



THE EFFECT OF AQUEOUS STEM BARK EXTRACT OF *ERYTHRINAMILDBRAEDII* ON CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

This study evaluated the curative effects of aqueous stem bark extract of *Erythrina mildbraedii* in CCL_4 induced liver damage. With the exception of group 1 (positive control), CCL_4 was administered at a dose of 150 mg/kg to groups II, III, IV and V. Administration of CCL_4 caused profound hepatic damage as indicated by elevation in serum levels of liver transaminases, alkaline phosphatase, total and direct bilirubin. There was depletion of serum proteins and albumin. Administration of aqueous stem bark extract of *Erythrina mildbraedii* at daily oral doses of 50, 100, 150mg/kg reversed these biochemical aberrations to a significant level. The curative effects of stem bark extracts of *Erythrina mildbraedii* were further substantiated by histopathological examination of the liver hepatocytes. The result of the study suggested that aqueous stem bark extract of *Erythrina mildbraedii* possesses hepatocurative effects.

Keywords: Carbontetrachloride, *Erythrina mildbraedii*, liver enzymes, hepatotoxicity.

INTRODUCTION

Liver is exposed to many potentially toxic substances via gastrointestinal tract from the diet, food additives, contaminants and drugs and is frequently a target in experimental animals. By virtue of its position, structure, function and biochemistry, the liver is especially vulnerable to damage from toxic compounds (Timbrell, 2009). As the major drug metabolizing and detoxifying organ in the body, the liver is subjected to potential damage from an array of pharmaceutical and environmental chemicals. Injury may result from direct toxicity via hepatic conversion of a xenobiotic to an active toxin or through immune mechanisms, usually by a drug or a metabolite acting as haptens to convert a cellular protein into an immunogen (Kumar *et al.*, 2006).

Medicinal plants are those plants that provide medicines to prevent disease, maintain health or cure sickness. In other words, these plants benefit virtually everyone on the earth. Plants provide the predominant ingredients of medicines in most traditional systems of healing and have been the source of inspiration for several new drug researches to major pharmaceutical companies (Maiti *et al.*, 1999). The plants that possess therapeutic properties or exact beneficial pharmacological effects on the body are generally designated as "medicinal".

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1993). They play a significant role in providing primary

health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicines.

Erythrina mildbraedii (*Minjirya* in Hausa) is a tree that grows up to 30 m in height and is native to West Africa. *Erythrina* species produce many secondary metabolites, some of which have a function of defence systems against pathogenic fungi and bacteria (Karthishwaran *et al.*, 2010). Ethnobotanical discovery process of sub-Saharan *Erythrina* has resulted in promising biological activities (Kone *et al.*, 2004; Nguyen *et al.*, 2010).

Many *Erythrina* species showed real potential for fighting pathogenic agents associated with public health problems in sub-Saharan Africa. For e.g. multidrug resistant pathogens are responsible for therapeutic failures (Kone and Kamanzi, 2009). This situation is serious because microbial infections are most frequent opportunistic diseases occurring during HIV/AIDS which affected many people in Africa. Moreover, during this infection, cancer and cardiovascular diseases, oxidative stress and generating free radicals is known to cause damage to cell and immune system of patients. Scientists are searching for new molecules that could provide alternative to conventional treatments (Coulidiati *et al.*, 2011).

High incidence of cancer, inflammation, and cardiovascular diseases are attributed to oxidative stress. Some sub-Saharan *Erythrina* species are used by traditional practitioners to treat cancer and inflammation. These plants were investigated for cancer chemo-protective agents and inhibitors of enzyme-borne diseases.

The studies were carried out on enzymes such as phospholipase C gamma 1, diacylglycerol acyltransferase (DGAT), protein tyrosin phosphatase 1 B (PTB 1B), ERK kinase, 5-lipoxygenase and 15-lipoxygenase. Inhibitors of these enzymes are proposed in the therapy of obesity, type 2 diabetes and cancer. Ethylacetate extract of *Erythrina mildbraedii* stem bark inhibited PTB 1B (Na *et al.*, 2006; Jang *et al.*, 2008). *Erythrina mildbraedii* extract showed anti-inflammatory activity and radical scavenging activity in 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assay (Njamen *et al.*, 2003).

