(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

Reversibility of the Biochemical Abnormality Linked to CCl_4-Induced Hepatotoxicity by Extracts/Fractions of *Tapinanthus bangwensis* and *Moringa oleifera* Leaves

Ihegboro Godwin O[1], Ononamadu Chimaobi J[1], Ihegboro Sandra N[2]and Jeph-Rolland Ifeanyichukwu,.C[1]*

[1]*Department of Biochemistry and Forensic Science, Faculty of Science, Nigeria Police Academy, Kano, Nigeria*

[2]*Department of Biochemistry, Faculty of Clinical Sciences, University of Lagos, Nigeria*

Abstract

Whenever toxicants are introduced into organisms, detrimental changes in biochemical indices are noticed, and requires urgent intervention. This study investigated the effects of the extracts/fractions of *Tapinanthus bangwensis* and *Moringa oleifera* leaves in carbon tetrachloride (CCl_4) induced toxicity in Wistar rats. The changes in the blood chemistry and reno-lipidemic parameters were assessed using haematopoietic method and spectrophotometer respectively. In the treated groups (TGs), the result demonstrated a decrease in the concentrations of creatinine, urea and serum electrolytes (Na^+, K^+, HCO_3^-, and Cl^-) compared to the untreated group (UTG). In addition, the result showed a decline in the levels of low-density lipoprotein (LDL-c), cholesterol and triglyceride in the treated group. However, high-density lipoprotein (HDL-c) and the haematological parameters progressively increased after treatment with the plans' extracts/fractions. According to the outcome of the kidney histology, the rats treated with the column fraction (CFTG) had normal glomeruli and Bowman's capsules, while those treated with CF3 and CF5 had moderate glomerular degenerative features, but the UTG had severe hydropic glomerular degeneration and obliterated convoluted tubular lumen. In comparison with the UTG, the result showed that the hepatic cells in the normal group and the CFTGs were well distributed. In conclusion, the administration of the plants' extracts/fractions may likely provide medicinal benefits in ameliorating CCl_4-induced toxicity in rat model.

Keywords: *Tapinanthus bangwensis*, *Moringa oleifera*, Biochemical indices, Haematology, Histology, Toxicants.

Email: *goihegboro@polac.edu.ng*

Received: July 12, 2023
Revised: August 8, 2023
Accepted: September 10, 2023
About the Journal: SJANS is a multidisciplinary science journal and the official journal of the Faculty of Science, Nigeria Police Academy, Kano, Nigeria

1.0 Introduction

Nature has bestowed upon man a plethora of medicinal plants with vast therapeutic purposes. In developing nations including Nigeria, about 87% of the human populace rely on plant for their therapy in cases where conventional treatment is unavailable, unaffordable, inaccessible and expensive (WHO, 2001; Mahmond *et al*., 2012). The positive therapeutic outcome attributed to medicinal plants is due to the great bioactive compounds diversity, which makes them a possible source for drug formulation (Lawal *et al*., 2015a; Piero *et al*., 2017). Several hepatotoxic agents including carbon tetrachloride have shown potentials as liver destroyer, affecting liver metabolism with consequential changes/abnormalities in the liver's biochemical parameters, renal parameters and blood chemistry, thereby providing vital information on the existence of oxidative stress factors, necrosis, and inflammation of visceral organs (Jurcik *et al*., 2007; Betancourt-Alonso *et al*., 2011). Carbon tetrachloride is a liver metabolite that generates trichloromethyl free radicals that bind covalently to membrane polyunsaturated lipids increasing lipid peroxides which induce oxidative stress with the resultant effect of pathological changes and damage (AL-Seeni *et al*., 2016, Jan *et al*., 2016, Yang *et al*., 2015, Kyung *et al*., 2007). Due to the enormous financial cost involved with organ transplantation and the adverse consequences associated with synthetic drugs, alternative remedy which lies in the use of medicinal plants has become necessary, because they have shown effectiveness in the treatment and management of a wide range of diseases with less toxicity. Many medicinal plants have been investigated for their anti-hepatotoxic property, however, there is the need for further Pharmacognostic

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

investigation. This study investigated the anti-hepatotoxic potentials of *Tapinanthus bangwensis* and *Moringa oleifera* in CCl_4-induced hepatotoxicity in Wistar rats, knowing that studies have demonstrated that these plants possess ethno-pharmacological properties like anti-inflammatory, anticancer, antimicrobial, hypertensive, anti-diabetic, antioxidants, but not limited to the aforementioned (Ihegboro *et al*., 2020). Taxonomically, African mistletoe (*T. bangwensis)* belongs to the *Loranthaceae* family, and a citrus-dependent plant. "The healing tree" is how the plant is referred to in English, while the core tribes in Nigeria (Hausa, Yoruba and Igbo) call it, *Kauci (Kanchi)*, *Afomo onisana* and *Awurusie*. With regards to *M. oleifera*, it is a drought-resistant deciduous shrub in the family of *Moringaceae*, that is designated as "miraculous tree or drumstick tree in English, and *Bagaruar maka (masar))*, *Idagbo monoye* and *Okochi egbu* in the Hausa, Yoruba and Igbo dialect respectively (Ihegboro *et al*., 2020).

2.0 Materials and Methods

2.1 Identification of the Plant Materials

The plant materials (*T. bangwensis* and *M. oleifera* leaves) were harvested in June, 2022, authenticated in the Pharmacognosy Department, University of Lagos and deposited in the University's herbarium with catalogue numbers of LUH 3856 and 4123 respectively.

2.2 Maceration extraction of Plant Materials

The steps included washing the leaves, air-drying for 21 days, and grinding them into powdered masses. About 1000 g each of the powdered leaves were dissolved in 2.0 L of 99% methanol, which were occasionally stirred and left for 2 days. A solid methanol extracts of *T. bangwensis* (MeCE 1; 62.5 g) and *M. oleifera* (MeCE 2; 75.0 g) were recovered after the filtrates were evaporated to concentrate at ambient room temperature.

