IN-VITRO ACARICIDAL EFFECTS OF *JATROPHA CURCAS*METHANOL SEED OIL EXTRACT ON *RHIPICEPHALUS (BOOPHILUS) DECOLORATUS*LARVAE AND ITS TOXICITY ON RABBITS

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ABSTRACT

The study evaluated the larvicidal effect of Crude Methanolic extract of *Jatrophacurcas* seed oil extract using Soxhlet apparatus on *Rhipicephalus (Boophilus) decoloratus*ticks. Different concentrations of 6.25, 12.5, 25 and 50 mg/ml were prepared from a stock (100 mg/ml) solution of the sample, control of 1% tween 80 and standard acaricide, Amitraz (positive control) were used. The mortalities were observed at time intervals. The experiment was performed in triplicate. The extract had an LC_{50} and $LC_{90}$of 2.88mg/ml and 6.76mg/ml respectively after 48h. Carbohydrates, cardiac glycosides, saponins, tannins, steroids and terpenes, alkaloids and flavonoids were detected. The extract exhibited a significant mean percentage mortality from the control across all the concentrations after 24h. The result confirms that *J. curcas* is a good alternative to be used as a larvicidal agent in the control of*Rhipicephalus (Boophilus) decoloratus*. Five groups of two (2) rabbits each were used to carry out haematologic and blood chemistry and histotological assessments of the effect(s) of graded levels of *J. curcas*methanolic seed extract topically applied on the skin of the rabbits for 2 weeks. 0.2mls of blood was collected from each rabbit on days 0, 7 and 14. Serum biochemical results of Mean AST, ALT and TP showed no significant changes compared with the control except for ALP values. Mean haematological parameters obtained (PCV, Hb, WBC and RBC) did not show any significant difference from the control and also falling within normal recommended ranges. Histopathological findings of the skin after the application of the pure crude revealed hyperkeratosis of the epidermis, infiltration of inflammatory cells and necrotic hair follicles. The splenic vessels were congested with widened red pulp. The liver showed distended sinusoid and congestion of the hepatic vessels with aggregation of inflammatory cells in the sinusoids. Haemosiderosis was seen with the 10% crude extract. This study reveals that the pure methanol extract of *J. curcas* seeds exhibited some toxic effect on the skin with prolonged use, but

generally at lower concentrations, it remarkably has mild effect because histopathologic finding showed no observable adverse effect. Biochemical and haematological evaluations showed mild effect or variation from the normal.

CHAPTER ONE

INTRODUCTION

1.1 General Background to the Study

Ticks are obligatory blood-sucking arachnid arthropods infesting a wide variety of animals including mammals, birds, reptiles and amphibians. They are vectors of disease agents (babesia, cowdria, anaplasma, etc) causing anaemia, dermatitis, paralysis, otocariasis as well as loss of production (Schimdt and Roberts, 1989). Two Families of ticks are well known and of veterinary importance, Ixodidae (hard ticks) and Argasidae (soft ticks) (Sonenshine, 1991; Luqman *et* al., 2007). Ticks are the most important ectoparasites of cattle, causing economic losses in terms of reduced productivity, fertility, diseases and death (Rajput *et al*., 2006). Besides being haematophagous, they also inflict notable dermatitis, predispose their host to dermatophilosis and other arthropod-borne infestations such as myiasis (Mtshali *et al*., 2004).

Tick control by use of chemical acaricides, is fraught with various problems like residues, environmental pollution and high cost, clearly demanding the need for alternative approaches (Ghosh *et al.,* 2007). Even though many plants extracts with promising acaricidal effects have been reported in literature, the feasibility of many of these extracts for the control of ticks infesting animals in field conditions has not been adequately studied (Rechav and Hay, 1992).

Jatropha curcas (*Euphorbiaceae* called pinion of India) is a species originating in the Indies and currently widespread in the villages of Tropical Africa (Adjanohoun *et al*., 1989). *J. curcas* grows in tropical and subtropical climates across the developing world, and is often cultivated as a hedge crop. It is easy to establish as it grows relatively quickly with high yield (Willis, 1967). It requires minimal water and nutrients, and is able to grow on barren land under harsh

conditions and poor quality or degraded land (Kandpal and Madan, 1995). It is a multipurpose, drought resistant tree and can be cultivated in areas of low rainfall (Pratt *et al.*, 2002). *J. curcas Linn* is the commonest specie found in Nigeria, but many species exist in different parts of the world. Oil from its seeds has been found useful for veterinary and medicinal purposes as insecticide, for soap production and as a fuel substitute (Gubitz *et al*., 1999). Names used to describe the plant vary per region or country. It is most commonly known as "physic nut". In Mali it is known as "pourghere", "bagani" in Ivory Coast and it is known as "tabanani" in Senegal. In Tanzania it is known as "makaen"/"mmbono" (Fact Foundation, 2006). In Nigeria it is known as "binidazugu/cinidazugu" and "lapalapa" in Hausa and Yoruba languages, respectively (Blench, 2003 and Blench, 2007).

Jatropha is one of the promising biodiesel plants. Oil from its seeds has been found useful for veterinary and medicinal purposes as insecticide, for soap production and as a fuel substitute (Gubitz *et al*., 1999). The antimicrobial and larvicidal activities of the leaves of the plant (Kalimuthu *et al.,* 2010; Kalimuthu *et al*., 2011), stem bark (Igbinosa *et al.,* 2009) and the insecticidal property (Adebowale and Adedire, 2006) of the *J. curcas* seed oil have been reported. Reports on the acaricidal activity of essential oils from various plants against ticks like *Boophilus annulatus* (Pirali-Kheirabadi and Teixeira da Silva, 2011) and *B. microplus*have been documented (Gazim *et al*., 2011). However, there are only few reports on acaricidal properties of *J. curcas.* Acaricidal activity of crude extracts from stem and leaves of different plants against the cattle ticks were also reported (Madzimure *et al.*, 2011). Jatropha seed is usually toxic when consumed because it contains toxic phorbol esters (Makkar *et al*., 1998). Thus, alternative use of the seed to derive /extract oil is more appropriate and beneficial than simply exploiting the seed as a food crop. Apart from its use as a biofuel, the Jatropha seed oil is reported to be used to

produce soap, medicine and pesticides (Shanker and Dhyani, 2006). Jatropha oil can be used as a fuel formulation in diesel engines directly and by blending it with methanol (Gubitz *et al*., 1999). The clear seed oil was used as diesel substitute during the World War II (Agarwal and Agarwal, 2007) and these tests conducted at Thailand during WW II.

1.2 Statement of Research Problem

Ticks are the most important ectoparasites of livestock in tropical and sub-tropical areas. Ticks do not only cause direct effect on animals by sucking blood. They are also responsible for the transmission of several important protozoan, rickettsial and viral diseases in man, companion and domestic animals (Soulsby, 1982). Besides their manifested abilities as vectors of disease causing organisms, the direct effects of ticks have substanstial economic importance since they prevent livestock from attaining maximum production (Kocan, 1995). The blood sucking activity of ticks (Ixodidae), irritation and destruction to hides and skins of infested animals, in addition to their role as vectors and intermediate hosts to a number of important protozoan, rickettsial, bacterial and viral diseases are well documented (Authur, 1970), with resultant negative implications on the total energy balance of the animal thereby resulting in decreased productivity (Byford *etal*., 1992).

Tick-borne diseases were again ranked high in terms of their impact on the livelihood of resource poor farming communities in developing countries (Perry *etal*., 2002; Minjauw and McLeod, 2003). This is particularly relevant in parts of Sub-Saharan Africa, Asia and Latin America where the demand for livestock products is increasing rapidly (Delgado *etal*., 1999). Tick bite marks damage the value of hides and skin up to 20–30% (Biswas, 2003). Ticks have also been reported as vectors of infectious agents and zoonotic diseases in humans (Petney *et al*., 2007).

Ticks are routinely controlled by use of chemical acaricideswhich however have serious limitations and cause environmental contamination and destruction to non-target organisms (Garcia-Garcia *et al*., 2000). These have stimulated research into alternative methods to control ticks by the use of natural products (Graf *et al*., 2004). The oil is a strong purgative and is used in treating skin ailments and rheumatism (Heller 1996; Marroquin *et al*., 1997). All parts of the plant have been shown to have insecticidal properties (Grainge and Ahmed, 1988; Consoli *et al*., 1989; Jain and Trivedi, 1997; Meshram *et al*., 1994), e.g.against insect/ pests like cotton bollworm, and on pests of pulses, potato and corn (Kaushik and Kumar, 2004). If effective as an acaricide, it may be a potent, eco-friendly vector control alternative.

1.3 Justification

The control of these tick species is therefore paramount if productivity of livestock is to be enhanced (George *etal.*, 1999). Ticks are routinely controlled by the use of chemical acaricides (eg Amitraz®, Flumethrin®, etc). Chemical acaricides cause serious environmental and food chain contaminations, in addition to the development of resistance by some tick species. The use of alternative means of controlling ticks is necessary so as to have a range of compounds with different modes of action to enable the rotational use of these chemicals in the management of existing problems of acaricidal resistance (Rechav and Hay, 1992).

Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). All parts of the plant—roots, stems, leaves seeds and fruits—have been widely used in traditional folk medicine in many parts of West Africa (Adamu *et al*., 2006) and their use for veterinary purposes has been practiced for a long time (Duke, 1985). *J. curcas* extracts are used in alternative medicine, e.g.

acaricidal activity (Dimri and Sharma, 2004), insecticidal properties (Jing *et al*., 2005; Georges *etal*., 2008). All parts of the plant have been reported to show insecticidal properties (Grainge and Ahmed, 1988; Consoli *et al*., 1989; Jain and Trivedi, 1997; Meshram *et al*., 1994) e.g against insect/ pests like cotton bollworm, and on pests of pulses, potato and corn (Kaushik and Kumar, 2004). *Jatropha curcas* contains higher steroids, terpenoids (mainly, phorbol ester), flavonoids and alkaloids (mainly, Jatrophine) compounds in the leaf and seed in comparison to other species of Jatropha (Krishnan and Paramathma, 2009). Studies by Makkar *et al*., 1997 as well as Goel *et al.*, 2007 have indicated that *J. curcas* seeds extract have a higher concentration of toxic phorbol esters and other compounds than in other parts of the plant including the leaves. Although plant based natural medicines/products are acclaimed to be safe, leading to a rise in it popularity, scientist advocate proper toxicological studies in other to ensure safety of these natural medicines/products before being used because it has been demonstrated that some poisons are also natural (Ansari and Inamdar, 2010). However, much work has not been done on evaluation of acaricidal properties of the seeds of *Jatrophacurcas*.

1.4 Aim of the Study

The study was aimed at evaluating the *in-vitro* acaricidal effects of *Jatropha curcas*methanolic seed extract on *Rhipicephalus* (*Boophilus*) *decoloratus* ticks and its toxicity on host animals using rabbits as models.

1.5 Objectives of the Study

The following were the objectives of this study:

1. determine the phytochemical constituents of the methanol seeds extract of *J. curcas*.

2. evaluate the effects of different concentrations of the methanol extract on larvae of *Rhipicephalus* (*Boophilus*) *decoloratus* ticks *in-vitro*.

3. determine the effects on hematological**,** biochemicalparameters and pathological effects on rabbits treated topically with the methanol seed extract of *Jatropha curcas*.

1.6 Research Questions

1. Does *Jatropha curcas* have various phytochemical constituents?
2. Does *Jatropha curcas* methanol seed extract have acaricidal activity on larval stage of *Rhipicephalus* (*Boophilus*) *decoloratus*?
3. Do the different concentrations of the methanol seed of *Jatropha curcas* extract have effect on the larvae of *Rhipicephalus* (*Boophilus*) *decoloratus* ticks *in-vitro*?
4. Does treatment with *Jatropha curcas* seed extract have effect on hematologic, biochemical parameters and pathology of rabbits?

CHAPTER TWO

LITERATURE REVIEW

2.1 Ticks

Ticks are obligate blood-sucking ecto-parasite arthropods of mammals, reptiles, amphibians and birds and are of medical and veterinary importance. They belonging to the Phylum Arthropoda (Hoogstraal, 1956) and make up the largest groups in the order Acarina. Ticks are the most widespread and important ecto-parasites of livestock in tropical and sub-tropical areas, and are responsible for severe economic losses in livestock (Rajput *et al*. 2006). The medical and economic importance of ticks is due to their ability to transmit diseases to humans and animals. They act as vectors of a wide variety of viral, prokaryotic and eukaryotic organisms which, when transmitted to mammalian hosts, may cause disease (Sonenshine, 1991).

2.2 Classification of Ticks

Phylogenetic studies based on morphological analysis and mitochondrial ribosomal DNA sequencing demonstrated that ticks belong to suborder Arachnida. These ectoparasites belong to the Phylum Arthropoda, Class Arachnida, Subclass Acari, Order Acarina and Suborder Ixodida, sharing the Order Parasitiformes with the suborders Holothyrida, Mesostigmata (commonly known as mites) and Opilioacarida (Barros-Battesti *et. al.*, 2006). The order Parasitiformes is divided into three families, Ixodidae (hard ticks), Argasidae (soft ticks) and Nuttalliellidae (an intermediate group) (Hoogstraal and Aeschlimann, 1982; Mans and Neitz, 2004).

There are approximately 879 known tick species worldwide. These species are grouped into 3 families: Argasidae or soft bodied (186 species), Ixodidae or hard bodied ticks (692 species) and Nuttalliellidae (1 species) (Barker and Murrell, 2004; Navas *et al*., 2009). Argasid ticks include four genera, namely *Argas*, *Carios*, *Ornithodoros* and *Otobius*, whereas the ixodid ticks consists of over 240 species in the genus *Ixodes* and over 440 species in the remaining genera (Horak *et al*., 2002).

Globally, the most important genera of hard ticks are *Amblyomma, Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus* and *Boophilus*. The genus *Boophilus* has being found to be phylogenetically related to genus *Rhipicephalus* by (Horak *etal*., 2002) and thus the five species of *Boophilus* are now considered as a subgenus of *Rhipicephalus* (Murrell *etal*., 2000; Horak *etal*., 2002).

2.3 Life-Cycle of *Rhipicephalus* (*Boophilus*)*decoloratus* (Koch, 1844)

In one-host ticks, the larvae that emerge from the eggs, three to four weeks after deposition at the earliest attach themselves to a host animal where they complete their entire development. Females lay about 2,500-4,000 eggs, that hatch between 14-145 days depending on environmental conditions. The parasitic period on the host ranges from 17-52 days (Lima *et al*., 2000). They moult to nymph then to adult and then copulate on the same individual host. Afterwards, they drop off and deposit their eggs on the ground. The entire development cycle takes mostly 19-21 days as a rule, with minimum of fifteen and maximum of 40 days, each stage taking one week (Seifert, 1996). In this tick species a stricter adaptation eliminates the need to drop to the ground for metamorphosis. All the instars occur on a single host. The larva and nymph molts on the same host at the point of their attachment to the host. There is only one parasitic phase (Douglas, 1969).

2.4 Distribution of *Rhipicephalus* (*Boophilus*)*decoloratus* (Koch, 1844)

Rhipicephalus (*Boophilus*) *decoloratus* also called African blue tick is found in continental Africa, wide spread in sub-Saharan Africa. In South Africa it is distributed throughout the wetter regions of the country. The tick occurs in parts of Namibia, Botswana and is also present in Mozambique, Zimbabwe, Angola, Zambia, Malawi, parts of Tanzania, Burundi, Uganda, Western Kenya and in the wetter highlands of Ethiopia. It is also found in parts of West Africa, such as Mali, Senegal and Nigeria. (Mason and Norval, 1980; FAO, 1984; Matthysse and Colbo, 1987; Norval, 1994). It is also widespread in Libya, Yemen and India (Walker and Olwage, 1987).

2.5 Host Range

B. decoloratus has a wide range of hosts. It affects cattle, frequently parasitizes horses, donkeys, sheep and goats and is commonly found on several wild ungulates including impala (*Tragelaphus strepsiceros*), eland (*Taurotragusoryx*) and sable (*Hippotragus niger*) (Spickett and Fivaz, 1992; Norval, 1994).

2.6 Medical and Veterinary Importance

The problem of ticks and tick-borne diseases has attracted attention of researchers for several decades and new data concerning the occurrence of ticks and the recognition of zoonoses in urban and rural areas are continuously being studied. Moreover, incidence of tick-borne diseases (TBDs) are on the increase worldwide (Colwell *et al*., 2011). Their veterinary importance is related to their blood-feeding, from which both their direct and indirect pathogenicity originates (Jongejan and Uilenberg, 2004). Ticks constitute the second vector group that transmit the major number of pathogens to humans, being surpassed only by culicid mosquitos (Socolovschi *et al*., 2009). Ticks are obligatory haematophagous ectoparasites of vertebrates and are responsible as

vectors or reservoirs in the transmission of pathogenic fungi (Dermatophilosis), protozoa (Babesiosis and Theileriosis), viruses (Crimean–Congo hemorrhagic fever, Powassan encephalitis, Kyasanur forest disease (KFD), Louping ill, Colorado tick fever, Omsk hemorrhagic fever (OHF), West nile virus (WNV), African swine fever virus (ASFV); tick-borne encephalitis (TBE), rickettsial (Rocky Mountain spotted fever, Brazilian spotted fever, Boutonneuse Fever or Mediterranean spotted fever, African tick bite fever, Human granulocytic anaplasmosis, Human monocytic ehrlichiosis, ehrlichiosis and anaplasmosis) and other bacterial diseases (lyme disease, tularemia, Q fever, relapsing fever and borreliosis) during their feeding process on the hosts (Sonenshine and Roe, 2014). Tick toxicosis caused by *Boophilus* (*Rh*.) *decoloratus* (Drummond, 1983). The cattle tick *Boophilus* (*Rh*.) *decoloratus* economically impacts cattle production by transmitting pathogens that cause Babesiosis (*B. bovis* and *B. bigemina*) and Anaplasmosis (*A. marginale*) (Peter *et al*., 2005).

2.7 Public Health Importance of *Rhipicephalus* (*Boophilus*)*decoloratus* (Koch, 1844)

Tick-borne diseases are common in the medical and veterinary clinical settings alerting to the importance of a one health approach to these diseases, thus unifying physicians and veterinarians in there surveillance for the management of zoonoses and communicable diseases common to man and animals (Dantas-Torres *et al*., 2012).

2.8 Control of Ticks

2.8.1 Chemical control

The most widely used method for the effective control of ticks is the direct application of chemical acaricides to the host animals. However, these acaricides are expensive and can be detrimental to the environment; also continuous use of these acaricides has led to the problem of resistance in these arthropods: hence their use should be minimized and integrated with

alternative approaches (Minjauw and McLeod, 2003). Conventional organophosphate, carbamate and pyrethroid acaricides are relatively inexpensive and broadcast delivery of these chemicals can be effective in reducing tick numbers within localized areas (Schulze *etal*. 1991, 2001a). The intensity of application of an acaricide should be done depending on whether the aim of application is to prevent disease transmission or to reduce tick damage and /or worry. Thus a choice can be made as to whether to adopt intensive or strategic acaricide application or other systems (Kemp, 1994). Although acaricides are effectively used to reduce transmission of tick-borne pathogens to livestock, repeated heavy applications to hosts can cause considerable mortality in non-target arthropods through environmental contamination (Gassner *etal*. 1997).

