

# Hepatoprotective and Nephroprotective Effects of Methanolic Extract of Different Parts of *Tamarindus Indica* Linn in Rats Following Acute and Chronic Carbon Tetrachloride Intoxication

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## Authors' contributions

This work was carried out in collaboration between both authors. Author SEA designed the study, wrote the protocol and interpreted the data while. Author MLL managed the literature searches, managed the laboratory analyses of the study and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** To investigate the hepatoprotective and nephroprotective potential of the methanolic extracts of the leaves, stem bark, seeds, fruit pulp, fruit bark and roots of *Tamarindus indica* Linn in acute and chronic rat model of organ injuries.

**Study Design:** The acute-injury model involved intraperitoneal pre-treatment with 10 mg/kg body weight of the extract for two days followed by intoxication with carbon tetrachloride at 0.6ml/kg, while the chronic injury model involved repeated intoxication with carbon tetrachloride (0.3ml/kg) at every 72 hourly intervals together with a concomitant 24 hourly administration of the extracts (5mg/kg) for twelve days, following initial CCl<sub>4</sub> intoxication at 0.6ml/kg.

**Place and Duration of Study:** Department of Biochemistry, Faculty of Science, Ahmadu Bello University Zaria, Kaduna State, Nigeria. January 2011-June 2011.

**Methodology:** In both acute and chronic experimental model, the rats were sacrificed at the end of

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the each experiment. Bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined from serum as indices of hepatic injuries while urea and creatinine were determined as markers of kidney damage.

**Results:** Extract treatment caused a significant ( $p < 0.05$ ) decrease in the activities of ALT from  $61.04 \pm 2.77$  U/l in  $\text{CCl}_4$  control group to between  $15.82 \pm 2.63$  and  $50.67 \pm 3.44$  U/l while AST activities were similarly lowered from  $100.57 \pm 3.27$  U/l to between  $25.10 \pm 1.48$  U/l and  $53.45 \pm 3.19$  U/l. There was also significant ( $p < 0.05$ ) decrease in the levels of bilirubin, urea and creatinine when compared to the  $\text{CCl}_4$  control. In general, extracts from the fruit pulp, the stem bark and fruit bark demonstrated better hepatoprotective and nephroprotective potential than those of the seed, root and leaves.

**Conclusions:** Various parts of *Tamarindus indica* possess hepatoprotective and nephroprotective properties to justify their usage in traditional medicine in Nigeria and some other developing countries.

**Keywords:** *Tamarindus indica*; hepatoprotective effect; nephroprotective effect; kidney damage; liver damage.

## 1. INTRODUCTION

Since pre-historical times, plant materials have widely been used for treatment of illness and diseases [1] in traditional societies of Africa and elsewhere [2]. Traditional medical practices on the African continent date as far back as 4000 years and were the sole medical system for healthcare delivery before the advent of orthodox or modern medicine. Even today, traditional medicine is still the predominant means of health care in developing countries where larger percentage of the total population is poor. Even in modern medicine, plants are the basis for the development of drugs because of their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds that produce definite physiological and pharmacological action in the body of living organisms [3]. Thus, a systematic search for useful bioactivities from medicinal plants such as *Tamarindus indica* Linn is now considered to be a rational approach in nutraceutical and drug development [4].

Tamarind (*Tamarindus indica* Linn) is a perennial commercial and ornamental herb belonging to the dicotyledonous family of Leguminosae which is known by several names such as Indian date (English), *tsamiya* (Hausa) and *tamrhind* (Arabic). It is a slow growing but long living plant (80-200 years) that averages 20-25m in height, 1m in diameter, and has a wide spreading crown with a short, stout trunk. It grows wild in many tropical and sub-tropical regions of the world, as it is well adapted to semi-arid tropical as well as humid tropical areas with seasonally high rainfall. *Tamarindus indica* is widely used in traditional medicine in Africa for the treatment of many diseases such as fever, dysentery, jaundice,

gonorrhoeal and gastrointestinal disorders [3]. For example, the pulp has been reported to be useful in the management of a number of ailments including the alleviation of sunstroke, *Datura* poisoning and the intoxicating effects of alcohol and cannabis [5]. It can be gargled for sore throats, dressing of wounds, is said to aid in the cure of malarial fever and in the restoration of sensation in cases of paralysis. Also, the fruits are reported to have anti-fungal and anti-bacterial properties [6] in many countries, while powdered seed husks or seed extracts is used in the treatment of boils, ulcer, diabetes and dysentery in Cambodia and India.

Scientific investigations have indeed confirmed the antibacterial, antifungal, hypoglycemic, anti-hypercholesterolemic and cytotoxic effects [3,1,7] of the *T. Indica* seeds and fruit pulp, which have also been demonstrated to enhance the bioavailability of drugs like ibuprofen in humans [4]. Furthermore, it has been found that ethanol and ethyl acetate extracts prepared from the seed coat exhibited anti-oxidative activity as measured by the thiocyanate and thiobarbituric acid (TBA) methods [8]. Similarly, Komutarin et al. [1] reported the *in vitro* and *in vivo* anti-inflammatory capacities of the pulp and seed extracts through modulation of nitric oxide production. However, there appears no systematic and comprehensive study evaluating the different parts of the plant for *in vivo* hepatoprotective and nephroprotective effects. Therefore this work seeks to establish the *in vivo* hepatoprotective and nephroprotective effects of different parts of *Tamarindus indica* in acute and chronic animal models of organ toxicity.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material Collection and Extraction

The various parts of *Tamarindus indica* (leaves, stem bark, root bark, and whole fruits) were carefully collected from a tree in Zaria Local Government area of Kaduna State, Nigeria. The plant was authenticated at the Herbarium Section of the Biological Science Department, Ahmadu Bello University, Zaria, Nigeria where a voucher number 900265 was assigned. The whole fruit was then carefully peeled off to obtain the fruit bark and subsequently the seeds separated from the pulp. Samples of leaves, stem bark, root bark, fruit bark and seeds were separately dried at room temperature and pulverized using mortar and pestle. They were then defatted with petroleum ether for 6 hrs and then extracted with methanol (4hrs x 2 times) using Soxhlex extractor. The combined methanol portions were taken to dryness *in vacuo* in desiccators and kept at -4°C until required.

The fruit pulp extracts were obtained by gentle warm maceration over a bath at 50°C. A weighed amount of the fruits were put into a beaker with 300ml methanol and allowed to stand for 2hrs. The mixture was then thoroughly macerated, and the seeds and debris picked out with the aid of laboratory tongs. Petroleum ether (300ml) was then added and the entire mixture was shaken intermittently for 2hours. The liquid suspension was then carefully decanted into a separating funnel while the remaining debris was discarded. The mixture in the separating funnel was then allowed to stand for clear separation of the two immiscible layers. The individual layers were then carefully run out and collected in a pre-weighed bottle. The process was repeated and the methanolic layer was combined and then dried *in vacuo* to obtain the dried extracts which were then stored in air tight dark glass bottles in a refrigerator at -4°C until required [4]. This elaborate procedure was necessitated by the fact that fruit pulp and the seed are so waxed together in a sticky manner that physical separation was otherwise, practically impossible.

