HEPATOPROTECTIVE AND TOXICOLOGICAL ASSESSMENT OF *SPONDIAS MOMBIN* L. (ANACARDIACEAE) IN RODENTS

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY

In the

Department of Pharmacology,
Faculty of Pharmacy and Pharmaceutical Sciences
College of Health Sciences

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AUGUST, 2010

DECLARATION

The experimental work described in this thesis was carried out at the Department of Pharmacology, KNUST. This work has not been submitted for any other degree.

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ABSTRACT

Spondias mombin L. aqueous leaf extract is claimed by some Ghanaian herbalists to be traditionally useful in the management of hepatitis related jaundice. Its role in the management has however not been validated. The extract is also traditionally used in the treatment of various ailments but there is little data on its safety or otherwise. The objectives of this study were to assess the possible hepatoprotective effect of the extract on carbon tetrachloride (CCl_4) - induced hepatotoxicity and to assess the possible general toxicity of the plant, in rodents.

The hepatoprotective assessment was determined biochemically (using Liver Function Test, LFTs), morphologically (histopathological) and functionally (using Pentobarbitone-induced sleep time) in rodents. In general toxicological assessment, the effects of the extract on haematological, biochemical, morphological and in organo-body ratio assessment were performed.

The extract was found to possess profound therapeutic ability as it decreased bilirubin levels from 5.2±0.1 µmol/L in the group treated with CCl_4 only to 4.2±0.5 µmol/L in the group that received CCl_4 and 1000 mg/kg of extract. Additionally, alanine aminotransferase (ALT) levels decreased from 219.7±30.02 U/L in CCl_4 only treated group to 40.25 ± 2.39 U/L in groups treated with CCl_4 and 1000 mg/kg respectively. This effect was clearly evident in histopathological studies of livers of rats. The therapeutic ability of the aqueous extract was comparable to Silymarin, a standard hepatoprotective agent. The extract was also found to be effective in both prophylactic and concomitant administrations.

Pentobarbitone (50mg/kg body weight) challenged rats that received 100-1500mg/kg of extract after liver injury had minimum sleeping times recorded. The extract shortened the pentobarbitone-induced sleeping time to 333.6 ± 18.3 minutes in groups treated with CCl_4 and 1500 mg/kg body weight, compared to 490.6 ± 36.9 minutes in CCl_4 only treated group. This reduction was comparable to the control (316.6 ± 28.2 minutes), and more significant than in the group that received CCl_4 and Silymarin (432.6±42.0 minutes).

Acute toxicity studies preliminarily carried out on the aqueous extract revealed no lethality. With the exception of food intake (that dose-dependently increased), no physical, physiological and behavioural effects on mice were observed in the study. The LD_{50} of the aqueous extract was found to exceed 5000 mg/kg body weight.

Sub-acute toxicity studies on the extract also showed no significant physical, physiological and behavioural effects. Haematological and biochemical studies also revealed no effect on rats administered with doses from 300 mg/kg to 1500 mg/kg body weight of extract. This effect was clearly confirmed by histopathological studies, as they showed no pathological difference in the target organs, livers and kidneys of treated rats when compared to those of the control rat group.

It is concluded that the aqueous leaf extract of *Spondias mombin* is safe at doses of 50-1500mg/kg in rats and mice. It is also hepatoprotective against CCl_4 induced liver damage. This hepatoprotective ability should have a role in the traditional use of the extract in the management of hepatitis related jaundice.

ACKNOWLEDGEMENT

All praise is due to the Almighty God for making it possible for me, taking me through it and ending it in success.

My sincere thanks goes to Dr. Charles Ansah, my supervisor for his guidance, understanding and tolerance, and to all the lecturers of the Pharmacology Department for their contribution in making my study at the department a good one.

I am especially thankful to the laboratory technicians, workers of the animal house and general office of the department especially Mr. T. Ansah, Mr. G. Ofei and Mr. Dokyi.

I wish to express my sincere gratitude to the Government of Ghana, KNUST and particularly to Mr. J. Hamenoo and Dr. Y. Edusei for their financial support.

My appreciation goes to all!

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1 Chapter 1 INTRODUCTION

1.1 GENERAL OVERVIEW

Treatment of diseases associated with the liver is very vital, and must be done with importance and extensive care. Many herbal remedies for liver diseases are known but only a few of them have been pharmacologically assessed for their efficacy. It is very important to assess natural products for their efficacy in the treatments they are used for. It is especially important to assess remedies for liver diseases due to the liver's fragility and relation to other vital organs, and its numerous physiological roles in the body (Elsebae *et al.*, 2008; Kiyosawa *et al.*, 1987; O'Reilly *et al.*, 1997).

In recent times, due to economic factors, people scramble for available, easily accessible and less costly medication, even with the slightest knowledge of efficacy, and minimum idea of toxicity. Herbal remedies to most people, are natural and thus non-toxic. Toxicity of natural remedies have however been reported, and scientifically proven hepatoprotective plants were found to contain hepatotoxins as well (Bramanti *et al.*, 1978; MacGregor *et al.*, 1989; Oshima, 1995). Thus, work on hepatoprotective herbal remedies remain a challenge (Schuppan *et al.*, 1999).

Spondias mombin is used as remedy for numerous ailments. Its antimicrobial, anthelmintic and cytotoxic actions (Abo *et al.*, 1999; Ademola *et al.*, 2005; Uchendu *et al.*, 2008) give caution for possible toxic action of the plant, though there have not been reports of toxicity of the extract. We were prompted by the claim of herbalists that the aqueous extract of *Spondias* leaves is effective in the management of jaundice to assess its hepatoprotective effects. The safety of the extract has also been generally assessed in rodents.

1.2 PHYSIOLOGICAL AND BIOCHEMICAL ROLES OF THE LIVER

The liver is the largest intra-abdominal solid organ in the human body. It performs various functions essential for the survival of an animal, such as involvement in the processing of fats and proteins from digested foods, the making of proteins essential for blood clotting, the storage of glycogen (which is fuel for the body, made from sugars), the regulation of water distribution between blood and tissues, the processing of some drugs taken into the body (such as acetaminophen and phenytoin), removal of toxins from the body, and the production of bile (which contains bile acids and bilirubin) into the gallbladder.

1.2.1 Management of body fuel

The liver has a unique ability of breaking down glycogen and gluconeogenesis to control blood glucose levels in cells. It removes phosphate from glucose-6-phosphate, forming free glucose to enter the blood. High amount of glucose is stored as glycogen during fed periods. It also synthesises glucose-6-phosphate from a variety of carbohydrates and from the carbon skeleton of many of the amino acids at a high rate during fasting period.

During fed period, the liver actively synthesises fat (triglyceride), by converting it to a transportable very low density lipoprotein (VLDL) and releasing into the blood for absorption mainly into adipose tissues as a way of storing excess calories for later use. In fasting period, the triglyceride is broken down into three molecules of free fatty acids and three carbon glycerol molecules. Two molecules of glycerol are converted into one of glucose for use as fuel.

Ketone bodies are important and preferred fuel for some muscle tissues such as heart. The liver solely converts fatty acids to ketone bodies. It degrades free fatty acids to acetyl coA, which in excess, two molecules react to form ketone bodies.

1.2.2 Protein metabolism and nitrogen excretion

About 90% of protein value is re-used in cell systems. This is maintained by synthesizing new molecules of amino acids and breaking down old ones. Amino acid degradation produces carbon structures such as acetyl coA and organic acids involved in the tricarboxylic acid (TCA) cycle. The liver converts excess amino acids into VLDL for storage in fed period, whilst in fasting period, amino acids are converted into proteins for use as fuel, or into other compounds. The liver also uses these amino acids in gluconeogenesis to produce energy.

1.2.2.1 Nitrogen excretion

Breakdown of amino acids releases the carboxylic acid group and the amino group. It is the sole and unique responsibility of the liver to synthesise urea with excess amino acids in its cells, release to the blood for transportation to the kidneys for concentration and excretion. The alanine cycle makes it possible for the nitrogen to get attached to alanine (synthesised from pyruvate, from glucose) for transportation to the liver and further absorption into liver cells. Nitrogen is released, pyruvate reformed for glucose synthesis and urea synthesised for excretion.

1.2.3 Regulation of water distribution

Water supply to cells is actively done by the circulatory system of heart and blood vessels, and follows the common law of flowing from a high concentrated region to a low concentrated region.

At cellular levels, maintenance of osmotic pressure in cells and blood vessels is complemented by the amount of the water-soluble protein, albumin present. Low albumin levels in blood may cause fluid to leak from blood vessels into tissues, resulting in oedema. The liver help maintain water balance by solely producing albumin and releasing into blood vessels to control the flow of water, and thus maintaining osmotic pressure.

1.2.4 Detoxification

Toxins are foreign bodies or substances which are harmful to the body, mainly by interfering with cellular processes and causing damage. Exposure to toxins is increasingly

becoming unavoidable. They are naturally occurring, from industrial chemicals, and in products we buy, use and even eat on a daily basis such as fertilizers and pesticides used in agricultural production, chemicals used in manufacturing plastics we use routinely in our homes, packaging materials, food additives, hair products body creams, etc. Even most pharmaceutical products and drugs meant to manage or treat health conditions are also potentially toxic.

These toxins are eliminated usually by modification of the compounds and excretion by the kidneys. Modification of toxic chemicals and compounds takes place in the smooth endoplasmic reticulum (SER) (Barritt *et al.*, 2008) where large amounts of different enzymes are found. The main modifying enzyme is a group of enzymes known as the cytochrome P_{450} enzymes, which absorbs light of particular frequencies, with a common function of modifying, mainly by oxidizing compounds. Liver cell contain very highly developed SERs, and thus contains large amounts of the body's cytochrome P_{450}. Complemented with its rich access to circulatory system makes it effective in the detoxification of foreign bodies. However, chronic exposure to some toxins, or brief exposure to high levels of some organic compounds could cause diseases to the liver.

1.3 CAUSES OF LIVER DISEASES

Though the liver is effective in the detoxification of foreign bodies, with its numerous but vital activities, the liver becomes a target organ for diseases, and is pre-disposed it to a high risk of liver diseases, causing hepatitis (a condition characterised by the destruction of liver cells and the inflammation of cells in the liver tissues), and subsequently, jaundice. There are various causes of liver diseases, generally resulting from viral or portozoal infections, excessive use of alcohol, drugs and xenobiotics (Ram, 1999).

Acute hepatitis may be caused by:

- Viral infections such as hepatitis A, hepatitis B, hepatitis C, hepatitis D and hepatitis E, and other viral diseases such as mononucleosis caused by cytomegalovirus (Joske, 1980),
- Severe bacterial infections,
- Amoebic infections,
- Increasing intake of medicines, e.g. acetaminophen (which can be hepatotoxic) and halothane (an anaesthetic),
- Poisoning from intake of alcohol, and fungal toxins, like toadstool poisoning.

Chronic hepatitis is mainly caused by:

- Contagious viral infections such as hepatitis B, hepatitis C and hepatitis D,
- Hepatotoxic medicines such as isoniazide (INH) (antituberculosis), methyldopa (Aldomet) adrenergic antihypertensive and tetracycline (an antibiotic),
- High alcoholic intake,
- Inborn metabolic disorders, such as Wilson's disease (disorder of the body's copper metabolism) and haemochromatosis (disorder of the body's iron metabolism),
- Liver cancer,
- Cirrhosis of the liver (Altlparmak *et al.*, 2005).

Incidence of the various disease conditions and its associated risks are high, and is therefore a major health problem (Harville *et al.*, 1963; Lavanchy, 2008; Parrilli *et al.*, 2007; Simonetti *et al.*, 1991).

1.4 EPIDEMIOLOGY OF LIVER DISEASES

Primarily, liver diseases with hepatitis compromises the storage and synthesis of glucose, affecting CNS function and mental processing, causes fatigue and general ailment and unwell being. It also disrupts storage and use of calories, causing fatigue and body mass wasting.

Since the liver is primarily responsible for amino acid metabolism and nitrogen removal from body, a diseased liver causes a decrease in glucose synthesis, affecting CNS function and causing fatigue; also causes accumulation of nitrogen waste with immense toxic effect on many tissues.

Additionally, effective water distribution is not maintained, causing oedema. There is also general alteration in cell function, causing general malaise and fatigue. Disease of the liver also causes malfunction in circulation, detrimental to survival.

Another major consequence of liver damage is a loss in the body's ability to detoxify and eliminate foreign substances, causing general toxification and general malaise.

Of the known hepatitis viruses, three can cause persistent infection and chronic hepatitis: the hepatitis B virus (HBV) (Lau *et al.*, 1993), the hepatitis C virus (HCV) (Gowans *et al.*, 2004)and the hepatitis delta (or hepatitis D) virus (HDV) (Hoofnagle, 1989). Hepatitis A and E cause acute, self-limited disease only.

Aside the effects hepatitis has on the liver, it also has an adverse effect on other vital organs such as the heart, with no known link. The combined infection of human cytomegalovirus and hepatitis C virus is reported to increase the risk of allograft vascular disease in heart transplant recipients (Dal Bello *et al.*, 1998). (Tsui *et al.*, 2009) shows that HCV seropositivity is found to be associated with inflammatory markers and heart failure events in persons with coronary heart disease.

Though vaccination for Hepatitis has been resorted to and its long-term reduction in cases has been appreciated, in Isreal for example, in Hepatitis A infections (Chodick *et al.*, 2008).

Incidence in the case of Argentina, even after vaccination which reduced incidence by 88% was detected to be 10.2/100,000 (Vacchino, 2008) and viral hepatitis infections still highly prevail in endemic populations such as Egypt, with the highest worldwide prevalence of HCV of 6->40%, and poses future morbidity and mortality risks (Lehman *et al.*, 2009).
Incidence of hepatitis B virus infections can also be found, though minute, among blood donors even in developed countries such as Japan (Tanaka *et al.*, 2008) and the United States of America (Zou *et al.*, 2009).

Drug-induced hepatitis remains a major concern to Medical practitioners (Barker *et al.*, 1976; Gungabissoon, 2003). In a survey, 13% of new patients who had anti-Tuberculosis administration had drug-induced hepatitis after treatment (Baghaei *et al.*, 2009). Epidemiological studies made in Tayside in a ten year period revealed 4992 patients with only viral hepatitis, of whom some were identified to have used drugs of potential abuse (Steinke *et al.*, 2002).

1.5 METHODS OF STUDYING HEPATIC DAMAGE

There are various ways by which hepatic damage could be assessed. Methods used are based on principles that govern mechanisms of liver damage and its manifestation. Assessment of biochemical parameters that are hindered in hepatic damage; presence of chemicals and chemical components that are in excess of the normal, evident in hepatic damage; assessment of the body's ability to metabolise certain chemicals managed by the liver are some methods used in assessing liver damage.

Hepatic damage usually manifest in jaundice. Jaundice is the yellowish staining of the skin and sclerae (the whites of the eyes) that is caused by high levels of the chemical bilirubin in the blood. When red blood cells get old, they are destroyed. Haemoglobin, the iron-containing chemical in red blood cells that carries oxygen, is released from broken down red blood cells, and the chemical that remains in the blood after the iron is removed is bilirubin. Bilirubin is a waste product removed from the blood by the liver.

After bilirubin has entered liver cells, they are conjugated to other chemicals, primarily glucuronic acid, and the conjugated bilirubin (bilirubin/glucuronate complex) is secreted into bile. This is eliminated through the intestines and the bladder.

Jaundice can occur when there is excessive bilirubin production (due to increased and/or rapid breakdown of red blood cells) for removal by the liver, as in haemolytic anaemia; a defect in the liver that inhibits bilirubin removal from the blood; or where there is cholestasis (a defect in the process of bilirubin conjugation, secretion, or flow of bile), such as

- a defect in the liver resulting in cholestasis,
- blockage of the hepatic ducts and/or the common hepatic duct that decreases the flow of bile from the liver into the gallbladder, as exhibited in cancers and inflammation of the hepatic ducts,
- blockage of the cystic and/or common bile duct that decreases the flow of bile from the gallbladder and liver, into the intestines, as in cancers, inflammation of the bile ducts and the presence of gallstones.

By evaluating the direct, indirect and total bilirubin levels, the extent of jaundice can be known, and hence the extent of liver damage (Khan, 2006).

In assessing the hepatoprotective ability of a drug in rodent models, determination of the liver enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamic transpeptidase (GGT), and bilirubin levels play a major role. These biochemical parameters reflect damage to the hepatic cells, and are determined clinically with Liver Function Tests (LFTs). ALP and GGT are however generally representative of the hepatobiliary system (The system of liver and the bile ducts). ALP is an enzyme, which is produced, and thus associated with the biliary tract. It is however not specific to the biliary tract because of its presence in bone and placenta. GGT though a more sensitive marker than ALP, may elevate even in minimal levels of liver damage. The enzyme AST also reflects damage to the hepatic cells, but is less specific for liver disease since it is present in minor quantities in red blood cells, cardiac, bone and brain cells, and

may be elevated in other conditions such as a myocardial infarct (heart attack). ALT is the enzyme produced within the cells of the liver. The level of ALT is increased in conditions where cells of the liver have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. The ratio of direct (conjugated) and/or indirect (unconjugated) bilirubin to total bilirubin is also significantly helpful in determining extent of liver damage.

In this experiment therefore, we would focus on the aminotransferases and bilirubin since they are quite specific liver function determinants.

The extent of damage can also be assessed by pentobarbitone-induced sleep time. Pentobarbitone is a chemical that depresses the central nervous system (CNS), and thus puts the body into an unconscious 'sleep' mode when administered. The chemical is however metabolized by the liver. Thus, the period it takes the body to recover from unconscious to conscious depicts the period it takes the liver to metabolise the chemical, and thus, the liver's functional ability.

Homogenous solutions could be made of blood and its light spectrum assessed with a Specrophotometer for various biochemical parameters evident in hepatic damage.

Histopathological analysis is another method of assessment useful after lethality due to hepatic injury. This involves excising the liver and making stained slides of liver segments, and observing under an electron microscope for changes in liver structure.

Scanning the liver with X-rays is sometimes used to examine the liver for abnormalities. This helps to assess changes in the liver such as inflammation.

1.6 INDUCTION OF HEPATIC DAMAGE

Liver injury is caused by various factors, such as viral infections, poisoning from alcohol, chronic bacterial infections and drugs, over periods of exposure. Experimentally, hepatic damage is induced using quick effective and reliable methods that give reproducible effects, with minimum lethality expectation (Jang *et al.*, 2008). This is usually attained experimentally by chemical means.

