HEPATOPROTECTIVE AND WOUND HEALING PROPERTIES OF METHANOLIC STEM BARK EXTRACT OF *FICUS PLATYPHYLLA* Del. Holl (MORACEA)

BY

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ZARIA – NIGERIA

MARCH, 2014

DECLARATION

I declare that the work in this thesis entitled "Hepatoprotective and wound healing properties of methanolic stem bark extract of *Ficus platyphylla* in rats" has been performed by me in the Department of Pharmacology and Therapeutics, under the supervision of Dr. A.U. Zezi, Professor I. Abdu-Aguye and Dr. A. Ahmed.

The information obtained from the literature has been duly acknowledged in the text and a list of references provided in this work. No part of this thesis has been previously presented for another degree or diploma at this or other Institution.

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CERTIFICATION

This thesis entitled "HEPATOPROTECTIVE AND WOUND HEALING PROPERTIES OF METHANOLIC STEM BARK EXTRACT OF *FICUS PLATYPHYLLA* IN RATS" by Raji Azeez Sheidu meets the regulations governing the award of the degree of Master of Science (Pharmacology) of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Abdulazeez Juniors: Muhammad Muhyseen and Muhammad Muhibb with their mother, Mrs. Nana Abdulazeez. Oh Allah! I seek your divine protection in my family.

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ABSTRACT

Ficus platyphylla (*Fp*) is a medicinal plant that is widely used in Northern part of Nigeria for the management of skin problems such as eczema and topical application of its gum (latex) to wounds. It is also used for treating malaria and psychosis. Its antioxidant, hepatoprotective and wound healing properties have been evaluated in wistar rats. The preliminary phytochemical screening of the methanolic stem bark extract of *Fp* was carried out using standard methods. The intraperitoneal and oral acute median lethal dose (LD_{50}) study of the methanolic stem bark extract of *Fp* was carried out using Lorke's method. Using spectrophotometric method the in vitro assay of 2, 2 DPPH with in vivo assay of Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) were used for the antioxidant screening. The effects of methanolic stem bark extract of *Fp* on carbon tetra chloride (CCl_4) induced hepatotoxicity in rats were evaluated using the assay of liver enzymes (Alanine amino-transaminases, ALT, Aspartate amino-transaminases, AST and Alkaline phosphatase, ALP) and histopathological studies of the liver. Excision wound healing model as described by Nayak *et al*., (2007) was used where excision wound measuring 1cm in diameter was created at the dorsal area of 30 wistar rats of both sex randomly divided into 6groups of 5 rats each. The animals in the same groups were also divided into different cages after excision so as to prevent cannibalism. Cream of methanolic stem bark extract of *Fp* was formulated in 1 %, 5 % and 10 % and applied topically with the base cream to the wounded area for 6 consecutive days and the area measured at 3 days interval for 11 days. Antimicrobial screening, zone of inhibition, minimum inhibition and minimum bacteriocidal concentrations of methanolic stem bark extract of *Fp* was

evaluated compared with Dermazin cream using agar and micro dilution methods. Phytochemical studies revealed the presence of carbohydrate, saponin, tannin, flavanoids, triterpenes and alkaloid with absence of anthraquinones. The median lethal doses (LD_{50}) in rats were found to be above 5000 mg/kg body weight orally and intra-peritoneally. The oxidative assay analysis showed significant decrease ($p < 0.001$) to prove its antioxidant activities. Also the liver enzyme analysis showed significant decrease respectively ($p < 0.05$, $p < 0.001$ and $p < 0.001$) with the histopathological study proved the hepatoprotective properties of methanolic stem bark extract of *Fp* and the protection was dose- dependent. The antimicrobial susceptibility results showed that the stem bark extract of *Ficus platyphylla* was effective against *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumonia and Salmonella typhi* with zone of inhibitions of 22, 24, 20, 27 and 20 (mm) respectively. The minimum inhibition concentrations (MIC) were 3.75 to 15 mg/L and minimum bacteriocidal concentrations (MBC) were 3.75 to 60 mg/L. Topical application of methanolic stem bark extract of *F. platyphylla* cream showed an overall significant decrease ($p < 0.001$) in wound measurement for 11 days possibly due to increased in wound retraction and reduction in the epithelization period. In conclusion these findings suggest that methanolic stem bark extract of *F. platyphylla* possess antioxidant properties, variable degree of hepatoprotection with strong wound healing effects that justify the ethno medicinal use of the plant in the management of liver disease, skin diseases like eczema and the healing of wound/ulcer when the plant is applied topically on wound.

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List of Abbreviations

AA%	Antioxidant Activities Percentages
ABUTH	Ahmadu Bello University Teaching Hospital
AL	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Amino Transaminases
AST	Aspartate Amino Transaminases
ARF	Acute Renal Failure
AOX	Antioxidant
BHA	Butylated Hydroxyanisole
B. NSc	Bachelor of Nursing Science
BHT	Butylated Hydroxytoluene
CAT	Catalase
CAM	Alternative or Complementary Medicine
cfu/ml	Colony forming unit/milliliter
CCl_4	Carbon Tetra Chloride
CKD	Chronic Kidney Disease
DNA	Deoxyribonucleic Acid
DPPH	2, 2 – Diphenyl-1-picrylhydrazyl
ECM	Extracellular Matrix
EC_{50}	Effective Concentration
EPO	Erythropoietin
Fe	Ferric Iron
FP	*Ficus platyphylla*
GPx	Glutathione Peroxide
GR	Glutathione Reductase
GSSG	Glutathione Disulfide
GSHJ	γ-Glutamycysteine Synthetase
Ha	Alternative Hypothesis
H and E staining	Haematoxylin and Eosin staining
H_2O_2	Hydrogen Peroxide

Ho	Null Hypothesis
IFCC	International Federation of Clinical Chemistry Reagent
$KBrO_3$	Potassium Bromate
LD_{50}	Median Lethal Dose
LDH	Lactose Dehydrogenase
LPO	Lipid Peroxidation
MBC	Minimum Bacteriocidal Concentration
MDA	Malondialdehyde
MIC	Minimum Inhibitory Concentration
mm	Millimeter
mg	Microgram
ml	milliliter
NADPH	Nicotinamide adenine dinucleotide phosphate
NBT	Nitro Blue Tetrazolium
NIPRD	National Institute of Pharmaceutical Research and Development
O_2^-	Superoxide anion radical
OH	Hydroxide
OH^-	Hydroxyl radical
OPC	Oligometric Proanthocyanidine
$OOCCl_3$	Trichloromethyl Peroxyl Radical
ORAC	Oxygen Radical Absorbance Capacity
PAF	Platelet Activation Factor
PCM	Paracetamol
PGE_2	Prostaglandin E_2
ROS	Reactive Oxygen Species
RNA	Ribonucleic Acid
SEM	Standard Error of Mean
SOD	Superoxide Dismutase
TBA	Thiobarbituric Acid
TB	Tuberculosis

TG	Transglutaminases
TM	Traditional medicine
TP	Total Protein
UV	Ultraviolet
US	United States
UV/VIS	Ultra-Violet Visualization
VEGF	Vascular Endothelial Growth Factor
W.H.O.	World Health Organization
>	Greater than
<	Less than
^0C	Degree Centigrade
μmol/l	Micro mole per liter

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Antioxidants play an important role of protecting the human body against the damage caused by reactive oxygen species (ROS) (Cheeseman and Scater, 2003). Reactive oxygen species are produced *in vivo* from various biochemical reactions and also from the respiratory chain as a result of occasional leakage (Donfack *et al.*, 2011). These are the main agents in lipid peroxidation which has gained a lot of recognition because of its involvement in the degenerative and pathological processes, such as aging (Burns *et al.*, 2001), cancer, coronary heart disease, Alzheimer's disease (Diaz *et al.*, 1997), neurodegenerative disorders, atherosclerosis, diabetes and inflammation (Chen *et al.*, 2006). Several anti-inflammatory, digestive, anti-neurotic, neuroprotective, and hepatoprotective drugs have recently been shown to have antioxidant and/or radical scavenging activities (Lin and Huang, 2002). The majority of antioxidant activities are due to the presence of flavones, isoflavones, flavanoids, anthocyanins, coumarin lignans, catechins and isocatechins (Aqil *et al.,* 2006).

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediate or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA. Further, some reactive oxygen species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling. In humans, oxidative stress is thought to be involved in the development of cancer (Halliwell and Barry 2007), Parkinson and Azheimer's diseases (Valko *et al*., 2007), atherosclerosis and heart failure (Singh *et al*., 1995), myocardial infarction(Ramond *et al*., 2011), fragile X syndrome (Diego *et al*., 2009), sickle cell disease (Amer *et al*,2006), ichen planus (Aly and Shahin, 2010), vitilgo (Africa and Kurutas, 2008),autism (James *et al*., 2004), and chronic fatigue syndrome (Gwen *et al*., 2005).

Liver being a vital organ in the body plays an important role in the detoxification of various drugs and xenobiotics in the body. The drug-induced liver disease accounts for 50% of all hospital admissions and 50% of all acute liver failure (McNally, 2005). It is the major cause of withdrawal of some approved drugs from the market (Ostapomicz *et al.,* 2002). It is an important target of toxicity of drugs, xenobiotics and oxidative stress. Drugs continue to be pulled from the market with disturbing regularity because of late discovery of hepatotoxicity. Such unexpected toxicities appear to be the consequences of the unique vascular, secretory, synthetic and metabolic features of the liver (Hartmurt *et al*., 2001).

Wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters none healing, under healing or even over healing of wound space. Therefore, the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences (Myer *et al.,* 1980). Growth factors like platelet derived growth factor (Mather *et al*., 1989) are necessary for the initiation and promotion of wound healing. Many substances like tissue extracts (Udupa *et al*., 1991), vitamin, minerals and a number of plants products (Dahanukar *et* *al.*, 2000) have been reported by various researchers to possess pro-healing effects. Wound healing herbs encourage blood clotting, fight infection and accelerate the healing of the wound (Raina *et al.*, 2008).

1.2 Statement of Research Problem

Numerous physiological and biochemical process in the human body may produce oxygen centered free radicals and other reactive oxygen species as by- products. Over production of this free radical can cause chronic diseases in human. This calls for the use of antioxidants but there are a number of synthetic phenolic antioxidants like the butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) being prominent examples (Vivek and Surenda, 2006). These compounds have been widely uses as antioxidants in food industry, cosmetics, and therapeutic industry. However, some physical properties of BHT and BHA such as their high volatility and instability at elevated temperature, strict legislation on the use of synthetic food additives, carcinogenic nature of some of antioxidants, and consumer preferences have shifted the attention of manufacturers from synthetic to natural antioxidants (Papas, 1999).

In the modern medicine of today 25% of drugs are directly or indirectly derived from higher plants (Singh *et al.*, 2011). The traditional system of medicine has incorporated several hepatoprotective plants like *Silybum marianum, Picrorrhiza kurroa, Tephrosia purpurea, Phyllantus amarus,* and *Asparagus racemosus* to treat hepatic body ailments (Singh *et al.*, 2011).

Many facets of wound healings require a delicate balance between oxidative stress and antioxidants under redox control. The normal physiology of wound healing depends on low levels of reactive oxygen species (ROS) and oxidative stress hence exposure to oxidative stress leads to impaired wound healing (Fitzmaurice and Isseroff, 2011).

Many plants have been identified for their antioxidant, hepatoprotective and wound healing properties by series of researches on medicinal plants but most of these medicinal plants have not been really subjected to studies in order to ascertain their effects on protecting these organs or otherwise. Such organs include the liver, kidney, spleen and the heart.

1.3 Justification of the study

Plants and plant substances have been the original medicines for humanity for most of human history. Medicinal plants have played a vital role in world health (Calixto *et al.*, 2000). Most cultures on earth have evolved rich systems of medicine focused on using plants to alleviate medical conditions from time immemorial. Modern science has recently focused on studying medical uses for plants and many plants have now been studied to justify scientifically their use in traditional medicine (Smith and Meline, 2000).

Several biological activities of the plants *Ficus platyphylla* have been reported (Sayed *et al*., 1991; Gamaniel *et al.*, 2000; Chindo *et al.*, 2003 and Danmalam *et al*., 2003), but the antioxidant, hepatoprotective and wound healings of *Ficus platyphylla* has not been studied. Studies of these effects will add more value to the therapeutic efficacy of *Ficus platyphylla.*

The pathogenesis of rheumatoid arthritis is thought to involve the production of free radicals at the side of inflammation (Dröge, 2002). Hence establishing the antioxidant property of *Ficus platyphylla* may provide pharmacological basis for its use in inflammation and pain.

In humans, oxidative stress is thought to be involved in the development of many diseases and organ damage (Shahin, 2010; Ramond *et al.,* 2011). Liver is an important target of toxicity of drugs, xenobiotics and oxidative stress (Hartmurt *et al.,* 2001).These provide rational for studying the effects of *Ficus platyphylla* on the liver and other major organs in the body.

Antioxidants are postulated to help control wound oxidative stress and thereby accelerating wound healing (Fitzmaurice and Isseroff, 2011). Antioxidants can act by scavenging reactive oxygen species thereby inhibiting their formation e.g. by blocking activation of phagocytes by binding transition metal ions and preventing formation of OH and/or decomposition of lipid hydro peroxides (Vivek and Surenda, 2006).Wound healing is also facilitated by the use of antimicrobial agents hence the need to study antimicrobial activity of *Ficus platyphylla.*

The majority of antioxidant activities are due to the presence of flavones, isoflavones, flavanoids, anthocyanins, coumarin lignans, catechins and isocatechins (Aqil *et al.*, 2006). Hence the need to determine the phytochemical constituents of *Ficus platyphylla.* This research therefore, seeks to investigate the antioxidant, hepatoprotective and wound healing properties of *Ficus Platyphylla* based on the earlier justifications.

1.3 Theoretical Framework

Around 80% of the population continues to use traditional medicine in Africa, Asia and Latin America and many governments in this region have incorporated traditional medicine practices to help meet their primary health care needs. In industrialized countries, almost half the population now regularly use some of traditional medicine (United states, 42%; France, 49%; Canada,70%) and considerable use exists in many developing countries (China, 40%; India, 70%; Chile, 71%; Colombia, 40%; up to 80% in African countries) (Bodeker and Kronenberg, 2002).

Several countries are developing Complimentary and Alternative Medicine (CAM) policies by introducing regulation, education, public financing and research funds. Higher per capita distribution of traditional practitioners in developing countries is an important reason for the widespread use of Traditional, Complimentary and Alternative Medicine (TCAM). Increase of chronic diseases, awareness about limitation of modern medicine, proven efficacy of TCAM systems in selected conditions, emerging interest in holistic preventive health, integrated approach to medical education and increasing awareness among Physicians are some of the reasons for renewed interest in traditional medicine (Bodeker *et al*., 2007).

1.5.1 Models for the studies

2, 2 – Diphenyl-1-picrylhydrazyl antioxidant assay (DPPH Assay):-

DPPH is a common abbreviation for the organic radical 2, 2 – diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder. It has two major laboratory research applications: one is a monitor of chemical reactions involving radicals most notably used in *in vitro* antioxidant assay (Sharma and Tej, 2009), and two is used as a standard to determine the position and intensity of electron paramagnetic resonance signal (Prokhorov and Fedorov, 1963).

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron that is responsible for the absorbance at 540nm and also for the visible deep purple colour. When DPPH accepts electron donated by an antioxidant compound, it is decolourized, which can be quantitatively measured from the change in absorbance (Goyal *et al*., 2010).

The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 540nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is express as effective concentration (EC_{50}) (Vani *et al*., 1997).

Superoxide oxide radical scavenging activity (SOD)

In vitro superoxide radical scavenging activity is measured by riboflavin/light/nitro blue tetrazolium (NBT) reduction. Reduction of NBT is the most popular method. The method is based on generation of superoxide radical by auto oxidation of riboflavin in presence of light. The superoxide radical reduces NBT to a blue coloured formaza that can be measured at 560nm. The capacity of extracts to inhibit the colour to 50% is measured in terms of EC_{50}. The superoxide radical can also be detected by oxidation of hydroxylamine, yielding nitrite which is measured in colorimetric reaction (Tarpey and Fridovich, 2001).

SOD destroys the free radical superoxide by converting it to peroxide that can in turn be destroyed by catalase (CAT) or glutathione peroxidase (GPx) reactions. SOD converts the highly reactive superoxide radical to the less reactive H_2O_2.

Thiobarbituric acid (TBA) or Microsomal Lipid Peroxidation assay:

TBA test is one of the most frequently used tests for measuring the peroxidation of lipids. Method involves isolation of microsomes from rats' liver and induction of lipid peroxides with ferric ions leading to the production of small amount of malonaldehyde (MDA). TBA reacts with MDA to form a pink chromagen, which can be detected spectrophotometrically at 532 nm (Lykkesfeidt, 2001).

However, the use of MDA as a marker for lipid peroxidation is controversial. MDA can be formed during eicosanoid metabolism and the analytical methods for measuring MDA are prone to artifactual errors (Janero, 1990).

Glutathione Peroxidase (GPx**) assay:**

This assay is carried out with an established procedure using hydrogen peroxide (H_2O_2) as a substrate. However, the assay can be carried out with cumene hydroperoxidase or ter-butyl hydroperoxide as the substrate instead of H_2O_2 to measure total GPx. This assay is an indirect, coupled assay for glutathione peroxidase. This assay takes advantage of glutathione disulfide (GSSG) formed by the enzymatic action of GPx and is generated by excess glutathione reductase (GR) in the assay. The action of GR is monitored by following the disappearance of the co-substrate NADPH at 340 nm. The assay recording of NADPH loss measures H_2O_2 reduction by GPx to an alcohol.

Catalase assay:-

This is measured by a spectrophotometric procedure measuring peroxide removal (Cullen *et al*., 2003). It is a direct assay with pseudo first order kinetics and is measured by the method of Beers and Sizer, 1952. The rate of peroxide removal by catalase is exponential. By the end of the assay, H_2O_2 is consumed and catalase is inactivated (Aebi and Lester, 1984). Catalase activity gels can also be used and it will be green-blue in colour with white broad band where the enzyme is present. Following separation of native protein, the catalase enzyme removes the peroxides from the area of the gel it occupies. Removal of peroxide does not allow for the potassium ferricyanide (a yellow substance) to be reduced to potassium ferrocyanide that reacts with ferric chloride to form a Prussian blue precipitate (Treadwell and Hall, 1948).

