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Hepato and Reno Protective Effect of the Methanolic Leaf Extract of *Punica Granatum L.* On Ccl$_4$ Exposed Wistar Albino Rats

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ABSTRACT: *This study therefore evaluated the hepato and reno protective effect of the methanolic leaf extract of Punica granatum against carbon tetrachloride (CCL_4) in wistar albino rats. In acute toxicity test, methanolic leaf extract of Punica granatum were administered orally using rats of both sexes weighing between 80-200g. fourty (40) albino rats were divided into eight (8) groups of five (5) rats each (A-H). groups A, B and C served as normal, negative and positive control groups repectively, groups D and E served as treatment groupsat doses of 200mg/kg and 400mg/kg body weights respectively, groups F, G and H are extract groups at 200mg/kg, 400mg/kg and 800mg/kg body weights respectively. In sub-acute study, the wistar albino rats were daily administered orally with methanolic leaf extract of Punica granatum at doses of 200, 400 and 800mg/kg body weights for 28days. They were weighed on the first day and after every 7days during treatment with the extract. Signs of toxicity were also observed after 28days. The rats were sacrificed and blood samples taken for full biochemical and haematological assessment and a histopathology of the liver and kidney. These assessments were carried out using different standard scientific methods described. No death was recorded within 24hours after oral administration at extract dose of 5000mg/kg body weight in acute toxicity (LD_{50}). In the sub-acute study, the extract did not exhibit any significant difference ($P<0.05$) on haemoglobin in all the tested doses. This study revealed that the administration of CCL_4 elevated serum levels of ALAT (151.25±5.25), ASAT (235.25±27.37), ALP (45.45±0.56), urea (47.22±6.10) and creatinine (1.61±0.17) whereas levels of albumin (15.40±0.05) and electrolytes (Na+, K and HCO_3) were decreased. The extract ameliorated the detrimental effects of CCL_4 and corrected all examined biomarkers toward the control values where ALAT (122.33±6.36), ASAT (198.33±1.33), ALP (34.10±5.40), urea (22.82±2.32) and creatinine (0.74±0.08) whereas levels of albumin (21.04±0.22) and electrolytes (Na+, K and HCO_3) at treatment group 200mg/kg. Treatment with extract at 400mg/kg body weight resulted in even more significant decrease in the elevated serum levels and increased levels in the parameters observed. Weights of internal organs (liver and kidney) were also recorded. Data was analysed and expressed as mean ±SD and statistically analysed using ANOVA with SPSS. Histopathological studies revealed no abnormalities in liver and kidney in treated Rats. The liver tissue displayed normal hepatocytes without any enlargement in sinusoidal vein, central vein, and portal triad in all treated groups compared to control (Plate 1). Kidney micrograph revealed normal architecture of glomerulus and Bowman's capsules with no degeneration, necrosis, or inflammation (Plate 2). Thus, histological evaluation indicated that the extract did not have any adverse effect on morphology of the tissues and these observations supported the biochemical results mentioned above. The present study concluded that methanolic leaf extract of Punica granatum plays a protective role against CCL_4 induced liver and kidney damage in rats.*

KEY WORDS: *Punica granatum*, CCL_4, LD_{50}, biochemical parameter, hematological parameter, histopathological parameter.

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INTRODUCTION

The pomegranate, botanical name '*Punica granatum',* is a fruit bearing deciduous shrub that contains thousands of health benefits. This fruit is mentioned three times in the holy Qur'an as it is grown in the garden of paradise, thus showing its advantage and privilages as fruits that are beneficial to human beings. In English terms, the pomegranate is known as the '*Pomegranate'* and '*al- Rumman'* or '*al- Rummanah'* in Arab.(Munirah, 2011.) in older times, the fruit considered in old testament of the Bible, the Jewish Torah, and the Babylonian Talmud as a sacred fruit conferring powers of fertility, abundance, and good luck.(Akhlaghi and Band, 2009.)
The pomegranate contains several major active components including flavonoids.(Jurenka, 2008; Marhari *et.al.,*2014; Suranto & Terbukti, 2011; Arun & Singh, 2012; Yasoubi, 2007). The benefits of flavonoids are as antibacterial, antiviral, anti-insecticide and anti-inflammatory while the tannin substances as hemostatic, antibacterial, antioxidant and anti-inflammatory (Prashanth *et.al.,*2001.) Carbon tetrachloride (CCl_4) is an extensively studied xenobiotic that induces lipid peroxidation and toxicity (Jeon *et al*., 2003). Liver cell injury induced by CCl_4 involves initially the metabolism of CCl_4 to trichloromethyl (CCl_3_) free radical by the mixed function oxidase system of the endoplasmic reticulum. The secondary mechanism could involve the generation of toxic products arising directly from CCl_4 metabolism or from peroxidative degeneration of membrane phospholipids, and causes functional and morphological changes in the cell membrane leading to accumulation of lipid-derived oxidants causing liver injury. Moreover, reactive oxygen intermediates (ROIs) generated in the hepatocytes as by-products of CCl_4 metabolism and excess of ROIs, oxidative stress, contribute to cell injury. CCl_4 induced damage also produces alteration in the antioxidant status of the tissues, which is manifested by abnormal histopathological changes. Several studies have previously demonstrated that antioxidants prevent CCl_4 toxicity, particularly hepatotoxicty, by inhibiting lipid peroxidation and increasing antioxidant enzyme activities (Kumaravelu *et al*., 1995). The increased cost and side effects of drugs associated with the treatment of Liver and Kidney diseases coupled with increase in morbidity necessitated the search for safer and protective measures to avoid toxicity. Different parts of *Punica granatum L.* were scientifically tested for their medicinal properties by previous workers worldwide, but little information is available on the leaves particularly in Nigeria. The aim of this study is to determine the hepato and reno protective effect of the methanolic leaf extract of *Punica Granatum L*. on some liver and kidney biochemical indices and organ histology in wistar albino rats.

