reported cases of toxicity in human were due to accidental consumption of the plant or intentional consumption for medicinal purposes. Also, animals do not usually eat toxic plant or its parts, but during unfavourable conditions, such as drought, animals might consume them due to acute shortage of feed (Oliveira *et al.*, 2008; Devappa *et al*., 2010). The important clinical signs observed in animals following consumption of *J. curcas* are diarrhoea, salivation, dyspnoea, depression, reduced water intake, weakness of the hind limbs, and recumbency before death. Histological findings revealed organ damages such as degeneration of liver and kidney cells due to consumption of *J. curcas* (Singh *et al*., 2010).

Despite the rising cases of *J. curcas* poisoning in human and animals, there is no antidote for the management.

1.3 Justification of the Study

Medicinal plants and herbal remedies are widely used especially in Africa because they are less expensive, accessible and abundant (Sofowora, 1993). In recent times, concerns have been raised over the lack of quality control and scientific facts for the efficacy and safety of herbal plants (Firenzuoli and Gori, 2007). Cautions have been raised regarding the potential adverse effects of herbal remedies including hepatotoxicity and nephrotoxicity (Seeff, 2007) even as it is known that medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively, or in synergy to improve health (Gurib-Fakim, 2006; Igbinosa, 2011).

Cases of plant poisoning are reported mainly due to overdose since most herbs lack therapeutic doses. Treatment for most plant poisonings is symptomatic and specific antidotes are used in only a few (Wolfle and Kowalewski, 1995; Krenzelok and Jacobsen, 1997). *Jatropha curcas* is a toxic plant with ethnomedical and industrial uses. Presently there is no specific antidote in the treatment of *J. curcas* poisoning only supportive therapy to relieve symptoms. This study aimed to investigate the efficacy of some antidotes for the management of *J. curcas* intoxication.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the study

The aim of this study is to investigate the toxicological effects of the hexane and methanol leaf and seed extracts of *J. curcas* in chicks and to determine appropriate antidotal therapy.

1.4.2 Specific objectives

1. To determine the presence or absence of toxic and anti-nutritional components in *J. curcas* leaves and seeds.
2. To determine the LD_{50} and observe signs of toxicity of the hexane and methanol leaf and seed extracts of *J. curcas*.
3. To investigate the therapeutic efficacy of commonly used antidotes for cyanide, organophosphate and heavy metal poisoning against *J. curcas* intoxication.

1.5 Statement of Research Questions

1. What toxic constituents are present in *J. curcas* leaves and seeds?
2. How toxic are the hexane and methanol leaf and seed extracts of *J. curcas* to chicks; and what are the signs of the toxicity?
3. Do routine antidotes for cyanide, organophosphate and heavy metals provide effective treatment against *J. curcas* leaf and seed intoxication?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Plant Poisoning

Plant poisoning refers to development of dose-related adverse effects following exposure to poisonous plants. Poisonous plants are plants that when touched or ingested in sufficient quantity can be harmful or fatal to an organism. Poisonous plants produce a variety of toxins which are classified based upon their molecular structure. The classes of toxins include alkaloids, terpenoids, tannins, cyanogenic glycosides, saponins and toxic amino acids. These toxins are generally called secondary metabolites (Da Rocha *et al*., 2001; Bent and Ko, 2004; Devappa *et al*., 2010).

2.1.1 Etiology of plant poisoning

Plant poisoning in animals is usually accidental and most frequently occurs during unfavourable conditions when pastures are poor due to drought and wild fires. Animals' consumption of hay contaminated with poisonous plants also is a cause of plant poisoning. In humans, it may be accidental or intentional. Accidental poisoning in humans maybe due to confusing poisonous plant with edible plants, contamination of food with poisonous plants, or by the use of plants as remedies (Mutschler *et al*., 2008; Rai and Lakhanpal, 2008; Devappa *et al*., 2010).

2.1.2 Clinical features and factors influencing plant poisoning

Poisonous plants can affect the entire spectrum of organ systems, with some plants having several toxic principles that affect different systems. Plant poisoning can be acute or chronic depending on amount of plant consumed and time of exposure to a poisonous plant. Acute poisoning occurs on exposure to a poison on one occasion or during a short period of time. Symptoms develop within a short period since exposure is high. Chronic poisoning occurs on long-term repeated or constinuous exposure to a poison where symptoms do not occur immediately or after each exposure. The patient gradually becomes ill, or becomes ill after a long latent period. Condition of a plant, its growth stage, part of the plant, the amount consumed, the species and susceptibility of the victims are factors that affect plant poisoning (Singh *et al*., 2010).

2.1.3 Phytotoxins

Plants contain a large number of biologically active chemicals. Some of these have been found to be extremely useful for treating various human and animal diseases (e.g. digitoxin, colchicine and atropine). However, some plant constituents produce adverse health effects following exposure. The presence of these toxic constituents in plants is believed to confer some degree of protection from plant predators such as insects and ruminants (Da Rocha *et al*., 2001; Bent *et al*., 2004). There are numbers of broad categories of toxicologically significant plant constituents. These include alkaloids, amino acids, peptides and proteins, glycosides, acids (e.g. oxalic acid), terpenes, phenolics tannins and essential oils. Essential oils are various steam-volatile, primarily lipophilic, organic plant metabolites stored in special plant organs (Devappa *et al*., 2010). Within each broad category, there is tremendous chemical heterogeneity.

2.2 Classification of Poisonous Plants

Plants may be classified according to their oral toxicity and mode of actions as determined in experimental animals.

2.2.1 Classification based on oral toxicity

This classification is based on amount of plant parts consumed with class I divided into two groups. Class Ia, which is extremely hazardous (5 mg or less per kg body weight) while the class Ib is highly hazardous (5 to 50 mg/kg body weight). The class II is moderately hazardous (50 to 500 mg/kg body weight). The class III is slightly hazardous (500 mg and more per kg body weight). It is important to recall that the dose is very important. Paracelsus (1493 – 1541) postulated in 1537 "sola dosis facet venenum" (it is the dose that makes a poison) besides inherent toxic properties (Mutschler *et al*., 2008; Wink and van Wyk, 2008).

2.2.2 Classification based on mode of actions

Toxins, which fall into the classes Ia, Ib and II interfere with central functions in an animal. The most poisonous substances are neurotoxins which affect the nervous system, followed by cytotoxins and metabolic poisons that disturb liver, heart, kidneys, respiration, muscles and reproduction. The classes based on mode of actions are:-

2.2.2.1 Neurotoxins

Neurotoxins can affect important ion channels of neuronal cells, such as Na^+, K^+ and Ca^{2+} channels, either by activating or inactivating them permanently. Both actions

will stop neuronal signal transduction and thus block the activity of the central nervous system (CNS) but also neuromuscular signaling (Wink, 2003; Alberts *et al.*, 2008*;* Mutschler *et al.*, 2008), which eventually leads to paralysis of both striated and smooth muscles of heart, lungs and skeleton. Cardiac glycosides represent a family of compounds that are derived from the foxglove plant (*Digitalis purpurea*). They are considered to be neurotoxins of class Ia because they are strong inhibitors of Na^+, K^+-ATPase, which is the most important ion pump in neuronal and other cells to maintain an ion gradient important for action potentials and transport mechanisms (Alberts *et al*., 2008; Mutschler *et al*., 2008). Neuroreceptors are another prime target for many alkaloids, which structurally resemble endogenous neurotransmitters, such as acetylcholine, dopamine, noradrenaline, serotonin, adrenaline, GABA or glutamate (Wink *et al*., 1998; Wink, 2000; Alberts *et al*., 2008; Mutschler *et al*., 2008). The neuroactive alkaloids can either function as agonists, which over stimulate a neuroreceptor or as antagonists, which would block a certain neuroreceptor. Agonists and antagonists can cause excitation, hallucinations, and general CNS disturbances, which would put a herbivore into deep sleep or coma, while higher doses would lead to death by either cardiac or respiratory arrest (Mutschler *et al*., 2008).

Physostigmine (also known as eserine from éséré, West African name for the Calabar bean) is a parasympathomimetic alkaloid, specifically, a reversible cholinesterase inhibitor. It occurs naturally in the Calabar bean. Such alkaloids inhibit the enzymes that break down neurotransmitters, such as cholinesterase (AChE) and monoamine oxidase (MAO). These toxins have similar toxic properties as secondary metabolites that are neuroreceptor agonists, since they would lead to a higher concentration of neurotransmitters in the synaptic cleft. Higher doses would lead to death by either

cardiac or respiratory arrest (Wink, 2003; Mutschler *et al*., 2008).

2.2.2.2 Inhibitors of cellular respiration

Cellular respiration, which takes place in mitochondria and generates Adenine triphosphate (ATP), is another vulnerable target in animals, since ATP is essential for all cellular and organ functions. Many plants such as sorghum, cassava and even some arthropods can attack this target with hydrogen cyanide (HCN), which binds to iron ions of the terminal cytochrome oxidase in the mitochondrial respiratory chain (Alberts *et al*., 2008; Mutschler *et al*., 2008). HCN does not occur in a free form, but is stored as cyanogenic glucosides in plant vacuoles. When plants are wounded, the cellular compartmentation breaks down and the content of the vacuoles get into contact with cytosolic enzymes, such as β-glucosidase and nitrilase. These enzymes hydrolyse the cyanogenic glycosides and the extremely toxic HCN is released. Also rotenoids (produced by some legumes) and some alkaloids can inhibit the mitochondrial respiratory chain. The diterpene, atractyloside is a potent inhibitor of the mitochondrial ADP/ATP transporter and thus inhibits the ATP supply of a cell (Wink and van Wyk, 2008).

2.2.2.3 Cytotoxins

Several poisons can be regarded as cytotoxins because they interfere with important cellular functions. An important target in this context is biomembranes, which have to control the import and export of metabolites and ions in cells (Alberts *et al*., 2008; Mutschler *et al*., 2008). Membrane fluidity and integrity can be severely disturbed by both steroidal and triterpenoid saponins. Saponins are usually stored as inactive bidesmosidic saponins in plant vacuoles; upon wounding and decompartmentation,

they are converted into the membrane-active monodesmosidic saponins, which are amphiphilic with detergent activities (Wink and van Wyk, 2008). Within cells, other important targets include several enzymes and proteins but also DNA/RNA and related processes.

Protein biosynthesis in ribosomes is vital for every cell and organism. A number of plant toxins inhibit ribosomal protein biosynthesis, such as the alkaloid emetine, from *Psychotria ipecacuanha*, amanitins from *Amanita phalloides* or a class of polypeptides, the lectins (Alberts *et al*., 2008; Mutschler *et al*., 2008). These lectins are extremely poisonous and an oral dose of 1 mg/kg body weight is enough to kill a human being. Parenteral administration even with 0.1 µg and less per kg body weight can be lethal (Wink, 2008c; Wink and van Wyk, 2008).

2.2.2.4 Alkylating and intercalating DNA toxins

A number of secondary metabolites are known to attack DNA and RNA, by either intercalation or alkylation. Intercalating compounds, such as β-carboline alkaloids, emetine, berberine, sanguinarine, athraquinones or furanocoumarins stabilise DNA and thus inhibit DNA replication (Schmeller *et al*., 1997; Wink, 2000). They can cause frame shift mutations, which drives a cell into apoptosis or which can cause malformations and even cancer. More common are alkylating compounds, which modify the DNA-bases in a covalent fashion. Known examples are pyrrolizidine alkaloids occurring in *Boraginaceae* and several *Asteraceae* (Wink and van Wyk, 2008). If alkylated DNA bases are not repaired, they can cause mutations and even cancer. They can induce abortion in a pregnant herbivore or cause malformation of their foetus(es).

2.2.2.5 Toxins of skin and mucosal tissues

Apart from internal organs, the skin and mucosal tissues of animals can be affected by several toxins. Common in members of the *Euphorbiaceae* and *Thymelaeaceae* are diterpenes, which resemble the endogenous signal compounds diacylglycerol (DAG), an activator of the key enzyme PKC (protein kinase C) (Alberts *et al*., 2008; Mutschler *et al*., 2008). These diterpenes are classified as phorbol esters and they also stimulate PKC. When in contact to skin, mucosal tissues or the eye they cause severe and painful inflammation, with ulcers and blister formation (Wink and van Wyk, 2008).

2.3 Diagnosis of Plant Poisoning

Diagnosis of plant poisoning in human and livestock depends on the history, clinical syndrome observed, post-mortem lesions, evidence that plants have been consumed or grazed and remains of toxic plants in the gastrointestinal tract. Where the toxic principle is known, confirmatory laboratory tests may be possible (McGaw and Eloff, 2005).

2.4 Treatment for Plant Poisoning

Unfortunately, there are few antidotal therapies for treating plant poisonings. The best approach for treating intoxicated animals often involves routine decontamination procedures such as induction of emesis (in appropriate species) and the administration of activated charcoal and a cathartic to hasten elimination of the plant from the gastrointestinal tract. In addition, symptomatic and supportive care needs to be provided. Obviously, continuous exposure to the suspected plant should be stopped.

For a few plant poisonings, specific antidotes may be indicated. For example, intoxication with plants containing belladonna alkaloids results in an anticholinergic syndrome that can be treated with physostigmine. Below are some toxic constituents present in some plants, their specific antidotes for various poisons and their dose schedule (Ellenhorn and Barceloux, 1988; Henry, 1995; Corbridge and Murray, 1998).

Table 2.1: Commonly Used Antidotes in Poisonings

Poison	Antidote	Dosage regimen
Anticholinesterases	Atropine	1-2 mg *i.v.* (*i.m.* or *s.c.* in less severely poisoned patients) repeated 2-4 mg every 5-10 min.
	Pralidoxime	1 gm *i.v.* (in 100 ml saline over 30 mins) repeated every 4 hours for 24 hours in severe cases.
Cyanide	Dicobalt edentate	300 mg *i.v.* over 3 minutes.
	Sodium nitrite	10 ml of 30% *i.v.* over 10 minutes.
	Sodium thiosulphate	50 ml of 25% solution in over 10 minutes.
	Hydroxocobalamin	May be up to 4 gm *i.v.*
	Oxygen	Administer in-spired oxygen till clinical recovery occurs.
Heavy metals (lead, mercury, arsenic)	DMSA (2,3-dimercapto-succinic acid)	30 mg/kg 8 hourly for 5 days then 20 mg/kg 12 hourly for 14 days.
	DMPS (Sodium 2,3 dimer capto-propane sulphonate).	Chronic: 100 mg 3 times a day. Acute: 250 mg every 4 hours for 24 hours then 250 mg every 6 hours for the next 24 hours.
	Sodium calcium-edentate	Up to 40 mg/kg twice daily by *i.v.* infusion repeated every 48 hours until level falls below toxic levels.
	Dimercaprol	Mercury: 2.5-3 mg/kg deep *i.m.* injection 4-hourly for 2 days, 2-4 times on third day. 1-2 times for up to 10 days.
	Pencillamine	Lead: 0.5-1.5 g per day orally for 1-2 months or until lead levels falls below toxic level.
Iron salts	Desferroxamine	In severe iron poisoning (> 90 mmol/L) up to 15 mg/kg per hour reduced to keep the total *i.v.* dose under 80 mg/kg in each 24 hours.

Source: (Ellenhorn and Barceloux, 1988; Henry, 1995; Corbridge and Murray, 1998)

2.5 Preventive Measures against Plant Poisoning

In human, adequate phytochemical screening of plants use 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 since they are more vulnerable. Herbivores had to find solutions to avoid extremely toxic plants or to detoxify their poison cocktails. Plants which are less toxic are eaten at least by some herbivores (Wink, 2007b, 2008b). The liver of animals, especially of herbivores or omnivores, has an 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 and urine (Alberts *et al*., 2008; Mutschler *et al*., 2008).

Another line of defence are ABC transporters (ATP Binding Cassette) (such as MDR; multiple drug resistance proteins), which are membrane proteins that can pump lipophilic xenobiotics, that have entered intestinal cells by free diffusion, back to the gut lumen (Wink, 2007a; Alberts *et al*., 2008; Mutschler *et al*., 2008). Herbivores have microorganisms in their intestine or rumen, which can help to degrade nutritional toxins (Aguiar and Wink, 2005a). Some herbivores have a rapid digestion, which would decrease the rate of toxin absorption. A few toxin eaters (e.g. parrots) are known to ingest clay (so-called geophagy), 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 (McGaw and Eloff, 2005).

2.6 *Jatropha curcas* Poisoning

2.6.1 Names of *Jatropha curcas*

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

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

English: Barbados nut, Chinese castor oil, fig nut, physic nut, pig nut, purging nut, wild oil nut e.t.c.