1.5.2 Hepatoprotective models

Carbon tetrachloride (CCl_4) model:-

Reactive Oxygen Species (ROS) and oxidative stress over-production can be initiated by a variety of factors, including exposure to xenobiotics such as acetaminophen, lead poisoning and carbon tetrachloride (CCl_4) (Ronsein *et al.*,2005). CCl_4 model is a reductive halogenations by cytochrome P450 2EI to the highly reactive trichloromethyl radical (CCl_3), which is subsequently converted into a trichloromethyl peroxyl radical ($OOCCl_3$) in the presence of oxygen (Goeptar *et al*., 1995). Removal of hydrogen atoms from the unsaturated fatty acids by the radicals tends to generates carbon-centered lipid radicals. The lipid radicals quickly add molecular oxygen to form lipid peroxyl radicals, thereby initiating the process of lipid peroxidation (LPO). Unless neutralized by radical scavengers, these peroxyl radicals in turn abstract hydrogen atoms from other lipid molecules, thereby propagating the process of lipid peroxidation (Recknagel *et al*., 1991).

The analgesic acetaminophen (paracetamol) causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose (Laura *et al*., 2003). It is activated by cytochrome P450 enzymes to a reactive metabolite that depleted glutathione (GSH) and covalently bound to protein. The repletion of GSH prevented the toxicity. More recently, the cytochromes 2E1, 1A2, 3A4, and 2A6 (Patten *et al*., 1993; Thummel *et al*., 1993; Chen *et al*., 1998) have been reported to oxidize acetaminophen to the reactive metabolites. Paracetamol has become a model toxin and a tool in biochemical and clinical toxicological research also; it is used as a model toxin for establishing the usefulness of in vitro models such as measuring the hepatoprotective activity of certain herbs indicated in ancient systems of medicine (Raman, Kumar and Surya, 2013).

Lead (Pb^{++}) is an environment pollutant which causes disturbances in the natural balance of the ecological system. Exposure to lead (Pb^{++}) mainly occurs from automobile exhaust in areas where leaded gasoline is still used and from drinking waters in areas where lead pipes are being used (Manuj, 2013). Pb^{++} is an environmental pollutant and metabolic poison with a variety of toxic effects with possible mechanism of its toxicity is the disturbance of pro-oxidant and antioxidant balance by generation of reactive oxygen species (ROS) (Manuj, 2013; Gurer and Ercal, 2000).This can evoke the oxidative damage of critical biomolecules such as lipids, proteins, and DNA. Exposure to lead is a dose-response relationship with changes in antioxidant enzyme levels and their activities (Wang *et al*., 2000). Pb++ is used as a hepatic toxicity model by exposing the animals to drinking water that contain the lead acetate thereafter measuring the effectiveness of certain drugs (e.g. Glycine) in treating the experimental lead intoxication using *in vitro* model (Contreras *et al*., 2011).

1.5.3 Wound healing model

Excision model:-

This involves the removal of a significant volume of the target tissue, and the filling of the void created allow more ample materials for determination of biochemical and histological parameters. The excision site can be biopsied to obtain cells, tissue, RNA, exudates, and histological specimens that have much more ample cross-sectional area and volume. Excision wounds can be covered with occlusive dressings, which retain the exudates as a means of assessing the status of various soluble factors in the wound environment, such as nutrients, procteinase, cytokines, and tissue degradation products.

This model is used to monitor the wound contraction and wound closure time. Wound contraction is being calculated as percentage reduction in wound area (Saha *et al.*, 1997; Nayak *et al*., 2007)

Incision model:-

This is the cutting of the skin or other tissue with a sharp blade resulting in rapid disruption of tissue integrity with minimal collateral damage. Other cutting devices like electrocautery blades and laser surgical devices may produce greater collateral damage but less bleeding. The amount of gape in the incision will depend on the factors like the amount of subcutaneous fat, the tensional forces on the wound site (i.e. the orientation of the incision), and the species.

In this model, incisions were made through the entire thickness of the skin. Here, surgical sutures are usually applied to the parted skin after the incision. Surgical were usually removed 10 days post incision and tensile strength measured.

$$\text{Wound contraction (100\%)} = \frac{\text{Initial wound size} - \text{Specific wound day size}}{\text{Initial wound size}} \times 100$$

Epithelization period is the measurement taken from the initial day to the day when the scab fell off from the wound surface exclusive of leaving a raw wound behind (Rashed, Afifi and Disi, 2003).

1.6. Aim and Objective

The main aim of the study was to scientifically evaluate the hepatoprotective and wound healing properties of methanolic stem bark extract of *Ficus platyphylla*.

1.6.1 Specific objectives.

The specific objectives for this study are:-

i. To establish the presence of phytochemical constituent of methanolic stem bark extract of *Ficus platyphylla.*

ii. To establish the safety of the use of *F. platyphylla* in traditional medicine.

iii. To evaluate the hepatoprotective properties of methanolic stem bark extract of *F. platyphylla* in wistar rats.

iv. To suggest possible mechanism of the wound healing and hepatoprotective properties of methanolic stem bark extract of *F. platyphylla*.

v. To evaluate the effects of the extract on the rat organs such as kidney, heart and spleen.

1.7 Research Hypothesis

Ho (Null Hypothesis):- The methanolic stem bark extract of *F. platyphylla* contains active constituents that are responsible for hepatoprotective and wound healing properties.

Ha (Alternative Hypothesis):- The methanolic stem bark extract of *F. platyphylla* does not contain active constituent that are responsible for hepatoprotective and wound healing properties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional Medicine and Medicinal plant

Traditional medicine is a cultural gem of various communities around the world and encompasses all kinds of folk medicine, unconventional medicine and indeed any kind of therapeutically available method that had been handed down by the tradition of a community or ethnic group (Adesina, 2013). Traditional medicine has been in use for thousands of years throughout the world for the treatment of illnesses. It has remained as the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities (Haile and Delenasaw, 2007).

The use of plants for medicinal purposes dates back to antiquity and has been very important in the health care delivery of nearly every nation (Oluduro and Omoboye, 2010). It is estimated by the World Health Organization that approximately 75-80 % of the world's population uses plant medicines either in part or entirety (Stephen, 1999). Plants are collected when available and are preserved usually by drying to eliminate moisture. Herbs were the first medicine used by pre- historic man. They are, therefore, part of every culture and tradition and have helped the development and growth of herbalism in Nigeria (Adesina, 2013).

Traditional medicine (TM) according to WHO,(1996) refers to the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness. It covers a wide variety of therapies and practices which vary from country to country and region to region. In some countries, it is referred to as "alternative or complimentary" medicine (CAM). Plants have been used in traditional medicine for several thousand years. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as *Ayurveda, Unani* and *Siddha*. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine (Pei, 2001).

Several plants with high antioxidant potentials shows significant dose-dependent hepatoprotective action against carbon tetra chloride (CCl_4) induced liver damage in both decreasing the levels of transaminases and bilirubin and in reducing the extent of morphological malformations of the liver (Radulovic *et al.*, 2012). Besides these properties, plants with antimicrobial action against several human pathogens in relation to its antioxidant activities especially in lipid peroxidation in reactive oxygen species had contributed greatly in wound healing potential of the plants (Raina *et al*., 2008).

According to Stephen (1999), the following plants proved to be important sources of potential therapeutic agents:-

i. *Allium sativum* (Garlic) which has been shown to reduce cholesterol by about 12% and reduce platelet adhesiveness.

ii. *Ginkgo biloba* - flavones glycosides ("ginkgolides") have been shown to reduce capillary fragility and decrease blood cell transit from inflamed capillaries. They have been shown to reduce "PAF"-platelet activating factor. These glycosides are also potent anti-oxidant

iii. *Tanacetum parthenium* (Fever few) contain parthenolides, a“sesquiterpene lactones”, which acts as a serotonin antagonist, inhibiting release of serotonin from platelets. Quite effective at lowering the frequency and severity of migraine headache.

All the plants listed above and numerous others not listed have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these photochemical have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases.

2.2 Antioxidants

Plants are potential sources of natural antioxidants. They produce various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive (Lu and Foo, 1995). In recent years much attention has been devoted to natural antioxidant and their association with health benefits (Arnous, *et al*., 2001). The terminal oxidant essential for respiration and other oxidative reactions in aerobic organisms is oxygen, which currently constitutes 21% of the atmosphere. During the reduction of molecular oxygen, reactive oxygen species (ROS) may be formed, including oxygen-centered free radicals (Anderson, 1999). These reactive species are implicated in many diseases including atherosclerosis, respiratory tract disorders, neurodegenerative diseases (Chen *et al*, 2006), inflammatory bowel disease, cancer and also in ageing (Burn, 2001).

Any atom or molecule that contains one or more unpaired electrons is a free radical. The unpaired electrons alter chemical reactivity, usually making the radical more reactive than the corresponding atom or molecule. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic (Mon *et al*., 2011). The free radicals are very reactive oxygen species ($O\text{-}O^-$,O-, ROO^-) produced as a result of an imbalance in the pro-oxidant/antioxidant homeostasis in the organism during the chemical reactions that contribute to the development and the maintenance of the cellular life (Mon *et al*., 2011).

In living tissues, ionizing radiation can split water to generate (the hydroxyl radical OH^-), which is extremely reactive at the site of formation. Many enzymatic and antioxidation reactions within the body produce superoxide anion radical (O^-_2), by addition of an electron to molecular oxygen. For example, phagocytic cells such as neutrophils, monocytes, macrophages and eosinophils, generate superoxide as part of their response to foreign organisms or particles. In chronic inflammation, this normal protective mechanism may become damaging (Grisham, 1994). Superoxide is poorly reactive but can take part in further reactions leading to the formation of more reactive species. For example, two superoxide radicals can combine to form hydrogen peroxide (H_2O_2) which can react with reduced metal ions (e.g. Fe^{2+}) to form OH. Superoxide may constitute further by reducing the metal ions which have become oxidized during the reaction (Mon *et al*., 2011).

Another physiological free radical is nitric oxide (NO^-), which is also made by phagocytes as well as the vascular endothelium, as a relaxing factor (Moncada and Higgs, 1993). Superoxide can react with nitric oxide to produce peroxynitrite

($O_2^- + NO \rightarrow ONOO^-$) which decomposes into toxic products including OH, nitrogen dioxide and nitronium ion (NO_2^+).

Reactive radicals such as OH^- attack all biological molecules including DNA, proteins and lipids. Lipid and lipoproteins are particularly susceptible to attack because hydrogen abstraction by a radical can initiate a devastating chain reaction, lipid peroxidation, which is implicated in atherosclerosis development (Witzum, 1994).

Under normal conditions, the body is equipped with defense mechanisms that scavenge reactive oxygen species (ROS) and protect the cell from oxidative damage. Antioxidants (AOX) are considered a promising therapeutic approach as they may be playing neuroprotective (preventing apoptosis) and neurodegenerative roles (Mon *et al*., 2011). The main characteristic of an antioxidant is its ability to trap free radicals.

In broader sense, antioxidants are grouped as exogenous and endogenous. The most important exogenous antioxidant is dietary phytochemical (such as polyphenols, quinones, flavanoids, catechins, coumarin, terpenoids) and the smaller molecules like ascorbic acid (vitamin C), alpha-tocopherol (vitamin E) and beta-carotene (Vitamin A). The endogenous group includes enzymes and trace elements (like superoxide dismutase, catalase, Zn, Mn and Cu,) and glutathione peroxide (Se). Proteins like albumin, transferring, ceruloplasmin, metallothionein and haptoglobin have antioxidant activity. The antioxidant processes occur in cytosol, mitochondria or in plasma (Mon *et al*., 2011).

Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is trend to substitute them with naturally occurring antioxidants. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing these free-radical-induced tissue injuries. Many plant extracts and phytochemical have shown to have free radical scavenging properties (Birdi *et al.*, 2006) but generally there is still a demand to find more information concerning the antioxidant potential of plant species.

Antioxidants enhance the healing of infected and non-infected wounds by reducing the damaged caused by oxygen radicals (Martin, 1996). Plant derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins, and proantocyanins could delay or prevent the onset of degenerative diseases because of their redox properties, which allow them to act as hydrogen donors, reducing agents, hydroxyl radicals (OH), or superoxide radicals (O_2) scavengers (Somashekar *et al.*, 2008).

2.3 Hepatic Protection

Liver play a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents (McNally, 2006). It is an important target organ of toxicity of drugs, xenobiotics and oxidative stress (Hartmurt *et al.*, 2001). It is important in the detoxification of foreign substances, secretion of bile for digestion, and in the metabolic functions of various nutrients including carbohydrates, proteins and fats (Mohammed *et al.*, 2010). Liver plays a major role in the removal of substances from the portal circulation which makes it susceptible to first and persistence attack by offending foreign compounds, culminating in liver dysfunction (Bodakhe and Ram, 2007). Liver diseases remained one of the major threats to public health and are a worldwide problem (Asha and Pushpangadan, 1998). Hence, chronic liver injury has serious medical consequences. A common chronic disease known as liver fibrosis may lead to end stage liver cirrhosis and liver cancer (Pelletier *et al*., 2009). Excessive consumptions of alcohol and viral infections are the most common risk factors for liver diseases in developed countries while environmental pollution, hepatic viruses, parasitic infections and chemotherapeutics are the main factors known to cause hepatic damage in developing countries (Alshawsh *et al*., 2011).

Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSHJ levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated (Mascolo *et al*., 1998).

Inspite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases (Maheswari *et al*., 2008). There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. Elimination of risk factors and alleviation of liver fibrosis are the most common approaches to prevent liver termination (Bataller and Brenner, 2005). Therefore, there is an urgent need for safe alternative therapeutics to treat liver pathology. Many natural products are being targeted for liver disease prevention and/or treatment (Muhammed *et al*., 2010). Liver protective plants contain a variety of chemical constituents like phenols, coumarins, monoterpens, glycosides, alkaloids and xanthenes (Bhawna and Kumar, 2009).

2.3.1 Hepatoprotective activity of some crude plant extracts

The aetiology of liver disease is diverse and a variety of plants has been reported in different journals to show hepatoprotective activity and this may prove efficacious for the management/treatment of liver diseases. Some of these plants are stated below:

Apium graveolens L. grows wild at the base of the North Western Himalayas and outlying hills in Punjab and in Western India. The seeds are used in India to treat bronchitis, asthma, liver and spleen diseases. Its hepatoprotective effect was tested against paracetamol and thioacetamide induced liver injury in rats. The results obtained after oral administration of the methanolic extract of the seeds of *A. graveolens* suggest that this plant has hepatoprotective action which may be due to its role as a membrane stabilizer (Singh and Handa, 1995)

Suja *et al*. (2004) reported the effect of the methanolic extract of *Heminthostachys zeylanica* L., hook rhizomes on carbon tetrachloride (CCl_4) induced liver damage in wistar rats. The results showed that significant hepatoprotective effect was obtained against CCl_4 induced liver damage, by oral administration of *H. zeylanica* methanolic extract as evident from decreased levels of serum enzymes and an almost normal architecture of the liver, in the treated groups, compared to the controls. This provides a rationale for the use of the plant in the management of liver diseases (Adewusi and Afolayan, 2010). Also a hepatoprotective activity of the leaf extract of *Achornea cordifolia* on acetaminophen induced toxicity *in vivo* has been reported (Olaleye *et al*., 2006).

Several other plants have been shown to have hepatoprotective properties like *Adansonia digitata* (Al-Qarawi *et al*., 2003), tuber roots of *Daucos carota* L (Bishayee *et al*., 1995), *Cassia occidentalis* L. (Jafri *et al*., 1999).

2.4 Renal Protection

Kidneys are the main part of the urinary system and are made up of millions of nephrons that act as individual filtering units and are complex structures themselves. They are organs that serve essential regulatory roles in most animals, including vertebrates and the invertebrates. The kidneys are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance and regulation of blood pressure (via maintaining salt and water balance). They serve the body as a natural filter of the blood, and remove wastes which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium, and they are also responsible for the reabsorption of water, glucose, and amino acids.

In addition to removing wastes, the kidneys release three important hormones namely:-

1. Erythropoietin, or EPO, which stimulates the bone marrow to make red blood cells.
2. Renin, which regulates the blood pressure

Calcitriol is the active form of vitamin D, which helps maintain calcium for bones and normal chemical balance in the body (Joshua, 2013).Most kidney diseases attack the nephrons, causing them to lose their filtering capacity. Damage to the nephrons can happen quickly, often as a result of injury or poisoning. But most kidney diseases destroy the nephrons slowly and silently. The two main causes of kidney diseases are the diabetes and high blood pressure. People with a family history of any kind of kidney problems are also at risk for kidney disease (Joshua, 2013).

2.4.1 Renal diseases

Acute renal failure (ARF) carry a mortality risk of 35%-70% with the higher risk found in those with co-morbidities, increased age, and oliguria (Brivet *et al*., 1996). Although ARF in the acutely ill patient may result from vascular, glomerular, interstitial, or obstructive injury, ischemic tubular injury is the final common pathway in the majority of cases. On these grounds “renal protection is clinically attractive concept and implies that renal failure is potentially reversible or preventable.

Diabetes is another disease that keeps the body from using glucose, a form of sugar. If glucose stays in the blood instead of breaking down, it can act like a poison. Damage to the nephrons from unused glucose in the blood is called diabetes kidney disease. Others like hypertension, glomerular disease, inherited and congenital kidney diseases, chronic kidney disease (CKD), etc have been identified clinically as diseases of the kidney.

Renal fibrosis is the principal process involved in the progression of chronic kidney diseases (Pradere *et al*., 2008), ureteral obstruction, malignant hypertension, severe diabetics condition, or chronic exposure to heavy metals (Al-Bayati *et al*., 2002). The development of renal fibrosis involves the progressive appearance of glomerulosclerosis, tubulointerstitial fibrosis, and changes in renal vasculature (Padere *et al*., 2008). At molecular levels, fibrosis can be defined as an excessive accumulation of extracellular matrices such as collagen and fibronectins (Padere *et al*., 2008). The presence of kidney fibrosis seems mostly to be viewed as an end point or marker of tissue or organ failure and loss of function (Cohen, 1995)

Many plants have been studied to have reno-protective properties. Research had showed the quality of bilberry extract in protecting the organism from renal damage caused by potassium bromated ($KBrO_3$) (Milan, 2008). Potassium bromate is an environmental pollutant, which can be formed as a by-product in the process of ozone purification of drinking water. Recent concerns have arisen from its discovery in bottles of mineral water both in the US and Nigeria. In addition to fears of carcinogenesis, potassium bromate may be toxic in other areas, affecting the kidneys in particular. The substance in fact causes the formation of free radicals which, in turn, many trigger harmful modification to cell membranes, proteins, enzymes and kidney cell DNA (Milan, 2008). Bilberry extract, by virtue of its exceptional properties, proved effective in reducing the kidney levels of various oxidative stress markers and increasing the capacity to absorb free radicals (Oxygen radical absorbance capacity- ORAC) in renal tissue (Milan, 2008).