MATERIALS AND METHODS

Chemicals

Reagents and chemicals used for the experiment are of analar grades. Assay kits for AST, ALT, ALP and Albumin were bought from RANDOX laboratories, India. All other reagents and chemicals were purchased from various manufacturers.

Plant collection and identification

The fresh leaves of pomegranate were collected from Botanical garden, University of Maiduguri, Borno State, Nigeria and it was identified by a plant taxonomist at the Department of Biological Sciences, University of Maiduguri, Nigeria.

Preparation of plant materials

Following collection, the fresh leaves of *Punica granatum Linn* was washed and shade dried. It was then ground into fine powder using mortar and pestle at the research laboratory, Biochemistry Department, University of Maiduguri, Borno State, Nigeria. It was sieved to remove debris and coarse plant materials. A powder form of the leaves was stored under laboratory condition prior to extraction.

Methanolic extract preparation

About 500g powder of *Punica granatum Linn* leaf was extracted with one liter of 70% methanol using Soxhlet extractor, the extract was then concentrated to dryness at low temperature on a rotary evaporator. The percentage yield of the extract was 13.54%.

Acute toxicity Study:

The acute toxicity study was conducted in accordance with Lorke's method (Lork, 1983). The study was conducted in two phases using a total of twelve albino rats. In the first phase, nine rats were divided into 3 groups of 3 rats each. Groups 1, 2 and 3 animals were given 10, 100 and 1000 mg/kg body weight (b.w.) of the extract, respectively, to possibly establish the range of doses producing any toxic effect. Each rat was given a single dose after at least 5 days of adaptation. In addition, a fourth group of three rats was set up as control group and animals in the group were not given the extract.

In the second phase, further specific doses (1600, 2900 and 5000 mg/kg b.w.) of the extract were administered to three rats (one rat per dose) to further determine the correct LD_{50} value.

Hepato and reno protective effect of PGMLE

***In vivo* Experimental Design**

Following acclimatization, a total of 40 healthy albino rats were divided into eight groups (A -H) of five animals each. The control group (A) was adminisrered with normal feed and water *ad libitum*, Negative control group (B) was administered with Carbon tetrachloride with No treatment, positive control group (C) was administered with a standard drug (Silymarin 6mg/kg) + Carbon tetrachloride, Treatment groups (D and E) were administered methanolic leaf extract of *Punica granatum* + Carbon tetrachloride at 2 different doses (200mg/kg and 400mg/kg body weights respectively), Extract groups (F, G and H) were administered with methanolic leaf extract of *Punica granatum* at different doses (200mg/kg, 400mg/kg and 800mg/kg body weights respectively). The administrations were done orally.

Serum Biochemical Parameter

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined by the Reitmen-Frankel colorimetric method (Reitman and Frankel, 1957), Alkaline phosphatase (ALP) was determined by Phenolphthalein monophosphate method (Klein *et al.,* 1960), Serum albumin was determined by the standard method described by Doumas, 1971. (liver damage) and Urea was determined by the modified method of (Searcy *et al.,* 1967) and Creatinine by the modified Jaffe method (Blass *et al.,* 1974) using the Quimica Clinica Applicada (QCA) test kit and electrolytes (Na+ and K+ were analyzed using flame emission spectrophotometric method (Tietz *et al.,* 1996) and HCO3 was estimated by titrimetric method of Davidson and Henry, 1970) (kidney damage) were analysed in the laboratory.

Determination of Haematological Parameters

Haematological parameter (Haemoglobin concentration) was determined every 4 days according to standard methods (Brown, 1976; Coles, 1986).