Nigeria: Hausa- *binidazugu*

Igbo- *wuluidu*

Yoruba- *lapa lapa*

2.6.2 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

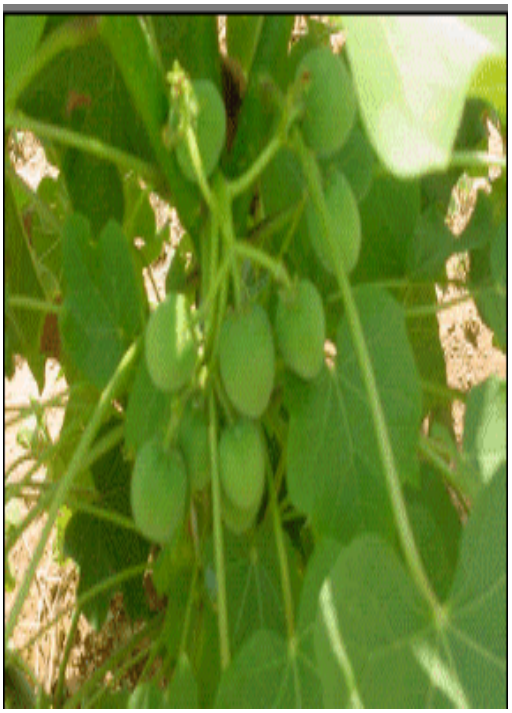
2.6.3 Characteristics of *Jatropha curcas*

2.6.3.1 Description of the plant

Jatropha curcas has thick glorious 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 ripe *Jatropha* fruits. The seed weight (per 1000) is about 727 gm (Raju and Ezradanam, 2002; Kochhar *et al*., 2008).



(a) ***Jatropha curcas*** plant



(c) ***Jatropha curcas*** fruits



(b) ***Jatropha curcas*** flowers



(d) ***Jatropha curcas*** seeds

Figure 2.1 (a-d): Photographs of various parts of *Jatropha curcas* Linn

2.6.3.2 Distribution

Jatropha curcas originates from Central America. From the Caribbean, *J. curcas* was probably distributed by Portuguese seafarers via the Cape Verde Islands and former Portuguese Guinea (now Guinea Bissau) to other countries in Africa and Asia. Today it is cultivated in almost all tropical and sub-tropical countries (Heller, 1996).

2.6.4 Uses of *Jatropha curcas*

Based on different literature, some of the uses of *J. curcas* are:

2.6.4.1. Uses as non-conventional energy crop

Jatropha oil is an environmentally safe, cost effective and renewable source of non-conventional energy. This makes it a promising substitute to hydro-power, diesel, kerosene, LPG, coal and firewood etc. The fuel properties of the *Jatropha* oil closely resembles with the diesel oil (Gubitz *et al.*, 1999; Rosenblum, 2000; Berchmans and Hirata, 2008).

2.6.4.2. Uses as an oil crop

Gubitz *et al*. (1999) reported that analysis of *J. curcas* seeds shows that it contains; moisture 6.62; protein 18.2; fat 38.0; carbohydrates 17.30; fibre 15.50; and ash 4.5%. The oil content is 35 to 40% in the seeds and 50 to 60% in the kernel (Gubitz *et al.*, 1999). The oil contains 21% saturated fatty acids and 79% unsaturated fatty acids (Gubitz *et al.*, 1999). It has also been found that there are some chemicals elements in the seeds which possess poisonous and purgative properties and render the oil in-edible for human consumption. It has also been stated that technologies are now

available, whereby it could be possible to convert *Jatropha* oil into edible oil which could prove to be a boon for developing countries (Gubitz *et al.*, 1999). The oil is obtained from decorticates seeds by expression or solvent extraction and is known in trade as *Jatropha*. In general, the oil is reported to be mixed with groundnut oil for adulteration. This indicates the possibilities of obtaining edible oil from *Jatropha* oil base (Gubitz *et al.*, 1999).

2.6.4.3. Industrial uses

Jatropha oil 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. It would also be advantageous to make use of *Jatropha* oil as hydraulic oil (Gubitz *et al.*, 1999; Mahanta *et al*., 2008).

2.6.4.4. Uses as medicinal plant

Most parts of the plant are used for the treatment of various human and veterinary ailments. The roots, stems, leaves, seeds and fruits of the plant have been widely used in traditional folk medicine in many parts of West Africa. The seeds of *J. curcas* have been used as a purgative, antihelminthic and abortifacient as well as for treating ascites, gout, paralysis and skin diseases. The seed oil of the plant has been used as an ingredient in the treatment of rheumatic conditions, itch and parasitic skin diseases, and in the treatment of fever, jaundice and gonorrhoea, as a diuretic agent and as a mouth-wash. The leaf has been used as a haemostatic agent and the bark as a fish

poison. A decoction of leaves and roots is given for diarrhoea. Similarly, roots are also reported to be used as antidote for snakebite. In certain African countries people are accustomed to chewing these seeds when in need of a laxative. *J. curcas* seeds have been found to be highly effective against *Strongyloides papillosus* infection in goats. It has also been suggested that *J. curcas* seeds could be a useful chemotherapeutic agent provided that it is active at a non-lethal dose (Gubitz *et al*., 1999; Thomas *et al*., 2008; Igbinosa *et al*., 2009; James *et al*., 2011; Oskoueian *et al*., 2011).

2.6.4.5. Uses as raw material for dye

The bark of *J. curcas* yields a dark blue dye which is reported to be used in Philippines for colouring cloth, finishing nets and lines. The dye may be extracted from leaves and tender stems and concentrated to yellowish syrup or dried to blackish brown lumpy mass. The dye imparts to cotton different shades of tan and brown which are fairly fast. Further research in this field can open up great possibilities (Gubitz *et al*, 1999).

2.6.4.6. Uses for enrichment of soil

Jatropha oil cake is rich in nitrogen, phosphorous and potassium and can be used as organic manure. This indicated the potential of this plant in initiating the process of reduction of surplus livestock maintain by the rural folk in India, mainly for the purpose of obtaining cow dung as manure. Tender branches and leaves are also used as manure for coconut trees. *Jatropha* oil cakes can, hopefully, replace synthetic fertilizers by undertaking plantations of *J. curcas* on wastelands. *J. curcas* leaves provide plentiful organic matter and increase the microbial activity including

earthworms which is an indication of ecological improvement of site ((Mahanta *et al*., 2008).

2.6.4.7. Uses as a feed stock

Jatropha leaves are used as feed for the tusser silkworm. The oil cake is rich in protein but contains some toxic principle and as such it is considered unfit for use as cattle feed. But it is reported that the poisonous principle appears to exist in the alcohol soluble fraction of the oil. With suitable research it could be possible to convert the non edible oil-cake into protein rich cattle and poultry feed on a massive scale (Gubitz *et al.*, 1999; Belewu *et al*., 2010).

2.6.4.8. Uses as insecticide/pesticide

The seeds are considered antihelemintic in Brazil. They are ground with palm oil and used as rat poison in Gabon. Aqueous extract of leaves is reported to have insecticidal properties. In Ghana, the leaves are used for fumigating houses against bed bugs. The ether extract shows antibiotic activity against Staphylococcus aureus and Escherichia coil. The juice of the whole plant is used for stupefying fish in Philippines (Gubitz *et al.*, 1999; Kaushik and Kumar, 2004; Boateng and Kusi, 2008).

2.6.4.9. Uses as profitable agro forestry crop

Owing to its multiple uses, there exists unlimited potential for extensive and convenient marketability of *Jatropha* oil, for indigenous as well as foreign markets. It has unlimited potential for import substitutions as well as export to other countries for cosmetic industry and as a vehicle fuel. Simple and cost effective technology of

growing *Jatropha* with or without irrigation makes it promising and profitable agro forestry crop both under rain fed and irrigated conditions ensuring optimal utilization of land, man power, water and financial resources. It is a crop with low capital investment, short gestation period, long productive period, unlimited employment potential in the rural areas, potential for certain productive assets, boosting of village based industries, providing nonconventional energy in a decentralized manner and above all having a potential for wastelands development (Gubitz *et al.*, 1999; Benge, 2006).

2.6.4.10. Uses as an ornamental plant

It has been found that the plant is grown in gardens for their ornamental foliage and flowers, mainly in Africa and America. It is also commonly grown as a live hedge around agricultural fields as it can be easily propagated by seeds or branch cutting and is not browsed by goats or cattle. It can be cut or cropped at any desired height and is well adapted for hedges around agricultural fields (Gubitz *et al.*, 1999).

2.7 Phytochemistry of *Jatropha curcas*

Phytochemical investigations on *J. curcas* resulted in the isolation of several classes of compounds, many of which expressed biological and toxic activities. All parts are considered toxic but in particular the seeds. Toxic and anti-nutritional components are present in *J. curcas* plant. Antinutrients are substances that by themselves, or their metabolic products generated in living systems, (1) interfere with food or feed utilization; (2) affect the health and reproduction of animals; and (3) produce death at high intake levels. The classes of toxins include alkaloids, terpenoids, tannins, cyanogenic glycosides, saponins, phytates and toxic amino acids. The main toxic

principle in *J. curcas* seeds, oil, and cake is the diterpene classified as phorbol esters (Devappa *et al*., 2010). There are two known genotypes of *J. curcas*, toxic and nontoxic. The nontoxic genotype is available only in Mexico (Makkar and Becker, 2009) and is devoid of phorbol esters.

I. Tannins

Tannins are phenolic substances associated with toxic and anti-nutritional effects including reduced food/feed intake, growth retardation, and impaired nutrient absorption. 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 (Parekh and Chanda, 2007). Tannins are found to be present in various parts of *J. curcas* plants (Makkar *et al*., 1998; Makkar and Becker, 2009).

II. Saponins

Saponins are steroid or triterpene glycoside compounds present in a variety of plants. In plants, saponins may serve as anti-feedants or help in protecting the plant against microbes and fungi. However, saponins are often bitter in taste, and thus, when present in high concentrations would reduce plant palatability in livestock (Oyi *et al*., 2007). *Jatropha curcas* saponins are nonhemolytic. In addition, the levels of saponins present in toxic and nontoxic varieties of *J. curcas* are almost similar. These observations suggest that *J. curcas* saponins are innocuous (Makkar *et al*., 1998).

III. Phytates

Phytic acid (phytate when in the salt form) is the principle 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 for consumers (Sudheer *et al*., 2004). Since non-ruminants cannot degrade phytate, their occurrence in feed reduces the availability of phosphorus to these animals. Phytates also form sparingly digestible phytate–protein complexes, thus reducing the availability of dietary protein (Oboh and Akindahunsi, 2003). On the other hand, ruminants utilize phytate because of phytase produced by rumen microorganisms. The effects of *J. curcas* phytate in animals have not yet been studied. Since the levels of phytate in the *J. curcas* kernel meal are high (ranging from 7.2 to 10.1%), efficient utilization as feed for monogastric animals would require the addition of phytase to feed (Oboh and Akindahunsi, 2003).

IV. Trypsin Inhibitors

Protease inhibitors are widespread antinutrient substances present in many plant derived nutritional ingredients (Norton, 1991), and potency is dependent upon origin and target enzyme. Trypsin inhibitors (TI) are known to decrease protein digestibility. Trypsin inhibitor (TI) activity in the *J. curcas* kernel meal of both toxic and non-toxic genotypes was found to be similar, ranging from 18.4 to 27.3 mg trypsin inhibited/g (Makkar *et al*., 1997). Makkar and Becker (1999) found that carp (*Cyprinus carpio*) fed diets containing *J. curcas* kernel meal of non- toxic genotype with 24.8 mg trypsin

inhibited/g and heat-treated (45 min, 121°C, 66% moisture) meal with 1.3 mg trypsin inhibited/g showed no marked differences in growth performance, indicating that fish were able to tolerate high levels of TI. However, feeding of unheated *J. curcas* kernel meal to monogastrics such as poultry, pigs, and fish other than carp may produce adverse effects since the levels of TI in *J. curcas* kernel meal are similar to that in raw soybean meal.

V. Cyanogenic Glycosides, Glucosinolates, and Amylase Inhibitors

These anti-nutrients were detected in *J. curcas* kernel meal or seeds. Sodium thiosulphate co-administered with glutathione has been determined to be effective in the management of subacute *J. curcas* seed oil intoxication in goats (Shukla and Singh, 2013). The presence of cardiac and cyanogenic glycosides has been determined in methanolic extract of *J. curcas* leaves (Ebuehi and Okorie, 2009).

VI. Lectins

Lectins are carbohydrate-binding (glyco) proteins and are ubiquitous in nature. Plant lectins when consumed by animals survive digestion in the GIT and bind to membrane glycosyl groups of the cells lining the GIT, producing a series of harmful local and systemic reactions. Lectins produce local reactions in the GIT by:

(1) Affecting the turnover and loss of gut epithelial cells,

(2) Interfering with nutrient digestion and absorption,

(3) Damaging the luminal membranes of the epithelium, and

(4) Modulating the immunological status of the digestive tract.

Systemically, lectins disrupt lipid, carbohydrate, and protein metabolism, promote enlargement and/or atrophy of internal organs, and alter the hormonal and immunological status. When consumed at higher concentration, lectins markedly threaten the growth and health of animals (Vasconcelos and Oliveira, 2004). The *J. curcas* kernel meal contains lectins at levels of 102 and 51 (1/mg of meal that produced hemagglutination per ml of assay medium) for toxic and nontoxic genotyopes respectively. Levels that are of similar order of magnitude are found in soybean meal (Makkar *et al*., 2007).

VII. Curcin

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. Thus curcin inhibit protein synthesis (Endo *et al*., 1987; Endo and Tsurugi, 1987). Curcin has protein translation inhibitory activity or N-glycosidase activity (Lin *et al*., 2003a; Lin *et al*., 2003b; Weike *et al*., 2006; King *et al*., 2009). Fang *et al*., (2005) found that curcin was specifically expressed in the endosperm and its calli, while it was not detected in root, stem, leaf, and leafstalk of *J. curcas* and their calli. In *J. curcas,* purified curcin inhibited cell-free translation in the reticulocyte lysate system with an IC50 (95% confidence limits) of 0.19 (0.11–0.27) nmol/L. Curcin was also reported to display antitumor activity, suggesting therapeutic importance (Lin *et al*., 2003b).

VIII. Diterpenes

Diterpenes are believed to be the most potent compounds synthesized by *Jatropha* species. Among the diterpenes, phorbol esters are the most toxic molecules in this

Jatropha species (Haas *et al*., 2002; King *et al*., 2009). 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* (Ahmed and Salimon, 2009). The phorbol esters are lipophilic, present mainly in oil, and when present in oil or kernel not affected by heat but can be hydrolyzed to less toxic substances extractable by either water or ethanol (Usman *et al*., 2009). In *J. curcas* the phorbol esters (mg/g dry matter) were present in kernels (2–6), leaves (1.83–2.75), stems (0.78–0.99), flowers (1.39–1.83), buds (1.18–2.10), roots (0.55), bark (outer brown skin) (0.39), bark (inner green skin) (3.08) and wood (0.09), but not in latex (Makkar and Becker, 2009). Phorbol esters are amphiphillic molecules 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 (PKC) function by increasing its affinity for phophatidylserine (PS)-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 are thus regarded as cocarcinogens (Horiuchi *et al*., 1988; Goel *et al*., 2007). In addition, Jing *et al*. (2005) isolated a phorbol type diterpene called jatropherol-I from *J. curcas* seed and oil. The concentration of jatropherol-I (molecular weight 322) is low (39 mg% seed weight) but highly toxic to silk worm larvae and mouse. Although *J. curcas* synthesizes many diterpenes, information on their biological activity remains scarce.

2.8 Heavy Metals Present in *Jatropha curcas*

Toxic elements from wastewater may contaminate agricultural soils, water supplies and environment and hence human food chain. The crops and the vegetables become contaminated either due to soil pollution or due to long exposure to polluted environment and accumulate unfavourable levels of metallic elements within them (Sobukola and Dairo, 2007). The uptake of metals by roots mainly depends on metal and soil characteristics and plant species etc. (Luo and Rimmer, 1995; Sresty and Rao, 1999; Sekhar *et al*., 2001).

Heavy metals consist of both biological essential and non-biological essential metals. Biological essential heavy metals include copper (Cu), nickel (Ni), iron (Fe) and zinc (Zn). Iron for instance forms an essential part of hemoglobin, a protein in our blood which transports oxygen from the lungs to other tissues. Although they are necessary, they become toxic at high concentrations. Non-biological essential heavy metals include lead (Pb), mercury (Hg), cadmium (Cd) and tin (Sn). They can be tolerated at low levels, but become toxic as well at higher concentrations. The order of toxicity (from low to high) has been suggested as follows: cobalt, aluminum, chrome, lead, nickel, zinc, copper, cadmium and mercury (Kennish, 1998).

