

Original Research Article

Protective Efficiency of *Pterocarpus Erinaceus* Leaves Extract in Carbon-tetrachloride-Induced Hepatic and Hematological Injuries in Rats

Abstract

Aims: Chemical toxicity is one of the major leading causes of tissues injuries, which impair the tissue's ability to maintain normal physiological functions. *Pterocarpus erinaceus* is a medicinal plant use as traditional remedy for the treatment of several disorders associated with tissues injuries. This study therefore aimed at investigating tissues protective efficiency of *Pterocarpus erinaceus* leaves extract in carbon-tetrachloride-induced hepatic and hematological injuries in rats.

Material & Methods: Leaves, stem and root of *Pterocarpus erinaceus* after collection were air-dried and pulverized. Each was extracted with methanol and the methanolic extracts were used. Acute toxicity and hepatoprotective studies against CCl₄ toxicity were conducted. Rats were grouped into; Group 1: Normal control (liquid paraffin, vehicle 1ml/kg), Group 2: Negative control (received 1ml/kg CCl₄), Group 3: Positive control (received 1ml/kg CCl₄ +100ml/kg Silymarin), Group 4-6: Extract treated rats (received 1ml/kg CCl₄ + varied doses of Extracts at 100, 200, and 400mg/kg body weight of rats). The treatment was done daily via oral means for 14 days.

Results: The results of the acute toxicity study showed *Pterocarpus erinaceus* extract safe with LD₅₀ greater than 5000mg/kg body weight. This study reveals *Pterocarpus erinaceus* able to ameliorate CCl₄ toxicity by minimizing damage to hepatic and hematological tissues. Histological study of the rats' hepatic cells further unveil the protective ability of the plant extract against severity of CCl₄-mediate hepatic architectural damage.

Conclusion: *Pterocarpus erinaceus* leaves possess components with tissue-protective properties hence its recommendation for further study to identify the active biomolecules.

Key words: Animal-study, CCl₄-toxicity, Tissues, Protection, Plant-extract

1. INTRODUCTION:

Jorum and Piero [1] stated that blood can act as a pathological and physiological indicator of animal health. Following absorption, substances (toxic chemical for instance) are carried by the blood to various organs, where they may exert harmful effects. High reactive metabolites produced by toxic chemicals can alter the hematological system of organisms and lower the

ability of blood to maintain homeostasis. Change of hematological components from normal levels represents the presence of toxicity or disease [2]

The existence of healthy tissue is based on the protection versus injury induced as a result of chemical toxicity [3]. The liver is term the most crucial organ that exhibit the vital role in safeguarding several physiological processes in the body. It is involve in several imperative functions, as metabolism, excretion, and storage. Liver provides a basic function in the detoxification of endogenous and exogenous intermediaries. Consequently, liver injuries is accompanied by crucial implications for the health of the affected person [4]. Liver injuries that are associated with toxic metabolites has been a major research focus by many scientific studies [5,6].

Liver injury due to chemical toxicity is responsible for about 5% of all hospital admissions and 50% of all acute liver failures [7]. Despite the growth in the production of agents with efficacies to reverse the damage induces on the liver, hepatic injuries still remain a global challenge with a serious concern by the health system. In this regard, exploration of more alternative therapeutic medicine without severe side effects is vigorously required. In line to this, therefore, herbal medicines ought to be re-evaluated as new dynamic therapeutic agents with minimal side effects [8]

The use of plants as alternative medicine is dated back to centuries, even before long recorded history [9]. People valued, appreciated the great diversity and importance of plants that are accessible to them [10]. As times passed by, people group have added the medicinal power of herbs in their field to its knowledge base [11]. Thus, in the exploration of many more reliable and safer liver protective agents, medicinal plants play a significant role. Medicinal plants being

an effective source of both traditional and modern medicines are gaining more ground for use in primary health care [12, 13]. Many plants and plant products have been recommended for use in the treatment of liver diseases. Among the plants are, *Silybum marianum* [14], *Picrorrhizakurroa* [15], and *Teptrosiapurpurea* [16] *Khaya senegalensis* [17,18, 19] among others.

The plant, '*Pterocarpus erinaceus* (Fabaceae)' is a tree found in the most tropical areas of Africa [20,21]. In West Africa, its leaves, stem bark, and roots have been reported as highly use for traditional remedies against inflammation, ulcer, pain in the joints, malaria-fever, and bacterial infections [22]. Various scientific studies had confirm *Pterocarpus erinaceus* ability to exhibit several biological activities as well as identification of several components. For example, analysis of *Pterocarpus erinaceus* aqueous extract has revealed the presence of catechin and epicatechin compounds and had also reported the inhibitory ability of the extract against γ -secretase activity [23]. The bark extract of *Pterocarpus erinaceus* was found to contain friedelin, lupeol, and epicatechin compounds and was able to exert anti-inflammatory, analgesic, and antioxidant activities in a study conducted by Ouedraogo *et al* [24].

In traditional medicine practice, *Pterocarpus erinaceus* has been claimed to be used in managing disorders related to tissue-injury, however, this has not been verified scientifically hence the aim of the present study to authenticate this claim. The present study therefore attempted to assess tissue-protective efficiency of *Pterocarpus Erinaceus* leaves extract in carbon-tetrachloride (CCl₄)-induce hepatic and hematological toxicity in rats. This was conducted using an animal model by exposing them to carbon-tetrachloride (CCl₄) toxicity, a chemical known to induce both liver and hematologic injuries followed by the administration of *Pterocarpus erinaceus* extract at varied doses.