Measurement of relative organ weight

Liver and kidney were carefully dissected out and weighed separately. The relative organ weight of each animal was then calculated as follows:

Relative organ weight = absolute organ weight (g)/body weight of rat on sacrifice day (g) × 100

Histopathological processing

The dissected Livers and Kidneys were grossly examined for surface smoothness, colour change, and any abnormal growth, and then immediately fixed in 10% neutral buffered formalin for histological examination. The Organs (Liver and Kidney) were processed using automated tissue processor. The tissues were embedded into paraffin blocks. The tissues were sectioned at 4 µm thickness and stained with hematoxylin and eosin (H&E) stain according to the histopathology laboratory work procedure and safety guidelines of UMTH Pathology Department using Haematoxylin and Eosin staining (Mkri *et al.,* 2011).

RESULTS

Acute Toxicity Test

Acute toxicity study conducted showed no sign of toxicity and/or mortality with the first phase dose of (10, 100 and 1000 mg/kg) and the second phase dose of 1600, 2900 and 5000 mg/kg of the methanolic leaf extract of *Punica granatum L.* administered orally. There was no sign of toxicity or death in rats during the 24hours of observation including autonomic effects (perspiration, defeaecation, inconsistent urination and salivation). In addition there was no sign of central nervous system intoxication (lethargy, drowsiness, restlessness, convulsions and coma). Therefore, the LD_{50} could not be calculated and it is possibly higher than 5000 mg/kg body weight. (Table1).

The change in mean body weight in rats (80 – 200g) after 28days are presented in Table 2. A general increase in physical activities, food and water intake were observed for all the animals during the feeding experiment. There was initial increase in weight which was sustained. Body weights of animals gradually increased in all groups during the 1st, 2nd and 3rd weeks of treatment and feeding but then suddenly dropped at the 4th week prior to induction with Carbon Tetrachloride due to the starvation for 24hours. Although, the change in body weights is not significantly different at $P<0.05$.

ALT which was elevated by Carbon tetrachloride in group 2 (Negative control) with a value of 151.25±5.25 was significantly reduced at $P<0.05$ by the plant extract at doses 200mg/kg and 400mg/kg with values of 122.33±6.36 and 125.33±5.36 respectively when compared with the normal control group indicating hepatoprotection, although, there is no significant difference between the positive control group 3 (silymarin) with value of 152.67±1.67 and the treatment group 4 at dose 200mg/kg. AST level was also elevated on the administration of CCL_4 with a higher value of 235.25±27.37 when compared to the normal control (89.33±12.99) and treatment groups at doses 200mg/kg and 400mg/kg (198.33±1.33 and 135.00±20.50 respectively) and ALP which was also elevated at Negative control group 45.45±0.56 when compared to the normal control and treatment groups was significantly reduced at $P<0.05$ by the plant extract at both 200mg/kg and 400mg/kg body weights. There was a large decrease in serum Albumin in Rats treated with Carbon tetrachloride (15.40±0.05). This indicates possible compromise in the synthetic capacity of the liver. *Punica granatum* methanolic leaf extract was able to prevent this and boost both the structural and functional integrity of the liver, this was seen in the treatment groups where levels were increased to 21.04±0.22 and 21.41±0.56 at doses of 200mg/kg and 400mg/kg respectively. (Table 3).

The decrease in creatinine level was found to be significant at $p<0.05$, only in the groups administered *Punica granatum* leaf extract at 200mg/kg and 400mg/kg (0.74±0.08 and 0.70±0.04 respectively) compared with the control (0.31±0.21) after 28 days. These levels where significantly increased at the negative control group (1.61±0.17). (Table 4).The administration of *Punica granatum* leaf extract at doses 200mg/kg and 400mg/kg body weight produced significant increase in Hb with values of 9.67±0.19 and 10.22±0.11 respectively which is similar to that of the normal control (10.78±0.62) as compared to the negative control which has a lower value of 8.17±0.44. (Figure 1).

Table 1.0: Lethal dose (LD_{50}) of the Methanolic Leaf Extract of *Punica granatum* in Wistar Strain Albino Rats.

Groups	Dose (mg/kg)	Mortality Ratio	% Survival
1st Phase			
1	10	0/3	100
2	100	0/3	100
3	1000	0/3	100
2nd Phase			
1	1600	0/1	100
2	2900	0/1	100
3	5000	0/1	100

The Lethal Dose (LD_{50}) was ≥ 5000 mg/kg body weight

Effect of the Methanolic Leaf Extract of *Punica granatum L.* on the Weekly Body weight in Wistar Strain Albino Rats exposed to Carbon tetrachloride.

The body weight of the animals gradually increased from week one to week three, but a slight decrease in the weight was observed after administering Carbon tetrachloride and starving the animals for 24 hours at week four (Table 2).

Table 2: Effect of the Methanolic Leaf Extract of *Punica granatum* on the Weekly Body weight in Wistar Strain Albino Rats (g) exposed to CCl_4 after 28 days of oral administration.