Carbon Tetrachloride once used extensively in dry cleaning and even as an anesthetic is primarily hepatotoxic, causing two different types of pathological effect. Its toxicity has been studied both from the biochemical and pathological viewpoints, and therefore the data available provide particular insight into mechanisms of toxicity. Carbon tetrachloride is a simple molecule which, when administered to a variety of species, causes centrilobular hepatic necrosis (zone 3) and fatty liver. It is a very lipid-soluble compound and is consequently well distributed throughout the body, but despite this, its major toxic effect is on the liver, irrespective of the route of administration (Timbrell, 2009). It is a classic example of a chemical activated by cytochrome P450 to form a highly reactive free radical. First, CCl₄ is converted to the trichloromethyl radical (CCl₃·) and then to the trichloromethylperoxy radical (CCl₃O₂·). Such radicals are highly reactive and generally have a small radius of action. For this reason the necrosis induced by CCl₄ is most severe in the centrilobular liver cells that contain the highest concentration of the P₄₅₀ isozyme responsible for CCl₄ activation. Typically free radicals may participate in a number of events, such as covalent binding to lipids, proteins, or nucleotides as well as lipid peroxidation. It is now thought that CCl₃·, which forms relatively stable adducts, is responsible for covalent binding to macromolecules, and the more reactive CCl₃O₂·, which is formed when CCl₃· reacts with oxygen, is the prime initiator of lipid peroxidation (Hodgson, 2010). The research work was carried out to evaluate the effect of aqueous stem bark extract of *Erythrina mildbraedii* on carbon tetrachloride induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant identification and Collection

Erythrina mildbraedii stem bark was collected from Song local Government Area of Adamawa State. It was identified and authenticated by a Botanist at the Botany unit of the Faculty of Science, Bayero University Kano, Nigeria

Extraction of Plant Materials

The stem bark of the plant collected was carefully washed in clean water, and then dried at room

temperature. They were pulverized to coarse powder and distilled water (500 cm³) was added to 100g of the powdered stem bark in a conical flask. The content of the flask was then shaken and the top was covered with aluminium foil and kept for 48 hours. The extract was then obtained by filtration using whatman No 1 filter paper. The filtrate was dried and then reconstituted for administration.

Experimental Animals

Twenty four (24) white albino rats (weighing 160 to 250g) were purchased from the Animal Room of the Biological Sciences Department, Bayero University, Kano. The rats were maintained under standard laboratory conditions and were allowed free access to both food and water throughout the period of the experiment.

The volume (cm³) of the stem bark aqueous extract given to each rat was determined by its weight and required dose as follows:

$$\text{Volume administered (cm}^3\text{)} = \frac{\text{weight of rat (kg)} \times \text{Required dose (mg/kg)}}{\text{Concentration of the extract (mg/cm}^3\text{)}}$$

Experimental Design

Twenty four (24) experimental rats were divided into eight groups of three (3) rats each. Liver toxicity was induced in groups II – VIII using CCl₄ according to Alhassan *et al.*, (2009).

Group I: normal rats (No extract, no CCl₄ administered)

Group II: CCl₄ administered no extract (Test Control)

Group III: were administered with aqueous stem bark extract of *Erythrina mildbraedii* (Dose; 50 mg/kg orally. Once daily for two weeks).

Group IV: were administered with aqueous stem bark extract of *Erythrina mildbraedii* (Dose; 100 mg/kg orally. Once daily for two weeks).

Group V: were administered with aqueous stem bark extract of *Erythrina mildbraedii* (Dose; 150mg/kg orally, once daily for two weeks).

Group VI: were administered with aqueous stem bark extract of *Erythrina mildbraedii* (Dose; 50mg/kg orally, once daily for four weeks).

Group VII: were administered with aqueous stem bark extract of *Erythrina mildbraedii* (Dose; 100mg/kg orally, once daily for four weeks).

Group VIII: were administered with aqueous stem bark extract of *Erythrina mildbraedii* (Dose; 150mg/kg orally, once daily for four weeks)

Blood and Tissue Samples Collection

Rats in groups I and II were sacrificed after 48hrs of CCl₄ administration to confirm lipid peroxidation and liver damage. Groups III- V were sacrificed after two weeks of extract administration, while groups VI- VIII were sacrificed after 4 weeks of extract administration. The liver from both control and test animals were excised, sliced and fixed in 10% formaldehyde solution and used for histological examination.