Also chronic exposure to Camium often causes damage to the kidney (Siddique, MF. 2010). *Momordica charantia* which is tendril bearing economically important medicinally vine belonging to the family *Cucurbitaceae* is a vegetable widely cultivated in tropical areas including West Africa and East Africa and have been shown to have reno-protection from the result of its serum urea and creatinine, and its histological examinations (Ajilore, *et al*; 2012)

Extract of Ginger has been showed to ameliorate kidney function parameters (Hamed *et al*, 2012). It is showed that treatment with ginger extracts after the selected rats have been injured with CCl_4 resulted in markedly decreased levels of LPO, PGE_2, Collagen and Kidney function tests, while increased levels of Glutathione (GSH), Superoxide Dismutase (SOD) and serum protein were also observed (Hamed *et al*., 2012).

2.5 Plants with Antimicrobial properties

An antimicrobial is an agent that kills microorganisms or inhibits their growth (Marjorie, 1999). Antimicrobials agents can be grouped according to the organisms they act primarily against. For example, antibacterial (commonly known as antibiotics) are used against bacterial and antifungal are used against fungi. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Marjorie, 1999). In many cases, these substances serve as plant defense mechanisms against predation by micro-organisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment.

Scientists are in search of new phytochemicals that could be developed as useful anti-microbial for treatment of infectious diseases. Currently, out of 80% of pharmaceuticals derived from plants, very few are now being used as anti-microbial (Ramar and Ponnampalam, 2010). Medicinal plants represent a rich source of antimicrobial agents. A wide range of medicinal plant parts (like root, stem, flower, fruit, twigs exudates and modified plant organs) are used for extracts as raw drugs and they possess varied medicinal properties (Mahesh and Satish, 2008). They are used to eliminate the infecting micro-organisms. The useful part of the plant should inhibit the micro-organism and exhibit greater selective toxicity towards the infecting micro-organism than the host cells. The mode of action for plant-derived agents should target biochemical features of the invading pathogens that are not possessed by the normal host cell. Sensitivity of the infecting micro-organism to a particular agent is some of the factors important for anti-microbial treatment (Ahmad and Beg, 2001).

2.5.1 Trends in the evaluation of Anti-microbial properties

Antibacterial screening of traditional medicinal plants has been the source of innumerable therapeutic agents. Random screening as a tool of discovering new biologically active molecules has been most productive. Chemotaxonomic considerations and target-directed screening also play a crucial role. Access to a large number of compounds/extracts must be well screened for a successful outcome. For example, ethanol extracts of 78 traditional medicinal plants from India are said to be useful for treating infectious diseases and this show bacterial and fungal activity at 1.6mg/ml. Antimicrobial and phytochemical studies revealed 45 Indian medicinal plants effective against multi-drug-resistance bacterial (Ahmad and Beg, 2001).

2.6 Wound Healing Properties

Wound healing whether from accidental injury or surgical intervention, involves the activity of an intricate network of blood cells, tissue types, cytokines, and growth factors. It involves a complex series of interactions between different cell types, cytokines mediators, and the extracellular matrix. It is a dynamic and complex process involving several events that will lead to blood clotting, healing of the wound area, and lastly closure of the wound (Pan, 2010). The cascade phases of normal wound healing include homeostasis, inflammation, proliferation, and remodeling. Each phase of wound healing is distinct, although the wound healing process is continuous, with each phase overlapping the next (Douglas and Alan, 2003).

Wound repair must occur in a physiologic environment conducive to tissue repair and regeneration. However, several clinically significant factors are known to impede wound healing, including hypoxia, tumors, metabolic disorders such as diabetes mellitus, the presence of debris and necrotic tissue, certain medications, and a diet deficient in protein, vitamins, or minerals. In addition, increased metabolic demands are made by the inflammation and cellular activity in the healing wound, which may require increased protein or amino acids, vitamins, and minerals (Stadelmann *et al.*, 1998).

Haemostasis and fibrin formation contribute to the formation of a wound scab following skin damage. This process facilitates repair by providing a matrix within which cell migration and angiogenesis takes place (Van Hinsbergh 2001).

Following injury, macrophages, which are normally present in tissue, increase in number, attracted by chemical messages released by the activation of the inflammatory process. They released the protein chemical messages, growth factors and growth stimulants needed to orchestrate the healing process. Macrophages have an essential role in the transition from wound inflammation to wound repair, the latter being characterized by the formation of granulation tissue (Clark, 1985).

Angiogenesis plays a central role in wound healing. Among many know growth factors, vascular endomethelial growth factor (VEGF) is believed to be the most prevalent, efficacious, and long-term signal that is known to stimulate angiogenesis in wounds

(Savita Khanna *et al.*, 2002). The wound site is rich in oxidants, such as hydrogen peroxide, mostly contributed by neutrophils and macrophages.

2.6.1 Phases of wound healing

Tissue injury initiates a response that first clears the wound of devitalized tissue and foreign material, setting the stage for subsequent tissue healing and regeneration. The initial vascular response involves a brief and transient period of vasoconstriction and homeostasis. A 5-10 minute period of intense vasoconstriction is followed by active vasodilatation accompanied by an increase in capillary permeability. Platelets aggregated within a fibrin clot secrete a variety of growth factors and cytokines that set the stage for an orderly series of events leading to tissue repair (Douglas and Alan, 2003).The second phase of wound healing, the inflammatory phase, presents itself as erythema, swelling, and warmth, and is often associated with pain. The inflammatory response increases vascular permeability, resulting in migration of neutrophils and monocytes into the surrounding tissue. The neutrophils engulf debris and micro-organisms, providing the first line defense against infection. Neutrophils migration ceases after the first few days post-injury if the wound is not contaminated. If this acute inflammatory phase persists, due to wound hypoxia, infection, nutritional deficiencies, medication use, or other factors related to the patient's immune response, it can interfere with inflammatory phase (Stadelmann et *al.*, 1998).In the late inflammatory phase, monocytes converted in the tissue to macrophages, which digest and kill bacterial pathogens, scavenge tissue debris and destroy remaining neutrophils. Macrophages begin the transition from wound Inflammation to wound repair by secreting a variety of chemotactic and growth factors that stimulate cell migration, proliferation, and formation of tissue matrix (Stadelmann *et al.*, 1998).

The proliferation phase is dominated by granulation tissue and epithelialization formation. The extent and its duration depend on the size of the wound. Growth factors and chemotactic released from platelets and macrophages stimulates the migration and activation of wound fibroblasts that produce a variety of substances essential to wound repair, including glycosaminoglycans (mainly hyaluronic acid, chondroitin-4-sulphate, dermatan sulphate, and heparin sulphate) and collagen (Stadelmann et al., 1998). These form an amorphous, gel-like connective tissue matrix necessary for cell migration (Douglas and Alan, 2003).

The final phase of wound healing is wound remodeling, including reorganization of new collagen fibers, forming a more organized lattice structure that progressively continues to increase wound tensile strength. The remodeling process continues up to two years, achieving 40-70% of the strength of undamaged tissue at four weeks (Stadelmann *et al.*, 1998).

Wound healing may be facilitated by oxygen therapy and topical oxygen involves local administration of pure oxygen. Antioxidants can act by scavenging reactive oxygen species thereby inhibiting their formation e.g. by blocking activation of phagocytes by binding transition metals ions and preventing formation of OH and/or decomposition of lipid hydro peroxides, by repairing damage(e.g. α- tocopherol repairing peroxyl radicals and so terminating the chain reaction of lipid peroxidation) (Vivek, 2006)

2.6.2 Wound healing progress monitoring

Wound healing progression depends solely on the depth, size, position, and cause of the wounds, as well as the physiological conditions of the animal or individual. The dorsal and dermal area used in this study for the wound excision was used in order to avoid excessive bleeding, severe infection, and possible complication due to the wound creation in deep tissue.

The early phase of the healing process involves homeostasis over the wound site, which provides protection for region being damaged and to prevent further damage. Plasma transglutaminases (TG) which are cross-linking enzymes that are involved in several phases of wound healing process and they are Factor XIIIa plays a significant role in the initial extracellular matrix during wound healings (Telci and Griffin, 2006). After the acute inflammatory response, the healing process starts to advance to the proliferation phase. In this phase, the epithelial cells start to express TG2 in order to initialize re-epithelialization process (Pan, 2010)

2.7 *Ficus platyphylla*

Ficus platyphylla Del. Holl belongs to the family Moracea and genus Ficus. It is a tree of 18m high and trunk 6m girth, often initially as epiphytic, with widely spreading crown; of open and wooded savannah; from Senegal to North and South Nigeria, and extending in the Sahel to Sudan and to Uganda.(Berg and Wiebes, 1992). Its habitat is in the savannah woodland often in rocky places, up to an altitude of 750m. It is locally known as “gamji” among the “Hausas” of Northern Nigeria and “Epo-obo” among the Yorubas in Southwestern Nigeria and is widely distributed throughout the savannah region of the West African Coast (Chindo *et al*., 2011).

The plant is widely used for Agric-horticulture i.e. for fence-post, poles sticks and shade trees, social as in ceremonial, religion, superstitions and magic bark while in medicine it is general use for healings, leprosy,-antidotes- venom stings, bites etc(Berg and Wiebes, 1992). The use of the bioactive substances of the extract has great potential in combating disease of microbial origin (Gandidaza and Gaza, 1993).

A number of *Ficus* species are used as food and for medicinal purposes (Mousa *et al*., 1994; De Amorin *et al*, 1999; Lansky *et al*., 2008). The biological activities of several species from the genus *Ficus* have already been proven; some few examples include: *Ficus platyphylla* (Chindo *et al*, 2003), *Ficus cordata* (Donfack *et al*., 2011), *Ficus racemosa* (Mandal *et al*., 2000) and *Ficus hirta* (Li *et al*., 2009).

Ficus platyphylla in its natural habitat

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Material

The plant was collected in Zaria, Kaduna state, Nigeria and was identified and authenticated by Mallam I. Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute of Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen (No. 4035) was deposited at NIPRD Herbarium for future reference. The stem bark of *Ficus platyphylla* was freed from sand particles by carefully scrapping with a spatula. It was chopped to pieces, air dried and milled into coarse powder using pestle and mortar. Extraction was carried out by cold maceration of 500g of the coarse powder with 2.5 L of 70 % $^{V}/_{V}$ aqueous methanol for 72 h, with constant shaking using the GFL shaker (No. 3017 GBh, Germany). The resultant mixture was filtered using Whatmann filter paper (No.1) and the filtrate was evaporated to dryness *in vacuo* at 40^{o}C using rotary evaporator to give a yield of 25% $^{w}/_{w}$ of the extract. Aliquot portions of the extract were weighed and dissolved in distilled water for use in the study.

3.2 Animals

Wistar rats of both sexes weighing 140-250g were gotten and housed in the Animal house, Department of Pharmacology and Therapeutics, A.B.U, Zaria under room temperature. They were all kept in plastic cages. The animals were fed on Vital Grower feeds (Vital feeds, Jos-Plateau State) and water ad *libitum*.

3.3 Drugs and Chemicals

The following chemicals and drugs were obtained and used for the studies:-

DPPH (Sigma-Aldrich, Germany), Chloroform (Sigma Chemical Co. USA), L-Ascorbic acid- (Sigma-Aldrich Co. LLC), Methanol (Synthetic colour chemical Industry, India), Molisch's reagent, Concentrated Sulphuric acid (H_2SO_4) (BDH Ltd Poole, England), Concentrated hydrochloric acid (BDH Ltd Poole, England), Ferric Chloride (BDH Ltd Poole, England),Silymarin tablets 140mg- film coated tablet (Micro Labs Ltd, India) and Lidnocaine Injection 2% (Erica Life Science Ltd. UK).

3.4 Equipment and other Materials

Animals cages, weighing balance, pestle and mortar, stop clock, syringes (1ml, 2 ml, 5 ml, 10 ml, 20 ml), beakers, plastics bottle, surgical gloves, conical flask, whatman filter papers, spatula, water bath, spectrophotometer etc.

3.5 Preliminary Phytochemical Screening of Methanolic stem bark Extract of *Ficus platyphylla*

Phytochemical screening of the methanolic stem bark extract of *F. platyphylla* was carried out as follows:-

3.5.1 Test for carbohydrates

Molisch's test:-

0.5 g of the stem bark extract of *F. platyphylla* was dissolved in 10 ml of methanol in a test tube. Few drops of Molisch's test solution were then added followed by 4 drops of concentrated sulphuric acid which was allowed to run down the side of the test without mixing. Appearance of purple (violet) ring at the interface as a result of the reaction between the molisch solution and concentrated sulphuric acid produced by dehydration of saccharides indicated the presence of carbohydrates (Silva *et al*, 2003).

3.5.2 Test for cardiac glycosides

a. Kella-Killiani Test:-

The extract (0.5 g) was dissolved in 4 ml of glacia acetic acid containing traces of ferric chloride. This was left for 1min and then transferred into a dry test tube. The test tube was held at an angle of 45^{o}C and 5 drops of concentrated sulphuric acid was added down the side of the test tube. On standing, a purple ring colour at the interface indicates the presence of cardiac glycosides (Evans, 2002).

b. Kedde's Test

1ml of 2% 3, 5- dinitrobenzoic acid in 95% methanol was added to 0.5 g of the methanolic stem bark extract of *F. platyphylla*. The solution was then made alkaline with 5% sodium hydroxide. On addition of 5% sodium hydroxide, an immediate violet colour was observed indicating the presence of cardenolides in the extract. This colour fades gradually through reddish- brown to brownish-yellow with the precipitation of a whitish crystalline solid indicating the presence of a lactones ring in the cardenolide (Rahman, 2010).

c. Salkowski test

0.5 g of the stem bark extract was dissolved in 2 ml of chloroform. 2 ml of sulphuric acid was then added carefully forming a lower layer. A reddish-brown colour at the interface was seen indicating the presence of a steroidal ring (i.e. aglycone portion of the cardiac glycoside) (Rahman, 2010)

3.5.3 Test for anthraquinones derivatives

Test for free anthraquinones (Borntrager's test)

Small portion of the stem bark extract of *F. platyphylla* was shooked with 10 ml of benzene and this was then filtered. 5 ml of 10% of ammonia solution was added to the filtrate and stirred for few minutes. Colourless ammonia layer indicate presence of anthraquinones (Rahman, 2010).

3.5.4 Test for Saponins

Frothing test:-

0.5 g of the stem bark extract of *F. platyphylla* was dissolved in10 ml of distilled water. This was then shaken vigorously for 30 s and allowed to stand for 30 min. Honey comb froth was formed for more than 30 min on standing showing the presence of saponin (Rahman, 2010).

3.5.5 Test for Steroid and Triterpenes (Lieberman-Burchards test)

2 ml of acetic acid anhydride was added to the stem bark extract of *F. platyphylla* and then shaken. 1ml of concentrated sulphuric acid was then added down side the tube to

form a lower layer. Purple colour indicating the presence of triterpenes only (Roger *et al*., 2006).

3.5.6 Test for Flavanoids

a. Shinoda test

0.5 g of the stem bark extract is added to 2 ml of 50% methanol and the mixture is then heated and metallic magnesium was added. 4 drops of concentrated hydrochloric acid was then added. Dark orange colour was observed showing presence of flavanoids glycones (Evan, 2002).

b. Sodium hydroxide test

0.5 g of the stem extract was added to little distilled water and aqueous sodium hydroxide. A dark yellow colouration was seen showing the presence of flavanoids (Evan, 2002)

3.5.7 Test for Tannins

a. Lead sub-acetate test

3-drops of lead acetate solution were added to solution of the methanolic stem bark extract of *F. platyphylla*. Brown precipitate was observed.

b. Ferric Chloride test

0.5ml of the methanolic stem bark extract of *F. platyphylla* was added to 10 ml of distilled water, it was then filtered. Few drops of ferric chloride solution were then added to the filtrate. Green precipitate was then seen (Evan, 2002).

3.5.8 Test for Alkaloids

a. Mayer's Test

Few drops of Mayer's reagent were added to sample of the extract in a test tube. This was then shaken vigorously and a dense precipitate was observed.

b. Dragendorff's test

Dragendorff's reagent was added to the extract. Orange-red precipitate was observed.

c. Wagner's test

Few drops of the above reagent were added to the methanolic extract of stem bark *F. platyphylla*. Reddish brown precipitate was seen showing presence of alkaloid (Evan, 2002).

3.6 Median Lethal Dose (LD_{50}) Determination in Rats.

LD_{50} determination was conducted using Lorke's method (1983) for both intra-peritoneal and oral routes in rats selected for the work. This method was carried out in two phases. In the first (initial) phase, 3 rats per group of different weights were treated with methanolic extract of *F. platyphylla* stem bark at a dose of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight orally and were observed for signs of toxicity and death for 24hours. In the second phase, 4 rats of different weights were administered with four (4) more specific doses of the extract at 1200 mg/kg, 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg body weight respectively based on the result of phase 1(i. e initial phase). The procedure was also repeated for intra-peritoneal route (i.p). The LD_{50} value was determined by calculating the geometric mean of the highest non-lethal dose (0/1) and lowest lethal dose (1/1) as shown in the formula below:-

$$LD_{50} = \sqrt{(\text{Highest non lethal dose}) \times (\text{Lowest lethal dose})}$$

3.7 Anti-oxidant Studies

3.7.1 Scavenging antioxidant activities using 2, 2 DPPH *Invitro* assay

The scavenging activity of methanolic stem bark of *Ficus platyphylla* for the radical 2, 2-diphenyl-2- picrylhydrazyl (DPPH) was determined using the method described by Goyal *et al* (2010). The DPPH solution was prepared in 95% methanol. Using 0.02 mg/ml, 0.4 mg of DPPH was dissolved in 20 ml of methanol in a test tube to prepare a stock solution of 50 mg/ml. Also using 0.1 mg/ml, 7.5 µg of the methanol stem bark extract of *Ficus platyphylla* was dissolved in 25 ml of 95% methanol to prepare the stock solution of 300 µg/ml in a test tube which were later divided into concentration of 10 µg – 280 µg using five test tubes. Finally 0.45 µg of ascorbic acid was dissolved in 15 ml of distilled water to prepared a stock solution of 30 µg/ml which were later divided into five test tube in a concentration of 1 µg – 28 µg. Freshly prepared DPPH solution was added to the test tubes and methanolic extracts of *Ficus platyphylla* were added followed by serial dilutions (10 µg-280 µg/ml) to every test tube such that the final volume was 2.5 ml and absorbance was measured at 517 nm wavelength after incubation for 15 min in the dark using Jen-way 6405 UV/VIS spectrophotometer at the multi-research laboratory, Department of Chemistry A.B.U, Zaria. Measurements were performed in triplicate. Ascorbic acid was used as a reference standard and dissolved in 95% methanol to make a stock solution with the same concentration (0.1 mg/ml). The control sample was prepared, which contained the same volume without any extract and 95% methanol was used as the blank percentage. All values were converted to percentage anti-oxidant activity using the formula below:-

$$\text{Antioxidant activity \% (AA \%)} = 100 - \left[\frac{A\,Sample - A\,Blank}{A\,Control}\right] * 100$$

A sample = Extract + DPPH, A blank = Extract + Methanol, A control = Methanol + DPPH.