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents:

All chemicals and reagents used for this study were of analytical grade. Chemicals and solvent were purchased from Sigma Chemical Co. (USA) and Merck (Germany) respectively. Different parameters analyzed in the present study were estimated using commercial kits following manufacturer's instructions.

2.2 Experimental Animals:

Thirty male Wistar Strain Albino rats weighing between (100-120g) was used for this study. The rats were purchased from the Animal House of University of Jos. The rats were allowed to acclimatize to the environment and were maintained on standard laboratory diet (Vita feed, Jos) and tap water for a period of two weeks. Animals were housed in clean cages under normal prevailing environmental condition. The Principles of laboratory animal care (NIH publication No. 8523, revised 1985) [25] were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of the University of Jos, Nigeria.

2.3 Plant Collection, Identification and Processing:

The leaves of *Pterocarpus erinaceus* was collected from Tulu Village of Toro Local Government in Bauchi State, it was then taken to the Plant Science Department of the University of Jos for identification. The leaves of *Pterocarpus erinaceus* was washed and air dried at room temperature. The sample was pulverized using laboratory mortar and pestle. The powdered sample was then place in bag and store in desiccator until required.

2.4 Extraction:

The powder of leaves of *Pterocarpus erinaceus* (500g) was soaked in 2.5 liters of methanol for 24 hours, after which was filtered using a piece of clean, sterile, white Muslin cloth to remove debris and filter on a Whatman No.1 filter paper. The filtrate was concentrated using a rotatory

evaporator and then evaporate to dryness using drying cabinet at 40⁰C as done by Saidu *et al.*[26].The dry crude methanolic extract was stored in an air-tired plastic containers and store in a refrigerator at 4⁰C until required.

2.5 Acute Oral Toxicity Study

The acute oral toxicity studies were conducted according to the method of Organization for Economic and Co-operation and Development for testing of chemicals [31]. The LD₅₀ was determined from the result of the study.

2.6 Tissue-Protective Study of Plant Extract

Induction of hepatic damage was done according to Guntupalli *et al* [32] method. Experimental rats were divided in to 6 groups of five rats each as showed below. Group 1: Normal control (liquid paraffin, vehicle 1ml.kg), Group 2: Negative control (received 1ml/kg CCl₄), Group 3: Positive control (received 1ml/kg CCl₄ +100ml/kg Silymarin), Group 4-6: Extract treated rats (received 1ml/kg CCl₄ + varied doses of Extracts at 100, 200, and 400mg/kg body weight of rats). The treatment was done daily via oral mean for 14 days period.

2.6.1 Effect of plant extract against CCl₄-induced hepatotoxicity

The method of Reitman and Frankel (1957) modified by Schmidt and Schmidt (1963) was used for evaluation AST and ALT activities. ALP was determined by the method of Wright *et al.* (1972). Serum albumin was measured by the method of Corcoran and Durnan (1977) while the method of Malloy and Evelyn, (1937) was used to estimate total bilirubin.

2.6.2 Effect of plant extract against CCl₄-Induced hepatic architectural damage

Histopathological test was conducted on liver tissues. The collected livers were kept for at least 24 h in the buffered formalin, then each one was dehydrated with alcohol, then embedded in paraffin wax, and cut into 4-5 cm thick sections, and stained with Haematoxylin-Eosin dye for

photomicroscopic observations. The microscopic features of the organs from each rat were compared with that of the control group.

2.6.3 Effect of plant extract against CCl₄-disrupt lipid metabolism

Serum total cholesterol (TC) was estimated by enzymatic Cholesterol oxidase peroxidase (CHO-POD) end point method of Allain *et al* (1974). HDL-C was determined by enzymatic method of Burstein *et al* (1970) while LDL-C was calculated using Friedewald formula (Friedewald *et al.*,

$$1972). \text{LDL - C (mg/dl)} = \text{TC} - (\text{HDL - C}) - \left(\frac{\text{TG}}{5} \right)$$

2.6.3 Effect of plant extract against CCl₄-induced hematotoxicity

Hematological parameters viz. Packed Cell Volume (PCV), Hemoglobin concentration, Red Blood Cells count (RBC), White Blood Cells count (WBC), Hematocrit (Hct), Platelets, Mean Cell Hemoglobin Concentration (MCHC) and Mean Cell Hemoglobin (MCH) were analysed using an automated hematological analyzer Sysmex XS800i (Sysmex corporation, USA) (Theml *et al.*, 2004).

3 Statistical Analysis

All data were expressed as mean \pm SEM. Differences among groups at various times of the experiment were subjected to a one-way analysis of variance (ANOVA) followed by Benferonimultiple comparison. Graph pad Instat were used for data analysis and P value of < 0.05 was considered as significant.

4 RESULTS

4.1 Acute Toxicity Oral Studies of *Pterocarpus erinaceus* Leaves Extract

Oral administration of 100-5000mg/kg of Methanolic leaves extracts of *Pterocarpus erinaceus* in albino rats did not produce any visible sign or symptoms of toxicity or mortality in the treated animals. Behavioral changes such as grooming, loss of appetite, salivation, fatigue, diarrhea and

refusal to eat and drink were not observed over the test period. Therefore, the result indicated that the LD₅₀ of Methanol leaves extracts of *Pterocarpus erinaceus* is greater than 5000mg/Kg.

4.2 Tissue-Protective Efficiency of *Pterocarpus erinaceus* Leaves Extract

4.2.1 Effects of Plant Extract against CCl₄-Toxicity on Liver Function

The results of liver function markers for rats administered carbon tetrachloride and methanolic leaves extract of *Pterocarpus erinaceus* is presented in Table 1. The result shows a significant increase in ALT, AST and ALP in carbon tetrachloride treated group (negative control) as compared with the normal control group as well as the various treated rat groups. A decrease in ALT, AST and ALP was recorded in rats administered Silymarin (standard drug) when compared with the negative group. In a similar manner, rats groups that received varied doses of the methanolic leaves extracts of *Pterocarpus erinaceus* also showed a significant decreases in ALT, AST and ALP activities but in a dose dependent manner.