Heavy metals, which can not be metabolized, persist in the body and exert their toxic effects by combining with one or more reactive groups (ligands) essential for normal physiological functions. Chelation therapy is an established treatment for heavy metal poisoning. Chelating agents, also known as heavy metal antagonists, form complexes with toxic heavy metals rendering them physiologically inactive and enhancing their excretion in the urine. Specific chelating agents include edetate calcium disodium

(EDTA), deferoxamine (Desferal), dimercaprol (BAL in oil) and penicillamine (Cuprimine, Depen) (Hodgson, 2004).

Yadav *et al*. (2009) reported that heavy metal contents in *J. curcas* grown in contaminated soils contain amount above the WHO/FAO permissible limit due to their ability to uptake this metals from the soil. Kabata and Pendias (2001) determined that Pb, Cd, Cr, Hg, Zn and Cu are the most common heavy metal contaminants. These heavy metals, when ingested by humans, may cause several health problems, such as anemia and skin allergies (Yadav *et al*., 2009).

2.9 Toxicity of *Jatropha curcas*

2.9.1 Toxicity in Human

All cases of systemic poisoning have resulted from ingestion of plant material (in most cases the seeds). *Jatropha* poisoning victims reported thus far have been children. This is mainly because of its attractive nature and pleasant taste. As the human toxic dose is not known, eating large quantities of any raw plant parts may produce severe toxicity. In some instances as few as three seeds have produced toxic symptoms. In others, consumption of as many as 50 seeds has resulted in relatively mild symptoms. There is one report where the ingestion of only one seed in an adult has produced toxic symptoms (Rai and Lakhanpal, 2008).

Nausea, vomiting and some signs of dehydration, with abdominal pain are the most common clinical manifestations and cause of admission of patients. Weakness can be explained by the dehydration. Cardiac rate seem unaffected though the mean heart

rate was 90 beats per minute. In severe *Jatropha* poisoning, these symptoms progress to hemorrhagic gastroenteritis and dehydration. Polydipsia can be extreme and salivation and sweating may occur. There may be skeletal muscle spasm. Intense hyperpnea is seen together with hypotension and electrocardiographic abnormalities. There may be CNS and cardiovascular depression (Singh *et al*., 2010). These symptoms suggest some cholinergic activities.

Majority of the study population hospitalized for *J. curcas* intoxication were discharged after recovery in less than or equal to 24 hours probably due to mild poisoning. The poisonous property of the *Jatropa* plant is mainly due to toxalbumin called curcin, ricin and cyanic acid, related to ricinoleic acid (Lucas and De Silva, 2006). In recent studies, phorbol esters have been identified as one of the main toxic agents in addition to the mentioned lectins (King *et al*., 2009). All cases of *J. curcas* intoxication in children reported were treated symptomatically with intravenous fluids, antispasmodics and antiemetics (Rai and Lakhanpal, 2008).

2.9.2 Toxicity in Sheep, Calves and Goats

Animals do not usually eat the *Jatropha* plant or its parts, but during unfavorable conditions, such as drought, animals might consume them due to acute shortage of feed. Most of the toxicity experiments were conducted using force-feeding of *Jatropha* plant materials.

The toxicity of powdered *J. curcas* seeds to young Nubian goats (5−8 months old; 8−22 kg body weight) was studied by administration through a stomach tube at doses from 0.25 to 10 g/kg/d. Doses at 5 and 10 g/kg/d were toxic with fatal consequences,

and goats died between 2 and 4 days. Liver biopsy showed considerable reduction in glycogen content (50–60%), fatty change, and necrosis of hepatocytes. The clinical signs observed were lack of appetite, reduced water consumption, diarrhoea, dehydration, sunken eyes, and decreased glucose levels and increased serum arginase and glutamate oxaloacetate transaminase activities. Postmortem studies revealed hemorrhage in the rumen, reticulum, kidneys, spleen, and heart, catarrhal or hemorrhagic abomasitis and enteritis, congestion and edema of the lung, and excessive fluid in serous cavities (Adam and Magzoub, 1975).

Oliveira *et al*., (2008) also reported that independent of the plant species (*J. curcas, J. glauca, J. aceroides, or J. gossypifolia*) the leaves of *Jatropha* species contain toxic compounds similar to those found in the seeds. The clinical symptoms included disturbances in the GIT, lung, and heart with microscopic alterations in liver and kidneys.

In another study, Nubian male goats (6−8 month old) were force-fed (using a stomach tube) with low concentrations of ground *J. curcas* seeds with daily oral doses of 0.25 or 1 g/kg/d. Overall, the animals lost body weight before death. At a dose of 1 g/kg/d, the onset of signs started on day 3 of the administration and included bloody diarrhoea, restlessness, lack of appetite, moaning, dyspnoea, ataxia, and recumbency prior to the death between 7 and 11 day. At the lower dose (0.25 g/kg/d), goats were observed with sunken eyes, deteriorating condition, pallor of the visible mucous membranes, and dehydration. Pathological diffuse or ecchymotic hemorrhage on the mucosa of the rumen, reticulum, and omasum, catarrhal or hemorrhagic abomasitis and enteritis, congestion or hemorrhage in lungs, heart, liver, kidneys, and spleen,

patchy pulmonary cyanosis and emphysema, and tracheal froth were observed in all animals before death (18-21 day). Other changes recorded were the fatty change of the centrilobular hepatocytes, degeneration or necrosis of the renal tubular cells, increase in serum aspartate aminotransferase (AST) activity, increase in urea concentration, and decrease in total protein and albumin levels reflecting hepatic and renal damage. The animals showed macrocytic norchromic anemia due to decrease in hemoglobin, packed cell volume, and red blood cells and rise in mean corpuscular volume without significant effect on the mean corpuscular hemoglobin concentration. The intensity in vascular changes of organs observed was dose dependent (Gadir *et al*., 2003).

Makkar and Becker (2010) observed that phorbol esters, the main toxin present in seeds and oil, cannot be degraded by rumen microbes, suggesting that ruminants are as susceptible to *Jatropha* as are monogastric animals.

All the studies just described indicate that ruminants cannot utilize *Jatropha* seeds or leaves as a feed. Their consumption may produce serious GIT, biochemical, histopathological and hematological disturbances in the body while higher levels of consumption produces mortality. However, information on dermal route exposure toxicity in ruminants or ocular toxicity is unavailable.

2.9.3 Toxicity in Mice and Rats

A number of toxicity studies have been conducted using mice and rats as animal models. In a study, feeding of *J. curcas* seeds (corticated and powdered) at 40 and 50% in the diet produced mortality in mice and severe clinical and pathological

symptoms before death. The important symptoms included impaired apetite, diarrhoea, accelerated respiration, difficulty in keeping their normal posture, and ruffled fur. Macroscopic findings in the intestine showed acute catarrhal enteritis and extravasation of blood in the lumen. The mucous membranes of the small intestine appeared swollen with fragile epithelial membrane. Congested liver, kidneys, and heart, and edema of lungs were observed. Microscopic findings demonstrated intestinal tract catarrhal enteritis. The exudate covering the intestinal mucosa consisted of mucus containing desquamated cells and leucocytes. Liver showed degenerated hepatocytes with pyknotic nuclei and contained little glycogen. The central veins and the surrounding sinusoids in some lobules were filled with blood. In the heart, nuclear degeneration of few cardiac muscle fibers, and in kidney, shrinkage of glomerular tuft and mild cellularity, due to the presence of polymorphonuclear cell infiltration and glomerular endothelial cells, were observed. The cells of the convoluted tubules showed fatty changes. There was congestion in the cortex and medulla, adjacent to the corticomedullary junction in kidney. In lungs, congestion of pulmonary alveolar capillaries and hemorrhage in alveoli was observed. The amount of *Jatropha* seeds in the diet influenced the degree of pathological abnormalities (Adam, 1974).

Similarly, *J. curcas* kernel meal containing phorbol ester concentration of 0.23 mg/g diet fed to 28-days-old male Wistar rats was found to be highly toxic. The mortality occurred within 8-10 day. All rats showed poor appetite and low diet intake, loss of body weight, difficulty in motor functions, and severe diarrhoea before death. The gross examination of vital organs indicated atrophy. Increases in kidney, heart, and

brain weights were observed, but microscopic observation of liver, kidney, heart muscle, and brain showed normal cellular architecture (Devappa *et al*., 2008).

The methanol extract of *J. curcas* seeds produced acute toxicity in rats when given intraperitoneally, with LD_{50} of 25.19 mg and the sublethal doses LD_{10} and LD_{20} of 10 and 13.80 mg. The haematological parameters were markedly affected. At 10 day (daily dose of 10 mg), the RBC counts were reduced by 51% (8.12 to 3.96 × 106/mm3), hemoglobin concentration fell by 39% (from 12.73 to 7.75 g/L), packed cell volume was lowered by 7.3% (from 49.5 to 45.9%), and high calculated mean corpuscular volume indicated macrocytic anemia (Oluwole and Bolarinwa, 1997).

The study of *J. curcas* oil fed to rats (90–130 g) exhibited severe inflammation of the intestine with an LD_{50} of 6 ml/kg body weight. Animals exhibited diarrhoea and hemorrhagic eyes, and autopsy showed inflammation of the GIT.

Topical application of petroleum ether extract (100 µl) of *J. curcas* oil produced marked erythema and edema on shaved dorsal rabbit skin, which later became necrotic and regenerated. In mice, topical application (50 µl) of the petroleum extract of *J. curcas* oil on the shaved dorsal skin demonstrated swelling of the face, hemorrhagic eyes, diarrhoea, and skin erythema before death, whereas in rats (50 µl), topical application showed edema and erythema at 4 h of the application on the shaved dorsal skin, which subsequently led to severe scaling and thickening of the skin. The stratum corneum demonstrated parakeratosis and thickening, and cellular infiltration was noted in the upper dermis.

Jatropha oil also produced hemolysis of rabbit RBC at 25 and 50 mg, and the petroleum ether-extracted fraction showed complete hemolysis at 1 mg in saline (Gandhi *et al*., 1995).

The methanolic extracts of *J. curcas* leaves were studied for their anti-schistosomal potency in albino rats (4−5 weeks old; weighing 15–20 g). The rats were infected each with 130–150 cercariae by tail immersion technique. At day 40 of post infection, the rats were administered orally with 2 mg each of methanolic extracts over 5 consecutive days at a daily rate of 0.1 ml containing 0.4 mg of extract per animal, and a control was administered with 0.5 ml of liquid paraffin. After 10 day post treatment, the mice had reduced liver weight and mean liver score (based on number of granulomata) (Adamu *et al*., 2007).

Further studies using higher doses of methanolic extracts of *J. curcas* leaves for their anti-schistosomal potency in male albino rats showed increased aspartate amino transferase activity and levels of serum creatinine, sodium, potassium, and iron, and reduction in urea and albumin concentrations in blood, leucopenia, and visceral color alterations. The mean LD_{50} value for male rats was 4–5 g/kg body weight and for female rats was 5 g/kg body weight (Mariz *et al.*, 2006).

The acetonitrile extract of *J. curcas* when administered to albino rats at an oral dose of 50 mg/kg body weight (single dose) produced mild toxicological, biochemical, and histopathological changes (Abd-Elhamid, 2004).

Similarly, purified phorbol esters fraction of *J. curcas* oil when force fed to male mice by intragastric administration were toxic with an LD_{50} of 27.34 mg/kg, and the LD_5 and LD_{95} were 18.87 and 39.62 mg/kg body mass, respectively. The physical observation of test animals showed depression, loss of body mass, closing of eyes, and watery anus. The pathological symptoms rose with increasing dosage. The symptoms included congestion of sinus hepaticus and pulmonary alveolar capillaries, haemorrhage of spleen, and renal glomerular atrophy. The animals tested at the highest dose of 36 mg/kg body weight showed necrosis of cardiac muscle fibers and anachromasis of cortical neurons, hyperemia and exudate in spleen, diffuse hemorrhage and exudates in lung, and renal glomerular sclerosis (Li *et al*., 2010).

The curcin exhibited varying degrees of toxicity in different modes of exposures. Curcin when injected into the stomach of mice was toxic with LD_{50} of 67.20 mg/kg upon oral exposure, with an LD_{50} of 104.7 mg/kg and subcutaneous exposure in mice having LD_{100} at 1.6 mg/kg after 9 days (Hua-mei *et al*., 2007). On i.p. administration of curcain, a proteolytic enzyme extracted and purified from *J. curcas* latex, mice showed an LD_{50} of 0.96 g/kg. On the other hand, curcain administered topically to mice displayed wound healing at 0.5 and 1% (w/w) in hydrophilic ointment (Nath and Dutta, 1992; Nath and Dutta, 1997). Rodents appear to be highly sensitive to oral or topical exposure to *Jatropha* species (seeds, extracts, or different plant parts).

2.9.4 Toxicity in Chicks

In chicks, toxicity studies have been reported for *J. curcas seeds*, whereas the toxicity by other parts of *J. curcas* plant or by other species of *Jatropha* is not known. *Jatropha curcas* seeds force-fed at 0.1 or 0.5% of the diet to brown Hisex chicks for 4

weeks showed growth depression, hepatonephropathies, and widespread hemorrhage and congestion. The biochemical changes included increase in serum sorbitol dehydrogenase, glutamate dehydrogenase, and gluatamate oxaloacetate transaminase activities, rise in potassium and phosphorus concentrations, and decrease in total protein and calcium concentrations. The total hepatic and cardiac lipid concentrations were significantly increased. Fatty liver, congested heart and intestines, and pale enlarged kidneys were the symptoms noted (El-Badwi and Adam, 1990; El-Badwi and Adam, 1992).

In another study, Brown Hisex chicks fed diets containing 0.5% *J. curcas* seeds showed a high incidence of mortality (El-Badawi *et al*., 1995). These results indicate higher susceptibility of Hisex chicks to *J. curcas* seeds, and these chicks may possibly be a reliable animal model to study *Jatropha* toxicity or to evaluate detoxification strategies being investigated to make *Jatropha* kernel meals or protein isolates nontoxic for incorporation in livestock diets (Makkar and Becker, 2009).

2.10 Management of *Jatropha curcas* Poisoning

The management of *Jatropha* poisoning is similar to most poisonous plants. Decontamination and enhanced elimination are indicated for all known or suspected poisonings. There is no antidote. Rehydration, either voluntary water ingestion or i.v. fluid administration, to counteract fluid lost due to vomiting and diarrhoea is critical. Treatment is essentially symptomatic and supportive. The more critical analyses and investigations are fluid and electrolytes, acid-base status, full blood count, and renal and hepatic function with monitoring level of consciousness. Specific therapy may be indicated for haemorrhagic gastrointestinal damage, skeletal muscle and

gastrointesinal spasm, excessive salivary secretions and haemoglobinuria. After substantial exposures to *J. curcas* plants, an observation period of up to 8 hours is advised (Rai and Lakhanpal, 2008; Singh *et al*., 2010). An antidote to treat *J.curcas* poisoning will be more effective and prevent adverse effect that results from multiple drugs used for symptomatic care.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Plant Materials

Jatropha curcas sample was collected from Institute of Agricultural Research (I.A.R.), Ahmadu Bello University (A.B.U.), Zaria in July, 2013 during the raining season. The plant was authenticated by a taxonomist (Mr Namadi Sanusi) at the herbarium section of the Department of Biological Sciences, (A.B.U.) Zaria, where a voucher specimen No.2370 is assigned to it.

3. 2 Experimental Animals

Day-old chicks (ranger cockerels, also known as Shika brown) were obtained from National Animal Production Research Institute (NAPRI) A.B.U., Shika, Zaria. They were maintained in batches of 20-30 chicks at a time in a room with constant lighting and floor litter consisted of wood shavings. The chicks were given pelleted chick feed and clean water *ad libitum* and kept for 7 days to acclimatize to laboratory condition before the experiment. Ethical clearance regarding the use of laboratory animals was obtained from the Department of Pharmacology and Therapeutics, A.B.U., Zaria.

3.3 Chemicals, Solvents and Drugs

Atropine sulphate, Methanol and Hexane (Sigma Aldrich, St. Loius USA), Distamine 125 mg (Alliance pharmaceuticals Ltd, UK), Infusion Normal Saline (Dana Pharmaceuticals Ltd, Nigeria), Sodium Nitrite, Sodium Thiosulphate and Edentate Calcium Disodium (Triveni Interchem Pvt. Ltd, Gujarat India)

3.4 Preparation of Plant Extracts

Leaves and seeds of *J. curcas* were collected in bulk for preparation of extracts. Extraction was done by the cold maceration method. The leaves and seeds were washed, air dried under shade, pulverized into coarse powder and weighed. Powdered leaves (500 g and 200 g) were separately extracted with hexane and methanol (80% v/v) while powdered seed (230 g) was extracted with methanol (80% v/v) for 48 hours with intermittent shaking at 2 hours interval. The hexane and methanol mixtures were filtered using Whatman No. 1 filter paper and the residues discarded. The filtrates (extracts) were dried at 40°C using water bath to get solid masses. The percentage yields of the extracts were calculated as follows:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of pulverized leaves (g)}} \times 100$$

Portions of the extracts were used for qualitative phytochemical screening, test for the presence or absence of heavy metals and antidotal therapy. Solutions of the plant extracts were obtained by dissolving appropriate quantities of the extracts in distilled water to obtain stock solutions of the extracts. For the hexane leaf and methanol seed extracts, two to three drops of tween 80 was added to dissolve the fat contents and obtain a suspension.

