



Hepato-protective Effect of Ethanol Extract of *Pterocarpus santalinoides* Leaf on Carbon Tetrachloride (CCl₄) Induced Albino Rats

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Key words: Hepato-protective, *Pterocarpus santalinoides*, aspartate aminotransaminase, alanine aminotransaminase, alkaline phosphatase and histological analysis.

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**Caribbean Journal of Science and
Technology**

ISSN 0799-3757

<http://caribjscitech.com/>

Abstract:

Hepato-protective effect of ethanol extract of *Pterocarpus santalinoides* leaf on CCl₄-induced hepatic damage in albino rats was carried out with a total of 25 rats. CCl₄ was administered in two groups; one group was not treated while the other groups were administered *Pterocarpus santalinoides* ethanol leaf extract at 100,200 and 400 mg/kg body weight of rats twice for 1 week followed administration of CCl₄ at the 7th day via intra-peritoneal route respectively. One group was not induced and received distilled water only. The Liver enzymes activities were determined from plasma transaminase activities of the rats. Twenty five albino rats were grouped into A, B and C. Group C was further subdivided into C₁, C₂ and C₃ respectively. CCl₄ was administered to all the groups except group A (Normal Control). Group B (Positive Control) was not treated while group C₁ to C₃ received ethanol extract of *P. santalinoides* leaf which was administered orally to the rats twice daily for 1 week at varying doses of 100, 200 and 400 mg/kg body weight respectively. The liver enzymes activities were determined using spectrophotometric method. The result revealed that there were significant (P<0.05) reductions in aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and alkaline phosphatase (ALP) activities in rats administered ethanol extract of *P. santalinoides* leaf in a dose dependent manner while significant(p<0.05) increase in the activities of AST, ALT and ALP were recorded in the group that received only CCl₄. The results of histological analysis showed that the ethanol extract of *P. santalinoides* leaf was able to prevent CCl₄ from damaging the liver architecture in a dose dependent manner. The results indicate that *P. santalinoides* leaf possesses hepato-protective property.

Introduction:

Medicinal plants constitute effective resources for both traditional and modern medicines. And herbal medicine has been shown to have genuine utility (Odeh and Tor-Anyiin, 2013). Extracts from plants have been utilized for their antifungal, antiviral and antibacterial activities globally (Odeh and Tor-Anyiin, 2013).

Pterocarpus santalinoides (L'Herit. ex DC, family- *Leguminosae: Papilionoideae*) is a plant believed to possess potent antibacterial properties in ethno-medicine (Odeh and Tor-Anyiin, 2013). It is a tree, 9–12 m tall with low straggling branches. The leaves are compound in nature and 5–9 leaflets, ovate-elliptic in shape, rounded at the base, glabrous, glossy, and rather coriaceous with about 8 pairs of prominent main lateral nerves looping away from the margin. *Pterocarpus santalinoides* also has leaf-stalk slender, glabrous stalk up to 10-20cm long and leaflet stalk stout 2-5mm long (Odeh and Tor-Anyiin, 2013). It grows along riverine forests of Africa and tropical South America. It is a native of Brazil, Cameroon, Ghana, Nigeria and Senegal. The ethno-medical use of leaves of *P. santalinoides* in the treatment of diarrhea and other gastrointestinal disorders has been scientifically proved (Anowai et al., 2012). The leaves are eaten as vegetable, wood is termite resistant, the bark contains tannins and dyes used for dyeing and the bark is also used as stomach ache remedy. The plant is variously known locally as *gundururu* or *gyadarkurmi* in Hausa, *maganchi* in Nupe, *ikyarakya* or *kereke* in Tiv, *gbengbe* in Yoruba, *okumeze* in Edo, *nturukpa* in Igbo, *nja* in Efik and *ugbampiegwu* or *uturukpa* in Igede (Odeh and Tor-Anyiin, 2013). It is commonly referred to as Red Sandal wood in English (Anowai et al., 2012).

Liver is one of the largest organs in human body and chief site for intense metabolism and excretion. So it has a role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Rajib et al., 2009). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, a healthy liver is a crucial factor for overall health and wellbeing. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailment like hepatitis, cirrhosis and alcoholic liver disease (Rajib et al., 2009).

Hepato-protection is the ability to prevent damage to the liver. Therefore the hepato-protective effect of a plant is its ability to protect the liver from damage. Hepato-toxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions. Toxins and drugs are among the basic pathogenic agents of acute liver failure in Western countries (Grattagliano et al., 2009). Nevertheless, chemical toxins (including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide) are often used as the model substances causing experimental hepatocyte injury in both in vivo and in vitro conditions. The pathogenesis of this damage involves all cell types in the liver via death and regeneration processes and progress to chronic hepatitis, fibrosis, cirrhosis and hepato-cellular carcinoma (Akram et al., 2012). Oxidative stress has been regarded as a major contributor to the development of various hepatic disorders (Albano, 2008; Ferret et al., 2001). The reactive oxygen species (ROS) are known to play a major role in either the initiation or progression of carcinogenesis by inducing oxidative stress (Sun, 1990; Gulcin, 2006). Oxidative stress plays a crucial role in the development of carbon tetrachloride (CCl₄) - induced hepato-toxicity (Sureshkumar and Mishra, 2006), and a connection between oxidative stress and lipid peroxidation has been reported (Kota et al., 2008).