Group	Initial	Week 1	Week 2	Week 3	Week 4
Normal control group	116.00 ±22.24[a]	121.80 ± 25.37[a]	128.07 ± 23.15[a]	137.17 ± 20.49[a]	140.07 ± 17.72[a]
Negative control group (CCl_4)	137.48 ± 11.33[a]	138.65 ± 13.33[a]	141.00 ± 13.88[a]	150.85 ± 14.53[a]	140.10 ± 11.96[a]
Positive control group (standard drug silymarin) + CCl_4	148.80 ± 9.72[a]	156.00 ± 9.45[a]	157.40 ± 9.96[a]	160.93 ± 10.44[a]	145.40 ± 10.91[a]
Treatment group (200mg/kg) + CCl_4	90.90 ± 15.22[a]	94.80 ± 17.44[a]	99.07 ± 16.72[a]	109.07 ± 17.27[a]	116.53 ± 17.89[a]
Treatment group (400mg/kg) + CCl_4	107.63 ± 27.42[a]	109.47 ± 27.42[a]	115.10 ± 25.61[a]	124.57 ± 23.61[a]	119.37 ± 17. 93[a]
Extract group (200mg/kg)	114.90 ± 33.45[a]	116.27 ± 33.77[a]	124.67 ± 31. 55[a]	130.80 ± 31 70[a]	133.73 ± 28.82[a]
Extract group (400mg/kg)	154.50 ± 26.50	176.10 ± 27.90	176.65 ± 28.25	178.05 ± 27.65	177.70 ± 21.50

Extract group (800mg/kg)	88.33 ± 7.52^{a}	84.27 ± 6.55^{ac}	96.57 ± 6.33^{ab}	106.07 ± 5.98^{ad}	110.60 ± 7.36^{b}

Values are presented as mean ± SEM, n = 5 along the raw
Values with different superscript horizontally are significantly different (P < 0.05)

Effect of the Methanolic Leaf Extract of *Punica granatum L.* on some serum Biochemical Parameters (Liver Function Test) in of Rats exposed to Carbon tetrachloride

The results of this study showed that Carbon tetrachloride caused the activities of AST, ALT and ALP at P< 0.05 and caused a reduction in the serum level of Albumin (P<0.05) compared to those of the control animals (Table 3). The methanolic leaf extract of *Punica granatum L.* reduced the levels of these enzymes and increase the level of Albumin to different extents at different doses of the extract to levels similar to those of the control group.

Table 3: Effect of the Methanolic Leaf Extract of *Punica granatum* Biochemical Parameters (Liver Function Test) on Wistar Strain Albino Rats (g) exposed to CCl_4 after 28 days of oral administration.

Groups	ASAT (µ/L)	ALAT (µ/L)	ALP (µ/L)	Albumin (g/dl)
Normal control group	89.33 ± 12.99^{ae}	63.67 ± 3.33^{a}	9.45 ± 0.84^{a}	50.60 ± 3.68^{af}
Negative control group (CCL_4)	235.25 ± 27.37^{b}	151.25 ± 5.25^{b}	45.45 ± 0.56^{b}	15.40 ± 0.05^{b}
Positive control group (standard drug silymarin) + CCl_4	209.00 ± 8.33^{cb}	152.67 ± 1.67^{cb}	35.41 ± 4.83^{c}	17.28 ± 0.31^{cb}
Treatment group (200mg/kg) + CCl_4	198.33 ± 1.33^{db}	122.33 ± 6.36^{d}	34.10 ± 5.40^{dc}	21.04 ± 0.22^{dc}
Treatment group (400mg/kg) + CCl_4	135.00 ± 20.50^{e}	125.33 ± 5.36^{ed}	32.82 ± 3.33^{ec}	21.41 ± 0.56^{ec}
Extract group (200mg/kg)	92.33 ± 10.53^{ae}	41.67 ± 4.10^{f}	12.13 ± 2.30^{a}	48.89 ± 3.78^{f}
Extract group (400mg/kg)	77.50 ± 18.50^{ae}	50.50 ± 8.62^{af}	9.66 ± 4.02^{a}	56.73 ± 0.82^{a}
Extract group (800mg/kg)	77.00 ± 10.69^{a}	51.00 ± 8.62^{af}	7.18 ± 0.90^{a}	55.32 ± 0.54^{a}

Values are presented as mean ± SEM, n = 5
Values with different superscript vertically down the group are significantly different from one another at P < 0.05. Values with the same superscript are not significantly different from one another.

Effect of the Methanolic Leaf Extract of *Punica granatum L.* on some Biochemical Parameters (Kidney Function Test) in serum of Rats exposed to Carbon tetrachloride

Table 4 shows that Carbon tetrachloride caused an increase in the serum Urea and Creatinine levels and abnormalities (Rise and fall) in the Electrolyte levels compared to control. The plant extract

was able to restore the concentrations to levels similar to that of the control group at different doses of the extract administration.