### 2.2 Chemicals and Reagents

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, urea and creatinine assay kits were obtained from Randox Laboratories Ltd., Ardmore, Antrim, United

Kingdom. Other reagents and chemicals were of analytical grade obtained from Sigma-Aldrich Company Ltd (USA).

### 2.3 Experimental Animals

Male albino rats weighing 150-200g (7-8weeks old) were obtained from the animal house of the National Research Institute for Chemical Technology (NARICT), Basawa, Zaria, Nigeria. They were acclimatized in a well ventilated room within the animal facility of the Department of Biochemistry, Ahmadu Bello University Zaria for two weeks before the commencement of the study. They were allowed free access to rat feeds (obtained from ECWA feed Ltd, Bukuru, Jos, Nigeria) and tap water *ad libitum* through the course of experiment. Animals were weighed and randomly assigned to each of 17 treatment groups (n=5). Permission was obtained from the University's Ethical Committee for laboratory use of the animals.

### 2.4 Animal Treatments

The study was carried out in two phases. The first phase involved the assessment of preventive potential of the extracts of different parts of *Tamarindus indica* in acute liver injury model. In this acute experimental model, the effects of the extracts were investigated in rats first by pre-treatment intraperitoneally with 10mg/kg body weight of the extract for two days followed by intoxication with carbon tetrachloride (CCl<sub>4</sub>) at 0.6ml/kg on the third day. This dose was selected because the study considered that to recommend any new compound as commercially and clinically useful as hepatoprotective agent, it must have be effective at lower or comparable doses to the antioxidant supplements that are currently available. Besides, previous sub-acute toxicity studies revealed safety of up to 500mg/kg dose, in addition to the fact that most part of the plants are consumed either as food or as traditional medicinal preparations.

In the second phase, the possible therapeutic potential of the extract in chronic liver injury was evaluated. This was carried out by repeated intoxication of rats with carbon tetrachloride (0.3ml/Kg) at every 72 hourly intervals with concomitant daily administration of the extracts (5mg/Kg) for twelve days, following initial CCl<sub>4</sub> intoxication at 0.6ml/kg. In all cases control groups treated with vitamin E alone, vitamin E + CCl<sub>4</sub>, CCl<sub>4</sub> only or solvent alone were also

included [9,10]. The use of vitamin E as standard antioxidant control is justified by work of other workers who investigated the antioxidant properties of other medicinal plants in the last decades [11,12].

For each phase, treatment for each plant part extract was divided into the following groups: Solvent only (corn oil); vitamin E only, Vitamin E + CCl<sub>4</sub>; extract only, extract + CCl<sub>4</sub> and CCl<sub>4</sub> only groups with 5 rats in each group. An untreated control group was also included. For the two acute intoxication, vitamin E as an oil gel was administered at a dose of 50mg/kg, while for low level chronic CCl<sub>4</sub> intoxication, vitamin E was administered at 10mg/kg, similar to the dose used in human subjects. These groupings were necessary to establish the preventive or therapeutic effect of the plant extracts on the organ damage caused by CCl<sub>4</sub> intoxication.

## **2.5 Animal Sacrifice and Tissue Collection**

Twenty four hours (24hrs) after the last treatment, all rats were sacrificed to permit maximum collection of blood that allowed triplicate analysis of all parameters for a statistically valid assessment. The sacrifice was performed under mild chloroform anaesthesia. Blood was collected directly at sacrifice; serum was separated after coagulating and centrifuging at 3000 rpm for 15 min and then stored at -20°C until required for analysis.

### **2.5.1 Determination of Aspartate Aminotransferase (AST) and Alanine amino-Transferase (ALT)**

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were determined independently as earlier described [13], using assay kits (Randox Laboratories Ltd). Exactly, 0.5µl of reagent 1 (which is made up of 100mM phosphate buffer pH 7.4, 100mM-aspartate or 200mM-alanine and 2mM α-oxoglutarate) was measured into a clean test tube containing 0.1ml of serum, mixed and incubated for 30 minutes at 37°C. Exactly, 0.5ml of reagent 2 (made up of 2mM 2,4-dinitrophenylhydrazine) was added, mixed and allowed to stand for 20 minutes at 25°C before 0.5ml of sodium hydroxide (0.4M) was added, mixed and absorbance was read against the reagent blank at 540nm after 5 minutes. The AST and ALT activities were determined from the standard calibration curve

prepared using the kits pre-validation data as provided by the manufacturers.

### **2.5.2 Determination of total, conjugated and unconjugated bilirubin**

Conjugated (indirect), unconjugated (direct) and total bilirubins were estimated as described by Sheirine and Safinaz [14] using assay kits (Randox Laboratories Ltd). These determinations were carried out based on the principles that bilirubin reacts with diazotised sulphanilic acid to form a blue coloured complex. Hence direct bilirubin was determined by its reaction with diazotised sulphanilic acid while total bilirubin is determined in the presence of caffeine, which aids release of albumin bound bilirubin. For total bilirubin (mg/dl), the absorbance of the sample against sample blank ( $A_{TB}$ ) was read at 560 nm and multiplied by a factor of 10.8, while for direct bilirubin the absorbance was read against the sample blank ( $A_{DB}$ ) at 530nm and multiplied by a factor of 14.4. Indirect bilirubin was obtained by difference between the total and direct bilirubin.

### **2.5.3 Determination of urea**

Urea was analysed based on the principle that in the presence of urease, urea in the serum is hydrolysed to ammonia which is trapped by Berthelot's reaction using analytical kits (Randox Laboratories), and measured spectrophotometrically as described by Stephen et al. [15]. In the method, 100µl of reagent 1 (which is made up of 116mM EDTA, 6mM sodium nitroprusside and 1g/l urease) was added into a clean test tube containing 10µl of serum. They were mixed and incubated at 37°C for 10 minutes. Then, 2.50ml of reagent 2 (made up of 120mM phenol) and 2.50ml of reagent 3 (made up of 27mM sodium hypochlorite and 0.14N sodium hydroxide) were added, mixed immediately and incubated at 37°C for 15 minutes before absorbance was taken at 540nm. Urea concentration was then calculated by simple proportion using the absorbance of a known concentration of the standard.