Biochemical Analysis

Blood samples were collected, allowed to clot and centrifuged at 2000 rpm for 10min to obtain the serum samples. For each serum sample, the level of alanine aminotransferase (ALT), aspartate amino transferase (AST) were determined using the method of Reitman and Frankel (1957), alkaline phosphatase (ALP) using the method of Rec (1972), direct bilirubin (DB) and total bilirubin (TB) by the method of Jendrassik and Grof (1938), total protein (TP) using the method of Tietz (1995) and albumin (ALB) using the method of Grant (1987) were analyzed in order to assess liver function.

Histopathology

Histological examinations were conducted using the method of Auwioro (2010). The liver tissues were fixed in multiple baths of ethanol, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Statistical Analysis

The data was statistically analysed using one-way analysis of variance (ANOVA) with P value < 0.01 considered to be significant. Graphpad InStat 3 software (2000). Version 3.05, Graphpad Inc was used for all the analysis.

RESULTS

The hepatocurative activity of aqueous stem bark extract of *Erythrina mildbraedii* was evaluated in the experimental animals two weeks and four weeks after inducement of liver damage. Table 1 shows the mean serum level of liver indices (AST, ALT, ALP, ALB, DB, TB and TP), for group of rats 48 hours after administration of 150mg/Kg CCl₄ (Group II) and

compared to that of normal control Group I which is to confirm inducement of hepatotoxicity and lipid peroxidation by the CCl₄.

The result obtained showed a significant increase (P<0.01) in the mean serum level of AST, ALT, ALP, DB and TB while that of TP and ALB decreases in the test control group when compared with the normal control rats.

After two weeks of oral administration of aqueous stem bark extract of *Erythrina mildbraedii* at a daily dose of 50mg/kg only the mean serum level of ALP significantly decreased (P<0.01) even though the level of AST, ALT, TB, DB decreased while that of ALB and TP increased but showed no significant difference (P<0.01) when compared with test control groups. However the groups administered with a daily dose of 100 and 150mg/kg had their mean serum levels of AST, ALT, ALP, DB and TB decreases significantly (P<0.01) when compared to test control rats but still higher than that of the normal control rats while that of ALB significantly increased (P<0.01) when compared with test control rats but lower than that of the normal control groups. The decrease in the mean serum levels of AST, ALT, ALP, DB, and TB and increase in the level of ALB was found to be dose dependent.

After four weeks of oral administration of aqueous stem bark extract of *Erythrina mildbraedii* the mean serum level of AST, ALT, ALP, DB and TB significantly decreased (P<0.01) when compared with test control groups while that of ALB and TP increased significantly (P<0.01) when compared to test control groups (Table 2). The mean serum level returned to near normal irrespective of the dose administered.

Plate 1 of the histopathology result showed section of liver of normal rats with hepatocytes arranged in cord radiating from central venules. Plate 2 showed area of the liver with necrosis, fibrosis and degeneration of hepatocytes due to damage caused by CCl₄ administration. Plate 3 showed section of the liver area with fibrosis after administration of 50mg/kg of the extract for two weeks. Plate 4 and 5 showed cross section of the liver with fat cells after administration of 100mg/kg and 150mg/kg of the extract for two weeks. Plates 6, 7 and 8 showed no significant pathology, they showed hepatocytes arranged in cord radiating from central venules after administration of varying doses of the extract for four weeks.

Table 1: Effects of oral administration of aqueous stem bark extract *Erythrina mildbraedii*(ASBEEM) on serum biochemical indices of CCl₄-induced hepatotoxicity in rats after two weeks.