Carbon tetrachloride is a known hepatotoxic agent used to induce liver injury due to its toxicity to hepatocytes that produces effects histopathologically similar to that of viral hepatitis. Its mechanism of action has been however obscure. Recent investigation conducted found mitochondria to be the main cell component attacked by the drug (Christie *et al.*, 1954). In this process, the tricarboxylic acid cycle is disorganized by the inhibition of the oxidation of citrate, malate, pyruvate and glutamate, 10 to 15 hours after administration of the drug. This is resulted in a disorganization of the enzyme systems. The mitochondria affected were found to lose pyridine nucleotides, and allow penetration of Co I, at abnormally fast rates, than in normal mitochondria.

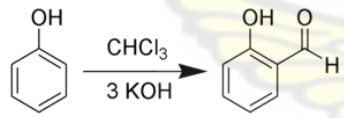
Acetaminophen (paracetamol), a widely used anti-pyretic analgesic, due to its availability and accessibility, upon repeated use or overdose becomes Hepatotoxic (Mahadevan *et al.*, 2006). This has been used experimentally to produce hepatic damage in rats. The injury is more pronounce when complemented by alcohol, since alcohol promotes the production of acetaminophen metabolites that are toxic.

In a study, the halothane-hypoxia (HH) model (adult male rats, phenobarbital induction, 1% halothane, 14% O_2, for 2 hrs); and the enflurane-hypoxia heating (EHH) model (phenobarbital-pretreated male adult rats, exposure to 1.5-1.8% enflurane at 10% O_2 for 2 hours with external heating); were assessed for efficiency for rat models of hepatotoxicity. The course of development of injury in the HH model resembled that of an oral dose of CCl_4. EHH resulted in an elevated serum glutamate pyruvate transaminase values and vacuolization of centrilobular hepatocytes (Lind *et al.*, 1985).

In another study, hepatic injury with the hepatotoxic drugs thioacetamide, dimethylnitrosamine, and carbon tetrachloride (CCl_4), for 12 weeks, through oral and intraperitoneal injection routes were compared in terms of the degree of fibrosis, reproducibility and histologic damage. Mice administered with 50% solution of CCl_4 (2 ml/kg orally) tolerated the entire induction period of 12 weeks, and histology shown that livers from animals administered with CCl_4 orally twice a week for 10 weeks was the most effective to achieve sufficient fibrosis highest reproducibility with acceptable animal survival (Jang *et al.*, 2008).

1.7 PHENOL – CARBON TETRACHLORIDE REACTION

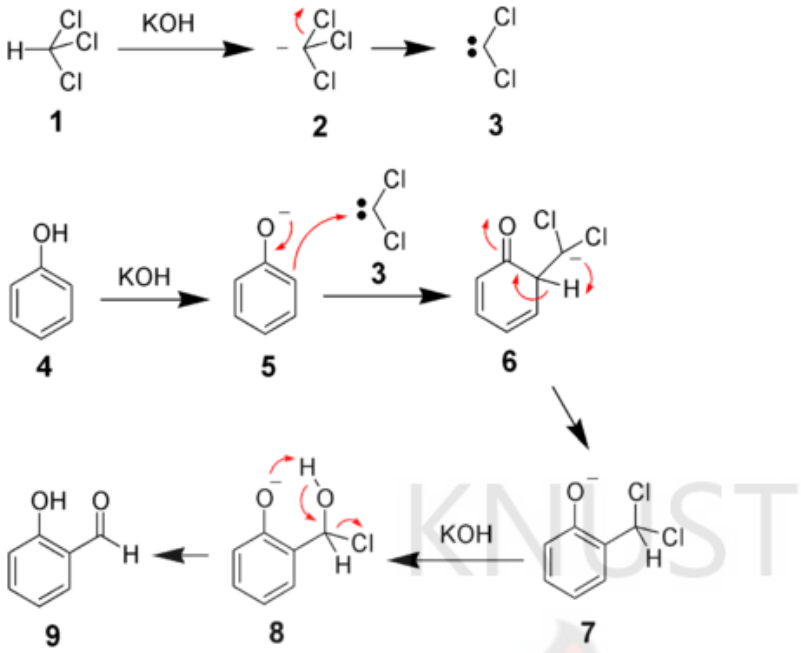
The reaction of phenols with carbon tetrachloride (CCl_4) is known as the Reimer–Tiemann reaction. In this reaction, the CCl_4 reacts with the phenol under a strong base to form Salicylaldehyde.



(Salicylaldehyde)

Mechanism of action:

Chloroform (1) reacts with a strong base to form a chloroform carbanion (2), which quickly alpha-eliminates to give dichlorocarbene (3). The dichlorocarbene then reacts in the ortho- and para- positions of the phenate (5 and 6) to give the dichloromethyl substituted phenol (7), which after basic hydrolysis forms the product salicylaldehyde (9).



Salicylaldehyde (9) is a known key precursor to a variety chelating agents.

1.8 CURRENT MANAGEMENT OF LIVER DISEASES

Management of liver diseases involves the management of jaundice (since bilirubin is a waste product which can be toxic to the system) and management of the root cause of the jaundice, then the cause of the liver disease. Hepatoprotectants used for the management of hepatitis varies from orthodox through homoeopathy to botanic medical therapies.

Silymarin, multivitamins, methionine, ursodeoxycholic acid and liver hydrolysate have also been used to manage liver diseases (Chamulitrat *et al.*, 2009; Crocenzi *et al.*, 2006).

Nitric oxide, a cytoprotectant, inhibits cell destruction by modulating heat shock proteins, S-nitrosylating caspases at their catalytic site cysteine residue, triggering the cGMP pathway, and preventing mitochondrial dysfunction (Abdel Salam *et al.*, ; Wang *et al.*).

Presently, there hasn't been found any synthetic hepatic damage remedy safe enough to give therapy effectively, yet without severe side effects (Ram *et al.*, 1999). In addition, no hepatic damage remedy has been found to give complete and perpetual cure to hepatic injuries, without relapses or resurfacing of the disease. Medicinal products used are found to give only symptomatic relief to patient with hepatic disorder without managing the fundamental cause to the symptoms (Ram *et al.*, 1999).

In Homoeopathy, liver injury, depending on its symptoms, cause and extent of damage, is managed by a variety of drugs, some of which are Bryonia, Mercurius, Podophyllum, Chelidonium and Digitalis.

Natural hepatoprotectants are used, and though not well investigated, are claimed to be effective in controlling hepatic disorders while limiting the side effects of the drug. Botanically, many hepatoprotectants have been reported. *Glycosmis arborea* extract was able to overcome the toxic effects of hepatotoxic agents in terms of lowering the levels of serum GPT, alkaline phosphatase necrosis of liver produced by carbon tetrachloride was reversed by the extract (Gomes *et al.*, 2003).

Intake of purple grape juice is found to possess antioxidant ability (El-Ashmawy *et al.*). Even its antioxidant ability is investigated to be more pronouncing in juices made from grapes cultivated with organic material than in purple grape juices of conventional sources (Dani *et al.*, 2008). This may predict the possible effects of biochemically functional foods. Dietary fish oil is also used to protect the liver from disorders, since it is known to contain omega-3 (Roy *et al.*, 2007). Studies with mice revealed that very long chain omega-3 PUFA like eicosapentaenoic and docosahexaenoic acid may act as preventive agent for hepatic cirrhosis.

New targets of hepatoprotectant still emerge, as the search of hepatoprotectants continues. In a recent report, the hepatic apelin system (Apelin is a peptide in serum that plays an important role in myophysiology and pathophysiology and inflammation), is evaluated (in rats with cirrhosis and ascites) for the involvement of the endogenous apelin system in the pathogenesis of the hepatic remodeling and complications that

occur in advanced liver diseases (Principe *et al.*, 2008). Findings predicted the hepatic apelin system as a novel therapeutic target in liver disease.

(Ram *et al.*, 1998) screened functionalized 1, 3-teraryls, synthesized through ring transformation of 6-aryl-3-carbomethoxy-4-methylthio-2H-pyran-2-one from arylketone for their hepatoprotective activity, and of them have demonstrated significant protection in animal hepatitis models.

In spite of the prevalence and increasing incidence and risk of hepatitis infections, there is however only a limited number of orthodox drugs and a limited number of botanical medicines with scientifically validated potential for the management of jaundice associated liver diseases. The use of herbal remedies for various ailments, folkloric or scientific has been of great usefulness to mankind. The search of botanical sources for hepatoprotective remedies to support the management of liver diseases will therefore be worthwhile.

Spondias mombin extract is a frequently used medication However, its specific role in the management of jaundice has not been validated scientifically. There is also little knowledge on the potential in-vivo toxicity of the plant. This study intends to establish the scientific basis and to validate the use of the plant in the treatment of jaundice, and evaluate its potential toxicity to ensure the safety of the remedy.

1.9 THE PLANT SPONDIAS MOMBIN

Spondias mombin L. (picture 1) (Family**:** Anacardiaceae), synonym *Spondias lutea,* commonly known as hog plum, yellow *mombin* or ubos. Locally called 'atoaa' in Ashanti, is a deciduous erect tree which grows to 15 - 20 meters tall with a trunk 60-75 cm wide. It has a grayish bark, slightly buttressed, thick, coursey trunk. The leaves are green, slender and tapest at the end, 15-30 cm long, most of which are shed prior to the production of numerous small, white fragrant flowers. The plant bears small (2 – 4 cm long, 1.5 – 2 cm

wide), green, plum-like shaped fruits which turn yellow upon ripening (Burkill, 1985; Gregory, 2000).

Picture 1: Picture of Spondias mombin tree branches with leaves and ripening fruits (http://www.rain-tree.com/Plant-Images/ubos-pic.htm)

Kingdom: Plantae

Phylum: Angiosperms

Order: Sapindales

Family: Anacardiaceae

Genus: *Spondias*

Species: *Spondias mombin*

1.9.1 Geographic distribution

The plant is found in the tropical Americas, including the West Indies, and has been naturalized in parts of Africa, including Ghana, and some parts of Asia (Burkill, 1985; Thomas, 1974).

1.9.2 Folkloric uses

It is traditionally known widely for the treatment of a variety of disease conditions. Its bark, leaves, roots and fruits are used in various ways. The leaves are used in the treatment of bacterial infections, the prevention and inhibition of the progression of viral infections, treatment of candida infections, and expelling parasites such as intestinal worms. It is also known to reduce anxiety, stop convulsions, calm and sedate, relieve pain,

and suppress cough. And also aid digestion and stimulate the uterus (Ademola *et al.*, 2005; Amadi *et al.*, 2007; Caceres *et al.*, 1995; Corthout *et al.*, 1988).

The bark is also used to reduce inflammation, relief pain, reduce spasms, kill fungi, kill bacteria, heal rashes, heal wound and stop bleeding. It is also used as a contraceptive (Uchendu *et al.*, 2008; Villegas *et al.*, 1997).

1.9.3 Biological actions of the plant

Antifertility activity of aqueous ethanolic leaf extract of *Spondias mombin* was reported (Uchendu *et al.*, 2008) to have anticonceptive but not abortifacient activity attributed to a direct action of the extract on the uterus.

Recent studies have proven its anthelmintic activity. In sheep, Ademola *et al*, (2005) reports the mean percentage feacal egg reduction in sheep drenched with extract of *Spondias mombin* to 15-100% in *Haenmonchus spp., Trichostrongylus spp., Oesophagostomunm spp., Strongyloides spp*. and *Trichuris spp*.

Spondias mombin also exhibited comparatively higher efficacy as an anthelmintic in studies with earthworm (Gbolade *et al.*, 2008) using paralysis time and death time.
The sedative, antiepileptic and antipsychotic effects are also documented (Ayoka *et al.*, 2006). Methanolic, ethanolic and aqueous extracts prolonged hexobarbital-induced sleeping time and reduced novelty-induced rearing (NIR) behaviour in both mice and rats.

On antimicrobial studies, extract of leaves of *Spondias mombin* exhibits wide spectrum antibacterial effects comparable to those of ampicillin and gentamycin (Abo *et al.*, 1999).
Antibacterial and molluscicidal activities is attributed to the long-chain salicylic acid derivatives isolated (Corthout *et al.*, 1994). These phenolic acids were shown to have pronounced antibacterial effect against *Bacillus cereus, Streptococcus pyogenes* and *Mycobacterium fortuitum*, and molluscicidal effect against the snail *Biomphalaria glabrata* (an intermediate host in the schistosome life cycle).

1.9.4 Chemical and nutrients composition of Spondias mombin

Recent quantitative analysis by Njoku (2007) revealed that leaves of *Spondias mombin* contains 3.82 %(w/v) tannins, 7.60 % saponins, 6.0 % alkaloids, 3.0 % flavonoids and 1.0 % phenols. The ethanolic extract of leaves and stems of *Spondias mombin* was found to contain the three phenolic acids: 6-(8'Z, 11'Z, 14'Z-heptadecatrienyl)-salicylic acid, 6-(8'Z, 11'Z-heptadecadienyl)-salicylic acid, and 6-(10'Z-heptadecenyl)-salicylic acid which are known to be responsible for the actibacterial and molluscicidal activity of the extract (Corthout *et al.*, 1994). Corthout (1992) also reports that the two Caffeoyl esters: 2-O-Caffeoyl-(+)-allohydroxycitric acid and chlorogenic acid butyl ester isolated, contributes to the antiviral action of the extract.

The extract contains potassium, sodium, calcium, phosphorus and magnesium (Njoku, 2007). The leaves are also rich in ascorbic acid and contain a good amount of niacin. It also contains riboflavin and thiamin (Njoku, 2007). SB-202742, a novel beta-lactamase inhibitor isolated from *Spondias mombin* (Coates *et al.*, 1994) may contribute to the antimicrobial activity of the leaves.

1.10 AIMS AND OBJECTIVES OF STUDY

The objective of the current study was

- To validate the possible prophylactic, therapeutic and concomitant use of *Spondias mombin* leaf extract in a rodent model of carbon tetrachloride-induced liver damage, and
- To assess the possible toxicity of *Spondias mombin* in rodents.

Specific objectives included:

- Determination of the LD_{50} of aqueous leaf extract of *Spondias mombin* in male and female mice.
- Determination of the LD_{50} of aqueous leaf extract of *Spondias mombin* in rats.

- Acute and sub-acute toxicity studies of aqueous leaf extract of *Spondias mombin* in rats:
    - The effects of aqueous leaf extract of *Spondias mombin* on haematological parameters,
    - The effects of aqueous leaf extract of *Spondias mombin* on biochemical parameters,
    - The effects of aqueous leaf extract of *Spondias mombin* on organo-body ratios of the target organs of toxicity; liver, kidneys, stomach and spleen,
    - Histopathological studies on the effects of aqueous leaf extract of *Spondias mombin* on the target organs of toxicity; liver and kidney.
- Induction of liver injury in rats using carbon tetrachloride (CCl_4), and investigation of the possible therapeutic, prophylactic and concomitant effects of aqueous leaf extract of *Spondias mombin* on the liver injury, based on the assessment of the levels of:
    - serum biochemical parameters alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamic transpeptidase (GGT), and total bilirubin, and
    - histopathological studies on slides of liver cells from extract - treated and control rats.
- Assessment of the effect of pentobarbitone on sleep-times of rats administered with both extract and carbon tetrachloride (CCl_4).

1.11 JUSTIFICATION OF PROJECT

Jaundice due to hepatitis is gradually becoming a problem of public health concern due to the changing lifestyle of people and increasing exposure to industrial hazardous chemicals. The indigenous African still uses herbal remedies in the management of various ailments. Personal communication with herbalists reveals that, in Ghana, in addition to the various uses of *Spondias mombin,* it is useful in the treatment of liver diseases including jaundice in parts of the Ashanti region. Its specific role in the management of jaundice has however not been validated scientifically. The hepatoprotective effects when established will justify the use and contribution of *Spondias mombin* in the management of hepatitis related jaundice.

Spondias mombin extract is a frequently used medication. However, there is also little knowledge on the potential toxicity of the plant. Establishing and documenting scientifically, the potential toxicity of the extract will ensure safety of the remedy.

2 Chapter 2 MATERIALS AND METHODS

2.1 ANIMALS

ICR mice (15 – 30 g) and Sprague dawley rats (100 – 250 g) of either sex were used. Animals were obtained from the Animal house of the Department of Pharmacology, KNUST or purchased from the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon. Purchased animals were quarantined and observed for 3 days before used. All animals were kept under normal day-night cycles, normal humidity levels, and room temperature, fed on a standard diet (normal commercial pellet diet from GAFCO, Tema) and provided with water *ad libitum.* All animals were healthy and kept in steel laboratory cages (34 X 47 X 18 cm) with wood shavings as bedding.

2.2 CHEMICALS AND REAGENTS

Carbon tetrachloride (CCl_4), Tragacanth, ethylenediaminetetraacetic acid (EDTA) and Formalin were purchased from BDH Chemicals Ltd, Poole, England. Silymarin was purchased from MADAUS, Paris, whilst olive oil was obtained from Bells, sons & co. Druggists, UK.

2.3 PLANT MATERIAL

Spondias mombin leaves were collected from the Kwame Nkrumah University of Science and Technology (KNUST) botanical gardens and its surroundings in December, 2006. Samples were authenticated at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana.

Leaves of *Spondias mombin* were washed with clean water, bench - dried and grounded comminuted using a Hammer mill. Aqueous extraction of sample was done by boiling 500g

of leaves in 5 litres of distilled water for 30 minutes on a hot plate. The supernatant was filtered by decanting and sieving, and the sample was subsequently re-boiled until there was maximum extraction. Maximum extraction was achieved with a final volume of 20 litres. Extract was then concentrated in a hot air oven at 60^0C and freeze-dried into dark brown powder. The yield was 6 %(w/w). Powdered extract was stored at room temperature in an enclosed rubber container.

2.3.1 Preparation of plant material for dosing

Sample suspensions were freshly prepared with 2% Tragacanth in distilled water, which served as drug vehicle, and for its controls. Suspensions were administered orally (p.o.). Volumes of extract administered did not exceed 2ml/kg body weight of the animal. Prepared suspensions were kept at -2 to + 8 ^{0}C for a maximum of one week.

2.4 QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *SPONDIAS MOMBIN*

The presence of glycosides in *Spondias* was tested by adding 5 ml of dilute sulphuric acid to 1 ml of the aqueous filtrate of the extract in a test tube, heated, cooled and neutralized with 20 % NaOH. 5 ml of Fehling's solution A and B was then added and observed for a brick red precipitate (Harbourne, 1984), (Sofowora, 1993)

To test for reducing sugars, 0.2 g of the *Spondias mombin* extract was dissolved in 5 ml of water and filtered. An equal volume (5 ml) of Fehling's A and B solutions were added to the filtrate, heated and observed for a red-brown precipitate (Sofowora, 1993).