### **2.5.4 Determination of creatinine**

Creatinine level was assayed using assay kits as described by Stephen et al. [15]. The analysis is based on the principle that creatinine in alkaline solution reacts with picric acid to form a coloured complex whose intensity is directly proportional to the creatinine concentration. In the method used, 0.2ml of serum was added into a clean test

tube containing 2.0ml of creatinine working reagent (made up of picric acid and sodium hydroxide). The mixture was shaken and the absorbance ( $A_1$ ) at 510nm was read after 30 seconds and at exactly 2 minutes later ( $A_2$ ). The creatinine concentration (mg/dL) was calculated by simple proportion from the standard using the difference between  $A_1$  and  $A_2$  in both cases.

## 2.6 Statistical Analysis

Results obtained were expressed as mean  $\pm$  SD. All analyses performed were in triplicates and data analyzed by analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 14 software with the confidence level set at 95% ( $P=0.05$ ).

## 3. RESULTS AND DISCUSSION

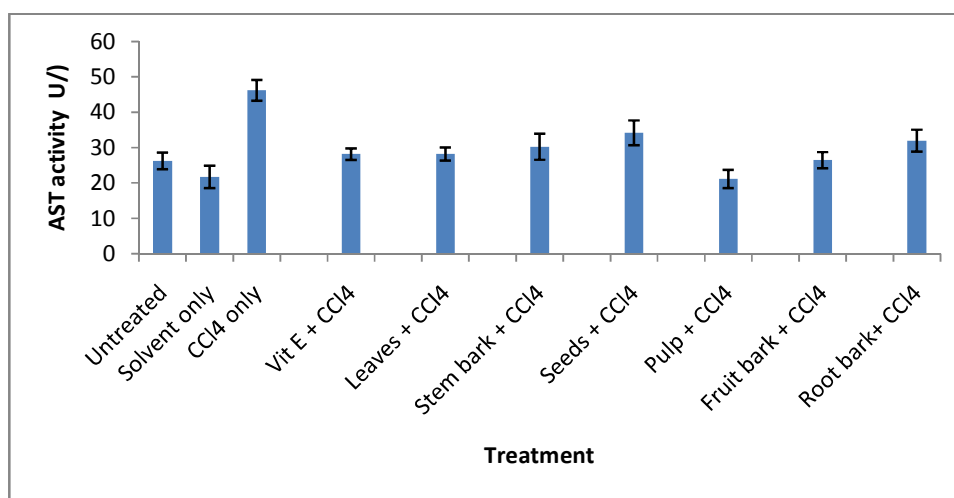
### 3.1 Results

Figs. 1-7 compares the effects of the extracts on the levels of some serum biochemical markers in rat's serum following two day pre-treatment with extracts (5mg/kg) while Figs. 8-14 compare the levels of biochemical parameters following twelve days therapeutic trials on chronic liver injury model.

In the acute model experiment, the activities of AST and ALT in the  $CCl_4$  only-treated control were statistically elevated ( $P=0.05$ ) above the untreated control and the extract pre-treated groups. Similarly, statistically significant lowering

( $P=0.05$ ) of AST and ALT levels were observed in the vitamin E - treated control as compared to the  $CCl_4$  control (Figs. 1 and 2). There was however no statistical significant change in the activity of these enzymes in the extract treated groups as compared to the solvent-treated group and the untreated control group (Figs. 1 and 2).

On the other hand, in the chronic model experiment, the levels of AST and ALT in the  $CCl_4$  only group were significantly ( $P=0.05$ ) elevated above the levels in the untreated control group. No such statistical ( $P=0.05$ ) differences exist when the untreated control group is compared with the extract-treated groups except groups treated with the leaves and root extracts (Figs. 8 and 9). However, in most of the groups intoxicated with  $CCl_4$  but administered methanolic extracts of different parts of *Tamarindus indica*, there was a significant ( $P=0.05$ ) decrease in the levels of the liver marker enzymes to levels that were not significantly ( $P=0.05$ ) different from that of the untreated control, but in some cases, even significantly ( $P=0.05$ ) lower than the untreated control, especially, in the case of stem and pulp extracts treated groups (Fig. 9). Similarly, the liver enzyme levels in the solvent and vitamin E - treated control showed no significant ( $P=0.05$ ) difference with the untreated control group. In general, the fruit pulps extract (Figs. 1 and 8) exhibited the best lowering effect in the levels of AST and ALT, while the leaves extract (Figs. 2 and 9) lowered the AST and ALT levels to the least extent.



**Fig. 1. Mean aspartate aminotransferase (AST) activity in serum of rats intoxicated with carbon tetrachloride (0.6ml/kg) following three days pre-treatment with methanol extract of *Tamarindus indica* (10.0mg/kg)**

The levels of total, conjugated and unconjugated bilirubin were significantly ( $P=.05$ ) elevated in the  $CCl_4$  control group above the untreated group in the acute model experiment (Figs. 3, 4 and 5). The reverse is however the case when the various extract or vitamin pre-treated groups were compared with the untreated control group (Figs. 3 and 5), where the bilirubin levels were not significantly elevated ( $P=.05$ ). The observed bilirubin lowering effect in the acute model experiment was also observed in the

experimental model of chronic liver injury (Fig. 10, 11 and 12). However, the decrease caused by the extracts in the levels of unconjugated bilirubin in the chronic injury model experiment was much more pronounced (Fig. 12), but the vitamin E treated control group showed the best bilirubin lowering potential, followed closely by the fruit pulp (Fig. 10) and stem bark (Fig. 12) extract treated groups, while the leaves treated group (Figs. 10, 11 and 12) showed the least potential.

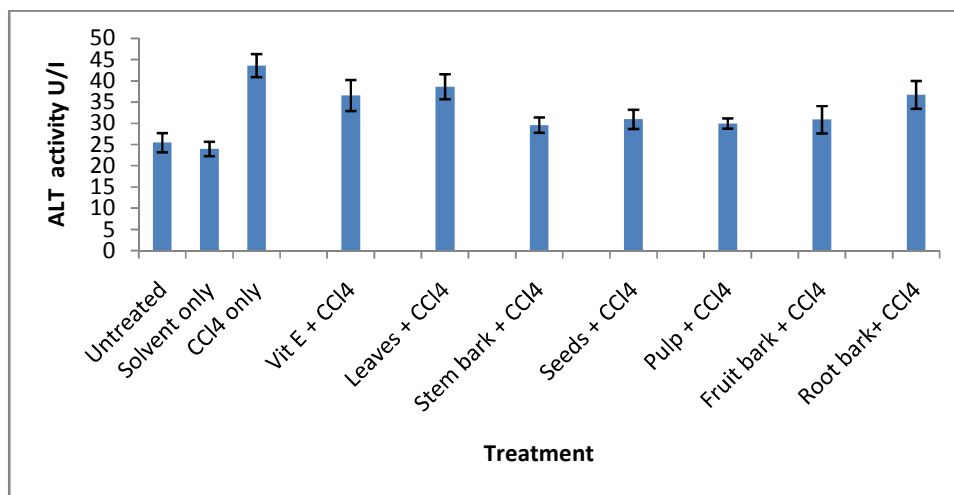


Fig. 2. Mean alanine aminotransferase (ALT) activity in serum of rats intoxicated with carbon tetrachloride (0.6ml/kg) following three days pre-treatment with methanol extract of *Tamarindus indica* (10.0mg/kg)