Serum bilirubin and albumin levels determined were also presented for the experimental animals. The negative control rats group (that is, rats administered carbon tetrachloride only) had their bilirubin and albumin levels increase when compared with normal control rats. Administration of silymarin (standard drug) causes a significant decrease in both the bilirubin and albumin levels. Also in the same vein, rats that were treated with methanolic leaves extracts of *Pterocarpus erinaceus* had their bilirubin and albumin levels decrease in a dose dependent manner.

4.2.2 Effects of Plant Extract against CCl₄-Toxicity on Hepatic Architecture

Photomicrograph of the liver sections of the experimental rats subjected to different treated is presented in Plate 1-VI. Photomicrograph of the liver sections of normal control (Group I), showing normal hepatocytes with no histopathological lesion (plate I) while that of CCl₄-

intoxicated rats (Group II) shows severe micro and macro vesicular steatosis (fatty change), (plate II). The histopathological architecture of liver sections of rats treated with the standard drug (Silymarin) in group III, shows very mild steatosis with normal hepatocytes at the background (plate III). In the rat's groups treated with varied doses of methanolic leaves extract, their liver photomicrograph showed a more or less normal lobular pattern with a mild degree of fatty changes in doses increase manner (plate IV-VI).

UNDER PEER REVIEW

Table 1: Assessment of Hepatoprotective Efficiency of *Pterocarpus erinaceus* Leaves Extract against Carbon-tetrachloride Induced Hepatic Injury

Groups/parameters	AST (U/l)	ALT (U/l)	ALP (U/l)	BLB (mg/dl)	ALB (mg/dl)
Group 1	66.6±1.29 ^a	28.8±1.71 ^a	167.0±1.41 ^a	0.25±0.01 ^a	4.5±0.15 ^a
Group 2	134.2±1.46 ^b	116.0±1.82 ^b	253.6±2.02 ^b	0.74±0.01 ^b	3.7±0.07 ^b
Group 3	74.4±1.66 ^{ab}	42.4±1.50 ^c	195.2±2.84 ^c	0.41±0.02 ^{ab}	4.3±0.09 ^a
Group 4	119.6±1.66 ^c	107.2±1.43 ^{ab}	225.4±2.94 ^{ab}	0.51±0.01 ^c	3.9±0.09 ^b
Group 5	97.6±1.54 ^d	84.8±1.07 ^d	203.4±1.44 ^d	0.58±0.01 ^c	4.8±0.14 ^{ab}
Group 6	83.0±1.14 ^e	82.0±2.55 ^d	183.6±2.14 ^c	0.69±0.01 ^b	5.0±0.17 ^{ab}

Values are expressed as mean ± SEM of five replicates. Mean values with different superscript letters(s) in a column are significantly different at P< 0.05.

Group 1: Normal control (liquid paraffin, vehicle 1ml/kg), Group 2: Negative control (received 1ml/kg CCl₄), Group 3: Positive control (received 1ml/kg CCl₄ +100ml/kg Silymarin), Group 4-6: Extract treated rats (received 1ml/kg CCl₄ + varied doses of Extracts at 100, 200, and 400mg/kg body weight of rats). The treatment was done daily via oral means for 14 days period. AST: Aspartate amino transferase, ALT: Alanine amino transferase, ALP: Alkaline phosphatase, ALB: Albumin, BLB: Bilirubin