3.5 Acid Digestion of Plant Samples

Half a gram of the *J. curcas* leaf and seed extracts were weighed into separate beakers for various heavy metals analysis. To each beaker, 2.5 ml of concentrated hydrochloric acid (HCl) 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 150^oC 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 cadmium, cobalt, chromium, copper, nickel, lead and zinc. The procedure used was described by AOAC (1995).

3.6 Phytochemical Screening

The extracts were subjected to qualitative phytochemical tests for plant secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins and terpenoids using standard procedures.

3.6.1 Test for alkaloids

a. **Mayer's test**: Few drop of the above reagent was added to sample of the extracts in a test tube and cream precipate indicates alkaloid.

b. **Dragendorff's test**: Few drop of this reagent was added to the extracts and red precipitate indicates the presence of alkaloid.

c. **Wagner's test:** Drops of this reagent was added to a small amount of the extract which precipitate indicates alkaloids (Sofowora, 1982).

3.6.2 Test for cardiac glycosides

a. **Kella-killiani test**: Extracts were dissolved in glacia acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45 degrees, 1 ml

of concentrated sulphuric acid was added down the slide. Purple ring colour at the interface indicates cardiac glycosides (Trease and Evans, 1983).

3.6.3 Test for fats and oils

a. **Sudan III test**: Small quantities of the extracts were added to water before adding 4 to 5 drops of Sudan III stain. A red precipitate or orange colouration indicates the presence of fats and oil.

b. **Paper/transluscent test**: Small quantities of the extracts were dropped on a piece of paper and the presence of a translucent grease spot indicates the presence of fats and oil (Trease and Evans, 1983).

3.6.4 Test for flavonoids

a. **Sodium hydroxide test**: About 0.5 g of extracts was dissolved in 10 ml of distilled water. Few drops of aqueous NaoH were added to 5 ml of the filtrate, a yellow colouration shows the presence of flavonoid (Trease and Evans, 1983).

3.6.5 Test for saponins

a. **Frothing test**: Half a gram of each extract was shaken with 2 ml of distilled water in a test tube. Frothing, which persisted on warming was taken as an evidence for the presence of saponins (Wall *et al*., 1952 and 1954).

b. **Lieberman-Burchards test**: Equal volume of acetic anhydride was added to the extract. 1 ml of concentrated sulphuric acid was added down side the tube. The colour change was observed immediately and later. Red, pink or purple

colour indicates the presence of triterpenes while blue or blue-green indicates steroids (Trease and Evans, 1983).

3.6.6 Test for tannins

a. **Ferric chloride test**: About half a gram of each extract was stirred in 10 ml of distilled water and then filtered. Few drops of ferric chloride solution were added to the filtrate. The appearance of a blue-black precipitate indicates hydrolyable tannins and green precipitate indicates the presence of condensed tannin (Trease and Evans, 1983).

3.7 Quantitative Determination of Anti-nutrients

The extracts were subjected to quantitative determination of anti-nutrients such as oxalate, phytate and tannins using standard procedures.

3.7.1 Determination of oxalate

The titrimetric method of Sanchez-Alonzo and Lachica (1987) was used in the determination of oxalate in hexane and methanol leaf extracts of *J. curcas*. Exactly one gram of the sample was placed in 250 cm^3 volumetric flask, 190 mL of distilled water and 10 cm^3 of 6 M HCL were added. The mixture was then warmed in a water bath at 90^oC for 4 hours and the digested sample centrifuged at 2,000 rpm for 5 min. The supernatant was diluted to 250 cm^3. Three 50 cm^3 aliquots of the supernatant was evaporated to 25 cm^3, the brown precipitate was filtered and washed. The combined solution and washings were titrated with concentrated ammonium solution in drops until the pink colour of methyl orange changed to yellow. The solution was then heated in a water bath to 900^oC and the oxalate was precipitated with 5% $CaCl_2$

solution was allowed to stand overnight and centrifuged. Precipitate was washed with hot 25% H_2SO_4, diluted to 125 mL with distilled water and titrated against 0.05 M $KMnO_4$.

Calculation: 1 mL 0.05 M $KMnO_4$ = 2.2 mg Oxalate

3.7.2 Determination of phytate

Spectrophotometric method of Trease and Evans (1989) was used in the determination of phytate. One gram of the extract was dissolved in 25 ml of 0.5 M HNO_3 and centrifuged at 4,000 rpm for 10 min. One millilitre of 0.03 M Ferric solution was added to the supernantant and left to stand for 15 min in order to allow chelation of the iron molecules by the indigenous plant phytate. At the end of the incubation, it was capped and heated for 20 min, 7.5 ml of distilled water was added to it and vortexed. Thereafter, 0.1 ml of 1.33 M NH_4SCN (Ammonium sulphocyanide) solution was added and absorbance read at 465 nm. The amount of phytate was extrapolated from a standard calibration curve for calcium phytate.

3.7.3 Determination of tannin

Spectrophotometric method of Trease and Evans (1989) was used in the determination of tannin. Five grams of the extract were added in 20 ml of warm water and filtered. 0.5 ml of the filtrate was added to 0.5 ml of 0.5M ferric solution in an alkaline medium and allowed to stand for 30 minutes for color development. The absorbance was read at 760 nm and the amount of tannin was extrapolated from a standard calibration curve for tannic acid.

3.8 Acute Toxicity Studies of the Extracts

The median lethal dose (LD_{50}) determination was used as indices to define the acute toxicity of *J. curcas*. The oral and intraperitoneal LD_{50} of the plant extracts were determined in chicks and mice using the Lorke's method (1983). The study was carried out in two phases. The chicks and mice were deprived of food for 3 to 4 hours prior to administration of the extracts respectively. In phase one, the chicks were assigned into 3 groups of 3 each. Groups 1, 2 and 3 were administered orally by gastric lavage or intraperitoneally with 10 mg/kg, 100 mg/kg and 1000 mg/kg of the extract. The chicks and mice were observed for clinical signs of toxicity for 2 hours and mortality for 24 hours.

In the second phase, three or four groups of one chick each were given *J. curcas* leaf and seed extract orally or intraperitoneally in geometrically increasing doses based on results from phase one. The animals were observed for clinical signs of toxicity for 2 hours and mortality for 24 hours. 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.

3.9 Antidotal Therapy against Acute *Jatropha curcas* Intoxication

The method described by Mousa (2009) and Mohammed *et al*. (2012) with slight modifications, was used for the experiment. Modifications were based on preliminary study, which revealed that 5 minute prophylactic administration of antidotes was better than 15 minutes interval if the optimum efficacy of an antidote was to be observed. For the prophylactic study (pre-treatment), the antidotes were administered five minutes before the extracts. While the antidotes were administered five minutes

after the extracts for the curative study (post-treatment). The therapeutic efficacies of various drugs in the management of acute *J. curcas* leaf and seed intoxication in chicks were assessed by two methods:

i. Determination of the 24 hours lethal doses (LD_{50}) of *Jatropha curcas* extract alone or with antidotes in chicks.

ii. Effect of antidotes on signs of acute *Jatropha curcas*-induced toxicosis in chicks.

3.9.1 Determination of 24 hours median lethal doses (LD_{50}) of *Jatropha curcas* extract alone or with antidotes.

The intraperitoneal LD_{50} of *J. curcas* extracts either alone or co-administered with sodium nitrite (25 mg/kg, *i.p.*); sodium thiosulphate (2.5 mg/kg, *i.p.*); penicillamine (30 mg/kg, *p.o.*); EDTA (80 mg/kg, *i.p.*) and atropine (20 mg/kg, *i.p.*) as prophylactic or therapeutic agents were determined by using 7-day old cockerels according to Lorke's method (1983).

Treatment with antidotes was done five minutes before (prophylactic) or after (curative) intraperitoneal administration of the plant extracts. The chicks were observed separately for the appearance of signs of acute toxicity within 2 hours and mortality for 24 hours. The LD_{50} values were determined to investigate and evaluate the protective effect of various drugs. The protective index (PI) was calculated as the ratio between the LD_{50} of extracts with and without treatment (Mousa, 2009; Mohammad *et al.*, 2012).

3.9.2 Effect of antidotes on signs of acute *Jatropha curcas* induced toxicosis in chicks

Prophylactic and curative potentials of various drugs against *J. curcas* intoxication were performed using 7-day old cockerels according to Mohammed *et al*. (2012). Thirty chicks were divided into 6 groups of 5 chicks each and the antidotes were administered five minutes before the extracts for prophylactic study. A similar procedure was adopted for the curative study but antidotes were administered five minutes after the extract. All the groups were given the extracts intraperitoneally at a dose of 135% LD_{50} values. Treatment with the normal saline or antidotes before or after the extract administration was done as follows:

Group I: as control, received normal saline (2 ml/kg, *i.p*.)

Group II: received sodium nitrite (25 mg/kg, *i.p*.)

Group III: received sodium thiosulphate (2.5 mg/kg, *i.p*.)

Group IV: received penicillamine (30 mg/kg, *p.o*.)

Group V: received EDTA (80 mg/kg, *i.p*.)

Group VI: received atropine (20 mg/kg, *i.p*.)

The time of administration of extracts and the signs of acute toxic manifestations were recorded. The time of death during the 2 and 24 h observation periods after extract intoxication were also recorded. The toxicity score was determined which indicates the severity of toxicosis (signs of poisoning) for each group and calculated by summing the grades of the percentage of occurrence of acute signs of poisoning (which were pecking, ataxia, escape attempt, crouching, closing of eyes, sleep and gasping) as follows: 1= 1-25%, 2= 26-50%, 3= 51-75% and 4= 76-100% (Mousa, 2009; Mohammad *et al*., 2012).

3.10 Antidotal Therapy due to Sub-acute *Jatropha curcas* Intoxication

The therapeutic potentials of various drugs against sub-acute *J. curcas* intoxication were performed using cockerels according to Tulsawani *et al*. (2005). Seventy chicks were divided into 7 groups of 10 birds each. All the groups receieved the extracts orally at a dose of 10% oral LD_{50} values except group one which received de-ionized water (5 ml/kg, *p.o*.) daily for 7 days. Treatment with the following was done on day 0, 3 and 7 of the experiment:

Group I: as control, received normal saline (2 ml/kg, *i.p*.) after d/water administration

Group II: received normal saline (2 ml/kg, *i.p*.) after extract administration

Group III: received sodium nitrite (25 mg/kg, *i.p*.) after extract administration

Group IV: received sodium thiosulphate (2.5 mg/kg, *i.p*.) after extract administration

Group V: received penicillamine (30 mg/kg, *p.o*.) after extract administration

Group VI: received EDTA (80 mg/kg, *i.p*.) after extract administration

Group VII: received atropine (20 mg/kg, *i.p*.) after extract administration

The normal saline or antidotes were administered five minutes after oral administration of deionized water or the extracts. The body weights of the chicks were recorded on day 0, 3 and 7. Twenty four hours after the last administration of extracts and antidotes (8^{th} day), blood samples were collected for haematological and biochemical analysis.

3.11 Haematological Parameters

Blood samples from 14-day old chicks (7 days post treatment) were collected from the jugular vein 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), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), platelet and hemoglobin (Hb) were then calculated.

3.12 Biochemical Parameters

The blood samples from the birds were collected from the jugular vein into non-heparinized tubes for analysis. Standard operating procedures (Afia and Momoh, 2006) were used to conduct assays for the following biochemical parameters. Creatinine, urea, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were then calculated.

3.13 Statistical Analysis

The statistical analysis of the data was done using SPSS version 20 software. Data for survival percentages and percentages of occurrence of acute signs of toxicity were analyzed using Fisher's exact probability test. The toxicity score for acute signs were analyzed using Kruskal-Wallis and then to Mann Whitney U-test. Data for the body weight, biochemical and haematological parameters were analysed using one way analysis of variance (ANOVA) followed by Dunnet t- test. Data were analyzed by comparing various groups with the de-ionized water and extract controls. Values of $P<0.05$ were considered statistically significant. The research results were calculated as mean, standard error of the mean (S.E.M) and percentages.

CHAPTER FOUR

4.0 RESULTS

4.1 Yields of the Plant Extracts

Three extracts were obtained from *Jatropha curcas* leaves and seeds. The Hexane leaf extract (HLE) of the plant was a greenish, oily, sweet smelling substance which was insoluble in water. The methanol leaf extract (MLE) of the plant was a greenish-black, water soluble solid, while methanol seed extract (MSE) was a brownish, oily substance only partially soluble in water. Yields of 3.69% w/w (HLE), 10.50% w/w (MLE) and 6.29% w/w (MSE) were obtained as shown in Table 4.1.

Table 4.1: Percentage Yield of *Jatropha curcas* Leaf and Seed Extracts

Extract	Weight of powdered material (g)	Weight of extract (g)	Percentage yield (%)
HLE	500	18.44	3.69
MLE	200	31.49	10.50
MSE	230	14.46	6.29

HLE= Hexane leaf extract, MLE=Methanol leaf extract and MSE=Methanol seed extract.

4.2 Qualitative Phytochemical Analysis

Preliminary phytochemical investigation revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins and terpenes in methanol leaf and seed extract but absent in hexane leaf extract. Phenol and tannins were present in hexane and methanol leaf extract but absent in methanol seed extract. Fats and oil present in hexane leaf and methanol seed extract were absent in methanol leaf extract of *J. curcas* as shown in Table 4.2.

Table 4.2: Phytochemical Constituents of N-Hexane Leaf Extract, Methanol Leaf Extract and Methanol seed Extracts of *Jatropha curcas*

Phytoconstituents	HLE	HMLE	HMSE
Alkaloid	-	+	+
Cardiac glycosides	-	+	+
Fats and oil	+	-	+
Flavonoids	-	+	+
Saponins	-	+	+
Tannins	+	+	-

Key: + = present; - = absent; HLE = Hexane leaf extract, MLE = Methanol leaf extract and MSE = Methanol seed extract.

4.3 Quantitative Anti-nutritional Analysis

The quantitative estimation of these secondary metabolites showed that methanol leaf extract has the higher amount of phytate, oxalate and tannins than the hexane leaf extract of *Jatropha curcas* as shown in table 4.3.

Table 4.3: Quantitative Phytochemical Analysis of Anti-nutritional Constituents of *Jatropha curcas* Leaf Extracts

	Anti-nutrients		
Extract	Phytate (g/dm^3)	Oxalate (ppm)	Tannin (ppm)
HLE	-	2.95	0.24
MLE	0.214	5.50	31.40

HLE = Hexane leaf extract, MLE = Methanol leaf extract

4.4 Heavy Metal Concentration in Plant Extracts

The heavy metal concentrations in *J. curcas* plant were variable among the extracts. The methanol seed extract showed the highest concentration in cadmium (0.070 ppm), cobalt (0.039 ppm), copper (3.073 ppm), nickel (0.448 ppm) and lead (0.198 ppm) followed by the methanol leaf extract with the exception of zinc with highest concentration in the hexane leaf extract (0.527 ppm). Chromium was found to be absent in all the extracts of the plant (Table 4.4).

Table 4.4: Heavy Metal Contents of *Jatropha curcas* Leaf and Seed Extracts

Extracts	Heavy metals						
	Cadmium	Cobalt	Chromium	Copper	Nickel	Lead	Zinc
HLE	0.007	0.000	0.000	0.100	0.039	0.035	0.527
MLE	0.011	0.012	0.000	0.153	0.219	0.070	0.493
MSE	0.070	0.039	0.000	3.073	0.448	0.198	0.001

HLE= Hexane leaf extract, MLE= Methanol leaf extract and MSE: Methanol seed extract. Values expressed in parts per million (ppm). Standard deviation was omitted because they approximated zero.

4.5 LD_{50} Determination and Acute Toxicity Studies

The (acute) 24 h oral LD_{50} value of methanol seed extract in 7-day old cockerels was found to be 1,100 mg/kg. The oral LD_{50} value for the hexane and methanol leaf extracts were found to be above 5,000 mg/kg in both chicks and mice as no mortality was observed at this dose. The (acute) 24 h intraperitoneal LD_{50} value in 7-day old chicks was also found to be lower in the methanol seed extract with 22 mg/kg followed by methanol leaf extract (74 mg/kg) and highest in hexane leaf extract (935 mg/kg). All the extracts were found to have lower LD_{50} values in chicks than mice as shown in Table 4.5. The chicks intoxicated with *J. curcas* showed signs of acute poisoning such as pecking, ataxia, escape attempts, closing of eyes, gasping, crouching and sleeping before death during 2-24 h observation period.