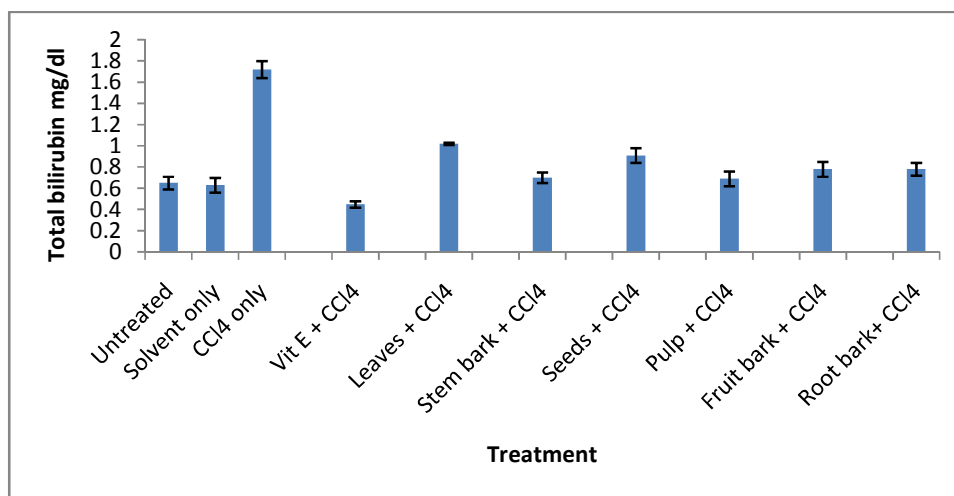
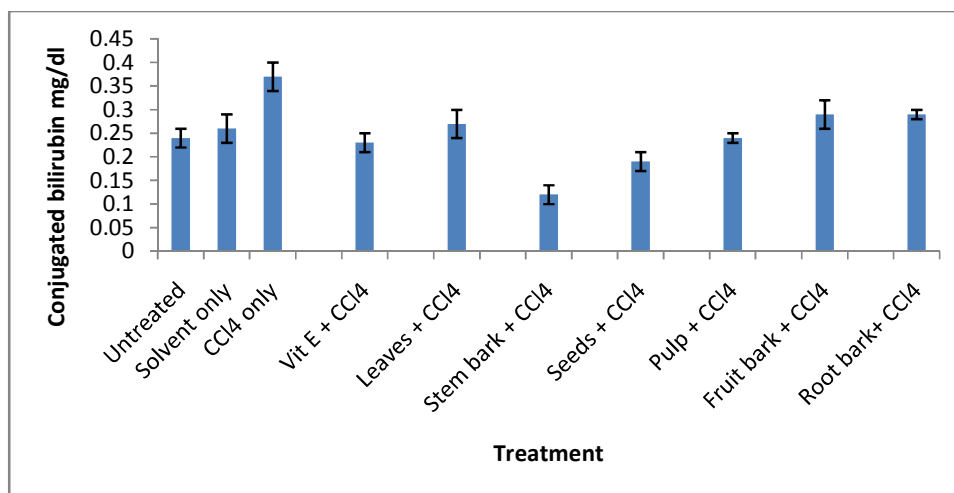
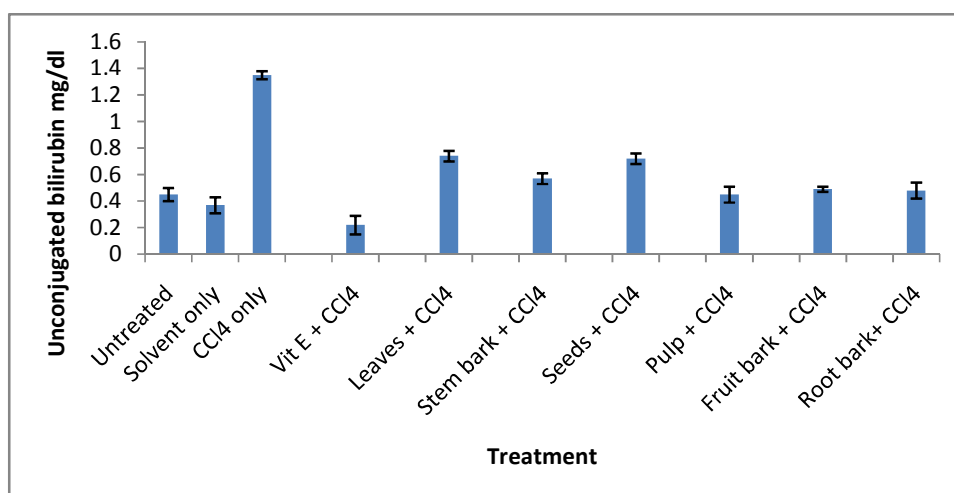


Fig. 3. Mean total bilirubin in serum of rats intoxicated with carbon tetrachloride (0.6ml/kg) following three days pre-treatment with methanol extract of *Tamarindus indica* (10.0mg/kg)



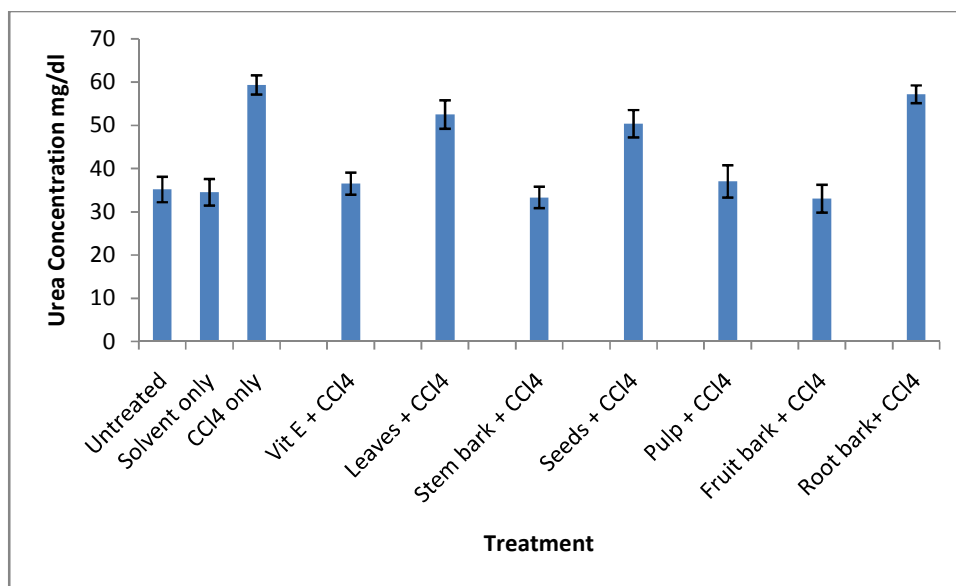
**Fig. 4. Mean conjugated bilirubin in serum of rats intoxicated with carbon tetrachloride (0.6ml/kg) following three days pre-treatment with methanol extract of *Tamarindus indica* (10.0mg/kg)**