2.8.2 Biological control

Natural enemies of ticks include insectivorous birds, parasitoid wasps, nematodes, *Bacillusthuringiensis* bacteria and deuteromycete fungi (largely *Metarhiziumanisopliae* and *Beauvariabassiana*) (Samish and Rehacek, 1999). Biological control agents have been investigated for their potential in controlling ticks. Interest in developing anti-tick biocontrol agents have increasingly being gaining momentum such as birds, parasitoides, entomopathogenic nematodes, entomopathogenic fungi, bacteria and baculoviruses (George *etal*., 2008). The future of biological products depends on the interest of the pharmaceutical industry and the price, which should be competitive with acaricides (Onofre *et al*., 2001). Strains of several nematode species including *Steinernemacarpocapsae* and *Heterorhabditisbacteriophora* induce mortality in engorged female *R. appendiculatus*, *R. avertis*, *Amblyommavariegatum*, *A. gemma* and *Boophilusdecoloratus* under experimental conditions (Kaaya and Hassan, 2000; Samish and Glazer, 2001). Unfed females and immature stages are however generally resistant (Kaaya and Hassan, 2000). The pathogenicity of these nematodes are generally restricted to engorged

adult female ticks (Samish and Glazer, 2001), probably due to ease of access through the genital pore or through stretched and thin cuticle. Mammals and birds typically consume ticks during self-grooming. For example, laboratory studies demonstrate that significant numbers of larval blacklegged ticks are consumed by white-footed mice (*Peromyscusleucopus*) during self-grooming (Shaw *etal*., 2003). Parasitoid wasps and flies are among the most effective agents in the biocontrol of insects (Kimberling, 2004). Although several bacterial species are pathogenic to ticks, the usefulness of bacteria as biocontrol agents is poorly studied. *Bacillusthuringiensis*, which is used as a biocontrol agent for many insects, is pathogenic to ticks, but apparently must be ingested to be effective (Samish and Rehacek, 1999; Zhioua *etal*., 1995). Because ticks tend to ingest only host blood, inducing ticks to ingest these bacteria seems impractical, and hence the prospects for *B. thuringiensis* as a biocontrol agent seem poor. Numerous laboratory assays of both *M .anisopliae* and *Be. bassiana* support their lethal effects on several tick species of epidemiological and veterinary importance. In addition, strong and demographically significant sublethal effects of these fungi on ixodid ticks have been noted by several researchers (Hornbostel *et al*., 2004). Certain *Stylosanthes* spp (tropical legumes) can kill or immobilize larval ticks and the use of these plants may simultaneously improve pasture quality (Fernandez-Ruvalcaba *et al*., 1999). *Brachiariabrizantha* has also been recorded to exhibit lethal effects to *Boophilus* larvae. Owing to development of acaricidal resistance and growing public concern about its residue in food chain, biological control is likely to play a substantial role in future integrated pest management programs for tick control (Samish *et al*., 2004; David, 2005). The full potential of biological agents in controlling ticks needs to be explored further.

2.8.3 Ecological control

Ecological measures within the grazing area also afford some degree of biological control over ticks (Young *et al*., 1988). Ecological control method is used for habitat and host linked treatment. Tick control in the habitat and vegetation requires modification of the plant cover by removal of vegetation that shelters ticks (Kirby, 2010). This system particularly aims at controlling the level of environmental infestation through pasture management and rotational grazing (Walker, 2011). Spelling and seasonal changes in cattle grazing areas in Australia and in Zambia respectively has been used as a tick control strategy and are believed to be responsible for a decrease in its burden (Walker *etal*., 2003), although these approaches are not always feasible due to land availability constraints and lack of compliance by farmers.

2.8.4 Genetic control

The application of chemical acaricides is the most common method used to control ticks but the resultant problems like resistance, residues in meat and milk, environmental pollution and high cost has prompted the need for alternatives (Ghosh, *etal*., 2007). Also, the arising need for better monitoring of the application systems and methods (Castro-Janer *et al*., 2010) to minimize these problems. Therefore, the study of the genetic resistance to ticks among different breeds of cattle can contribute to the development of alternative control methods (Ibeli *et al*., 2011). It is widely known that *Bosindicus* cattle are more resistant to ticks than are *Bostaurus* animals. For, instance, resistance to *Boophilus* spp varies from breed to breed, but is generally greater in Zebu cattle (*Bosindicus*) than in European cattle (*Bostaurus*) (O' Kelly and Spiers, 1976). There are great differences between these two breeds of cattle with regard to their susceptibility to parasitism by cattle ticks (Bianchin *et al*., 2007). Studies are intensifying into the crossing of these two groups, aiming to obtain animals that are more resistant to the conditions found in tropical countries and also enhance good meat production (Ibelli *etal*., 2012). Nguni cattle also

harbours fewer *A. hebraeum*, I. *decoloratus* and *Hyalomma* spp. ticks than Bonsmara and Hereford (*B. taurus*) cattle (Spickett *et al*., 1989). Nguni, Africander and Brahman cattle were also more resistant to *B. decoloratus* than those of the Santa Gertrudis and Simmental breeds (Rechav and Kostrzewski, 1991). In Zimbabwe, the indigenous Mashona oxen of the Sanga type were observed to be more resistant to *B. decoloratus* than crossbred Africander x Sussex oxen (Fivaz *et al*., 1992).

2.8.5 Immunological method

An extremely attractive way of controlling ticks is to introduce resistance to these ectoparasites through immunization. The feasibility of controlling tick infestations through immunization of hosts with selected tick antigens was demonstrated with the development of vaccines that reduced *Boophilus* spp. infestations on cattle (de la Fuente and Kocan, 2003; Willadsen, 2004). Experiments done with B. *microplus* Bm86-based vaccines demonstrated cross-protection against B. *annulatus* and B. *decoloratus* infestations and conferred partial protection against *Hyalomma* and *Rhipicephalus* spp. (Fragoso and Ortiz *et al.,* 1998; de la Fuente *et al.,* 2000; de Vos*etal*, 2001) The first ever commercial anti-anthropod vaccine used against *Boophilusmicroplus* (Tellam *et al*., 1992; Willadsen and Jongejan, 1999) has been developed from a concealed antigen, a gut glycoprotein (Bm86). These commercial vaccines are TICKGARD™ and TICKGARD™Plus which are used in Australia and GAVAC™, manufactured in Cuba (Rand *et al*., 1989, Rodriguez *et al*., 1994).

2.8.6 Alternative control measure

Ethno-veterinary plants are being used for tick control in Africa and other developing countries, this is important since a greater proportion of livestock farmers are small-scale and most of them are in rural areas where cultural practices are still preserved. These plant extracts are being

prepared or developed by farmers rather than scientists due to lack of finance to purchase synthetic acaricides which force them to depend on traditional methods of controlling ticks (Liang *et al*., 2003). Various natural bioactive products with acaricidal activity (botanical and microbial pesticides, essential oils, horticultural spray oils, mycopesticides) have become important alternatives to synthetic acaricides (Copping and Duke, 2007; Faria and Wraight, 2007). Essential oils extracted from various plants have shown a significant acaricidal activity (Aslan *et al*., 2004; Choi *et al*., 2004; Miresmailli *et al*., 2006).

2.9 Jatropha Plant

Jatropha curcas (Linnaeus) or physic nut, is a multipurpose drought resistance small tree or shrub of the family *Euphorbiaceae*, producing seeds containing oil (Jongschaap *et al*, 2007). It is a hardy shrub that can grow on poor soils and areas of low rainfall (from 250 mm a year) hence its being promoted as the ideal plant for small farmers (Sarin *et al*.,2007; Foidl *et al*., 1996). The plant is known by various names in different languages as:-

In English: physic nut or purging nut; in Dutch: purgeernoot or schijtnoot; in Arabic: hab el meluk (Makkar and Becker, 1999).

In Nigeria it is known as “Binidazugu/Cinidazugu”, “Olulu-idu/Uru” and “Lapa lapa” in Hausa, Igbo and Yoruba languages, respectively (Blench, 2003 and Blench, 2007).

2.10 Taxonomic Hierarchy and Botanical Description

The genus Jatropha belongs to the:

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Euphorbiales

Family: Euphorbiaceae

Genus: Jatropha Linnaeus.

Species: *Jatrophacurcas* Linnaeus (Cronquist, 1981)

It belongs to the tribe Joannesieae in the Euphorbiaceae family and globally, there are about 170 known species.Linnaeus (1753) was the first to name the physic nut Jatropha L. in "Species Plantarum" and this is still valid today. The genus name Jatropha derives from the Greek word jatros (doctor) and trophe (food), which implies medicinal uses. The physic nut, by definition, is a small tree or large shrub, which can reach a height of three to five meters, but under favourable conditions it can attain a height of 8 or 10m. The plant shows articulated growth, with a morphological discontinuity at each increment. The branches contain latex. Normally, five rootsare formed from seedlings, one central and four peripheral. A tap root is not usually formed by vegetatively propagated plants. Leaves five to seven lobed, hypostomatic and stomata are of paracytic (Rubiaceous) type. The trees are deciduous, shedding the leaves in dry season. Flowering occurs during the wet season and two flowering peaks are often seen, i.e. during summer and autumn. In permanently humid regions, flowering occurs throughout the year. The inflorescence is axillary paniculate polychasial cymes. The plant is monoecious and flowers are uni-sexual; occasionally hermaphrodite flowers occur and pollination is by insects (Dehgan and

Webster, 1979). A flower is formed terminally, individually, with female flowers (tricarpellary, syncarpous with trilocular ovary) usually slightly larger and occurs in the hot seasons.

In conditions where continuous growth occurs, an unbalance of pistillate or staminate flower production results in a higher number of female flowers. Ten stamens are arranged in two distinct whorls of five each in a single column in the androecium, and in close proximity to each other. In the gynoecium, the three slender styles are connate to about two-thirds of their length, dilating to massive bifurcate stigma (Dehgan and Webster, 1979). The rare hermaphrodite flowers can be self-pollinating. The flowers are pollinated by insects especially honey bees. Each inflorescence yields a bunch of approximately 10 or more ovoid fruits. With good rainfall conditions nursery plants may bear fruits after the first rainy season, and directly sown plants after the second rainy season. Three, bivalved cocci is formed after the seeds mature and the fleshy exocarp dries .The seeds mature about 3–4 months after flowering. The seeds are black and the seed weight per 1000 is about 727g, there are 1375seeds/kg in the average.

Jatropha curcas has thick globious 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. After pollination by insects, 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 727gm (Raju and Ezradanam, 2002; Kochhar *et al*., 2008).

2.11 Cultivation of *Jatropha* Plant

Jatropha curcasLinnaeus has various socio-economic benefits which makes it more economical when cultivated on commercial scale. The best soils for *Jatropha* are aerated sands and loams of at least 45 cm depth (Gour, 2006). Heavy clay soils are less suitable and should be avoided, particularly where drainage is impaired, as *Jatropha* is intolerant of waterlogged conditions *Jatropha* can be propagated from seeds and through vegetative method from cuttings. Seeds or cutting can be directly planted in the main field. Otherwise, seedlings grown in poly bags are transplanted in the main field. A hectare of Jatropha plantation is reported to yield 2.5-3.5 tonnes of seeds in the third year and increases sharply to 5000-12,000 tonnes per hectare from the sixth year onwards (Henning, 1996). With good soil, higher rainfall and optimal management practices, there are reported yields of 5.0 tonnes per hectare (Achten *etal*., 2008). (Jongschaap *etal*., 2007) calculated a theoretical potential seed yield of between 1.5 to 7.8 tonnes per ha under optimal conditions.

2.12 Distribution

Jatropha curcas is believed to be a native of tropical America. From the Caribbean where the species was already used by the Mayas (Schmook and Serralta-Peraza, 1997), *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 as hedge crop and for traditional uses (Heller, 1996; Kumar and Sharma, 2008). It will grow under a wide range of rainfall regimes from 250 to over 1200 mm per annum (Katwal and Soni, 2003). It is well adapted to arid and semi-arid conditions and grows almost anywhere except waterlogged lands, even on gravelly, sandy and saline soils. It can thrive on the poorest stony soil. It can grow even in the crevices of rocks. The leaves shed during the winter months form mulch around the base of the plant. The organic matter from shed

leaves enhances earthworm activity in the soil around the root-zone of the plants, which improves the fertility of the soil.). In low rainfall areas and in prolonged rainless periods, the plant shed sits leaves as a counter to drought. Its water requirement is extremely low and it can withstand long periods of drought by shedding most of its leaves to reduce transpiration loss.

Jatropha is also suitable for preventing soil erosion and shifting of sand dunes. It grows on well-drained soils with good aeration and is well adapted to marginal soils with low nutrient content (Openshaw, 2000). On heavy soils, root formation is reduced. Jatropha is a highly adaptable species, but its strength as a crop comes from its ability to grow on very poor and dry sites.

2.13 Uses of *Jatropha curcas*

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

2.13.1 Uses asfeed stuff

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).

2.13.2 Enrichment of soil

Jatropha oil cake is rich in nitrogen (6%), phosphate (2.7%) and potassium (0.94%) and can be used as organic manure (Agbogidi *et al*., 2013). The leaves and branches are also used as manure for coconut trees and to provide plant with organic matter thereby increasing microbial activities that helps the decomposition including earthworms which is an indication of ecological improvement of site (Mahanta *et al*., 2008).

2.13.3 Usesas an oil crop

The 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% (Gubitz *etal.*, 1999). 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 chemical elements in the seeds which possess poisonous and purgative properties and render the oil non edible for human consumption. It is also been stated that technologies are now available, whereby it could be possible to convert Jatropha oil into an edible oil which could prove to be a boom 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 *etal*., 1999).

2.13.4 Land reformation

The plant also has the potential of regaining marginal and degraded soil by re-anchoring the soil with its substantial root system and reducing possibility of erosion (Agbogidi and Ekeke, 2011). This results in recycling nutrients from deeper soil layers, providing shadow to the soil and thereby reducing the risk of erosion and desertification. Concept of the reclamation of marginal soils are reported (Spaan *et al*., 2004), although the oil production is not proven to be at commercially subsistent level (Francis *et al*., 2005), the use of *J. curcas* oil as bioenergy to replace petrol, diesel will protect the environment because it will reduce dependency on firewood and also serve as hedge crop against erosion (Agbogidi *et al*., 2013).

2.13.5 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 Seed oil content of this

plant is about 40% higher than the typical oil crops such as soybean and rape (Gubitz *etal*., 1999; Deore and Johnson, 2008). 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 of productive assets; boosting of village based industries; providing nonconventional energy in a decentralized manner and above all having a potential for wastelands development (Gubitz *etal.*, 1999; Benge, 2006). Thus, J. curcas has been considered as a strategic plant resource in many countries (Carvalho *etal*., 2008).

2.13.6 Industrial uses

Jatropha oil has very high saponification value and is 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 the villages of China 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. The protein content of *Jatropha* oil cake may be used as raw material for plastics and synthetic fibres. It would also be advantageous to make use of *Jatropha* oil as hydraulic oil (Gubitz *et al*., 1999; Mahanta *etal*., 2008).

The bark of *J. curcas* is reported to yield a dark blue dye which is used in Philippines to colour clothes, finishing for 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).

The use of *Jatropha* oil by local soap industry is one of the most economically attractive alternative for the oil. The glycerin by-product of the trans-esterification process of *Jatropha* oil can be used in making high quality soap, it is refined and sold at varying prices, depending on its purity. The soap has positive effect on the skin and is therefore marketed for medicinal purposes (Berchmans and Hirata, 2008).

2.13.7 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 *etal*., 1999).

2.13.8 Non-conventional energy crop

Jatropha oil is an environmentally friendly, 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).

2.13.9 Medicinal plant

Most parts of the plant are used for the treatment of various medicinal and veterinary ailments (Dukes, 1988). The roots, stems, leaves, seeds and fruits of the plant have been widely used in traditional folk medicine in many parts of West Africa (Adamu, *et al*., 2006). The plant has

medicinal value because it has bioactive phytochemical constituents that produce definite physiological actions on human bodies (Maikhube, 2006). The seeds are used as antihelminthic and as purgatives, with the leaves as haemostatics (Watt and Breyer-Brandwijk, 1962).

2.13.10 Insecticide/Pesticide/Acaricide

The oil and aqueous extract from oil has potential as an insecticide. For instance it has been used in the control of insectpests of cotton including cotton bollworm and on pests of pulses, potato and corn (Kaushik and Kumar, 2004). (Methanolextracts of Jatropha seed (which contains biodegradable toxins) are being tested in Germany for control of bilharzia carrying snails). **(**The seeds are considered antihelmintic 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 *Staphylococcusaureus* and *Escherichiacoli*. 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). Adebowale and Adedire (2006) demonstrated ovicidal activity, egg and larval mortality in seed beetle (*Callosobrochusmaculastus*). Ethanol extract of leaves of *J. curcas* may be as useful for developing a safe and ecofriendly therapeutic agent to combat the problems of tick *Rhipicephalus* (*Boophilus*) *annulatus* and tick-borne diseases (Juliet *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the Pharmacology Laboratory of the Department of Veterinary Pharmacology and Toxicology, the Clinical Pathology Laboratory of the Department of Veterinary Pathology and also Entomology Laboratory of the Department of Veterinary Parasitology and Entomology all at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria which is situated within latitude 11° 3' 0" North, longitude 7° 42' 0" East (Mortimore, 1970).

3.2 Preparation of *Jatropha curcas* Seeds

Jatropha curcas seeds were obtained from the National Research Institute for Chemical Technology, Zaria. The plant was taken to the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria for identification and voucher specimen referencing number 22873 was given. The seeds were de-shelled and pulverized to powdered form using mortar and pestle.

3.3 Extraction

Five hundred grams (500 gm) of the seed powder was extracted in a Soxhlet apparatus using 1500 ml of methanol (as a solvent) for 8 hours. The methanol extract obtained was concentrated using a water bathto obtain an oily extract. The concentrate obtained was stored in a refrigerator till when needed for used.

3.4 Phytochemical Screening

Phytochemical analysis of the plant extract was conducted to determine the presence of the following; carbohydrates, alkaloids, steroids/terpenes, saponins, flavonoids, cardiac glycosides, and tannins using standard procedures as described by Trease and Evans (2002).

3.4.1 Test for carbohydrates (molisch test)

Two (2 ml) of the extract solution in a test tube, was added a few drops of Molisch reagent, followed by the addition of concentrated (100%) sulphuric acid down the side of the test tube to form a lower layer. A reddish coloured ring at the interphase indicated the presence of carbohydrate (Trease and Evans, 2002).

3.4.2 Test for cardiac glycosides (keller-kiliani test)

Two (2 ml) of the extract solution in a test tube was dissolved in 1 ml of 0.1 M glacial acetic acid containing traces of 1% ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acidwas added down the side of the test tube to form a layer at the bottom and the interphase carefully observed for purple-brown ring.This color change indicated the presence of deoxy sugars while a pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Trease and Evans, 2002).

3.4.3 Test for steroids/terpenes (liebermann-burchard test)

Two (2) ml of the extract solution was added to an equal volume of 0.1M acetic acid anhydride and mixed gently. One (1) ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour changes were observed immediately and over a period of one hour. Blue to blue-green colour in the upper layer and a reddish, pink or purple color at the interphase indicated the presence of steroids and terpenes (Trease and Evans, 2002).