2.3 Solvent-Partitioning Extraction

The methanolic extract (approximately 20g) of *T. bangwensis* was further extracted using different resulting mixtures (ethylacetate-water and acetone-water) in a separating funnel. After shaking and being allowed to stand, the organic layers were collected, concentrated, and the solid extracts recovered were labelled as ETF 1 (8.07 g) and ACF 1 (5.74 g). Following the above procedure, the methanolic extract of *M. oleifera* yielded ETF 2 (6.32 g) and ACF 2 (4.18 g) respectively.

2.4 Column Chromatographic Extraction

The pharmacologically active extract (ETF 1) was further extracted by the Vivex *et al*., 2016 method. A column glass (r = 1.2cm, h = 6.0 cm) was packed with 100 g of silica gel (mesh size: 60-120) dissolved in hexane solvent, and layered with a prepared slurry particle. Different eluting mixtures of varying concentrations were prepared from solvents of various polarity index (hexane, ethylacetate, methanol and water) was used for the extraction. Column fractions numbering about one hundred were collected and concentrated by evaporation at room temperature. Applying the thin layer plate (TLP), six column fractions were recovered based on the retention factor.

2.5 Experimental Animals

The OECD, (2000) guideline was adopted. The healthy male Wistar rats (90 g in weight) were acclimatized for 2 weeks under standard environmental conditions (Temperature, 25^0C; Photo cycle: 12:12hour light-dark and Humidity: 55%), and afterward, were induced with 120mg/kg body weight of carbon tetrachloride solvent in olive oil.

2.6 Experimental Design

The rats were classified as follows:

Group A: Rats received food and potable water (Normal group)

Group B: Rats were exposed to 120 mg/kg BW CCl_4 in olive oil intraperitoneal (Positive control group)

Group C: Rats were treated with 250 mg/kg BW methanol extract of *T. bangwensis* daily for 7 days after exposure to 120 mg/kg BW CCl_4 once.

Group D: Rats were treated with 250 mg/kg BW ethylacetate extract of *T. bangwensis* daily for 7 days after exposure to 120 mg/kg BW CCl_4 once.

Group E: Rats were treated with 250 mg/kg body BW acetone extract of *T. bangwensis* daily for 7 days after exposure to 120 mg/kg BW CCl_4 once.

Group F: Rats were administered with 250 mg/kg BW methanol extract of *M. oleifera* daily for 7 days after exposure to120 mg/kg BW CCl_4 once.

Group G: Rats were administered with 250 mg/kg BW ethylacetate extract of *M. oleifera* daily for 7 days after exposure to 120 mg/kg BW CCl_4 once.

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

Group H: Rats were administered with 250 mg/kg BW acetone extract of *M. oleifera* daily for 7 days after exposure to 120 mg/kg BW CCl_4 once.

Note: The above experimental design was followed to evaluate the pharmacological activity of the six column fractions administered at 100mg/kg BW for 7days.

2.7 Blood preparation for Analyses

At the end of the 7th day of oral treatment, the animals were subjected to a 12-hour fast prior to sacrifice. The blood collected were centrifuged at a speed of 3000 revolutions per minute for 5mins to obtain a supernatant.

2.8 Determination of Kidney Function Indices

The urea concentration was evaluated by the method described by Fawcett *et al.*, 1960; while the concentrations of creatinine, chloride ion (Cl^-) and potassium ion (K^+) were determined by Henry, 1974. Serum bicarbonate (HCO_3^-) and sodium ion (Na^+) concentrations were estimated from the procedure outlined by Orrester *et al.*, 1960 and Trinder 1951.

2.9 Determination of Lipid Profile and Haematological Parameters.

The method of Bachoric, 2000 was used for the estimation of the low-density lipoprotein (LDL-c), whilst the method of Albers *et al*., 1978 was adopted for high density lipoprotein (HDL-c) and triacylglycerol determination, the Allain *et al*., 1974 method was applied for the estimation of cholesterol (CHOL) level. Finally, the Hematology analyzer evaluated the various haematological parameters (Mindary BC 2800 3 Diff CBC Machine, China).

2.10 Histological Examination of the rats' organs

The organs removed from the animals were firstly washed in physiological saline solution, followed by fixation in 10% neutral buffered formalin solution. They were further dehydrated in ethanol (50–100%), cleared in xylene and embedded in paraffin. A cross sectional area of 5µm thickness was cut out, stained with hematoxylin and eosin dye and examined under a microscope (Olympus IX71) for possible pathological changes. The above procedure was obtained from the method of Mohammad *et al*., 2018.

Statistical Analysis

Data were obtained in triplicates and analyzed as Mean ± SD using SPSS (version 23.0). Further analyses were carried out using ANOVA and Tukey multiple comparison test (TMCT) at significance limit considered at $p < 0.05$

3.0 Equations

$$\text{Creatinine} = \frac{\Delta\text{Abs of Test}}{\Delta\text{Abs of Std}} \times \text{Conc of Std}$$

$$\text{Urea} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std}} \times \text{Conc of Std}$$

$$\text{Electrolytes} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std}} \times \text{Conc of Std}$$

$$\text{Lipid level} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std}} \times \text{Conc of Std}$$

Where, ΔAbs stands for change in absorbance
Std stands for standard

4.0 Results and Discussion

4.1 Anti-renal effects of the extracts/ fractions of the plants.

Table 1 reflected the effect of the samples on renal parameters. Both the Urea and creatinine levels increased in the untreated group (UTG) compared to the normal group (NG) and the treated group (TG). There was no significant difference between the UTG and the TGs, rather, a significant difference existed between NG, TGs and UTG. In comparison with UTG, a declined in the levels of bicarbonate (HCO_3^-) and potassium (K^+) was recorded in NG and TG. Additionally, significant difference existed in all the groups, except between NG and UTG. Furthermore, the levels of sodium (Na^+) and chloride (Cl^-) in the NG and TGs decreased compared to UTG, as no considerable difference occurred between the UTG and TGs but a significant difference was found between NG and other groups. Considering Table 4, decreased in the levels of urea and creatinine was observed in the TGs and NG compared to UTG, in addition, a significant differences existed in all the groups. Finally, NG and TGs had a reduction in Na^+ level compared to UTG, moreover, a significant difference existed between the UTG and the TGs. The activities of the kidney ranges from hormonal secretion, homeostasis and excretion (Mishral, 2012), nonetheless, it becomes damaged when exposed to carbon tetrachloride including the hepatic cell (Mohamed *et al*., 2019, Hasan *et al*., 2018). As explained above, increased in the serum electrolytes (Na^+, Cl^- and K+), indicated a predisposal risk outcome such as myocardial infarction, hypertension and stroke as well as increased renal water retention occasioned by increased water transport from the intracellular