Groups/Dose(mg/kg)	AST(U/L)	ALT(U/L)	ALP(U/L)	TP(g/L)	ALB(g/L)	DB(Umol/L)	TB(Umol/L)
I (Normal rats)	23.33±1.53	12.00±1.00	110.33±1.53	67.33±2.08	43.33±5.51	7.70±1.15	18.33±0.75
II CCl ₄ (150mg/kg)	60.00±2.65*	44.00±6.25*	273.67±4.93*	37.33±3.51*	25.33±5.51*	20.40±0.78*	37.10±2.44*
III ASBEEM(50mg/kg)	44.67±4.04	29.00±6.00	245.00±3.61 ^c	43.67±6.66	33.67±2.31	12.87±0.85	27.87±1.57
IV ASBEEM(100mg/kg)	41.00±1.00 ^a	24.67±1.53 ^b	191.67±12.74 ^c	51.33±2.08	38.00±1.73 ^e	10.87±1.16 ^f	25.50±0.89 ^g
V ASBEEM(150mg/kg)	32.00±2.00 ^a	19.00±1.00 ^b	177.00±1.00 ^c	52.00±4.36	37.33±2.08 ^e	11.60±0.98 ^f	21.07±2.01 ^g

Results are expressed as mean±SD for three determinations, n=3

Values with asterisk are significantly different at P<0.01 when compared with normal rats.

Values in the same column bearing similar superscript are significantly different at P<0.01 when compared with the test control groups

Table 2: Effects of oral administration ASBEEM on serum biochemical indices of CCl₄-induced hepatotoxicity in rats after four weeks.

Groups/Dose(mg/kg)	AST(U/L)	ALT(U/L)	ALP(U/L)	TP(g/L)	ALB(g/L)	DB(Umol/L)	TB(Umol/L)
I (Normal rats)	23.33±1.53	12.00±1.00	110.33±1.53	67.33±2.08	43.33±5.51	7.70±1.15	18.33±0.75
II CCl ₄ (150mg/kg)	60.00±2.65*	44.00±6.25*	273.67±4.93*	37.33±3.51*	25.33±5.51*	20.40±0.78*	37.10±2.44*
III ASBEEM(50mg/kg)	23.67±4.04 ^h	14.00±2.00 ⁱ	123.00±11.79 ^j	60.33±2.52 ^k	40.00±1.00 ^l	9.77±0.57 ^m	21.47±2.08 ⁿ
IV ASBEEM(100mg/kg)	25.67±3.01 ^h	13.80±0.72 ⁱ	117.00±4.36 ^j	59.33±2.30 ^k	40.67±2.52 ^l	9.90±1.35 ^m	22.60±1.90 ⁿ
V ASBEEM(150mg/kg)	26.00±1.00 ^h	12.27±0.58 ⁱ	113.67±7.76 ^j	66.33±3.79 ^k	41.00±8.00 ^l	9.53±2.55 ^m	22.17±2.82 ⁿ

Results are expressed as mean±SD for three determinations, n=3

Values with asterisk are significantly different at P<0.01 when compared with normal rats.

Values in the same column bearing similar superscript are significantly different at P<0.01 when compared with test control group

Plate 1 shows photomicrograph of cross section of liver of normal rats. Plate 2 showed the section of the liver administered with 150mg/kg body weight of CCl₄. Plates 3, 4 and 5 showed cross section of the

liver administered with varying doses of ASBEEM for two weeks. Plates 6, 7 and 8 showed cross section of the liver treated with varying doses of ASBEEM for four weeks.

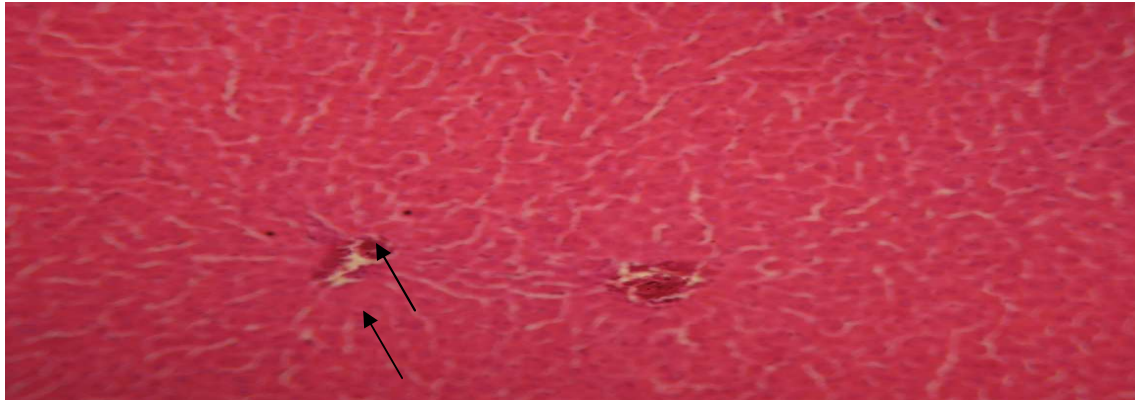


Plate1: Section of the liver of normal rats shows hepatocytes arranged in cord radiating from central venules. H & E STAIN X 10