Table 4.5: LD_{50} of *Jatropha curcas* Leaf and Seed Extracts in Chicks and Mice

Extract	Animal	Age (days)	LD_{50} [*p.o.*] (mg/kg)	LD_{50} [*i.p.*] (mg/kg)
HLE	Cockerel	1	>5,000	894
	Cockerel	7	>5,000	935
	Mice	Adult	>5,000	1,386
MLE	Cockerel	1	>5,000	89
	Cockerel	7	>5,000	74
	Mice	Adult	>5,000	775
MSE	Cockerel	7	1,100	22

HLE= Hexane leaf extract, MLE= Methanol leaf extract and MSE= Methanol seed extract.

4.6 Effect of Antidotes on LD_{50} of Various Extracts of *Jatropha curcas* Intoxicated Chicks

Administration of various antidotes as pretreatment (-5min) or post-treatment (+5min) mainly increased the intraperitoneal LD_{50} of HLE, MLE and MSE of *J. curcas* as show in Table 4.6, 4.7 and 4.8.

4.6.1 Effect of antidotes on LD_{50} of n-hexane leaf extract of *Jatropha curcas* intoxicated chicks

The LD_{50} value of HLE of *J. curcas* without treatment (control) was determined to be 835 mg/kg. Pre-treatment with *i.p*. administration of sodium thiosulphate and EDTA produced the maximum protection index of 1.66 (66% increase in LD_{50}) against HLE of *J. curcas* followed by 1.31 (31%) produced by penicillamine pre-treatment, sodium thiosulphate post-treatment and atropine pre-treatment and post-treatment compared to the control. Sodium nitrite post- treatment reduced the LD_{50} (775 mg/kg) below the control value of 835 mg/kg as shown in Table 4.6.

Table 4.6: Effect of Antidotes on LD_{50} of N-Hexane Leaf Extract of *Jatropha curcas* Intoxicated Chicks

Treatment	Pre-treatment			Post-treatment		
	LD_{50}	PI	Change (% LD_{50})	LD_{50}	PI	Change (% LD_{50})
Control	835	-	-	-	-	-
Ext+SN	894	1.07	7.18	775	0.93	- 7.18
Ext+STS	1,386	1.66	66.04	1,100	1.31	31.28
Ext+PNA	1,100	1.31	31.28	894	1.07	7.18
Ext+EDTA	1,386	1.66	66.04	1,100	1.31	31.28
Ext+Atropine	1,100	1.31	31.28	1,100	1.31	31.28

Control (Extract + Saline), Ext (Extract), SN (Sodium nitrite), STS (Sodium thiosulphate), PNA (Penicillamine), EDTA (Ethylene diamine tetra acetic acid).
Antidotes or saline were administered 5 minutes before (pre-treatment) or after (post-treatment) (+5 min) *i.p*. administration of extracts.
PI (Protection Index) = LD_{50} of extracts in protected chicks/LD_{50} of control (835 mg/kg). Change (% LD_{50}) = Percentage difference between treated and control groups.

4.6.2 Effect of antidotes on LD_{50} of methanol leaf extract of *Jatropha curcas* intoxicated chicks

The LD_{50} value of MLE of *J. curcas* without treatment (control) was found to be 74 mg/kg. Pre-treatment with atropine, pre-treatment and post-treatment with sodium thiosulphate produce the maximum protection index of 1.60 against MLE of *J. curcas* intoxication compared to control. Pre-treatment with penicillamine and EDTA and post-treatment with atropine gave protection index of 1.35. Sodium nitrite marginally protected when given as pre-treatment with protection index of 1.21 as did EDTA post-treatment. Also, post-treatment with sodium nitrite with protective index of 0.95 prove to be lower than the control as observed with HLE (Table 4.7).

Table 4.7: Effect of Antidotes on LD_{50} of Methanol Leaf Extract of *Jatropha curcas* Intoxicated Chicks

Treatment	Pre-treatment			Post-treatment		
	LD_{50}	PI	Change (% LD_{50})	LD_{50}	PI	Change (% LD_{50})
Control	74	-	-	-	-	-
Ext+SN	89	1.21	20.72	71	0.95	- 4.56
Ext+STS	118	1.60	59.70	118	1.60	59.70
Ext+PNA	100	1.35	34.97	77	1.05	4.55
Ext+EDTA	100	1.35	34.97	89	1.21	20.72
Ext+Atropine	118	1.60	59.70	100	1.35	34.97

Control (Extract + Saline), Ext (Extract), SN (Sodium nitrite), STS (Sodium thiosulphate), PNA (Penicillamine), EDTA (Ethylene diamine tetra acetic acid).
Antidotes and saline were administered 5 minutes before (pre-treatment) or after (post-treatment) *i.p*. administration of extracts.
PI (Protection Index) = LD_{50} of extracts in protected chicks/LD_{50} of control (74 mg/kg). Change (% LD_{50}) = Percentage difference between treated and control groups.

4.6.3 Effect of antidotes on LD_{50} of methanol seed extract of *Jatropha curcas* intoxicated chicks

The LD_{50} value of MSE of *J. curcas* without treatment (control) was determined to be 28 mg/kg. Pre-treatment with sodium thiosulphate produced protection index of 2.00 while sodium nitrite produced protection index of 1.73. The protection index for sodium thiosulphate and atropine as post-treatment were each 1.73 when compared to the control. Pre-treatment with penicillamine and post-treatment with sodium nitrite did not offer reasonable protection with protection index of 0.79 and 1.12 when compared to the control as shown in Table 4.8.

Table 4.8: Effect of Antidotes on LD_{50} of Methanol Seed Extract of *Jatropha curcas* Intoxicated Chicks

Treatment	Pre-treatment			Post-treatment		
	LD_{50}	PI	Change (% LD_{50})	LD_{50}	PI	Change (% LD_{50})
Control	28	-	-	-	-	-
Ext+SN	49	1.73	73.23	32	1.12	11.81
Ext+STS	57	2.00	100.04	49	1.73	73.23
Ext+PNA	22	0.79	- 20.93	28	1.00	0.00
Ext+EDTA	32	1.12	11.81	32	1.12	11.81
Ext+Atropine	32	1.12	11.81	49	1.73	73.23

Control (Extract + Saline), Ext (Extract), SN (Sodium nitrite), STS (Sodium thiosulphate), PNA (Penicillamine), EDTA (Ethylene diamine tetra acetic acid).
Antidotes and saline were administered 5 minutes before (pre-treatment) or after (post-treatment) *i.p*. administration of extracts.
PI (Protection Index) = LD_{50} of extracts in protected chicks/LD_{50} of control (28 mg/kg). Change (% LD_{50}) = Percentage difference between treated and control groups.

4.7 Effect of Antidotes on Signs of Acute *Jatropha curcas* Intoxication in Chicks

The doses for various extracts used for the study were 135% of the intraperitoneal LD_{50} value in chicks. The dose for hexane leaf extract was determined to be 1,262 mg/kg, methanol leaf extract (100 mg/kg) and methanol seed extract (30 mg/kg). The chicks intoxicated with *J. curcas* with or without treatment showed signs of acute poisoning such as pecking, ataxia, escape attempts, crouching, closing of eyes, sleeping, respiratory distress, before death during the 2-24 h observation period.

4.7.1 Effect of antidotes on signs of acute n-hexane leaf extract of *Jatropha curcas* intoxication in chicks

4.7.1.1 Effects of antidotes as pre-treatment on signs of acute n-hexane leaf extract of Jatropha curcas intoxicated chicks

In the pre-treatment study, chicks of the control group showed 40% and 20% of survival after 2 h and 24 h of HLE of *J. curcas* poisoning (1,262 mg/kg, intraperitoneally). Also, the control expressed most signs of acute poisoning with a toxicity score of 27 (Table 4.9). All the antidotes given as pre-treatment at 5 minutes before HLE of *J. curcas* intoxication increased the percentage survival to 100% from 60% after 2 h of intoxication. Penicillamine increased the survival after 24 h to 60%, while other antidotes had no effect or caused a decrease when compared to the control. All the antidotes decreased the percentages of occurrence of acute signs but significant decrease to 20% occurred in ataxia when treated with sodium nitrite and EDTA, Escape attempt (20%) by atropine, Gasping (20%) by sodium thiosulphate and EDTA. All the antidotes significantly decreased the toxicity score when compared to the control group (Table 4.9).

Table 4.9: Effects of Antidotes as Pre-treatment on Signs of Acute N-Hexane Leaf Extract of *Jatropha curcas* Intoxicated Chicks

Group	% Survival (2 h)	% Survival (24 h)	The percentages of occurrence of acute signs of toxicity							Toxicity score
			Pecking	Ataxia	Escape attempt	Crouching	Eye closing	Sleeping	Gasping	
Control	40	20	100	100	100	80	100	60	100	27
Ext+SN	100	20	60	20*	80	20	100	40	40	17*
Ext+STS	100	20	60	60	80	40	100	20	20*	18*
Ext+PNA	100	60	60	60	60	20	100	40	40	18*
Ext+EDTA	100	20	40	20*	60	20	100	20	20*	13*
Ext+Atropine	100	0	60	60	20*	60	100	20	80	19*

Control (Extract + Saline), Ext (Extract), SN (Sodium nitrite), STS (Sodium thiosulphate), PNA (Penicillamine), EDTA (Ethylene diamine tetra acetic acid).
n = 5 (number of chicks per group); *Significantly different from the control group at $p<0.05$; Data for survival percentages and percentages of occurrence of acute signs of toxicity was analyzed using Fisher's exact test while grades of toxicity score were analyzed using Kruskal-Wallis and then Mann Whitney U-test.

4.7.1.2 Effects of antidotes as post-treatment on signs of acute n-hexane leaf extract of Jatropha curcas intoxicated chicks

In the post-treatment study, chicks of the control group showed 40% and 20% survival after 2 h and 24 h of HLE of *J. curcas* poisoning (1,262 mg/kg, intraperitoneally). Also, the control expressed most signs of acute poisoning with a toxicity score of 27 (Table 4.10). All the antidotes given as post-treatment at 5 minutes after HLE of *J. curcas* intoxication increased the percentage survival of chicks compared to control. Sodium thiosulphate, penicillamine and atropine given as post-treatment showed the highest percentage increase to 100% from 40% for the control after 2 h of intoxication. The antidotes had no effect on percentage survival after 24 h of intoxication compared to the control. Sodium thiosulphate and penicillamine significantly decreased pecking to 20% compared to 100% in the control group. Atropine significantly ($p<0.05$) decreased gasping to 20% from 100% observed in control. All the antidotes insignificantly decreased the toxicity scores with the exception of sodium nitrite which had no effect when compared with the control (Table 4.10).

Table 4.10: Effect of Antidotes as Post-treatment on Signs of Acute N-Hexane Leaf Extract of *Jatropha curcas* Intoxicated Chicks

Group	% Survival (2 h)	% Survival (24 h)	The percentages of occurrence of acute signs of toxicity							Toxicity score
			Pecking	Ataxia	Escape attempt	Crouching	Eye closing	Sleeping	Gasping	
Control	40	20	100	100	100	80	100	60	100	27
Ext+SN	60	20	100	60	100	100	100	80	100	27
Ext+STS	100	20	20*	100	100	40	100	20	60	19
Ext+PNA	100	20	20*	100	100	40	100	0	40	17
Ext+EDTA	80	0	80	100	100	60	100	80	40	25
Ext+Atropine	100	20	80	100	40	80	100	60	20*	22

Control (Extract + Saline), Ext (Extract), SN (Sodium nitrite), STS (Sodium thiosulphate), PNA (Penicillamine), EDTA (Ethylene diamine tetra acetic acid).
n = 5 (number of chicks per group); *Significantly different from the control group at $p<0.05$; Data for survival percentages and percentages of occurrence of acute signs of toxicity was analyzed using Fisher's exact test while grades of toxicity score were analyzed using Kruskal-Wallis and then Mann Whitney U-test.

4.7.1.3 Effect of antidotes as pre-treatment or post-treatment on the toxicity score of acute n-hexane leaf extract of Jatropha curcas intoxicated chicks

EDTA pre-treatment produced the highest percentage decrease in toxicity scores (51.85 %) followed by sodium nitrite pre-treatment and penicillamine post-treatment (37.04%) when compared with control. Sodium nitrite post-treatement produced the least effect with no decrease in toxicity score when compared to the control. The pre-treatment group had less toxicity scores compared to the post-treatment groups as shown in Figure 4.1 and Appendix A.

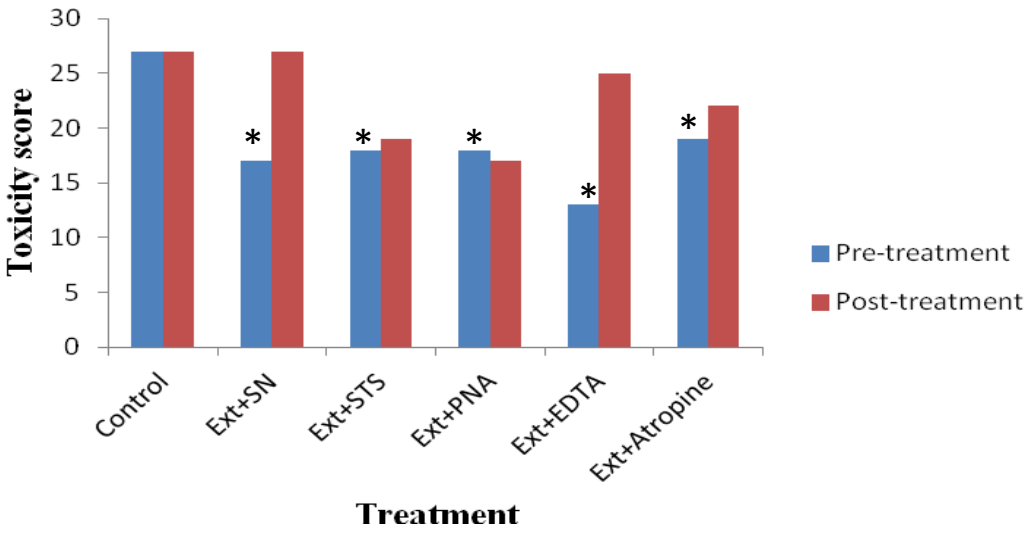


Figure 4.1 Effect of antidotes as pre-treatment or post-treatment on the toxicity score of acute n-hexane leaf extract of *Jatropha curcas* intoxicated chicks

All groups received extracts. Control group was treated with Saline (*i.p.*); n = 5 chicks per group. *Significantly different from the respectively control groups, $p<0.05$; Data analyzed using Kruskal-Wallis and then Mann Whitney U-test.

4.7.2 Effect of antidotes on signs of acute methanol leaf extract of *Jatropha curcas* intoxicated chicks

4.7.2.1 Effect of antidotes as pre-treatment on signs of acute methanol leaf extract of Jatropha curcas intoxicated chicks

All the antidotes given as 5 min pre-treatment before MLE of *J. curcas* (100 mg/kg, intraperitoneally) intoxication increased the percentage survival to 100% with the exception of EDTA (80%) which had no effect when compared to the control value of 80% survival after 2 h of intoxication. Also, all the antidotes increased percentage survival from 20% observed in control to 40% with the exception of EDTA which produced no effect after 24 h of MLE of *J. curcas* intoxication. All the antidotes decreased the percentages of occurrence of acute signs of poisoning. Sodium thiosulphate and atropine groups significantly ($p<0.05$) decreased the toxicity scores to 17 and 14 compared to the control value of 26 (Table 4.11).

Table 4.11: Effect of Antidotes as Pre-treatment on Signs of Acute Methanol Leaf Extract of *Jatropha curcas* Intoxicated Chicks

Group	% Survival (2 h)	% Survival (24 h)	The percentages of occurrence of acute signs of toxicity							Toxicity score
			Pecking	Ataxia	Escape attempt	Crouching	Eye closing	Sleeping	Gasping	
Control	80	20	60	100	100	80	100	60	80	26
Ext+SN	100	40	20	60	100	40	100	20	80	19
Ext+STS	100	40	20	60	80	40	100	20	40	17*
Ext+PNA	100	40	20	100	80	40	100	20	60	19
Ext+EDTA	80	20	20	80	80	40	100	20	40	18
Ext+Atropine	100	40	0	40	60	40	100	0	60	14*

Control (Extract + Saline), Ext (Extract), SN (Sodium nitrite), STS (Sodium thiosulphate), PNA (Penicillamine), EDTA (Ethylene diamine tetra acetic acid).
n = 5 (number of chicks per group); *Significantly different from the control group at $p<0.05$, Data for survival percentages and percentages of occurrence of acute signs of toxicity was analyzed using Fisher's exact test while grades of toxicity score were analyzed using Kruskal-Wallis and then Mann Whitney U-test.