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

to extracellular space or arterial water volume rise (Mohamed *et al.*, 2019). The decrease in the serum electrolytes suggested that the plants may have the capacity to improve tubular reabsorption, which had been attested to by previous report (Hasan *et al*., 2018). Both creatinine and urea are more kidney specific, although, they could serve as renal and liver indicators. It is worthy of note, that creatinine and urea are by-products of creatinine phosphate and protein metabolism respectively (Adedapo *et al*., 2009). An increase in creatinine and urea levels above the clinically recommended value, may indicate glomerular dysfunction, defective creatinine biosynthesis, increased amino acid deamination, renal disorders, muscular protein depletion and excretion dysfunction. Therefore, creatinine and urea reduction after oral treatment, indicated that the plants may increase glomerular permeability transition (GPT), thereby resulting to an increase in renal clearance (Banfi, 2006, AL-Seeni *et al*., 2016).

4.2 Haematological potential of the extracts/fractions of the plants.

The haematological result as shown in Table 2, indicated an increase in the concentrations of haematological parameters in the TGs compared to the UTG. However, a considerable difference ($p < 0.05$) among the different groups was observed. The haematological activity of the column fractions (CFs) as shown in Table 3, indicated increase in the blood cell concentrations in the TGs compared to the UTG. For instance, the white blood cells (WBCs) and their differentials increased in the TGs more than in the UTG. In addition, red blood cells (RBC), haemoglobin (HGB), packed cell volume (HCT), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin count (MCHC) were increased in the TGs compared to the UTG. Finally, there was a reduction in the concentration of platelet count (PLT) and mean platelet volume (MPV) compared to the TGs. A substantial significant difference ($p < 0.05$) between the NG and other groups were observed, as well as between the UTG and the TGs. Haematological indices, do not only provide information on the physiological and nutritional status, it also indicated the pathological condition of an organism (Jurcik, 2007, Betancourt-Alonso *et al.*, 2011, Odeghe *et al*., 2012). Experimental evidence suggested that exposure to drugs, plant extract and carbon tetrachloride, cause considerable changes (decrease) in haematological parameters (Fatima 2017, Tilak *et al*., 2007, Essawy *et al*., 2010, Ononamadu *et al*., 2020). For example, low immunoglobulin (WBC) and lymphocytic count, revealed defective immune response (Lawal *et al*., 2015b), whilst decrease in differential RBC, signified anemic conditions (Peters *et al*., 2011, Ozkan *et al*., 2012). However, the present result reflects that the plants had immunomodulatory effect by possibly increasing antibody generation during phagocytosis (Bashiru *et al*., 2005a, Okunlola *et al*., 2012) and stimulating erythropoietin activity thereby promoting erythropoiesis (Mishral, 2012, Meral, 2013). Furthermore, the decrease in PLT and MPV, may result in either action of uremic toxins following renal failure or underproduction of platelet or platelet over-storage (Witters *et al*., 2008). In addition, PLT and MPV reduction could indicate defective blood clotting mechanism with consequential hemorrhagic effect (Lawal *et al*., 2005b). Nevertheless, the plants exhibited thrombopoietic effect by improving both PLT and MPV levels as shown in the documented results.

4.3 Anti-lipidemic activity of the fractions of ethylacetate extract of *T. bangwensis* leaves.

As shown in Table 4, the level of low density lipoprotein (LDL-c) decreased in the NG and TGs compared to UTG, however, the effect of CF2 was higher. Also, cholesterol (CHOL) and triglyceride (TRIG) was reduced remarkably in the NG and TGs compared to UTG. Nevertheless, CHOL and TRIG increased appreciably in the groups treated with CF1 and CF2 respectively compared to the other treated groups. In comparison with UTG, the high density lipoprotein (HDL-c) increased in the NG and TGs, except in the group treated with the CF 0. An increase in TRIG, CHOL and LDL-c, and a decrease in HDL-c levels, arises due to the inhibition of lipid β-oxidation, increased triglyceride biosynthesis from acetyl COA, increased fatty acid esterification and decreased lipid excretion (Weber *et al*., 2003, Fernadez, 2005) or active translocation of fat from adipose tissues to the hepatocyte domain (Weber *et al*., 2003) The decreased in these lipid parameters, may be attributed to the antioxidant role of the plants via lipid chelation, and the inhibition of lipid reabsorption by insoluble complex, thereby improving lipid excretion.(Ihegboro *et al*., 2020, Iheanacho *et al*., 2008, Ihegboro *et al*., 2020).

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

Table 1: Effects of the plants extracts on Kidney Function Indices in CCl_4-induced Wistar rats.