Test for saponins was also done by adding 0.2 g of the extract to 5 ml of water in a test tube, after which the mixture was shaken and allowed to stand, and observed for the presence of a froth which does not break readily upon standing. (Trease, 1983)

Presence of flavonoids was assessed by first adding 5 ml of dilute ammonia solution to 2 ml of the aqueous filtrate of the *Spondias* extract, then slowly adding concentrated H_2S0_4

to the resultant solution and observing for a yellow coloration (Harbourne, 1984), (Sofowora, 1993).

To test for the presence of tannins, 0.5 g of the extract was dissolved in 25 ml of water and filtered. 1 ml of the extract was made up to 10 ml with water, and 2 drops of 1 % ferric chloride solution was added and observed for a blue - black precipitate (Sofowora, 1993).

2.5 LD_{50} DETERMINATION AND ACUTE TOXICITY STUDIES.

The LD_{50} and acute toxicity of the *Spondias* extract was determined separately for male and female ICR strain mice, and subsequently determined for Sprague Dawley rats.

2.5.1 LD_{50} and Acute toxicity in male mice

Thirty - five male mice were grouped into 7 (n = 5). All animals were starved overnight of food, with water *ad libitum* on day 0. Group 1, the control group received 2 % Tragacanth at 2 ml/kg body weight. Groups 2, 3, 4, 5, 6 and 7 received 50 mg/kg, 100 mg/kg, 500 mg/kg, 1000 mg/kg, 3000 mg/kg and 5000 mg/kg body weight of *Spondias*, p.o. respectively, on day 1, after which feeding was resumed. Volumes of extracts administered did not exceed 2ml/kg body weight. All animals were closely observed for 24 hours, for lethality, general behavioural, physiological and pharmacological changes. Animals were kept and further observed for 14 days, for possible late toxicity of the extract.

2.5.2 LD_{50} and Acute toxicity in female mice

Thirty - five female mice were grouped into 7 (n = 5). All animals were starved overnight of food, with water *ad libitum* on day 0. Group 1, the control group received 2 % Tragacanth at 2 ml/kg body weight. Groups 2, 3, 4, 5, 6 and 7 received 50 mg/kg, 100 mg/kg, 500 mg/kg, 1000 mg/kg, 3000 mg/kg and 5000 mg/kg body weight of *Spondias*, p.o. respectively, on day 1, after which feeding was resumed. Volumes of extracts

administered did not exceed 2ml/kg body weight. All animals were closely observed for 24 hours, for lethality, general behavioural, physiological and pharmacological changes. Animals were kept and further observed for 14 days, for possible late toxicity of the extract.

2.5.3 LD_{50} and Acute toxicity in rats

Eighteen rats were grouped into 6 (n = 3). All animals were starved overnight of food, with water *ad libitum*. Groups 2, 3, 4, 5 and 6 received a single dose of 100 mg/kg, 500 mg/kg, 1500 mg/kg, 3000 mg/kg and 5000 mg/kg body weights of *Spondias mombin* respectively. Volumes of extracts administered did not exceed 2ml/kg body weight. Group 1 received 2 % Tragacanth at 2 ml/kg body weight, and served as the control. Feeding continued after dose administration. All animals were closely observed for 24 hours, for general behavioural, physiological and pharmacological changes, as well as lethality. Animals were kept and further observed for 14 days, for possible late toxicity of the extract.

2.6 SUB-ACUTE TOXICITY STUDIES

Twenty female rats were grouped into 4 (n = 5). All animals were starved overnight of food, with water *ad libitum* on day 0. On days 1 through to day 14, group 1 served as control and received 2 % Tragacanth (2ml/kg) daily. Groups 2, 3, and 4 received 300 mg/kg, 1000 mg/kg and 1500 mg/kg body weight of *Spondias*, daily p.o. respectively. Volumes of extracts administered did not exceed 2ml/kg body weight. During this period, animals were observed for general behavioural, physiological and pharmacological changes, as well as lethality. On days 8 and 15, body weights of the animals were taken. Blood samples were taken for haematological and biochemical analysis on day 15. All animals were subsequently euthanised, and samples of the target organs of toxicity, liver and kidney taken for histopathological analysis.

2.6.1 Haematological analysis

Blood samples were collected via veinous puncture into sterile sample tubes containing the anticoagulant, EDTA. Blood haemoglobin concentration (HB), Red blood cell (RBC) count, White blood cell (WBC) count, Haematocrit (HCT), Mean haemoglobin concentration (MHC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) as well as Platelet (PLT) count were analysed using an automated analyser, Cell Dyne, model 331430, Abbott laboratories, IL USA.

2.6.2 Assessment of serum biochemical parameters

Blood was collected via veinous puncture into sterile sample tubes without anticoagulant, allowed to settle, and separated by centrifuging at 500 rpm for 10 minutes. Supernatant serum was collected and analysed for levels of the liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamic transpeptidase (GGT), using an automated analyser, ATAC 8000 (Elan Diagnostics, CA USA). Total, direct and indirect bilirubin levels were also determined.

2.6.3 Organo-body ratios

At the end of the experiment, animals were weighed and euthanised. The target organs, liver, kidneys, spleen and stomach were excised from individual animals and weighed. The ratio of organ to body weights were then computed and statistically analysed.

2.6.4 Histopathological analysis

The target organs of toxicity, liver and kidney excised from animals and weighed were stored in 1 % formal saline solution, for tissue sections and subsequent examination.

2.7 HEPATOPROTECTIVE STUDIES

This was carried out in 3 phases: therapeutic, prophylactic and concomitant. In all phases, Silymarin was used as a standard hepatoprotective agent in one of the groups. Liver injury was induced as recommended by Jang *et al*. (2008) for one week.

2.7.1 Therapeutic effect of Spondias mombin in rat models of carbon tetrachloride-induced liver injury

In this study, liver injury was induced before administration of extract. Induction of liver injury was done by oral administration of 50 % carbon tetrachloride (CCl_4) in olive oil at 2 ml/kg body weight, every other day for 1 week. 20 female rats were divided into 4 groups (n=5). In week 1, liver injury was induced in groups 2, 3 and 4. Group 1, the negative control group, received olive oil (2ml/kg). Forty-eight hours after administration of last dose, administration of extract commenced. Extract was administered daily p.o. 14 days. Groups 2 and 3 received 300mg/kg and 1000mg/kg body weight of *Spondias* extract, while group 4 received Silymarin at 25mg/kg body weight, daily. Volumes administered did not exceed 2ml/kg body weight. Group 1 received 2 % Tragacanth (2ml/kg). Twenty-four hours after the last dose of treatment, blood samples were taken from animals for biochemical analysis. All animals were subsequently euthanised, and the livers excised for histopathological examination.

2.7.1.1 Assessment of serum biochemical parameters

Blood was collected via veinous puncture without anticoagulant, allowed to settle, and separated by centrifuging at 500 rpm for 10 minutes. Supernatant serum was collected and analysed for levels of the liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamic transpeptidase (GGT), using an automated analyser, ATAC 8000 (Elan Diagnostics, CA USA). Total, direct and indirect bilirubin levels were also determined.

2.7.1.2 Histopathological analysis

All animals were euthanised after experiments, and the target organ, liver was excised from animals, weighed and stored in 1 % formal saline solution, for tissue sections and subsequent examinations.

2.7.2 Prophylactic assessment of Spondias mombin in rat models of carbon tetrachloride-induced liver injury

Liver injury in this experiment was induced 1 week after commencement of treatment with extract and standard drug reference. Twenty female rats of were divided into 4 groups (n = 5). Group 1 received 2 % Tragacanth and olive oil (at 2ml/kg) and served as the negative control. In the first week, groups 2 and 3 received 300 mg/kg and 1000 mg/kg body weight of *Spondias* extract, while group 4 received Silymarin at 25mg/kg body weight orally, every day. Volumes administered did not exceed 2ml/kg body weight. Group 1 received 2 % Tragacanth at 2 ml/kg. Liver injury was induced by oral administration of 50 % carbon tetrachloride in olive oil at 2 ml/kg body weight, every other day for 1 week. Administration of extract and reference drug doses continued in week 2, coupled with the induction of liver injury in groups 2, 3 and 4, and administration of olive oil at 2 ml/kg body weight in group 1 every other day, for a week.

Twenty-four hours after the last administration, blood samples were taken from animals for biochemical analysis. All animals were subsequently euthanised, and the livers excised for histopathological examination.

2.7.2.1 Assessment of serum biochemical parameters

Blood was collected via veinous puncture without anticoagulant, allowed to settle, and separated by centrifuging at 500 rpm for 10 minutes. Supernatant serum was collected and analysed for levels of the liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamic transpeptidase (GGT), using an automated analyser, ATAC 8000 (Elan Diagnostics, CA USA). Total, direct and indirect bilirubin levels were also determined.

2.7.2.2 Histopathological analysis

All animals were euthanised after experiments, and the target organ, liver was excised from animals, weighed and stored in 1 % formal saline solution, for tissue sections and subsequent examinations.

2.7.3 Assessment of the concomitant effect of Spondias mombin and liver injury with carbon tetrachloride in rats

This experiment was conducted to assess the hepatoprotective ability of the extract when administered at the same time carbon tetrachloride injury is incurred.

Twenty female rats were grouped into 4 (n = 5). Group 1 served as the negative control and received 2 % Tragacanth and olive oil (2ml/kg). Liver injury was induced concomitantly with daily administration of minimum and high doses of extract, and reference drug for one week. Induction of liver injury was done by oral administration of 50% carbon tetrachloride in olive oil at 2 ml/kg body weight, every other day for 1 week.

Groups 2 and 3 received 300 mg/kg and 1000 mg/kg body weight of *Spondias* respectively, whilst group 4 received Silymarin at 25 mg/kg body weight. Volumes administered did not exceed 2ml/kg body weight. Twenty-four hours after the last administration, blood samples were taken from animals for biochemical analysis. All animals were subsequently euthanised, and the livers excised for histopathological examination.

2.7.3.1 Assessment of serum biochemical parameters

Blood was collected via veinous puncture without anticoagulant, allowed to settle, and separated by centrifuging at 500 rpm for 10 minutes. Supernatant serum was collected and analysed for levels of the liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamic transpeptidase (GGT), using an automated analyser, ATAC 8000 (Elan Diagnostics, CA USA). Total, direct and indirect bilirubin levels were also determined.

2.7.3.2 Histopathological analysis

All animals were euthanised after experiments, and the target organ, liver was excised from animals, weighed and stored in 1 % formal saline solution, for tissue sections and subsequent examinations.

2.8 EFFECT OF *SPONDIAS MOMBIN* EXTRACT ON PENTOBARBITONE-INDUCED SLEEPING TIME IN RATS

Male rats were put in 7 groups (n = 5). Extract was administered p.o. daily for 9 days at 300 mg/kg, 500 mg/kg, 1000 mg/kg and 1500 mg/kg body weight in groups 3, 4, 5 and 6 respectively. Volumes administered did not exceed 2ml/kg body weight. Group 7 received Silymarin at 50 mg/kg body weight daily, whilst groups 1 and 2 received 2 % Tragacanth (2ml/kg). During this period, carbon tetrachloride (CCl_4) was administered (2ml/kg) on days 3, 5 and 7 to groups 2, 3, 4, 5, 6 and 7. Group 2 served as positive control (for CCl_4). Group 1 received olive oil (2ml/kg) and served as the negative control. Six hours after the last administration of doses, all animals were challenged with a single dose of pentobarbitone at 50mg/kg body weight. Pentobarbitone was administered intraperitonealy (i.p). The time it took each animal to lose its righting reflex was recorded as the time between injection with pentobarbitone and the loss of the righting reflex. Sleeping time was measured as the time between the fall of sleep (as measured by loss of the righting reflex) and the time of wake (as measured by the regain of righting reflex).

2.9 STATISTICAL ANALYSIS

Results are expressed as mean ± standard error of the mean (S.E.M.), and the number of observations is represented by 'n'. One way Analysis of Variance (ANOVA) was used to compare group data, followed by Tukey's multiple comparison test ($p < 0.05$ was considered significant). The statistical package used was Graph pad Prism 5.00.288.

3 Chapter 3 RESULTS

3.1 PHYTOCHEMISTRY OF *SPONDIAS MOMBIN*

A general test for glycosides and reducing sugars were positive. Further phytochemical tests showed that *Spondias* leaf extract contained tannins, saponins and flavonoids (Table 1).

Table 1: Presence of phytochemicals in aqueous leaf extract of *Spondias mombin*.

TEST	OBSERVATION	COMMENT
Glycosides	A brick red precipitate observed	Glycosides +
Tannins	A blue-black precipitate observed	Tannins ++
Saponins	A persistent froth which did not break upon standing observed	Saponins +++
Reducing sugars	A reddish-brown precipitate was observed on heating	Reducing sugars +
Flavonoids	The resultant solution turned yellow	Flavonoids +++

+, ++ and +++ indicates presence of trace, medium and substantial amount of corresponsive phytochemical respectively.

3.2 LD_{50} AND ACUTE TOXICITY STUDIES IN MALE MICE

No lethality was observed in all 7 groups of male mice for the period of the experiment. The single dose administration of *Spondias mombin* did not show any significant changes in behavioural, physiological and pharmacological activities. There was however, a dose-dependent increase in the food intake (19.00 ± 1.00g to 34.00 ± 8.72 g) of animals treated with 50 – 5000 mg/kg, compared to the control group (18.67 ± 0.33 g) which received the drug vehicle at 2 ml/kg body weight, from day 2 of experimental period (Figure 1A). Further observation of animals for 14 days did not reveal any signs of late toxicity of the extract.

3.3 LD_{50} AND ACUTE TOXICITY STUDIES IN FEMALE MICE

Female mice administered with single doses of *Spondias* (50 – 5000 mg/kg) orally, similarly did not show any significant changes in behavioural, physiological and pharmacological activities. There was however, a dose-dependent increase in the food intake (19.33 ± 0.88 to 34.67 ± 8.67g) of grouped animals treated with 50 – 5000 mg/kg, compared to the control group (19.00 ± 0.00g) which received the drug vehicle at 2 ml/kg, from day 1 of experimental period (Figure 1B). There were no signs of late toxicity in mice when observed for the 14 day period. No lethality observed.

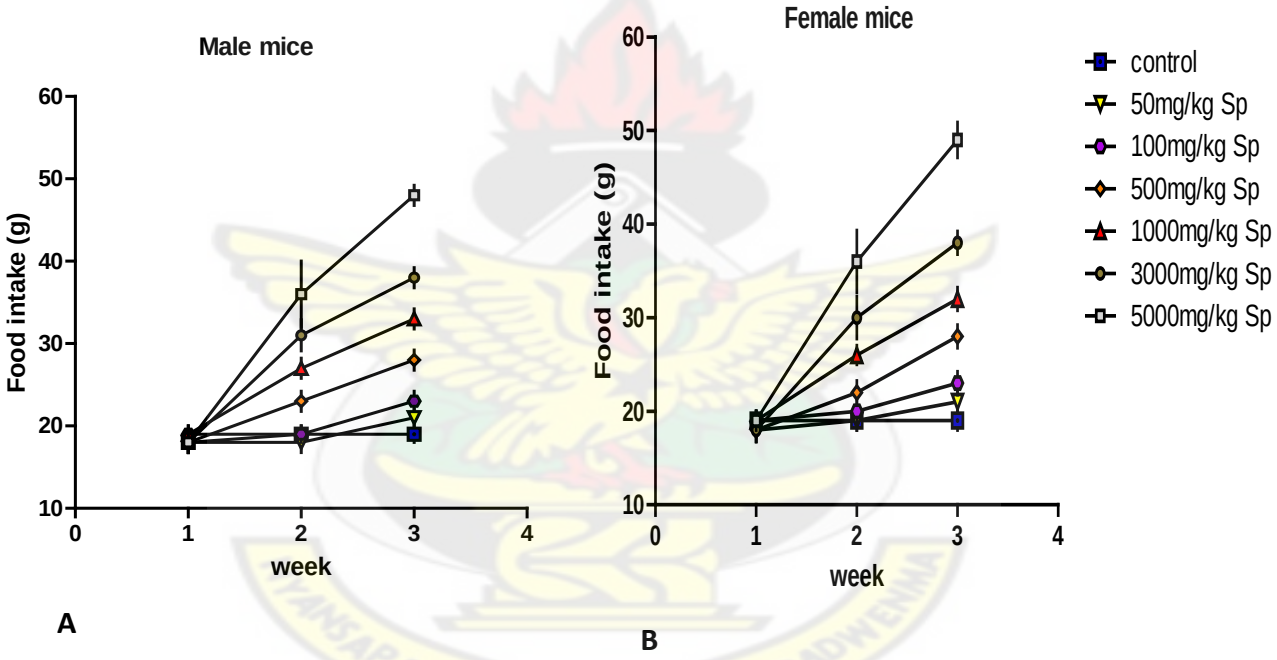


Figure 1: Food intake pattern of (A) male mice and (B) female mice. Animals received a single dose of 50 – 5000 mg/kg body weight of *Spondias* orally. Values are presented as mean + SEM.

3.4 LD_{50} AND ACUTE TOXICITY STUDIES IN RATS

No lethality was observed in rats administered with single doses of *Spondias* (100 - 5000 mg/kg body weight) p.o. Animals did not show any significant changes in behavioural, physiological and physical activities. There were no signs of late toxicity in rats when observed for a further 14 day period.

3.5 SUB-ACUTE STUDIES IN RATS

3.5.1 Haematological parameters

There was no significant difference in blood haemoglobin concentration (HB), red blood cell (RBC) count, haematocrit (HCT), mean haemoglobin concentration (MHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), Procalcitonin (PCT) as well as platelet (PLT) count (Figure 3). The white blood cell (WBC) count however showed a significant decrease at doses of 300 mg/kg ($22.53 \pm 4.61 \times 10^9$/L at $p < 0.01$) and 1000 mg/kg ($30.88 \pm 2.47 \times 10^9$/L at $p < 0.05$) of extract compared to the control ($46.65 \pm 0.35 \times 10^9$/L) by Tukey's test. The extract however showed a dose dependent decrease in WBC counts compared to control (Figure 3H).

3.5.2 Serum biochemical parameters

After a 14 day administration of doses of *Spondias*, levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamic transpeptidase (GGT), total bilirubin, albumin and total protein, in all groups treated with the *Spondias* extract (300 mg/kg, 1000 mg/kg and 1500 mg/kg body weight) were comparable to that of the control group (Figure 2). There was no significant change in serum biochemical parameters.