Carbon tetrachloride (CCl₄) is widely used to induce liver damage because it is metabolized in hepatocytes by cytochrome P₄₅₀, generating a highly reactive carbon-centered trichloromethyl radical, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis (Fang et al., 2008; Weber et al., 2003). In Nigeria, many plants are used in traditional medicine as antimicrobial agents, blood boosters, hepato-protective/therapeutic and so on but only few are documented. Plants based system of traditional medicine has continued to play an essential role in health care in so many cultures. The increased use of plant derived products as alternatives to orthodox or synthetic drugs and increasing awareness of beneficial effects of natural products has resulted in increased interest in

alternative therapies. The aim of the present study was to examine the hepato-protective effect of *Pterocarpus santalinoides* on CCl₄-induced acute hepatic injury in albino rats.

Materials And Methods:

Materials

Equipment and Instrument:

The equipment and instruments are of analytical standards.

Chemicals and Reagents: The chemicals and reagents are of analytical grades

Collection of Plant Materials

Fresh leaves of fully grown *Pterocarpus santalinoides* were collected from Mgbalukwu in Onicha local government area of Ebonyi State between the month of August and September, 2014. The plant sample was identified by a taxonomist, Prof. S. S. Onyekwelu of the department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. A voucher specimen was deposited at the herbarium in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria, for reference purposes.



Figure 1: Leaves and Flowers of *Pterocarpus santalinoides* Tree

Preparation of *Pterocarpus santalinoides* Leaves Extracts

Fresh leaves of *Pterocarpus santalinoides* were collected and air-dried under ambient temperature. The dried leaves were grinded into powdered form using blender. Exactly 200g of grinded *Pterocarpus santalinoides* leaves were soaked in 1500ml of ethanol and were allowed to stand for 24hrs. The mixtures were filtered using clean sieve cloth and the filtrates evaporated forming substance pastry in nature. The extract was kept in a dried clean container and stored in a refrigerator.

Experimental Animals

Fifteen Wister male albino rats weighing between 60 –100g (4-6-weeks old) were obtained from the animal house of the faculty of Veterinary Medicine University of Nigeria, Nsukka. They were acclimatized for seven days in

stainless steel cages under good laboratory conditions. They were fed with commercial poultry growers mash feed (Vital feed[®], Jos, Nigeria). Clean water was provided daily and access was free. The animals were weighed using triple beam weighing balance. Handling, management and use of animals for the experiment were as such that allowed minimal stress. Faculty of Science, Ebonyi State University, Abakaliki Animal Ethical Committee approved the animal study.

Experimental Design

The animals were divided into 5 groups of 3 each (A, B, C₁, C₂ and C₃). The albino rats were then subjected to the following treatment for 7 days.

Group A: Distilled water for 7 days

Group B: Distilled water for 7 days + CCl₄ 1ml on the 7th day.

Group C₁: 100 mg/kg body weight of ethanol leaf-extract of *Pterocarpus santalinoides* for 7 days + CCl₄ 1ml on the 7th day

Group C₂: 200 mg/kg body weight of ethanol leaf-extract of *Pterocarpus santalinoides* for 7 days + CCl₄ 1ml on the 7th day

Groups C₃: 400 mg/kg body weight of ethanol leaf-extract of *Pterocarpus santalinoides* for 7 days + CCl₄ 1ml on the 7th day

Food was withdrawn 12 hours before carbon tetrachloride administration via intra-peritoneal cavity to enhance the acute liver damage in animals of groups B, C₁, C₂ and C₃. The animals were sacrificed 24 hours after the administration of CCl₄. Blood samples were collected for assay of AST, ALP and ALT marker enzymes. The liver was immediately isolated and washed with normal saline. The liver was then subjected to histo-pathological examination.

Blood Collection and Preparation

After overnight fast, the animals were sacrificed on the 8th day under mild chloroform anaesthesia and blood was obtained via femoral vein. Blood samples were transferred into plain centrifuge tubes and allowed to clot at room temperature. They were then centrifuged within 1 hour of collection at 4000x g for 10min on a Centrifuge to separate the sera from the clot. The resultant sera samples were stored frozen at -20°C. Prior to liver enzyme assay, frozen sera were completely thawed and well mixed and all reagents were allowed to attain room temperature.

Determination of Liver Enzymes and Alkaline phosphatase

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) were determined by method described by Carl and Edward, 1999 and Reitman and Frankle, 1957.

Histo-pathological Examination Histological analysis was done using standard method.

Data Analysis

Results were expressed as mean standard deviation. The one-way analysis of variance (ANOVA) was used to analyze the data followed by post-hoc tests. The results are considered significant at P<0.05.

Results:

The result of liver enzyme levels in CCl₄ induced hepatic damage in albino rats treated with *Pterocarpus santalinoides* ethanol leaf extract showed a significant (P<0.05) decrease in levels of AST, ALT and ALP in a dose dependent manner as shown in Figure 2, 3 and 4.