Parameters/	UREA (mM^{-1})	CREAT (mg/dL)	HCO_3(mEq/l)	CL^-(mEq/l)	K^+(mEq/l)	Na^+(mEq/l)
Normal	25.67±2.08^{a}	0.87±0.15^{a}	22.00±1.00^{a}	108.30±1.16^{a}	4.97±0.95^{a}	145.70±5.86^{a}
CCl_4 only	35.33±2.08^{bc}	1.37±0.21^{ac}	26.67±2.52^{ac}	116.70±3.06^{bc}	5.73±0.29^{bc}	159.00±5.57^{ac}
CCl_4+MeCE1	24.00±3.61^{bc}	0.80±0.10^{bc}	23.67±1.53^{bd}	107.30±3.06^{bc}	4.73±0.64^{bd}	146.70±7.23^{bd}
CCl_4+ETF1	19.67±2.52^{bc}	0.70±0.10^{bc}	23.66±1.16^{bd}	105.30±0.58^{bc}	4.80±0.26^{bd}	138.00±4.58^{bc}
CCl_4+ACF1	19.33±4.93^{bc}	0.77±0.12^{bc}	24.33±1.16^{bd}	103.00±1.73^{bc}	4.53±1.05^{bd}	140.30±3.79^{bc}
CCl_4+MeCE2	24.00±5.00^{bc}	0.90±0.10^{bc}	24.33±1.16^{bd}	107.00±2.00^{bc}	4.53±0.12^{bd}	143.30±2.89^{bc}
CCl_4+ETF2	21.33±1.53^{bc}	1.00±0.10^{bc}	25.00±1.73^{bd}	104.00±5.57^{bc}	4.40±0.36^{bd}	141.00±2.00^{bc}
CCl_4+ACF2	23.67±5.51^{bc}	0.90±0.10^{bc}	24.33±0.58^{bd}	107.70±4.93^{bd}	4.70±0.87^{bd}	142.30±3.22^{bc}

The triplicate values were expressed as Mean ± SD. Same superscript indicated no significant difference between the normal group and other groups and between the CCl_4 untreated group and the CCl_4 treated groups at $p< 0.05$. **Keys**: MeCE 1=Methanol extract of *T. bangwensis*, ETF 1=Ethylacetate extract of *T. bangwensis*, ACF 1= Acetone extract of *T. bangwensis*, MeCE 2=Methanol extract of *M. oleifera*, ETF 2=Ethylacetate extract of *M. oleifera* and ACF 2= Acetone extract of *M. oleifera*

Table 2: Effects of the plants extracts on Haematological Parameters in CCl_4 induced Wistar rats.

Parameters	Normal	CCl_4 only	CCl_4+Me CE1	CCl_4 +ETF1	CCl_4 +ACF1	CCl4+MeCE2	CCl_4 +ETF2	CCl_4+ ACF2
WBC(10^9L)	13.93±2.76^{a}	12.63±5.95^{bc}	17.87±5.20^{bd}	16.00±6.09^{bd}	14.73±1.36^{bd}	14.20±3.86^{bd}	15.53±3.68^{bd}	13.13±3.58^{bd}
RBC(10^{12}L)	6.55±2.34^{a}	5.77±1.11^{bc}	6.16±0.20^{bd}	6.95±0.58^{bd}	6.48±0.26^{bd}	7.04±0.61^{bd}	6.51±0.21^{bd}	8.67±0.44^{bc}
LYMP (%)	52.03±13.88^{a}	34.77±8.47^{bc}	30.50±1.39^{ad}	53.67±3.62^{ad}	50.10±4.59^{bd}	51.43±1.91^{bd}	41.00±10.28^{bd}	24.60±1.05^{bd}
HGB(g/dl)	17.70±1.55^{a}	13.17±2.24^{bc}	9.70±0.35^{bd}	15.07±1.59^{bd}	14.00±0.69^{bd}	14.53±1.25^{bd}	13.67±1.95^{bd}	17.10±1.73^{bd}
MCH(pg)	25.03±1.44^{a}	18.53±1.66^{bc}	20.03±2.14^{bd}	19.00±1.39^{bd}	19.47±1.14^{bd}	20.60±2.76^{bd}	23.97±1.18^{bd}	30.13±8.38^{bd}
MCHC(g/dl)	35.93±6.99^{a}	35.10±5.01^{bc}	26.77±8.89^{bd}	36.40±9.01^{bd}	35.27±9.64^{bd}	40.83±8.18^{bd}	32.00±4.78^{bd}	26.37±6.08^{bd}
MCV(fL)	54.37±8.61^{a}	50.50±7.63^{bc}	57.10±3.64^{bd}	53.83±8.26^{bd}	57.37±11.68^{bd}	50.20±7.16^{bd}	63.23±8.06^{bd}	60.70±1.68^{bd}
PLT(L)	841.00±42.58^{a}	536.33±143.31^{bc}	433.33±295.60^{bd}	207.67±78.14^{ad}	731.00±167.15^{ad}	540.33±54.88^{bd}	767.00±322.86^{bd}	1151.67±105.08^{bc}
MPV(fL)	6.60±1.21^{a}	6.70±0.89^{bc}	6.30±0.17^{bd}	6.07±1.36^{bd}	5.87±1.01^{bd}	6.23±1.08^{bd}	6.40±1.70^{bd}	7.63±0.55^{bd}
PCT (%)	0.41±0.35^{a}	0.30±0.06^{bc}	0.28±0.19^{bd}	0.37±0.23^{bd}	0.38±0.03^{bd}	0.34±0.08^{bd}	0.23±0.22^{bd}	0.22±0.16^{bd}

The triplicate values were expressed as Mean ± SD. Same superscript indicated no significant difference between the normal and other groups and between the CCl_4 untreated group and between the CCl_4 untreated group and the CCl_4 treated groups at $p< 0.05$.. **Keys:** WBC=White blood cell; RBC = -Red blood cell; LYMP=Lymphocyte; HGB=Hemoglobin; MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Count; MCV=Mean cell volume; PLT=Platelet count; MPV=Mean platelet volume; PCT= Plateletcrit.

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

Table 3: Haematological potential of the fractions of ethylacetate extract of *T. bangwensis leaves* in CCl_4 induced Wistar rats.