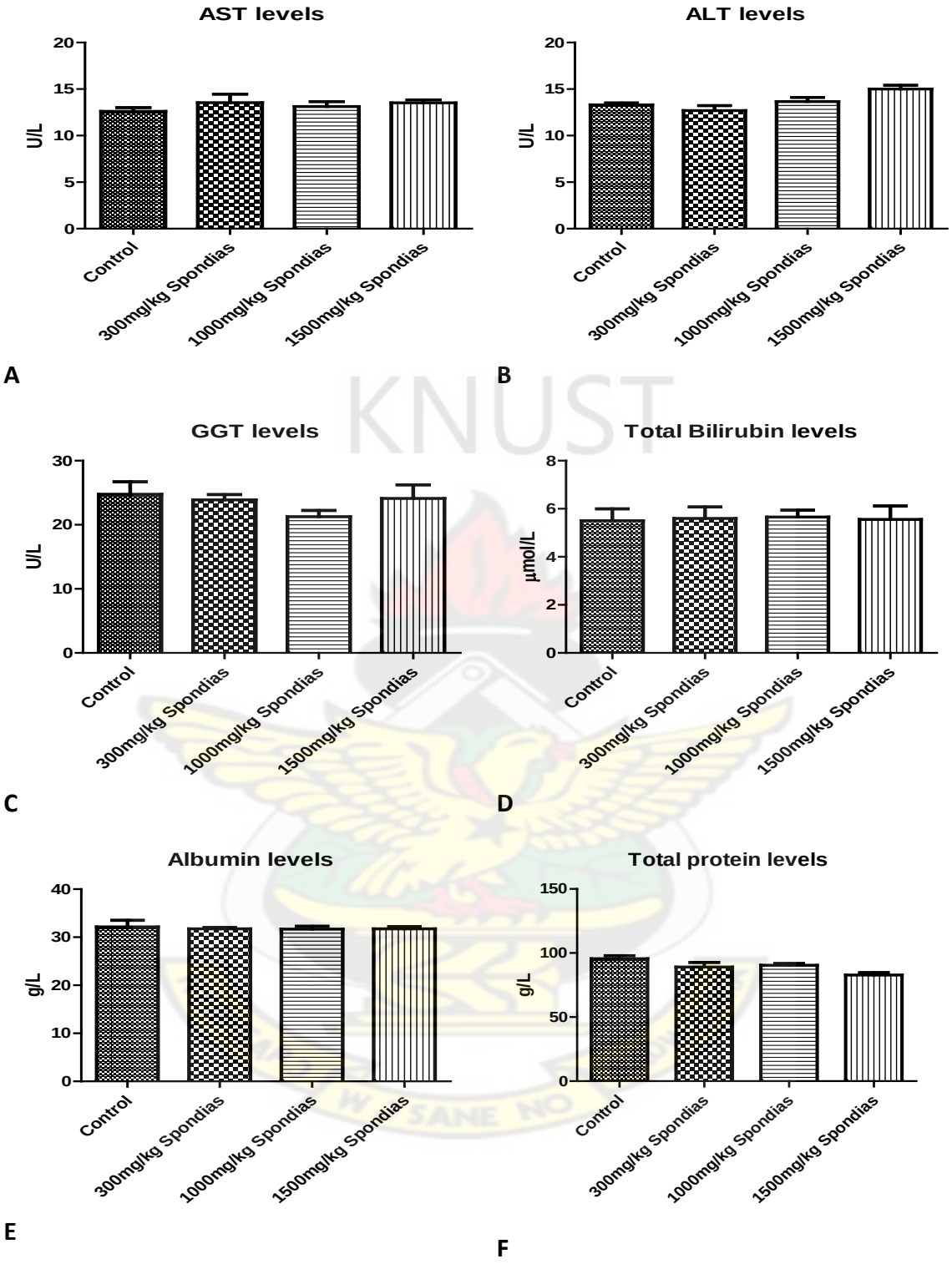


Figure 2: Effect of Spondias on biochemical parameters in rats that received 300 - 1500 mg/kg *Spondias* daily for 14 days. The levels of levels of alanine aminotransferase (ALT)(A), aspartate aminotransferase (AST)(B), gamma glutamic transpeptidase (GGT)(C), total bilirubin (D), albumin(E) and total protein (F) levels. Values are presented as mean ± SEM.

3.5.3 Relative weights of organs

There was no change in the organ – body weight ratios in the animals over the period of treatment. Ratios were comparable to the control, with the exception of the group administered with 300 mg/kg, which showed slightly decreased ratios (Table 2).

Table 2: Effect of *Spondias* on relative organ to body weights in rats treated with extract for 14 days.

	ORGANO-BODY RATIOS (%)			
TREATMENTS	liver	kidney	stomach	spleen
Control	2.27±0.10	0.79±0.12	0.74±0.07	0.27±0.02
300mg/kg *Spondias*	1.79±0.06	0.54±0.04	0.51±0.02	0.22±0.01
1000mg/kg *Spondias*	2.01±0.08	0.64±0.04	0.59±0.03	0.24±0.01
1500mg/kg *Spondias*	1.99±0.07	0.61±0.04	0.67±0.04	0.25±0.01

Values are expressed as mean ± SEM

3.5.4 Histopathological examination

Examination of histopathological slides of the liver confirmed no cellular damage in all groups of animals, compared to the control (Panel 1). Cells of liver and kidneys were within normal limits, with only mild reactive changes in cells of rats that received 300 mg/kg body weight of extract (see original pathology report, Tables 3 and 4).

Kidney cells observed also showed no cellular damage. Cells were within normal limits, with mild reactive changes in cells of group rats that received 300mg/kg body weight of extract (see original pathology report, Table 4). Panel 2 shows high power photomicrographs of kidney cells of various rat groups.

Table 3: Histopathology report on liver cells of rats in sub-acute toxicological studies of *Spondias mombin*

GROUP	HISTOLOGY OF LIVER CELLS
Control:	Within normal limits
300mg/kg *Spondias*:	Within normal limits
1000mg/kg *Spondias*:	Within normal limits
1500mg/kg Spondias:	Within normal limits

Indices assessed:

- Reactive changes
- Apoptosis
- Necrosis
- Inflammation
- Steatosis

Table 4: Histopathology report on kidney cells of rats in sub-acute toxicological studies of *Spondias mombin*

GROUP	HISTOLOGY OF KIDNEY CELLS
Control:	Mild psammoma calcification, mild glumerulonephritis with regional ischemic change (Within normal limits)
300mg/kg *Spondias:*	Within normal limits
1000mg/kg *Spondias:*	Within normal limits
1500mg/kg *Spondias:*	Within normal limits

Indices assessed:

- Reactive changes
- Apoptosis
- Necrosis
- Inflammation
- Steatosis

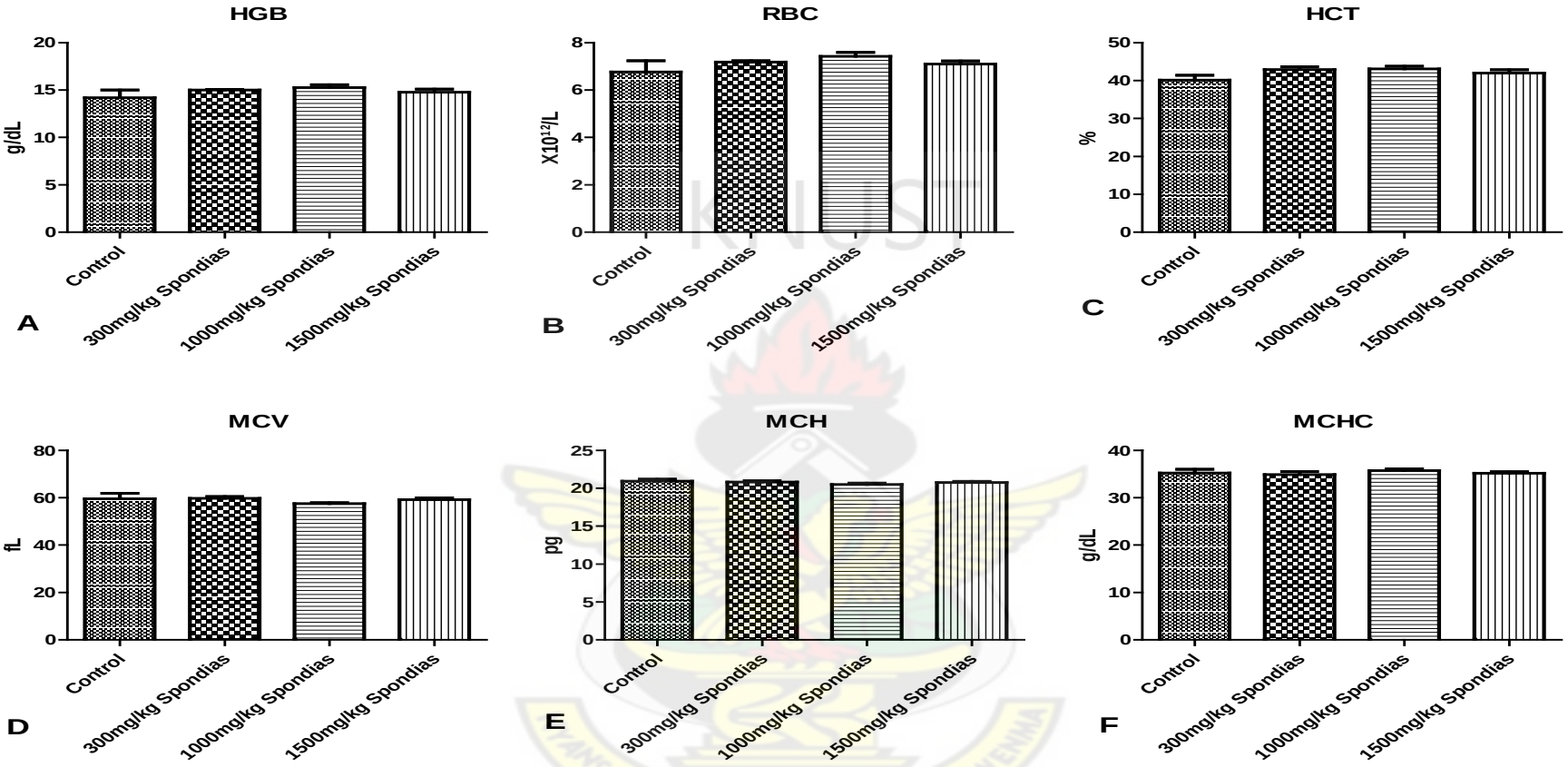


Figure 3A - F: Effect of Spondias on haematological parameters in rats. Animals received doses orally for 14 days. The blood haemoglobin concentration (HB) (A), red blood cell (RBC) count (B), haematocrit (HCT) (C), mean corpuscular volume (MCV) (D), mean haemoglobin concentration (MHC) (E) and mean corpuscular haemoglobin concentration (MCHC) (F) levels. Values are represented as mean + SEM. * indicates significance ($p < 0.05$), and ** indicates significance ($p < 0.01$), compared to control group by Tukey's test.

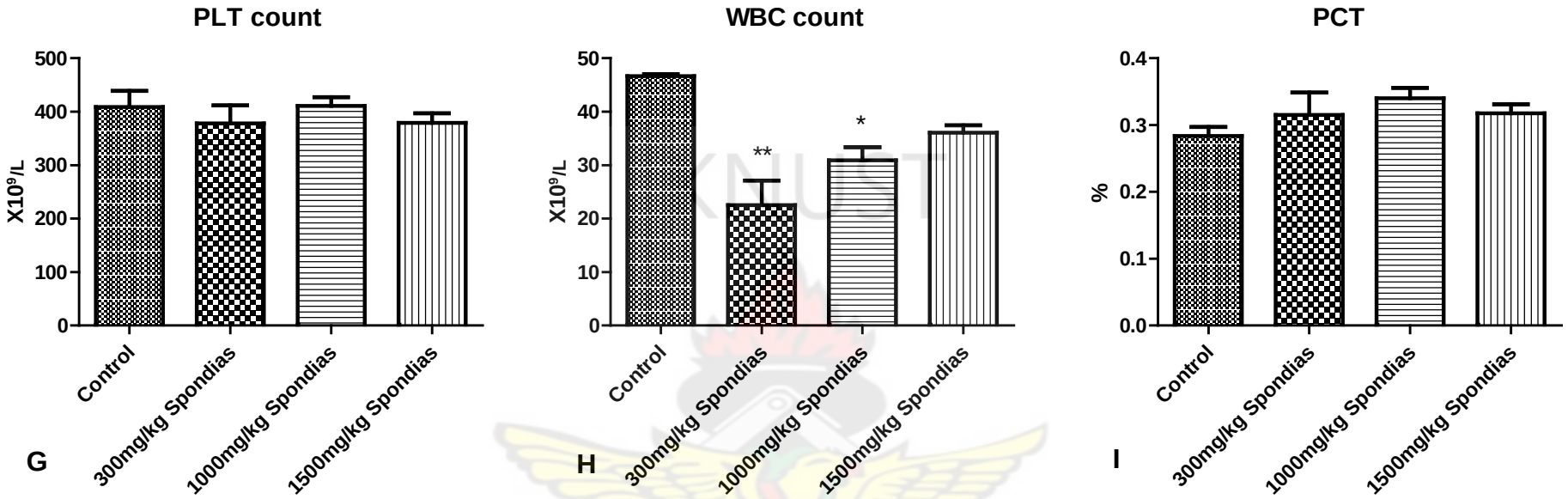
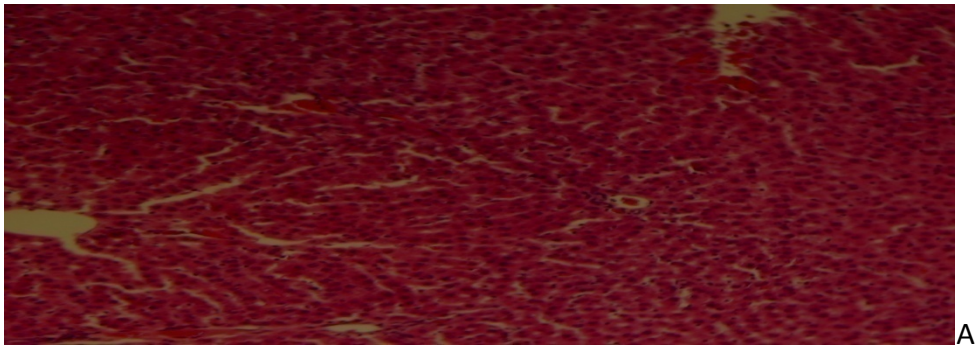


Figure 3G – I: Effect of Spondias on haematological parameters in rats. Animals received doses orally for 14 days. The blood platelet (PLT) count (G), white blood cell (WBC) count (H) and Procalcitonin (PCT) levels (I). Values are represented as mean + SEM. * indicates significance ($p < 0.05$), and ** indicates significance ($p < 0.01$), compared to control group by Tukey's test.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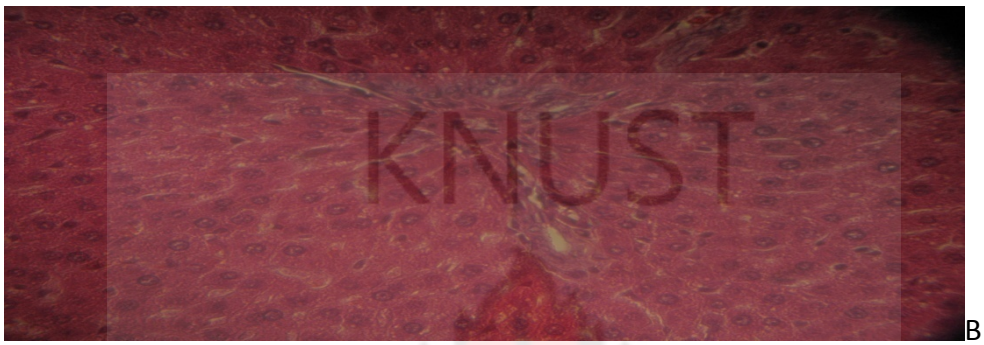