4.7.2.2 Effect of antidotes as post-treatment on signs of acute methanol leaf extract of Jatropha curcas intoxicated chicks

Five minutes post-treatment with EDTA and atropine increased the percentage survival of chicks to 100% after 2 h of MLE of *J. curcas* intoxication. Sodium nitrite, sodium thiosulphate and penicillamine showed no effect when compared to 80% survival for the control group after 2 h of intoxication. Sodium thiosulphate increased the percentage survival of chicks to 40% from 20% for the control group after 24 h of intoxication. The other antidotes produced no effect on percentage survival of chicks when compared to control after 24 h of extract intoxication. All the antidotes either insignificantly ($p<0.05$) decreased or had no effect on the percentages of occurrence of acute signs of poisoning when compared to the control. Though all the antidotes decreased the toxicity scores but EDTA and atropine significantly decreased it to 17 and 18 when compared to the control value of 26 as shown in Table 4.12.

Table 4.12: Effect of Antidotes as Post-treatment on Signs of Acute Methanol Leaf Extract of *Jatropha curcas* Intoxicated Chicks

Group	% Survival (2 h)	% Survival (24 h)	The percentages of occurrence of acute signs of toxicity							Toxicity score
			Pecking	Ataxia	Escape attempt	Crouching	Eye closing	Sleeping	Gasping	
Control	80	20	60	100	100	80	100	60	80	26
Ext+SN	80	0	60	100	80	60	100	40	80	24
Ext+STS	80	40	40	80	80	40	100	40	60	21
Ext+PNA	80	20	40	80	100	40	100	60	60	22
Ext+EDTA	100	20	20	100	60	60	100	0	40	17*
Ext+Atropine	100	20	40	60	80	40	100	20	40	18*

Control (Extract + Saline), Ext (Extract), SN (Sodium nitrite), STS (Sodium thiosulphate), PNA (Penicillamine), EDTA (Ethylene diamine tetra acetic acid).
n = 5 (number of chicks per group); *Significantly different from the control group at $p<0.05$, Data for survival percentages and percentages of occurrence of acute signs of toxicity was analyzed using Fisher's exact test while grades of toxicity score were analyzed using Kruskal-Wallis and then Mann Whitney U-test.

4.7.2.3 Effect of antidotes as pre-treatment or post-treatment on the toxicity score of acute methanol leaf extract of Jatropha curcas intoxicated chicks

Atropine pre-treatment show the highest percentage decrease of toxicity scores (46.15%) followed by sodium thiosulphate pre-treatment and EDTA post-treatment (34.62%) when compared with control. Sodium nitrite post-treatement produced the least decrease of 7.69% when compared to the control. The pre-treatment group had less toxicity scores compared to the post-treatment groups with the exception of EDTA as shown in Figure 4.2 and Appendix B.

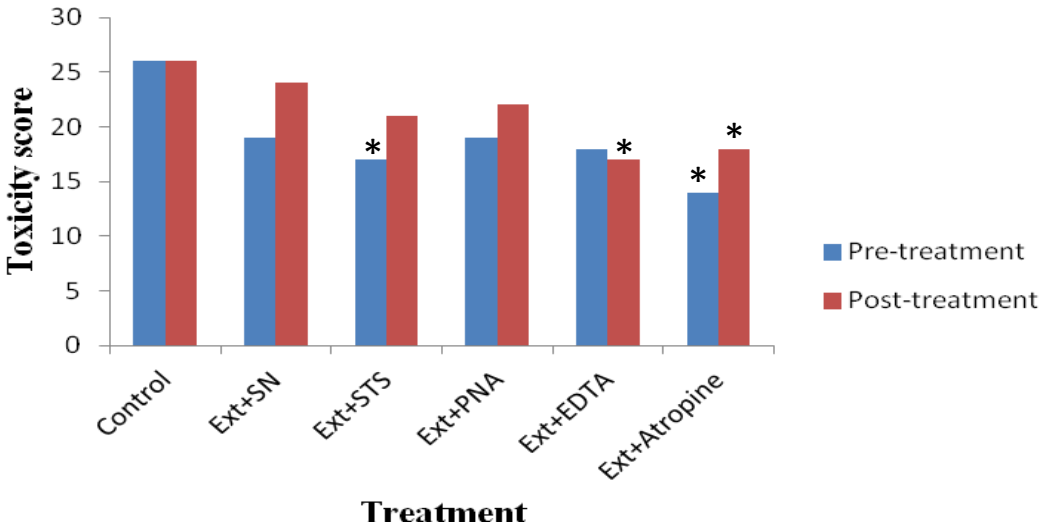


Figure 4.2. Effect of antidotes as pre-treatment or post-treatment on the toxicity score of acute methanol leaf extract of *Jatropha curcas* intoxicated chicks

All groups received extracts. Control group was treated with Saline (*i.p.*). n = 5 chicks per group. *Significantly different from the respectively control groups, $p<0.05$; Data analyzed using Kruskal-Wallis and then Mann Whitney U-test.

4.7.3 Effects of antidotes on signs of acute methanol seed extract of *Jatropha curcas* intoxicated chicks

4.7.3.1 Effects of antidotes as pre-treatment on signs of acute methanol seed extract of Jatropha curcas intoxicated chicks

All the antidotes given as pre-treatment at 5 minutes before MSE of *J. curcas* (30 mg/kg, intraperitoneally) intoxication produced insignificant ($p<0.05$) increase in percentage survival compared to control group of 60% and 20% survival after 2 h and 24 h of intoxication. Sodium thiosulphate and atropine produced the highest percentage survival (80%) followed by sodium nitrite with 60% survival when compared to control (20%) after 24 h of intoxication. All the antidotes produced a decrease in acute signs of toxicity. Sodium nitrite and atropine produce a significant decrease in crouching to 20% from 100% observed in control group. Sodium thiosulphate and atropine also produced a significant decrease in gasping to 20% from 100% observed in control group. All the antidotes produced a significant decrease in toxicity score when compared to control as shown in Table 4.13.

Table 4.13: Effect of Antidotes as Pre-treatment on Signs of Acute Methanol Seed Extract of *Jatropha curcas* Intoxicated Chicks

Group	% Survival (2hrs)	% Survival (24hrs)	The percentages of occurrence of acute signs of toxicity							Toxicity score
			Pecking	Ataxia	Escape attempt	Crouching	Eye closing	Sleeping	Gasping	
Control	60	20	80	100	100	100	100	80	100	28
Ext+SN	80	60	40	40	100	20*	100	40	40	17*
Ext+STS	100	80	40	100	80	40	100	20	20*	18*
Ext+PNA	100	40	40	100	80	60	100	40	40	21*
Ext+EDTA	100	40	40	80	80	40	100	20	40	19*
Ext+Atropine	100	80	20	60	100	20*	100	20	20*	15*

Control (Extract + Saline), Ext (Extract), SN (Sodium nitrite), STS (Sodium thiosulphate), PNA (Penicillamine), EDTA (Ethylene diamine tetra acetic acid)
n = 5 (number of chicks per group); *Significantly different from the control group at $p<0.05$, Data for survival percentages and percentages of occurrence of acute signs of toxicity was analyzed using Fisher's exact test while grades of toxicity score were analyzed using Kruskal-Wallis and then Mann Whitney U-test.

4.7.3.2 Effect of antidotes as post-treatment on signs of acute methanol seed extract of Jatropha curcas intoxicated chicks

All the antidotes given as post-treatment at 5 minutes after MSE of *J. curcas* (30 mg/kg, intraperitoneally) intoxication produced insignificant ($p<0.05$) increase in percentage survival compared to control group of 60% and 20% survival after 2 h and 24 h of intoxication. EDTA and sodium thiosulphate produced the highest percentage survival of 80% and 60% from 20% for the control group after 24 h of intoxication. All the antidotes decreased the acute signs of toxicity when compared to control. Atropine produced a significant decrease in sleeping while EDTA on gasping. Sodium thiosulphate, EDTA and atropine significantly decreased the toxicity score to 22, 14 and 18 compared to control value of 28 as shown in Table 4.14.

Table 4.14: Effect of Antidotes as Post-treatment on Signs of Acute Methanol Seed Extract of *Jatropha curcas* Intoxicated Chicks

Group	% Survival (2 h)	% Survival (24 h)	The percentages of occurrence of acute signs of toxicity							Toxicity score
			Pecking	Ataxia	Escape attempt	Crouching	Eye closing	Sleeping	Gasping	
Control	60	20	80	100	100	80	100	80	100	28
Ext+SN	80	20	80	80	100	60	100	40	80	25
Ext+STS	100	60	60	100	80	60	100	20	60	22*
Ext+PNA	80	20	80	100	100	60	100	40	80	25
Ext+EDTA	100	80	40	40	80	20	100	20	0*	14*
Ext+Atropine	100	40	60	80	100	20	100	0*	40	18*

Control (Extract + Saline), Ext (Extract), SN (Sodium nitrite), STS (Sodium thiosulphate), PNA (Penicillamine), EDTA (Ethylene diamine tetra acetic acid).
n = 5 (number of chicks per group); *Significantly different from the control group at $p<0.05$, Data for survival percentages and percentages of occurrence of acute signs of toxicity was analyzed using Fisher's exact test while grades of toxicity score were analyzed using Kruskal-Wallis and then Mann Whitney U-test.

4.7.3.3 Effect of antidotes as pre-treatment or post-treatment on the toxicity score of acute methanol seed extract of Jatropha curcas intoxicated chicks

EDTA post-treatment produced the highest percentage decrease of toxicity scores of 50% followed by atropine (46.43 %) and sodium nitrite (39.29 %) pre-treatment when compared with control. The pre-treatment group had less toxicity scores compared to the post-treatment groups with the exception of EDTA as shown in Figure 4.3 and Appendix C.

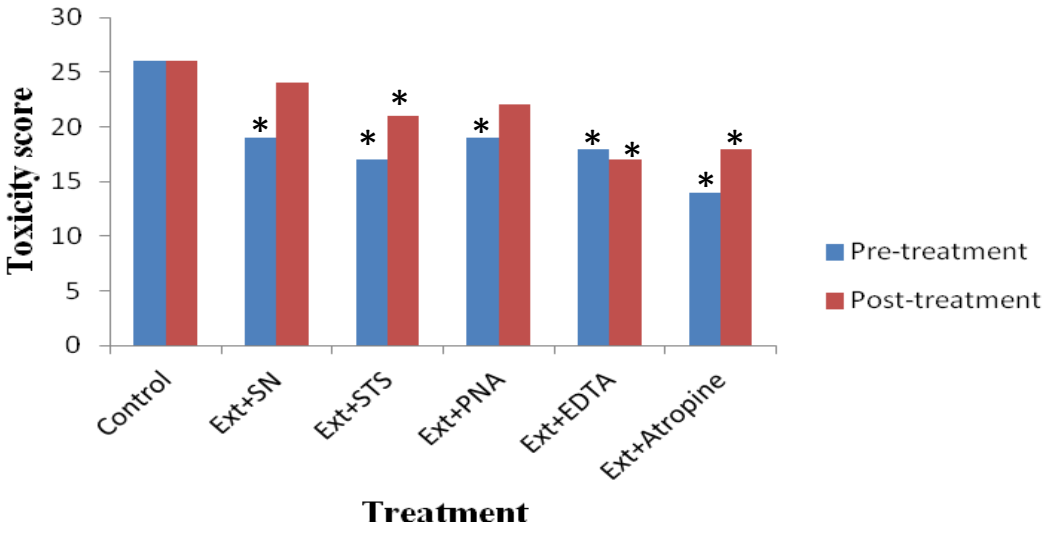


Figure 4.3. Effect of antidotes as pre-treatment or post-treatment on the toxicity score of acute methanol seed extract of *Jatropha curcas* intoxicated chicks

All groups received extract. Control group was treated with Saline (*p.o.*). n = 5 chicks per group. *Significantly different from the respectively control group at $p<0.05$; Data analyzed using Kruskal-Wallis and then Mann Whitney U-test.

4.8 Antidotal Therapy due to Sub-acute *Jatropha curcas* Intoxication

For the sub-acute study, the dose of methanol leaf and seed extracts orally administered were 500 mg/kg and 110 mg/kg respectively for seven consecutive days.

4.8.1 Effect of methanol leaf and seed extracts of *Jatropha curcas* intoxication on the body weight of chicks in the presence or absence of antidotes

4.8.1.1 Effect of methanol leaf extracts of Jatropha curcas intoxication on the body weight of chicks in the presence or absence of antidotes

The body weight (grams) of chicks on day 0, 3 and 7 for the group treated with saline after methanol leaf extract administration (saline treated group) were 35.30 ± 1.42, 40.00 ± 1.62 and 46.50 ± 2.13. No significant ($p<0.05$) change was observed in the body weight of the antidotes treated groups when compared with the saline treated group as shown in figure 4.4 below.

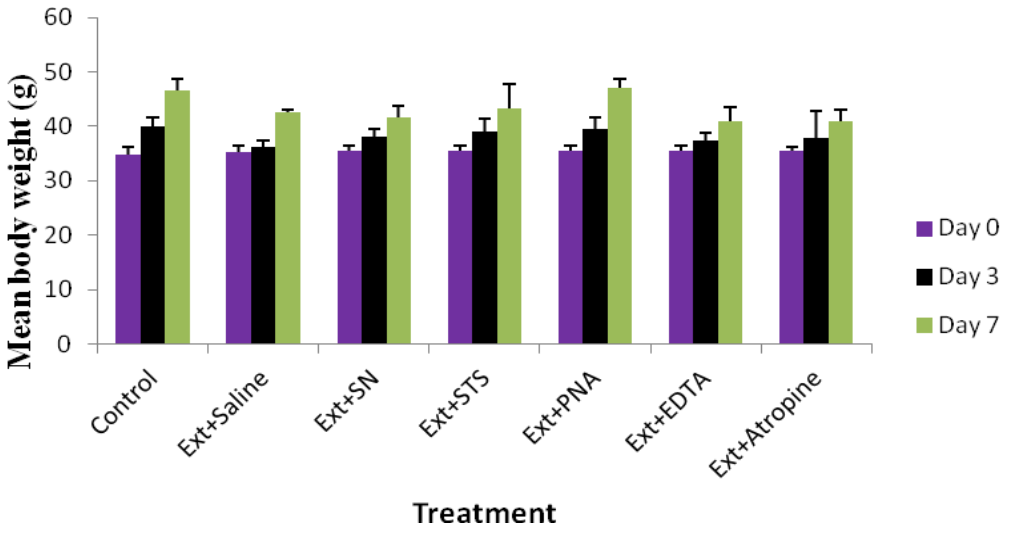


Figure 4.4. Effect of methanol leaf extract of *Jatropha curcas* intoxication on the body weight of chick in the presence or absence of antidotes

n = 6 (number of animals); Values are expressed in mean ± S.E.M.;
Data analyzed using one way Anova and Dunnett's post hoc test;
No significant difference between antidotes treated group from the control group and saline treated group at $p<0.05$

4.8.1.2 Effect of methanol seed extracts of Jatropha curcas intoxication on the body weight of chicks in the presence or absence of antidotes

The body weight (grams) of chicks on day 0, 3 and 7 for the group treated with saline after methanol seed extract administration (saline treated group) were 39.70 ± 1.44, 41.14 ± 1.24 and 49.00 ± 2.56, respectively. No significant ($p<0.05$) change was observed in the body weight of the antidotes treated groups when compared with the saline treated group as shown in figure 4.5 and Appendix E.