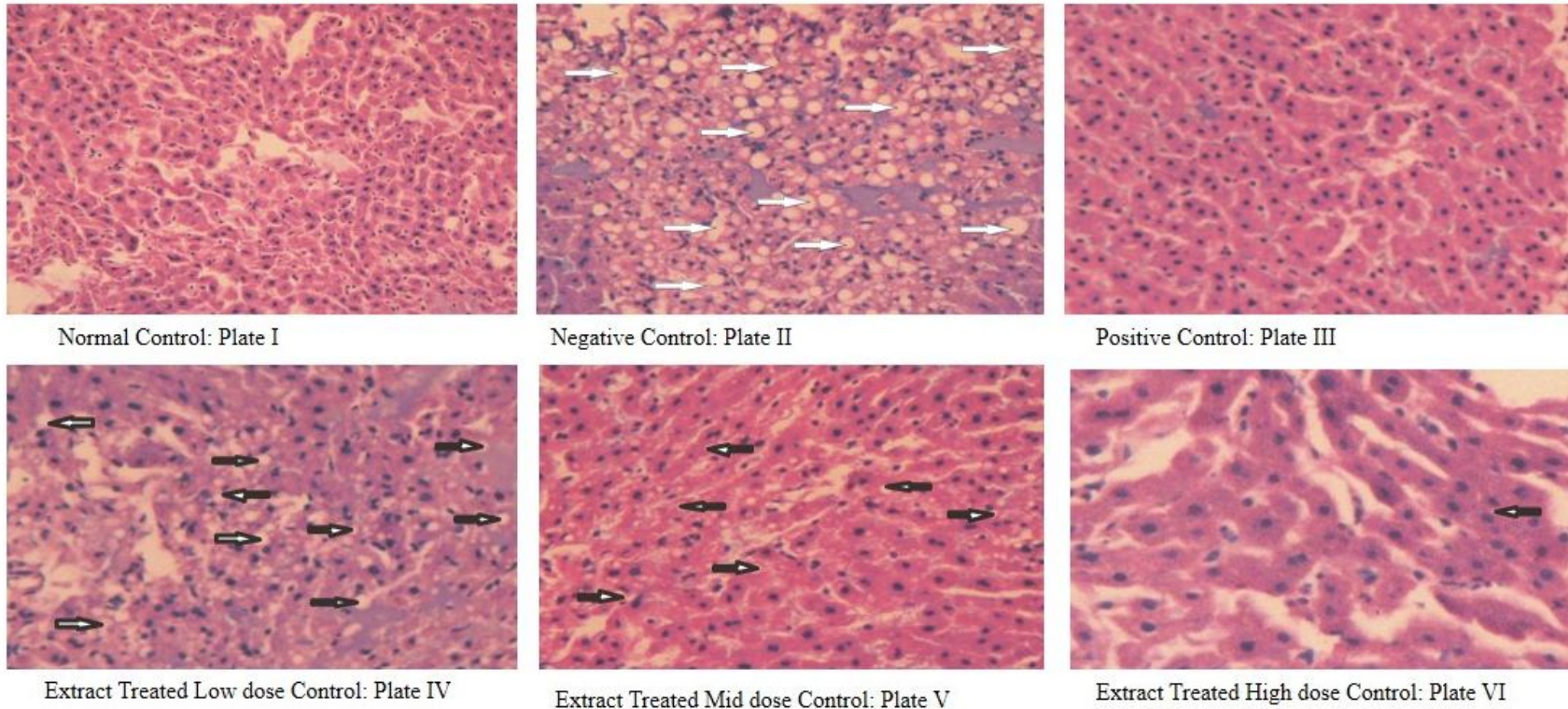


Plate I-VI. Photomicrograph of the Liver Sections of Experimental Rats Subjected to Different Treated. Hematoxylin and Eosin (H&E) x 400.

Plate I: normal hepatocytes with no histopathological lesion; plate II: severe micro and macro vesicular steatosis (fatty liver); plate III: mild fatty liver with normal hepatocytes at the background; Plate IV: macro vesicular steatosis (fatty liver) and peripheral hepatocellular necrosis. ; plate V: moderate steatosis (fatty liver), and plate VI: showing mild steatosis (fatty liver) at portal areas but no lesion.

Group 1: Normal control (liquid paraffin, vehicle 1ml.kg), Group 2: Negative control (received 1ml/kg CCl₄), Group 3: Positive control (received 1ml/kg CCl₄ +100ml/kg Silymarin), Group 4-6: Extract treated rats (received 1ml/kg CCl₄ + varied doses of Extracts at 100, 200, and 400mg/kg body weight of rats). The treatment was done daily via oral means for 14 days period

4.2.3 Effects of Plant Extract against CCl₄ on Lipid profile

The serum lipid levels of rats administered carbon tetrachloride and methanolic leaves extract of *Pterocarpus erinaceu* is presented in Table 2. The result show a significant increase in TG, T.CHO and LDL-C levels of rats treated with carbon tetrachloride (negative control). Increase in the TG, T.CHO and LDL-C were minimized in rat's group received Silymarin (standard drug) in a significant manner when compared with the values from the negative control rats. In rat's groups treated with the methanolic leaves extracts of *Pterocarpus erinaceus*, a decrease in TG, T.CHO and LDL-C was recorded also. Assessment of HDL-C level in the negative control rats showed a decrease while in the treated rats groups with either the standard drug or the methanolic leaves extracts of *Pterocarpus erinaceus* there was a significant increase.

4.2.3 Effects of Plant Extract against CCl₄ on Hematological Components

The result of hematological parameters of rats administered carbon tetrachloride and methanolic leaves extract of *Pterocarpus erinaceus* is presented in Table 3. The results showed a significant changes in Hb, WBC, RBC, PLT and Hematocrit in rats received carbon tetrachloride only as compared with those rat's groups that received silymarin or methanolic leaves extracts of *Pterocarpus erinaceus*. In the treated rats groups, a reverse in the alteration of the parameters: Hb, WBC, RBC, PLT and Hemotocrit were recorded in a manner close to the normal control rats. The study also observed a significant changes in MCHC and MCH in the negative control rats compared the silymarin and plant extract treated rats.

Table 2: Effects of *Pterocarpus erinaceus* Leaves Extract against Carbon tetrachloride Toxicity on Lipid Profile of Rats

Groups/parameters	TG (mg/dl)	T.CHO (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Group 1	71.2±0.86 ^a	79.0±1.41 ^a	46.2±1.16 ^{ab}	44.6±0.93 ^a
Group 2	133.8±1.07 ^b	123.8±1.36 ^b	31.6±1.21 ^b	140.8±0.58 ^b
Group 3	73.4±1.81 ^a	84.4±1.89 ^c	41.8±0.58 ^{ab}	48.8±1.39 ^a
Group 4	104.0±1.70 ^{ab}	97.0±1.30 ^{ab}	43.4±0.93 ^{ab}	116.0±2.07 ^{ab}
Group 5	80.0±1.58 ^c	87.4±1.72 ^c	44.6±1.69 ^{ab}	92.8±1.66 ^c
Group 6	69.0±1.23 ^a	74.8±2.63 ^a	48.0±1.82 ^{ab}	104.2±1.69 ^d

Values are expressed as mean ± SEM of five replicates. Mean values with different superscript letters(s) in a column are significantly different at P< 0.05.