Parameters	Normal	CCl_4 only	CCl_4+F0	CCl_4+F1	CCl_4+F2	CCl_4+F3	CCl_4+F4	CCl_4+F5
WBC x 10^9 (L)	10.77±1.85 ^{a}	7.90±0.30 ^{bc}	8.83±1.88 ^{bc}	8.4±0.66 ^{bd}	5.97±2.23 ^{bd}	4.67±1.70 ^{ad}	8.57±0.42 ^{bd}	7.37±0.92 ^{bd}
RBC x 10^{12}(L)	5.42±1.09^{a}	3.79±0.21^{bc}	3.63±0.57^{bd}	5.80±1.04 ^{bd}	4.65±1.13^{bd}	4.50±1.13^{bd}	4.15±0.17 ^{ad}	3.05±0.87^{bd}
LYMP (%)	79.8±2.67 ^{a}	66.97±2.95 ^{bc}	37.87±3.56 ^{bd}	67.10±24.4 ^{bd}	67.43±25.15 ^{bd}	75.3±7.38 ^{bd}	77.27±8.79 ^{bd}	97.8±6.82 ^{bd}
HGB (g/L)	12.23±0.40 ^{a}	12.30±0.90 ^{bc}	10.30±0.70 ^{bd}	12.07±0.68 ^{bd}	12.3±0.56 ^{bd}	10.33±1.92 ^{bd}	12.13±0.40 ^{bd}	9.57±2.77 ^{bd}
MCH (pg)	27.03±3.67 ^{a}	23.75±4.34 ^{bc}	28.73±3.99 ^{bd}	21.33±5.19 ^{bd}	26.47±1.95 ^{bd}	23.53±4.54 ^{bd}	29.2±0.35 ^{bd}	31.27±0.40 ^{bd}
MCHC(g/l)	32.4±1.47 ^{a}	29.7±2.2 ^{bc}	31.73±1.45 ^{bd}	29.93±3.25 ^{bd}	32.2±1.06 ^{bd}	32.93±2.71 ^{bd}	33.67±0.71 ^{bd}	31.13±0.63 ^{bd}
MCV (fl)	71.07±8.64 ^{a}	81.25±8.35 ^{bc}	87.53±9.17 ^{bd}	70.93±9.24 ^{bd}	82.3±4.33 ^{bd}	71.71±8.82 ^{bd}	86.87±1.29 ^{bd}	94.67±6.30^{ad}
PLT (L)	924.69±101.11 ^{a}	776.5±6.5 ^{bc}	809.33±72.14 ^{bd}	980±67.10 ^{bd}	1076.3±44.73 ^{bd}	975.67±50.72 ^{bd}	969.67±72.18 ^{bd}	316.00±20.40 ^{bd}
MPV (Fl)	7.27±0.15 ^{a}	5.53±4.79 ^{bc}	7.65±0.05 ^{bd}	7.73±0.21 ^{bd}	7.3±0.36 ^{bd}	7.87±0.25 ^{bd}	7.33±0.45 ^{bd}	9.07±0.68 ^{bd}
PCT (%)	0.42±0.37 ^{a}	0.33±0.37 ^{bc}	0.59±0.01 ^{bd}	0.23±0.39 ^{bd}	0.41±0.36 ^{bd}	0.12±0.21 ^{bd}	0.44±0.38 ^{bd}	0.28±0.16 ^{bd}
HCT (%)	37.83±2.93 ^{a}	41.8±6.1 ^{bc}	32.4±0.8 ^{bd}	40.43±2.66 ^{bd}	38.3±0.51 ^{bd}	31.37±5.25 ^{bd}	35.97±1.31 ^{bd}	29.57±7.19 ^{bd}

The triplicate values expressed as Mean ± SD. Same superscript indicated no significant difference between the normal and other groups and between the CCl_4 untreated group and between the CCl_4 untreated group and the CCl_4 treated groups at $p< 0.05$. Keys: WBC=White blood cell; RBC=Red blood cell; LYMP=Lymphocyte; HGB=Hemoglobin; MCH=Mean corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Count; MCV=Mean cell volume; PLT=Platelet count; MPV= Mean Platelet volume; PCT=Plateletcrit; HCT= Packed cell volume

Table 4: Lipo-renal effect of the fractions of ethylacetate extract of *T. bangwensis leaves* in CCl_4 induced Wistar rats.

Parameters	LDL (mg/dL)	HDL (mg/dL)	CHOL(mg/Dl	TRIG(mg/dl)	CREAT(mg/Dl)	UREA(mg/dL)	Na^+(mEq/l)
Normal	51.2±3.80 ^{a}	146.00±6.95 ^{a}	211.53±13.85 ^{a}	164.13± 1.29^{a}	1.17±0.46 ^{a}	34.57±3.06 ^{a}	104.47±0.70^{a}
CCl_4 only	97.57±5.79 ^{bc}	129.83±5.09 ^{bc}	242.33±3.85^{bc}	175.67±9.74 ^{bc}	1.40±0.00 ^{bc}	39.27±2.37 ^{bc}	120.53±0.57^{bc}
CC l_4+F0	96.4±2.05 ^{bd}	126.7±6.84 ^{bd}	221.53±23.29 ^{bd}	166.55±8.46 ^{bd}	1.30±0.44 ^{bd}	32.13±1.85 ^{bc}	116.13±0.25^{bd}
CC l_4+F1	71.8±6.48 ^{bd}	140.9±6.99 ^{ad}	241.6±11.15 ^{bd}	170.73±6.13 ^{bd}	1.23±0.15 ^{bd}	38.33±1.62 ^{bd}	118.07±0.06^{bd}
CC l_4+F2	114.93±3.75 ^{bd}	134.83±9.57 ^{bd}	238.47±13.4 ^{bd}	173.53±2.68 ^{bd}	1.33±0.12 ^{bd}	33.73±2.05 ^{bd}	118.40±0.10^{bd}
CC l_4+F3	61.97±6.72 ^{bd}	135.70±4.68 ^{bd}	226.27±13.07 ^{bd}	173.43±12.89 ^{bd}	1.23±0.29 ^{bd}	38.67±3.00 ^{bd}	118.37±0.47^{bd}
CC l_4+F4	54.83±9.65 ^{bd}	133.13±4.76 ^{bd}	203.83±19.98 ^{bd}	161.47±1.85 ^{bd}	0.97±0.12 ^{bd}	33.07±1.67 ^{bd}	112.77±0.23 ^{bd}
CC l_4+F5	64.03±5.48 ^{bd}	136.83±4.02 ^{bd}	212.8±13.50 ^{bd}	157.10±8.64 ^{bd}	1.30±0.17 ^{bd}	34.43±1.50 ^{bd}	117.57±0.21^{bd}

The triplicate values were expressed as Mean ± SD. Same superscript indicated no significant difference between the normal and other groups and between the CCl_4 untreated group and the CCl_4 treated group at $p < 0.05$.