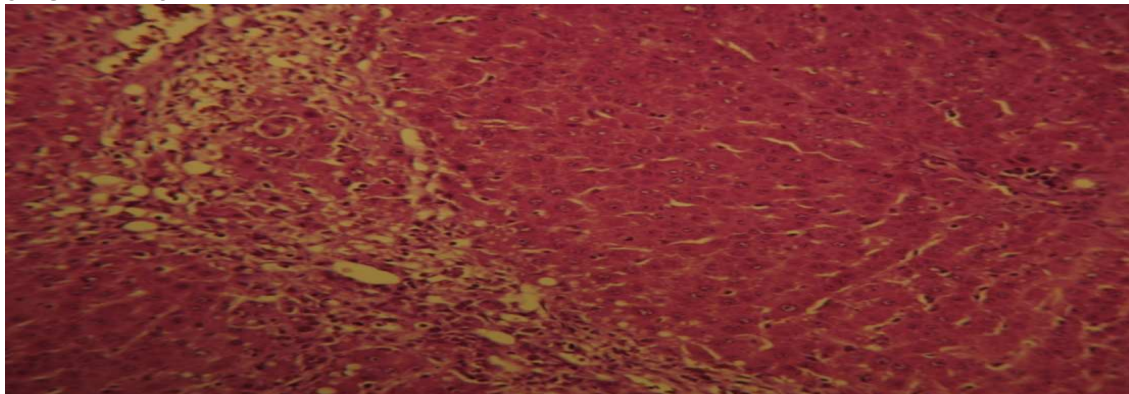


Plate 2: Section of the liver shows area of necrosis, fibrosis and degeneration of hepatocytes after CCl₄ 150mg/kg administered to induce liver damage. H & E STAIN X 10

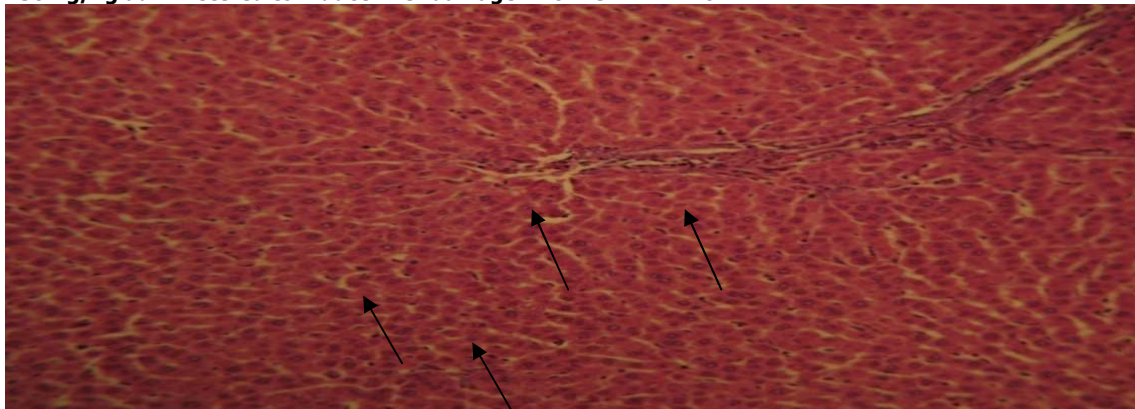


Plate 3: Section of the liver shows area of fibrosis after ASBEEM 50mg/kg administered for two weeks. H & E STAIN X 10