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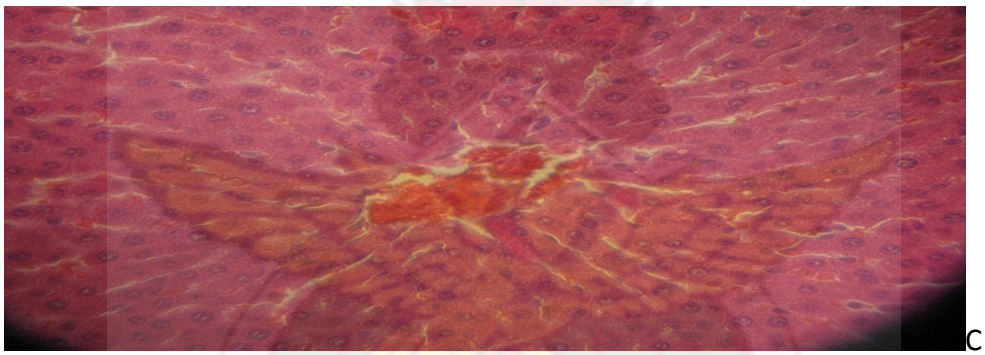
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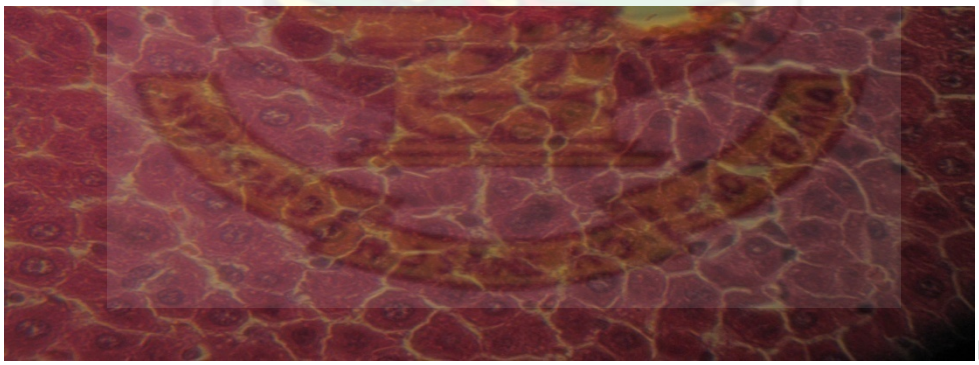
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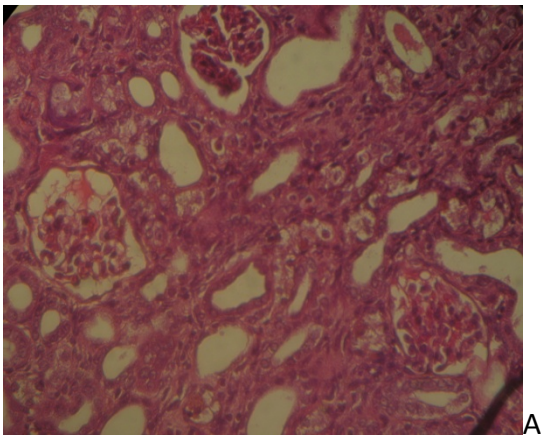
Panel 1: Photomicrographs, X 250 (original) of liver cells of (A) control rat that received drug vehicle, (B) rat that received 300 mg/kg *Spondias mombin*, (C) rat that received 1000 mg/kg *Spondias mombin* and (D) rat that received 1500 mg/kg *Spondias mombin* for 14 days.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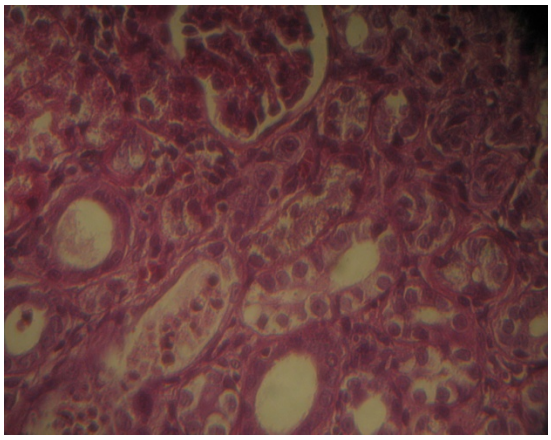
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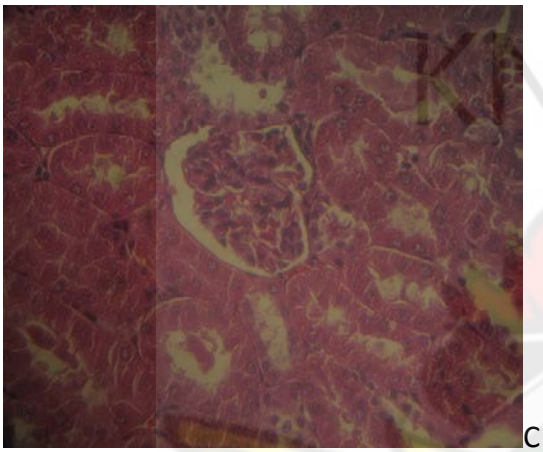
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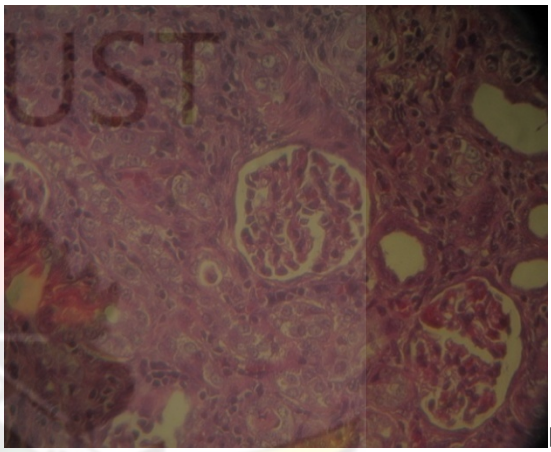
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Panel 2: Photomicrographs, X 250 of kidney cells of (A) control rat that received drug vehicle for 14 days, (B) rat that received 300 mg/kg *Spondias mombin*, (C) rat that received 1000 mg/kg *Spondias mombin* and (D) rat that received 1500 mg/kg *Spondias mombin*, for 14 days.

3.6 THERAPEUTIC EFFECT OF *SPONDIAS MOMBIN* IN RAT MODELS OF CARBON TETRACHLORIDE-INDUCED LIVER INJURY

Results from clinical biochemistry assessment reveals that *Spondias mombin* extract did not show significant alterations in the levels of gamma glutamic transpeptidase (GGT), and shows slight alterations in bilirubin, which was very evident in the indirect bilirubin (unconjugated bilirubin) levels (Figure 5). Aspartate aminotransferase (AST) compared to the vehicle - treated control group however showed a significant decrease at 1000 mg/kg dose level of extract compared to the CCl_4 – only treated control (Table 5). There was also a significant ($p < 0.0001$) decrease in the levels of alanine aminotransferase (ALT) in all treated groups comparable to the vehicle control and reference groups, compared to the CCl_4 – only treated control (Table 5, Figure 4). The total protein level in blood was compromised, but did not show effect on the synthesis of albumin, which showed a comparable level to the negative control at 1000 mg/kg of the extract.

Table 5: Effect of *Spondias* on serum biochemical parameters in rats treated with *Spondias* and reference drug for 14 days, after administration of CCl_4

TREATMENTS	LEVEL OF SERUM BIOCHEMICAL PARAMETER					
	AST U/L	ALT U/L	GGT U/L	Total bilirubin µmol/L	Albumin(g/L	Total protein(g/L)
Control	138.5±1.5	48.5±0.5***cc	5.0±0.0	3.4±0.0	35.1±0.51	75.76±5.28
CCl_4 only	139.4±6.5	219.7±30.02	5.5±0.5	5.2±0.1	31.68±0.75*	61.2±0.64**
CCl_4 +300mg/kg *Spondias*	138.7±7.2	44.56±2.01***cc	5.46±0.23	5.9±0.6	33.0±0.83	61.6±0.85**
CCl_4+1000mg/kg *Spondias*	114.9±1.7*cc	40.25±2.39***cc	5.425±0.27	4.2±0.5	35.38±0.57**cc	65.03±0.84
CCl_4 +Silymarin	111.3±0.7**cc	38.04±0.88***cc	5.28±0.19	5.06±0.16	34.9±0.54*cc	64.04±0.35*
p value	0.0015	<0.0001	0.7929	0.0489	0.0030	0.0035

Values are expressed as mean ± SEM. *, ** and *** indicates significance at $p < 0.05$, $p < 0.01$ and $p < 0.0001$ respectively compared to the negative control. *cc, **cc and ***cc indicates significance at $p < 0.05$, $p < 0.01$ and $p < 0.0001$ respectively compared to the CCl_4 control group.

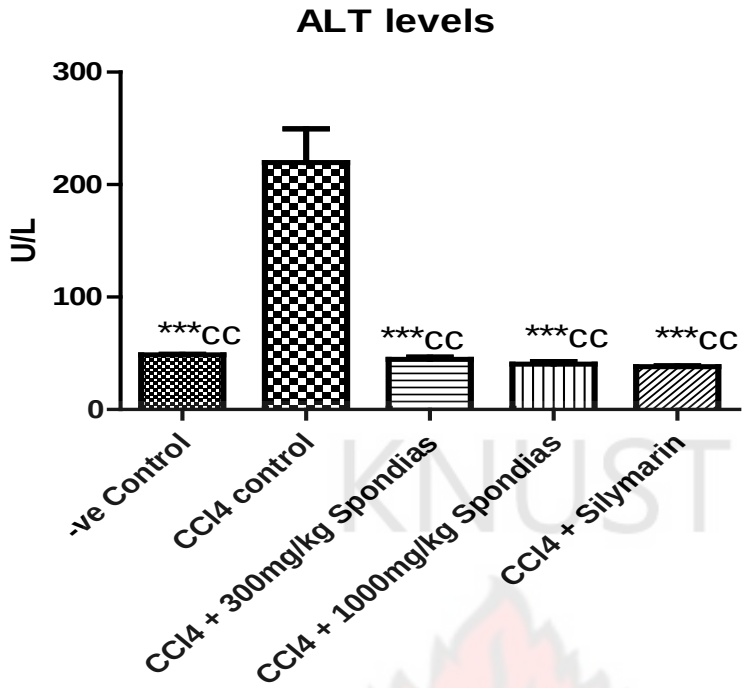


Figure 4: Effect of *Spondias* on serum alanine aminotransferase (ALT) in rats treated with *Spondias* and reference drug (Silymarin) for 14, days after administration of CCl4. Values are presented as mean ± SEM. ***cc indicates significance at $p < 0.0001$ compared to the CCl4 control group.

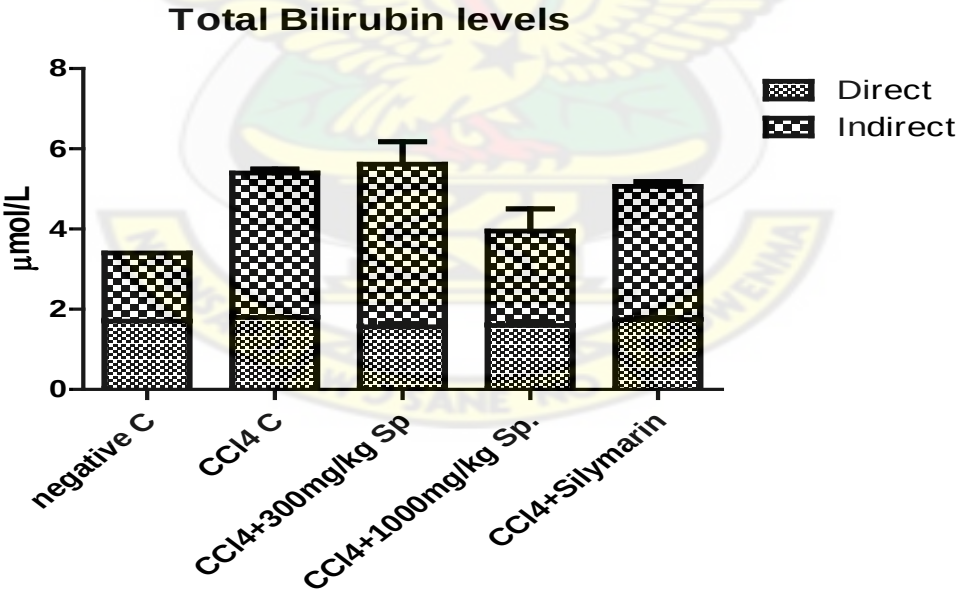


Figure 5: Effect of *Spondias* on bilirubin levels in rats treated with *Spondias* and reference drug (Silymarin) for 14, days after administration of CCl4. Values are presented as mean ± SEM. Interaction between parameters is significant at $p = 0.0202$. The level of indirect bilirubin decreased comparable to the control at 1000 mg/kg body weight of *Spondias*.

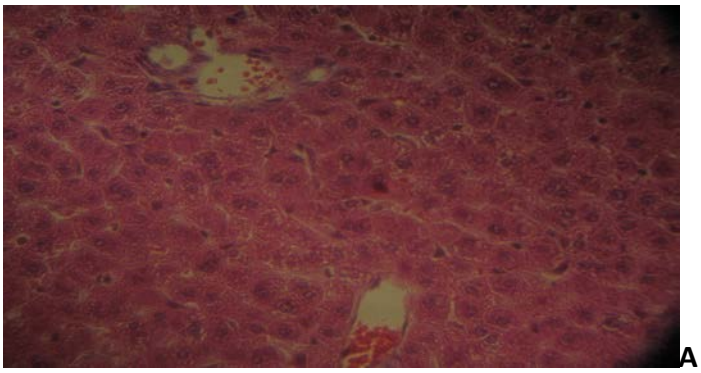
Histopathological examination of cells from all groups of experimental rats revealed that groups treated with 300 mg/kg, 1000 mg/kg body weight of *Spondias* and the Silymarin showed no signs of cell damage. The cells were within normal limits, with mild reactive changes, focal portal lymphocytic infiltrate and cell congestion due to cell recovery processes compared to the CCl_4 control group (Panel 4). Extract treated groups showed significant cell recovery comparable to the vehicle control (Panel 3). The CCl_4 - treated control group however showed mild to moderate zone 2 and 3 steatosis with increased mitosis and degenerative changes and apoptosis (see original pathology report, Table 6).

Table 6: Histopathology report on liver cells of rats in therapuetic assessment of *Spondias mombin*

GROUP	HISTOLOGY
Control:	Within normal limits.
CCl_4 only:	Moderate zone two and three steatosis, degenerative changes, apoptosis.
CCl_4+300mg/kg *Spondias*:	Mild reactive change, otherwise normal.
CCl_4+1000mg/*kg Spondias*:	Within normal limits
CCl_4+Silymarin:	Within normal limits.

Indices assessed:

- Reactive changes
- Apoptosis
- Necrosis
- Inflammation
- Steatosis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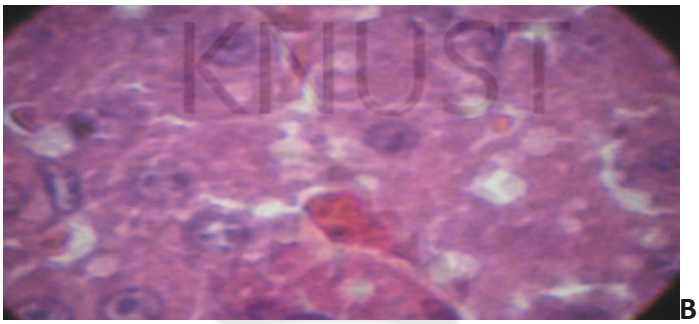

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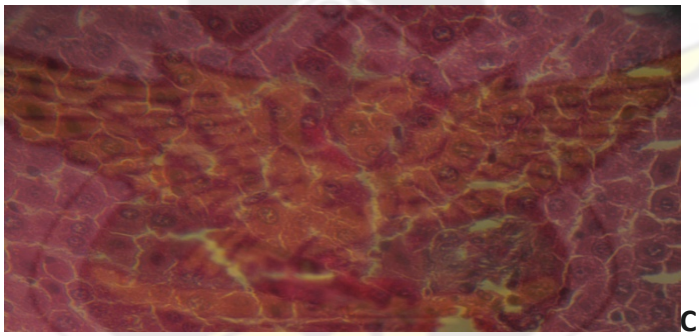
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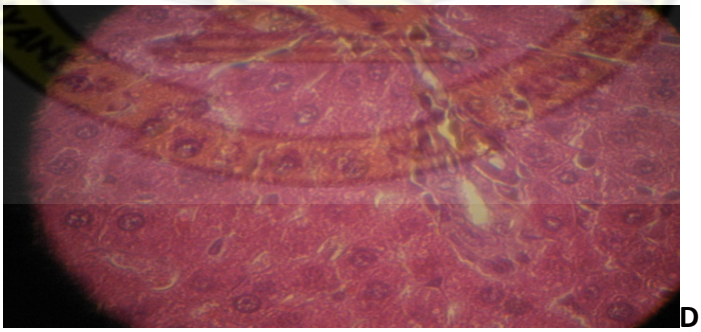
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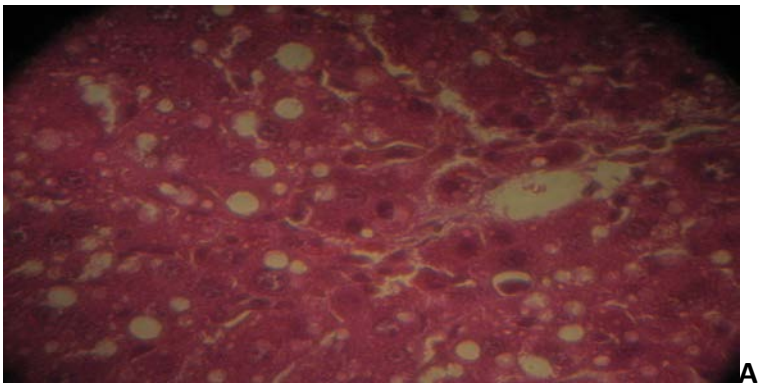
Panel 3: Photomicrographs, X 250 of liver cells of rats of the negative control group that received only vehicles (A), rats that were induced with carbon tetrachloride and treated with 300 mg/kg *Spondias* (B), 1000 mg/kg *Spondias*(C) and Silymarin (reference drug at 25 mg/kg) (D).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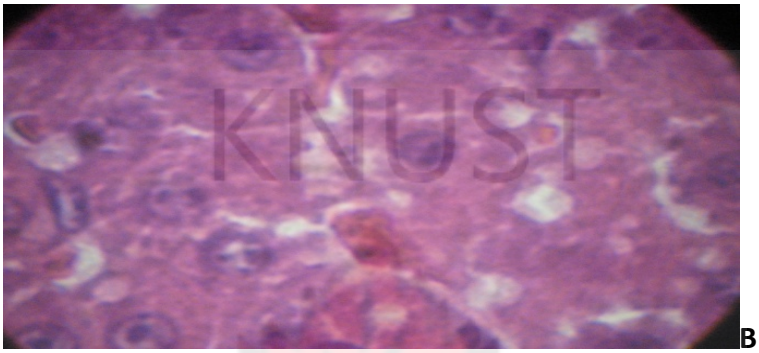
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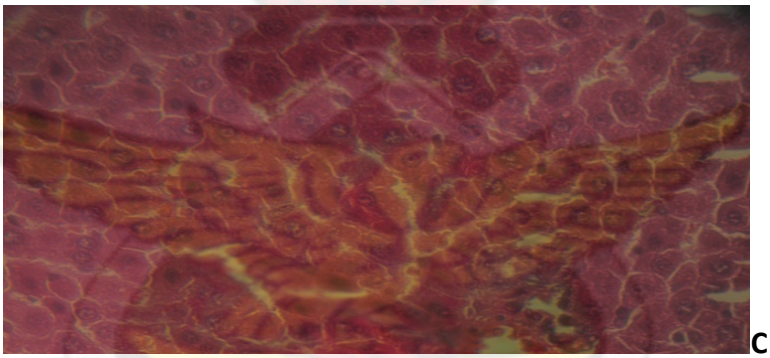
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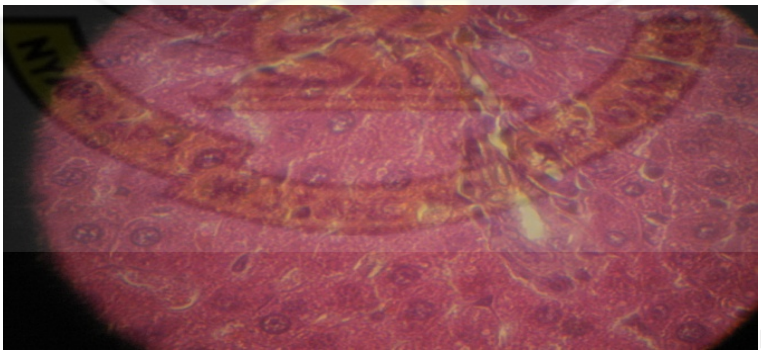
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Panel 4: Photomicrographs, X 250 of liver cells of rats of the positive control group that were administered with carbon tetrachloride only (A), rats that were induced with carbon tetrachloride and treated with 300 mg/kg *Spondias* (B), 1000 mg/kg *Spondias* (C) and Silymarin (reference drug at 25mg/kg) (D*).*

3.7 PROPHYLACTIC ASSESSMENT OF *SPONDIAS MOMBIN* IN RAT MODELS OF CARBON TETRACHLORIDE-INDUCED LIVER INJURY

Spondias mombin extract showed highly significant reduction in aspartate aminotransferase, AST (to 114.6 ± 1.6 U/L) at a dose of 300 mg/kg body weight, compared to the CCl_4 control (135.9 ± 4.0 U/L). This was comparable to the negative control (120.1 ± 2.8 U/L). The extract also significantly lowered the levels of alanine aminotransferase, ALT dose-dependently from 117.6 ± 0.6 U/L to 114.6 ± 2.6 U/L (CCl_4 and 300mg/kg to CCl_4 and 1500mg/kg *Spondias* groups, respectively), compared to the CCl_4 control group (119.1 ± 0 .8 U/L). The reduction was comparable to the negative control (110.2 ± 0.6 U/L) and the Silymarin (104.3 ± 5.1 U/L) groups (Table 8).