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

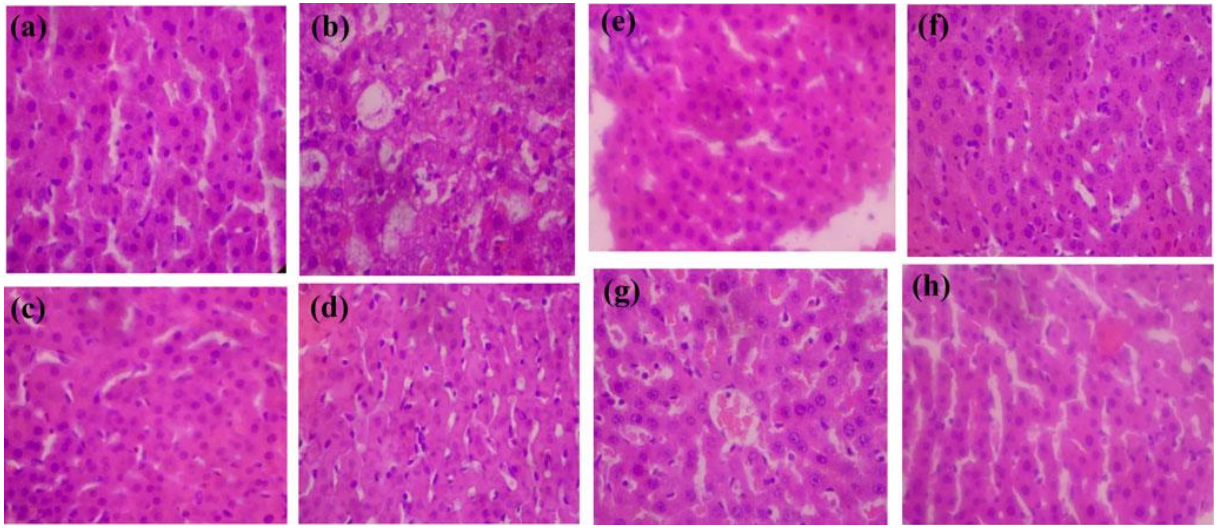


Figure 1: Photomicrographs indicating the effect of the fractions (CFs) of ethylacetate extract of *T. bangwensis leaves* on liver histology in CCl_4-induced Wistar rats (H & E 100). **Keys:** (a) Normal hepatocyte distribution and no fatty changes observed (normal group), (b) fatty changes (or liver steatosis) observed (positive control group), and (c - h) Normal hepatocyte distribution and no fatty changes observed in the CCl_4 treated group with CF0, CF1, CF2, CF3, CF4, and CF5.

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

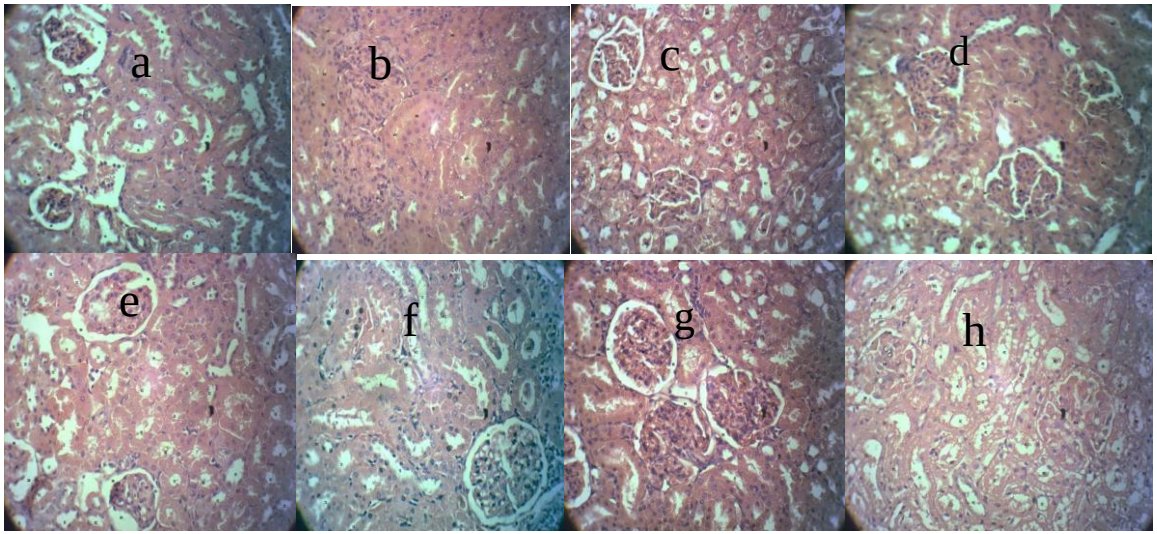


Figure 2: Photomicrographs indicating the effect of the fraction of ethylacetate extract of *T. bangwensis leaves* on kidney histology in CCl_4 induced Wistar rats. (H & E x 100) **Keys:** (a): Normal glomeruli, Bowman's capsule and convoluted tubules (Normal control) (b) Severe hydropic glomerular degeneration and obliterated convoluted tubular lumen (positive control group) and (c, e and g)): Normal glomeruli and Bowman's capsule with group treated with CF0, CF1, CF2 and CF4, (f and h): moderate tubular degeneration was observed in CF3 and CF5 respectively.

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

4.4 Effect of the fractions of ethylacetate extract of *T. bangwensis* leaves on the organ histology.

Figure 1, revealed an improved hepatocyte distribution without fatty changes in the NG and TGs as opposed to liver steatosis identified in the UTG. In Figure 2, severe glomerular, Bowman's capsule and convoluted lumen architecture was observed in the UTG compared to the normal glomeruli and Bowman's capsules that were seen in the TGs. Nonetheless, moderate tubular degeneration was observed in the CF3 and CF5 treated groups

5.0 Conclusion

Liver toxicity induced by toxicants, may be further compounded when treated with synthetic drugs. However, the present study demonstrated an improved activity in renal clearance/permeability, lipid excretion and haematopoiesis when the plants' extracts/fractions were administered compared to the CCl_4 untreated rats. The above outcome, may likely be attributed to the ability of the plants to stimulate hematopoietic and organ restorative activity. In conclusion, the resulting outcome, suggested that the plants may reverse pathological disorder induced by trichloromethyl free radicals in experimental rats.