3.4.4 Test for saponins (frothing test)

About 10 ml of distilled water was added to 0.5 ml of the extract and shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and then observed for 30 minutes. A honeycomb froth that persisted for 10-15 minutes indicated the presence of saponins (Trease and Evans, 2002).

3.4.5 Test for tannins (ferric chloride test)

To five (5 ml) of the extract solution, 3-5 drops of 10% ferric chloride solution was added. A green-black precipitate indicates the presence of condensed tannins while blue or brownish-blue precipitate indicated the presence of hydrolysable tannins (Trease and Evans, 2002).

3.4.6 Test for alkaloids

About 0.5 ml of the extract was stirred in 5 ml of 1% aqueous HCl on water bath and then filtered. Equal portion of the filtrate were taken into three test different tubes and the following tests conducted.

3.4.6.1 Meyer’s test

To the first portion of the extract solution, a few drops of Meyer's reagent were added. A creamywhite precipitate indicated the presence of alkaloids (Trease and Evans, 2002).

3.4.6.2 Wagner's test

Few drops of Wagner's reagent were added to the second portion of the extract solution. The presence of reddish-brown precipitate indicated the presence of alkaloids (Trease and Evans, 2002).

3.4.6.3 Draggendoff's test

To the third portion of the extract solution, a few drops of Draggendoff's reagent were added. An orange brown precipitate indicated the presence of alkaloids (Trease and Evans, 2002).

3.4.7 Test for flavonoids

3.4.7.1 Shinoda test

About 0.5 g of extract solution was dissolved in 1-2 ml of 50% methanol and then heated. Metallic magnesium chips and a few drops of concentrated HCl were added. Appearance of red colour indicated presence of flavonoiuds (Trease and Evans, 2002).

3.4.7.2 Sodium hydroxide test

Few drops of 10% sodium hydroxide were added to 2 ml of extract solution in a test tube. Yellow coloration indicated the presence of flavonoids (Trease and Evans, 2002).

3.4.8 Test for free anthracene derivatives (*bontrager's* test)

Two (2 ml) of the extract solution in a dry test tube, 5 ml of chloroform was added and shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution. Appearance of a bright pink colour in the aqueous (upper) layer indicates the presence of free anthraquinones (Trease and Evans, 2002).

3.4.9 Test for combined anthracene derivatives (*modified bontrager's* test)

Two (2 ml) of the extract solution in a test tube was boiled with 5 ml of 10% hydrochloric acid for 2-3 minutes. This would hydrolyse the glycoside to yield aglycones, which are soluble in hot water. This was then filtered to remove any coarse particle and the filtrate was cooled and extracted with 5 ml of benzene. The benzene layer was pipetted off and shaken gently in a test tube with half of its volume of 10% ammonium hydroxide. The change of colour from rose pink to cherry red in lower ammonia layer is indicative that the mixture contained combined anthraquinones derivative (free or in combined state) (Trease and Evans, 2002).

3.5 Stock Preparation and Preparation of Different Concentrations

One gram of the concentrated seed extract of the pulverized seed of *J. curcas* was dissolved in 10 ml of 1% Tween 80 (as an emulsifying agent) and kept as stock (100 mg/ml) solution. This stock solution was used to prepare the desired concentrations through serial dilution to obtain dilutions of 50, 25, 12.5 and 6.25 mg/ml for exposure to the *Rhipicephalus (Boophilus) decoloratus* larvae.

3.6 Tick Collection

Engorged females of *Rhipicephalus* (*Boophilusdecoloratus*) were collected from naturally infested cattle by hand picking from Fulani cattle in herds near The Nigerian Defence Academy (NDA) at Mando, Kaduna and brought to the Entomology Laboratory of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

3.7 Rearing of Ticks

The engorged ticks were cleaned with 10% ethanol-soaked cotton wool and rinsed with distilled water. Each engorged female tick was placed in a sterile plain plastic tube with the open end plugged with cotton wool. The properly labeled plastic tubes were then maintained in an incubator in the laboratory at 28 ± 2°C and a relative humidity of 84 ± 5% as described by Mattioli and Cassama (1995); George *et al*., (2001) and Natala *etal*., (2002) to create a suitable condition until egg-laying was completed. After egg laying, the dead female ticks were removed from the universal bottles, the eggs were incubated under the same laboratory conditions for the eggs to hatch. The larvae that emerged from the eggs were used for the bioassay test after 14-20 days post hatching.

3.8 Evaluation of *Jatropha. curcas* Seed for Acaricidal Properties

Petri dishes (9cm in diameter) with one sheet of Whatman® filter paper of the same size inside each of the petri dishes were used for the experiment. One milliliter of 50, 25, 12.5 and 6.25 mg/ml of *J. curcas* seed extract, with 1% tween 80 and Amitraz (negative and positive controls

respectively), were evenly soaked (using syringe and needle) on different filter papers of each petri dish. Then about 60 larval ticks were put inside each petri dish with the help of a soft paint brush and then monitored for mortality at 6, 12, 18, 24 and 48 hours.

3.9 Larvicidal Bioassay Groups

Group A were treated with 50 mg/ml, Group B with 25 mg/ml, Group C with 12.5 mg/ml, Group D with 6.25 mg/ml, Group E 1% tween 80 while Group F were treated with Amitraz (1.4 ml/ liter of water; manufacturer's specification). Each treatment group was carried out in triplicate. Larvae were considered dead when on being agitated were unable to exhibit paddling of the legs or immobile when agitated. Corrected mortality was obtained using Abbot's formula for control mortality;

$$\text{Corrected mortality (\%)} = \frac{M_{observed} - M_{control}}{100 - M_{control}} \times 100$$

Where; $M_{control}$ = Mortality in control

$M_{observed}$ = Observed treatment mortality

3.10 Mortality

Observations to determine the mortality of *R.* (*Boophilus*) *decolaratus* larvaewas made at 6, 12, 18, 24 and 48 hours in the toxicity assay. Larvae of *R.* (*Boophilus*) *decolaratus* were considered dead when there was no sign of movement or response to external stimuli on agitation or the palps of the ticks when seen pointing outwards. Death was recorded against time. It was converted to probit kill table. Probit value was used against the log of concentration of the methanol extracts of *J. curas* to find their lethal concentration LC_{50} (Finney, 1971). The probit kill table value was used to convert the percentage mortality and plotted against the log concentration. A horizontal line of 50% mortality was drawn. The point where the mortality line met the regression line was drawn to the log concentration and the antilog read as the LC_{50} of the extract.

3.11 Toxicity Studies

3.11.1 Experimental animals

Ten adult rabbits were purchased and treated with Amprolium 250 WSP® Kepro BV Holland for the control of Coccidiosis; 1g was admistered in 1liter of water (100 g sachet in 100 liters of drinking water for 5 days, Kepro BV), Tetranor® (Oxytetracycline 10%, 20 mg/Kg body weight, Jubaili Ltd) and Vitamix® (150 g in 200 liters of drinking water for 5 days, Global Organics Ltd) and adequately fed with grower mash.

3.11.2 Animal preparation and grouping

The rabbits, after being conditioned and treated against infectious agents, were randomly assigned to 5 groups of 2 animals each and then placed in different cages. The animals in each group were tagged as follows: Group 1(1A, 1B); Group 2(2A, 2B); Group 3(3A, 3B); Group 4(4A, 4B) and Group 5(5A, 5B).

Prior to the topical treatment of the rabbits with the various concentrations of the extract, an area of the trunk of the rabbits of about 10 × 10 cm on one side of the abdomen of each of the rabbits in all the groups were shaved, with a sharp sterile fresh razor blade and cleaned with distilled water.The animals were allowed 24 hours after shaving before the topical application of the test materials(crude methanol extract of *J. curcas* seed) at 100%, 10%, 1%, and 1% tween 80 for 14 days. 0.2 mls of blood was collected from the ear vein of each rabbit on days 0, 7 and 14 for serum biochemical and histopathological studies.

3.11.3 Treatment groups

Group A were treated with 100% crude extract, Group B were treated with 10% of the crude extract, Group C were treated with 1% of the crude extract, Group D were treated with 1% tween 80 while Group E were untreated control group.

3.11.4 Serum biochemical and haematological studies for toxicity

3.11.4.1 Blood and serum collection

Before, during and after exposure of the rabbits to the extract for 14 days, blood samples (0.2 mls of blood from each rabbit at every collection) were collected for biochemical and haematological analyses.

3.11.4.2 Determination of serum alkaline phosphatase

1ml alkaline buffer was placed into a test tube with 1ml phenyl phosphate substrate. The mixture was incubated for a minute at 37±2°C. 1 ml of serum from the rabbits was added into the tubes in the incubator, thoroughly mixed and further incubated at 37±2°C for 5 minutes. The test tubes

were then removed and 0.8 ml of 0.5 NaOH (Sodium hydroxide), 1.2 ml of 0.5 NaHC03 (Sodium bicarbonate), 1.0 ml of Potassium ferriccyanide (K3FeCN) and 1.0 ml of 4-amino antipyrine added. For standard: 1.2 ml of buffer was added to 1.0ml of phenol and treated as test after incubation.

Blank: 1.2ml of buffer +1.2ml of water and treated as test after incubation. Instrument was zeroed with blank. Serum alkaline phosphate = T- TB X W (King Armstrong unit/100ml) S-b Absorbance was read at 510nm wavelength. Multiplying the obtained king Armstrong unit value by 7.1 gave the nativity in IU.

3.11.4. 3 Determination of serum aspartate aminotransferases (AST) and alanine phosphatase (ALP)

Prior to the topical treatment of the rabbits with the various concentrations of the extract, an area of the trunk of the rabbits of about 10 × 10 cm on one side of the abdomen of each of the rabbits in all the groups were shaved, with a sharp razor blade and cleaned with distilled water. The animals were allowed 24 hours after shaving before the topical application of the test materials (crude methanol extract of *J. curcas* seed) at 100%, 10%, 1%, and 1% tween 80 for 14 days.

Blood samples were obtained from the rabbits for determination of haemogram and blood chemistry as described by Aatish, *et al*., (2007). Blood samples were taken at day 0, day 7 and day 14 of the experiment for determination of haemogram and blood chemistry.

3.12 Histopathology

One rabbit from each of the groups was sacrificed and the skin, liver, kidney and spleen samples were obtained and processed for histopathological studies.

3.13 Data Analyses

Data from mortality was analyzed using ANOVA (Graphpad prism version 5). P-value of less than 0.05 was statistically significant. Regression coefficient between the probit kill and log concentration of the extract was used to determine the LC_{50} after the toxicity bioassay (Finney, 1971).Analysis of variance (ANOVA) and Duncan multiple range tests (DMRT) (1955) were used to test for differences between levels of treatments and to separate means respectively.

CHAPTER FOUR

RESULTS

4.1 Extraction and Percentage Yield of the Extract

The 500g pulverized seeds of *J. curcas* which was extracted with methanol as a solvent and it yielded 196.35g (39.27%) of oil.

4.2 Phytochemical Screening

The results of qualitative phytochemical screening of crude methanol extract of *J. curcas* are shown in Table 4.1. The following phytochemicals were present in seed extract of *J. curcas*; carbohydrates, cardiac glycosides, saponins, tannins, steroids and terpenes, alkaloids and flavonoids. Anthraquinones were not detected.

4.3Mortality of the Larvae of *Rhipicephalus* (*Boophilus*) *decolaratus* to Different Concentrations of Methanol Extract of *Jatropha curcas*Seeds

The percentage mortality of *Rhipicephalus* (*Boophilus*) *decoloratus*larvae using methanol extracts of *J. curcas* exposed to toxicity test for 48 hours are presented in Table 4.2. The results showed that the mortality was time and concentration dependent. At the lowest concentration of 6.25mg/ml the mortality values were lower when compared to highest concentration of 50mg/ml. Time and concentration had significant effects on the mortality of *Rhipicephalus* (*Boophilus*) *decolaratus* exposed to methanol extracts of *J. curcas* seedat $P \leq 0.05$.

The larvicidal bioassay of *Jatropha curcas*methanol seed extract showed that at 6h, 50 mg/ml (63.41±4.82) and 25 mg/ml (58.79±9.95) were significantly different ($p<0.05$) from the control. At 12h, 50 mg/ml (85.13±5.66), 25 mg/ml (86.70±4.57) and 12.5 mg/ml (23.42±2.86) were significantly different ($p<0.05$) from the control. At 18h, 50 mg/ml (92.79±3.18), 25 mg/ml (93.70±3.80) and 12.5 mg/ml (35.81±6.60) were significantly different ($p<0.05$) from the control. At 24h and 48h, all the concentrations (50-6.25 mg/ml) showed significant difference ($p<0.05$) from the control.

4.4Larvicidal Effect of Various Concentrations of *Jatropha curcas* Methanol Seed Extract At Different Times Compared to Reference Acaricide (Amitraz®)

Larvicidal bioassay of the methanol extract of *J. curcas* seed against *R.(Boophilus) decoloratus* carried out at concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml, at 6h, 12h, 18h, 24h and 48h of exposure and results recorded as shown in Table 4.2.

At 6h, all the concentrations, 50 mg/ml (63.41±4.82) down to 6.25 mg/ml (8.27±2.67) showed significant difference from the positive control, ($p<0.05$). At 12h, all the concentrations, 50 mg/ml (85.13±5.66) down to 6.25 mg/ml (9.13±3.18) were significantly different from the positive control, ($p<0.05$). At 18h, concentrations of 12.5 mg/ml (35.81±6.60) and 6.25 mg/ml

(17.24±3.74) showed significant difference from the positive control, ($p<0.05$). At 24h, concentrations 12.5 mg/ml (47.54±8.15) and 6.25 mg/ml (29.15±1.26) were significantly different from the positive control, ($p<0.05$). At 48h, concentrations of 12.5 mg/ml (76.85±4.89) and 6.25 mg/ml (66.26±1.47) were significantly different from the positive control, ($p<0.05$).

The results showed that at 18h, 24h and 48h at concentrations of 50 mg/ml (92.79±3.18, 97.19±2.06, 100±0.00) and 25 mg/ml (93.70±3.80, 95.50±3.38, 97.35±1.61) respectively there were no significant difference (p□ 0.05) from the positive control while the other concentrations of 12.5 mg/ml and 6.25 mg/ml showed significant difference ($p<0.05$) from the positive control all through 6h, 12h, 18h, 24h and 48h respectively.

Table 4.1 Results of Qualitative Phytochemical Screening of *Jatropha curcas*Methanol Seed Extract

Constituent(s)	Test	Reference	Observation	Inference
Carbohydrates	Molisch	Trease and Evans,2002	Reddish colour ring	+
Cardiac glycosides	Keller-kiliani	"	Purple brown colouration	+
Steroids and terpenes	Liebermann-Burchard	"	Reddish colouration	+
Saponins	Frothing	"	Persistent (honey comb) froth	+
Tannins	Ferric chloride	"	Brownish-blue ppt	+
Alkaloids	Meyer's	"	Creamy white ppt	+
"	Wagner's	"	Reddish brown ppt	+

"	Draggendoff's	"	Orange brown ppt	+
Flavonoids	Shinoda's	"	Red colouration	+
"	Sodiun hydroxide	"	Yellow colouration	+
Free Anthracene Derivative	Bontrager's	"	Yellow colouration	-
Combined Anthracene	Modified			
Derivative	Bontrager's	"	Yellow colouration	-

+ Test substance present; - Test substance absent.

Table 4.2 Mean Percentage Mortality of *Rhipicephalus* (*Boophilus*) *decoloratus* Larvae treated with *Jatrophacurcas* Methanol Seed Extract

Treatment (Exposure) Time (h)	Control(1% Tween 80)	Concentration of Extract (mg/ml) 6.25	12.5	25	50
6	4.09 ± 1.06^{a}(%)	8.27 ± 2.67^{a}(%)	12.73 ± 1.24^{a}(%)	58.79 ± 9.95^{b}(%)	63.41 ± 4.82^{c}(%)
12	5.57 ± 0.74^{a}"	9.13 ± 3.18^{a} "	23.42 ± 2.86^{b} "	86.70 ± 4.57^{c} "	85.13 ± 5.66^{d} "
18	5.57 ± 0.74^{a} "	17.24 ± 3.74^{a} "	35.81 ± 6.60^{b} "	93.70 ± 3.80^{c} "	92.79 ± 3.18^{d} "
24	5.57 ± 0.74^{a} "	29.15 ± 1.26^{b}	47.54 ± 8.15^{c} "	95.50 ± 3.38^{d} "	97.19 ± 2.06^{e} "
48	6.27 ± 0.23^{a} "	66.26 ± 1.47^{b}	76.85 ± 4.89^{c} "	97.35 ± 1.61^{d} "	100 ± 0.00^{e} "

Means in the same row with different superscripts are significantly different to the control ($p\leq0.05$)

Table 4.3 Corrected Mean Percentage Mortality of *Rhipicephalus* (*Boophilus*) *decoloratus* Larvae treated with *Jatrophacurcas* Methanol Seed Extract at Various Exposure Periods

Exposure Time (h)	Concentration of extract (mg/ml)			
	6.25	12.5	25	50
6	4.36±1.87(%)	9.01±1.15(%)	57.03±5.51[a](%)	61.85±2.94[a](%)
12	3.77±1.96 ”	18.90±1.80[a]”	85.92±2.66[a]”	84.25±3.20[a] ”
18	12.36±2.24 ”	32.02±3.67[a]”	93.33±2.27[a]”	92.36±1.96[a] ”
24	24.97±1.00[a]”	44.46±4.45[a]”	95.23±2.06[a]”	97.02±1.40[a] ”
48	64.00±0.85[a]	75.30±2.56[a] ”	97.17±0.92[a] ”	100.0.±0.00[a] ”

Means in the same row with superscripts are significantly different to the control (P<0.05).

Table 4.4: Mean Percentage Mortality of *Rhipicephalus* (*Boophilus*) *decoloratus* Larvae treated with *Jatrophacurcas* Methanol Seed Extract and Compared to Standard (Control)

Treatment (Exposure) Time (h)	Amitraz (12.5%)	Concentration of Extract (mg/ml)			
		6.25	12.5	25	50
6	100±0.00^{a}(%)	8.27±2.67^{b}(%)	12.73±1.24^{c}(%)	58.79±9.95^{d}(%)	63.41±4.82^{e}(%)
12	100±0.00^{a,,}	9.13±3.18^{b,,}	23.42±2.86^{c,,}	86.70±4.57^{d,,}	85.13±5.66^{e,,}
18	100±0.00^{a,,}	17.24±3.74^{b,,}	35.81±6.60^{c,,}	93.70±3.80^{a,,}	92.79±3.18^{a,,}
24	100±0.00^{a,,}	29.15±1.26^{b,,}	47.54±8.15^{c,,}	95.50±3.38^{a,,}	97.19±2.06^{a,,}
48	100±0.00^{a,,}	66.26±1.47^{b,,}	76.85±4.89^{c,,}	97.35±1.61^{a,,}	100±0.00^{a,,}

Means in the same row with different superscripts are significantly different to the control ($p \leq 0.05$)

4.5 Determination of Lethal Concentration Required to kill 50% (LC_{50}) and 90% (LC_{90}) of *R. (Boophilus) decoloratus* Larvae using Methanol Extract of *Jatropha curcas* Seed for the Various Hours of Exposure

The percentage mortalities were converted to probit values using probit tables whilethe concentrations were converted to log values and plotted on the X-axis with the probit values on the Y-axis. Where 50 % or 90% mortality line met the regression line was read off as LC_{50} or LC_{90} values for the methanol seed extract of *J. curcas* (Appendix6). As the time increased, so did the LC_{50} or LC_{90} of the extract decreased.