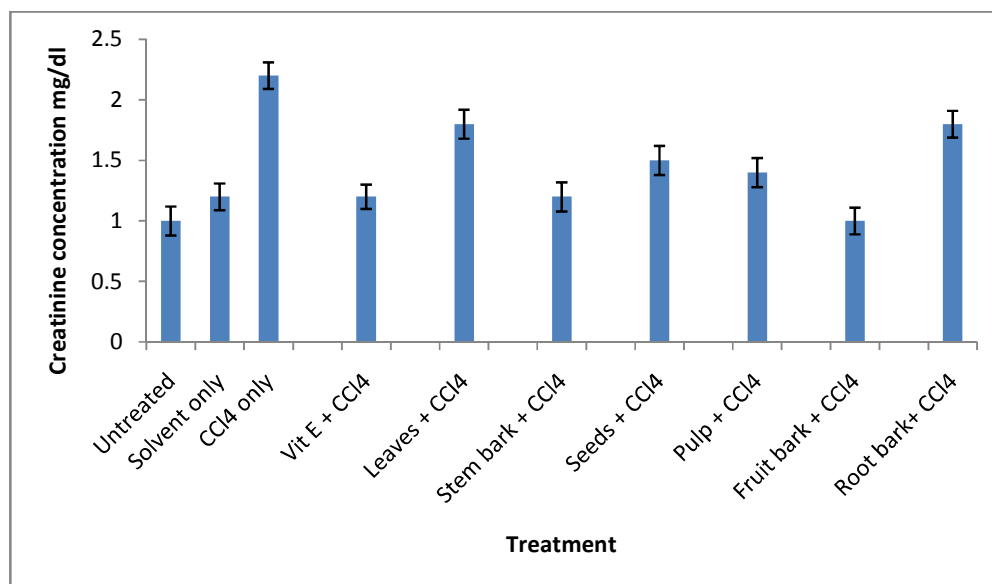
**Fig. 5. Mean unconjugated bilirubin in serum of rats intoxicated with carbon tetrachloride (0.6ml/kg) following three days pre-treatment with methanol extract of *Tamarindus indica* (10.0mg/kg)**

The kidney function indicators, urea and creatinine also showed varied responses to the different treatment across the groups in the acute model experiments. There were elevations in levels of urea in the CCl<sub>4</sub> only treated group when compared to the untreated control (Figs. 6

and 7). However, in the extract pre-treated groups, there were no such significant ( $P=0.05$ ) elevation when compared with the untreated control, except in the case of the leaves and root extract treated groups which showed significant ( $P=0.05$ ) elevation in the level of urea (Fig. 6).

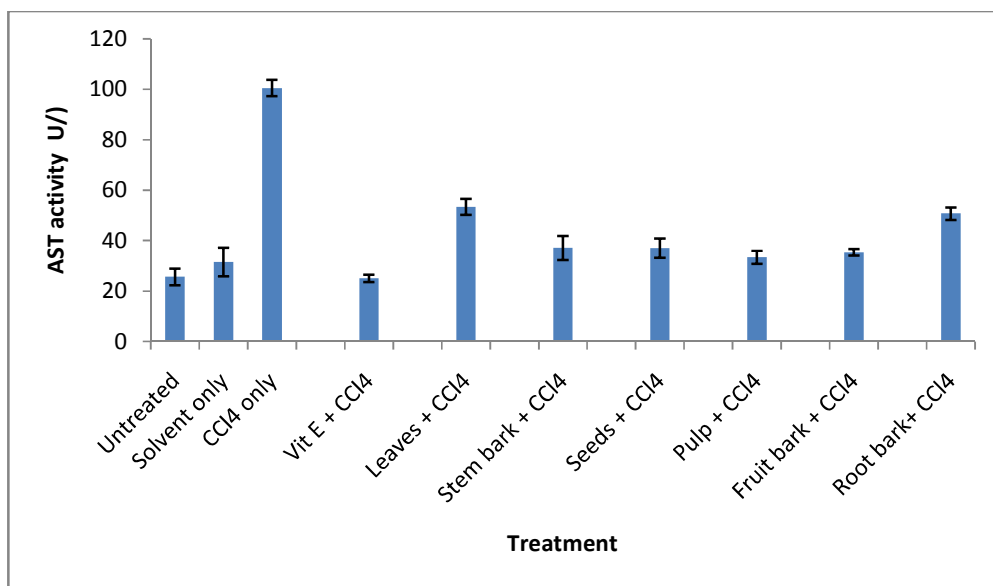


**Fig. 6.** Mean urea concentration in serum of rats intoxicated with carbon tetrachloride (0.6ml/kg) following three days pre-treatment with methanol extract of *Tamarindus indica* (10.0mg/kg)

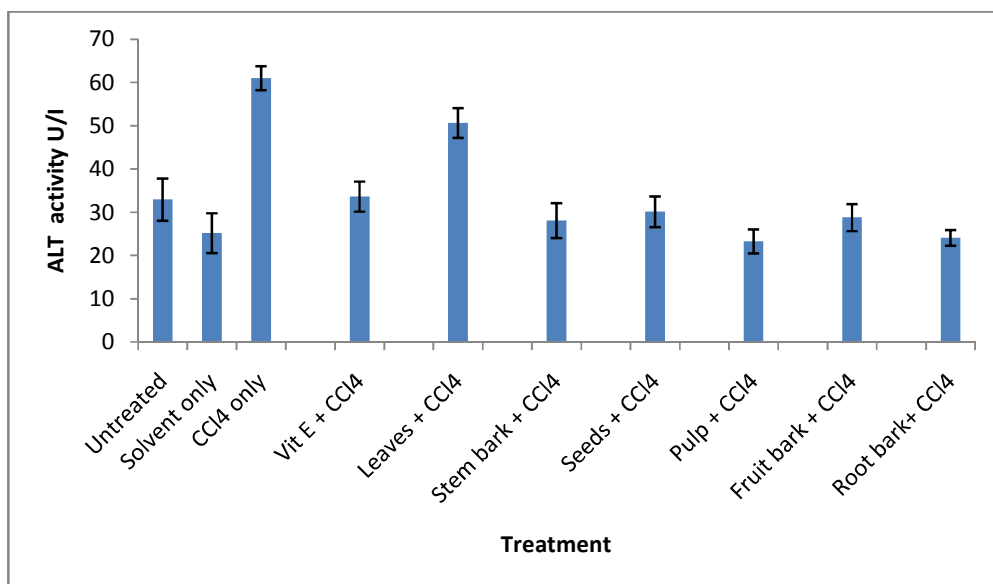


**Fig. 7.** Mean creatinine concentration in serum of rats intoxicated with carbon tetrachloride (0.6ml/kg) following three days pre-treatment with methanol extract of *Tamarindus indica* (10.0mg/kg)