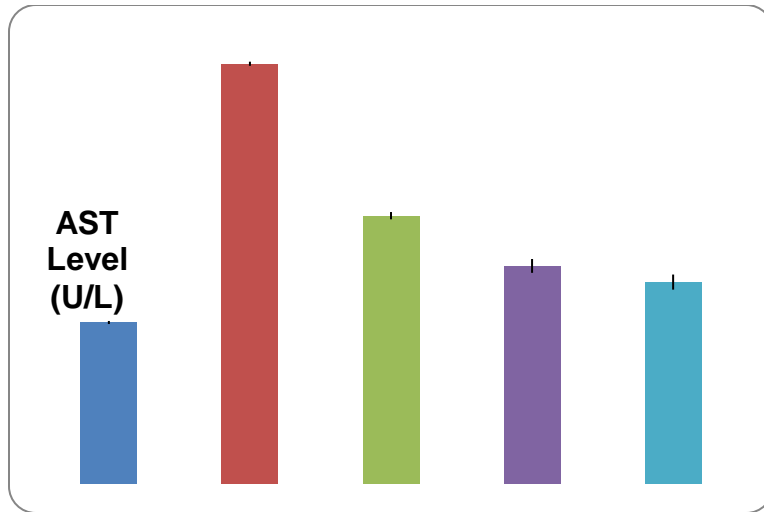


Figure 2: AST level in CCl₄ hepatic damage in Albino Rats Treated with Ethanol leaf extract *Pterocarpus santalinoides*

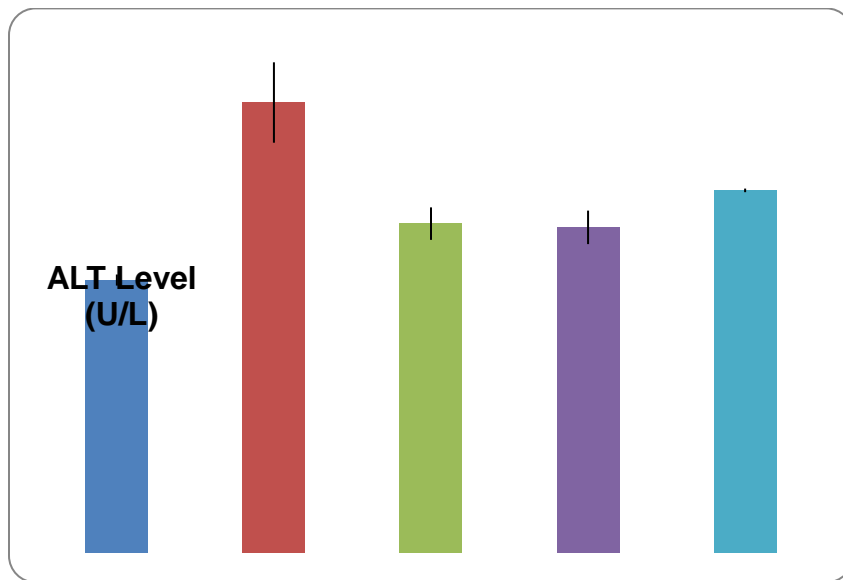


Figure 3: ALT level in CCl₄ hepatic damage in Albino Rats Treated with Ethanol leaf extract *Pterocarpus santalinoides*

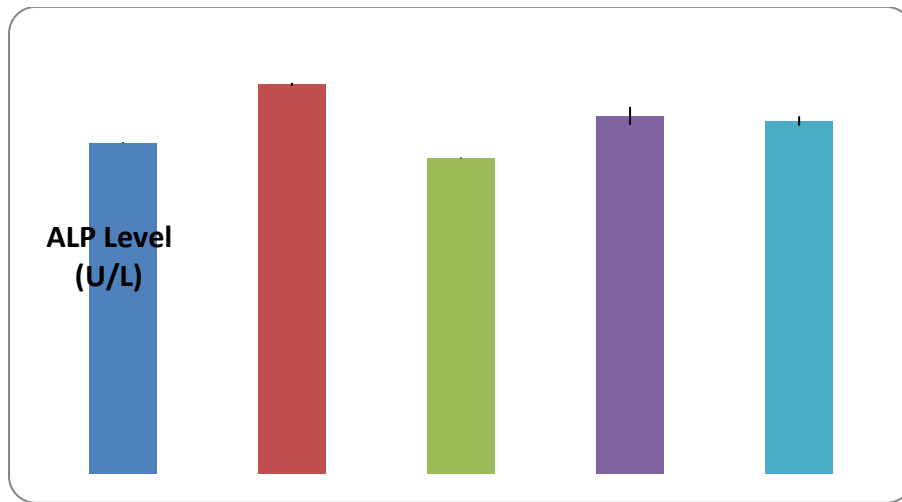


Figure 4: ALP level in CCl₄ hepatic damage in Albino Rats Treated with Ethanol leaf extract *Pterocarpus santalinoides*