Histopathological examination of slides of liver sections revealed that the extract dose-dependently alleviated the degree of cellular damage, evident in the elimination of extensive fatty changes and reduction in cell necrosis (Panels 5 and 6). At 1000 mg/kg of extract, fibrosis was not detected. Cells showed very mild necrosis, otherwise, normal (see original pathology report, Table 7).

Table 7: Histopathological report on liver cells of rats in the prophylactic assessment of *Spondias mombin*

GROUP	HISTOLOGY
Control:	Within normal limits (of mild fatty change and inflammation).
CCl_4 only:	Mild to Extensive fatty change, fibrosis and inflammation.
CCl_4+300mg/kg *Spondias*:	Mild to Moderate fatty change, mild inflammation, very mild necrosis and fibrosis.
CCl_4+1000mg/kg *Spondias*:	Moderate fatty change, very mild necrosis; otherwise normal.
CCl_4+1500mg/kg *Spondias*:	Mild fatty change, mild inflammation and fibrosis; very mild necrosis.
CCl_4+Silymarin:	Moderate fatty change, mild inflammation and necrosis, very mild fibrosis.

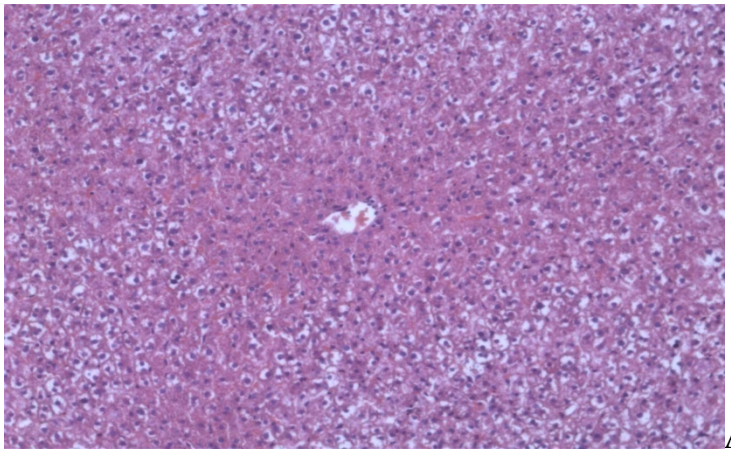
Features assessed:

- Periportal fibrosis
- Central fibrosis
- Necrosis
- Inflammation
- Cytoplasmic
- Vacuolation/fatty degeneration

Table 8: Effect of *Spondias* on serum biochemical parameters in rats treated with *Spondias* and Silymarin (for 7 days) before and during the administration of CCl_4 (prophylactic).

TREATMENTS	SERUM BIOCHEMICAL PARAMETER							
	AST(U/L)	ALT(U/L)	ALP(U/L)	GGT(U/L)	Total bilirubin(µmol/L)	Indirect bilirubin(µmol/L)	Albumin(g/L)	Total protein(g/L)
Control	120.1±2.8**cc	110.2±0.6	284.5±17.6	5.22±0.4	4.8±0.6	2.40±0.27	40.22±1.52	66.74±1.83
CCl_4 only	135.9±4.0	119.1±0.8	249±0.6*	4.46±0.3	5.68±0.4	3.00±0.08	34.22±1.24	64.14±0.89
CCl_4 +300mg/kg *Spondias*	114.6±1.6***cc	117.6±0.6	251.1±1.2	4.18±0.4	5.85±0.1	3.08±0.08	35.76±1.19	66.24±0.48
CCl_4+1000mg/kg *Spondias*	116.8±1.9***cc	115.3±2.1	240.6±4.5**	4.76±0.1	5.08±0.2	2.82±0.09	36.6±1.89	66.4±0.93
CCl_4+1500mg/kg *Spondias*	118.2±1.6**cc	114.6±2.6	237.6±4.7**	4.98±0.4*	5.1±0.4	2.46±0.20	42.4±1.17**CC	67.5±1.09
CCl_4 +Silymarin	110±4.1***cc	104.3±5.1	220.5±5.7***	3.6±0.4*	5.45±0.5	2.8±0.16	36.5±0.96	67.63±0.81
P value	<0.0001	0.0047	0.0007	0.0398	0.4157	0.0348	0.0031	0.2987

Values are expressed as mean ± SEM. *, ** and *** indicate significance at $p < 0.05$, $p < 0.01$ and $p < 0.0001$ respectively, compared to control. *cc indicates significance at $p < 0.05$ compared to the CCl_4 control group. **cc indicates significance at $p < 0.01$ compared to the CCl_4 control group and ***cc indicates significance at $p < 0.0001$ compared to the CCl_4 control group.



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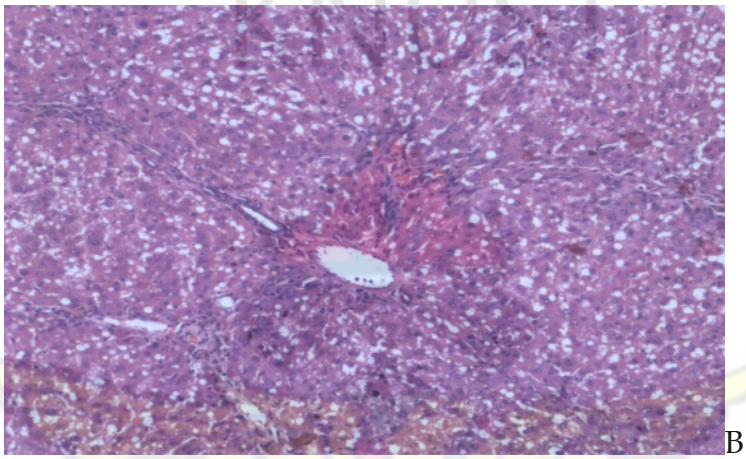

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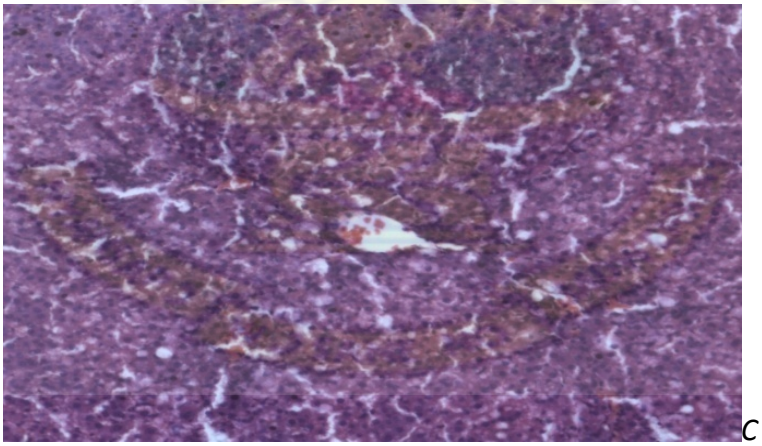

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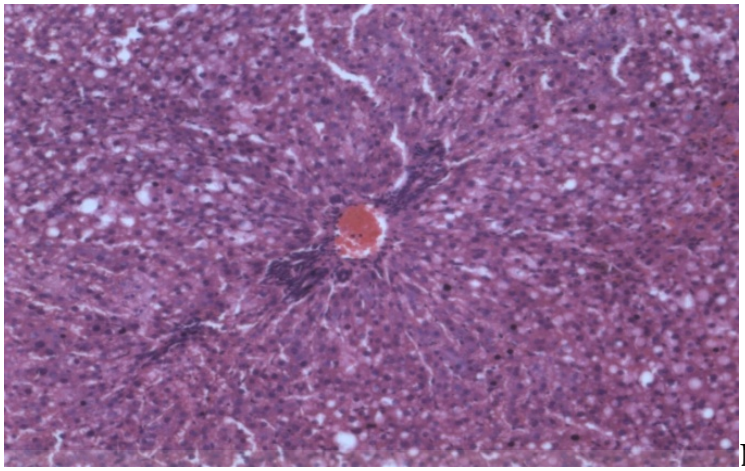
Panel 5 (A –C): Photomicrographs, X 100 of cells of rats of the the negative control group that received only drug vehicle (A), rats that were treated with 300 mg/kg *Spondias* (B) and 1000 mg/kg *Spondias*(C); and induced with carbon tetrachloride.



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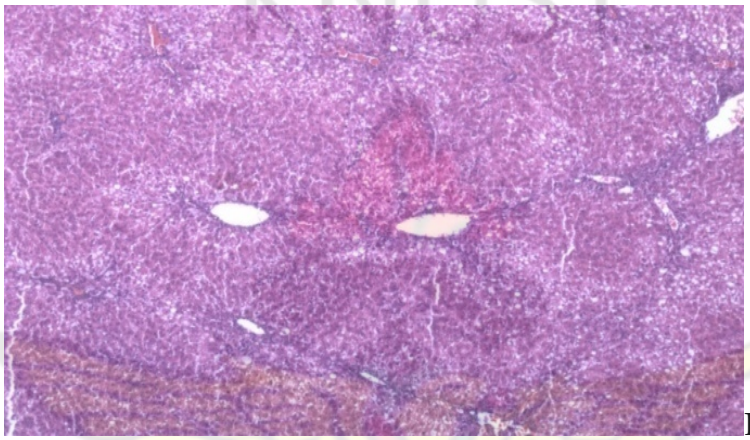

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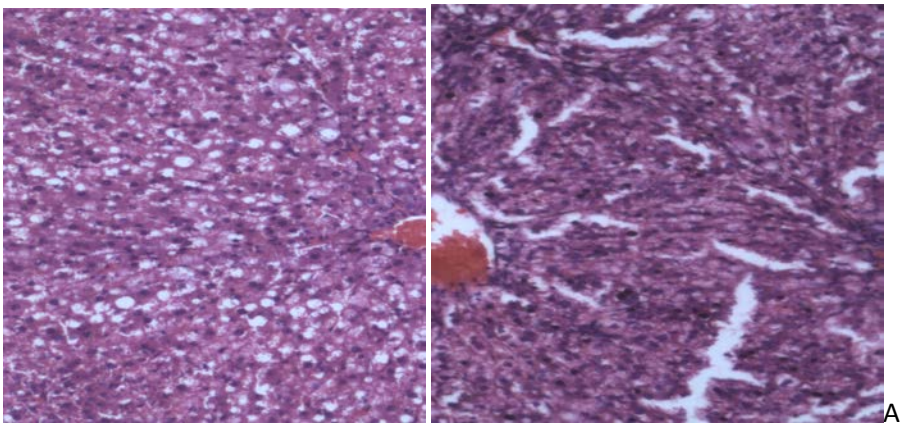
Panel 5 (D –E): High power micrographs, X100 of cells of rats that were treated with 1500 mg/kg *Spondias* (D) and Silymarin (reference drug at 25 mg/kg) (E); and induced with carbon tetrachloride.



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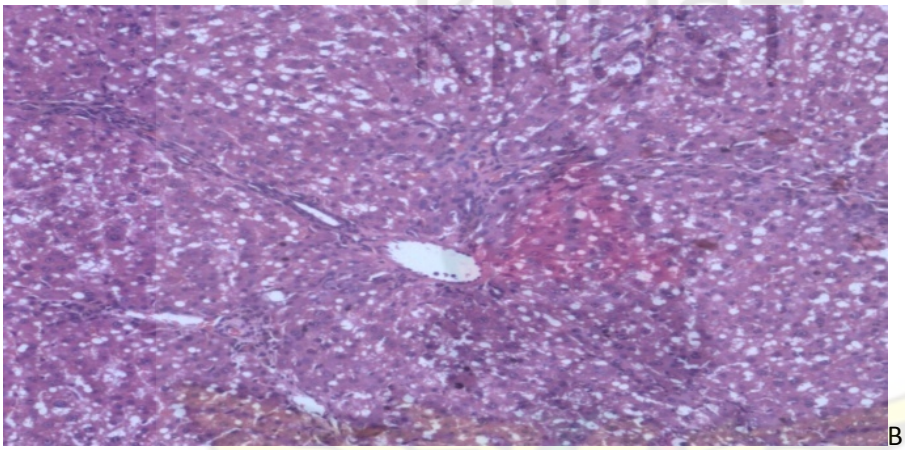

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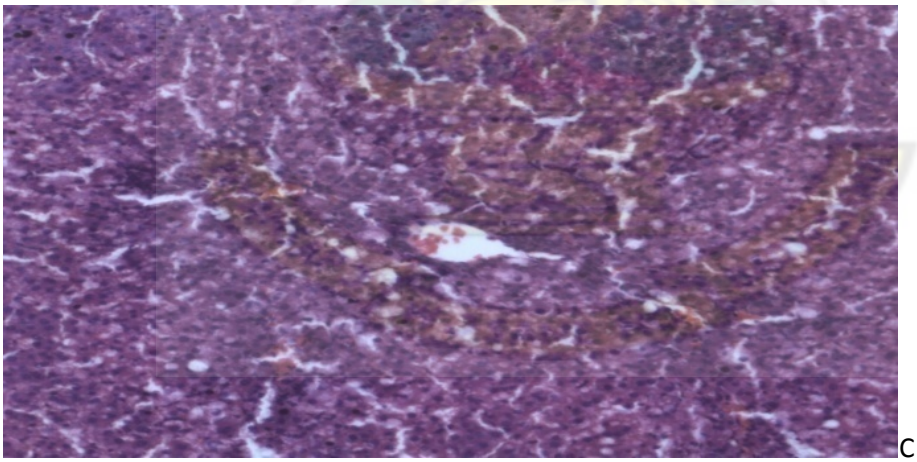

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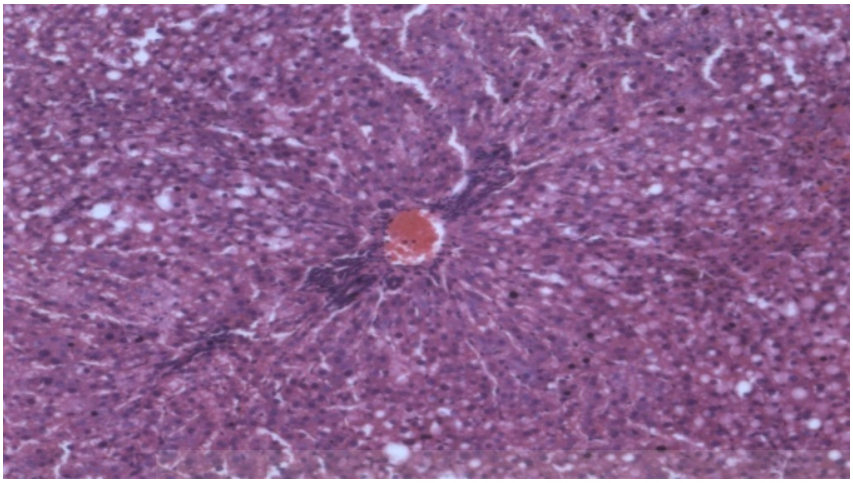
Panel 6 (A – C): High power micrographs, X100 of cells of rats of the positive control group that were administered with carbon tetrachloride only (A), rats that were treated with 300 mg/kg *Spondias* (B) and 1000 mg/kg *Spondias*(C); and induced with carbon tetrachloride.



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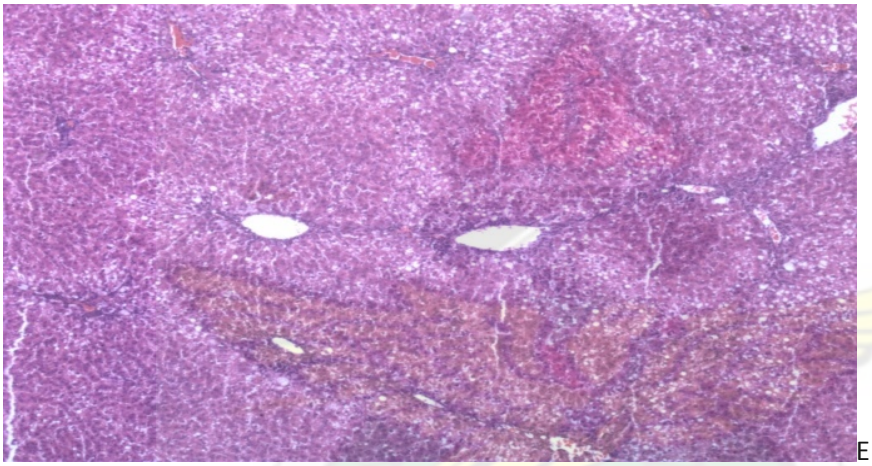

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Panel 6 (D – E): High power micrographs, X100 of cells of rats that were treated with 1500 mg/kg *Spondias* (D) and Silymarin (reference drug at 25 mg/kg) (E); and induced with carbon tetrachloride.