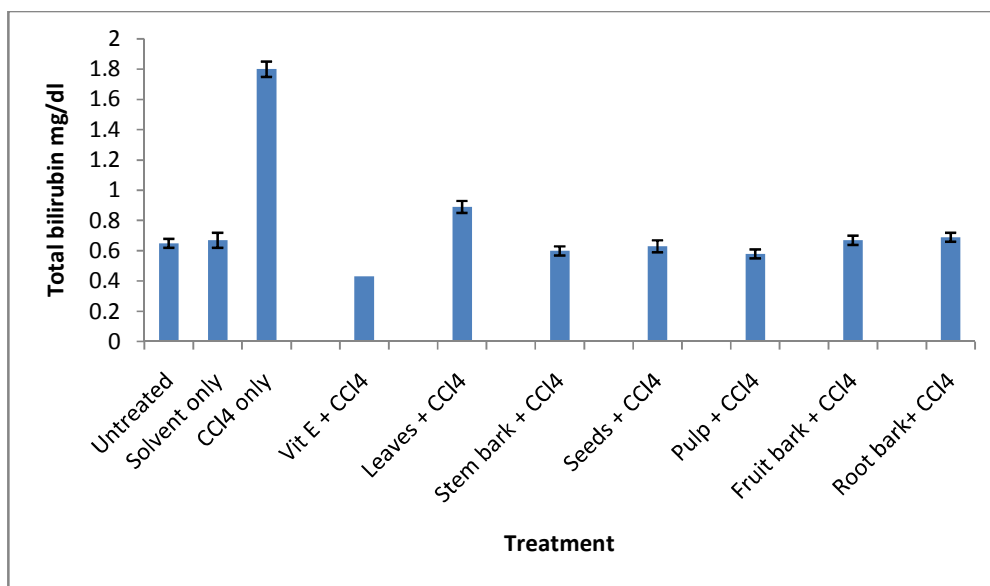




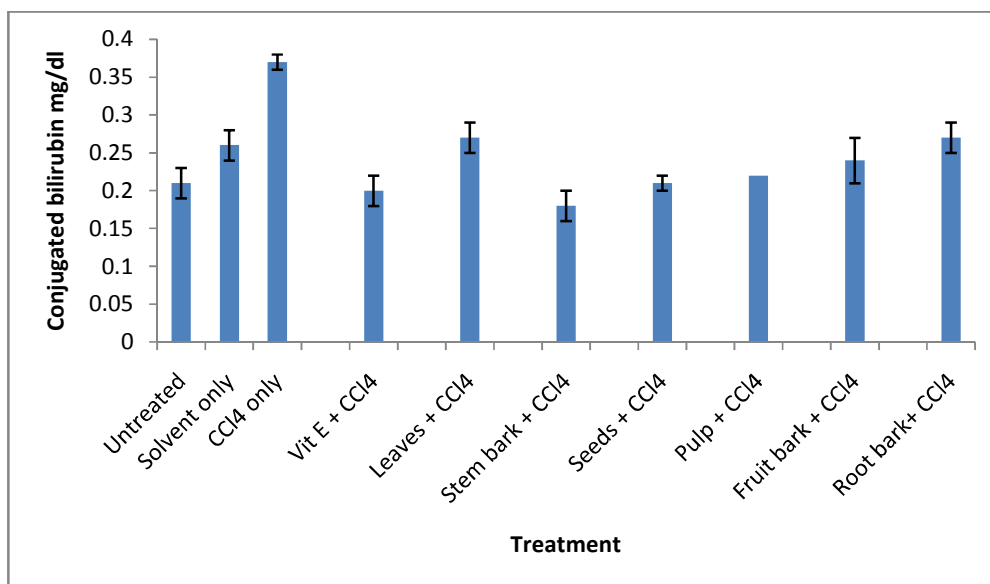
**Fig. 8. Mean aspartate aminotransferase (AST) activity in serum of rats following daily intraperitoneal administration of *Tamarindus indica* extract (5mg/kg) with 72 hourly injection of carbon tetrachloride (0.3ml/kg) for 12 days**



**Fig. 9. Mean alanine aminotransferase (ALT) activity in serum of rats following daily intraperitoneal administration of *Tamarindus indica* extract (5mg/kg) with 72 hourly injection of carbon tetrachloride (0.3ml/kg) for 12 days**



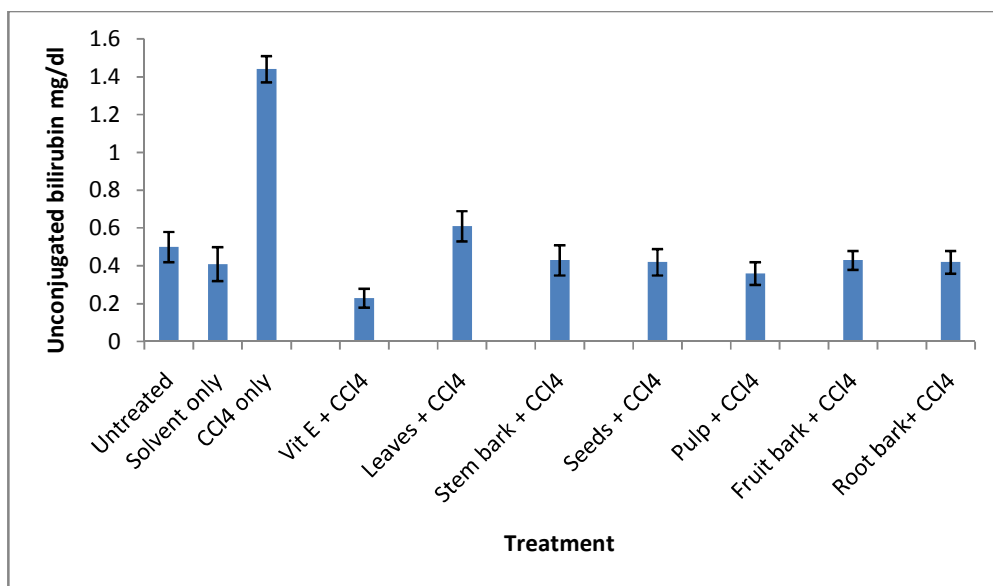
**Fig. 10.** Mean total bilirubin in serum of rats following daily intraperitoneal administration of *Tamarindus indica* extract (5mg/kg) with 72 hourly injection of carbon tetrachloride (0.3ml/kg) for 12 days