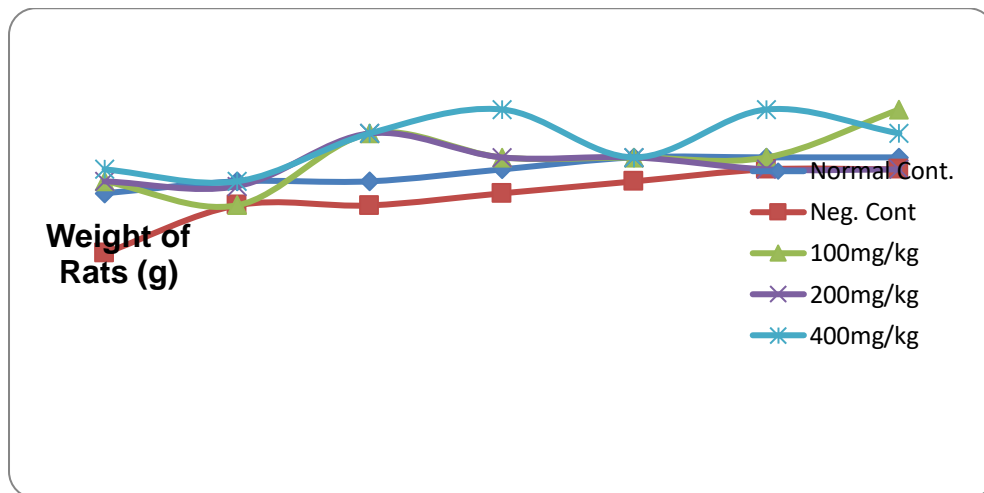


Figure 5: Weight of CCl₄ hepatic damage in Albino Rats Treated with Ethanol leaf extract *Pterocarpus santalinoides*

Histology of liver of normal control animals (Group A) showed normal liver cells with well-defined cytoplasm (Plate 1), whereas the group treated with CCl₄ only (Group B) showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, Kupffer cells hyperplasia, karyorrhexis, inflammatory cells and necrotic cells (Plate 2). The hepato-protective effect of the ethanol extract of *Pterocarpus santalinoides* showed weak activity at 100mg/kg dose (Figure 1, 2 and 3), moderate activity at 200mg/kg (Plate 3) and a good activity at 400mg/kg (Plate 4). The histological appearances of the *P. santalinoides* extract treated groups were quite similar to that of the healthy control group (Group A) and the tissue damage and necrosis were of a lesser extent than that of the CCl₄ group (Plate 2)

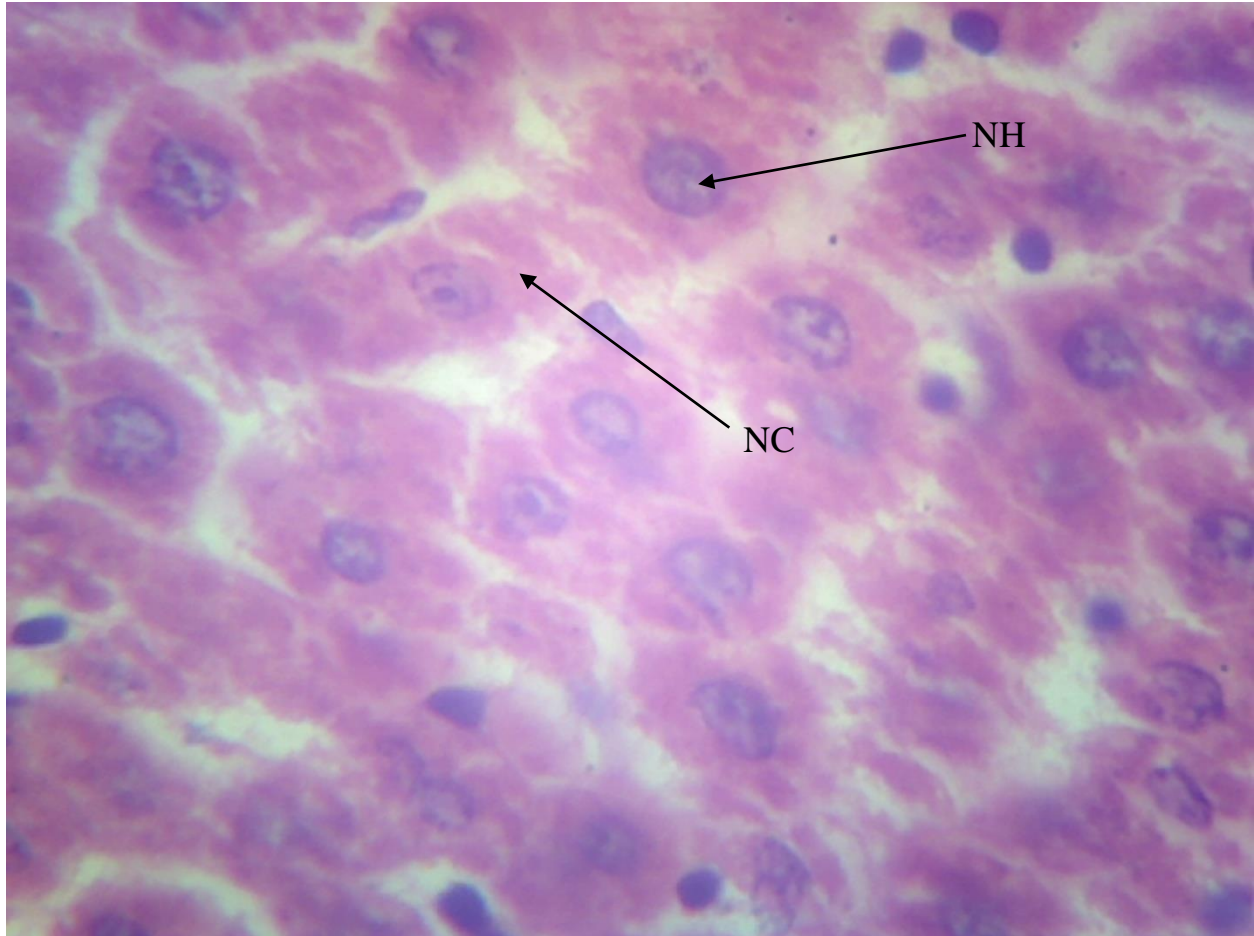


Plate1: Photomicrogram of healthy control liver stained with H/E (X 600) shows normal liver hepatocyte (NH), and the normal cytoplasm (NC). The overall architecture of the liver section appears normal.