The actual decrease in absorbance induced by the test compounds (*Ficus platyphylla*) was compared with the positive controls (Ascorbic acid). The EC_{50} value was calculated using the dose inhibition curve and the co-efficient of regression (R^2).

3.7.2 *In vivo* methods for antioxidant and hepatoprotective studies

In vivo antioxidant and hepatoprotective studies were conducted using the method described by Shashank *et al.*, 2011 with modifications. Wistar rats weighing between 140-250 g were divided into six groups with each group having six animals. Group I was served as control and received normal saline 0.9% solution for 14 days. Group II to VI were daily injected with carbon tetrachloride, CCl_4 at dose of 0.1 ml/kg/day i.p for 14 days. Group III served as the standard group and administered Silymarin (100 mg/kg/day p.o.). Group IV, V and VI were treated with methanol stem bark extract of *F. platyphylla* in the doses of 100 mg, 200 mg and 400 mg/kg/day p.o. (as per acute toxicity studies) for 14 days respectively. All the animals that survived after 14 days post-treatment were sacrificed under light anesthesia. The blood samples were collected separately into sterilized dry centrifuge tubes and were allowed to coagulate for 30 min at room temperature. The clear serum was then separated at 2500 rpm for 10 min at the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital and biochemical investigations were carried out to assess liver function parameters like: - Alanine Transaminases (ALT), Aspartate Transaminases (AST), Alanine Phosphatase (ALP) and antioxidant parameters namely Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx).

3.7.2.1 *Assay Protocol for Glutathione Peroxidase (GPx) Antioxidant Activity Measurement*

GPx activity was done in a micro-plate assay. All the reagents used were brought to room temperature (37^0C). Micro-plate was removed from plastic bag and 50 µL of working NADPH was added to each well. Then 50 µL of working H_2O_2 was added to each well. The procedure was allowed for 1min on standing and it was then monitored at a wavelength of 340 nm for 30 min with a recording interval of 30 s. The concentration of H_2O_2 was then calculated from the absorbance using the following expression:

H_2O_2 Mm = (Absorbance 340 nm x 1000) / 39.4 mol. Where 39.4 $mol^{-1}cm^{-1}$ is the molar extinction coefficient for H_2O_2 (Christine and Joseph, 2011).

3.7.2.2 *Assay protocol for Superoxide Dismutase (SOD) Antioxidant Activity Measurement*

230 µL of assay buffer was added to each well and 10 µL each were added to blank and sample each respectively. It was then shaken and incubated for 2 min. 10 µL of hematoxylin reagent was added to begin the reaction using multi- channel pipette. Shaker

instrument was used to mix them quickly and immediately absorbance was taken at 560 nm every 10 s. The concentration of H_2O_2 was then calculated from the absorbance using the following expression:

H_2O_2 Mm = (Absorbance 560 nm x 1000) / 39.4 mol. Where 39.4 $mol^{-1}cm^{-1}$ is the molar extinction coefficient for H_2O_2 (Christine and Joseph, 2011).

3.7.2.3 *Assay Protocol for Malondialdehyde (MDA) Antioxidant Activity Measurement*

10 µL Butylated hydroxytoluene (BHT) reagents were added to micro-centrifuge vial and 250 µL of the sample vial was added. Also 250 µL acid reagents and 250 µL Thiobarbituric acid (TBA) reagent were added to the vial. It was then vigorously shaken and incubated for 60 min at $60^{O}C$. It was then centrifuged at 10,000 rpm for 3 min. The reactions were then transferred to cuvette and a spectrum was recorded from 560 nm. The concentration of H_2O_2 was then calculated from the absorbance using the following expression:

H_2O_2 Mm = (Absorbance 560 nm x 1000) / 39.4 mol. Where 39.4 $mol^{-1}cm^{-1}$ is the molar extinction coefficient for H_2O_2 (Christine and Joseph, 2011).

3.7.2.3 *Assay Protocol for Catalase (CAT) Antioxidant Activity Measurement*

15 µL of diluted sample was pipette to clean Ultra Violet (UV) micro-plate and 290 µL of assay cocktail was added to the well where all were mixed quickly using reader's shaker function. Results were immediately recorded at an absorbance of 340 nm every 2 s. The concentration of H_2O_2 was then calculated from the absorbance using the following expression:

H_2O_2 Mm = (Absorbance 340 nm x 1000) / 39.4 mol. Where 39.4 $mol^{-1}cm^{-1}$ is the molar extinction coefficient for H_2O_2 (Christine and Joseph, 2011).

All the above *in vivo* antioxidant activities (MDA, SOD, CAT and GPx) were carried out in Department of Chemical Pathology, ABUTH, Zaria.

3.8 Liver Enzyme Assay

The liver enzyme tests were carried out in the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria using an automated machine called “Express plus Clinical Chemistry Analyzer manufactured in Germany”.

The liver enzymes that were analyzed were Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP).

3.8.1 Aspartate aminotransferase (AST):-

For AST - International Federation of Clinical Chemistry (IFCC) Reagent: - Bayer Diagnostics AST Reagent (IFCC) was used.

The principle of this application is based on a modification proposed by the International Federation of Clinical Chemistry (IFCC)

L-aspartate + 2- oxoglutarate $\longrightarrow$ oxalacetate + L-glutamate

Oxalacetate + NADH + H^+ $\longrightarrow$ L-malate + NAD+ + H_2O

250 µL of reagent volume was added to 25 µL sample volume and incubated at 37^oC and readings were taken at every 100 second (Reitman and Frankel, 1957).

3.8.2 Alanine aminotransferase (ALT)

For ALT, (IFCC) Reagent: - Bayer diagnostic ALT reagent and following method of IFCC was used.

The principle of this application is $\longrightarrow$ pyruvate + L-glutamate

Pyruvate + NADP + H^+ $\longrightarrow$ Lactate + NAD^+

250 µL of reagent volume was added to 25 µL sample volume and this was incubated at 37^{o}C where readings were then taken at every 100 s (Reitman and Frankel, 1957).

3.8.3 Alkaline Phosphatase (ALP)

For ALP (IFCC) Reagent modality: - Bayer Diagnostics Alkaline Phosphatase (IFCC) was used. The principle of this is based on the kinetic procedure reported by Bowers and McComb, 1966 as modified, and by the International Federation of Clinical Chemistry (IFCC).

P-Nitrophenyl Phosphate + H_2O $\longrightarrow$ p- nitrophenol + H_3PO_4

300 µL of reagent volume was added to 6 µL of sample volume and was incubated at 37^{o}C and readings were taken at every 60 s (Bessey *et al*., 1967).

3.9 Antimicrobial Assay

The antimicrobial activity of methanolic extract of stem bark of *F. platyphylla* was determined using the following pathogenic microorganisms *(Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Proteus mirabilis, Pseudomonas florescence, Klebsiella pneumonia and Salmonella typhi*). The microbes were obtained from the Directorate of Research and Development, Nigeria Institute of Leader Research and Technology (NILEST), Zaria.

0.6 g of *Ficus platyphylla* extract was weighed and dissolved in 10 ml of distilled water so as to obtain a concentration of 60 mg/ml. This was the initial concentration used to check the antimicrobial activities of the plant. Mueller Hinton agar was the

medium used as the growth medium for the microbes. The medium was prepared according to manufacturer's instruction sterilized at 121^{o}C for 15 min. The medium was poured into sterile petri-dish, the plates were then allowed to cool and solidify.

Diffusion method was the method employed for the screening of the extract. The sterilized medium was then seeded with 0.1 ml of the standard inoculums of the test microbes. The inoculum was spread evenly over the surface of the medium with a sterile swab. By the use of a standard cork borer of 6 mm in diameter a well was cut at the center of each inoculated medium. 0.1 ml of the solution of the extract in concentration of 60 mg/ml was then introduced into each well on the inoculated medium. The inoculated plates were incubated at 37^{0}C for 24 hr, after which each plate was observed for zone of inhibition of growth. The zone was measured with a transparent ruler and the results recorded in millimeter (Rocksin *et al*., 2007).

3.9.1 Minimum inhibition concentration

The minimum inhibitory concentration of *F. platyphylla* extract was carried using broth dilution method. Mueller Hinton broth was prepared, 10 ml was dispensed into test tubes and was sterilized at 121^{O}C for 15 min, and the broth was then allowed to cool. MC-farland's turbidity standard scale number 0-5 was prepared to give turbid solution.

Normal saline was prepared, 10mls was dispersed into sterile test tube and the test microbe was inoculated and incubated at $37^{o}C$ for 6 hr. Dilution of the test microbe in the normal saline was done until the turbidity marched that of the MC- Farland's standard by visual comparison; at this point the test microbe has a concentration of about $1.5x\ 10^{8}$ cfu/ml. Two fold serial dilution of the *F. platyphylla* extract in the sterilized broth was made to obtain the concentration of 60 mg/ml, 30 mg/ml, 15 mg/ml, 7.5 mg/ml and 3.75 mg/ml. The initial concentration was observed by dissolving 0.6 g of the *F. platyphylla* extract in 10 ml of the sterile broth. On obtaining the different concentrations of the *F. platyphylla* in the broth, 0.1 ml of the test microbe in the normal saline was then inoculated into the different concentrations. Incubation was made at $37^{0}C$ for 24 hr after which the test tube was observed for turbidity (growth), the lowest concentration of the *F. platyphylla* in the broth which shows no turbidity was recorded as the MIC (Hannan, 2000).

3.9.2 Minimum bacteriocidal concentration (MBC)

The MBC was carried out to check whether the test microbes were killed or only the growth was inhibited. The Mueller Hinton agar was prepared, sterilized and was poured into sterile petri- dishes and the plate were allowed to cool and solidify.

The content of the MIC in the serial dilutions were then sub-cultured onto the prepared medium, incubation was made at $37^{0}C$ for 24 hr, after which each plate was observed for colony growth. The MBC was the plate with the lowest concentration of the extract without colony growth (Hannan, 2000).

3.9.3 Preparation of cream for wound healing activities

The method was adapted as described by Schumacher (1967b) where the sorbitan monostearate and stearic acid were melted in the liquid paraffin and cool at 54^{o}C.The initial quantity of the extract used was 30 g and the weight of the beaker used when empty was 25.65 g. The dissolution of all the quantity used was in 10 ml. The methanolic stem bark extract of *F. platyphylla* in different concentrations of 1 %, 5 % and 10 % were determined. For 1 %, it was calculated as 1/30 x 100 and this gave 3.3 g which was weighed and mixed with propylene glycol 4.02 % $^{W}/_{W}$, glycerin 4.13 % $^{W}/_{W}$ and 10 ml of distilled water, all heated in one beaker till dissolution and this were allowed to cool to a room temperature. This procedure was also repeated for 5 % and 10 % respectively. Others like acetyl alcohol 3.56 % $^{W}/_{W}$, stearic acid 4.80 % $^{W}/_{W}$, olive oil 5.78 %, span 60 1.78 % $^{W}/_{W}$ and tween 80 0.75 % $^{W}/_{W}$ were also heated in different beaker and all finally dissolved, stirred together until it cooled. The creams were then separated in different transparent container for onward use for the wound healing activities.

3.9.3.1 *Method for wound healing activities*

An excision wound healing model was used as described by Nayak *et al*., 2007. The rats were weight-matched and placed into six (6) group (n=5 per group), but the rats in the same group were also separated in different cages after excision and subsequent application of cream prepared for wound healing at the dorsal region. The dorsal fur of the animal was shaved with a razor blade and the area of the wound to be created was outlined on the back of the animals using permanent marker. All rats used for the experiment were anaesthetized with lidnocaine at the region of the excision and a full thickness excision wound of 1cm in width was created along the markings using toothed forceps, a surgical blade and pointed scissors in Department of Pharmacology Laboratory, A.B.U Zaria. Group I rats served as control and was treated with base cream only, while groups II, III, IV were treated with cream extract of 1 %, 5 % and 10 % $w/_{w}$ of the animal in each groups measured. Group V served as synergism group and was treated with the cream 1 % silver sulfadiazine (dermazin^{R}) + 10 % extract $w/_{w}$. Group VI served as standard and was treated with 1% silver sulfadiazine (dermazin^{R}) only. All the treatment lasted for eleven (11) days and wound area measurements were taken on days 3, 5, 7, 9, and 11 following the initial wound using transparent ruler. All the animals were left for observation for two (2) weeks post complete wound closure and healing for any observable signs of adverse reaction.

3.10 Histopathology Analysis

Heart, kidneys and the liver were removed from the group representative rats, weighed examined and stored in 10% buffered formalin for 48 hrs for histopathological studies. The harvested organs fixed in 10% buffered formalin were grossed and placed in tissue cassettes. They were loaded into tissue processor machine where they underwent further fixation in formalin, dehydration in gradient alcohol from 70% to absolute. The tissue were later impregnated with paraffin and later made into tissue blocks. The paraffin embedded tissues were sectioned with microtome and placed on labelled frosted slides. After removing the paraffin with xylene, reverse dehydration took place in absolute to 70% alcohol and stained with haematoxylin and eosin stains. The slides were examined under the microscope and photomicrographs of the section were taken (Ruifrok and Johnson, 2001).

CHAPTER FOUR

4.0 RESULTS

4.1 Preliminary Phytochemical Screening Results.

The results of preliminary phytochemical screening shows the presence of carbohydrate, cardiac glycoside, saponin, flavanoids, triterpenes, tannins, alkaloids but anthraquinones was absent (Table 4.1)

Table 4.1 Phytochemical constituents of methanolic stem bark extract of *Ficus platyphylla*

Constituents	Remarks
Carbohydrate	Present
Saponins	Present
Tannins	Present
Flavanoids	Present
Triterpenes	Present
Alkaloid	Present
Anthraquinones	Absent

4.2 Median Lethal Dose (LD_{50}) Determination

The intraperitoneal and oral lethal dose LD_{50} values of the methanolic stem bark extract of *Ficus platyphylla* in rats were found to be all above 5000 mg/kg body weight (Table 2).

Table 4.2 Median Lethal Dose (LD_{50}) values of the methanolic extract of *Ficus platyphylla*.

Route of Administration	Animal Species	LD_{50} values (mg/kg body weight)
Intraperitoneal	Rats	>5000
Oral	Rats	>5000

4.3 Antioxidant Studies

4.3.1 Effect of *Ficus platyphylla* on 2, 2- DPPH

The DPPH scavenging activities of methanolic stem bark extract of *Ficus platyphylla* showed that there was absorption band at 517 nm in visible region and DPPH solution decolourized as the colour changes from deep violet to light yellow. Based on regression coefficient (R^2) of the resulting models, it was observed that methanolic stem bark extract of *Ficus platyphylla* had strong positive effects on scavenging 2,2-DPPH with an EC_{50} of 36.40 calculated from $y = 0.8009x – 3.6563$ (as showed in Fig 1, where $x = 50$) and R^2 of 0.9847 as compared with ascorbic acid of EC_{50} of 75.78 also calculated from $y = 1.6204x – 5.2403$ (as showed in Fig 2, where $x = 50$) with R^2 of 0.8764 (Tables 4.3 and 4.4, Fig 1 and 2).

Table 4.3 Effect of methanolic stem bark extract of *Ficus platyphylla* on 2, 2- DPPH *in vitro* inhibition

Treatment MSBEFP (μg/ml)	Mean AA (%)
280	75.01 ± 1.45
125	97.20 ± 0.30
50	36.80 ± 3.80
25	09.50 ± 4.80
10	09.97 ± 2.70

Key: MSBEFP- methanolic stem bark extract of *Ficus platyphylla*, AA- antioxidant activities

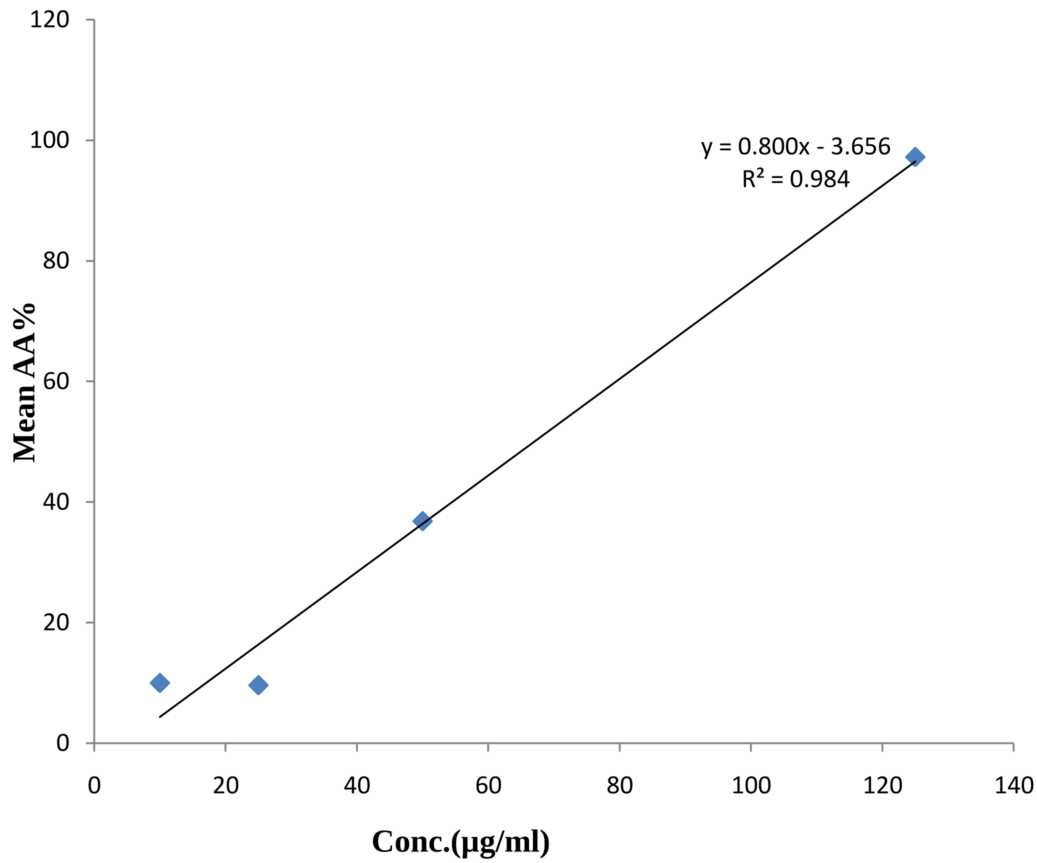

Figure 1: Coefficient regression graph of methanolic stem bark extract of *Ficus platyphylla* on DPPH Inhibition

Key: AA-Antioxidant activities, Conc.-Concentration

Table 4.4 Effect of Ascorbic acid on 2, 2- DPPH *Invitro* Inhibition.