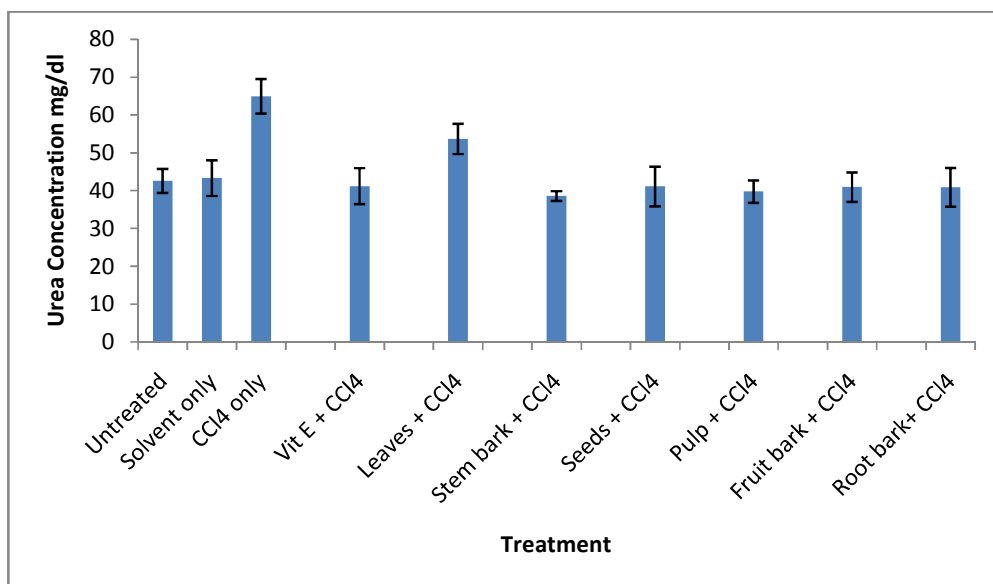
**Fig. 11.** Mean conjugated bilirubin in serum of rats following daily intraperitoneal administration of *Tamarindus indica* extract (5mg/kg) with 72 hourly injection of carbon tetrachloride (0.3ml/kg) for 12 days

In the chronic injury model experiment, the CCl<sub>4</sub> only control group showed statistically ( $P=.05$ ) elevated levels of urea and creatinine when compared to the untreated control (Figs. 13 and 14). However, no such elevations existed when the CCl<sub>4</sub> intoxicated group treated with extracts

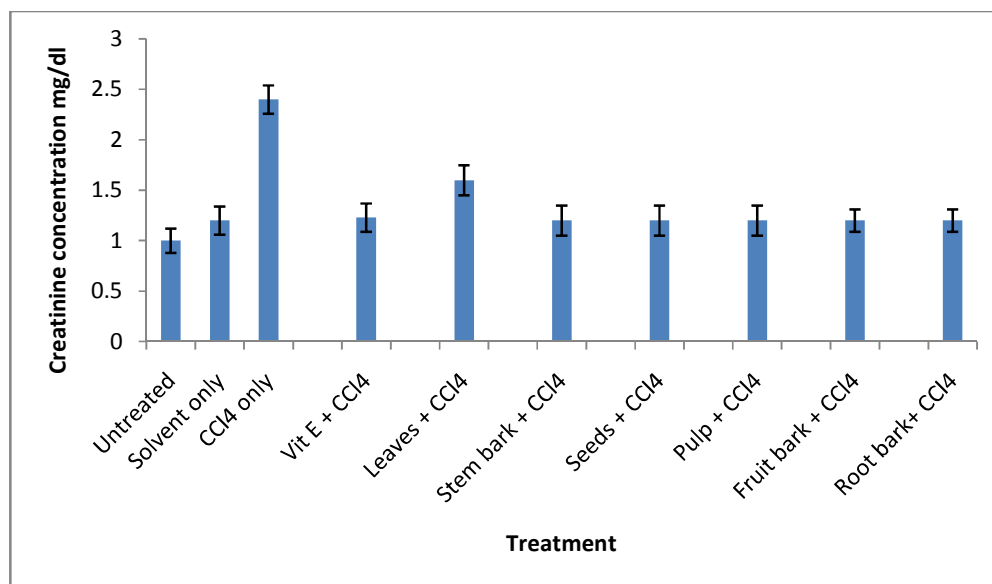
were compared with the untreated or solvent-treated group. Groups treated with only stem (Fig. 14), seed (Fig. 13), pulp (Fig. 14) or fruit bark (Fig. 13) extracts were particularly and significantly ( $P=.05$ ) lower than the untreated control and the vitamin E-treated group.



**Fig. 12. Mean unconjugated bilirubin in serum of rats following daily intraperitoneal administration of *Tamarindus indica* extract (5mg/kg) with 72 hourly injection of carbon tetrachloride (0.3ml/kg) for 12 days**



**Fig. 13. Mean urea concentration in serum of rats following daily intraperitoneal administration of *Tamarindus indica* extract (5mg/kg) with 72 hourly injection of carbon tetrachloride (0.3ml/kg) for 12 days**



**Fig. 14. Mean creatinine concentration in serum of rats following daily intraperitoneal administration of *Tamarindus indica* extract (5mg/kg) with 72 hourly injection of carbon tetrachloride (0.3ml/kg) for 12 days**

### 3.2 Discussion

The results obtained generally showed a statistically significant ( $P=0.05$ ) lowering in the levels of aspartate aminotransferase, alanine aminotransferase, bilirubin, urea and creatinine in the extracts-treated groups as compared to the CCl<sub>4</sub> control, both in the ameliorative (chronic) and in the preventive (acute) model experiments. In contrast, no statistical ( $P=0.05$ ) difference were observed in most instances between the extract-treated groups and the vitamin E-treated group or the untreated control group (Figs. 3-7, 12-14), suggesting that the extracts were able to counteract the hepatotoxic effects of CCl<sub>4</sub> by restoring the functional integrity of the liver and the kidney.