Authors Contribution

Ihegboro Godwin Okwudiri conceptualized, supervised and prepared the manuscript, Ononamadu Chimaobi James analyzed the data while Ihegboro Sandra and Jeph-Rolland IfeanyiChukwu C assisted in the laboratory work. All authors revised and edited the manuscript, and then authorized the final submission.

Conflict of Interest

The authors declare no conflict of interest in this study.

Acknowledgements

Our sincere appreciation goes to Mr. Maicah Chijoke, for his immense technical contribution.

Funding

The authors declare that TETFund, Abuja provided financial support for this research

References

Adedapo, A.D.A., Osude, Y.O., Adedapo, A.A. (2009). “Blood pressure lowering effect of *Adenanthera pavonina* seed extract on normotensive rats,” *Records of Natural Products*. 3 (2), 82–89.

Albers, J.J., Warnick, G.R., Chenng, M.C. (1978) Quantitation of high-density Lipoprotein. Lipids. 13, 926-932.

Allain, C.C., Poon, L.S., Chan, C.S. (1974). *Enzymatic determination of total serum cholesterol*. Clin. Chem, 20 (4), 470-475.

AL-Seeni, M.N., EL-Rabey, H.A., Zamzami, M.A. (2016). The hepatoprotective activity of olive and *Nigella sativa* oil against CCl_4 induced hepatotoxicity in male rats. *BMC Complemt Altern. Med*. 16, 438.

Bachoric, P. (2000). *Measurement of Low-density Lipoprotein. In: Handbook of Lipoprotein Testing*. Rilai, Warnick and Dominiczak), 2nd edition, AACC press. 2000, 245-263.

Banfi, G., Del-Fabbro, M. (2006). Relation between serum creatinine and body mass index in elite athletes of difference sport disciplinees. *Br. J. Sport. Med*. 40, 675-678.

Bashiru, L., Shittu, O.K., Busari, O.K, Sani, S., Aizha, M.I.(2005a). Safety evaluation of giant African land snails (*Archachatina maginata*) haemolymph on haematological and biochemical parameters of albino rats. *J. Adv. Med. Pharmaceut. Sci*. 3, 122- 130.

Betancourt-Alonso, M.A., Orihuela, A., Aguirre, V., Vazquez, R., Flores-Perez, I. (2011). Changes in behavioural and physiological parameters associated with *Taenia pisifornis* infection in rabbits (*Oryctolagus cuniculus*) that may improve early detention of sick rabbits. *World Rabbit Sci*. 19, 21- 30.

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

Essawy, A.E., Hamed, S.S., Abdel-Moneim, A.M., Abou-Gabal, A.A., Alzergy, A.A. (2010). Roles of black seeds (*Nigella sativa*) in ameliorating CCl_4 induced hematotoxicity in Swiss albino rats. *J. Med. Plants. Res*. 4, 1977-1986.

Fatima, R., Tarek, R. (2017). Protective effects of *Teucrium polium* aqueous extract and ascorbic acid on haematological and some biochemical parameters against CCl_4 induced toxicity in rats, *Biomed. Pharmacotherapy*. 91, 43-48.

Fawcett, J.K., Scott, J.E. (1960). A rapid and precise method for the determination of urea. J. Clin. Pathol. 13, 156-159.

Fernandez, M.L., West, K.L. (2005). Mechanisms by which dietary fatty acids modulate plasma lipids. *J. Nutr*. 135(9), 2075-2078.

Hasan, S.Y., Gamal, A.S., Ahmed, I.F., Maged, S.A., Mohd, N.A., Attab, N.A., Mohammad, A.S. (2018). Protective role of Aerial parts of *S. villosa* Alcoholic extract against CCl_4 induced cardiac and renal toxicity in rats. *Int. J. Pharmacol*. 14(7), 1001-1009.

Henry, R.F. (1974). *Clinical Chemistry Principle and Techniques*, 2nd Ed., Harper and Row, Hagerstown. 608-712.

Iheanacho, K.M.E., Udebani, A.C., Ihegboro, G.O. (2008). Phytochemistry and effect of aqueous leaf extract of mistletoe (*Tapinanthus bangwensis*) on serum glucose and cholesterol concentrations in normoglycemic rats. *Nig. J. Biochem. Mol. Biol*. 23(2), 35-39

Ihegboro, G.O., Alhassan, A.J., Afor, E., Ononamadu, C.J., Owolarafe, T.A., Imam, A.A., Salawu, K., Ibrahim, A., Mohamed, M.S. (2020). Hepatocurative potentials of *Tapinanthus bangwensis* and *Moringa oleifera* in carbon tetrachloride induced hepatotoxicity wistar rats. *Ife J. Sci*. 22(2), 1-14.

Ihegboro, G.O., Alhassan, A.J., Ononamadu, C.J., Sule, M.S. (2020). Identification of bioactive compounds in ethylacetate fraction of *Tapinanthus bangwensis* Leaves that ameliorate CCl_4-induced hepatotoxicity in wistar rats. *Toxicol Res. Appl*. 4, 1-10.

Ihegboro, G.O., Alhassan, A.J., Ononamadu, C.J., Owolarafe. T.A., Sule, M.S. (2020). Evaluation of biosafety potentials of *Tapinanthus bangwensis* and *Moringa oleifera* using *Allium cepa* model. *Toxicol. Report*. 7, 671-679.

Jan, S., Khan, M.R. (2016). Protective effects of *M. buxifolia* fruit on renal toxicity induced by CCl_4 in rats. *BMC Complement Altern. Med*. 16, 289.