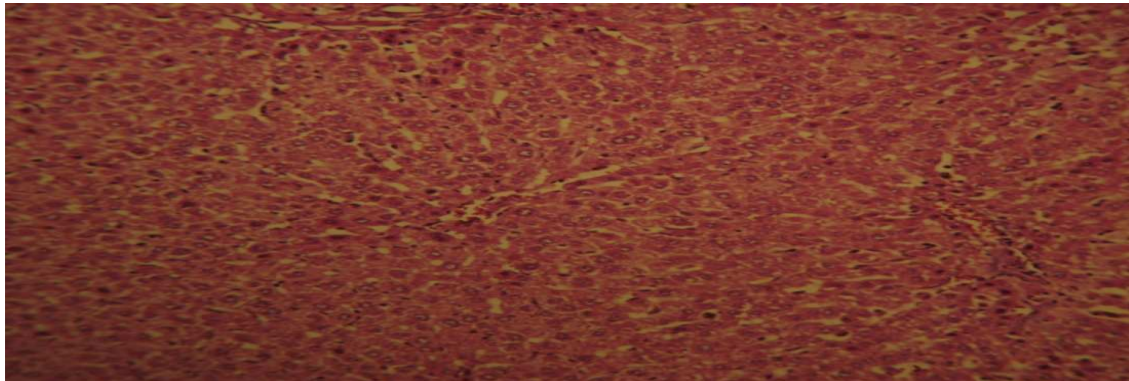


Plate 4: Section of the liver area of fat cells after ASBEEM 100mg/kg administered for two weeks. H & E STAIN X 10

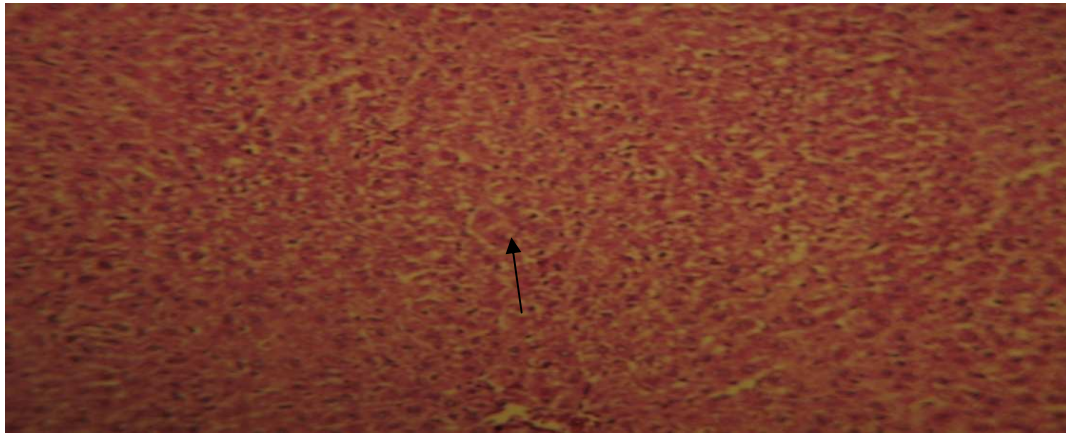


Plate 5: Section of the liver area of fat cells after ASBEEM 150mg/kg administered for two weeks. H & E STAIN X 10

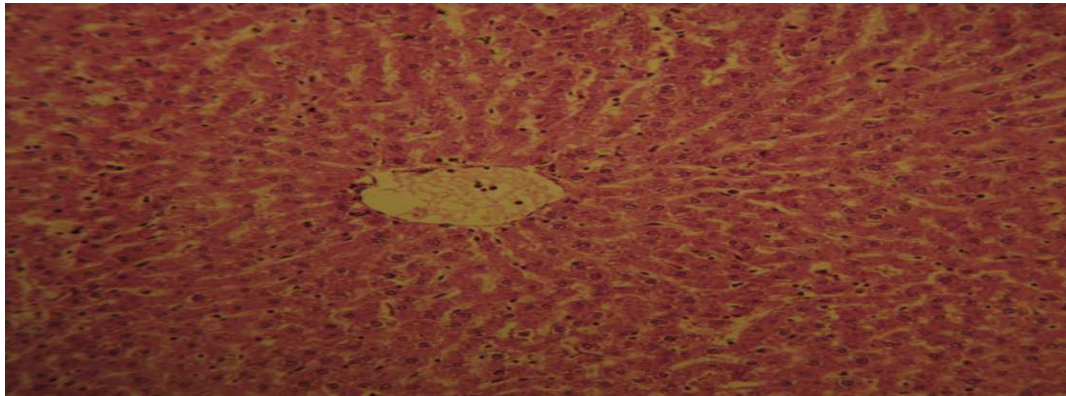


Plate 6: Section of the liver shows no significant pathology, they showed hepatocytes arranged in cord radiating from central venules after ASBEEM 50mg/kg administered for four weeks. H & E STAIN X 10