4.6 Biochemical and Haematological Parameters of Rabbits treated with Different Concentrations of Methanol Extract of *Jatropha curcas* Seed Oil

The results on Biochemical Parameters of rabbits treated with 100% CE, 10% CE, 1% CE, 1%T80 and NT are shown in Tables 4.6 – 4.9. Mean AST (u/l)values ranged from 10.5±3.5 to 18.5±6.5 (Table 4.6). Mean ALT (u/l) values ranged from 5.0±0.0 to 17.5±6.5 (Table 4.7). Mean ALP (u/l) values ranged from 131.0±20.0 to 259.5±3.5 (Table 4.8).The Mean Values of ALP showed significant differences ($P < 0.05$) on Day 0 at 100% CE (145.0±35.0), 1% CE (137.0±23.0) and Day 7 at 100% CE (152.0±44.0).10% CE (202.0±1.0), 1% CE (131.0±20.0) and 1%T80 (194.5±6.5) compared to the control. Mean TP (g/dl) values ranged from 4.4±0.4 to 6.4±0.6 (Table 4.9).

The results on Haematological Parameters of rabbits treated with 100% CE, 10% CE, 1% CE, 1%T80 and NT are shown in Tables 4.10 – 4.13. Mean PCV (%) values ranged from 28.5±0.5 to 41.5±2.5 (Table 4.10). Mean Hb (g/dl) values ranged from 9.45±0.15 to 13.8±0.8 (Table 4.11). Mean WBC ($\times 10^9$/L) values ranged from 3.9±0.3 to 25.3±13.4 (Table 4.12).The Mean Values of

WBC showed significant differences ($P < 0.05$) on Day 7 at 100% CE (6.4±1.1), 10% CE (11.5±1.3) and 1%T80 (4.65±2.55) and Day 14 at 10% CE (21.05±1.95) compared to the control. The Mean RBC ($\times 10^{12}$/L) values ranged from 4.7±0.1 to 7.0±0.6 (Table 4.13).

Table 4.5 Summary of the LC_{50} and LC_{90} of the Methanol Extract of *Jatroha curcas* Seed at the Various Exposure Times

Duration of Exposure Time(h)	LC_{50}(mg/ml)	LC_{90}(mg/ml)
6	21.38	245.47
12	17.38	169.82
18	15.13	131.83
24	13.18	104.71
48	2.88	6.76

Table 4.6 Means ± SEM of AST (u/l) of Rabbits treated with Different Concentrations of Methanol Extract of *J. curcas* Seed Oil

Concentration Groups	Means ±SEM of AST(u/l) on Treatment Days		
	Day 0	Day 7	Day 14
100%CE	15.5±4.5	15.5±2.5	10.5±3.5
10%CE	14.5±0.5	16.5±0.5	14.0±6.0
1%CE	15.5±3.0	18.0±1.0	12.5±6.5
1%T80	12.0±2.0	13.0±3.0	18.5±6.5
NT	18.0±2.0	18.5±0.5	12.0±1.0

100%CE = 100% crude extract

10%CE = 10% crude extract

1%CE = 1% crude extract

1%T80 = 1% Tween 80

NT = Non- Treated

Table 4.7 Means ± SEM of ALT (u/l) of Rabbits treated with Different Concentrations of Methanol Extract of *J. curcas* Seed Oil

Concentration	Means ±SEM of ALT(u/l) on Treatment Days		
Groups	Day 0	Day 7	Day 14
100%CE	15.0±4.0	12.5±6.5	7.5±2.5
10%CE	10.0±0.0	10.5±2.5	17.5±6.5
1%CE	7.0±1.0	6.0±2.0	12.0±3.0
1%T80	5.0±0.0	7.5±1.5	15.0±5.0
NT	11.0±1.0	10.5±0.5	7.0±3.0

100%CE = 100% crude extract

10%CE = 10% crude extract

1%CE = 1% crude extract

1%T80 = 1% Tween 80

NT = Non- Treated

Table 4.8 Means ± SEM of ALP (u/l) of Rabbits treated with Different Concentrations of Methanol Extract of *J. curcas* Seed Oil

Concentration	Means ±SEM of AST(u/l) on Treatment Days		
Groups	Day 0	Day 7	Day 14
100%CE	145.0±35.0*	152.0±44.0*	224.5±18.5
10%CE	189.0±3.0	202.0±1.0*	207.0±6.0
1%CE	137.0±23.0*	131.0±20.0*	213.5±7.5
1%T80	191.5±11.5	194.5±6.5*	191.5±20.5
NT	221.0±21.0	259.5±3.5	209.5±11.5

Means in the same row with asterix are significantly different from the control ($P < 0.05$).

100%CE = 100% crude extract

10%CE = 10% crude extract

1%CE = 1% crude extract

1%T80 = 1% Tween 80

NT = Non- Treated

Table 4.9 Means ± SEM of TP (g/dl) of Rabbits treated with Different Concentrations of Methanol Extract of *J. curcas* Seed Oil

Concentration	Means ±SEM of TP(g/dl) on Treatment Days		
Groups	Day 0	Day 7	Day 14
100%CE	6.0±0.0	5.5±0.5	5.5±0.5
10%CE	6.3±0.3	6.4±0.6	6.0±0.2
1%CE	6.4±0.6	6.1±0.1	5.5±0.1
1%T80	6.1±0.1	5.8±0.2	5.1±1.1
NT	5.4±0.4	5.5±0.5	4.4±0.4

100%CE = 100% crude extract

10%CE = 10% crude extract

1%CE = 1% crude extract

1%T80 = 1% Tween 80

NT = Non- Treated

Table 4.10 Means ± SEM of PCV (%) of Rabbits treated with Different Concentrations of Methanol Extract of *J. curcas* Seed Oil

Concentration	Means ±SEM of PCV (%) on Treatment Days		
Groups	Day 0	Day 7	Day 14
100%CE	41.5±2.5	36.0±4.0	31.5±0.5
10%CE	39.0±1.0	39.0±3.0	32.0±2.0
1%CE	37.5±6.5	32.5±5.5	28.5±0.5
1%T80	41.0±3.0	32.0±7.0	39.0±2.0
NT	34.0±4.0	37.5±3.5	29.0±4.0

100%CE = 100% crude extract

10%CE = 10% crude extract

1%CE = 1% crude extract

1%T80 = 1% Tween 80

NT = Non- Treated

Table 4.11 Means ± SEM of Hb (g/dl) of Rabbits treated with Different Concentrations of Methanolic Extract of *J. curcas* Seed Oil

Concentration Groups	Means ±SEM of Hb (g/dl) on Treatment Days		
	Day 0	Day 7	Day 14
100%CE	13.8±0.8	11.95±1.35	10.45±0.15
10%CE	12.95±0.35	13.0±1.0	10.65±0.65
1%CE	12.45±2.15	10.8±1.8	9.45±0.15
1%T80	13.6±1.0	10.65±2.35	12.95±0.65
NT	11.3±1.3	12.45±1.15	9.65±1.35

100%CE = 100% crude extract

10%CE = 10% crude extract

1%CE = 1% crude extract

1%T80 = 1% Tween 80

NT = Non- Treated

Table 4.12 Means ± SEM of WBC ($\times 10^9$/L) of Rabbits treated with Different Concentrations of Methanol Extract of *J. curcas* Seed Oil

Concentration	Means ±SEM of WBC ($\times 10^9$/L) on Treatment Days		
Groups	Day 0	Day 7	Day 14
100%CE	6.4±1.6	6.4±1.1*	13.1±4.0
10%CE	7.7±0.3	11.5±1.3*	21.05±1.95*
1%CE	6.45±0.65	16.3±4.2	14.2±1.9
1%T80	12.0±6.0	4.65±2.55*	13.9±2.4
NT	3.9±0.3	25.3±13.4	10.1±0.6

Means in the same row with asterix are significantly different from the control ($P < 0.05$).

100%CE = 100% crude extract

10%CE = 10% crude extract

1%CE = 1% crude extract

1%T80 = 1% Tween 80

NT = Non- Treated

Table 4.13 Means ± SEM of RBC ($\times 10^{12}$/L) of Rabbits treated with Different Concentrations of Methanol Extract of *J. curcas* Seed Oil

Concentration Groups	Means ±SEM of AST ($\times 10^{9}$/L) on Treatment Days		
	Day 0	Day 7	Day 14
100%CE	6.95±0.35	5.85±0.75	5.25±0.05
10%CE	6.55±0.25	6.5±0.5	5.35±0.45
1%CE	6.3±1.1	5.7±0.7	4.7±0.1
1%T80	7.0±0.6	5.4±1.1	6.45±0.45
NT	5.7±0.6	6.1±0.6	5.1±0.9

100%CE = 100% crude extract

10%CE = 10% crude extract

1%CE = 1% crude extract

1%T80 = 1% Tween 80

NT = Non- Treated

4.7 Gross Effect of *Jatropha curcas* Seed Oil Extract on the Skin of Rabbits

J. curcas oil topically applied on the skin (100% crude extract) produced marked erythema and edema on the skin of the rabbit. The erythema sets in on the 5^{th} day, the skin became necrotic, degenerated and scaling off the skin and adjourning hair.



Plate 1: Marked erythemaof the shavedskin portionapplied withthe100% methanol extract of *Jatropha curcas*.



Plate 2: Necrotised, degenerated and scaling of the skin and adjourning hair of rabbit applied
with 100% methanol extract of *Jatropha curcas*.

4.8 Postmortem Findings

4.8.1 Histopathologic findings

The histopathologic findings in the organs of rabbits exposed to various percentage concentrations of crude methanol extract of *J. curcas* methanol seed oil extract and non-treated (control) group to ascertain the toxicity (safety) of the extract are as presented in plates 3 to 18:

Plate 3; section of the skin in the non-treated group showing no observable microscopic lesion.

Plate 4; section of the skin in the 1% *J. curcas* seed methanol extract treated group showing no observable microscopic lesion.

Plate 5; section of the skin in the 10% *J. curcas* seed methanol extract treated group showing no observable microscopic lesion.

Plate 6; section of the skin in the 100% *J. curcas* seed methanol extract treated group showing hyperkeratosis of the epidermal tissues, necrotic hair follicles and infiltration of inflammatory cells.

Plate 7; section of the liver in the non-treated group showing no observable microscopic lesion.

Plate 8; section of the liver in the 1% *J. curcas* seed methanol extract treated group showing no observable microscopic lesion.

Plate 9; section of the liver in the 10% *J. curcas* seed methanol extract treated group showing no observable microscopic lesion.

Plate 10; section of the liver in the 100% *J. curcas* seed methanol extract treated group showing congested vessels and aggregation of inflammatory cells.

Plate 11; section of the kidney in the non-treated group showing no observable microscopic lesion.

Plate 12; section of the kidney in the 1% *J. curcas* seed methanol extract treated group showing no observable microscopic lesion.

Plate 13; section of the kidney in the 10% *J. curcas* seed methanol extract treated group showing no observable microscopic lesion.

Plate 14; section of the kidney in the 100% *J. curcas* seed methanol extract treated group showing no observable microscopic lesion.

Plate 15; section of the spleen in the non-treated group showing no observable microscopic lesion.

Plate 16; section of the spleen in the 1% *J. curcas* seed methanol extract treated group showing no observable microscopic lesion.

Plate 17; section of the kidney in the 10% *J. curcas* seed methanol extract treated group showing haemosiderin deposits.

Plate 18; section of the kidney in the 100% *J. curcas* seed methanol extract treated group showing no observable microscopic lesion.

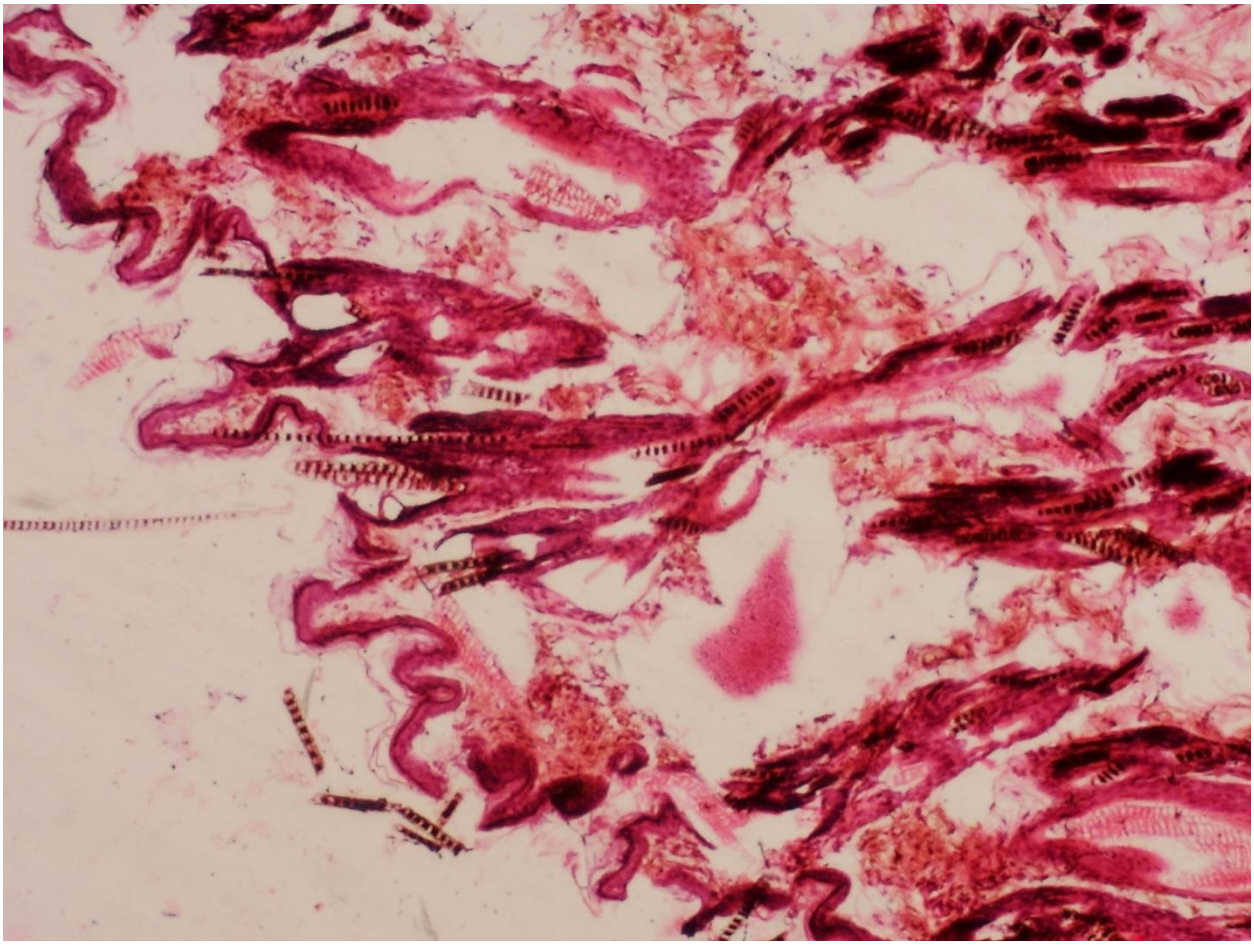


Plate 3: Photomicrograph of a section of the skin of rabbit in the non-treated group (control).
No observable microscopic lesions seen (H & E, ×400)

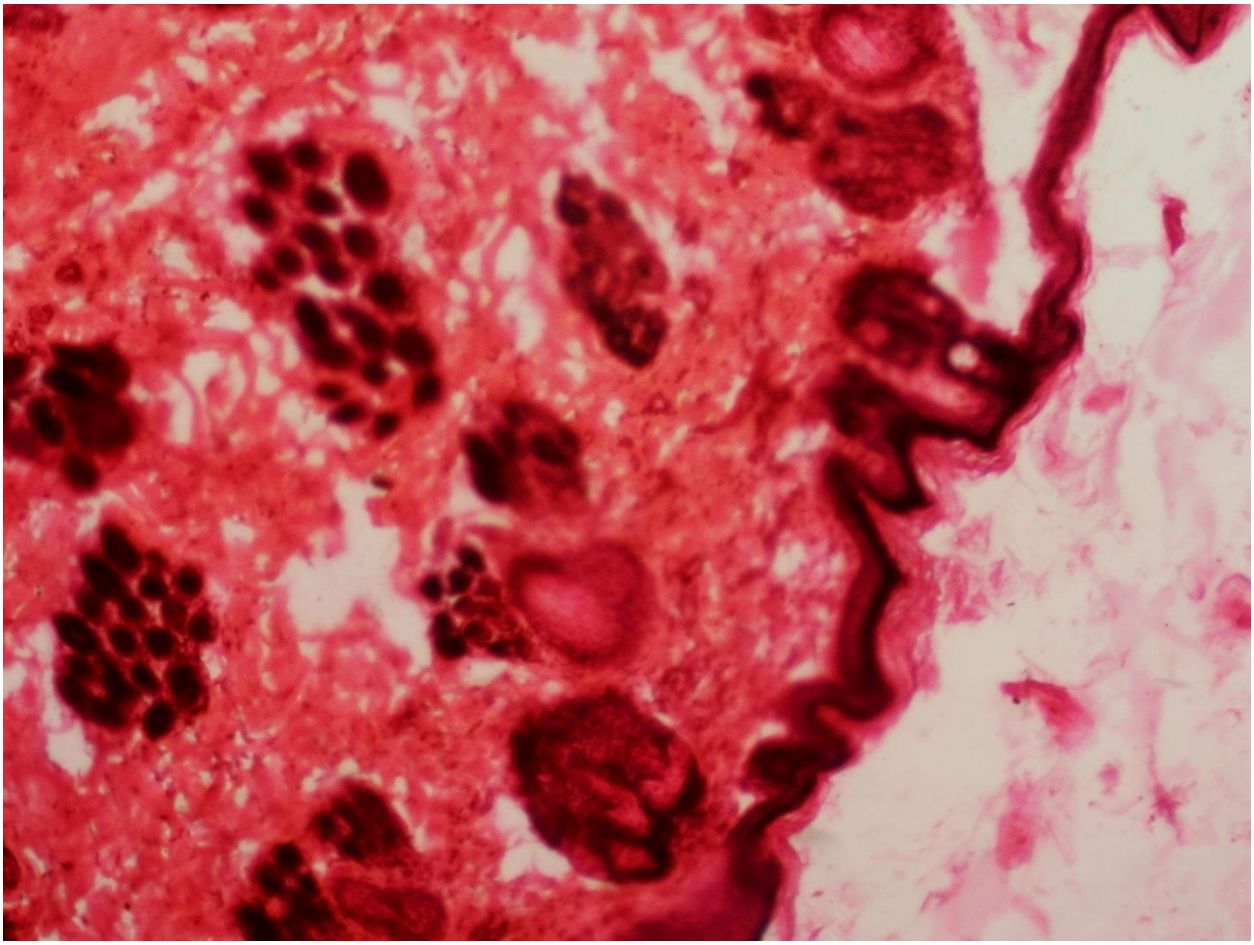


Plate 4: Photomicrograph of a section of the skin of rabbit treated with 1% methanol extract
of *J. curcas* seed. No observable microscopic lesions seen (H & E, ×400)