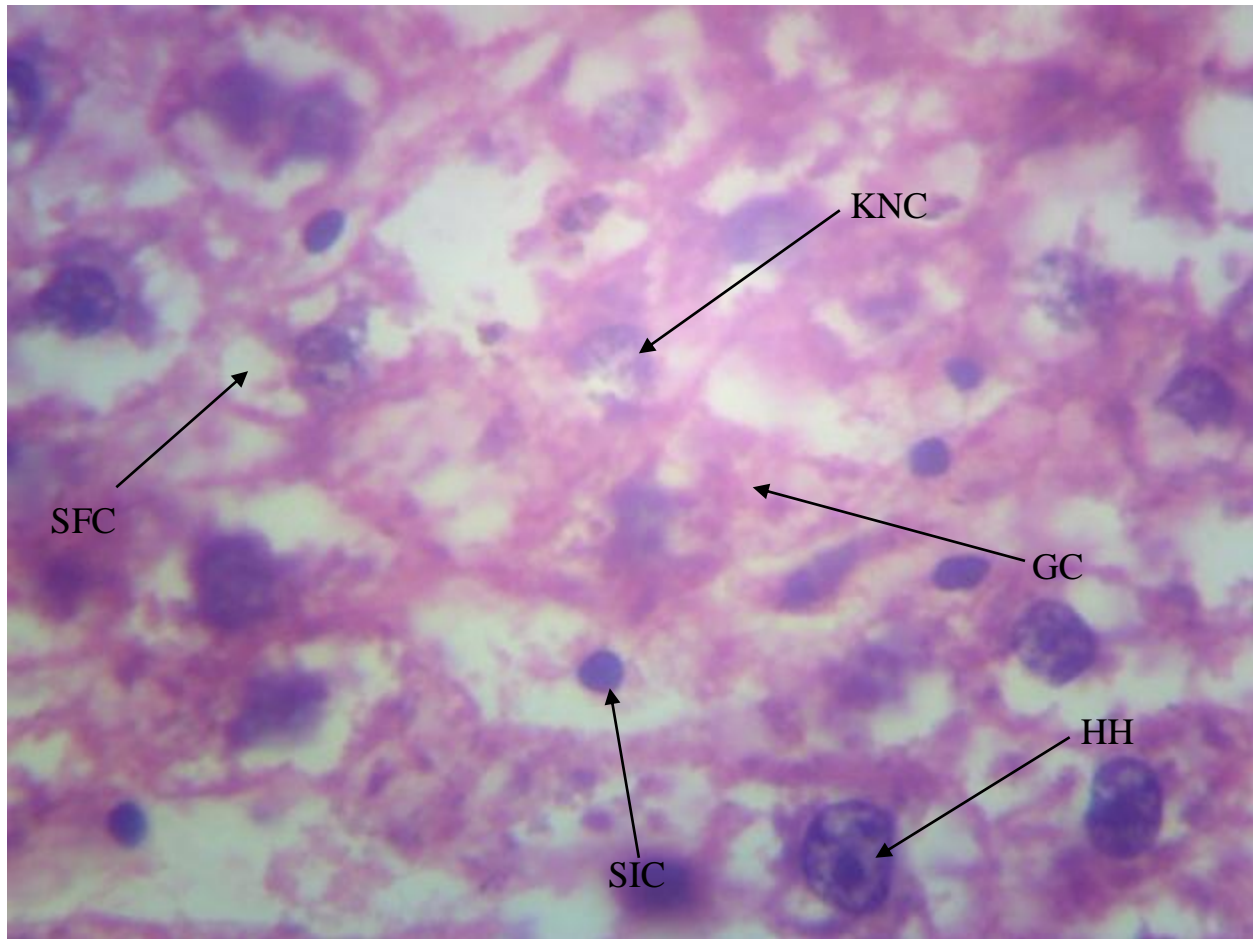


Plate 2: Photomicrogram of CCl₄ induce liver section stained with H/E (X 600) shows hyperplased hepatocyte (HH), granulated cytoplasm (GC), server fatty change (SFC), severe inflammatory cells (SIC) and karyorrhexis leading to necrosis of Cell (KNC)