Treatment Ascorbic acid (μg/ml)	Mean AA (%)
28	44 ± 3.50
12.5	4.6 ± 0.30
5	4.8 ± 0.70
2.5	3.3 ± 0.60
1	5.2 ± 0.70

Key: AA- antioxidant activities

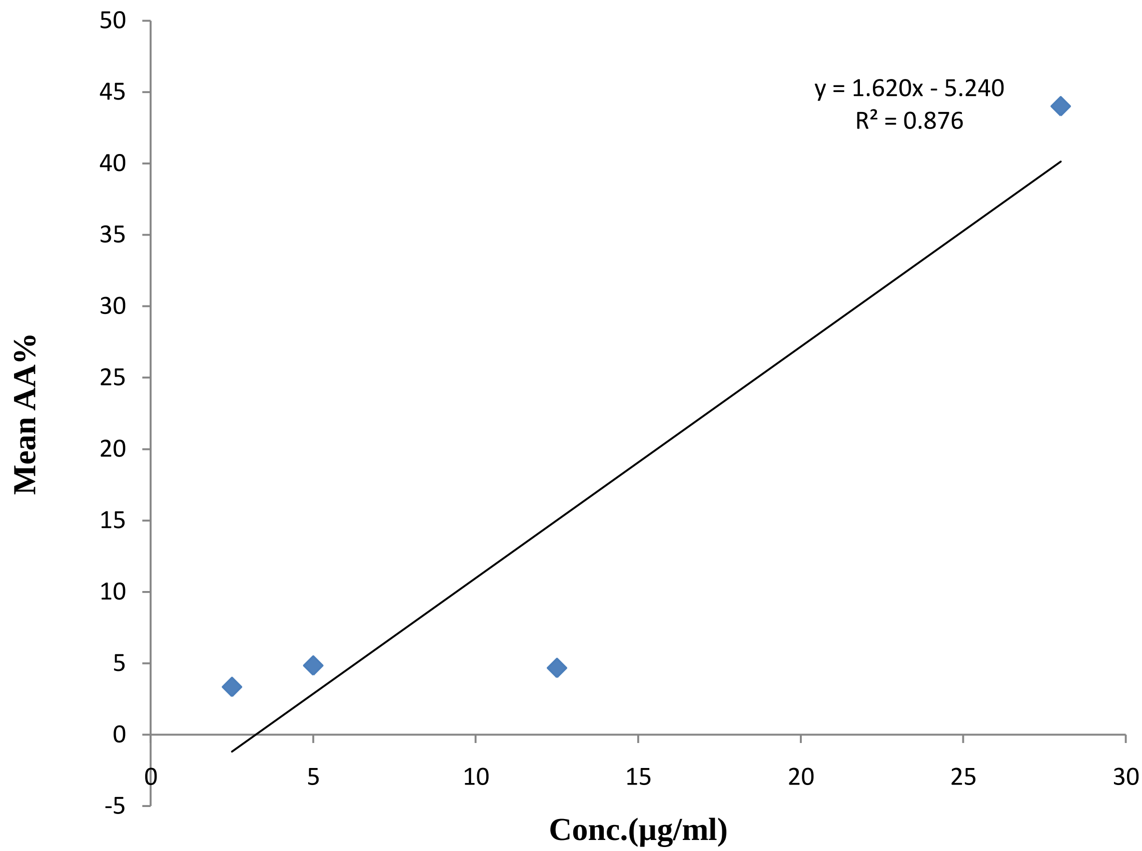

Figure 3: Coefficient regression graph of Ascorbic acid on DPPH inhibition

Key: AA- Antioxidant activities, Conc.- Concentration

4.3.2 Effect of methanolic stem bark extract of *Ficus platyphylla* on Oxidative parameters in carbon tetrachloride treated rats for 14 days

The methanolic stem bark extract of *Ficus platyphylla* does not significantly have effect on the MDA antioxidant property i.e. the lipid peroxidation decomposition ($p > 0.05$) where α value of the MDA analysis is 0.06. The methanolic extract of *Ficus platyphylla* stem bark significantly have significant $p < 0.001$) but dose dependent of antioxidant properties because the analysis showed that the 100 mg/kg body weight of the rat has $p < 0.5$. Also the 200 mg dose per kg body weight of the rat had $p < 0.05$ but has slight significant increase to that of 100 mg and the highest dose used 400 mg/kg body weight had significant increase of $p < 0.001$. The standard Silymarin offered SOD antioxidant of $p < 0.001$. The methanolic extracts of *Ficus platyphylla* ranging from 100 mg- 400 mg/kg body weight have statistical increase $p < 0.001$ of CAT properties. The standard Silymarin offered CAT antioxidant protection of $p < 0.001$. The methanolic stem bark extract of *Ficus platyphylla* ranging from 100 mg- 400 mg/kg body weight have increase statistical difference $p < 0.001$ of GPx properties. The result shows that the extract has the ability to catalyze the reduction of various peroxidase to quantify the enzyme activity of glutathione Peroxidase. The standard Silymarin offered 95% ($p < 0.05$) GPx antioxidant protection (Table 4.4).

Table 4.4.1 Effect of methanolic stem bark extract of *Ficus platyphylla* on Oxidative parameters in carbon tetrachloride treated rats for 14 days

Treatment	MDA (nmol/ml)	SOD (U/gHb)	CAT (U/gHb)	GPx (U/gHb)
Normal Saline	1.70 ± 0.07	2.04 ± 0.13	50.0 ± 1.41	44.0 ± 0.71
CCl_4 Only	1.46 ± 0.08	1.58 ± 0.12	44.4 ± 1.03	39.6 ± 0.51
CCl_4 + Silymarin	1.52 ± 0.04^{ns}	1.48 ± 0.10^{c}	43.4 ± 1.17^{c}	39.8 ± 1.28^{c}
CCl_4 + Ext. 100 mg/kg	1.72 ± 0.06^{ns}	1.60 ± 0.07^{a}	42.8 ± 1.07^{c}	33.6 ± 1.03^{c}
CCl_4 + Ext. 200 mg/kg	1.76 ± 0.12^{ns}	1.46 ± 0.10^{b}	41.40 ± 1.08^{c}	35.0 ± 0.71^{c}
CCl_4 + Ext. 400 m/kg	1.64 ± 0.05^{ns}	1.30 ± 0.07^{c}	40.0 ± 0.71^{c}	37.0 ± 0.71^{c}
df	5, 24	5, 24	5, 24	5, 24
f	2.53	6.09	9.98	19.10
α	0.06^{ns}	0.001^{b}	0.001^{b}	0.001^{b}

KEY: Values are expressed in Mean ± SEM, n=6, ns=not significant, df=degree of freedom, f=frequency, α=significant values, a=$p<0.05$, c=$p<0.001$, One Way ANOVA.

4.4 The Effect of Methanolic stem bark extract of *Ficus platyphylla* on some organs weight from Carbon Tetrachloride treated Rats for 14 days

The extract significantly protected both the liver and the kidney at $p < 0.001$ and $p < 0.05$ but did not have significant effect on the heart and the spleen because the CCl_4 has no negative effects on both the organs. The weight of the liver and the kidney changed significantly but there were no change in the weight of the spleen and the heart. This is to show that the metabolism of the toxin CCl_4 begins in the liver and next in the kidney because of toxin elimination. Here the result showed that there is greater protection from the extract, *Ficus platyphylla* in both the liver and that of the kidney having significant decrease of $p < 0.001$ and $p< 0.05$ respectively from One Way ANOVA and post-hoc Duncan test after the administration of CCl_4 to all the animals for 14 days.

Table 4.4.2 Percentage (%) effect of methanolic stem bark of *Ficus platyphylla* on Oxidative parameters in carbon tetrachloride treated rats for 14 days

Treatment	%MDA	%SOD	%CAT	%GPx
Normal Saline	17.3	21.5	19.1	34.0
CCl_4 Only	14.9	16.6	16.9	30.7
CCl_4 + Silymarin	15.5	15.6	16.6	30.7
CCl_4 + Ext. 100 mg/kg	17.6	16.8	16.3	26.0
CCl_4 + Ext. 200 mg/kg	18.0	15.4	15.8	27.1
CCl_4 + Ext. 400 mg/kg	16.7	13.7	15.3	28.7
df	10,4		10,4	
f	85.022		29.89	
α	0.001		0.001	

Key: Values expressed in percentages (%), n=6, df=degree of freedom, f=frequency, α=significant values, One Way ANOVA.

Table 4.5 The effect of methanolic extract of *Ficus platyphylla* stem bark on some organs weight in carbon tetrachloride treated rats for 14 days

Treatment	Mean $\pm$ SEM g/body weight			
	Heart	**Spleen**	**Liver**	**kidney**
Normal Saline	0.62 ± 0.16	1.22 ± 0.16	6.18 ± 0.47	1.16 ± 0.08
CCl_4 Only	0.73 ± 0.05	0.94 ± 0.12	8.20 ± 0.21	1.40 ± 0.10
CCl_4 + Silymarin	0.64 ± 0.07	0.80 ± 0.22	7.32 ± 0.23^{c}	1.34 ± 0.15^{a}
CCl_4 + Ext. 100 mg/kg	0.60 ± 0.02	0.86 ± 0.12	6.11 ± 0.25^{c}	1.25 ± 0.10^{a}
CCl_4 + Ext. 200 mg/kg	0.55 ± 0.03	0.98 ± 0.17	6.07 ± 0.44^{c}	1.01 ± 0.05^{a}
CCl_4 + Ext. 400 mg/kg	0.58 ± 0.02	1.09 ± 0.06	6.61 ± 0.33^{c}	1.08 ± 0.07^{a}
df	5, 30	5, 30	5, 30	5, 30
f	2.20	1.01	6.47	2.60
α	0.08^{ns}	0.43^{ns}	0.000^{c}	0.046^{a}

Key: Values are expressed in Mean ± SEM, n=6, ns=not significant, df=degree of freedom, f=frequency, α=significant values, a=p<0.05, c=p<0.001. One Way ANOVA.

4.5 The effect of methanolic stem bark extract of *Ficus platyphylla* on liver enzymes in carbon tetrachloride treated rats for 14 days

The analysis showed that there was statistical decrease ($p < 0.05$, $p < 0.001$ and $p < 0.000$) in the level of AST, ALT and ALP enzymes in animals treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of the extract and concurrent administration of CCl_4 (0.1 ml/kg) (groups 4, 5 and 6). Meanwhile, the overall results as analyzed by one way ANOVA showed that there were statistical differences ($p < 0.05$ and $p < 0.001$) (Table 4.6).

Table 4.6 The effect of methanolic stem bark extract of *Ficus platyphylla* on liver enzymes in carbon tetrachloride treated rats for 14 days

Treatment	ALT	AST	ALP
	Mean ± SEM (IU/L)		
Normal Saline	33.4 ± 2.73	26.2 ± 2.22	58.4 ± 2.46
CCl_4 Only	42.4 ± 1.81	36.2 ± 2.35	66.6 ± 0.67
CCl_4 + Silymarin	43.2 ± 1.36^c	38.6 ± 2.01^c	68.6 ± 1.50^a
CCl_4 + Ext. 100 mg/kg	43.2 ± 3.10^c	40.4 ± 2.79^c	65.0 ± 0.84^a
CCl_4 + Ext. 200 mg/kg	51.0 ± 1.30^c	42.8 ± 1.39^c	66.4 ± 2.98^a
CCl_4 + Ext. 400 mg/kg	42.8 ± 1.28^c	36.4 ± 1.21^c	64.0 ± 1.51^a
df	5, 24	5, 24	5, 24
f	7.33	7.71	3.60
α	0.001	0.001	0.014

Keys: Values are expressed in Mean ± SEM, n=5, ns=not significant, df=degree of freedom, f=frequency, α=significant values, $p < 0.05^a$, $p < 0.000^c$ ALT = Alanine Transaminases; AST= Aspartate Transaminases; ALP= Alanine Phosphatase, One Way ANOVA.

4.6.1 Effect of methanolic stem bark Extract of *Ficus platyphylla* on the Histopathology of Liver, in Carbon Tetrachloride Treated Rats for 14 Days

There were differences in the histopathology of the liver in the different groups where 100 mg/kg, 200 mg/kg and 400 mg/kg of the methanolic extract of *Ficus platyphylla* were administered per kg body weight of the animals for 14days with concurrent administration of the CCl_4 (0.1 ml/kg) as showed in the photomicrograph below as compared with the group treated with standard drug Silymarin (Plate II - VII).

4.6.2 Effect of methanolic extract of *Ficus platyphylla* on the histopathology of kidney, in carbon tetrachloride treated rats for 14 days

There were differences in the histopathology of the kidney in the different groups where 100 mg/kg, 200 mg/kg and 400 mg/kg of the methanolic stem bark extract of *Ficus platyphylla* were administered per kg body weight of the animals for 14days with concurrent administration of the CCl_4 (0.1 ml/kg) as showed in the photomicrograph below as compared with the group treated with standard drug Silymarin (Plate VIII - XIII).

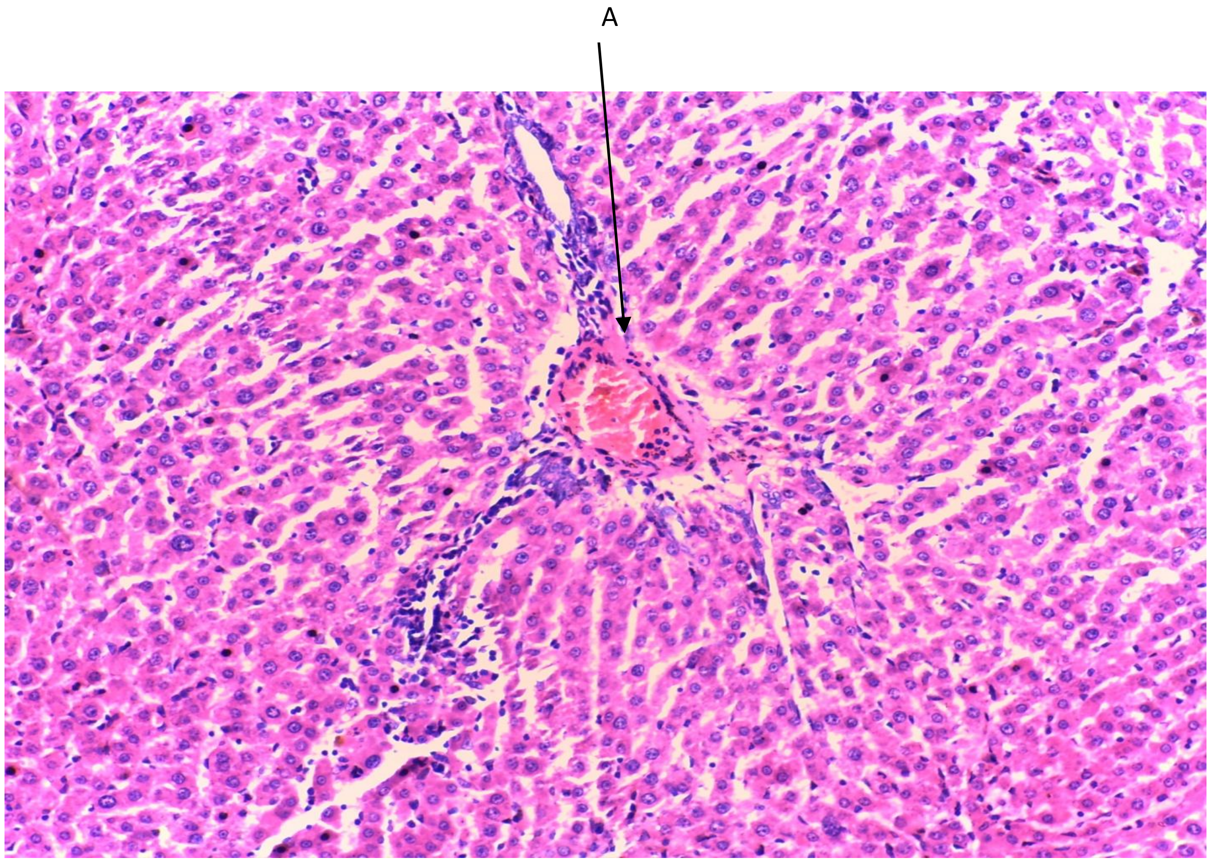

Plate II (liver): Normal hepatocytes with the central vein visualized in normal saline treated group for 14 days. Magnification x 10 (H and E stain)   A:- Represent the central hepatic vein of the liver

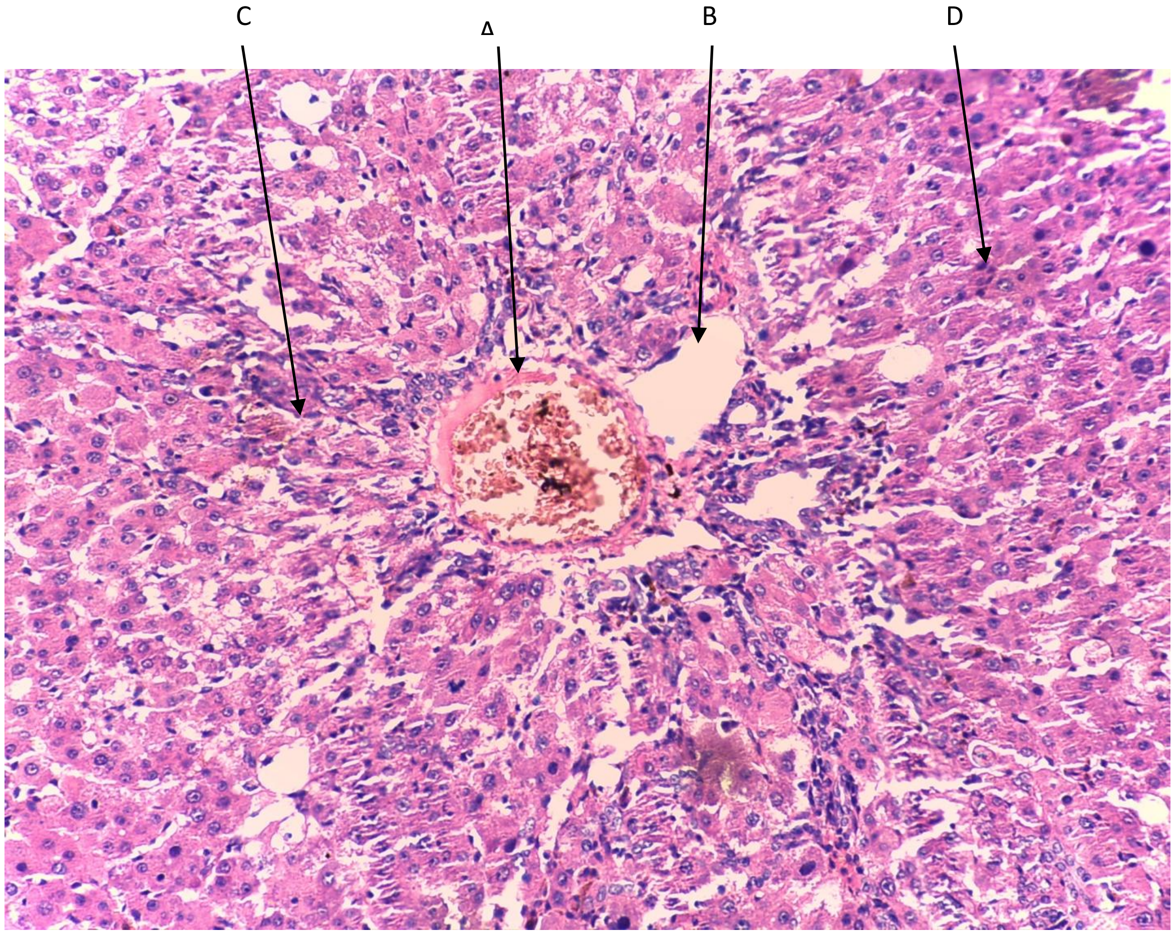

Plate III (liver)**:** Hepatitis with apoptosis bodies and necrosis of the hepatocytes with massive destruction of the liver cell in CCl_4 treated rats for 14 days. Magnification x 10 (H and E stain)

A= Central hepatic vein, B= Necrosis of the hepatocyte, C= apoptosis and D= severe hepatitis.