Table 4: Effect of the Methanolic Leaf Extract of *Punica granatum* Biochemical Parameters (Kidney Function Test) on Wistar Strain Albino Rats exposed to CCl_4 after 28 days of oral administration.

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Na^+	K^+	HCO_3^-
Normal control group	10.74 ± 1.22^{a}	0.31 ± 0.21^{a}	139.33 ± 0.33^{a}	3.10 ± 0.06^{a}	26.00 ± 0.58^{a}
Negative control group (CCL_4)	47.22 ± 6.10^{b}	1.61 ± 0.17^{a}	136.50 ± 0.29^{b}	3.50 ± 0.06^{b}	20.00 ± 0.58^{b}
Positive control group (standard drug silymarin) + CCl_4	30.17 ± 2.21^{c}	2.96 ± 1.97^{cb}	143.50 ± 0.29^{c}	3.68 ± 0.20^{cb}	23.00 ± 0.58^{c}
Treatment group (200mg/kg) + CCl_4	22.82 ± 2.32^{dc}	0.74 ± 0.08^{a}	138.50 ± 0.29^{ad}	3.00 ± 0.06^{a}	25.50 ± 0.58^{ac}
Treatment group (400mg/kg) + CCl_4	20.72 ± 1.20^{ac}	0.70 ± 0.04^{a}	139.00 ± 0.58^{a}	4.40 ± 0.11^{d}	22.50 ± 0.87^{dc}
Extract group (200mg/kg)	15.27 ± 1.38^{ac}	0.52 ± 0.09^{a}	137.00 ± 1.15^{db}	3.90 ± 0.06^{ec}	25.50 ± 0.29^{a}
Extract group (400mg/kg)	13.31 ± 0.00^{ac}	0.33 ± 0.10^{a}	143.00 ± 1.00^{ec}	3.45 ± 0.15^{ab}	22.50 ± 1.50^{ebc}
Extract group (800mg/kg)	15.01 ± 1.49^{ac}	0.69 ± 0.15^{a}	138.50 ± 0.87^{ad}	4.45 ± 0.14^{fd}	25.00 ± 1.15^{ac}

Values are presented as mean ± SEM, n = 5
Values with different superscript vertically down the group are significantly different ($p < 0.05$)

Effect of the Methanolic Leaf Extract of *Punica granatum* on Hematological parameter (Hemoglobin) on serum of Rats exposed to Carbon tetrachloride

Administration of Carbon tetrachloride caused a significant decrease in the hemoglobin level due to damage caused to the cells by either inhibiting or decreasing the synthesis of the hemoglobin. The methanolic leaf extract of *Punica granatum L.* thus increased the hemoglobin levels to a value similar to that of the control group ($P<0.05$) by deteliorating the effect of carbon tetrachloride as shown in Figure 1.