This is because cellular damages are usually accompanied with leakage of intracellular enzymes into the blood, and as such the levels of these enzymes in the serum can be measured as indicators of cell damage [14,16,17]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are among such enzymes. Alanine aminotransferase is found in the liver and is markedly elevated in hepatitis and other liver diseases. Oxidants like free radicals are capable of causing peroxidative degradation of cellular membranes and endoplasmic reticulum which are rich in polyunsaturated fatty acids. Hence, oxidative damage is implicated in

most disease processes such as cardiovascular disease, cancer, inflammatory conditions, asthma, liver diseases and muscular degeneration [16] through destruction of the hepatic cellular membrane by altering the cellular permeability of hepatocytes leading to elevated levels of serum biochemical parameters like ALT, AST and bilirubin. Similarly these radicals can cause oxidative damage of the kidney leading to elevated levels of urea and creatinine [18]. Hence many workers consider the reverse of this phenomenon as indices of hepatoprotective and nephroprotective activities [9,18].

Thus, the significant ( $P=0.05$ ) elevation in the levels of ALT and AST ( Figs. 1, 2, 8 and 9) observed in the CCl<sub>4</sub> control when compared to the untreated group can be attributed to cellular damages caused by CCl<sub>4</sub> intoxication. These elevations were clearly more evident in the chronic model experiment than it was in the acute model experiment, probably because of the repeated long term CCl<sub>4</sub> intoxication in the latter. In the acute model experiment, the extract-treated and the vitamin-E treated groups showed significant decrease compared to the CCl<sub>4</sub> control, indicating the protective effects of the extracts and vitamin E against CCl<sub>4</sub>-induced organ damages (Figs. 1 and 2). Similarly, the reversal and maintenance of lowered AST and ALT levels even after chronic intoxication with CCl<sub>4</sub> in the chronic model experiment suggests

strong hepatoprotective and therapeutic effects of the extracts. Comparatively the effects exhibited by the stem, pulp, fruit bark and seeds extracts were more pronounced than those shown by the root and leaves (Figs. 1, 2, 7 and 8).

Bilirubin is excreted by the liver, and hence any interference with the normal liver functions affects its rate of excretion. Thus, elevated levels of bilirubin is used as an index of liver function as high levels of unconjugated bilirubin suggests liver malfunction or there is excessive breakdown of haemoglobin [17,19]. The results obtained showed a clearly significant ( $P=0.05$ ) increase in the levels of total and unconjugated bilirubin in  $\text{CCl}_4$  treated animals, which are markedly reduced by pre-treatment (Figs. 3, 4 and 5) or concomitant treatment (Figs. 10, 11 and 12) with the methanolic extract of the stem, pulp, fruit bark or seed. Although this was true and consistent for both the acute and preventive model experiments, it can be saliently observed that the effect on the unconjugated bilirubin in the chronic (curative) model experiment was more pronounced (Fig. 10). This appears to demonstrate the abilities of the extracts to restore and maintain liver functions even under chronic toxicological condition. In comparative terms, the stem bark, fruit pulp and fruit bark extracts exhibited the best bilirubin lowering potential and hence possibly better hepatoprotective and curative capacity.

The kidney is a target organ for xenobiotics metabolism, second only to the liver, and hence substances like carbon tetrachloride that are toxic to the liver are also known to be potentially toxic to the kidney [8,20], whose principal function is the excretion and elimination of wastes like urea and creatinine. Thus, impairment of the kidney function leads to decreased clearance rate of urea and creatinine which conversely results in their elevated levels within the serum [9,18,10,11,12]. The study revealed a significantly ( $P=0.05$ ) elevated levels of urea and creatinine in the  $\text{CCl}_4$  groups which were significantly ( $P=0.05$ ) reduced by pre-treatment or administration of either stem, fruit pulp or fruit bark extracts strongly suggesting their nephroprotective abilities (Figs. 6 and 7). Interestingly, these significant ( $P=0.05$ ) decreases in urea and creatinine levels in the extracts-treated groups were more clearly observed in the chronic experimental model (Figs. 13 and 14). In the acute experimental model, the urea and creatinine levels were markedly decreased only

in the stem bark, fruit pulp and fruit bark extracts treated groups (Figs. 6 and 7), suggesting better that these parts contained substances with better ameliorative capacities, while in the chronic experimental model, all the extracts except the leaves demonstrated good capacity for protection and amelioration of kidney damage (Figs. 13 and 14).

Researches into plant foods and plants with medicinal properties have received increased interest in recent years. For instance, various reports have indicated that many plant foods and medicinal plants including *Vernonia amygdalina* [18], *Labisiapumila* [21], *Spirulina maxima* [22], *Hibiscus esculentus* [10], *Moringa oleifera* [23], *Anogeissus leiocarpus* [8] are known to contain antioxidant constituents with demonstrated capacity to chemoprevent oxidative stress-related diseases. Other workers have shown that under *in vitro* conditions seeds of *T. indica* exhibited antioxidant effect [24]. The present findings have thus proven that *Tamarindus indica* has potent capacity to prevent and ameliorate oxidative damage to the liver and kidney.

The organ protective effect of *Tamarindus indica* may be related to the presence of antioxidant compounds in the different methanolic extracts of the plant parts. For instance, polar solvent extracts of tamarind seeds and pulp revealed the presence of compounds with possible antioxidant potential and demonstrable anti-microbial, anti-diabetic and anti-cancer potential [1,8,7]. Methanolic extracts of plants have been reported to have phenolics, which have several biological activities and health benefits, including antioxidant roles as a major constituent [1]. Similarly, the presence of polyphenols was reported in *Tamarindus indica* fruits [3,7] with a profile dominated by proanthocyanidins in various forms, catechin, procyanidin B2, epicatechin, procyanidin trimer, procyanidin tetramer, procyanidin pentamer, procyanidin hexamer and other compounds in less quantity [1,24]. Hence, the organ protective and therapeutic effects exhibited by different parts of *Tamarindus indica* may be ascribed to these phytoconstituents [25].

Thus, the reversal of elevated levels of AST, ALT, bilirubin, urea and creatinine by the extracts of various parts of *Tamarindus indica* clearly illustrates the organ-protective and therapeutic potential of different parts of the plant. It is worth noting however, that the stem and fruit extracts showed strongest organ protective activities

against CCl<sub>4</sub> induced damage while the roots and leaves showed the least potency. This observation appears to justify the use of the stem, seed and fruit to treat jaundice, boils and stroke respectively in traditional medicine, while the low activity of the roots and leaves might explain the reason for their poor usage in traditional medical practice [15].

#### 4. CONCLUSION

In conclusion, amongst the various parts, the fruit pulp, stem bark, fruit bark and seeds extracts showed the most effective hepatoprotective and nephroprotective potentials, while the roots and the leaves are the least respectively. The findings suggest that *Tamarindus indica*, a widely available and consumed plant resource in tropical Africa and Asia could serve as a cheap source of naturally occurring drugs and antioxidants for pharmaceutical and nutraceutical industries, but the exact bioactive substance responsible for the observed activity as well as the mechanism of the biological protection and therapeutic capacities would require further elaboration.

#### ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) 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.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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