A

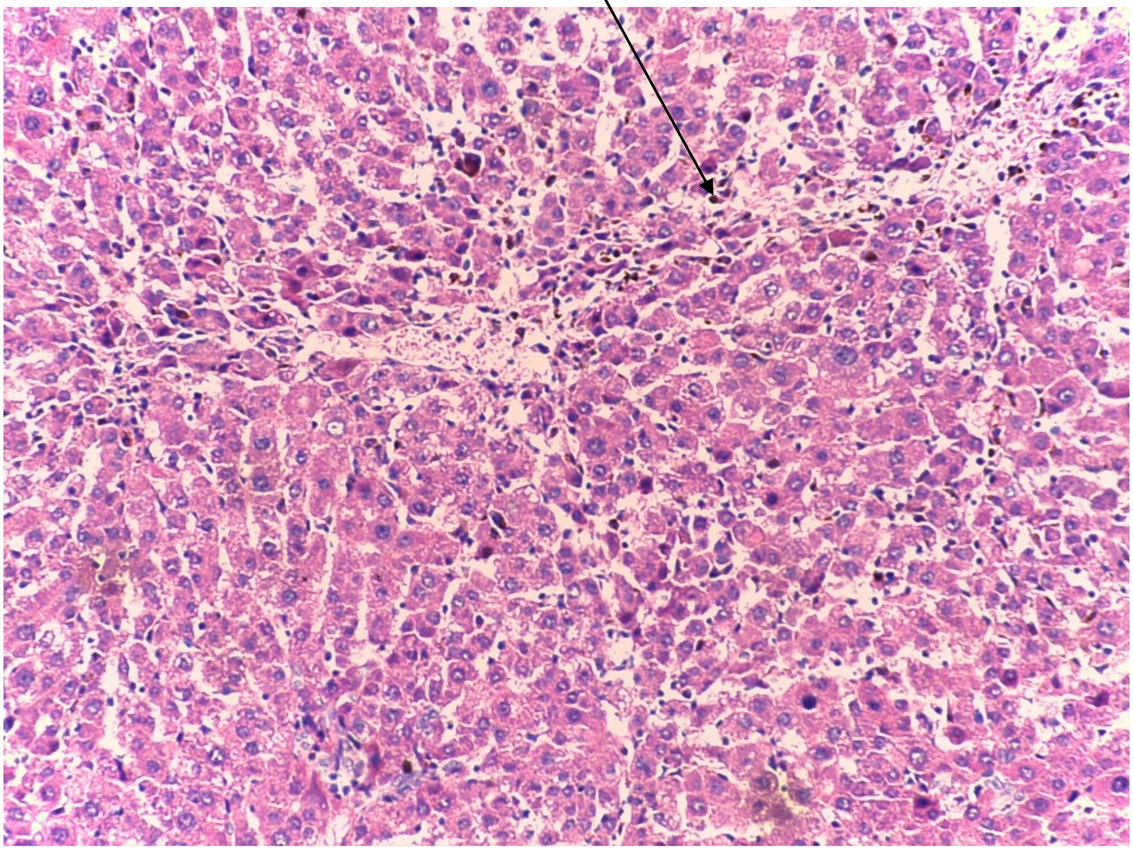

Plate IV (liver): Bridging necrosis of the hepatocytes with mild protection of the liver cells noticed in CCl_4 + Silymarin treated rats for 14 days. Magnification x 10 (H and E stain).A == Represent bridging necrosis

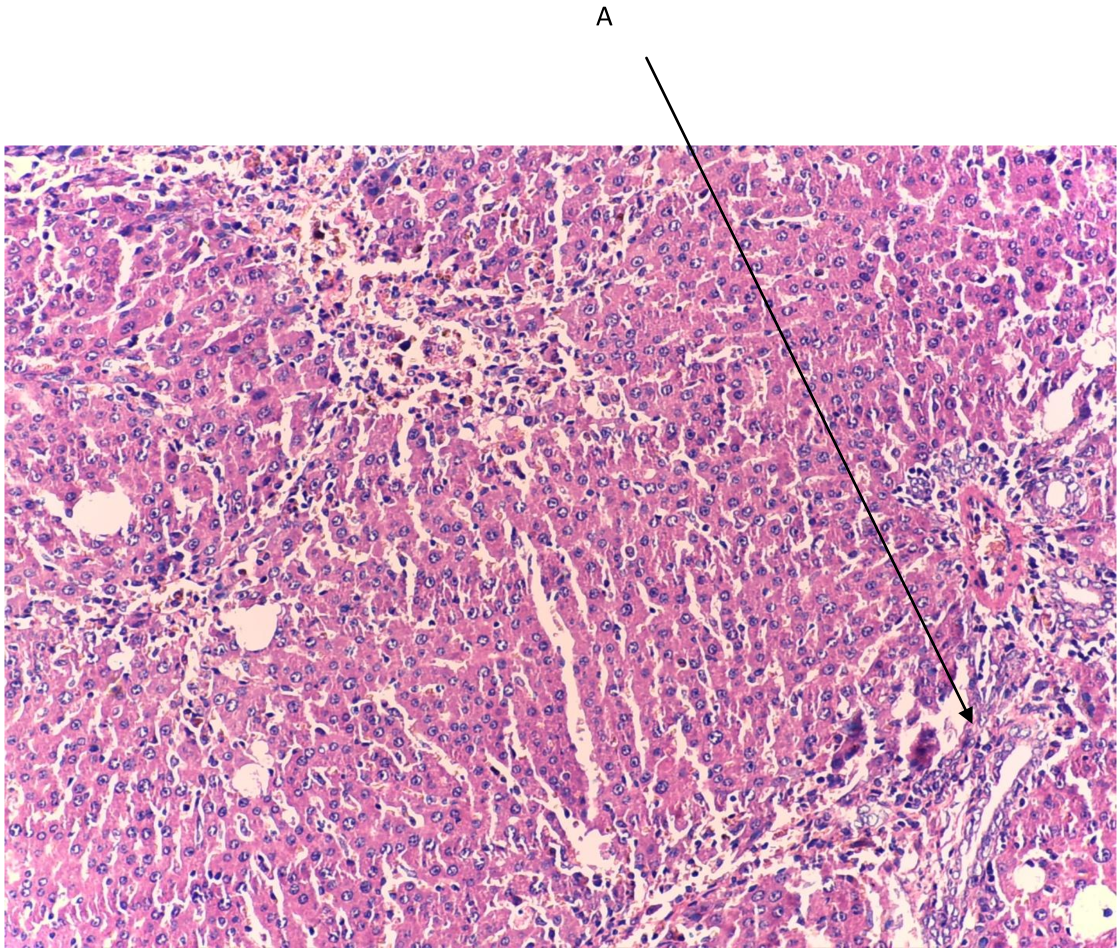

Plate V (liver): Peri-portal hepatitis with inflammation within the portal tract and bridging necrosis in rats treated with CCl_4 + methanolic stem bark extract of *Ficus platyphylla* 100 mg/kg for 14 days. Magnification x 10 (H and E stain). A== Represent bridging necrosis and peri-portal hepatitis.

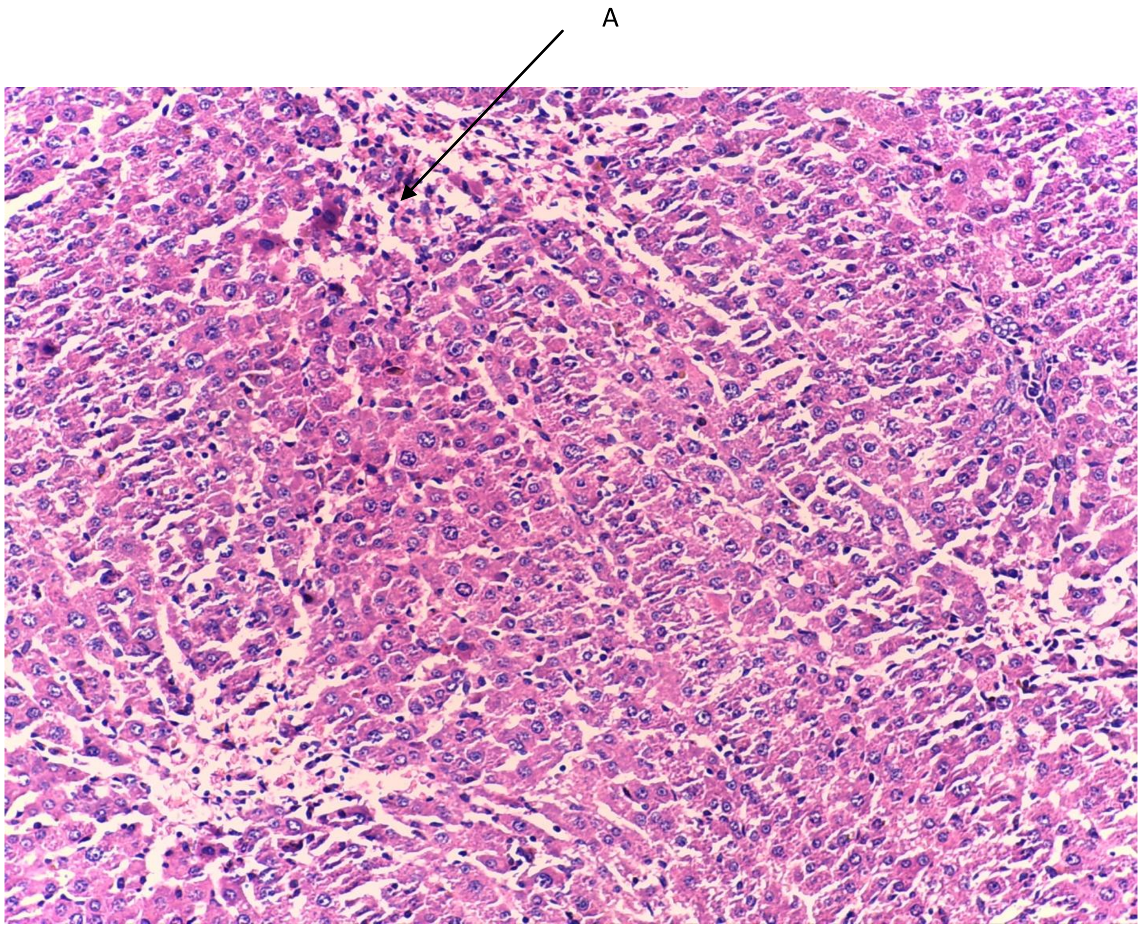

Plate VI (liver): Diffused mild hepatitis with inflammatory cell within the hepatocytes in CCl4 + methanolic stem bark extract of *Ficus platyphylla* 200 mg/kg treated rats for 14 days. Magnification x 10 (H and E stain). A== Represent Inflammation of the hepatocytes.

A

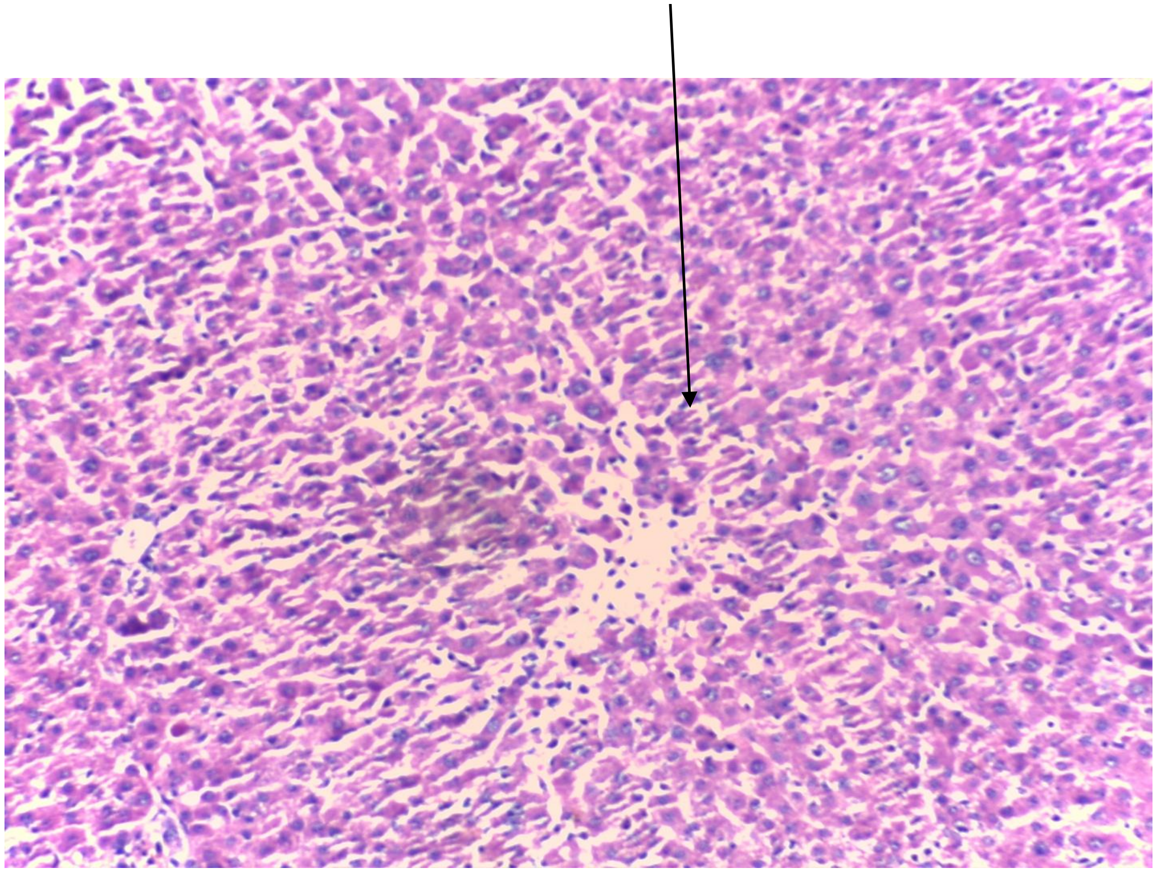

Plate VII (liver): Mild inflammation of hepatocytes with preserved cyto-architectures in CCl_4 + methanolic stem bark extract of *Ficus platyphylla* 400 mg/kg treated rats for 14 days.