3.8 ASSESSMENT OF THE CONCOMITANT EFFECT OF *SPONDIAS MOMBIN* AND LIVER INJURY WITH CARBON TETRACHLORIDE (CCl_4) IN RATS

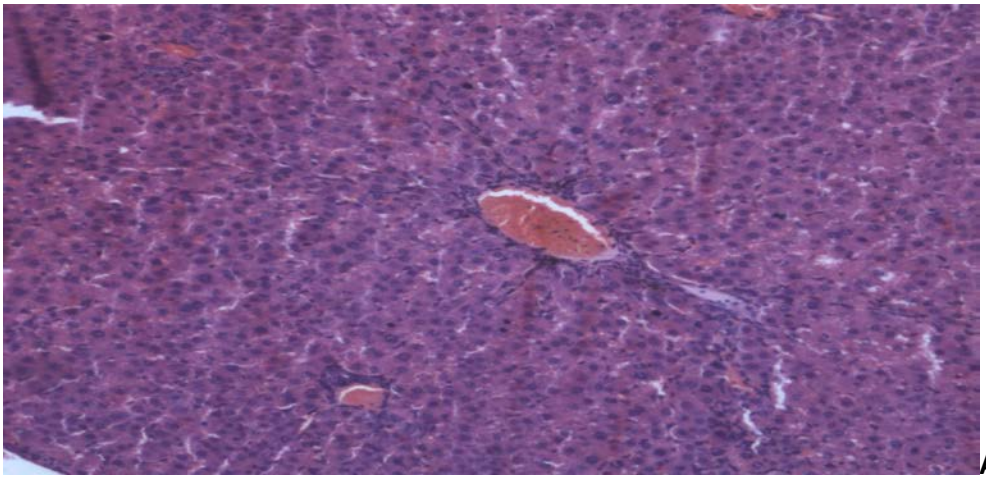
The *Spondias* extract decreased the levels of aspartate aminotransferase (AST) dose dependently from 176.2 ± 46.1 U/L to 135.6 ± 83.5 U/L (300 to 1500 mg/kg respectively), compared to the CCl_4 control (374.8 ± 64.7 U/L). This decrease was comparable to the negative control (147.1 ± 6.3 U/L), but not significant by Tukey's test (Table 9). Alanine aminotransferase levels were also decreased, but not significantly. There was however a significant difference in the levels of the biliary tract parameters, ALP and GGT, in treated groups and controls. This effect was predominant at 1000 mg/kg of extract, and comparable to Silymarin.

Histopathological results suggest that *Spondias mombin* extract was able to eliminate cell necrosis, minimize fibrosis and reduce fatty degenerations extensively at dose of 1000 mg/kg (Panels 7 and 8).

Table 9: Effect of *Spondias* extract on serum biochemical parameters. Doses were administered concomitantly with carbon tetrachloride injury induction.

TREATMENTS	SERUM BIOCHEMICAL PARAMETERS						
	AST(U/L)	ALT(U/L)	ALP(U/L)	GGT(U/L)	Total bilirubin µmol/L	Albumin(g/L	Total protein(g/L)
Control	147.1±6.3	55.04±3.12	791±94.4	1.66±0.44	1.86±0.38	39.68±0.55	60.9±1.60
CCl_4 control	374.8±64.7	226.5±32.7	1577±158.4*	8±2.35*	3.58±0.69	37±0.71	66.28±1.69
CCl_4 + 300mg/kg *Spondias*	176.2±46.1	126.8±44.4	1077±216.7	3.32±1.79	2.33±0.48	39.84±0.02	67.3±2.73
CCl_4 + 1000mg/kg *Spondias*	152.1 ±74.4	113.8±55.9	982.1±147.6	1.62±0.72*cc	2.4±0.59	39.56±1.01	67.96±2.09
CCl_4 + 1500mg/kg *Spondias*	135.6±83.5	133.6±69.6	1288±182.4	5.56±0.90	2.32±1.08	41.34±1.20	79.16±9.01
CCl_4 + Reference drug	177.4±51.7	155.1±43.5	913.7±169.2	2.28±0.85	2.74±1.28	42.12±0.92	74.26±3.85*cc
p value	0.0626	0.2044	0.0316	0.0147	0.7770	0.0540	0.0882

Values are represented as mean ± SEM. *cc indicates significance at $p < 0.05$ compared to the CCl_4 control.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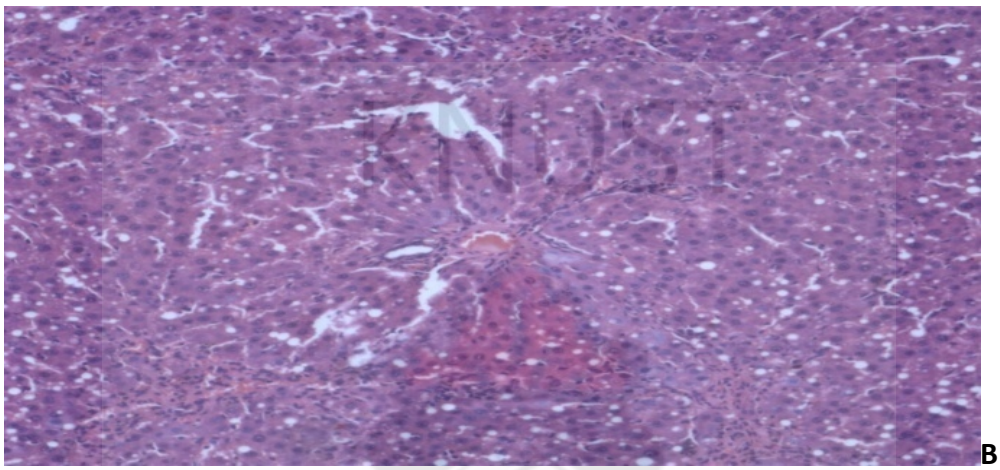
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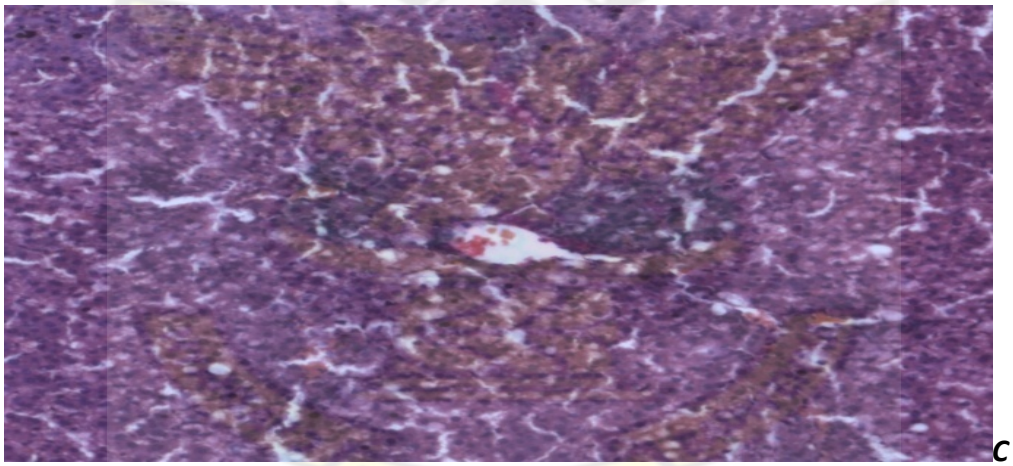
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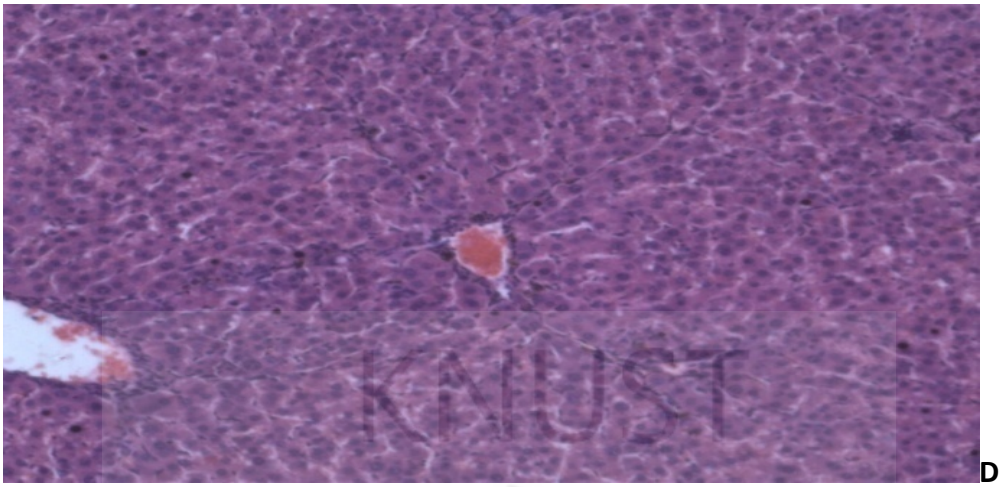
Panel 7 (A –C): High power micrographs, X100 of cells of control rats (A), rats that were treated with carbon tetrachloride and 300 mg/kg *Spondias* (B) and rats treated with carbon tetrachloride and 1000 mg/kg *Spondi* concomitantly.



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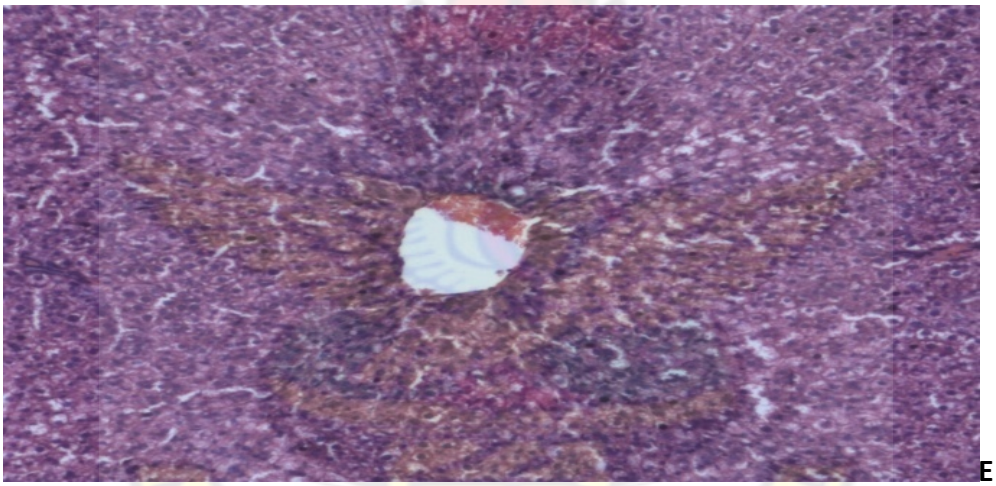
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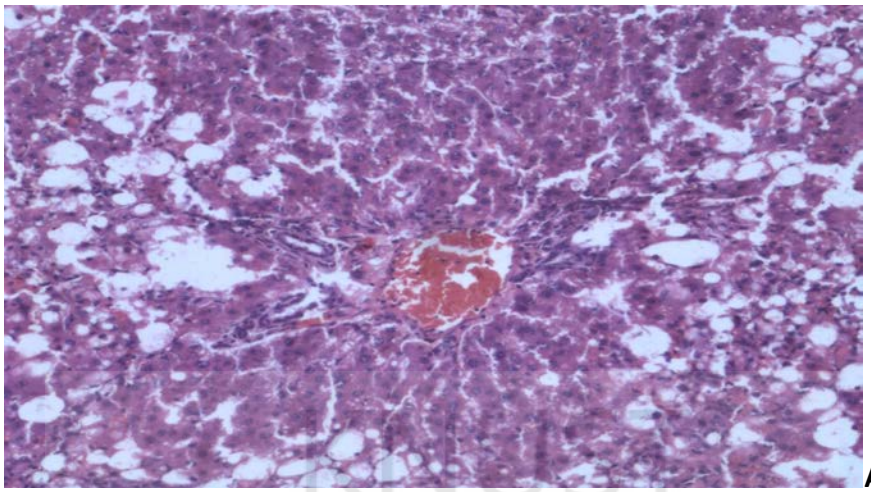
Panel 7 (D –E): High power micrographs, X100 of cells of rats that were treated with carbon tetrachloride and 1500 mg/kg *Spondias* (D) and carbon tetrachloride and Silymarin (reference drug at 25 mg/kg) (E), concomitantly.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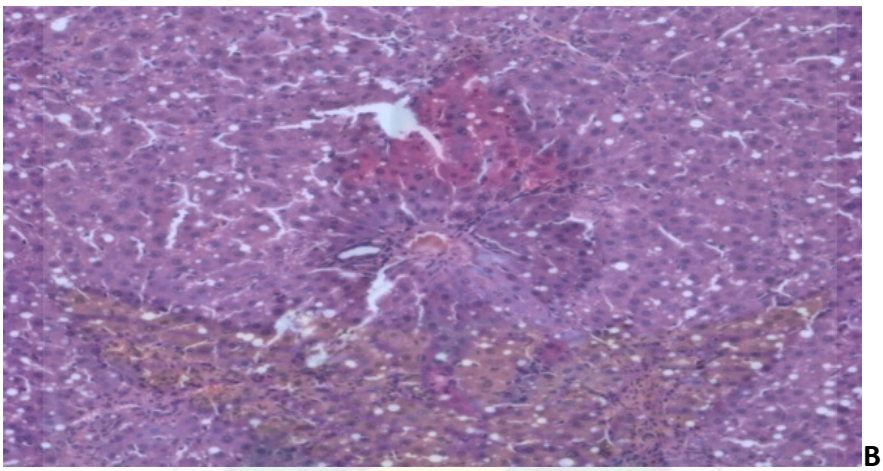
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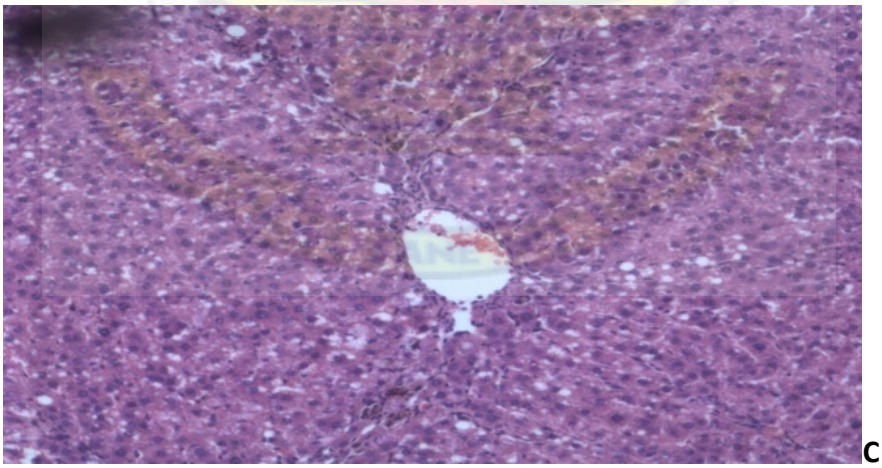
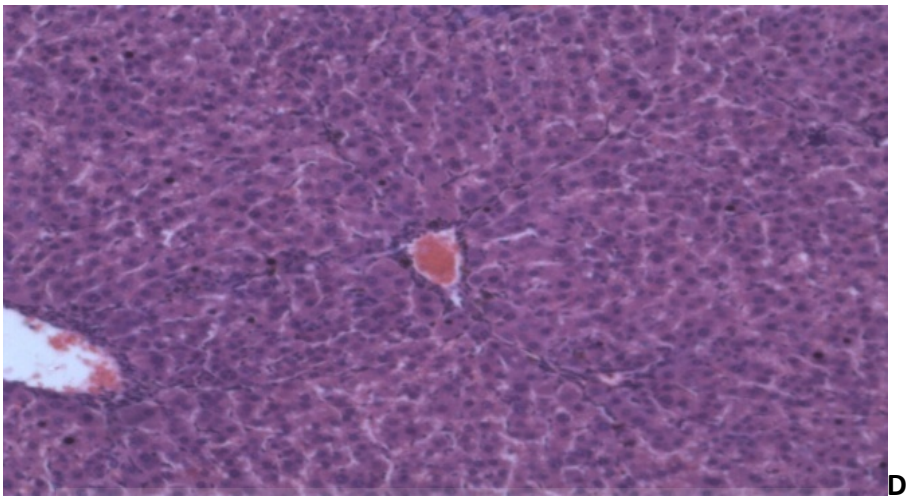
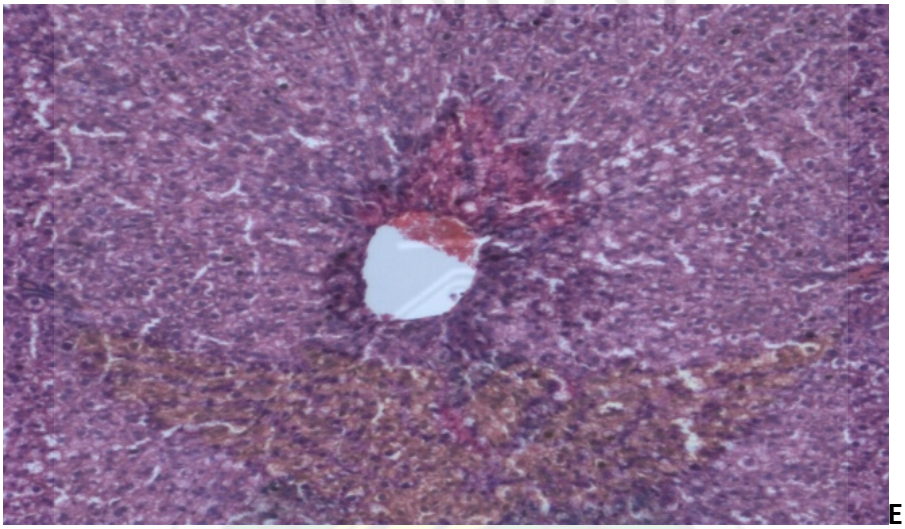

C

Panel 8 (A – C): Photomicrographs, X 100 of cells of rats of the positive control group that were administered with carbon tetrachloride only (A), rats that were treated with carbon tetrachloride and 300 mg/kg *Spondias* (B) and carbon tetrachloride and 1000 mg/kg *Spondias* (C); concomitantly.



D



E

Panel 1 (D – E): High power micrographs, X 100 of cells of rats that were treated with carbon tetrachloride and 1500 mg/kg *Spondias* (D) and carbon tetrachloride and Silymarin (reference drug at 25 mg/kg) (E) concomitantly.

3.9 EFFECT OF *SPONDIAS MOMBIN* AQEUOUS LEAF EXTRACT ON PENTOBARBITONE-INDUCED SLEEPING TIME IN RATS

The extract showed a dose-dependent decrease in sleep time, compared to the CCl4 control group when rats were challenged with pentobarbitone. The extract at a dose of 1500 mg/kg showed a sleep time (measured as the time between the loss and gain of the righting reflex of each rat) comparable to the negative control (Figure 6). Figure 8 shows the trend of sleep-time in the various rat groups.