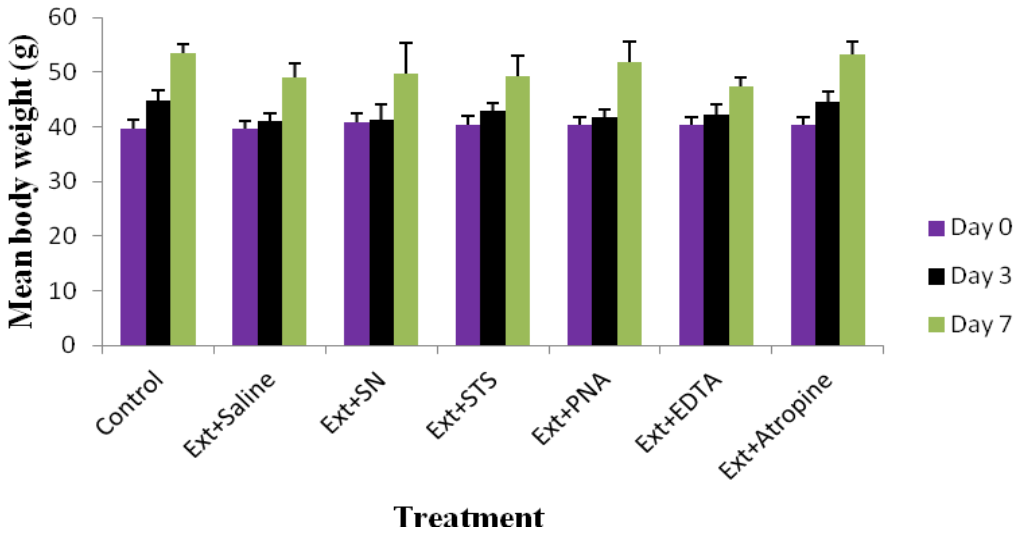


Figure 4.5. Effect of methanol seed extract of *Jatropha curcas* intoxication on the body weight of chicks in the presence or absence of antidotes

n = 6 (number of animals); Values are expressed in mean ± S.E.M.;
Data analyzed using one way Anova and Dunnett's post hoc test;
No significant difference between antidotes treated group from the control group and saline treated group at $p<0.05$

4.8.2 Effect of methanol leaf and seed extracts of *Jatropha curcas* intoxication on haematological indices in the absence or presence of antidotes in chicks

4.8.2.1 Effect of methanol leaf extract of Jatropha curcas intoxication on haematological indices in the absence or presence of antidotes in chicks

The red blood cells (10^{12}/L), white blood cell (10^{9}/L), mean cell volume (fL), mean cell haemoglobin concentration (g/L), platelet (10^{9}/L), haemoglobin (g/L) and haematocrit (%) levels in extract co-administered with saline treated group (saline treated group) were 2.32 ± 0.01, 245.10 ± 5.78, 120.50 ± 0.45, 408.33 ± 3.48, 22.67 ± 4.26, 113.33 ± 0.88 and 26.17 ± 0.73. The saline, sodium thiosulphate, penicillamine and atropine treated groups significantly ($p<0.05$) increased the WBC while sodium nitrite treated group significantly decreased the parameter when compared to the control. Sodium nitrite and EDTA treated groups significantly decreased WBC when compared with saline treated group. The saline and antidotes treated groups significantly increased the MCV when compared to the control. The saline and antidotes treated groups increased MCHC, PLT, Hb and HCT but sodium nitrite significantly decreased MCHC when compared to the control. EDTA treated group significantly increased the RBC and MCV while EDTA and sodium nitrite significantly decreased the WBC and MCHC when compared to saline treated group as shown in table 4.15 below.

Table 4.15: Effect of Methanol Leaf Extract of *Jatropha curcas* on Haematological Indices in the Absence or Presence of Antidotes in Chicks

Treatment	RBC (10^{12}/L)	WBC (10^{9}/L)	MCV (fL)	MCHC (g/L)	PLT (10^{9}/L)	Hb (g/L)	HCT (%)
Control	2.08±0.08	217.17±1.39	104.17±0.98	396.67±4.67	14.33±0.67	87.00±5.51	21.87±1.19
Ext+Saline	2.32±0.01	245.10±5.78[a]	120.50±0.45[a]	408.33±3.48	22.67±4.26	113.33±0.88	26.17±0.73
Ext+SN	1.92±0.44	201.03±1.68[ab]	117.60±1.08[a]	373.33±8.25[ab]	29.33±2.40	108.67±6.23	24.40±5.26
Ext+STS	2.24±0.03	236.00±2.48[a]	117.60±0.50[a]	393.33±5.24	17.33±0.33	105.00±1.53	27.10±0.06
Ext+PNA	2.44±0.02	236.47±2.33[a]	114.67±0.90[a]	394.00±3.00	14.33±2.33	111.67±0.67	27.97±0.47
Ext+EDTA	2.18±0.01[b]	215.60±2.60[b]	113.70±2.58[ab]	386.33±2.73[b]	17.33±2.33	98.00±3.46	25.43±0.90
Ext+ Atropine	2.34±0.08	236.97±6.50[a]	118.77±2.26[a]	402.67±1.20	18.67±0.67	104.00±5.03	27.80±2.32

n = 6 (number of animals); Values are expressed in mean ± S.E.M.;
Data analyzed using one way Anova and Dunnett's post hoc test;
[a]Significantly different from the control group at $p<0.05$
[b]Significantly different from the saline treatedgroup at $p<0.05$

4.8.2.2 Effect of methanol seed extract of Jatropha curcas intoxication on haematological indices in the absence or presence of antidotes in chicks

The red blood cells (10^{12}/L), white blood cell (10^{9}/L), mean cell volume (fL), mean cell haemoglobin concentration (g/L), platelet (10^{9}/L), haemoglobin (g/L) and haematocrit (%) levels in chicks treated with methanol seed extract co-administered with saline (saline treated group) were 2.01 ± 0.14, 225.93 ± 7.39, 110.73 ± 1.14, 392.67 ± 4.91, 20.33 ± 1.45, 94.33 ± 6.23 and 23.97 ± 1.31 respectively. Sodium thiosulphate, penicillamine, EDTA and atropine significantly ($p<0.05$) increased the MCV when compared to the control group. Penicillamine significantly increased PLT and Hb when compared to the control. No significant effect was observed in the parameters when the treated groups were compared with saline treated group as shown in table 4.16 below.

Table 4.16: Effect of Methanol Seed Extract of *Jatropha curcas* on Haematological Indices in the Absence or Presence of Antidotes in Chicks

Treatment	RBC (10^{12}/L)	WBC (10^{9}/L)	MCV (fL)	MCHC (g/L)	PLT (10^{9}/L)	Hb (g/L)	HCT (%)
Control	2.08±0.08	217.17±1.39	104.17±0.98	396.67±4.67	14.33±0.67	87.00±5.51	21.87±1.19
Ext+Saline	2.01±0.14	225.93±7.39	110.73±1.14	392.67±4.91	20.33±1.45	94.33±6.23	23.97±1.31
Ext+SN	1.97±0.39	226.47±7.34	108.53±2.97	403.33±6.36	19.00±3.06	99.33±3.71	21.17±3.82
Ext+STS	2.14±0.05	229.37±2.36	113.80±1.30[a]	390.67±0.88	17.67±1.45	98.00±1.73	22.87±1.68
Ext+PNA	2.24±0.07	232.47±2.36	116.20±1.10[a]	396.00±2.65	21.67±0.88[a]	106.67±1.76[a]	24.33±0.90
Ext+EDTA	1.88±0.04	209.77±1.42	112.37±0.56[a]	394.00±1.00	13.33±1.45	84.00±1.00	21.30±0.30
Ext+ Atropine	2.02±0.24	213.90±6.24	112.93±2.05[a]	402.00±0.58	19.33±1.33	95.00±7.23	22.77±2.53

n = 6 (number of animals); Values are expressed in mean ± S.E.M.;
Data analyzed using one way Anova and Dunnett's post hoc test;
[a]Significantly different from the control group at $p<0.05$,
[b]Significantly different from the saline treated group at $p<0.05$.

4.8.3 Effect of methanol leaf and seed extracts of *Jatropha curcas* on biochemical variables in cockerels in the absence or presence of antidotes

4.8.3.1 Effect of methanol leaf extract of Jatropha curcas on biochemical variables in cockerels in the absence or presence of antidotes

The biochemical parameters (ALP, ALT, AST and Urea) of methanol leaf extract co-administered with saline group (saline treated group) showed significant ($p< 0.05$) increase when compared with the control. Sodium thiosulphate, EDTA and atropine significantly decreased the level of ALT when compared to the saline treated group. Sodium nitrite, sodium thiosulphate and atropine significantly decreased the level of AST when compared with the saline treated group. Atropine significantly decreased the level of urea when compared to saline treated group. Oral treatment with penicillamine brought an insignificant decrease in all biomarker while sodium nitrite significantly elevates the level of creatinine when compared to control and saline treated group as shown in table 4.17 below.

Table 4.17: Effect of Methanol Leaf Extract of *Jatropha curcas* on Biochemical Variables in Cockerels in the Absence or Presence of Antidotes

Treatment	Liver Function Tests			Kidney Function Tests	
	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	Urea (mmol/L)	Creatinine (µmol/L)
Control	56.50 ± 3.57	26.75 ± 3.64	23.25 ± 2.69	2.08 ± 0.17	44.50 ± 2.60
Ext+Saline	69.83 ± 3.16[a]	46.33 ± 2.44[a]	45.17 ± 2.30[a]	2.92 ± 0.12[a]	40.67 ± 2.40
Ext+SN	76.00 ± 2.28[a]	41.33 ± 1.86[a]	35.00 ± 1.37[ab]	2.82 ± 0.11[a]	69.00 ± 1.41[ab]
Ext+STS	67.71 ± 2.30[a]	37.43 ± 2.06[ab]	31.00 ±2.61[b]	2.53 ± 0.11	45.71 ± 3.15
Ext+PNA	66.60 ± 2.40	41.60 ± 1.36[a]	41.20 ±1.50[a]	2.62 ± 0.14	40.33 ± 0.67
Ext+EDTA	68.40 ± 1.12[a]	37.80 ± 1.62[ab]	41.20 ± 2.40[a]	2.76 ± 0.19[a]	35.80 ± 1.16
Ext+Atropine	70.00 ± 2.65[a]	31.75 ± 3.44[b]	29.50 ± 2.53[b]	2.00 ± 0.20[b]	40.50 ± 5.50

n = 6 (number of animals); Values are expressed in mean ± S.E.M.;
Data analyzed using one way Anova and Dunnett's post hoc test;
[a]Significantly different from the control group at $p<0.05$;
[b]Significantly different from the saline treated group at $p<0.05$.

4.8.3.2 Effect of methanol seed extract of Jatropha curcas on biochemical variables in cockerels in the absence or presence of antidotes

The biochemical parameters (ALT and AST) of methanol seed extract co-administered with saline (saline treated group) showed significant ($p< 0.05$) increase when compared with the control. Sodium thiosulphate significantly decreased the level of ALT and AST when compared to the saline treated group. Also, sodium nitrite significantly increased the level of creatinine when compared to control and saline treated groups as shown in Table 4.18.

Table 4.18: Effect of Methanol Seed Extract of *Jatropha curcas* on Biochemical Variables in Cockerels in the Absence or Presence of Antidotes

Treatment	Liver Function Tests			Kidney Function Tests	
	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	Urea (mmol/L)	Creatinine (µmol/L)
Control	60.00 ± 1.00	26.75 ± 3.64	23.25 ± 2.69	2.20 ± 0.15	42.33 ± 2.03
Ext+Saline	69.00 ± 3.61	41.25 ± 3.61[a]	42.33 ± 2.40[a]	2.58 ± 0.13	41.67 ± 2.60
Ext+SN	74.00 ± 2.98[a]	39.20 ± 2.15[a]	36.80 ± 2.27[a]	2.88 ± 0.14[a]	62.33 ± 2.03[ab]
Ext+STS	68.00 ± 0.95	32.00 ±1.91[b]	31.40 ± 2.38[b]	2.60 ± 0.09	46.60 ± 3.20
Ext+PNA	65.75 ± 2.56	45.33 ± 2.85[a]	48.67 ± 3.18[a]	2.83 ± 0.16[a]	35.00 ± 2.00
Ext+EDTA	68.60 ± 3.46	38.40 ± 1.36[a]	40.00 ± 2.17[a]	2.48 ± 0.07	34.67 ± 2.19
Ext+Atropine	71.80 ± 1.59[a]	37.20 ± 1.50[a]	33.80 ± 2.24[a]	2.22 ± 0.13	41.25 ± 1.65

n = 6 (number of animals); Values are expressed in mean ± S.E.M.;
Data analyzed using one way Anova and Dunnett's post hoc test;
[a]Significantly different from the control group at $p<0.05$;
[b]Significantly different from the saline treated group at $p<0.05$.

CHAPTER FIVE

5.0 DISCUSSION

Preliminary phytochemical screening of the leaf and seed extracts of *J. curcas* revealed the presence of toxins or anti-nutrients such as glycosides, tannins, saponins. The presence of triterpenoids, volatile oils, alkaloids, flavonoids, saponins and tannins in the leaves and seeds *J. curcas* grown in Southern part Nigeria were previously reported (Uche and Aprioku, 2008; Akinpelu *et al*., 2009; Igbinosa *et al*., 2009). However, solvent and geographical location of the plant used in the present study was different. Previous researchers reported the toxic nature of aqueous and petroleum ether fractions of *J. curcas* in laboratory animals and reported the effect of geographical variation on *J. curcas* toxicity (Oluwole and Bolarinwa, 1997; Mariz *et al*., 2006; Adamu *et al*., 2007; Ebtisam and Hamadttu, 2014).

Quantitative phytochemical estimation revealed high contents of oxalate, phytate and tannins on the methanol leaf extract when compared to the hexane leaf extract of *J. curcas*. The essence of estimating the concentrations of these secondary metabolites is to establish and advice on the quantity one can consume at a time. The higher the concentration of these metabolites, the more dangerous they become to health. Thus, the methanol leaf extract is more toxic, than the hexane leaf extract. Oxalate, phytate and tannins chelate mineral ions such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{3+}, and Fe^{3+}, resulting in these ions becoming unavailable for consumers (Sudheer *et al*., 2004). These anti-nutrients also form complexes with protein, thus reducing the availability of dietary protein which can result to kwashiorkor or marasmus (Oboh and Akindahunsi, 2003). Hui (1992) reported that intake of 5 g or more of oxalic acid could be fatal to humans,

while Munro and Basir (1969) estimated the threshold of oxalate toxicity in man to be 2-5 g/ 100 g of the sample. The high content of oxalate in some plants prevents even herbivores from feeding on such plants (Frutos *et al*., 1998; Duncan *et al*., 2000). The level of phytate and tannins in the extract was below the level of 7.2-10.1% reported in *J. curcas* kernel meal. Consumption of large amount of the *J. curcas* leaves can lead to intake of toxic amounts of phytate and tannins. For efficient utilization as feed for monogastric animals, it would require the addition of phytase to feed (Oboh and Akindahunsi, 2003; Makkar and Becker, 2009).

In the present study, heavy metals were found present in HLE, MLE and MSE of *J. curcas* in ascending order of concentration. The concentration of lead, cadmium, copper, zinc and chromium present in the extracts were found to be below WHO/FAO (2007) permissible daily dietary limit of 5.0, 0.2, 40.0, 60.0, and 2.3 mg/kg respectively (Crentsil *et al*., 2011). The intake of large quantities of this plant material for medicinal purposes may lead to consumption of heavy metals above the daily dietary limit. Heavy metals can be tolerated at low levels, but become toxic at higher concentrations (Kennish, 1998). Yadav *et al.* (2009) reported that heavy metal contents in *J. curcas* grown in contaminated soils contained quantities above the WHO/FAO permissible limit due to their ability to uptake this metals from the soil. The consumption of materials with high concentration of such heavy metals may cause several health problems including anaemia and skin allergies (Yadav *et al*., 2009). Previous investigations have shown that anti-nutrients and heavy metals are responsible for *J. curcas* toxicity and acknowledged the mechanism of action of their toxicity (Richardson *et al*., 1985; Duffus and Duffus, 1991; Makkar *et al*., 1998; Fang *et al*., 2005; Pereira and Famadas, 2006; Fernandes and Freitas, 2007; Goel *et al*.,

2007; Ribeiro *et al*., 2007; Magadum *et al*., 2009; Makkar and Becker, 2009; Fernandez-Salas *et al*., 2011; Abiri, 2012). Few other studies have also reported the therapeutic efficacy of some antidotes especially sodium thiosulphate against *J. curcas* intoxication in laboratory animals (Shukla and Singh, 2013).

The CNS and respiratory effects due to *J. curcas* intoxication have been previously reported in chicks, rodents and goats (El-Badwi and Adam, 1990; El-Badwi and Adam, 1992; Mariz *et al*., 2006; Adamu *et al*., 2007; Abiri, 2012; Shukla and Singh, 2013). The similar signs observed might be due to the anti-nutrients or heavy metals present in *J. curcas* plant. Among the anti-nutrients, phorbol esters are the most toxic molecules in this *Jatropha* species and might be responsible for most of the CNS effects observed in chicks due to its stimulating effect on protein kinase-C thereby enhancing intracellular signal transduction process (Haas *et al*., 2002; King *et al*., 2009; Usman, *et al*., 2009; Shukla and Singh, 2013). Previous study on insecticidal property of *J. curcas* reported that jatropherol-1 (diterpenoid) isolated from the plant decreased the activity of mid-gut acetylcholinesterase, esterase and carboxylesterase in insects by activation of protein kinase-C (Jing, 2005; Jing *et al*., 2005). Singh and Singh (2002a) reported that aqueous solutions of the leaves and stem bark extracts of *J. gossypiifolia* were found to kill fishes by inhibition of acetylcholinesterase.