Magnification x 10 (H and E stain). A== Represent mild inflammation of the hepatocytes

A

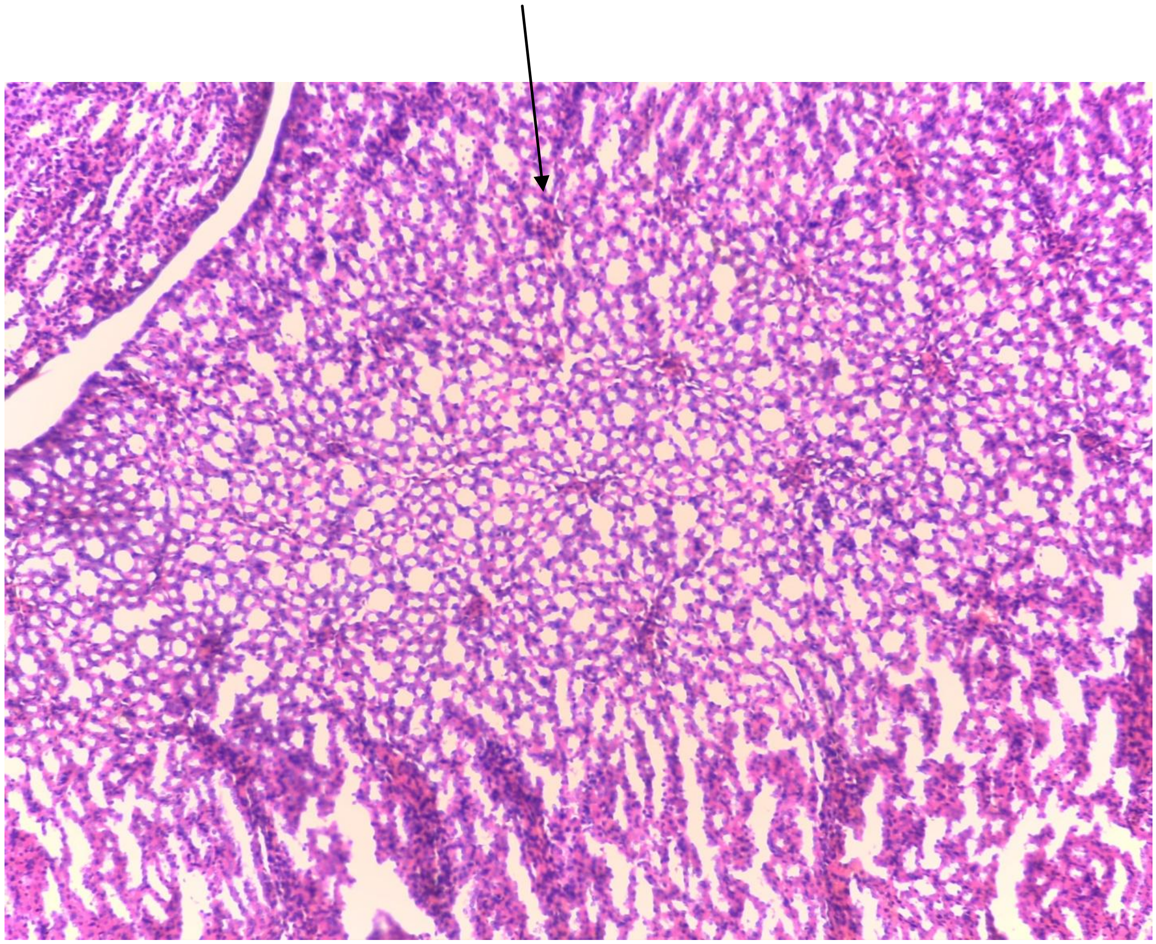

Plate VIII:- Normal kidney. Unremarkable renal tubules. No cell destroyed. Magnification x 10 (H and E stain). A== Represent unremarkable renal tubules with cell intact

A

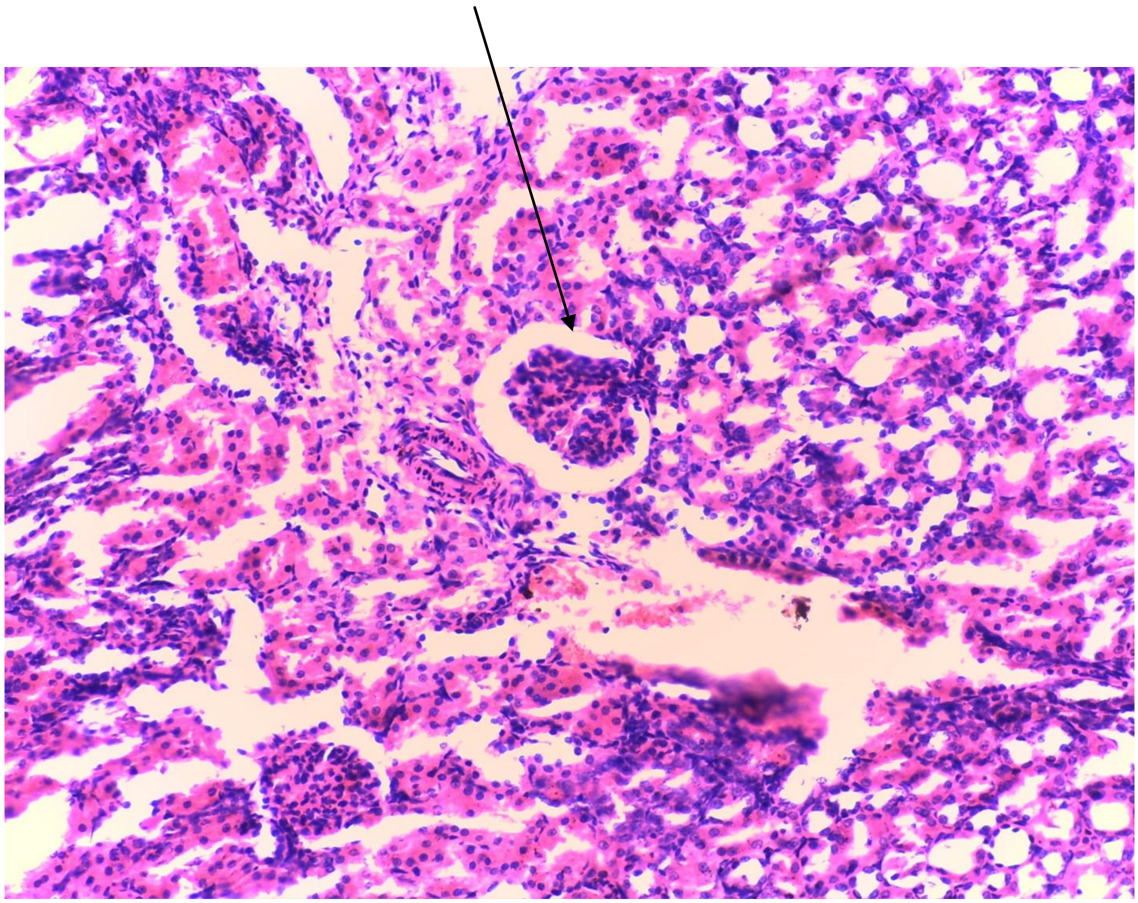

Plate IX :- Kidney of CCl_4 treated rats for 14 days – There is increase glomerular cellular proliferations. Architectures are preserved. Magnification x 10 (H and E stain). A== Represent Glomerular cell

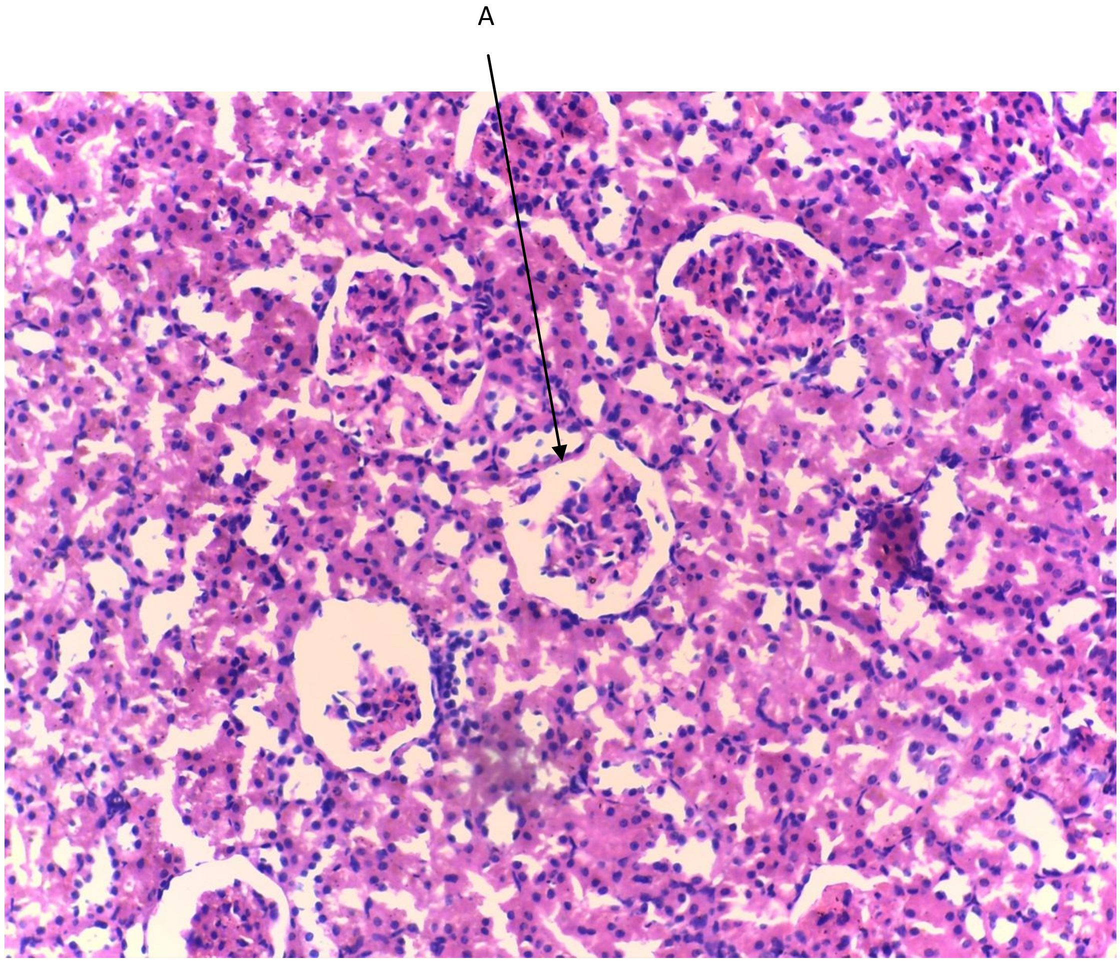

Plate X : Kidney of CCl_4 + Silymarin treated rats for 14 days – Here there are normal glomeruli visualized with good tubular structures. Magnification x 10 (H and E stain). A== Represent Normal glomerular

A

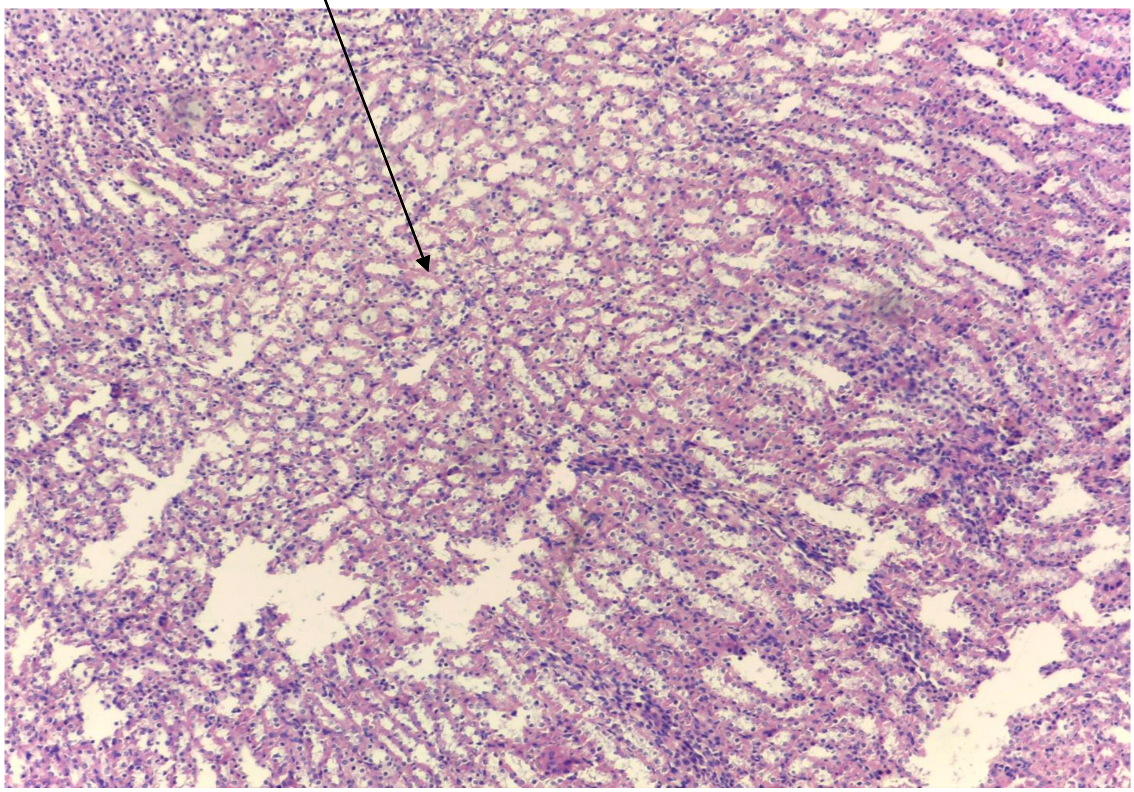

Plate XI : Kidney of CCl_4 + methanolic stem bark extract of *Ficus platyphylla* 100 mg/kg treated rats for 14 days. Normal looking tubules. Magnification x 10 (H and E stain).

A== Represent normal looking tubules

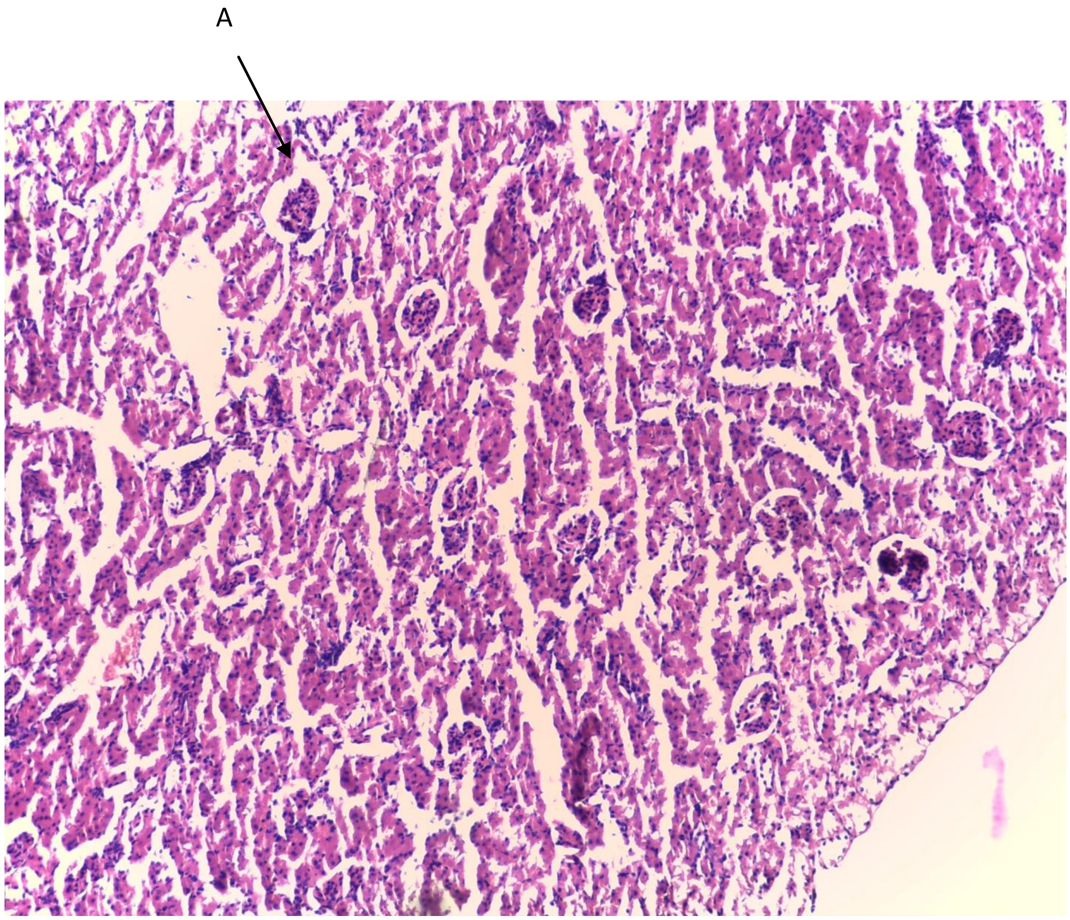

Plate XII : Kidney of CCl_4 and methanolic stem bark extract of *Ficus platyphylla* 200 mg/kg treated rats for 14 days – Normal renal tubules. Magnification x 10 (H and E stain). A== Represent Normal renal tubules

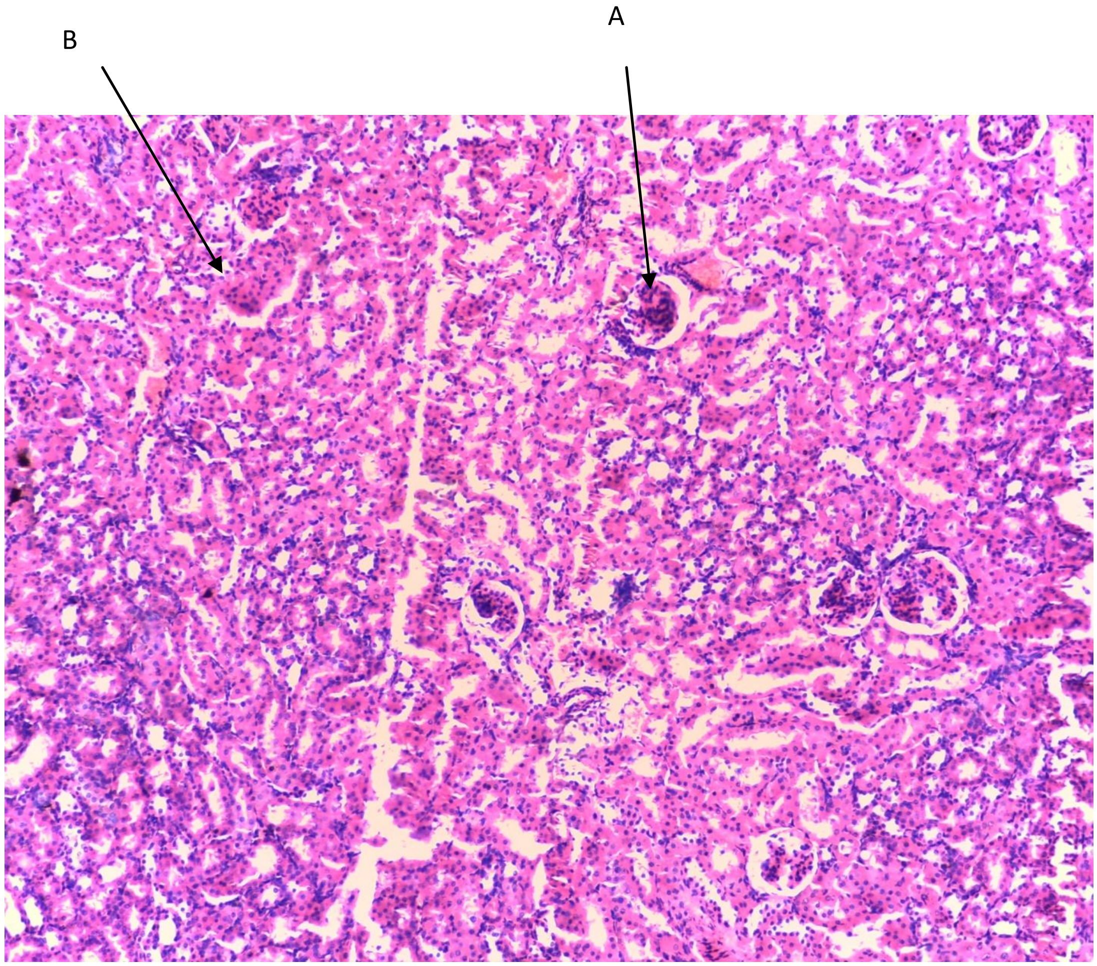

Plate XIII : Kidney of CCl_4 and methanolic stem bark extract of *Ficus platyphylla* 400 mg/kg treated rats for 14 days – Normal glomeruli and tubules. Magnification x 10 (H and E stain). A== Normal glomerular, B== Normal tubule

4.7 ANTIMICROBIAL STUDIES

4.7.1 Antimicrobial susceptibility effect of methanolic extract of Ficus platyphylla stem bark and dermazin

The results of antimicrobial susceptibility effects of methanolic extract of *Ficus platyphylla* stem bark and dermazin against the tested organisms (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Escherichia coli, Proteus mirabilis, Pseudomonas flourescense, Klebsiella pneumonia* and *Salmonella typhi*) showed that the microbes *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumonia* and *Salmonella typhi* are sensitive to *F. platyphylla* but the microbes *Streptococcus pyogenes, Proteus mirabilis* and *Pseudomonas flourescense* were resistance to *F. platyphylla.*

Meanwhile, the microbes *Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumonia* and *Salmonella typhi* were sensitive to dermazin but *Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis* and *Pseudomonas flourescense* were resistance to dermazin (Table 4.7).