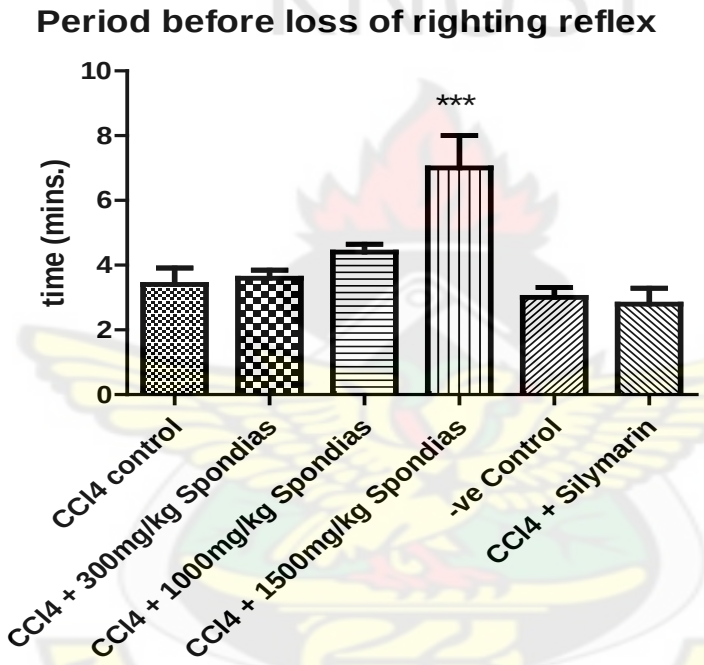


Figure 6: Effect of *Spondias on t*ime taken to effect sleep in rats when administered with pentobarbitone 6 hours after last administration of drug doses. Values are presented as mean ± SEM of time between administrations of pentobarbitone and lose of the righting reflex. *** indicates significance at $p < 0.001$.

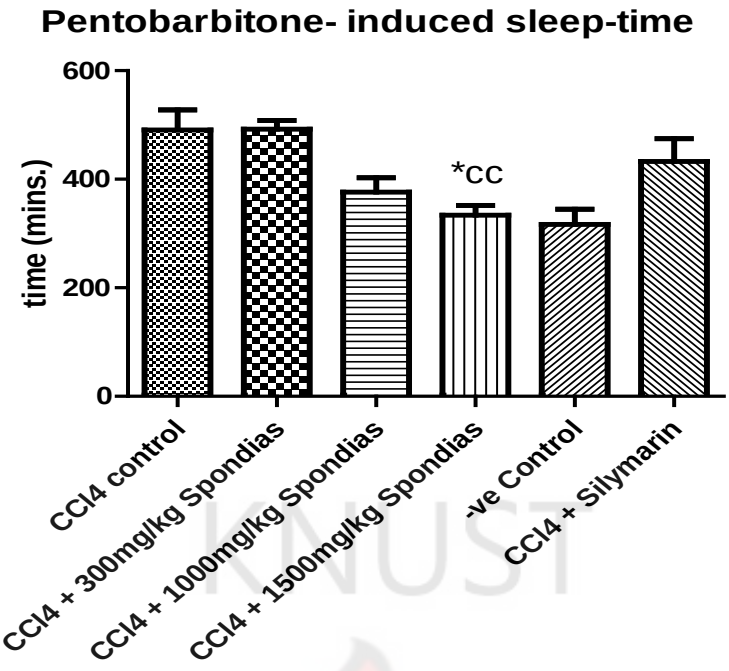


Figure 7: Effect of *Spondias* on pentobarbitone - induced sleeping time in rats. Animals received last dose of extracts and reference drug 6 hours before the pentobarbitone challenge. Values are presented as mean ± SEM of time between lose and gain of the righting reflex. *cc indicates significance ($p < 0.05$) compared to the CCl4 control.

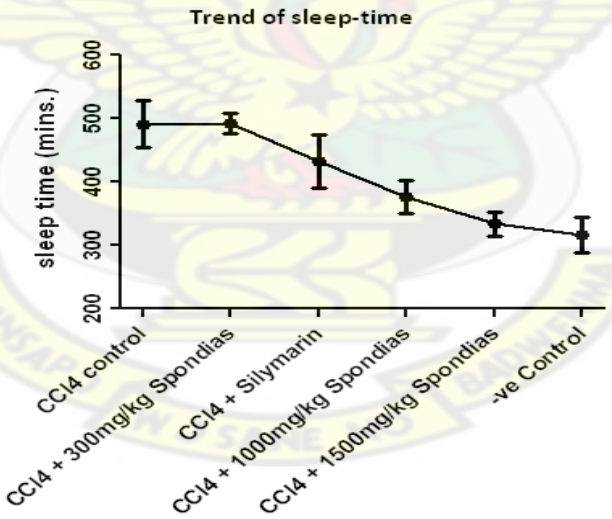


Figure 8: Graph showing the trend in pentobarbitone-induced sleep-time in *Spondias mombin* extract groups and reference drug (Silymarin) group, compared to the negative and positive controls.

At a dose of 500 mg/kg body weight *Spondias mombin*, animals shown a distinct deviation from the trend observed. Compared to the time for the loss of righting reflex in the negative control group (3.00± 0.32 minutes) and in the positive control (CCl_4 only treated) group (3.40 ±0.51 minutes), there was a highly significant increase in the time taken for rats to lose the righting reflex when challenged with pentobarbitone after administration of 500mg/kg extract and CCl_4 (53.80 ±13.06 minutes) (Figure 9). The pentobarbitone-induced sleep time was also significantly reduced from 490.6 ±36.9 minutes in CCl_4 only treated group and 316.6 ±28.2 minutes in the negative control group to 175.6± 63.41 minutes in the group that received CCl_4 and 500mg/kg *Spondias* before the pentobarbitone challenge (Figure 10).

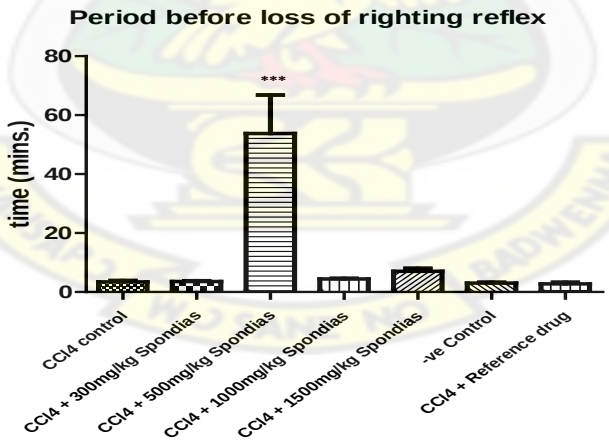


Figure 9: Effect of *Spondias* on time taken to effect sleep in rats when administered with pentobarbitone 6 hours after last administration of drug doses. Values are presented as mean ± SEM of time between administration of pentobarbitone and lose of the righting reflex. *** indicates significance at $p < 0.001$ compared to all groups.

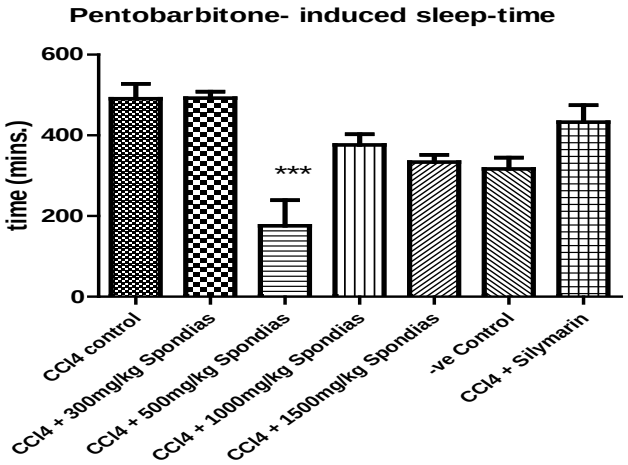


Figure 10: Effect of *Spondias* on pentobarbitone-induced sleeping time in rats. Animals received last dose of extracts and reference drug 6 hours before the pentobarbitone challenge. Values are presented as mean ± SEM of time between loss and gain of the righting reflex. *** indicates significance at $p < 0.001$ compared to the control.

4 Chapter 4 DISCUSSION

Treatment of diseases associated with the liver is very vital, and must be done with importance and extensive care. Many herbal remedies for liver diseases are known but only a few of them have been pharmacologically assessed for their efficacy. It is very important to assess natural products for their efficacy in the treatments they are used for. It is especially very important to assess remedies for liver diseases due to the liver's fragility and relation to other vital organs, and yet its numerous vital roles detrimental to the survival of a person.

In recent times, due to economic factors, people are in need of available, easily accessible and less costly medication, even with the slightest knowledge of efficacy, and minimum idea of toxicity. It is believed by most people that since herbal remedies are natural, they are non-toxic. Toxicity of natural remedies have however been reported. Even scientifically proven hepatoprotective plant was found to contain hepatotoxins as well (Bramanti *et al.*, 1978; MacGregor *et al.*, 1989; Oshima, 1995). Thus, work on hepatoprotective herbal remedies remain a challenge (Schuppan *et al.*, 1999).

Spondias mombin is used as remedy for numerous ailments. Its antimicrobial, anthelmintic and cytotoxic actions give caution for possible toxic action of the plant, though there have not been reports of toxicity of the extract. The fact still lies that herbal remedies are not adequately monitored. We were also prompted by the claim of herbalists that extract of *Spondias* leaves is effective in the management of jaundice, to assess its efficacy in the treatment and yet, its suspected toxicity.

In assessing the therapeutic effect of the extract, *Spondias mombin* aqueous leaves extract decreased the levels of total bilirubin, greatly evident in the indirect (unconjugated) bilirubin levels, suggesting that the hepatoprotective effect it has on the liver act not on the liver's ability to conjugate bilirubin, but on its ability to assemble bilirubin. The liver however would have shown defectiveness in conjugating

bilirubin under intense damage of hepatocytes exhibited by extensive periportal fibrosis, central fibrosis, necrosis, inflammation and cytoplasmic vacuolation/fatty degeneration; which was not exhibited by the liver injury induced in the present study.

The estimated levels of the serum biochemical parameters that are diagnostic markers of extent (Ansari, 1991) and position of the liver injury also offered complementary explanation to the hepatoprotective ability of *Spondias*. The extract lowered significantly the level of alanine aminotransferase (ALT), which is the most precise determinant for assessing hepatoprotectivity in biochemical analysis, even at a dose of 300 mg/kg body weight. This effect suggests that the liver' ability is further enhanced at this dose level. Histopathological examination of cells revealed that though its hepatoprotective effect did not show in bilirubin levels at dose 300 mg/kg, on cellular levels, the effect is comparable to the control.

In prophylactic studies, *Spondias mombin* extract showed a significant reduction in aspartate aminotransferase, AST levels at a dose of 300 mg/kg body weight. The extract also significantly lowered the levels of alanine aminotransferase, ALT, dose-dependently. These reductions were comparable to the control and the reference drug. The level of bilirubin also lowered at a dose of 1000 mg/kg body weight. Examination of histopathological slides of liver of rats in various groups confirms its hepatoprotective ability. Presence of mild necrosis and fibrosis in liver cells of rats treated with doses is not comparable to the control, though slides of the CCl_4 only treated rats shown more extensive cell damage compared to control. This extent of damage was also seen in slides of rats treated with the reference drug. These suggest that *Spondias mombin* can prevent liver injury by CCl_4, when administered for a longer period.

When given concomitantly with carbon tetrachloride, *Spondias mombin* reduced the levels of aspartate aminotransferase and alanine aminotransferase at 300 mg/kg. These reductions were however not significant by Tukey's test. Histopathological examination confirms a reduction in cellular features, showing rectification. These

changes were prominent at 1000 mg/kg, and were comparable to the control. These suggest that the extract was able to reverse the injurious effects of CCl_4, or did not enable CCl_4 to cause pronounced injury in the cells, also evident in serum aminotransferase levels. If administered for a longer period, the extract could prevent completely, liver injury from CCl_4.

The effect of *Spondias mombin* on pentobarbitone-induced sleeping time was assessed using all three phases of assessing the hepatoprotective ability (prophylactic, concomitant and therapeutic) of the extract. When challenged with pentobarbitone, groups that received *Spondias mombin* extract dose dependently reduced sleeping time significantly to normal at a dose of 1500 mg/kg body weight. Pentobarbitone is a central nervous system (CNS) – depressant, metabolized by the liver (Leander *et al.*, 1982). The liver's metabolism rate was elevated by treatment with the extract. This suggests that in totality, *Spondias mombin* aqueous leaves extract is a good hepatoprotective agent.

At 500 mg/kg body weight dose level of *Spondias* however, the extract interestingly, highly significantly reduced sleep time in rats, while prolonging the time taken to lose the righting reflex. Thus, at this dose level, the extract could prevent the pentobarbitone from hypno-sedating the animals completely, and if sedation occurred, cleared pentobarbitone from the system in minimal period. This suggests that the extract, at a dose of 500 mg/kg b. wt., may have inhibited some enzymatic reactions, prolonging the time for the loss of the righting reflex; or have altered the porosity in the membrane structure of liver cells, by de-mobilizing free radicals that cause peroxidation of membrane phospholipids, regulating the entry of pentobarbitone.

When assessed the possible acute toxicity to mice, there was no lethality observed at 5000 mg/kg body weight, no physical changes were also observed. Behaviourally, the *Spondias* extract was found to dose-dependently affect the amount of food intake and subsequently, body weights when administered a single dose to both male and female

mice. This could be attributed to a physiological change, leading to an increase in appetite for food. This was not observed in rats when administered single doses. This could be due to species – specific difference with regards to response to *Spondias mombin* aqueous leaf extract.

In sub-acute studies, daily administration of different doses of *Spondias mombin* extract for 14 days in rats had minimal effect. There was no significant change in the levels of the serum biochemical parameters AST, ALT, GGT, and bilirubin compared to the control group, though there was slight decrease in GGT levels (with the exception of 1500 mg/kg b. wt), synthesis of albumin was not least compromised, confirming no effect of extract on the metabolic roles of the liver. Histopathological analysis of liver cells confirms minimal effect of extract on liver and kidney at all doses. The slight decrease in GGT would therefore postulate an enhanced biliary tract. The extract therefore has a high safety on the target organs, liver and kidney.

Evaluation of haematological indices also revealed minimal effect by the extract, except for white blood cell (WBC) count which showed a dose-dependent (300 - 1500 mg/kg) significant increment towards the control. At a low dose of 300 mg/kg body weight of the *Spondias*, the extract showed a reduced count of WBC compared to the control. This is further increased at 1000 mg/kg and 1500 mg/kg body weights of the *Spondias* extract. This suggests that the extract has a positive effect on WBC count at high doses. WBCs are immunological cells that help fight the invasion of foreign bodies and disease infections in the system. The extract increasing the WBC count at high doses suggests that *Spondias* extract may enhance the immune system by elevation of levels of WBCs.

Although there were slight decreases in the relative weights of the liver, kidney, stomach and spleen, to the body in the various doses, compared to the control, the decreases were however only significant at 300 mg/kg body weight of extract in liver, kidney and stomach. Despite the decrease in the relative weight of liver, the levels of the serum alanine aminotransferase and aspartate aminotransferase were normal in

all groups compared to the control. Bilirubin levels were also found to be normal in all groups compared to the control. This shows that the liver's ability to produce ALT and synthesise bilirubin were not compromised, indicating that the liver functioned adequately, and the extract had minor effect on the liver at all doses (from 300 mg/kg through 1000 mg/kg to 1500 mg/kg body weights).

The anti-tumor/anti-carcinogenic (Bang SC, 2005; Kang *et al.*, 2005; MacDonald *et al.*, 2005; Sakagami *et al.*, 1990) and antioxidant (Gulcin *et al.*, 2004) properties of saponins and tannins reported could be a major contributing factor for the extracts ability to reverse damage. The ability of tannins to stop bleeding and help heal wounds is due to its astringent property that makes it anti-microbial, by being toxic to microbes. Flavonoids are also attributed with the properties of anti-allergic, anti-cancer (Ferguson PJ, 2004), antioxidant, anti-inflammatory and anti-viral. Their presence in *Spondias mombin* leaves extract explains its hepatoprotective effect against carbon tetrachloride-induced liver injury.

Further analysis revealed the presence of reducing sugars in the extract. There is therefore a possibility of this effect on liver and/or kidney cells, most likely at low doses, causing the anonymous effect at low doses. A low dose of the extract could also result in reduced WBCs if that is recognized as foreign bodies. This is reflected in the minor increment in Procalcitonin (PCT) levels which have been reported to bring about apoptosis in pancreatic cells (Kaneto *et al.*, 1996), at a low dose (of 300 mg/kg body weight). At the same dose level, the presence of phenols in minor quantities (Njoku, 2007) could trigger possible interactions between the tetrachloride and phenols when in low quantities. This interaction is explained (1.7), to form a final product, salicylaldehyde, which is known to be a key precursor of a number of chelating agents. The formation of chelating agents could affect membranous structures, inhibiting effective absorption and pernetration of the test drug into cells.

When extract administered concurrently with CCl_4, as seen in experiments to assess the concomitant and prophylactic effect of the extract with CCl_4, the hepatoprotective

effect is minimal, and highly dose-specific. At the minimum dose, caution must therefore be taken in the administration of safe doses.

Alkaline phosphatase (ALP) and gamma glutamic transpeptidase (GGT) shown an elevation in concomitant assessment at 1500 mg/kg, and GGT also elevated in the prophylactic assessment at a high dose of 1500 mg/kg, without elevations in the aminotransferases. This suggests a problem with the biliary tract at very high dose when treatment is being administered with CCl_4.

Administering 1000 mg/kg body weight of *Spondias mombin* could be most effective in all cases of hepatoprotectivity.

5 Chapter 5 CONCLUSIONS

The study conducted in this project reveals that:

- *Spondias mombin* has profound therapeutic ability on liver injury induced by carbon tetrachloride.
- *Spondias mombin* possesses adequate prophylactic ability against carbon tetrachloride-induced liver injury.
- *Spondias mombin* has slight hepatoprotective ability when administered concomitantly with carbon tetrachloride.
- The hepatoprotective ability of the extract, assessed at the three levels of therapeutic, prophylactic and concomitant are individually dose – specific.
- Administering 1000 mg/kg body weight of *Spondias mombin* could be most effective in all cases of hepatoprotectivity.

Sub-acute toxicological studies also showed that:

- *Spondias mombin* caused an increase in food intake and body weights.
- Significant reduction in white blood cell count at a dose of 300mg/kg suggests that treatment with very low doses, though may be effective in protecting the liver, should be recommended with caution.

In acute toxicity studies, the LD50 was found to be well beyond 5000mg/kg in mice and rats.

Thus,

- *Spondias mombin* is hepatoprotective, and
- It is non-toxic

Suggestions for further work

1. Reproductive toxicity of the extract could be assessed for possible effect of prolonged use of the extract, since treatment of liver diseases takes a long period of time.

2. The anomaly observed at a dose of 500mg/kg of the extract (see Figures 9 and 10), on the study of Pentobarbitone-induced sleep time, is interesting and needs further investigation to determine the possible effect of the extract at this dose, on the Central Nervous System (CNS)

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