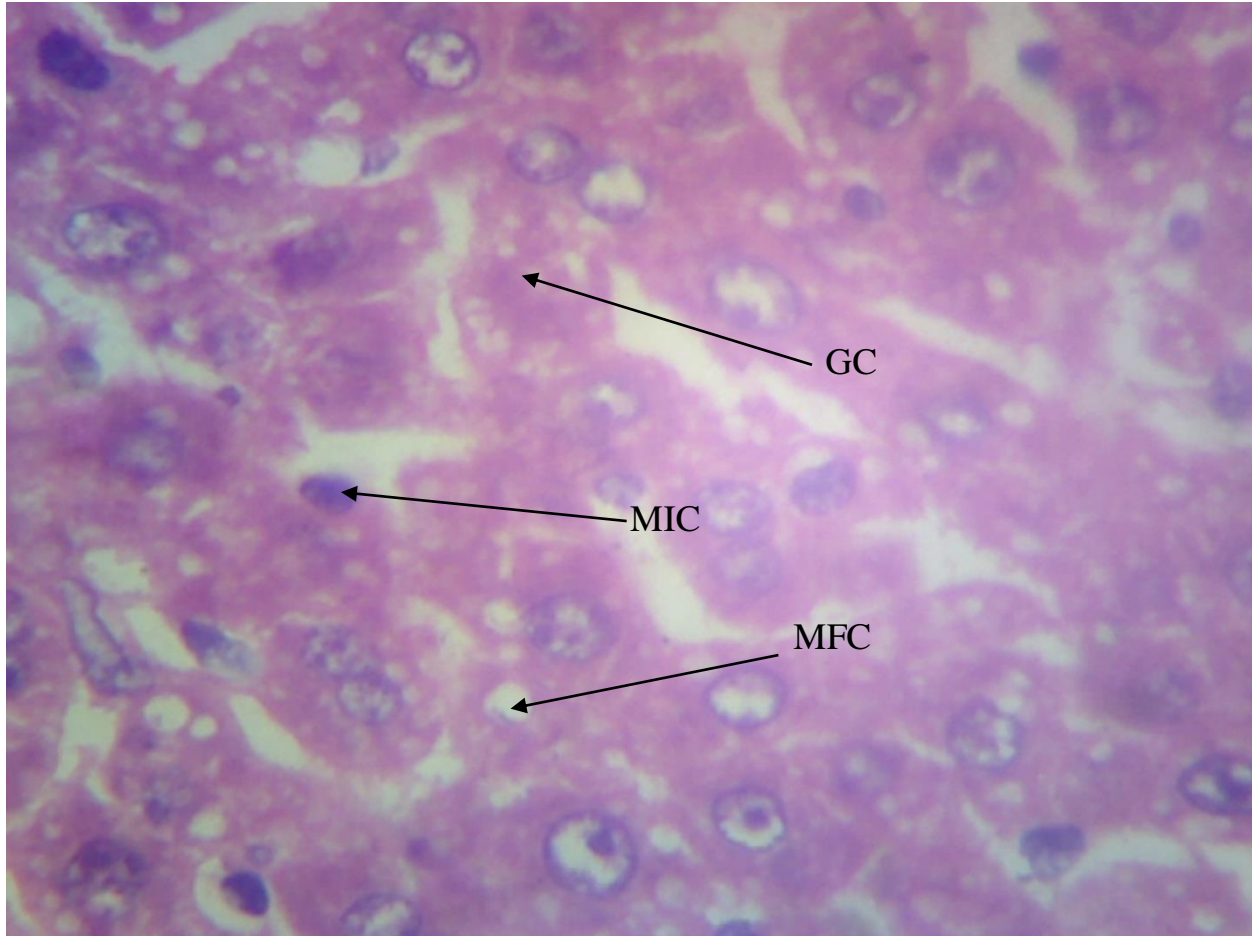


Plate 3: Photomicrogram of liver section treated with 100mg/kg weight of ethanol leaf extract of *Pterocarpus santalinoides* stained with H/E (X 600) shows mild fatty change (MFC), mild inflammatory cells (MIC) and granulated cytoplasm (GC)