Group 1: Normal control (liquid paraffin, vehicle 1ml/kg), Group 2: Negative control (received 1ml/kg CCl₄), Group 3: Positive control (received 1ml/kg CCl₄ +100ml/kg Silymarin), Group 4-6: Extract treated rats (received 1ml/kg CCl₄ + varied doses of Extracts at 100, 200, and 400mg/kg body weight of rats). The treatment was done daily via oral means for 14 days period. TG: Triglycerides, T.CHO: Total cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol.

Table 3: Effects of *Pterocarpus erinaceus* Leaves Extract against Carbon tetrachloride-induced Hematotoxicity in Rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
PCV (%)	54.2±0.73 ^a	45.2±1.50 ^b	55.2±1.66 ^b	47.8±1.56 ^b	54.2±1.39 ^a	56.4±2.16 ^a
HB (g/dl)	14.2±0.86 ^a	7.1±0.25 ^b	14.8±0.58 ^a	10.0±0.70 ^{ab}	12.4±0.51 ^a	14.4±0.50 ^a
MCHC(g/dl)	44.2±1.43 ^a	56.6±1.36 ^b	47.4±2.02 ^a	41.6±1.63 ^a	35.8±1.42 ^{ab}	42.8±2.49 ^a
MCH (pg)	19.6±0.93 ^a	25.8±1.16 ^b	18.6±0.93 ^a	14.8±0.66 ^{ab}	18.8±1.16 ^a	17.0±0.84 ^a
WBC(×10 ³) cells/μL	7216±4.52 ^a	2503.6±1.75 ^b	5530.0±8.52 ^c	5113.4±3.95 ^{ab}	6510.4±3.2 ^d	7080.0±21.2 ^e
RBC(×10 ⁶) cells/μL	6.9±0.17 ^a	3.7±0.18 ^b	6.7±0.16 ^c	5.6±0.12 ^{ab}	6.3±0.10 ^c	7.1±0.16 ^a
PLT (×10 ³) cells/μL	425.2±1.77 ^a	80.2±1.56 ^b	380.0±1.70 ^{ab}	145.0±3.23 ^d	240.2±1.77 ^c	311.8±3.23 ^e
Hematocrit (%)	35.4±1.21 ^b	16.8±1.28 ^a	37.6±1.03 ^b	25.8±0.86 ^{ab}	36.4±0.87 ^b	37.6±1.08 ^b

Values are expressed as mean ± SEM of five replicates. Mean values with different superscript letters(s) in a column are significantly different at P< 0.05.

Group 1: Normal control (liquid paraffin, vehicle 1ml/kg), Group 2: Negative control (received 1ml/kg CCl₄), Group 3: Positive control (received 1ml/kg CCl₄ +100ml/kg Silymarin), Group 4-6: Extract treated rats (received 1ml/kg CCl₄ + varied doses of Extracts at 100, 200, and 400mg/kg body weight of rats). The treatment was done daily via oral means for 14 days period. PCV: Packed cell volume, RBC: Red blood cell count, WBC: White blood cells count, PLT: Platelet, Hb: Haemoglobin.

5 DISCUSSION

Medicinal plants are known to possess components with curative potentials of certain biological activity [37]. These component are referred to as active principles or phytochemical substances [38]. In an attempt to assess tissue-protective effect of the leaves of *Pterocarpus erinaceus*, acute toxicity study was conducted where the study found the leaves extract to be safe with LD₅₀ greater than 5000mg/kg body weight. This was followed by an in vivo study where rats were administered carbon tetrachloride alongside plant extract and various parameters in relation to hepatic and hematological toxicity were assayed.

Acute Toxicity can be described as the adverse effects following oral administration of a substance that results either from a single or multiple exposures in a short space of time usually within 24 hours [39]. Determination of LD₅₀ (Lethal dose that would kill 50 % of the tested population) is usually the first step in the evaluation of toxic characteristic of a substance [40]. According to Ukwuani *et al.* [41], acute toxicity study is an initial appraisal of toxic manifestations and is one of the initial screening experiments performed with all compounds. The result of the acute oral toxicity of methanol leaves extract of *Pterocarpus erinaceus* suggested to be greater than 5000 mg/kg body weight is an indication of its safety.

The participation of liver in a variety of metabolic activities including biotransformation and excretion of chemical agents makes it more vulnerable and susceptible to toxicity from those agents. To assay in vivo tissue-protective potential of *Pterocarpus erinaceus* leaves extract, rats were exposed to carbon-tetrachloride (CCl₄) toxicity. The toxicity of CCl₄ on the tissue arose when it undergoes biotransformation by cytochrome P450 in the hepatic endoplasmic reticulum to form a highly reactive and unstable trichloromethyl radical [42]. The latter in the presence of

oxygen is metabolized to peroxides and chloroform, which overwhelm the antioxidant capacity of the liver, leading to oxidative denaturation of unsaturated fatty acids of lipid membranes and thereby causing severe tissue damage and membrane leakage [43, 44].

Carbon tetrachloride is a well-established hepatotoxin and it is the best-defined animal model of chemical induced hepatotoxicity [45]. Alteration in the activities of aspartate and alanine transaminases, alkaline phosphatase, albumin and bilirubin estimated in serum samples as biomarkers of liver function in this study is evidenced that CCl₄ induces liver injury. Increase alterations in the serum liver function markers in the negative control rats suggest uninterrupted damage pose by CCl₄. Treating rats with *Pterocarpus erinaceus* leaves extract seem to ameliorate CCl₄-induced hepatic injury by reversing the changes in liver function markers toward normalcy.