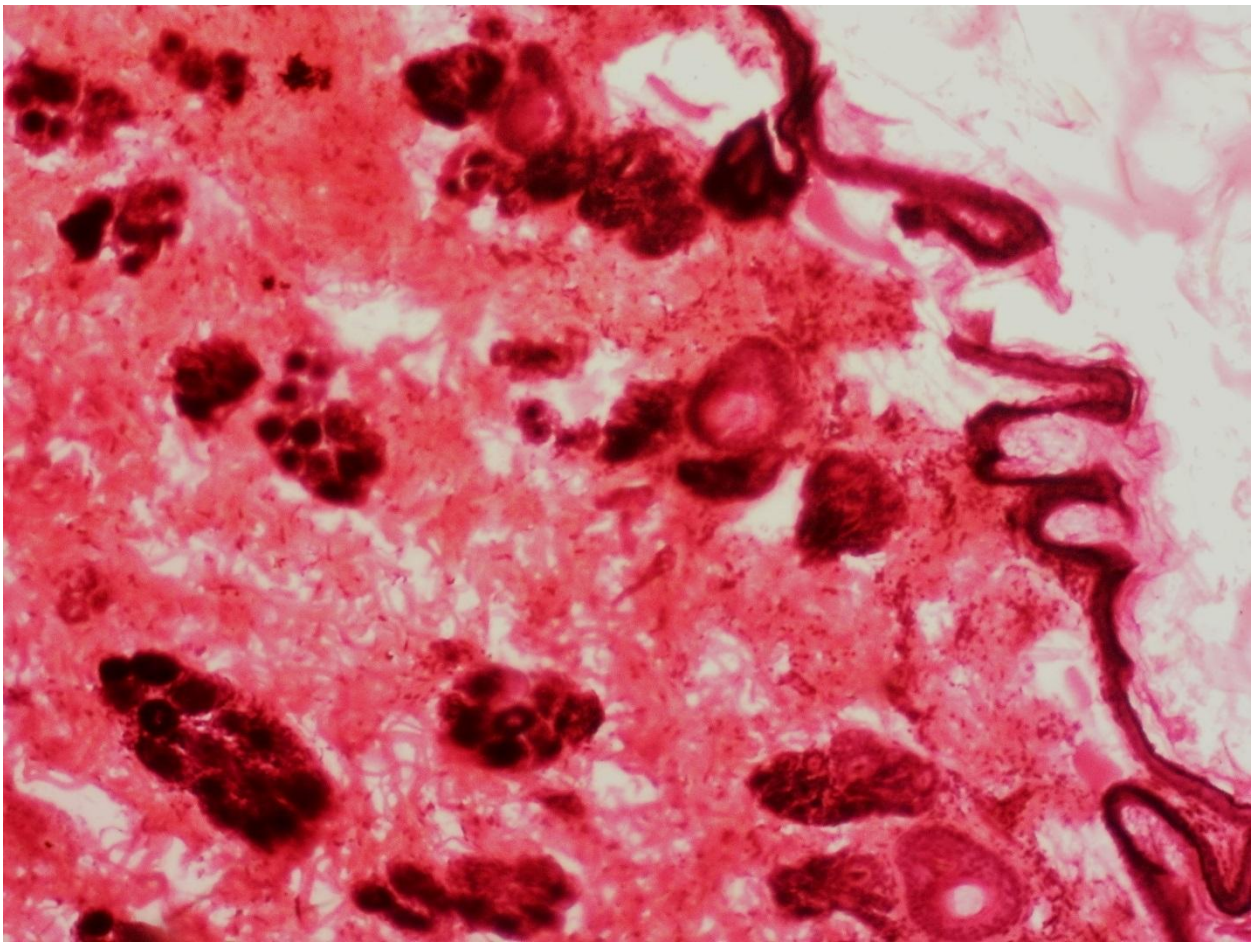


Plate 5: Photomicrograph of a section of the skin of rabbit exposed to the 10% methanol seed extract of *J. curcas* treated group. No observable microscopic lesions seen (H & E, ×400)

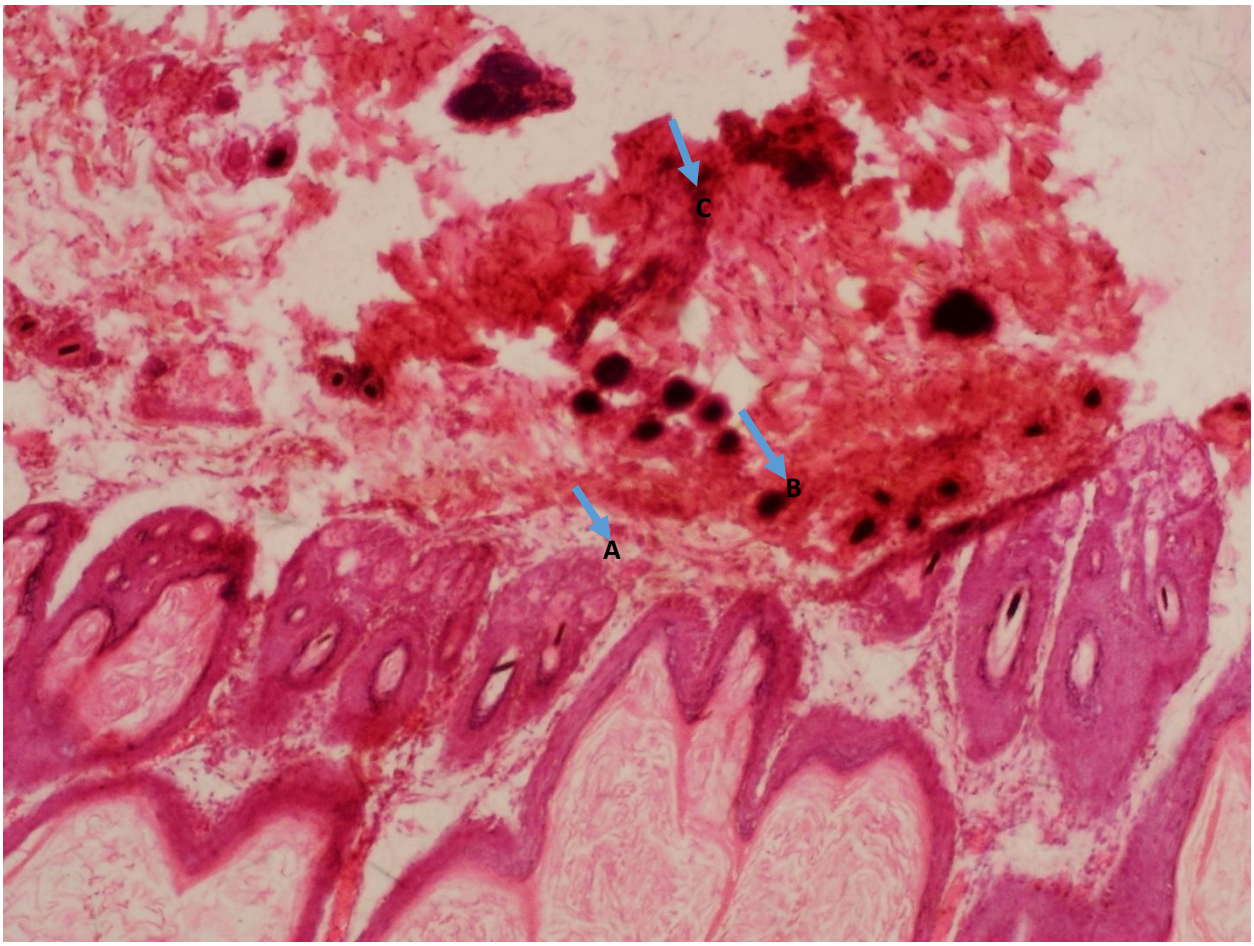


Plate 6: Photomicrograph of a section of the skin of rabbit exposed to the 100% methanol seed extract of *J. curcas* treated group. Note the hyperkeratosis of the epidermal tissue (A), Necrotic hair follicle (B), Infiltration of inflammatory cells (C) (H & E, ×400)

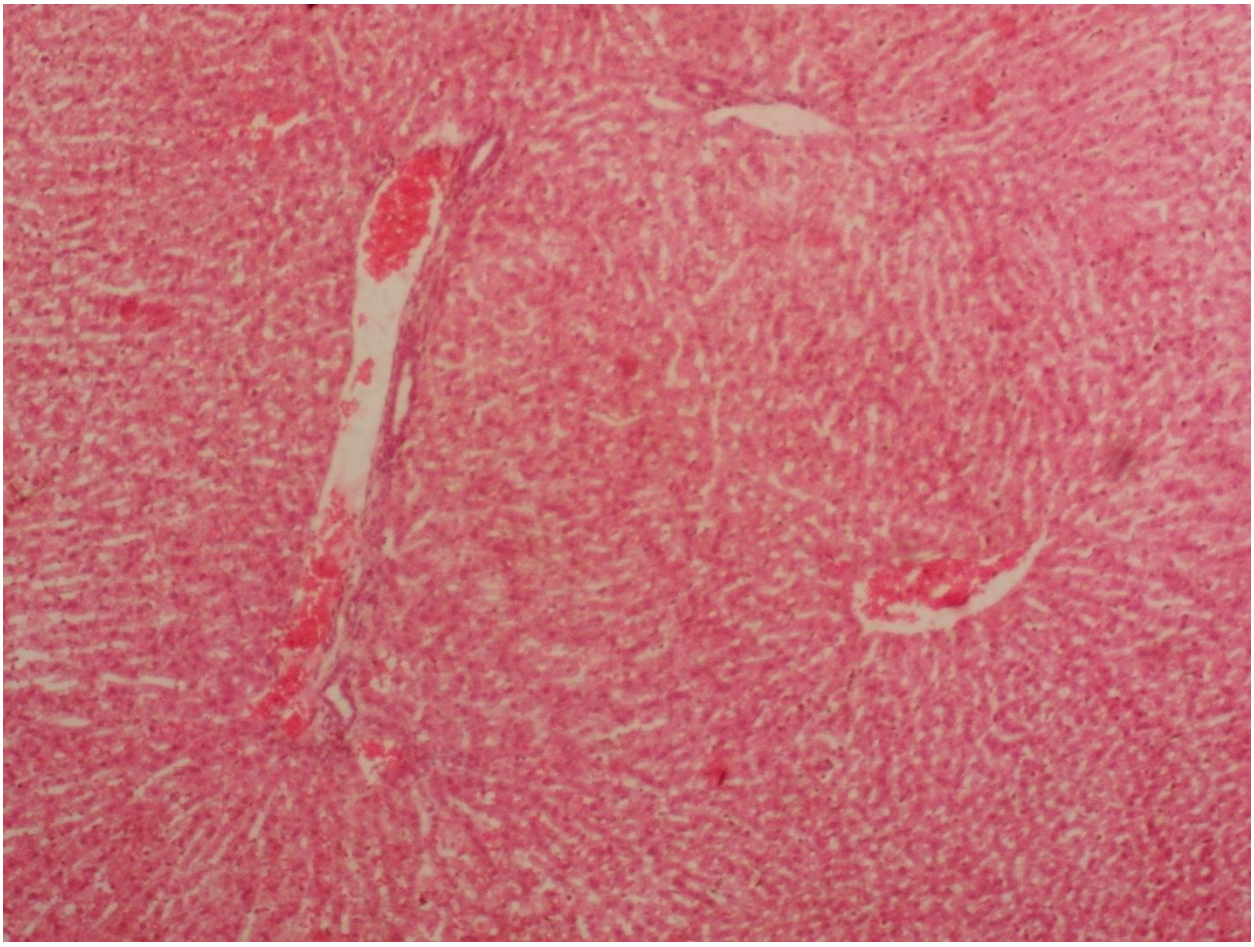


Plate 7: Photomicrograph of a section of the liver of rabbit in the non-treated group (control).
Noobservable microscopic lesions seen (H & E, ×400)

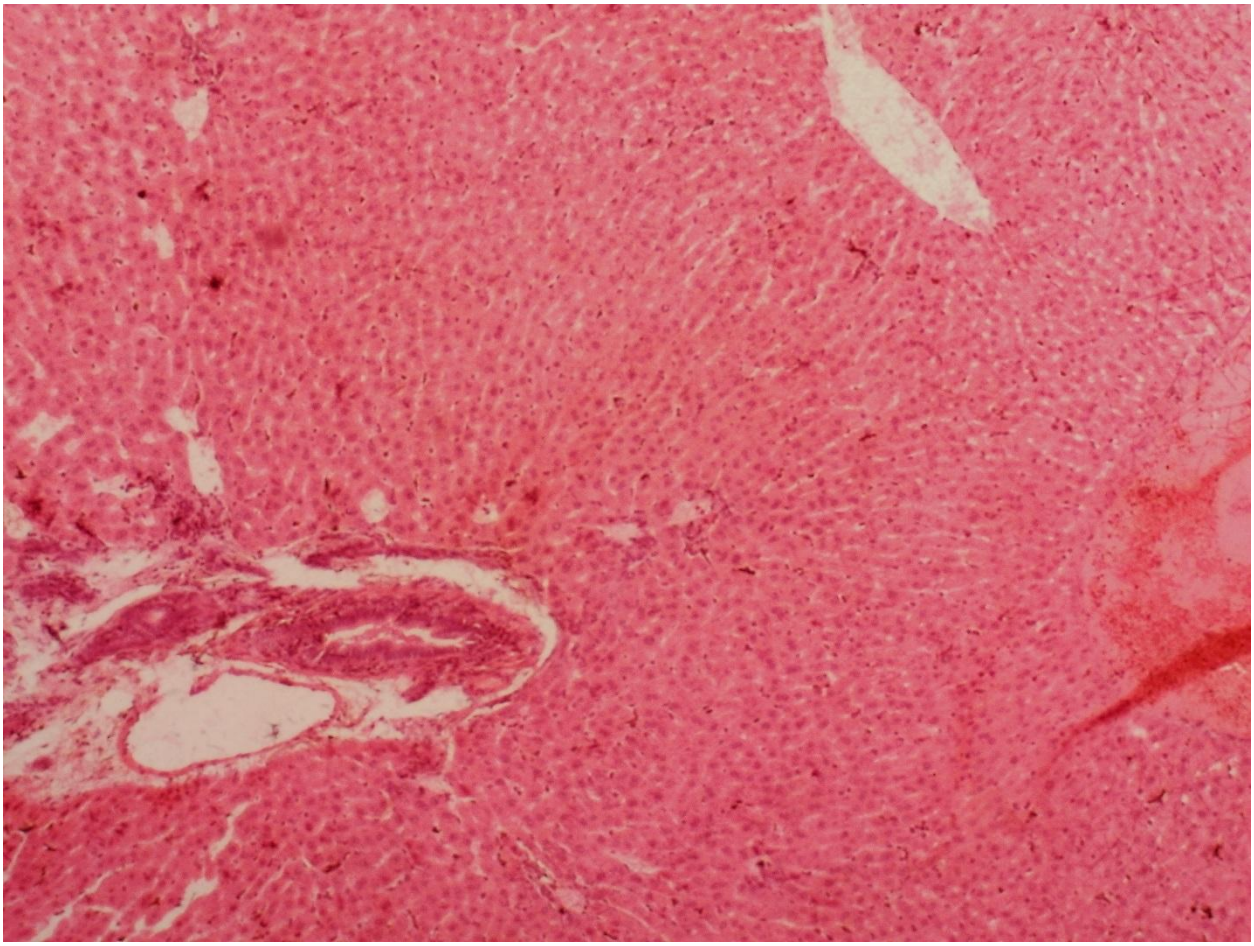


Plate 8: Photomicrograph of a section of the liver of rabbit exposed to the 1% methanol extract of *J. curcas* seed treated group. No observable microscopic lesions seen (H & E, ×400)

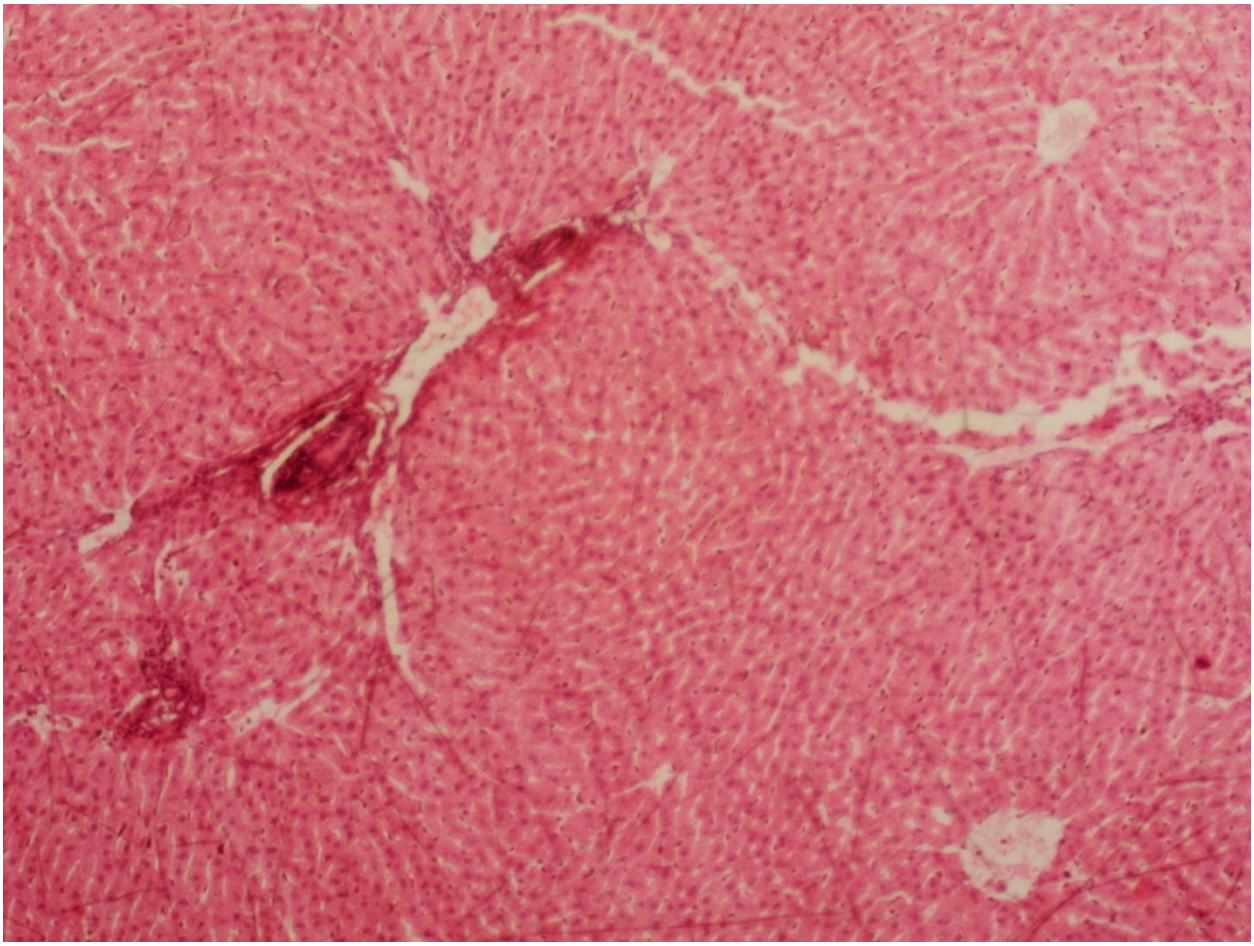


Plate 9: Photomicrograph of a section of the liver of rabbit exposed to the 10% methanol extract of seed of *J. curcas* treated group. No observable microscopic lesions seen (H & E, ×400)