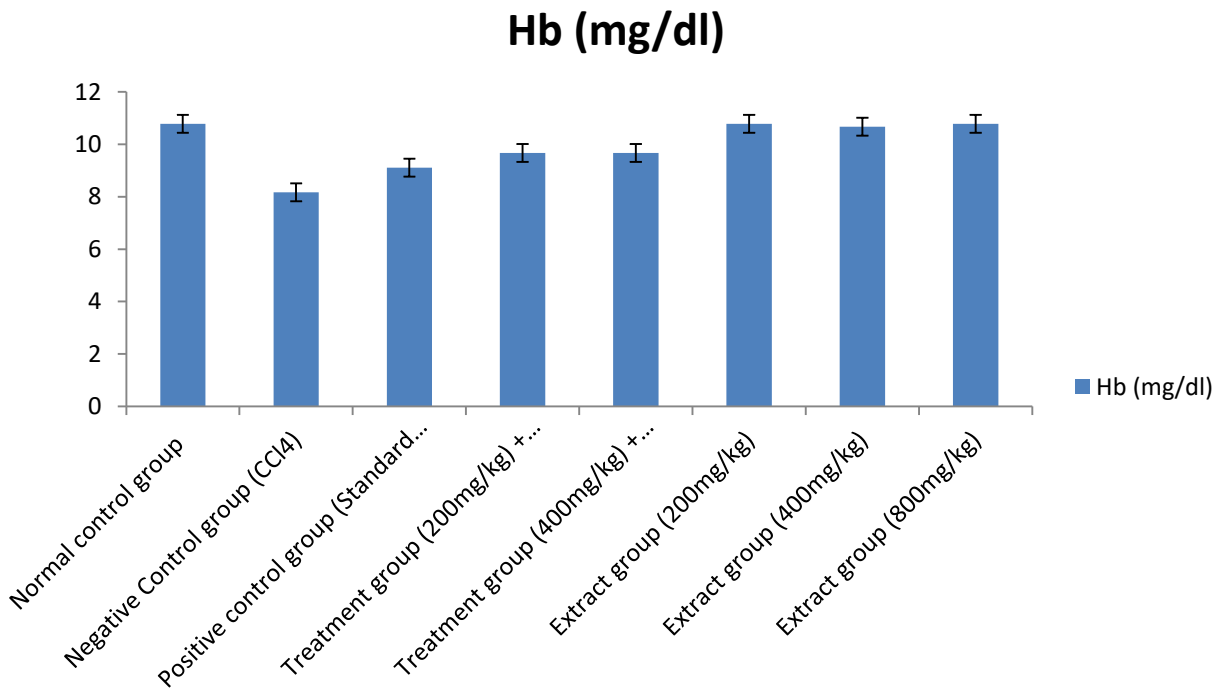

Figure 1.0
Effect of *Punica granatum* Methanolic Leaf Extract on Hematological parameter (Hemoglobin) in Rats exposed to carbon tetrachloride after 28days of extract administration.

Effect of the Methanolic Leaf Extract of *Punica granatum* on Organ Weight (Liver and Kidney).
Table 5 showed that there is significant difference in all the groups.

	Liver		Kidney	
Groups	Absolute Organ Weight	Relative Organ Weight	Absolute Organ Weight	Relative Organ Weight
Normal control group	3.87±0.16[a]	3.17±0.21[a]	0.89±0.05[a]	0.70±0.02[b]
Negative control group (CCL_4)	4.28±0.34[a]	3.21±0.06[b]	0.75±0.12[a]	0.56±0.01[a]
Positive control group (standard drug silymarin) + CCl_4	4.07±0.42[a]	2.91±0.07[b]	0.71±0.09[a]	0.50±0.01[b]

Treatment group (200mg/kg) + CCl_4	4.79±0.18[a]	4.48±0.21[a]	0.96±0.03[a]	0.89±0.04[a]
Treatment group (400mg/kg) + CCl_4	4.88±0.11[a]	4.25±0.12[a]	1.05±0.07[a]	0.90±0.01[b]
Extract group (200mg/kg)	3.71±0.16[a]	3.04±0.95[b]	0.98±0.09[a]	0.82±0.03[a]
Extract group (400mg/kg)	4.77±0.48[a]	3.32±0.46[b]	0.88±0.12[a]	0.55±0.00[a]
Extract group (800mg/kg)	3.41±0.13[a]	3.05±0.07[a]	0.79±0.05[a]	0.79±0.05[a]

Values are expressed as mean ±SEM, n=3
Values with different superscript along the raw are significantly different (P<0.

Plate 4.1: Histopathology of the Liver

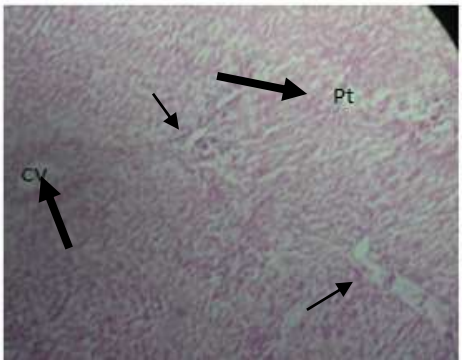

Plate I: Group A (Control group)
Photomicrograph of Rat liver showing Portal Triad (Pt) and Central Vein (CV) and clear sinosides. No histopathological effect. H&E ×100.

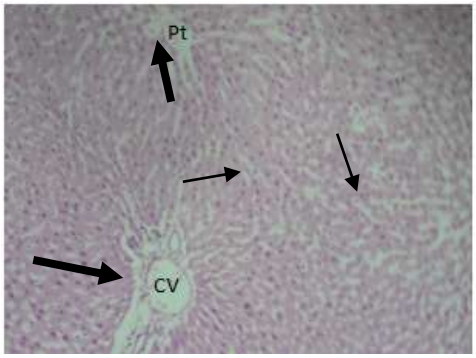

Plate III: Group C (Positive control group)

Photomicrograph of Rat liver showing Portal Triad (Pt) and Central Vein (CV) and mild sinosidal haemorrhage. H&E ×100.

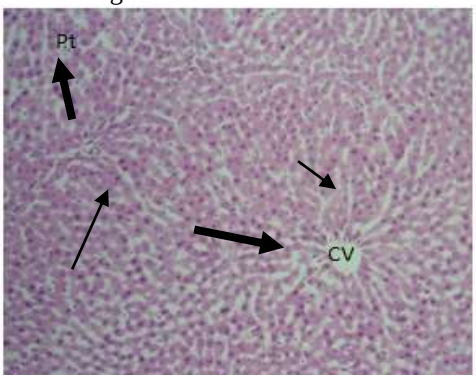

Plate V: GROUP E (Treatment group 400mg/kg)
Photomicrograph of Rat liver showing Portal Triad (Pt) and Central Vein (CV) and clear sinosides. No histopathological effect. H&E ×100.