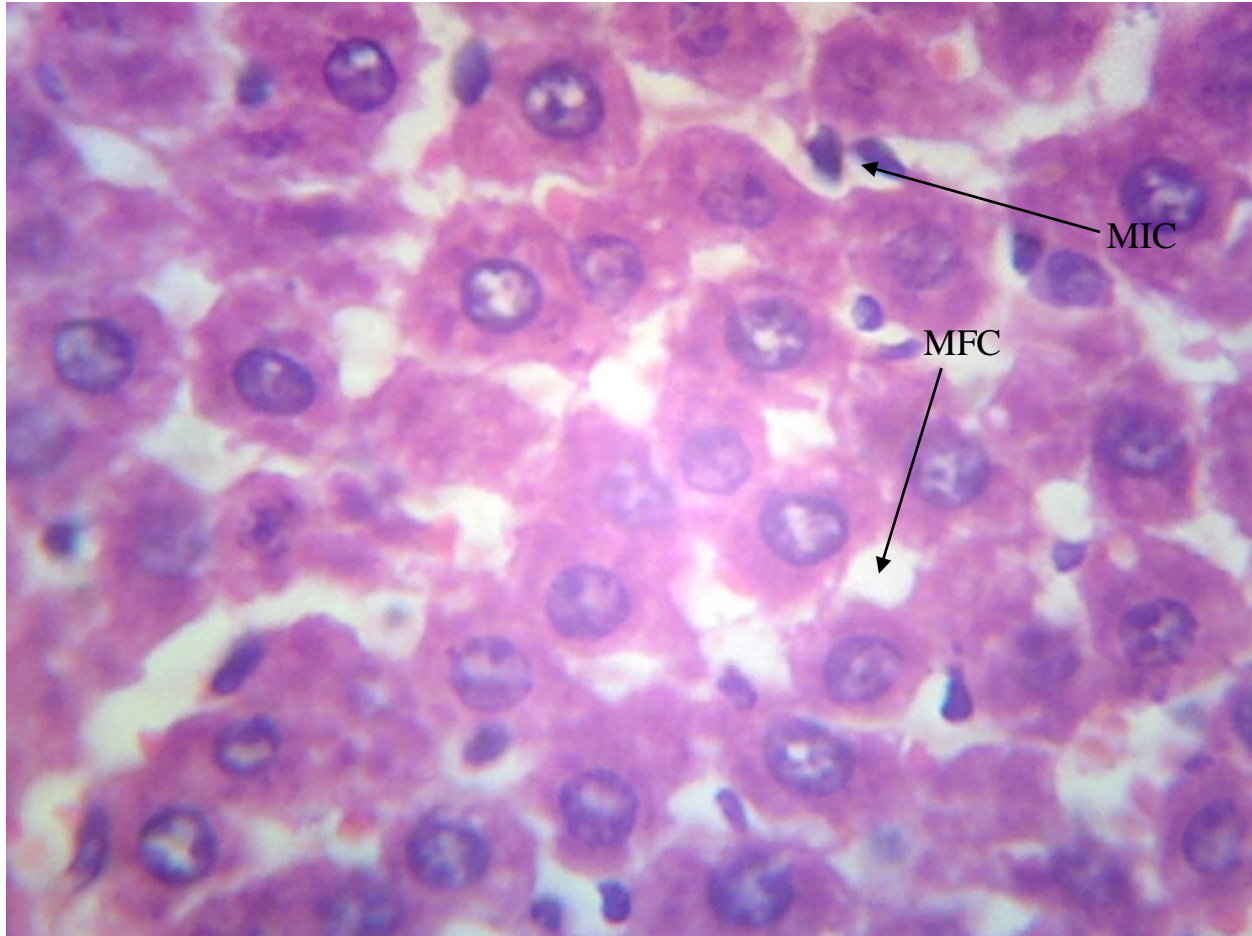


Plate 4: Photomicrogram of liver section treated with 200 mg/kg weight of ethanol leaf extract of *Pterocarpus santalinoides* stained with H/E (X 600) shows mild fatty change (MFC) and mild inflammatory cells (MIC)