Carbon tetrachloride is reported to induce steatosis (fatty liver), this is said to be done via multiple events. One major role involves the impairment in the transfer of triacylglycerols as very low density lipoproteins from the liver to the circulation [46], the second part has to do with disruption in the balance between lipid biosynthesis and catabolism [47]. CCl₄ effect on the lipid portions of the erythrocytes may attribute to the changes in the serum lipid profile of the experimental rats recorded in the present study. [48] in their work has observed dyslipidemia due to the abnormal levels and proportions of lipids in the blood as well as disturbed lipoprotein metabolism. Improvement in the changes of lipid proportion in rats received extract and standard drug toward normalcy suggests possible effect of the *Pterocarpus erinaceus* leaves extract against CCl₄ causing imbalance between lipid synthesis and catabolism.

Alteration in hematological indices of rats following CCl₄-induced tissue damage recorded in this study is an indication of pancytopenia. This is evidenced by a great decrease in red blood

cell count, hematocrit ratio, packed cell volume, HB concentration values and platelets count in rats received CCl₄ only. Alterations in hematological parameters of rats have been reported earlier with CCl₄ [49]. Exposure of erythrocyte to chemicals and some drugs has been reported to be associated with erythrocyte depletion and hemolytic anemia [50]. The reduction in erythrocytes count, haematocrit ratio, HB level and microcytic-hypochromic recorded in the present study could be attributed to altered hematopoiesis, depletion of erythrocytes, and distraction in the erythropoiesis rate and their facilitated removal from circulation as a result of the toxicity of CCl₄. Reduced rate of pancytopenia or erythrocyte depletions in rats received plant extract or standard drug alongside CCl₄ suggests possible protective effect of the *Pterocarpus erinaceus* leaves extract against CCl₄-toxicity. This results is in line with the findings reported by Madthi *et al* [49], that plants components has ability to counteract toxic effects of Carbon tetrachloride on the hemopoietic tissues.

High significant decrease of WBCs count and increases in MCHC and MCH in rats intoxicated with CCl₄ may indicate lymphopenia. The abnormal hematologic parameter changes in total and differential leukocytes count caused by CCl₄ may be attributed to the inflammatory response by the tissues. As a defensive mechanism of immune system, studies has found that, treatment of rats with CCl₄ cause a release of neutrophil pool into circulation [51, 52]. Reversal of these changes by concomitant administration of *Pterocarpus erinaceus* leaves extract may be an indication that the leaves possess component with anti-inflammatory properties.

In vivo studies of CCl₄ have shown that it causes steatosis (fatty liver) [46]. Fatty liver was observed in this study from the liver tissues of rats in the CCl₄ control group and extract treated rats in different degrees. Changes in degree of fatty liver formation in the extract-treated rats suggest the ability of *Pterocarpus erinaceus* leaves extract to protect the liver against the toxicity

of CCl₄ in a dose increasing manner. It is interesting to note that, *Fabaceae*, the plant family which *Pterocarpus erinaceus* belongs has been reported among the traditional and pharmacological uses plants for hepato-protection against CCl₄ [53].

CONCLUSION

The study have affirmed that CCl₄ induced tissues damage in albino rats, and that, methanol leaves extract of *Pterocarpus erinaceus* able to minimize the severity of CCl₄ toxicity. Findings from this study have aid in validating the traditional use of *Pterocarpus erinaceus* leaves in the treatment of cellular damage due to chemical toxicants. The plant's leaves may possess components with tissue-protective properties hence its recommendation for further study to identify the active biomolecules that could be develop as agent to improve cytoprotection.

References:

- [1].Jorum O H, and Piero N M. Haematological effects of dichloromethane-methanolic leaf extracts of *Carissa edulis* (Forssk.) Vahl in normal rat models. *J Hematol Thromboembolic Dis* 2016;04. doi: 10.4172/2329-8790.1000232.
- [2].Owoade AO, Adetutu A, Olorunnisola OS. Hematological and biochemical changes in blood, liver and kidney tissues under the effect of tramadol treatment. *J Alcohol Drug Depend* 2019;07:1–7. doi: 10.35248/2329-6488.19.7.326.
- [3].Mugoni, V., Postel, R., Catanzaro, V., De Luca, E., Turco, E., Digilio, G., et al. Ubiad1 is an antioxidant enzyme that regulates eNOS activity by COQ10 synthesis. *Cell*, 2013,152:504–518.
- [4].Ilyas, U., Katare, D.P., Aeri, V., Naseef, P.P. A review on hepatoprotective andimmunomodulatory herbal plants. *Pharmacognosy Reviews*, 2016, 10: 66–70
- [5].Beretta, G and Facino, R.M. Recent advances in the assessment of the antioxidant capacity of pharmaceutical drugs: from in vitro to in vivo evidence. *Analytical and Bioanalytical Chemistry*, 2010,398: 67–75.
- [6].Niki E. Assessment of antioxidant capacity in vitro and in vivo. *Free Radic Biol Med*. 2010, 49(4):503-15. doi: 10.1016/j.freeradbiomed.2010,04.016.
- [7].Ostapowicz G, Fontana RJ, Schiødt FV, Larson A, Davern TJ, Han SH, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States". *Ann Intern Med*. Dec. 2002,137 (12): 947–54. doi:10.7326/0003-4819-137-12-200212170-00007.