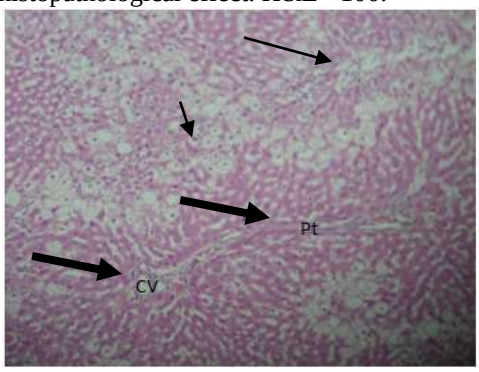

Plate II: Group B (Negative control group)

Photomicrograph of Rat liver showing Portal Triad (Pt), Central Vein (CV), Sinosidal haemorrhage and multi-binocular cells (small arrows) H&E ×100.

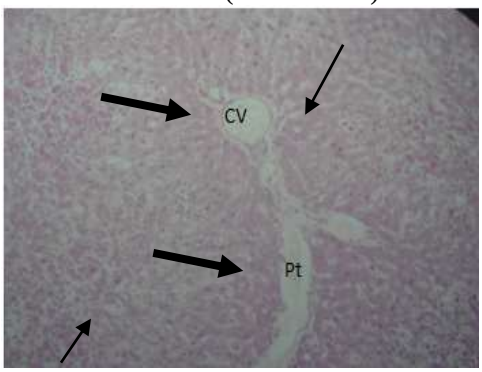

Plate IV: GROUP D (Treatment group 200mg/kg)
Photomicrograph of Rat liver showing Portal Triad (Pt) and Central Vein (CV) and clear sinosides. No histopathological effect. H&E ×100.

Plate 4.2: Histopathology of the Kidney

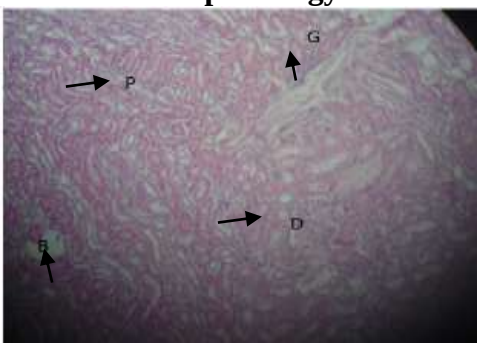

Plate I: Group A (Normal control group)
Photomicrograph showing normal features. That is no significant damage detected. Indicators: Bowman's capsule (B), glomerulus (G), proximal collecting tubule (P), distal collecting tubule (D). H&E ×100.

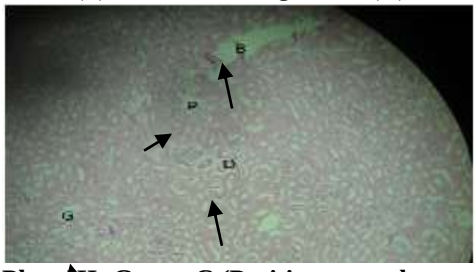

Plate III: Group C (Positive control group)
Photomicrograph showing mild interstitial inflammation, mild glomerula degeneration. Indicators: Bowman's capsule (B), glomerulus (G), proximal collecting tubule (P), distal collecting tubule (D). H&E ×100.

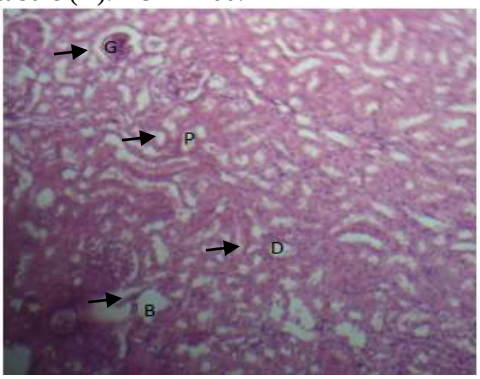

Plate V: Group E (Treatment group 400mg/kg)
Photomicrograph showing normal features. That is no significant damage detected. Indicators: Bowman's capsule (B), glomerulus (G), proximal collecting tubule (P), distal collecting tubule (D). H&E ×100.

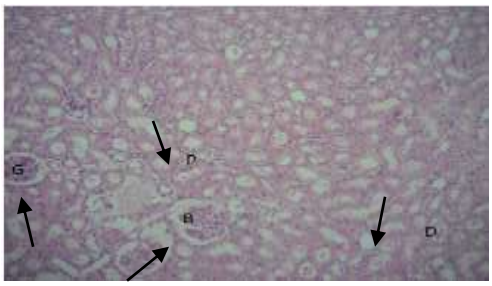

Plate II: Group B (Negative control group)
Photomicrograph showing interstitial inflammation, glomerula degeneration. Indicators: Bowman's capsule (B), glomerulus (G), proximal collecting tubule (P), distal collecting tubule (D). H&E ×100.