Shukla and Singh (2013) reported the presence of cyanogenic and cardiac glycosides in the seeds oil of *J. curcas* and these may be responsible for the observed neuromuscular (twitching) and respiratory effect in chicks. Cyanogenic glycosides are known to release free cyanide on enzymatic or acid hydrolysis and acute cyanide intoxication mainly occurs due to cytotoxic anoxia following inhibition of cytochrome

oxidase, a respiratory chain enzyme (Wink and van Wyk, 2008). Many studies have also shown the risk of cyanide poisoning after prolonged occupational exposure or consumption of food containing cyanogenic glycosides (El-Ghawabi *et al*., 1975; Philbrick *et al*., 1979; Osuntokun, 1980; Jackson, 1988; Kamalu, 1993; Adewusi and Akindahunsi, 1994; Okolie and Osagie, 1999).

The LD_{50} value of the extracts in chicks is lower than in mice, which indicates a greater sensitivity of chicks to the extracts which may be due to absence or poor development of the blood brain barrier in chicks. The acute toxicity study in 7-day old chicks showed that the *i.p*. LD_{50} of HLE, MLE and MSE were 935 mg/kg, 74 mg/kg and 22 mg/kg while that of oral LD_{50} value was 1,100 mg/kg for MSE and above 5,000 mg/kg for HLE and MLE. Agaie *et al*. (2007) reported that substance with an *i.p*. LD_{50} > 1,000 mg/kg as non-toxic and substance with *p.o*. LD_{50} of >5,000 mg/kg are regarded as practically non-toxic. The result of the LD_{50} values in chicks indicated that the *i.p*. administration of all the extracts were toxic while only the oral administration of MSE was slightly toxic.

The methanol seed extract, methanol leaf extract and hexane leaf extract were toxic to chicks in decending order. The toxicity of these extracts might be due to the presence of higher concentrations of the anti-nutrients mainly the phorbol esters and also heavy metals in seeds. Makkar *et al*. (1998) reported that phorbol esters are the main toxins in *J. curcas* oil and seeds. Phorbol esters are present in the leaves, stem, flower and root of *J. curcas* and therefore the consumption of this plant in any form, oil, seed, seed cake or extracts is toxic to animals, and elicits severe pathological symptoms (Makkar and Becker, 2009).

Ethylene diamine tetra-acetic acid (EDTA), sodium thiosulphate and atropine were more effective antidotes than sodium nitrite and penicillamine against HLE of *J. curcas* intoxication. This might be due to high concentration of cyanogenic glycoside and the efficacy of EDTA as smooth muscle relaxant in averting cardiac and respiratory depression (Alberts *et al*., 2008; Mutschler *et al*., 2008).

Atropine and sodium thiosulphate were more effective among the antidotes against MLE of *J. curcas* intoxicated chicks. This might be due to the antidotal effects of atropine and sodium thiosulphate on the phorbol esters and hydrogen cyanide present in the extract (Watt and Breyer-Brandwijk, 1962; Haas *et al*., 2002; Makkar and Becker, 2009; Shukla and Singh, 2013).

Sodium thiosulphate and atropine were more effective among the antidotes against MSE of *J. curcas* intoxicated chicks. This might be due to cyanogenic glycoside and phorbol esters being the major anti-nutrients responsible for its toxicity (Haas *et al*., 2002; Makkar and Becker, 2009; Shukla and Singh, 2013).

The sub-acute exposure of chicks to MLE and MSE of *J. curcas* showed signs of intoxications similar to those reported in chicks, goats, mice and rats fed with seed or leaf of *J. curcas* plant (Adam, 1974; Liberalino *et al*., 1988; Rakshit *et al*., 2008). In this study, the signs of MLE and MSE of *J. curcas* intoxication observed were more obvious on the saline treated group when compared with the groups treated with antidotes after intoxication with the extracts. The results suggest that the antidotes reduced the degree of seriousness of the toxic signs associated with oral administration of *J. curcas* (Shukla and Singh, 2013).

Blood parameters are good indicators of physiological, pathological and nutritional status of an animal and changes in haematological parameters have the potential to show the impact of therapeutic drug testing and toxicological factors (Gadir *et al*., 2003). In the present study, methanol leaf extract increased RBC, Hb, HCT and MCHC compared to control, which might be attributed to increased haemopoiesis (Choudhari and Deshmukh, 2007). Significant increase in WBC observed in saline and antidotes treated groups after methanol leaf extract intoxication might be attributed to stimulation of the immune system by the extract to generate antibodies against diseases (Soetan *et al*., 2013). These effects suggested the therapeutic efficacy of methanol leaf extract of *J. curcas*. All the antidotes did not provided significant effect when compared to the saline treated group. Sodium nitrite and EDTA proved to be toxic with significant decrease in MCHC and WBC below saline treated group.

The methanol seed extract intoxication on chicks did not produce significant effect on the haematological parameters when compared to the control. Though, decreases in RBC, MCHC with increase in MCV in the saline treated group suggest mild macrocytic anaemia. These suggested the toxic nature of methanol seed extract of *J. curcas* (Oluwole and Bolarinwa, 1997). The significant increase in MCV observed in sodium thiosulphate, penicillamine, EDTA and atropine treated groups when compared with control indicated the presence of macrocytic anaemia. The antidotes, especially sodium thiosulphate provided insignificant increase in haematological parameters (RBC, Hb, MCHC and WBC) when compared to the saline treated group. These may suggest the therapeutic efficacy of the antidote (Shukla and Singh, 2013). There were no major effects observed on the haematological parameters when chicks were administered with methanol seed extracts of *J. curcas*.

Specific serum makers are indicative of hepatic and renal dysfunction. High levels of serum creatinine are found in renal dysfunction or muscle injury and urea is a waste product of protein breakdown (Sodipo *et al*., 2012). In the presence of hepatic injury, such as that produced by hepatotoxins, the transport function of the cells is impaired, leading to leakage of hepatic enzymes (such as AST and ALT) from the cell cytoplasm into serum (Usha *et al.*, 2008). In the present study, the biochemical parameters (ALP, ALT, AST and urea) of methanol leaf extract intoxication treated with saline group (saline treated group) showed significant ($p<0.05$) increase when compared with the control. Also, the biochemical parameters (ALT and AST) of methanol seed extract treated intoxication treated with saline group showed significant ($p< 0.05$) increase when compared with control. The increase in liver and kidney enzymes is an indication of nephrotoxic and hepatotoxic potential of *J. curcas*. Previous researchers had reported that the nephrotoxicity and hepatotoxicity might be due to curcin anti-nutrient (Kaneko 1989; Tietz, 1994; Philip *et al*., 1995; Aniagu *et al*., 2004; Nabil *et al*., 2011). Other studies reported curcin might be responsible for liver and kidney damage in goats due to its ability to cause damage in structural and cellular integrity of the hepatocytes and centrilobular necrosis which in turn increased the leakage of the liver specific enzymes (Lin *et al*., 2010; Shukla and Singh, 2013). The present data correlate with previous report that leaf and seed extracts of *J. curcas* increased hepatic and renal enzymes such as ALP, ALT, AST, urea and creatinine in goats, chicken, mice and rats (Adam and Magzoub, 1975; El-Badwi and Adam, 1990; El-Badwi and Adam, 1992; Gadir *et al*., 2003; Oliveira *et al*., 2008).

In the sub-acute study of antidotal efficacy against methanol leaf extract of *J. curcas* intoxication in chicks, treatment with sodium thiosulphate, EDTA and atropine

significantly decreased the level of ALT when compared to the saline treated group. Sodium nitrite, sodium thiosulphate and atropine significantly decreased the level of AST when compared with the methanol leaf extract treated with saline group. Atropine significantly decreased the level of urea when compared to saline treated group. In the study using methanol seed extract, treatment with sodium thiosulphate significantly ($p<0.05$) decreased the level of ALT and AST when compared to the saline treated group. Also, sodium nitrite significantly increased the level of creatinine when compared to the saline treated group. The decrease in biochemical enzymes offered by the respective antidotes when compared to the saline treated group is an indication of their ameliorative potentials against *J. curcas* induced hepatic and renal injury. Sodium thiosulphate proved to be most effective in reducing the biochemical enzymes compared to other antidotes. The increase in ALP and Urea above the levels of the saline treated group when treated with sodium nitrite is an indication that treatment with the antidote is not safe against *J. curcas* intoxication.

Sodium nitrite and sodium thiosulphate are standard drugs used in the management of cyanide poisoning. Their effectiveness against *J. curcas* intoxication is probably due to their ability to neutralize cyanide present in *J. curcas* (Shukla and Singh, 2013). The negative results associated with the use of sodium nitrite in the management of *J. curcas* intoxication might be due to formation of cyanomethemoglobin which shift cytotoxic hypoxia caused by cyanide to an anaemic hypoxia accompanied by hypotension (Baumeister *et al*., 1975; Graham *et al*., 1977). Penicillamine and EDTA have the ability to neutralize heavy metals, ensure vasodilation, and prevent cardiac arrest and also respiratory collapse due to *J. curcas* (Lamas *et al*., 2013; Escolar *et al*., 2014). Atropine as a muscarinic antagonist might probably prevent the cholinergic

symptoms such as tremor and ataxia associated with phorbol esters present in *J. curcas* (Jing, 2005; Jing *et al*., 2005; Rai and Lakhanpal, 2008).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The presence of anti-nutrients and heavy metals in *Jatropha curcas* may be responsible for its toxic nature. The hexane leaf extract (HLE), methanol leaf extract (MLE) and methanol seed extract (MSE) were toxic to chicks in ascending order. Sodium nitrite, sodium thiosulphate, penicillamine, ethylene diamine tetra-acetic acid (EDTA) and atropine used as protective or ameliorative agents provided protective or ameliorative effect against acute HLE, MLE and MSE of *J. curcas* intoxication. However, sodium nitrite had no ameliorative effect on acute HLE and MLE of *J. curcas* intoxication. Likewise, penicillamine had no protective or ameliorative effect on acute methanol seed extract of *J. curcas* intoxication. The MLE and MSE of *J. curcas* were toxic to chicks following seven days daily oral administration of the extracts. The MSE proved to be more toxic than MLE on the chicks. Sodium nitrite, sodium thiosulphate, penicillamine, ethylene diamine tetra-acetic acid and atropine provided ameliorative effect against sub-acute MLE and MSE of *J. curcas* intoxication in chicks. However, sodium nitrite and EDTA significantly decreased WBC and MCHC when used as antidotes against methanol leaf extract intoxication in chicks.

Contributions of the current research to the body of literary knowledge

1. Heavy metals and anti-nutrients are present in *J. curcas* seeds and leaves located in Zaria, North-Western part of Nigeria.

2. *J. curcas* plant located in Zaria, North-Western part of Nigeria is toxic.

3. Sodium nitrite, sodium thiosulphate, penicillamine, ethylene diamine tetra-acetic acid (EDTA) and atropine are effective antidotes against acute and sub-acute *J. curcas* intoxication in chicks.

6.2 Conclusion

This study revealed the presence of anti-nutrients and heavy metals in *J. curcas* plant. Sodium nitrite, sodium thiosulphate, penicillamine, ethylene diamine tetra-acetic acid and atropine provided protective and ameliorative effect against acute and sub-acute *J. curcas* intoxication. Sodium thiosulphate and atropine proved to be more effective antidotes and may be used in the management of *J. curcas* intoxication.

6.3 Recommendations

The recommendations for further research on this study include:

i) Isolation and purification of the toxic principles in the extracts of *J. curcas*.

ii) Establishment of the exact mechanism of toxic action of the extracts and the isolated toxic principles.

iii) Analysis of the dose-response relationships between the extracts and antidotes before the utilization of any of these drugs in the management of *J. curcas* intoxication.

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APPENDICES

Appendix A: Effect of Antidotes as Pre-treatment or Post-treatment on the Toxicity Score of Acute N-Hexane Leaf Extract of *Jatropha curcas* Intoxicated Chicks

Treatment	Toxicity score (pre-treatment)	Change in toxicity scores (%)	Toxicity score (post-treatment)	Change in toxicity scores (%)
Control	27	0.00	27	0.00
SN	17*	37.04	27	0.00
STS	18*	33.33	19	29.63
PNA	18*	33.33	17	37.04
EDTA	13*	51.85	25	7.41
Atropine	19*	29.63	22	18.52

Antidotes and saline were administered 5 min before or after administration of HLE of *J. curcas* (1,262 mg/kg, *i.p.*); n = 5 (number of chicks per group); *Significantly different from the control group at $p<0.05$; Data for survival percentages and percentages of occurrence of acute signs of toxicity was analyzed using Fisher's exact test while grades of toxicity score were analyzed using Kruskal-Wallis and then Mann Whitney U-test.

APPENDIX B: Effect of Antidotes as Pre-treatment or Post-treatment on the Toxicity Score of Acute Methanol Leaf Extract of *Jatropha curcas* Intoxicated Chicks

Treatment	Toxicity score (pre-treatment)	Change in toxicity scores (%)	Toxicity score (post-treatment)	Change in toxicity scores (%)
Control	26	0.00	26	0.00
SN	19	26.92	24	7.69
STS	17*	34.62	21	19.23
PNA	19	26.92	22	15.38
EDTA	18	30.77	17*	34.62
Atropine	14*	46.15	18*	30.77

Antidotes and saline were administered 5 min before or after administration of MLE of *J. curcas* (100 mg/kg, *i.p.*); n = 5 (number of chicks per group); *Significantly different from the control group at $p<0.05$; Data for survival percentages and percentages of occurrence of acute signs of toxicity was analyzed using Fisher's exact test while grades of toxicity score were analyzed using Kruskal-Wallis and then Mann Whitney U-test.

APPENDIX C: Effect of Antidotes as Pre-treatment or Post-treatment on the Toxicity Score of Acute Methanol Seed Extract of *Jatropha curcas* Intoxicated Chicks

Treatment	Toxicity score (pre-treatment)	Change in toxicity score (%)	Toxicity score (post-treatment)	Change in toxicity score (%)
Control	28	0.00	28	0.00
SN	17*	39.29	25	10.71
STS	18*	35.71	22*	21.43
PNA	21*	25.00	25	10.71
EDTA	19*	32.14	14*	50.00
Atropine	15*	46.42	18*	35.71

Antidotes and saline were administered 5 min before or after administration of MSE of *J. curcas* (30 mg/kg, *i.p.*); n = 5 (number of chicks per group); *Significantly different from the control group at $p<0.05$; Data for survival percentages and percentages of occurrence of acute signs of toxicity was analyzed using Fisher's exact test while grades of toxicity score were analyzed using Kruskal-Wallis and then Mann Whitney U-test.

APPENDIX D: Effect of Methanol Leaf Extract of *Jatropha curcas* Intoxication on the Body Weight of Chicks in the Presence or Absence of Antidotes

Treatment	Body weight (g)		
	Day 0	Day 3	Day 7
Control	34.80 ± 1.42	40.00 ± 1.62	46.50 ± 2.13
Ext+Saline	35.30 ± 1.14	36.25 ± 1.03	42.50 ± 0.56
Ext+SN	35.50 ± 1.00	38.00 ± 1.50	41.63 ± 2.20
Ext+STS	35.50 ± 0.96	39.00 ± 2.41	43.33 ± 4.36
Ext+PNA	35.60 ± 0.96	39.50 ± 2.08	47.00 ± 1.78
Ext+EDTA	35.40 ± 0.96	37.29 ± 1.60	41.00 ± 2.63
Ext+Atropine	35.40 ± 0.91	37.75 ± 5.01	41.00 ± 1.95

n = 6 (number of animals); Values are expressed in mean ± S.E.M.;
Data analyzed using one way Anova and Dunnett's test;
No significant different from the control group at $p<0.05$,
No significant different from the saline treated group at $p<0.05$.

APPENDIX E: Effect of Methanol Seed Extract of *Jatropha curcas* Intoxication on the Body Weight of Chicks in the Presence or Absence of Antidotes

Treatment	Body weight (g)		
	Day 0	Day 3	Day 7
Control	39.60 ± 1.62	44.80 ± 1.82	53.50 ± 1.66
Ext+Saline	39.70 ± 1.44	41.14 ± 1.24	49.00 ± 2.56
Ext+SN	40.80 ± 1.66	41.29 ± 2.88	49.80 ± 5.51
Ext+STS	40.40 ± 1.68	42.86 ± 1.55	49.17 ± 3.82
Ext+PNA	40.40 ± 1.44	41.83 ± 1.40	51.83 ± 3.66
Ext+EDTA	40.40 ± 1.46	42.14 ± 1.92	47.33 ± 1.78
Ext+Atropine	40.40 ± 1.48	44.57 ± 1.78	53.33 ± 2.16

n = 6 (Snumber of animals); Values are expressed in mean ± S.E.M.;
Data analyzed using one way Anova and Dunnett's test;
No significant different from the control group at $p<0.05$,
No significant different from the saline treated group at $p<0.05$.