- [8]. Tong, J., Yao, X., Zeng, H., Zhou, G., Chen, Y., Ma, B., et al. Hepatoprotective activity of flavonoids from *Cichorium glandulosum* seeds in vitro and in vivo carbon tetrachloride-induced hepatotoxicity. *Journal of Ethnopharmacology*, 2015, 174, 355–363.
- [9]. Jamshidi-Kia, F., Z. Lorigooini, et al. Medicinal plants: Past history and future perspective." *Journal of herbmed pharmacology*, 2018, 7(1).
- [10]. Li, G. and H. X. Lou. Strategies to diversify natural products for drug discovery." *Medicinal research review*, 2018, 38(4): 1255-1294.
- [11]. Dereli, F. T. G., M. Ilhan, et al. New Drug Discovery from Medicinal Plants and Phytoconstituents for Depressive Disorders." *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*, 2019, 18(2): 92-102.
- [12]. Santos, P.R.V., Oliviera, A.C.X., Tomassini, T.C.B. Control microbiogicode products fitorapicos. *Rev. fam. Bioquim*, 1995, 31: 35-38
- [13]. Oteng Mintah, S., Asafo-Agyei, T., Archer, M.-A., Atta-Adjei Junior, P., Boamah, D., Kumadoh, D., ... Agyare, C. Medicinal Plants for Treatment of Prevalent Diseases. *IntechOpen*. 2019, doi: 10.5772/intechopen.82049
- [14]. Wallace, K., Burt, A.D. and Wright, M.C. Liver fibrosis. *Biochemical Journal*, 2008, 411: 1-18.
- [15]. Liu, C.T., Chuang, P.T., Wu, C.Y., Weng, Y.M., Wenlung, C. and Tseng, C.Y. Antioxidative and in vitro hepatoprotective activity of *Bupleurum kaoi* leaf infusion. *Phyther. Res.*, 2006, 20(11): 1003-1008.
- [16]. Opoku, A.R., Ndlovu, I.M., Terblanche, S.E., and Hutchings, A.H. In vivo hepatoprotective effects of *Rhoicissustridentata* subsp. *cuneifolia*, a traditional Zulu medicinal plant against carbon tetrachloride-induced acute liver injury in rats. *South Africa Journal of Botany*, 2007, 73(3): 372-377.
- [17]. Baytop, T. Therapy with medicinal plants in turkey, nobel tip Basimevi, Istanbul, 1999, Turkey.
- [18]. Mhya DH, Umar, IA., and Onyike E. Anti-peroxidative and Biochemical Protective Activity of *Khaya senegalensis* Stem Bark Extract on Rats Fed Pesticide-infused Feed. *International Journal of Pharma Sciences and Research*, 2014a, 5(07): 385-390. <https://www.ijpsr.info/docs/IJPSR14-05-07-024.pdf>
- [19]. Mhya DH, Umar, I.A., and Onyike, E. Cytoprotection by *Khaya senegalensis* Extract on Rats Fed Pesticide-Infused Feed. *International Journal of Toxicological and Pharmacological Research*, 2014b, 6(4): 6(4): 75-79 <https://ijtp.com/volume6issue4/>
- [20]. Tittikpina, N.K., Nana, F., Fontanay, S., Philippot, S., Batawila, K., Akpagana, K., et al.. Antibacterial activity and cytotoxicity of *Pterocarpus erinaceus* extracts, fractions and isolated compounds. *J. Ethnopharmacol.* 2018, 212, 200–207. <https://doi.org/10.1016/j.jep.2017.10.020>.
- [21]. Tittikpina, N.K., Atakpama, W., Pereki, H., Nasim, M., Ali, W., Fontanay, S., et al. Capture of plants with interesting biological activities: a case study. *OpenChem*. 2017, 15, 208–218. <https://doi.org/10.1515/chem-2017-0024>.
- [22]. Noufou, O., Anne-E, H., Claude, W.O.J., Richard, S.W., André, T., Marius, L., J. Biological and phytochemical investigations of extracts from *Pterocarpus erinaceus* (Fabaceae) root barks. *Afr. J. Tradit. Complement. Altern. Med.* 2017, 14, 187–195. <https://doi.org/10.21010/ajtcam.v14i1.21>.

- [23]. Hage, S., Stanga, S., Marinangeli, C., Octave, J.N., Dewachter, I., Quetin-Leclercq, J., et al. Characterization of *Pterocarpus erinaceus* extract and its gamma-secretase inhibitory properties. *J. Ethnopharmacol.* 2015, 163, 192–202. <https://doi.org/10.1016/j.jep.2015.01.028>.
- [24]. Ouédraogo, N., Sawadogo, R.W., Tibiri, A., Bayet, C., Lompo, M., Hay, A.E., et al. Pharmacological properties and related constituents of stem bark of *Pterocarpus erinaceus* (Fabaceae). *Asian Pac. J. Trop. Med.* 2012, 5, 46–51. [https://doi.org/10.1016/S1995-7645\(11\)60244-7](https://doi.org/10.1016/S1995-7645(11)60244-7).
- [25]. National Institute of Health (NIH). Principles of Laboratory Animal Care. NIH Publication. 1985; No. 85-23 Revised.
- [26]. Saidu, Y., Bilbis, L.S., Lawal, M., Isezuo, S.A., Hassan, S.W. and Abbas A.Y. Acute and sub-chronic toxicity studies of crude aqueous extract of *Albizia chevalieri* harms. *Asian Journal of Biochemistry*, 2007, 2(4): 224-236.
- [27]. Harborne, J.B. phytochemical methods, London. Chapman and Hall, Ltd., 1073, 49-80.
- [28]. Trease, G.E. and Evans, W.C. Pharmacognosy. 11th ed. 1989. Brailliar. Tiridel Can. Macmillian.
- [29]. Sofowora, A. Medicinal Plants and Traditional Medicines in Africa. New York: Chichester John, Wiley & Sons. 1993, pp. 97–145.
- [30]. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol.* 2011, 48(4):412-22. doi: 10.1007/s13197-011-0251-1.
- [31]. OECD. Organization for economic co-operation and development. OECD guidelines for testing chemicals, acute up and down procedure no. 2001, 425: 1-2
- [32]. Guntupalli, M.M.R., Chandana, V.R., Palpu, P., and Anine, S. Hepatoprotective effects of *rubiadin*, a major constituent of *rubiacordifolia* Linn. *Ethnopharmacology*, 2006, 103: 484-490.
- [33]. Sinha, K.A. Colorimetric Assay of Catalase. *Analytical Biochemistry*, 1972, 47, 389-394. [http://dx.doi.org/10.1016/0003-2697\(72\)90132-7](http://dx.doi.org/10.1016/0003-2697(72)90132-7).
- [34]. Ellman GL. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, 1959, 82: 70-77
- [35]. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys.* 1984, 21(2):130-2. PMID: 6490072.
- [36]. Okhawa H, Ohishi, N and Yagi K. Assay for lipid peroxidation in animals by thiobarbituric acid reaction. *Analytical Biochemistry*, 1999, 95:351-358.
- [37]. Oladeji O. The Characteristics and Roles of Medicinal Plants: Some Important Medicinal Plants in Nigeria. *Nat Prod Ind J.* 2016, 12(3):102
- [38]. Mhya, DH and Mankilik M. Phytochemical Screening of Aqueous Extract of *Luffa aegyptiaca* (Sponge gourd) Leave Sample from Northern Nigeria: A Short Communication. *International Journal of Pharma Sciences and Research*, 2014, 5(07): 344-345. <http://www.ijpsr.info/abstract.php?file=14-05-07-018>
- [39]. IUPAC. Compendium of chemical terminology, 1997, 2nd edition (the golden book)
- [40]. Ogbuehi, I.H., Ebong, O.O. and Obianime, A.W. Oral acute toxicity (LD₅₀) study of different solvent extracts of *Abrus precatorius* Linn leaves in Wistar rats. *European Journal of Experimental Biology*, 2015, 5(1):18-25.