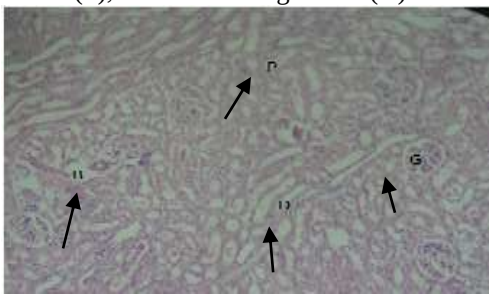

Plate IV: Group D (Treatment group 200mg/kg)
Photomicrograph showing normal features. That is no significant damage detected. Indicators: Bowman's capsule (B), glomerulus (G), proximal collecting tubule (P), distal collecting tubule (D). H&E ×100.

DISCUSSION

The leaf extract of *Punica granatum* Linn. did not show any toxic effects because doses up to 5000 mg/kg did not cause death or alter the behaviour of normal animals. According to Lorke's (1983), any substance that is not toxic at 5000 mg/kg is considered relatively safe. The plant extract was therefore considered to be safe at doses tested as shown in Table 1.

The value of the liver function test depends on the specificity for damage as well as their sensitivity (Okonkwo *et al.,* 1997, Sodipo *et al*., 2009). Although, serum levels of both AST and ALT become elevated when disease processes affect the liver integrity, ALT is the more liver specific enzyme and therefore generally more specific to changes in activity levels than AST (Kachmar and Moss, 1976; Sodipo *et al.,* 2009). Also, AST is highly concentrated in several tissues including the heart, muscle, liver, skeletal muscle and kidney while ALT has its highest concentration in the liver (Kaneko and Cornelius, 1971; Wilkinson, 1976; Okonkwo *et al.*, 1997; Nduka, 1997; Mayne, 1998; Atangwho *et al.,* 2007, Sodipo *et al.,* 2009). Therefore, a measure of ALT in serum is of greater diagnostic specificity in confirming or excluding liver damage. Since the decrease in ALT in this study was significant after 28 days of administration compared with the control, then there may not be any likelihood of liver damage by the methanolic leaf extract of *Punica granatum*.

The kidney is responsible for filteration of the blood, and when there is a problem with the kidney, urea and creatinine which are supposed to be filtered into the urine accumulates in the blood (Al-Yahya *et al.,* 2013, Mahmoud *et al.,* 2015). The increase in the levels of serum Creatinine could be due to impaired urine formation or excreation, irrespective of wether the causes are prerenal, renal or post renal in origin (Kaplan *et al.,* 1988; Gidado *et al.,* 2001).The concentration of urea and creatinine in the serum of Rats administered Carbon tetrachloride drastically increased. *Punica granatum* leaf extract lowered the serum urea and creatinine concentrations to levels similar to those of the control group.

Similarly, there were significant differences in Hb concentration as observed between control and treated groups as shown in Figure 1. Hb was determined in rats due to their roles in providing reliable information concerning haematological changes toxicants could cause. Hb concentration has been associated with health indices and is of diagnostic significance in routine clinical evaluation of the state of health (Muyibi *et al.,* 2000). According to (Onyeyilli *et al.,* 1998), administration of an agent can result in loss of blood cells and/or inhibition of blood cell synthesis and decrease in such hematological parameters in experimental animals has been associated with anemia. The above result suggests the nontoxicity of *Punica granatum* leaf extract in Rats. A similar observation was reported by (Ping *et al.,* 2013), after oral administration of *Euphorbia hirta, Cacia papaya, Petrooselinum crispum, and Lygodium flexuosum.*

There were no significant changes in organ weight and relative organ weight of liver and kidney with respect to the body weight as well (Table 5). (Kluwe, 1981) documented that the increase in

organ weight had been observed to be a relative sensitive indicator of nephrotoxicity. Thus, *Punica granatum* leaf extract did not induce any toxic effect on the liver and kidney going by this indicator. Histopathological studies revealed no abnormalities in liver and kidney in treated Rats. The liver tissue displayed normal hepatocytes without any enlargement in sinusoidal vein, central vein, and portal triad in all treated groups compared to control (Plate 1). Similar type of observation was also seen by (Bello *et al.*, 2016) in Rat liver. Kidney micrograph revealed normal architecture of glomerulus and Bowman's capsules with no degeneration, necrosis, or inflammation (Plate 2), which are comparable with the study made by (Ping *et al.*, 2013) and (Nabukenya *et al.*, 2014). Thus, histological evaluation indicated that the extract did not have any adverse effect on morphology of the tissues and these observations supported the biochemical results mentioned above. Therefore, it is concluded that *Punica granatum* leaf extract did not produce any toxic effect in Wistar Albino Rats.

CONCLUSIONS

This study shows that the methanolic leaf extract of *Punica granatum* can ameliorate liver and kidney damage posed by exposure to Carbon tetrachloride.

Recommendation

It is recommended that further studies should be carried out to on the hematological effects of this plant.

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