Jurcik, R., Suregova, K., Hanusova, E., Massanyi, P., Ryban, L., Chrenek, P. (2007). Evaluation of haematological, biochemical and histopathological parameters of transgenic rabbits. *J. Vet. Med.. Ser*. 54, 527- 531.

Kyung, J.L., Jea, H.C., Hye, G.J. (2007). Hepatoprotective and antioxidant effects of the coffee diterpenes kahweol and cafestol on carbon tetrachloride-induced liver damage in mice. *Food. Chem. Toxicol*. 45, 2118-2125.

Lawal, B., Shittu, O.K., Abubakar, A.N., Umar, B.M., Ibrahim, A., Haruna, G.M.(2015a). Biochemical evaluation in wister rats (*Rattus novergicus*) following chronic exposure of methanol leaf extract of *Telfairia occidentalis., J. Pharm. Biomed. Sci*. 5: 740- 744.

Lawal, B., Shittu, O.K., Abubakar, A.N., Haruna, G.M., Saidu, S., Ossai, P.C.(2015b). Haematopoietic effect of methanol extract of Nigerian honey bee (*Apis mellifera*) propolis in mice. *J. Coastal Life Med*, 3, 648- 651.

Mahmond, A., Mahmond, A., Quireshi, R.A. (2012). Antimicrobial activities of three species of family Mimosaceae. *Pakistan. J. Pharmaceutic Sci*. 25:203-206.

Meral, L., Kameri, M. (2013). Effects of *Nigella sativa* and *Urtica diocial* L on selected mineral status and haematological values on CCl_4 treated rats. *Biol. Trace Elem. Res. Winter*. 96, 263-270.

Mishral, N., Tandon, V.L. (2012). Haematological effect of aqueous extract of

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245

ornamental plants in male Swiss albino mice. *Vet. World*. 5, 19- 23.

Mohamed, A.E., Haddad, A.E., Wesam, E., Mohamed, I.S., Fahad, M.A. (2019). The methanolic extract of *Moringa oleifera* attenuates CCl_4 induced hepatonephrotoxicity in the male rat. *Biochemical Res*. 30(1), 365.

Mohammad, M., Yavar, M., Mir-Mehdi, C.A., Shokofeh, B., Lotfollah, R., Alireza, M. (2018). Renoprotective effects of the methanolic extract of *Tanacetum parthenium* against carbon tetrachloride-induced renal injury in rats. *Avicenna J. Phytomed*, 8(4), 370-379.

Odeghe, O.B., Uwakwe, A.A., Monago, C.C. (2012). Some biochemical and haematological studies on the methanolic extract of *Anthocleisia gramdiflora* stem bark. *Int. J. Applied Sci. Technol*. 2, 58-65.

OECD, (2000) Acute oral toxic class method. Guideline 423, adopted 23.03, 1996. In: Eleventh Addendum to the OECD guidelines for the Testing of chemicals. Paris, June 2000.

Okunlola, D.O., Olorunisomo, A.O., Aderinola, A.O., Agboola, A.S., Omole, O.G. (2012). Haematology and serum quality of red Sokoto goats fed Baobab (*Adansonia digita*) fruit meal as supplement to guinea grass (*Panicum maximum*). Proceedings of the 17th Annual Conference of Animal Science Association of Nigeria, 427- 433.

Ononamadu, C.J., Alhassan, A.J., Ibrahim, A., Imam, A.A., Ihegboro, G.O., Owolarafe, T.A, Ezeigwe, O.C., Atiku, M.K., Sule, M.S. (2020). Toxicological study of aqueous methanol solvent fraction of methanol extract of *Dacryodes edulis* leaves. *Toxicol Reports*. 7, 909-918.

Orrester, J.S., Diamond, G., Chatterjee, K., Swan, H.J. (1960). Determination of serum bicarbonate. *New. Engl. J. Med*, 295(24), 1356-1362.

Ozkan, C., Kaya, A., Akgul, Y. (2012). Normal values of haematological and some biochemical parameters in serum and urine of New Zealand white rabbits. *World Rabbit Sci*. 20, 253- 259.

Peters, S.O., Gunn, H.H., Imumorin, I.G., Agaviezor, B.O, Ikeobi, C.O.N. (2011). Haematological studies of frizzled and naked neck genotype of Nigerian native chickens. *Trop. Anim. Health Prod*, 43, 631- 638.

Piero, N.M., Njagi, M.J., Kibiti, M.C., Nageranava, J.N.J., Njagi, N.E. (2012). Herbal management of Diabetes mellitus: A rapidly expanding research Avenue., *Int. J. Current Pharmaceutic Res*. 4(2), 1-4.

Tilak, K.S., Veeralah, K., Reju, M.P. (2007). Effects of ammonia, nitrite and nitrate on haemoglobin content oxygen consumption of freshwater fish, *Cuprinus carplo* (Linnaeus). *J. Enviro. Biol*. 28(11), 45-47.

Trinder, P. (1951). A rapid method for the determination of sodium in serum. Analyst, 76, 596-599.

Vivex, K.B., Rajib, M., Jae, G.P. (2016) Isolation and purification of plant secondary metabolites using column chromatographic techniques. *Bangladesh. J. Pharmacol*. 11, 844-848.

Weber, L.W., Boll, M., Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes, CCl_4 as a toxicological model. *Crit. Rev. Toxicol*. 332), 105-136.

World Health Organization. (2001). Legal status of traditional medicine and complememtary/alternative medicine: A worldwide review, WHO/EDM/TRM/2001.2. World Health Organization, Geneva, Switzerland. 2001.

Witters, P., Freson, K., Verslype, C., Peerlinck, K., Hoylaerts, M., Nevens, F. (2008). Review article: Blood platelet number and function in chronic liver disease and cirrhosis. *Ailment. Pharmacol. Ther*. 27, 1017-1029.

Yang, C., Gong, X., AL, Q. (2015). 5-aminoimidazole-4-carboxamide-1-β-Dribofuranoside alleviates CCl_4 induced acute hepatitis in mice, *Int Immuno pharmacol*. 25, 393-399.

(Print) ISSN 3027-284X Online: E-ISSN 3026-9245