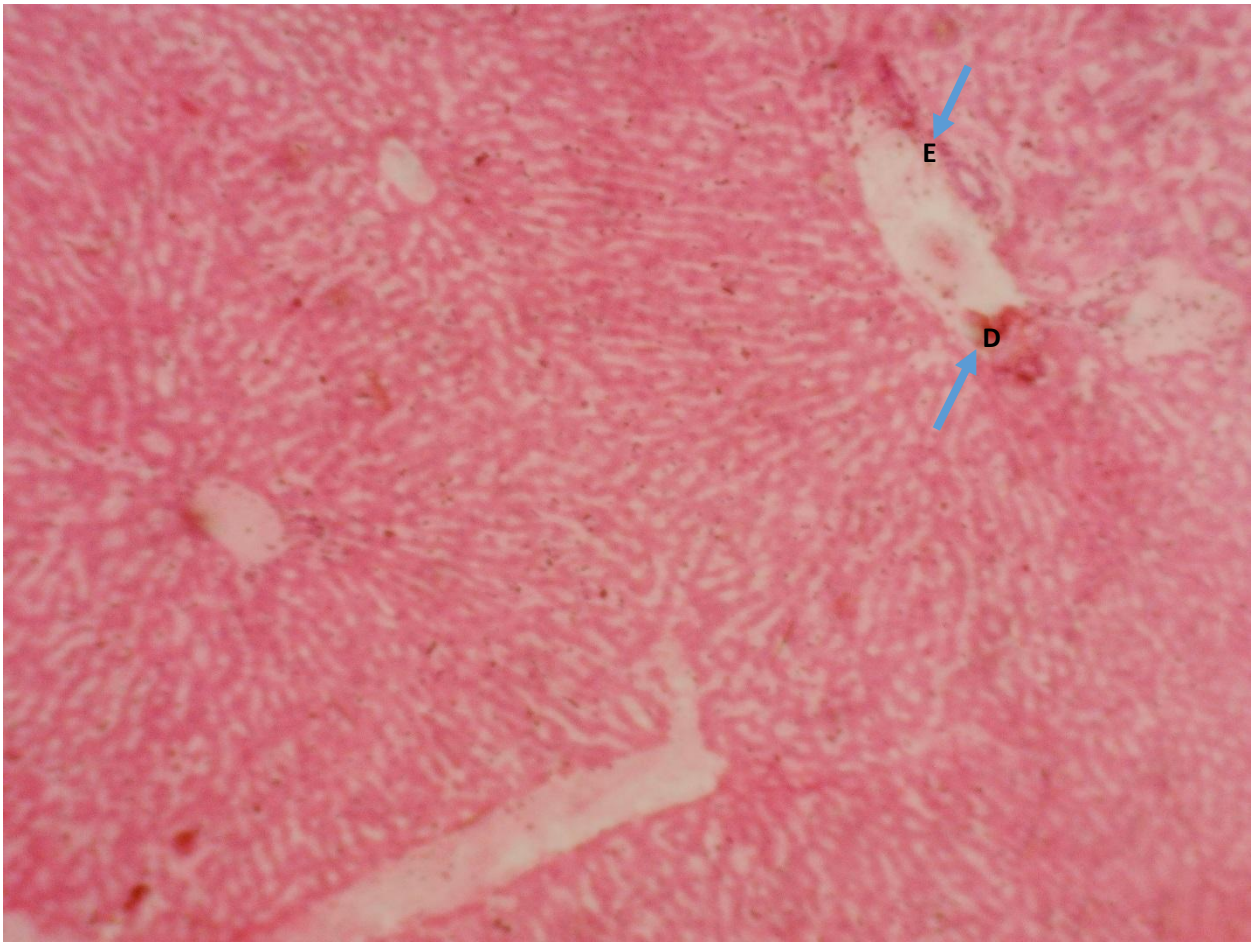


Plate 10: Photomicrograph of a section of the liver of rabbit exposed to the 100% methanol extract of seed of *J curcas* treated group. Note congested vessels (D) aggregation of inflammatory cells (E). (H & E, ×400)

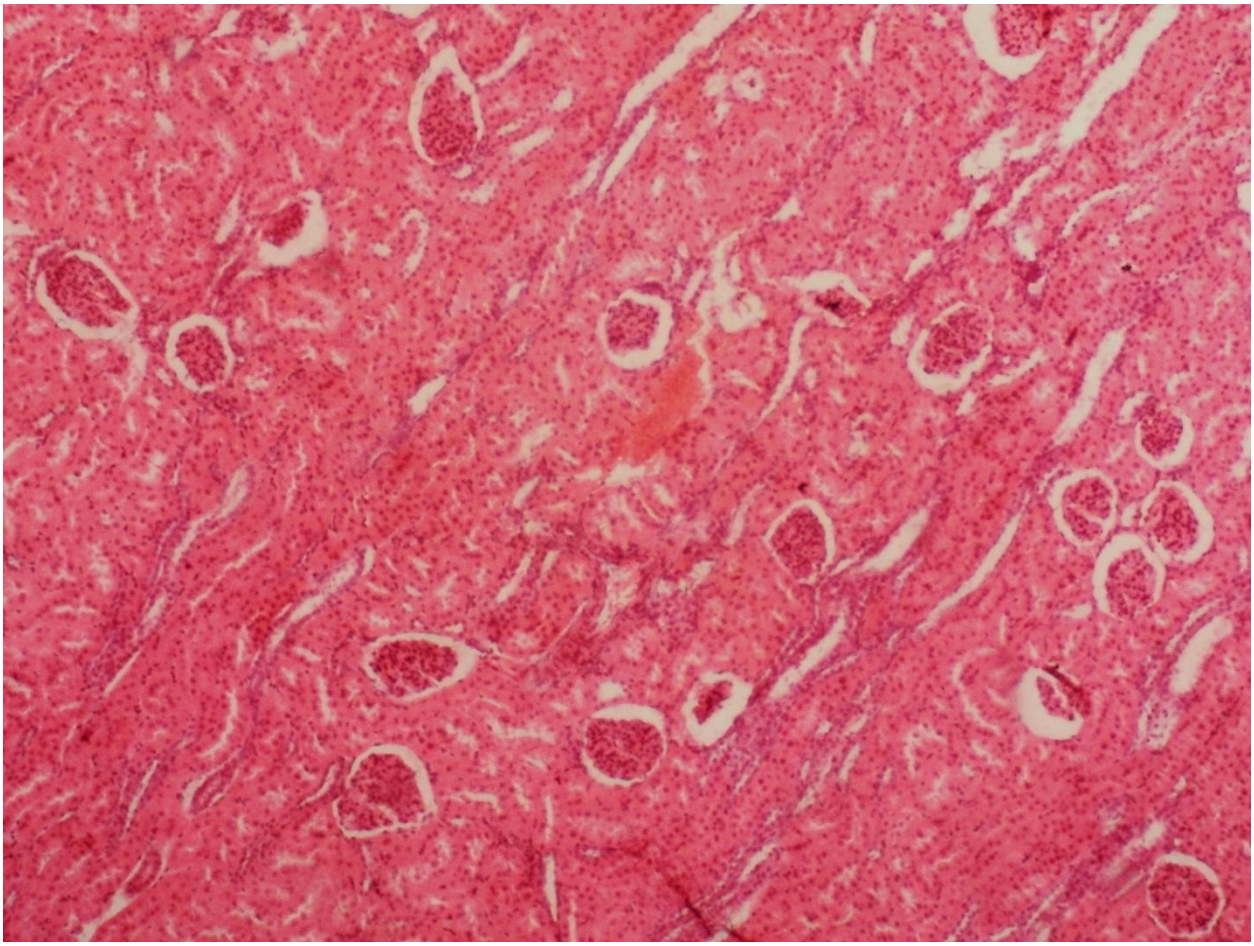


Plate 11: Photomicrograph of a section of the kidney of rabbit in the non-treated group (control). No observable microscopic lesions seen (H & E, ×400)

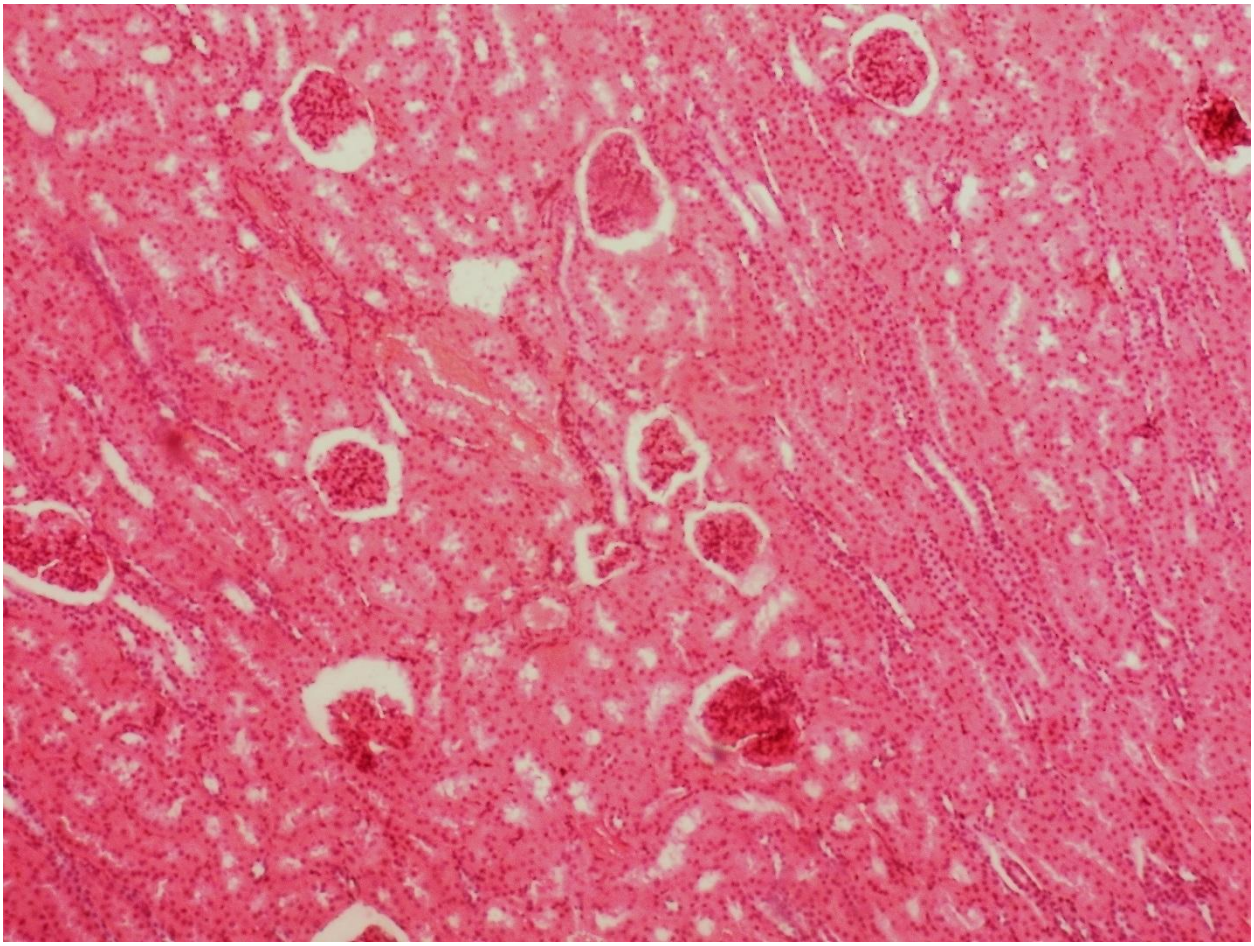


Plate 12: Photomicrograph of a section of the kidney of rabbit exposed to the 1% methanol extract of *J. curcas* seed treated group. No observable microscopic lesions seen (H & E, ×400)

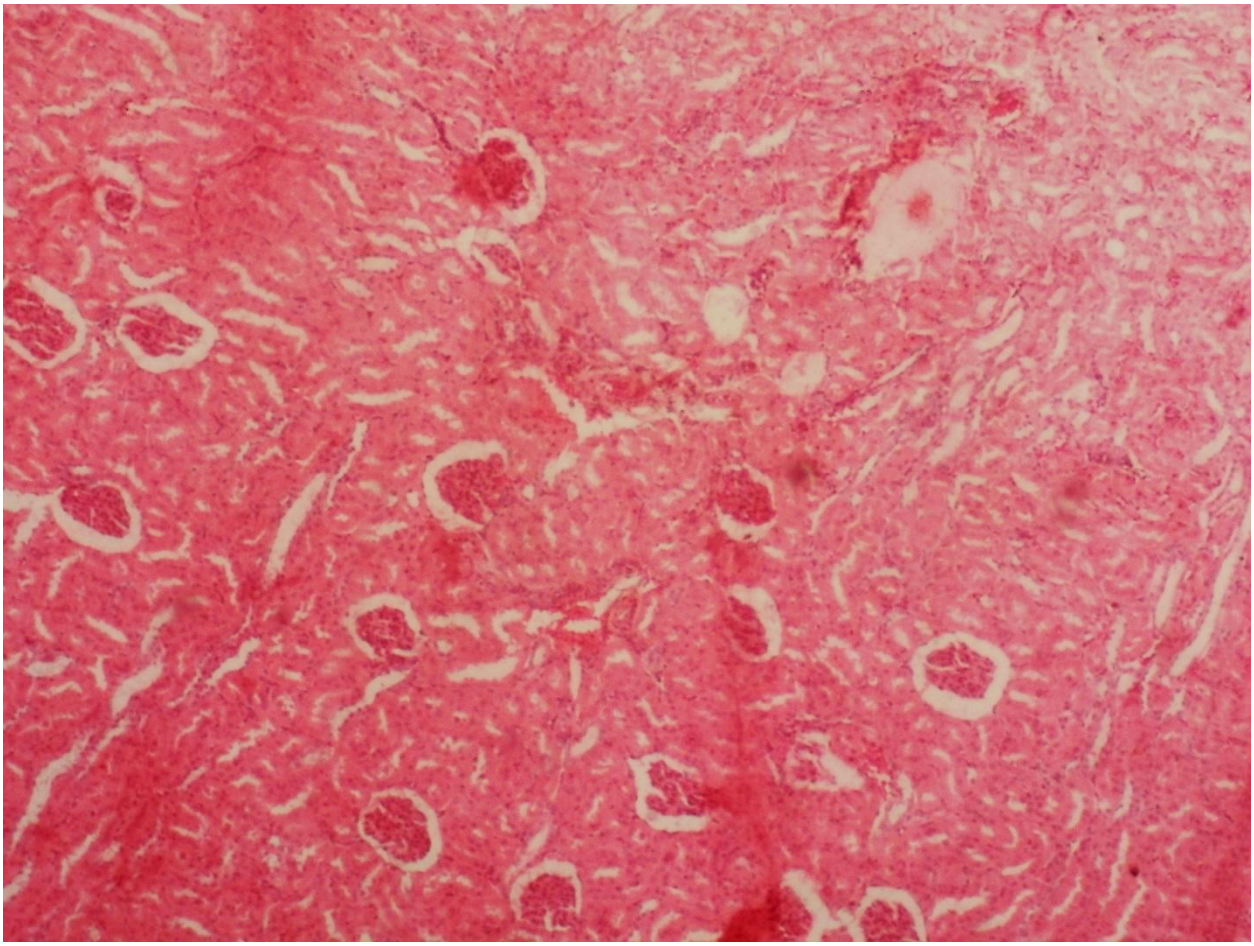


Plate 13: Photomicrograph of a section of the kidney of rabbit exposed to the 10% methanol
extract of seed of *J. curcas* treated group. No observable microscopic lesions seen
(H &E, ×400)

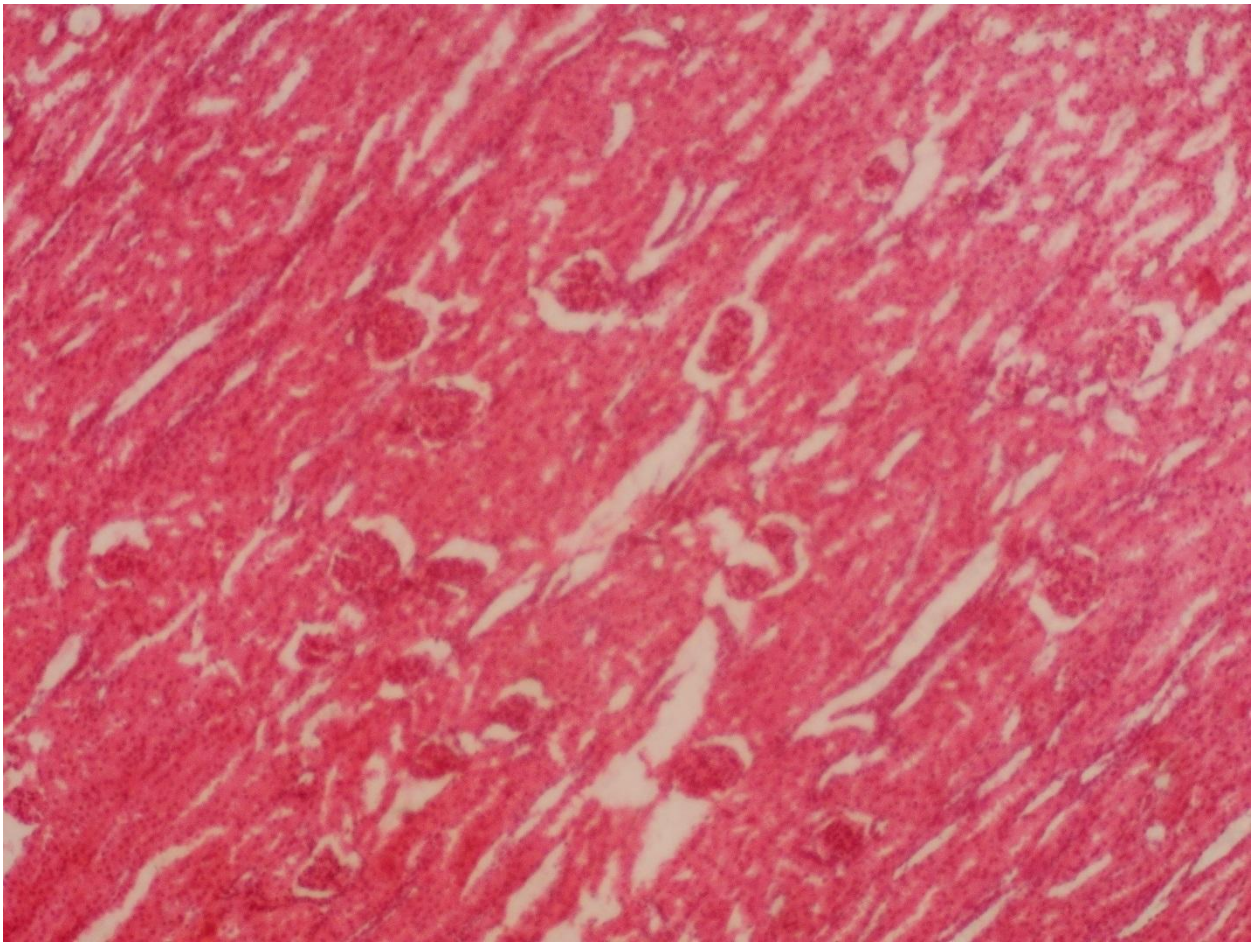


Plate 14: Photomicrograph of a section of the kidney of rabbit exposed to the 100% methanol
extract of seed of *J. curcas* treated group. No observable microscopic lesions seen
(H & E, ×400)