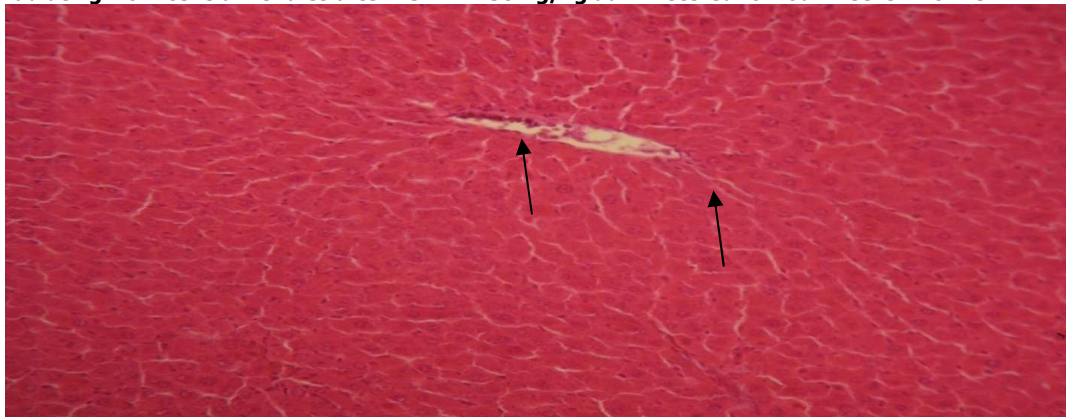


Plate 7: Section of the liver shows no significant pathology, they showed hepatocytes arranged in cord radiating from central venules after ASBEEM 100mg/kg administered for four weeks. H & E STAIN X 10

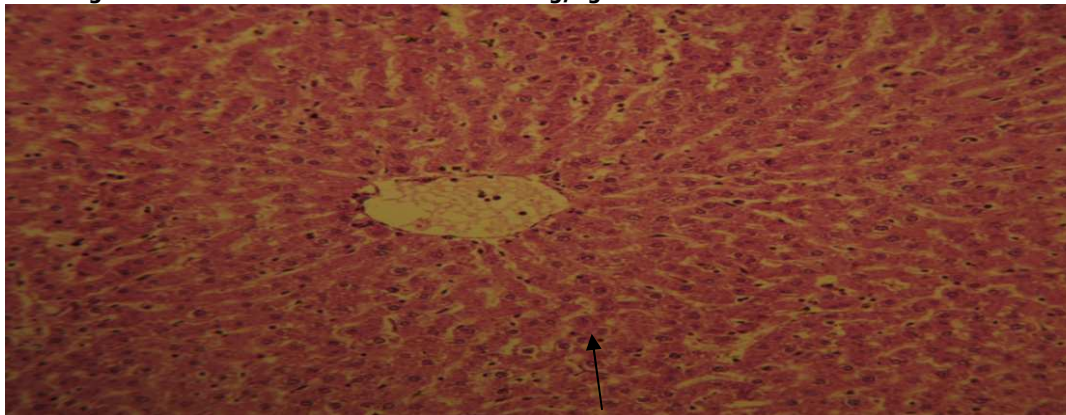


Plate 8: Section of the liver shows no significant pathology, they showed hepatocytes arranged in cord radiating from central venules after ASBEEM 150mg/kg administered for four weeks. H & E STAIN X 10

DISCUSSION

Liver injury induced by CCl₄ is the best-characterized system of xenobiotic –induced hepatotoxicity and is a commonly used model for the screening of medicinal and toxicological activities of drugs (Brautbar and Williams, 2002; Brent and Rumack, 1993). Information in Table 1 showed that CCl₄ administration caused significant elevation of ALT, AST, ALP, direct bilirubin and total bilirubin by inducing hepatic damage in test control group when compared to normal group.