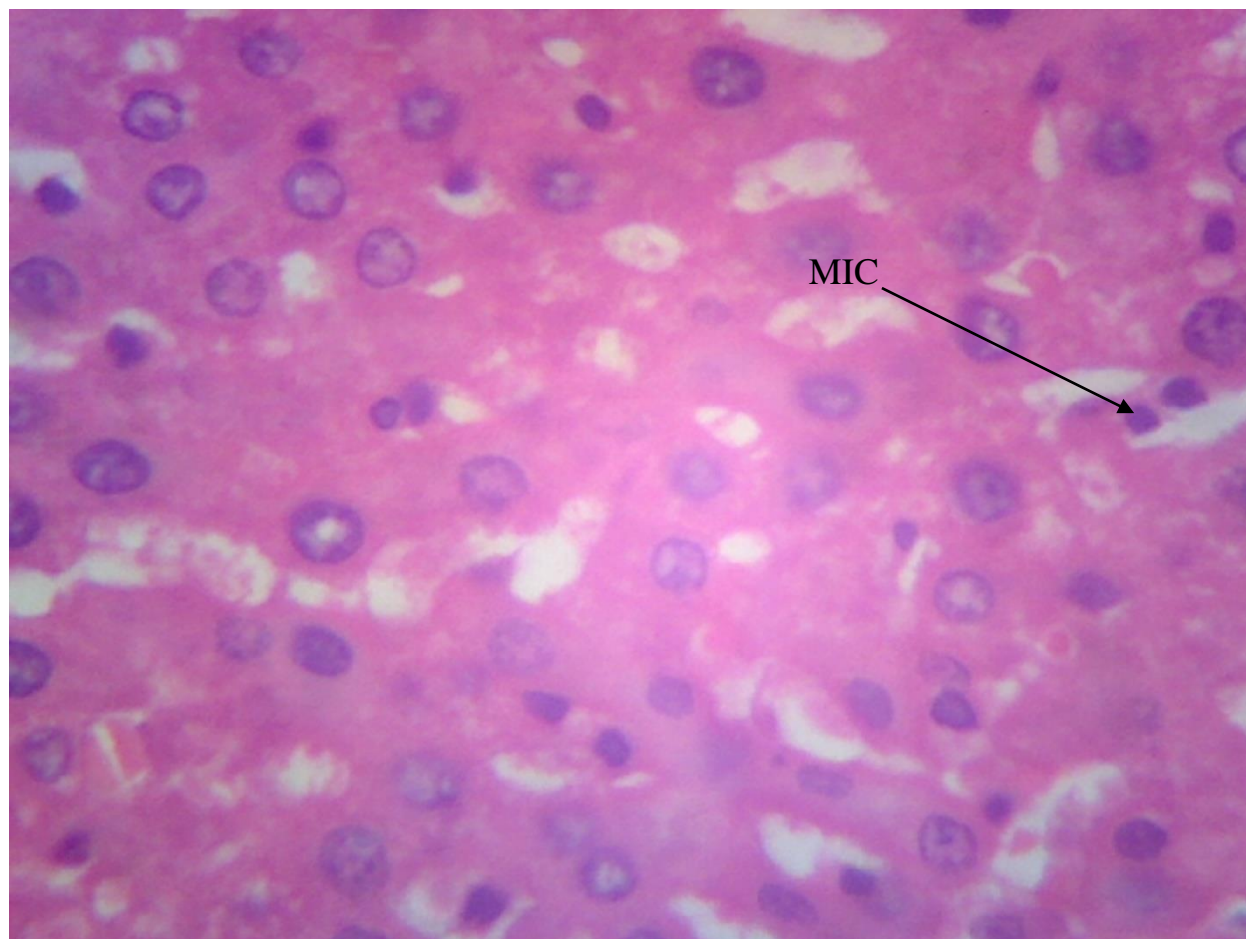


Plate 5: Photomicrogram of liver section treated with 400mg/kg weight of ethanol leaf extract of *Pterocarpus santalinoides* stained with H/E (X 600) shows mild inflammatory cells (MIC) otherwise normal

Discussion

This study demonstrates the hepato-protective effects of *P. santalinoides* ethanol leaf extract against CCl₄-induced liver injury in rats. Results indicated that *P. santalinoides* ethanol leaf extract was able to suppress the hepatotoxicity of CCl₄ and thus exhibiting its hepato-protective effect. Levels of liver marker enzymes in serum are used as diagnostic markers of hepatic injury. One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes, such as transaminases and serum alkaline phosphatase in the circulation after CCl₄ administration (Akram *et al.*, 2012). CCl₄ is found to produce free radicals, which affect the cellular permeability of hepatocytes leading to elevated levels of liver enzymes (Kumar *et al.*, 2009). The administration of *P. santalinoides* extract may be responsible for the low levels of liver enzymes obtained in the treated groups. Similar results have been obtained with pretreatment of rats with some plant extracts. Some includes the methanol extract of the leaf and bark of *Glycosmi spentaphylla* (Rajib *et al.*, 2009). And also the petroleum ether, aqueous and methanol extracts of *Jatropha gossypifolia* showed a similar result (Bipin *et al.*, 2009). The result was in correlation with the report of Aja *et al.* (2013) which showed that the administration of aqueous, ethanolic and methanolic extracts of *Moringa oleifera* seeds at 100, 200, 400, and 800 mg/kg body weight significantly decreased ($p < 0.05$) the levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in a dose dependent manner. The

hepato-protective activity of these plants were found to be dose dependent and the effect been exhibited best at the highest dose administered.

The histological results reported in this study confirmed the biochemical results and indicated that CCl₄ induced severe histological changes in the hepatic tissues. Similar histological changes in the liver have been documented previously (Bilgin et al., 2011; Cetin et al., 2011). The acute hepato-toxic effects induced by CCl₄ administration were confirmed with histopathology examinations, revealing extensive hepato-cellular degeneration and necrosis, fatty changes, inflammatory cell infiltration, karyorrhexis, hyperplasia and ground like cytoplasm. The hepatocytes showed vacuolar degeneration and nuclear pleomorphism. In contrast, the histological results showed that treatment with ethanol extract of *P. santalinoides* leaf effectively protected rat's against CCl₄-induced hepatic toxicity. Pre-treatment with *P. santalinoides* prevented the necrosis and the other histo-pathological changes induced by CCl₄ treatment.

The hepato-protective effects of various plants parts against CCl₄ induced hepato-toxicity have been studied so far. Some includes berberine is an active compound in *Coptidisrhizoma* (Yibin et al., 2010), cinnamon ethanolic extract (Akram et al., 2012), *Nigella sativa* seeds (Amina et al., 2012) among others. Numerous studies noted that CCl₄ is widely used to induce liver damage because it is metabolized in hepatocytes by cytochrome P₄₅₀, generating a highly reactive carbon centered trichloromethyl radical, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis (Fang et al., 2008; Weber et al., 2003; Ashok et al., 2001; Bahceioglu et al., 1990; Aleynik et al., 1997; Halliwell and Gutteridge, 1998). The hepato-protective action of some of pantothenic acid against CCl₄ induced hepato-toxicity is thought to be due to its ability to scavenge free radicals. Also Hwang et al. (2002) reported that berberine exhibited antioxidant property by its ability to quench free radicals of 1, 1-diphenyl-1-picrylhydrazyl, decrease the leakage of lactate dehydrogenase and ALT and prevent the formation of malondialdehyde. It is therefore possible that the hepato-protective activity of *P. santalinoides* ethanol leaf extract may be due to its anti-oxidant activity.

Conclusion:

The present bio-chemical and histological results proved that leaf of *P. santalinoides* possess potential to protect the liver tissue against oxidative damages and could be used as an effective protector against CCl₄-induced liver damages. The Anti-oxidant activity of *P. santalinoides* could be further studied to determine if its hepato-protective activity stems from its anti-oxidant activity.

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