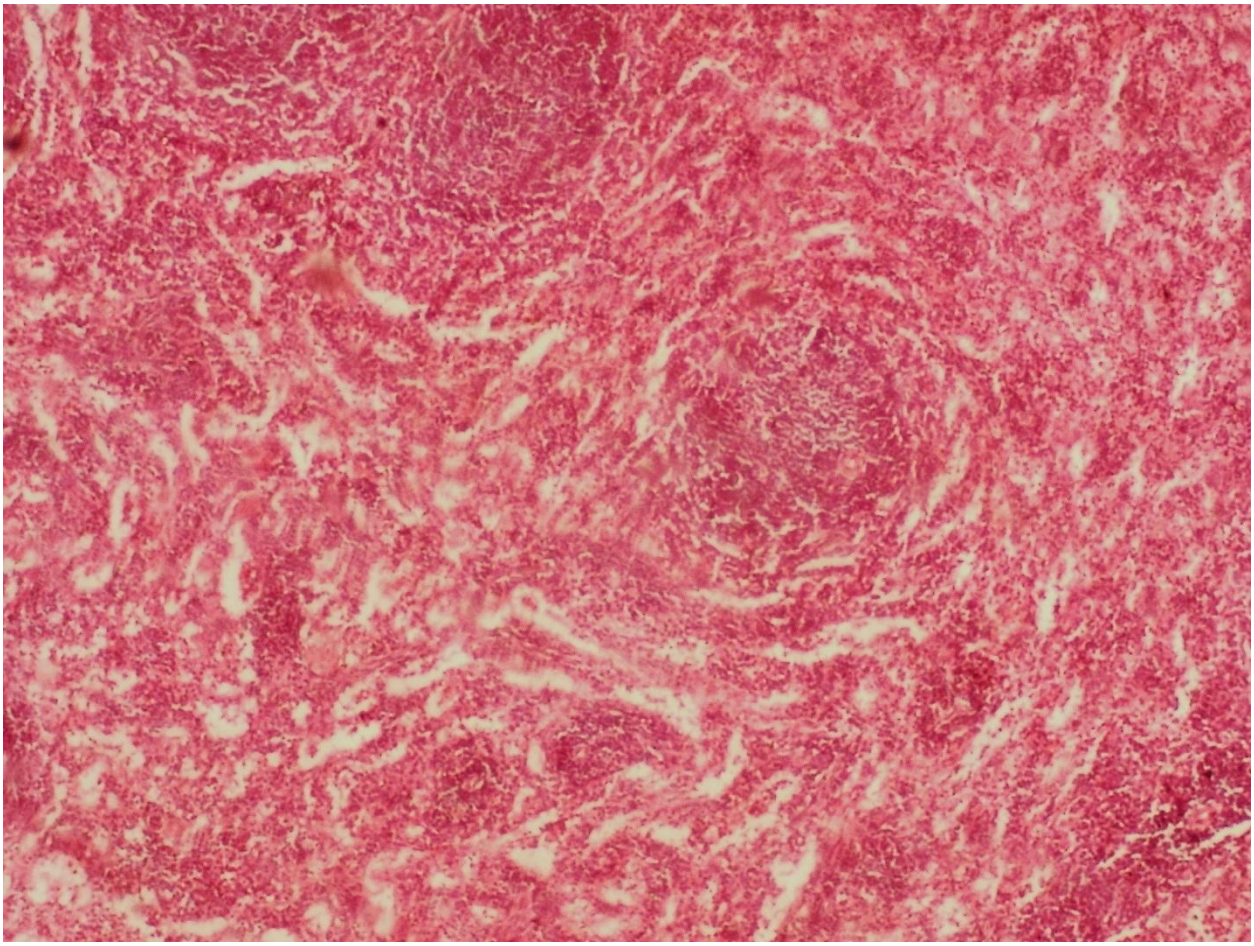


Plate 15: Photomicrograph of a section of the spleen of rabbit in the non-treated group (control). No observable microscopic lesions seen (H & E, ×400)

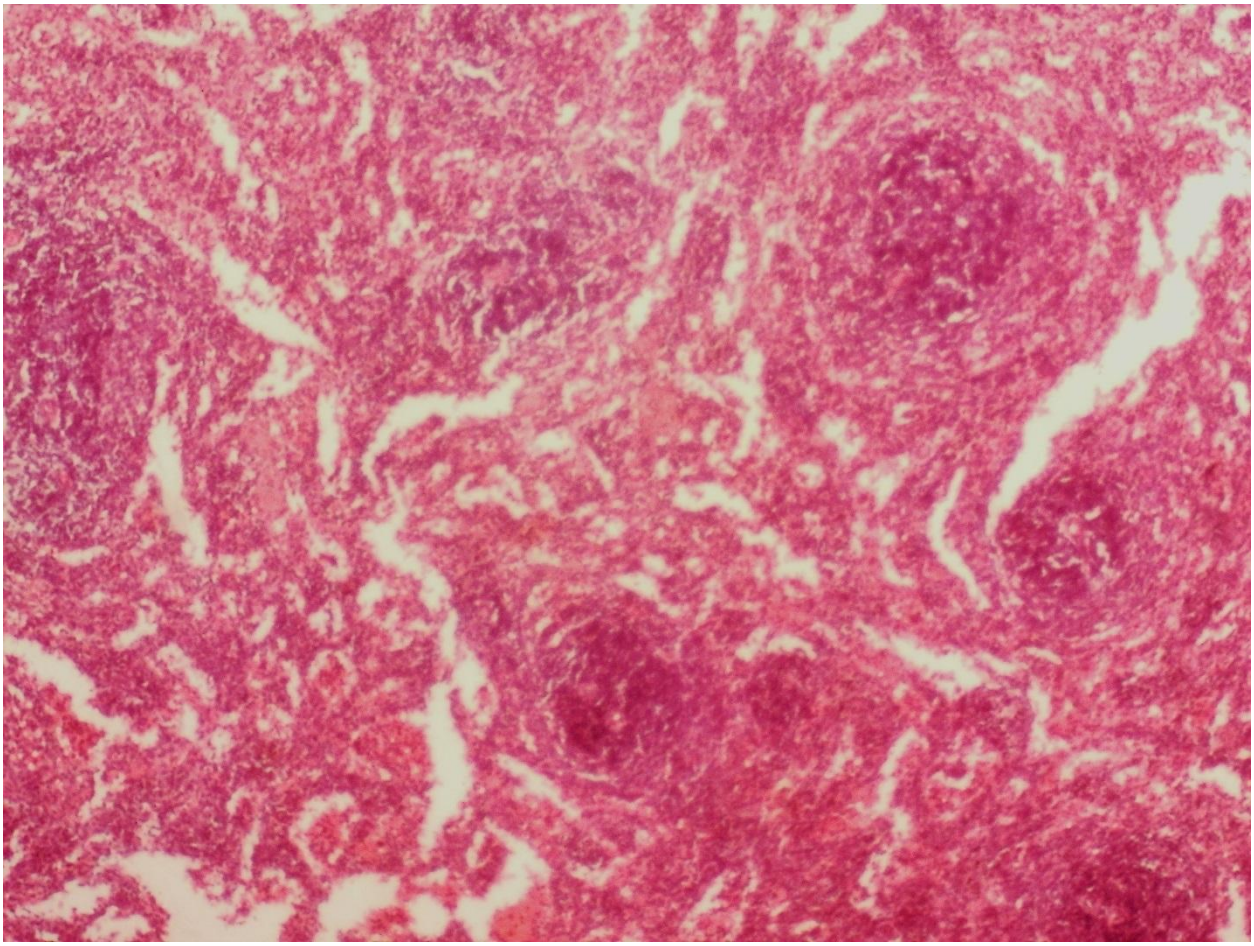


Plate 16: Photomicrograph of a section of the spleen of rabbit exposed to the 1% methanol extract of seed of *J. curcas* treated group. No observable microscopic lesions seen (H &E, ×400)

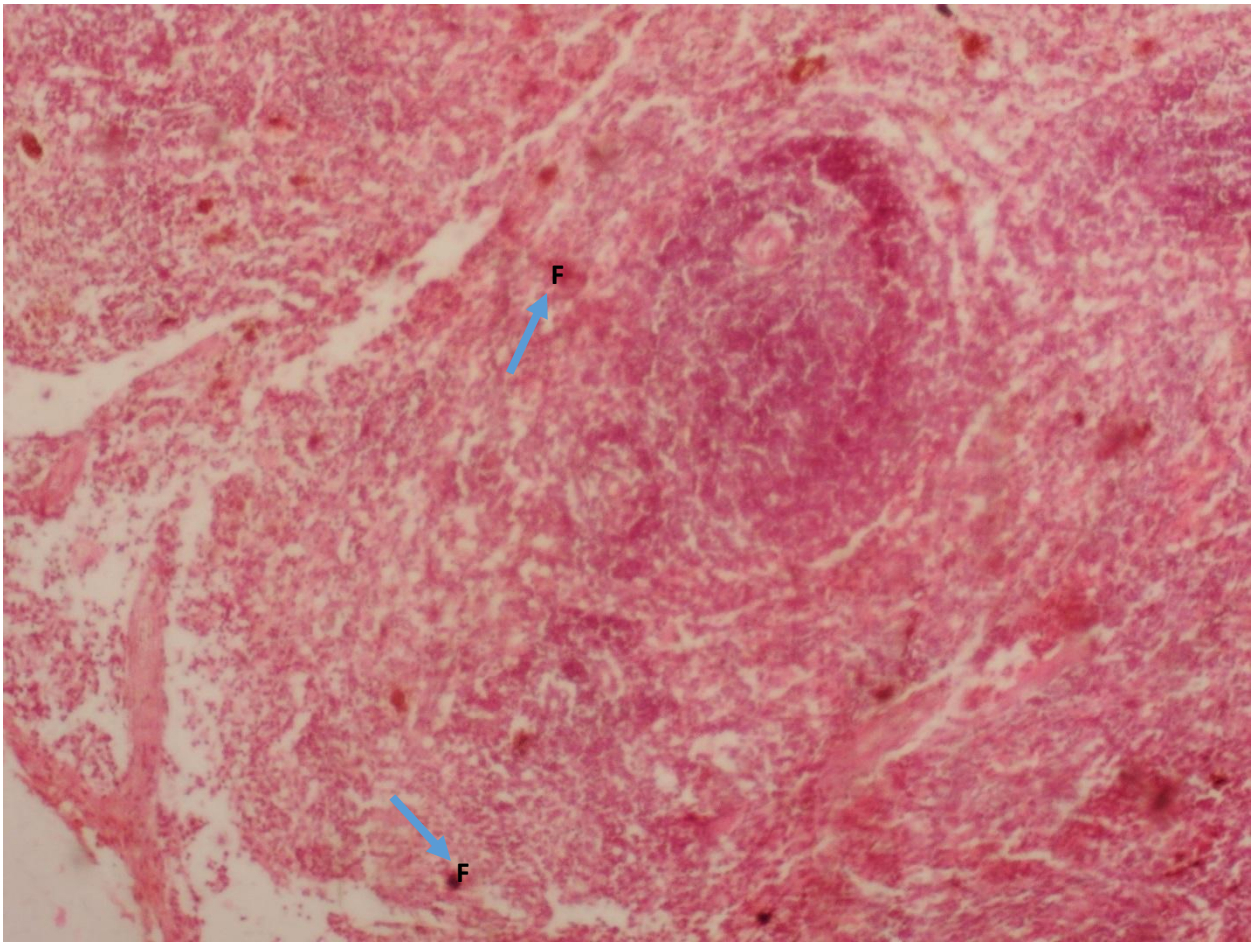


Plate 17: Photomicrograph of a section of the spleen of rabbit exposed to the 10% methanol extract of seed of *J. curcas* treated group. Note the haemosiderin deposits. (F) (H & E, ×400)

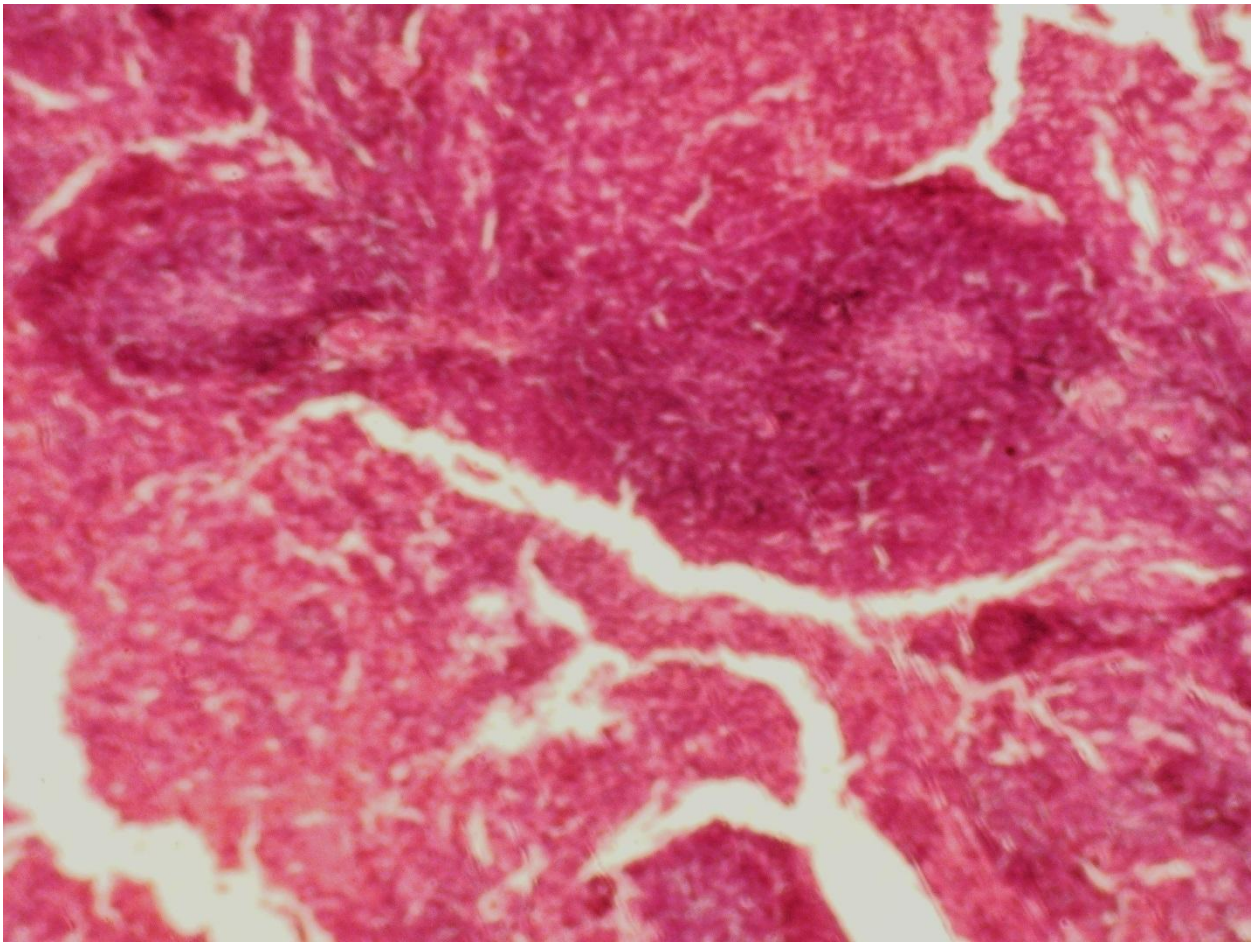


Plate 18: Photomicrograph of a section of the spleen of rabbit exposed to the 100% methanol
extract of seed of *J. curcas* treated group. No observable microscopic lesions seen
(H & E, ×400)

CHAPTER FIVE

DISCUSSION

In this study, 500g of *J. curcas* seeds was extracted using methanol as solvent giving a percentage yield of 39.27% which agrees with reports of Gubitz *et al*, (1999) and Adebayo *et al*, (2011) who obtained 39.7%, though fell short of 66.4%, recorded by Adebowale and Adedire (2006). The phytochemical screening in this study revealed the presence of carbohydrates, cardiac glycosides, saponins, tannins, steroids and terpenes, alkaloids and flavonoids. This shows that the seeds are as rich in phytochemicals as other parts of the plant as reported by other workers. This agrees with the report by Igbinosa *et al*. (2009) on stem bark extract of *J.* curcas that revealed similar findings. However, Abdulhamid *et al.* (2013) did not isolate tannins, flavonoids and phenols from *J. curcas* seed oil in a similar work done in Kebbi, Nigeria. This could be due to variation in geographical location, soil type, method of extraction and solvent system used which has been shown to affect plant metabolites (Ekpendu *et al*., 2000). Terpenes, alkaloids, tannins, flavonoids and phenolic compounds are the most effective and important bioactive compounds of plants as insecticide (Mann, 2012) and can be used to control ticks (Dimri and Sharma, 2004).

Redfren *et al*., (1982) reported that some phytochemicals act as toxicants which generally kill different life stages of the insect while various other phytochemicals interfere with growth and metamorphosis. In this study, *Jatropha curcas* seed extract exhibited acaricidal activity against *R. (Boophilus) decoloratus* larvae in a concentration and time dependent manner. This toxicity could be attributed to the presence of many chemical ingredients such as alkaloids, steroids, flavonoids, saponins, tannins and triterpenes. Alkaloids are known to possess medicinal and

pesticidal properties (Fantassi, *et al.,* 2014). These compounds have been reported in all parts of the plant but are more abundant in its seeds (Haas and Mittelbach, 2000). However several works have shown that the major factor responsible for *J. curcas* toxicity is the high concentration of phorbol esters (tetracyclic diterpenoids)in the seeds (Goel *et al*., 2007). Saponins are not easily degraded during extraction as they remain hydrophilic and may have contributed to the toxicity of the crude methanolic extracts (Rug and Ruppel, 2000). Saponins exhibit surfactant property by foaming soap-like foams, aswellas altering the permeability of cell walls and hence exert toxicity on all organized tissues (Moyo *et al.,* 2012). The antiovipositional and ovicidal effects of *J. curcas* against *Callosobruchusmaculatus* was earlier demonstrated by Adebowale and Adedire (2006) where 92% inhibition effect was reported. It is speculated that suffocation and / or lethal chemical poisoning due to Jatropha oil application prevented the adult emergence from the bruchid, *C. maculatus*. The acaricidal properties exhibited by *J. curcas* seed extract was less efficacious than that of Amitraz (Amitik®), a standard synthetic acaricide recommended by WHO, which kill larval ticks of *Rhipicephalus (Boophilus) decoloratus*) at lower concentration compared to the extract. The results obtained by use of the extract was satisfactory in exhibiting acaricidal effects.