Hepatic injury caused by CCl₄ is due to the generation of its reactive trichloromethyl radical (CCl₃*) by the action of microsomal Cytochrome P₄₅₀ enzyme (CYP2E11). This highly reactive free radical readily reacts with molecular oxygen to form the trichloro methyl peroxy radical (CCl₃O₂). Both trichloro and peroxy radicals can bind to cellular proteins and lipids, initiating lipid peroxidation and liver damage. Direct and total bilirubin levels were elevated in CCl₄ treated rats, caused either due to an increased production, decreased secretion from the liver or blockage of the bile ducts (Bun *et al.*, 2006). Bilirubin is derived from the regular degradation of haemoglobin from the red blood cells and excreted from the liver in the bile. It is a chemical normally present in the blood in small amounts and used by the liver to produce bile. When the liver cells are damaged, they may not be able to excrete bilirubin in the normal way, causing a build-up of bilirubin in the blood and extracellular fluid (Saukonen *et al.*, 2006).

Liver is an important site for the synthesis of many serum proteins and the levels of serum proteins are decreased in hepatic diseases. The oxidative damage of some amino acids is considered as the major cause of metabolic dysfunction in hepatic damage (Bandyopadhyay *et al.*, 1999). Serum albumin is the main plasma protein produced in the liver, a clinically useful indicator of hepatic synthetic function (Sturgill and Lambert 1997)

Liver injury is associated with a decline in the concentration of serum albumin, and hypoalbuminemia has been considered to be consequence of a decreased rate of hepatic synthesis of the essential protein in both humans and animals (Ruot *et al.*, 2002). Accordingly, the serum albumin and total protein levels were determined in the present study. The CCl₄-treated rats exhibited significantly lower serum total protein and albumin levels compared with the normal control rats. However, administration of ASBEEM increased these serum levels to a normal control range. This suggests that ASBEEM possesses ability to promote protein synthesis that plays a significant contributory role in hepatoprotective activity, which in turn has been shown to enhance proliferative process and the production of hepatocytes (Awang, 1993).

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(Recknagal *et al.*, 1989). When cell membrane of hepatocytes is damaged, a variety of enzymes such as ALT, AST and ALP are released into the blood from the cytosol (Mitra *et al.*, 1998). A rise in serum transaminases is a sensitive indicator of damage to hepatocytes even if there is no evident hepatic impairment. The increase in ALT, AST and ALP activities observed in the test control group is an indication of the extensive liver damage induced by CCl₄. Administration of ASBEEM causes a significant and dose dependent decrease in the level of AST, ALT and ALP when compared to test control group after two weeks, however administration of ASBEEM after four weeks completely abolished the alterations induced by CCl₄ in activities of serum alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase (Table 2). These suggests that aqueous stem bark extract of *Erythrina mildbraedii* might possess hepatoprotective effect.

The histopathology studies of liver showed fatty degeneration and loss of normal structure of hepatocytes in CCl₄ administered rats, in comparison with the normal control. The extract treated groups showed regeneration of hepatocytes and reduction of fatty changes in hepatocytes. It has been reported by previous findings that CCl₄ causes necrosis (Sun *et al.*, 2001), fibrosis (Natsume *et al.*, 1999), mononuclear cell infiltration (Natsume *et al.*, 1999), steatosis and degeneration of hepatocytes, increase in mitotic activity (Teocharis *et al.*, 2001) and cirrhosis (Zalatnai *et al.*, 1991) in liver. It has also been reported that CCl₄ causes apoptosis in the liver (Shi *et al.*, 1998; Sun *et al.*, 2001). Therefore, histopathological findings in the liver due to CCl₄ administration are in agreement with previous studies that CCl₄ may cause fatty liver due to disturbance in normal lipid homeostasis by an increase in the esterification of free fatty acids to form triglycerides, phospholipids and other fatty acid esters with increase in cholesterol synthesis (Boll *et al.*, 2001).

Phytochemical screening of ASBEEM has revealed the presence of alkaloids, flavonoids, steroids, glycosides, saponins and tannins. Phytoconstituents like the flavonoids (Baek *et al.*, 1996), saponins (Tran *et al.*, 2001) and alkaloids (Vijayan *et al.*, 2003) are known to possess hepatoprotective activity. The presence of flavonoids in our extract may be responsible for its antioxidant effect and thus hepatoprotective activity.

CONCLUSION

The results of this study showed the hepatocurative effect of aqueous stem bark extract of *Erythrina mildbraedii* on CCl₄ induced liver damage. The hepatocurative effect was found to be dose dependent and increases with duration of treatment with the extract.

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