Table 4.7 Antimicrobial susceptibility effect of methanolic extract of *Ficus platyphylla* and dermazin

Test Organism	*Ficus platyphylla*	Dermazin Cream
Staphylococcus aureus	Sensitive	Resistance
Streptococcus pyogenes	Resistance	Resistance
Corynebacterium ulcerans	Sensitive	Sensitive
Escherichia coli	Sensitive	Sensitive
Proteus mirabilis	Resistance	Resistance
Pseudomonas flourescense	Resistance	Resistance
Klebsiella pneumoniae	Sensitive	Sensitive
Salmonella typhi	Sensitive	Sensitive

4.7.2 Zone of inhibition (mm) of test microbes by methanolic extract of *Ficus platyphylla* and dermazin

The results of zone of inhibitions of the methanolic stem bark extract of *Ficus platyphylla* against the tested microbes (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Escherichia coli*, *Proteus mirabilis, Pseudomonas flourescense*, *Klebsiella pneumoniae* and *Salmonella typhi*) showed respectively as 22, 0, 24, 20,0, 0, 27 and 20 all in millimeters (mms). Also for dermazin against the microbes (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Escherichia coli*, *Proteus mirabilis*, *Pseudomonas flourescense, Klebsiella pneumonia* and *Salmonella typhi*) are respectively 0, 0, 32, 29, 0, 0, 35 and 27 (Table 4.8).

Table 4.8 Effects of methanolic extract, *Ficus platyphylla* and dermazinon zone of inhibition on test microbes

	Zone of Inhibition (mm)	
Test organisms	*Ficus platyphylla*	Dermazin
Staphylococcus aureus	22	0
Streptococcus pyogenes	0	0
Corynebacterium ulcerans	24	32
Escherichia coli	20	29
Proteus mirabilis	0	0
Pseudomonas fluorescence	0	0
Klebsiella pneumonia	27	35
Salmonella typhi	20	27

4.7.3 Minimum inhibitory concentration and antimicrobial susceptibility effects of *Ficus platyphylla* on the test microbes

The results showed that *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli* and *Salmonella typhi* were inhibited by the stem bark extract of *Ficus platyphylla* at 15 mg/ml turbidity and of slight growth at 7.5 mg/ml with moderate turbidity at 3.75 mg/ml respectively. *Streptococcus pyogenes, Proteus mirabilis* and *Pseudomonas flourescense* were not inhibited by the extract. Lastly, *Klebsiella pneumoniae* has the minimum inhibition concentration of 7.5 mg/ml when treated with the extract and turbidity of 3.5mg/ml was also observed (Table 4.9).

Table 4.9 Minimum inhibitory concentration and antimicrobial susceptibility effect of *Ficus platyphylla against test microbes*

	MIC (mg/ml)				
Test Organism	60	30	15	7.5	3.75
Staphylococcus aureus	-	-	0□	+	++
Streptococcus pyogenes	-	-	0□	+	++
Corynebacterium ulcerans	-	-	0□	+	++
Escherichia coli	-	-	0□	+	++
Proteus mirabilis	-	-	-	-	-
Pseudomonas flourescense	-	-	-	-	-
Klebsiella pneumonia	-	-	-	0□	+
Salmonella typhi	-	-	0□	+	++

Key: - = No turbidity (no growth), 0□ = Minimum Inhibitory Concentration, + = Turbidity (high growth), ++ = Moderate Turbidity, MIC = Minimum Inhibitory Concentration

4.7.4 Minimum bacteriocidal concentration of methanolic stem bark extract of *Ficus platyphylla* on test microbes

The results showed that the methanolic stem bark extract of *Ficus platyphylla* can kill the microbes *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumoniae and Salmonella typhi* at a minimum bacteriocidal concentration of 30 mg/ml, 30 mg/ml, 60 mg/ml, 15 mg/ml and 60 mg/ml respectively. This means that the extract will kill these microbes completely at this concentration stated in their respective regions. The extract has no effects on *Streptococcus pyogenes, Proteus mirabilis, Pseudomonas flourescence and Klebsiella pneumoniae* (Table 4.10).

Table 4.10 Minimum bacteriocidal concentration of *Ficus platyphylla* on test microbes

	MBC (mg/ml)				
Test Organism	60	30	15	7.5	3.75
Staphylococcus aureus	-	0□	+	++	+++
Streptococcus pyogenes	-	-	-	-	-
Corynebacterium ulcerans	-	0□	+	++	+++
Escherichia coli	0□	+	++	+++	++++
Proteus mirabilis	-	-	-	-	-
Pseudomonas flourescense	-	-	-	-	-
Klebsiella pneumonia	-	-	0□	+	++
Salmonella typhi	0□	+	++	+++	++++

Key: - =No turbidity i.e. no growth, 0□ = Minimum bactericidal concentration, + = Scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth.

4.8 Effects of Topical Application of stem bark extract of *Ficus platyphylla* cream on Excision Wounds in rats treated for 11 days

The topical application of 1 % stem bark extract of *Ficus platyphylla* cream showed that there was no statistical difference ($p > 0.05$) at day 3 but there was statistical decrease ($p < 0.001$) of the cream extract 5 % and 10 % in respect to the wound area respectively compared to the base cream. The 5 % and 10 % showed 100 % effectiveness in the healing of the wound. The standard 1 % dermazin offered effectiveness of 100 % also at day 3. At day 5, Extract 1 % cream preparation had statistical decrease ($p < 0.05$) while that of 5 %, 10 % cream extract had statistical difference ($p < 0.001$). This showed that at day 5, 1 % cream extract had 95 % wound healing effectiveness while 5 % and 10 % had 100 % wound healing effectiveness. The standard dermazin 1 % had also 100 % wound healing effectiveness. At both day 7 and 9, there was statistical difference ($p < 0.001$) showing 100 % wound healing effectiveness of all the percentage cream extracts.

At day 11, there was statistical difference ($p < 0.05$) in all the cream percentage extract use (Table 4.11).

Table 4.11 Effects of Topical Application of *Ficus platyphylla* cream extract on excision wound in rats treated for 11days

Cream	Reduction in Diameter of Wound (mm)				
(% $^{w}/_{v}$)	Day 3	Day 5	Day 7	Day 9	Day 11
Base	0.86 ± 0.02	0.68 ± 0.04	0.56 ± 0.04	0.42 ± 0.02	$0.30 \pm .03$
Ext. 1 %	0.80 ± 0.03^{ns}	0.52 ± 0.04^{a}	0.36 ± 0.02^{c}	0.12 ± 0.01^{c}	0.06 ± 0.02^{a}
Ext. 5%	0.56 ± 0.05^{c}	0.38 ± 0.04^{c}	0.22 ± 0.02^{c}	0.13 ± 0.03^{c}	0.06 ± 0.02^{a}
Ext. 10%	0.48 ± 0.04^{c}	0.24 ± 0.03^{c}	0.16 ± 0.02^{c}	0.10 ± 0.03^{c}	0.12 ± 0.10^{a}
Ext. 10% + 1% Dermazin	0.82 ± 0.04^{ns}	0.62 ± 0.04^{ns}	0.36 ± 0.02^{c}	0.18 ± 0.01^{c}	0.12 ± 0.02^{a}
1% Dermazin	0.56 ± 0.05^{c}	0.38 ± 0.04^{c}	0.22 ± 0.02^{c}	0.13 ± 0.01^{c}	0.06 ± 0.02^{a}
df	5,24	5,24	5,24	5,24	5,24
f	16.90	21.13	30.32	29.04	4.11
α	0.000	0.000	0.000	0.000	0.008

Key: Values are expressed in Mean ± SEM, n=5, ns=not significant, df= degree of freedom, f=frequency, α= degree of significant, $p < 0.05^{a}$, $p < 0.001^{b \text{ and } c}$

One Way ANOVA

CHAPTER FIVE

5.0 DISCUSSION

The phytochemical screening of methanolic extract of *Ficus platyphylla* stem bark revealed the presence of flavanoids, tannin, cardiac glycosides, alkaloids, carbohydrate and triterpenes which might be responsible for the observed antioxidant, hepatoprotective and wound healing activities of the extract. Also the minimum inhibitory concentration (MIC) and the minimum bacteriocidal concentration (MBC) of the methanolic extract of *Ficus platyphylla* stem bark in combination with phytochemical constituent might also be responsible for its significant and concentration dependent reduction in wound area.

Antioxidant effects have been observed with the presence of flavanoids and tannins which is responsible for the free radical scavenging effects observed in plants (Ayoola *et al*; 2008). There are also mechanisms of action of alkaloids through inhibition of peroxidation which shows the antioxidant properties of the plant derivatives (Kessler *et al*; 2003).

Antioxidant properties of methanolic extracts of some Nigerian plants on nutritional stress showed that DPPH scavenging abilities have high percentage inhibitions in the Nigerian plants e.g. Ebolo and water leaf. Free radicals are known to be a major factor in biological damages caused by oxidative stress (Omoregie and Osagie, 2012).

Hanif *et al*., (2005) reported that the extract of *Zingiber officinale* may exerts its anti-cancer effects by replacing the function of SOD, GPx, CAT and MDA in removing superoxide radicals and hydrogen peroxide that cause oxidative damage to cells of human liver in form of cancer in comparing to normal liver cell proving its antioxidant properties.

The intra-peritoneal and oral values of LD_{50} of the extract *Ficus platyphylla* stem bark were greater than 5000 mg/kg body weight in rats suggesting that the extract is non-toxic intraperitoneally and orally.

The DPPH test showed the reactivity of the test compound with a stable free radical. The DPPH gives a strong absorption band at 517 nm in visible region. As the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourized as the colour changed from deep violet to light yellow. The degree of reduction in absorbance measurement is an indicative that the extract had a radical scavenging property hence its antioxidant power. The methanolic stem extract of *Ficus platyphylla* exhibited a significant concentrated dependent inhibition of DPPH activity ($p < 0.001$), with a 50 % inhibition (EC_{50}) at a concentration of 67 µg/ml. The extract had showed to be potent as compared with vitamin C with a maximum inhibition of 97.2 % at 125 µg/ml and 44 % for vitamin C at 28 µg/ml.

This result suggests that the stem bark extract of *Ficus platyphylla* posses' antioxidant activities as measured by the DPPH scavenging activities which can counteract the oxidative stress/damage induced by the intra-peritoneal administration of CCl_4 in destroying the liver. This is consistent with the work of Ayoola *et al*., (2008).

There MDA showed no significant antioxidant property $p > 0.05$. The 100 mg/kg, 200 mg/kg and 400 mg/kg dose of methanolic stem bark extract of *Ficus platyphylla* had 17.6

%, 17.9 % and 16.7 % lipid peroxidation decomposition respectively. The standard Silymarin provide 15.5 % lipid peroxide decomposition (Table 4.4.2).

SOD has powerful anti-inflammatory and antioxidant activities. SOD is a highly effective experimental treatment of inflammation in colitis and it also ameliorate Cis-platinum induced nephrotoxicity in rodents. They also have chemo-protective role as demonstrated by Hanif *et al*., (2005). The ability of the extract to have hepatoprotective property after intra-peritoneal administration of CCl_4 might be as a result of its ability to decrease reactive oxygen species generation and oxidative stress and, thus, inhibits endothelial activation proving its antioxidant property. It showed statistically significant decrease of $p < 0.001$.

It was noted that other antioxidant enzymes parameter like CAT and GPx activities have antioxidant significant of $p < 0.001$. The 100, 200 and 400 mg/kg body weight of CAT antioxidant activities showed 16.3 %, 15.80 % and 15.30 % respectively compared with the standard Silymarin which showed 16.6 % protection while that of the GPx antioxidant activity was 26.0 %, 27.1 % and 28.7 % protection. The standard Silymarin has 30.70% antioxidant activity showing conformity with the result of Hanif *et al* (2005) in determination of hepatoprotective property of *Zingiber officinale*.

Meanwhile, the antioxidant activity had distinctive fluctuation as compared to the standard Silymarin probably because of the dosage used.

There were no statistically significant differences in the level of ALT, AST and ALP enzymes with 100 mg/kg and 200 mg/kg compared to the CCl_4 groups as shown in Table 4.6 during the 14days studies.

However, there was statistically significant decrease ($p < 0.001$) in 400 mg/kg dose group but with fluctuation as compared to the CCl_4 group with the enzymes ALT, AST and ALP. This result indicates that the extract has protective effects on the liver of the animals but in higher doses.

The histopathological results showed that the liver of the rats that received 100 mg/kg of the extract, *Ficus platyphylla* had peri-portal hepatitis and inflammation within the portal tract compared with standard Silymarin 100 mg/kg which showed bridging necrosis of the hepatocytes during the 14 days studies. The liver were protected to some extend from the damage being imposed through the administration of CCl_4 (0.1ml/kg) on the experimental rats at doses 200 and 400 mg/kg body weight.

Also the liver of the rats that received 200mg/kg of the extract, *Ficus platyphylla* showed mild hepatitis with inflammatory cell within the hepatocytes comparing to 100 mg/kg that showed complete peri-portal hepatitis. This showed that the protection for 200 mg/kg increases on the liver from the damage experienced during the administration of CCl_4 (0.1ml/kg) on the experimental rats.

However, the liver administered with 400 mg/kg extract, *Ficus platyphylla* showed mild inflammation of the hepatocytes with no hepatitis or necrosis and the cyto-architectures are highly preserved. The result showed that the hepatoprotection from the extract used was dose dependent on the dosage as also showed from the liver enzyme (ALT, AST and ALP) results.

There were no pathological features observed in other organ like the spleen and the heart except the kidneys which showed increased in glomerular cellular proliferation in the group administered with CCl_4 only but the architectures are preserved.

The extract *Ficus platyphylla* stem bark exhibited significant activities on the degree and rate of wound closure compared to the group where only base cream were used. The extract having influences ($p < 0.001$) on the degree and rate of closure of the wound from day 3 to 11 after treatment.

The presence of pathogenic organisms such as *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumonia and Salmonella typhi* in wounds normally may lead to infection of wounds which may results in the formation of chronic wounds (Frantz, 2005). The study showed that the extract exhibited strong broad spectrum antimicrobial activity against these pathogens. The topical application of antimicrobial agents/extract is an efficient method of microbial populations destruction because of the availability of the active agents at the wound site which led to enhanced wound healing activity (Nayak, *et al*; 2007).

The extract *Ficus platyphylla* stem bark had significant influence on the rate of wound closure based on different days of treatment of the wounds with the extract compared with the base cream treated group.

The 5 % cream extract had the same strength compared to the standard 1% silver sulphadiazine at day 3. At day 5 there were significant wound contraction ($p < 0.001$) which showed the same in day 7 and day 9 and wound closure of 81.56 %, 80.85 % and

88.9 % for 1 % cream extract respectively. Also there was different percentage wound closure for 5% and 10 % cream extract which showed significance statistically.

The wound of the animals treated with base cream showed no statistical differences compared to the cream extracts (1 %, 5 % and 10 % cream preparations) and 1% silver sulphadiazine (reference/standard) treated wounds. This may also indicate that the components of the methanolic base cream did not interfere with the activity of the extract and hence the enhanced effects of the stem bark methanolic extract of *Ficus platyphylla* which may be primarily due to the bioactive principles present in the extract. The wound healing effects of the extract may be due to the various phytochemical constituents that were present in the extract. Tannins such as proanthocyanidins and other tannins including tannic acid, geranin and furosin are known to facilitate wound healing (Agyare *et al*; 2013). The extract also contain flavanoids and saponins and these secondary metabolites have been found to enhance wound healings and hence the enhanced wound healing effects of the extract may be attributed to their phytochemical constituents. The above findings may support the claims that wound healing process may be due to their direct action on the wound repair process, phytochemical bioactive present and antimicrobial effects or the combination of the above effects (Shetty *et al*., 2006).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

Based on the results obtained on this work, the use of the plant *Ficus platyphylla* in traditional medicine is relatively safe. Wound healing and hepatoprotective properties of F. platyphylla have been established. The stem bark extract can be used in the treatment of malaria and any oxidative stress caused to the liver (as hepatoprotective) without the fear of being injurious to organ and other associate organ like the kidney most especially. The possible mechanism of the above effects might be due to anti-oxidant and antimicrobial effects of the methanolic stem bark extract of F. platyphylla. However, there is mild hepatocytes changes in the liver based on the dose used hence the extract is dose dependent and may not be specific with people having chronic liver damage.

It is therefore recommended that other parameters (like total protein, bilirubin level and albumin) in conjunction with AST, ALT and ALP be investigated together during the administration of CCl_4 or Paracetamol induced hepatic damage and for longer days especially in the differences seen in liver protection from this work. Sub-chronic and chronic toxicity are recommended.

Further research like histopathology of the wound area and wound healing monitoring using near infrared fluorescent fibrinogen are needed in order to have detailed mechanism of the wound healing effects of the plant.

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APPENDICES

Appendix 1 Calculation of Percentage yield of the methanolic stem extract of *F. platyphylla*

The crude drug of 500g was extracted two times using 2.5 liters of pure methanol.

First extraction:

Weight of crude drug that was extracted = 500g

Weight of the crude extract obtained = 155.50g

$$\text{Percentage yield} = \frac{\text{Weight of the crude extract}}{\text{Weight of the crude drug}} \times 100$$

$$= \frac{155.5}{500} \times 100$$

$$= 31.1\%$$

Second extraction:

Weight of crude drug that was extracted = 500g

Weight of the crude extract obtained = 120.50g

$$\text{Percentage yield} = \frac{\text{Weight of the crude extract}}{\text{Weight of the crude drug}} \times 100$$

$$= \frac{120.5}{500} \times 100$$

$$= 24.1\%$$

Appendix 2 Lethal dose (LD50) determination on methanolic stem bark extract of *F. platyphylla*

		First phase (n=3)		Second phase (n=1)		LD_{50} (mg/ml)
Species	Route of Admin.	Doses used (mg/kg)	Quantal mortality	Doses used (mg/kg)	Quantal mortality	
Rats	Oral	10	0/3	1600	0/1	
		100	0/3	2900	0/1	>5000
		1000	0/3	5000	0/1	
	Intraperitoneal	10	0/3	1600	0/1	
		100	0/3	2900	0/1	>5000
		1000	0/3	5000	0/1	

Note:- LD50 = $\sqrt{(\text{Highest non lethal dose}) \times (\text{Lowest lethal dose})}$