The use of*J. curcas*methanol seed extract on rabbits recorded no mortality. The effects of seed extract of *J. curcas* used in this study differed from the work of Gandi *et al*. (1995) who reported erythema within 24 hours of application as opposed to this work in which erythema appeared on day 5 post application. This could be attributed to the fact that Gandhi used the isolated toxic fraction (phorbol esters) which has irritant effect (Adolf *et al*., 1984) as opposed to this study where crude extract was used. The common manifestations seen on topical application of (isolated) phorbol esters on rabbits, mice and rats were erythema, oedema, necrosis, diarrhea,

scaling and thickening of skin (Gandi *et al*., 1995). Some of the clinical symptoms did not manifest during the present work because it was a crude extract (not isolated phorbol ester), duration of application and the various diluted concentrations.

Haematological parameters do serve as baseline information for comparison in determining the physiological and health status of the rabbits (Daramola, *et al.,* 2005). Packed Cell Volume (PCV) which is also known as haematocrit (Ht or HCT) or erythrocyte volume fraction (EVF) is the percentage (%) of red blood cells in blood (Purves *et al.,* 2003). There was no significant difference in mean PCV values of tested rabbits compared to the control. All the variationsseen may be attributed to normal physiological changes associated with growth. This finding is similar to observations made on rabbits treated with *Triplochitonscleroxylon* extract. (Prohp *etal*., 2006, 2008).

Haemoglobin concentration (Hb) values in this study was within the range of 9.3 -19.3 g/dl which is for healthy rabbits (Mitruka and Rawnsley, 1977). This haematological constituent (Hb) reflect the physiological responsiveness of animals to their internal and external environments, which include weather, feeding, etc (Esonu *et al*., 2001). There was no significant difference in mean Hb values of test rabbits compared to the control.

The significant levels of WBC noticed here may be from the marked increase in mean WBC value on day 7 in the control group (non-treated rabbits). This elevated values may indicate the existence of recent stressors (fear from venipuncture) or probably infection usually with bacteria (Ahamefule *et al.,* 2008). However, Antai *et al*., (2009) did not observe significant effect on WBC in rats treated using *Gonglonema latifolium*. *J. curcas* extract had cardiac glycosides which elicit anti-inflammatory properties that increases WBC.

The red blood cell counts recorded in this study were within the normal range (3.7-7.5 $\times 10^6$ /mm^3) for healthy rabbits(Mitruka and Rawnsley, 1977; Hewitt *et al*., 1989). This means that the extract did not have any deleterious effect on erythropoietic tissues of the rabbits. The result agrees with the report of Adeyemo and Sani, (2013) that showed Packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC) within the values reported byMuhammad and Oloyede,(2009). Except for the WBC values on day 7 which exhibited significant differences, which is due to the marked increase in the control group, indicating some challenge probably due to stress factors in the control group.

There was no significant differences in mean AST, ALT and TP values of the test rabbits compared to the control. Thus, this established the fact that the biochemical parameters reflected the physiological status of the rabbits (Iheukwuemere *et al*, 2006). Elevations in AST, ALT and ALP are usually secondary to tissue damage. This is because such damage results in the leakage of these enzymes from their intracellular stores into plasma. ALT is most prevalent in the liver whereas AST may also be found in heart, skeletal muscles and liver to nearly the same level. Significant increases in the levels of transaminases commonly accompany liver diseases such as toxic hepatitis, acute liver necrosis or hepatic cirrhosis. Increases in AST are often seen in hemolytic anaemia, myocardial infarction and cholestatic diseases of the liver (Mayne, 1994; Wallach, 1996).

Histopathological examination of the tissues (skin, liver, kidney and spleen) in the group treated with 100% crude extract revealed hyperkeratosis of the epidermis, necrotic (destroyed) hair follicles, infiltration of the inflammatory cells, distended sinusoids with congested hepatic vessels having aggregation of inflammatory cells. Splenic vessels showed congestion and widening of the red pulp. For the other groups, the tissues showed normal histoarchitecture,

though haemosiderosis was seen in the spleen of group treated with 10% crude extract treated group in the spleen. This may be indicative of haemolytic anaemia in the rabbit involved. Orhue *etal.*, (2008) using *Jatrophatanjorensis* leaves in rabbits administered orally, reported mild necrosis of liver, kidney. In this study these lesions were not observed in these organs. This probably may be due to the route of administration, the species involved, the method of extraction, concentration of toxic principle(s) in the crude extract. Also, Azubike *etal.*, (2015) using Jatropha leaves administered orally in mice produced pathologic effects ie fatty vacuoles in the liver hepatocyte and haemorrhages in the spleen.

Thus from this work, the clinical signs exhibited show that Jatropha seed extract have phorbol esters, which may have acted singly or in combination with other toxic principles in the plant to elicits the symptoms or observations seen in the rabbits administered with the extract.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study showed that:

1. The crude extract from *Jatropha curcas* methanol seed extract contains many bioactive chemical compounds including carbohydrates, cardiac glycosides, saponins, tannins, steroids and terpenes, alkaloids and flavonoids .
2. These compounds exhibited very good in-vitro acaricidal effect on the larvae of *R.* (*Boophilus*) *decoloratus*.
3. The various concentrations of the extract showed acaricidal activity at a concentration dependent gradient and also the level of significance increased with increase in exposure time.
4. At 25mg/ml and 50mg/ml after 18h, the extract exhibited acaricidal activity as potent as that of Amitraz® the recognized acaricide.
5. LC_{50} and LC_{90} of 2.88mg/ml and 6.76mg/ml respectively were obtained after 48h.
6. *Jatropha curcas* seed extract can be safely used as an acaricide because biochemical, haematological and histopathologic findings in the experimental animals revealed no serious (grave) side effects?

6.2 Recommendations

- Results obtained from this study suggest that the compounds in *Jatropha curcas* methanol seed extract exhibit potent acaricidal activity. Thus, in the search for a cheaper,

safer, selective, biodegradable and natural acaricide, further studies needs to be done to identify the particular compound(s) causing this acaricidal activity.

- Need for further investigation and isolation of the compounds for acaricidal products development.
- Need to determine the mode of action of the active ingredients in methanol extract of *J. curcas*seed and their residual activities and their possible effects on non-target arthropods.

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APPENDICES

APPENDIX 1: Ticks in the Incubator



APPENDIX 2: Soxhlet Extraction Apparatus



APPENDIX 3: Materials Used for Larvicidal Bioassay



APPENDIX 4: *Jatrophacurcas* Plant



APPENDIX 5: Probit Analysis Calculations for the Various Hours

Probit calculation table for larvae of *R.* (*Boophilus decoloratus*) after 6 Hours of exposure to *J. curcas* methanol seed extract

Conc(mg/ml)	Log_{10} Conc	Total No. of Ticks	No. of Dead	% Mortality	Corr. % Mortality	Probit
0	-	42	2	5	-	-
6.25	0.8	59	5	8	3	3.12
12.5	1.1	60	8	13	8	3.59
25	1.4	39	23	59	57	5.18
50	1.7	53	34	64	62	5.28

Probit calculation table for larvae of *R.* (*Boophilus decoloratus*) after 12 hours of exposure to *J. curcas* methanol seed extract

Conc(mg/ml)	Log_{10} Conc	Total No. of Ticks	No. of Dead	% Mortality	Corr. % Mortality	Probit
0	-	42	2	5	-	-
6.25	0.8	59	7	12	7	3.52
12.5	1.1	60	14	23	19	4.12
25	1.4	39	34	87	86	6.08
50	1.7	53	45	85	84	5.99

Probit calculation table for larvae of *R.* (*Boophilus decoloratus*) after 18 hours of exposure to *J. curcas* methanol seed extract

Conc(mg/ml)	Log_{10} Conc	Total No. of Ticks	No. of Dead	% Mortality	Corr. % Mortality	Probit
0	-	42	2	5	-	-
6.25	0.8	59	10	17	13	3.87
12.5	1.1	60	21	35	32	4.53
25	1.4	39	36	92	92	6.41
50	1.7	53	49	92	92	6.41

Probit calculation table for larvae of *R.* (*Boophilus decoloratus*) after 24 hours of exposure to *J. curcas* methanol seed extract

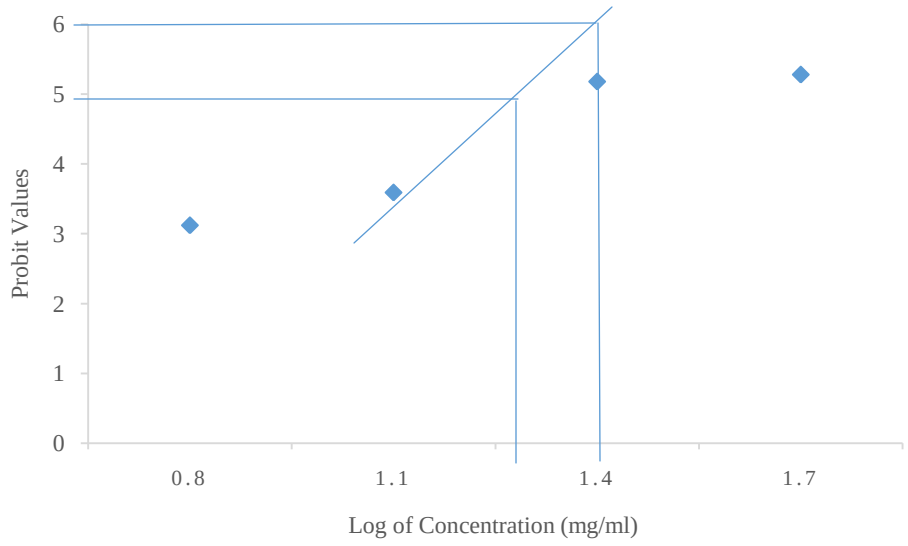
Conc(mg/ml)	Log10 Conc	Total No. of Ticks	No. of Dead	% Mortality	Corr. % Mortality	Probit
0		42	2	5		
6.25	0.8	59	17	29	25	4.33
12.5	1.1	60	28	47	44	4.85
25	1.4	39	37	95	95	6.65
50	1.7	53	51	96	96	6.75

Probit calculation table for larvae of *R.* (*Boophilus decoloratus*) after 48 hours of exposure to *J. curcas* methanol seed extract

Conc(mg/ml)	Log10 Conc	Total No. of Ticks	No. of Dead	% Mortality	Corr. % Mortality	Probit
0		42	3	7		
6.25	0.8	59	39	66	63	5.33
12.5	1.1	60	46	77	75	5.67
25	1.4	39	38	97	97	6.88
50	1.7	53	53	100	100	0

APPENDIX 6: Probit Analysis Graphical Representation for the Various Hours

APPENDIX 6.1: Probit Analysis Graphical Representation at 6 Hours

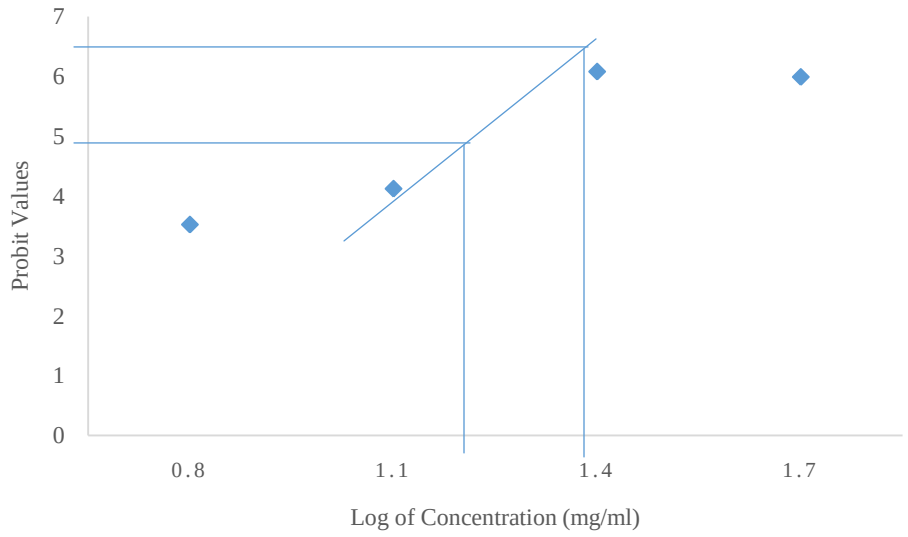


Probit Analysis for Determination of LC_{50} and LC_{90} of *Jatropha curcas* methanol seed extract exposed to *Rhipicephalus* (*Boophilus*) *decoloratus* larvae after 6 hours.

Probit Value: percentage mortality converted through probit table.

Log of concentration: the various concentration of the extracts converted through Log table.

APPENDIX 6.2: Probit Analysis Graphical Representation at 12 Hours

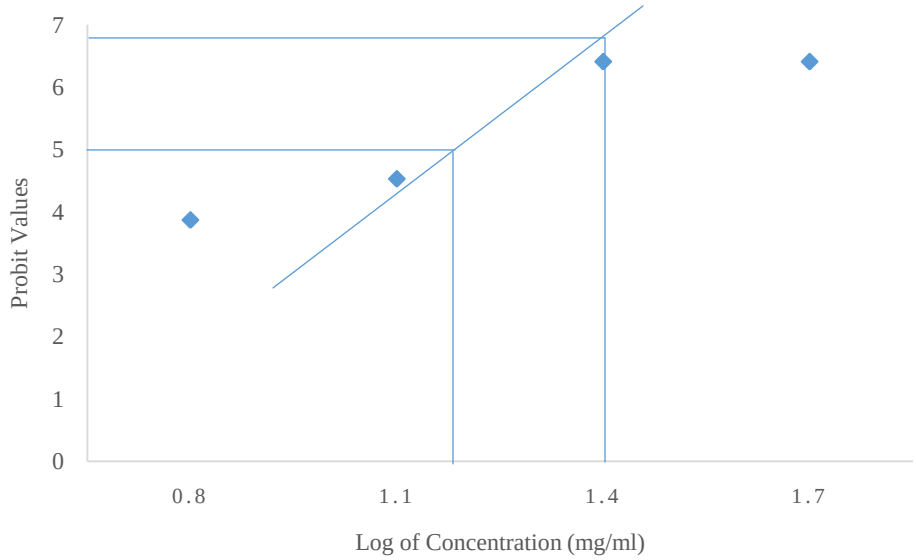


Probit Analysis for Determination of LC_{50} and LC_{90} of *Jatropha curcas* methanol seed extract exposed to *Rhipicephalus* (*Boophilus*) *decoloratus* larvae after 12 hours.

Probit Value: percentage mortality converted through probit table.

Log of concentration: the various concentration of the extracts converted through Log table.

APPENDIX 6.3: Probit Analysis Graphical Representation at 18 Hours

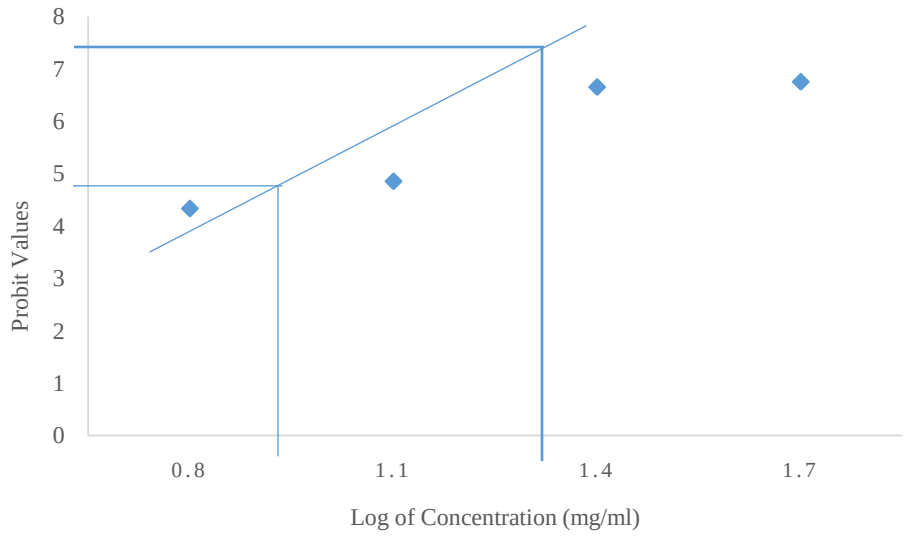


Probit Analysis for Determination of LC_{50} and LC_{90} of *Jatropha curcas* methanol seed extract exposed to *Rhipicephalus* (*Boophilus*) *decoloratus* larvae after 18 hours.

Probit Value: percentage mortality converted through probit table.

Log of concentration: the various concentration of the extracts converted through Log table.

APPENDIX 6.4: Probit Analysis Graphical Representation at 24 Hours

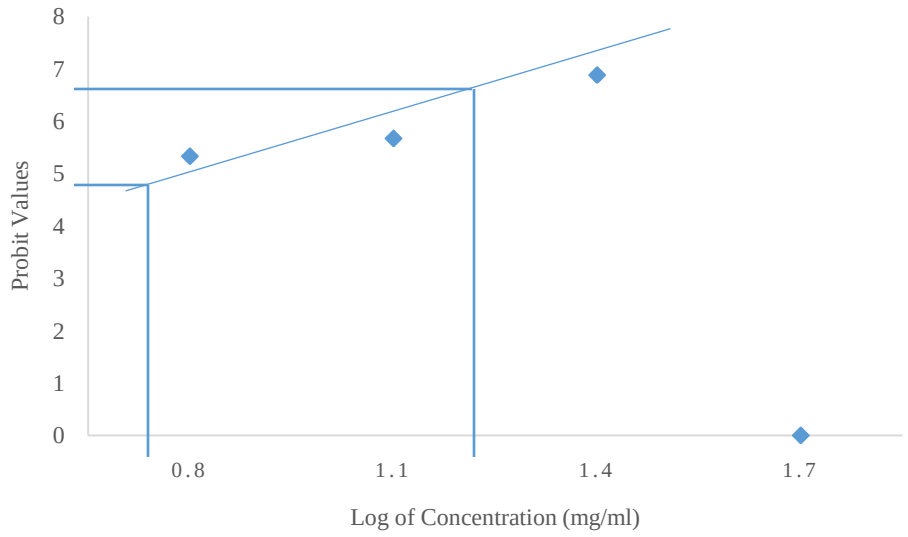


Probit Analysis for Determination of LC_{50} and LC_{90} of *Jatropha curcas* methanol seed extract exposed to *Rhipicephalus* (*Boophilus*) *decoloratus* larvae after 24 hours.

Probit Value: percentage mortality converted through probit table.

Log of concentration: the various concentration of the extracts converted through Log table.

APPENDIX 6.5: Probit Analysis Graphical Representation at 48 Hours



Probit Analysis for Determination of LC_{50} and LC_{90} of *Jatropha curcas* methanol seed extract exposed to *Rhipicephalus* (*Boophilus*) *decoloratus* larvae after 48 hours.

Probit Value: percentage mortality converted through probit table.

Log of concentration: the various concentration of the extracts converted through Log table.