- [41]. Ukwuani, A.N., Abubakar, M.G., Hassan, S.W. and Agaie, B.M. Toxicological studies of hydromethanolic leaves extract of *Grewiacrenata*. *International Journal of Pharmaceutical Sciences and Drug Research*, 2012, 4(4): 245-249.
- [42]. Li, Xiao-Xi & Zheng, Qing-Chuan & Wang, Yong & Song, Ming-Xing. (2014). Theoretical insights into the reductive metabolism of CCl₄ by cytochrome P450 enzymes and the CCl₄-dependent suicidal inactivation of P450. *Dalton transactions* (Cambridge, England 2014, 43. 10.1039/c4dt02065k.
- [43]. Weber, J.S., Francis, M.S., John, S., Michael, L.Z. Cell membrane disruption as a result of hepatic toxicity, *International Journal of Pharmaceutical Sciences*, 2003, 36: 21-30
- [44]. Tamara R. Knight, Marc W. Fariss, Anwar Farhood, Hartmut Jaeschke, Role of Lipid Peroxidation as a Mechanism of Liver Injury after Acetaminophen Overdose in Mice, *Toxicological Sciences*, Volume 76, Issue 1, November 2003:229–236, <https://doi.org/10.1093/toxsci/kfg220>
- [45]. Powers HR. Riboflavin (vitamin B-2) and health. *Am J Clin Nutr*, 2003, 77, (6):1352-1360. <https://doi.org/10.1093/ajcn/77.6.1352>
- [46]. Poli G., Gravela E., Albano E. and Dianzani M. U. Studies on fatty liver with isolated hepatocytes II. The action of carbon tetrachloride on lipid peroxidation, protein and triglyceride synthesis and secretion. *Exp. Molec. Path.* 1979,30, 116-127.
- [47]. Boll M., Weber L. W. D., Becker E. and Stampfl A. Pathogenesis of carbon tetrachloride-induced hepatocyte injury. Bioactivation of CCl₄ by cytochrome P450 and effects on lipid homeostasis. *Z. Naturforsch.* 2001, 56c, 111-121.
- [48]. Aderibigbe MA, Obafemi TO, Olaleye MT *et al.* Effects of gender, age and treatment duration on lipid profile and renal function indices in diabetic patients attending a teaching hospital in South-Western Nigeria. *Afr Health Sci.*, 2018, 18:900. doi: [10.4314/ahs.v18i4.8](https://doi.org/10.4314/ahs.v18i4.8)
- [49]. Madthi AS. Al-Diwan MA. AL-Jadaan SAN. Hematological Profile Of Rats Treated With Quercetin Derivative Against Carbon Tetrachloride (CCl₄) Toxicity. *Bas.J.Vet.Res.*2018,17(2):70-84
- [50]. Beutler E Hemolytic anemia due to chemical and physical agents. In Beutler E, Coller BS, Lichtman MA, Kipps TJ, Seligsohn U (eds): *Williams Hematology*, 6th edition, New York , 2001, pp. 629–632.
- [51]. Patrick-Iwuanyanwu KC, Wegwu MO, Ayalogu EO. Prevention of CCl₄-Induced Liver Damage by Ginger, Garlic and Vitamin E. *Pak J Biol Sci.*; 2007,10: 617-621
- [52]. Saba AB, Oyagbemi AA and Azeez OI. Amelioration of carbon tetrachloride-induced hepatotoxicity and haemotoxicity by aqueous leaf extract of *Cnidioscolus aconitifolius* in rats. *Nig. J. Physiol. Sci.* 2010, 25: 139 – 147.
- [53]. Ugwu, C.E., Suru, S.M. Medicinal plants with hepatoprotective potentials against carbon tetrachloride-induced toxicity: a review. *Egypt Liver Journal*, 2021,11:88 <https://doi.org/10.1186/s43066-021-00161-0>