

PROPHYLACTIC EFFECTS OF METHANOLIC LEAF EXTRACT OF *Momordica balsamina* AGAINST CCL₄ INDUCED LIVER INJURY IN WISTAR RATS

ABSTRACT

Introduction: Liver injury can result from various causes, including alcohol consumption, viral infections (such as hepatitis), autoimmune diseases, metabolic disorders, and exposure to certain chemicals, drugs or toxins. It can manifest as inflammation, fatty liver disease, cirrhosis, or acute liver failure, depending on the cause and severity. Treatment often involves addressing the underlying cause, lifestyle changes (such as avoiding alcohol or certain medications), and sometimes medication to support liver function or manage specific conditions. Early detection and management are crucial for preventing further damage and promoting liver health. Studies have reported the effects of *Momordica balsamina* on liver injury.

Aim: This study aim to evaluate the prophylactic effect of methanolic leaf extract of *Momordica balsamina* against CCL₄-induced liver injury in Wistar Rats.

Methodology: The fresh leaves of *Momordica balsamina* were purchased at Marina Market, Sokoto, the plant was air dry at room temperature for 2 weeks and the air-dried leaves were processed with methanol to obtain methanolic extract. An acute toxicity study of *M. balsamina* extract was conducted with six doses (10, 100, 1000, 1600, 2900 and 5000 mg/kg) to evaluate its safety. Phytochemical analysis of the extract was carried out to detect the presence or absence of carbohydrates, saponins, flavonoids, tannins, phenols, protein, alkaloids, cardiac glycosides and steroids. A total of 42 Wistar rats (170±20g) of either sex roughly of the same age (8-10 weeks) were used for the study. After two weeks of acclimatization, the rats were randomly divided into six groups of seven rats each; group 1 (normal control) treated with distilled water and vital feeds; groups 2 (Negative control) CCl₄ treated with liver injury not on treatment; group 3 (Standard control) CCl₄ liver injury treated with 50 mg/kg Silymarin; group 4 (500 mg/kg *M. balsamina* + 2mL CCl₄); group 5 (1000 mg/kg *M. balsamina* + 2mL CCl₄) and group 6 (1500 mg/kg *M. balsamina* + 2mL CCl₄) for 4 weeks. On the last day, the rats were anaesthetized, and blood and liver organ were collected for biochemical and histomorphology study.

Results: The prophylactic effects of methanolic leaf extract of *Momordica balsamina* against CCl₄-induced liver injury were evaluated on liver function tests (LFTs) and Malondialdehyde (MDA). This study shows that *Momordica balsamina* extract significantly ($P < 0.05$) decreased the level of serum AST, ALT, ALP, TB and DB positively by inhibiting their raise at dose-dependent manner compared to the negative control, equally, the extract increased the serum level of Albumin and total protein when compared to the negative control. On the other hand, the extract shows significant reduction of serum level of Malondialdehyde (MDA) near to normal at varying dose. The finding of the prophylactic effect of methanolic leaf extract of *Momordica balsamina* on histology shows significant improvement on liver cell with notable recovery and appearance of the histological architecture of the hepatocyte at the highest dose (1500 mg/kg+2mL CCl₄) compared to negative control group.

Conclusion: The presence of phytochemical compounds and antioxidant properties as well as a gene that is responsible for its prophylactic effect may be one of the mechanisms through which the plant extract was able to exert prophylactic effect on CCl₄ induced liver injury in Wistar rats. This study suggests that *M. balsamina* extract may be considered as an affordable and non-invasive treatment option for liver injury in human.

Keywords: Liver function test (LFTs), Aspartate transaminase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total bilirubin (TB), Direct bilirubin (DB), Albumin (Alb), Total protein (TP) and Malondialdehyde (MDA).

1.0 INTRODUCTION:

“Liver is one of the largest internal organ of the human body that is weighing about 1.2-2.5 kg in an average adult individual that is located between absorptive surface of gastrointestinal tract, as an organ, it offer a wide range of functions including metabolic function, secretory function, storage function, heat production function, synthetic function, haemopoietic function, defensive and detoxification function as well as production of biochemical that are necessary for digestion” [1]. It is central target of the toxicity of drug, xenobiotic and oxidative stress because of an important role it plays in metabolism and relationship to the gastrointestinal tract.

“Liver injury can result from various causes, including alcohol consumption, viral infections (such as hepatitis), autoimmune diseases, metabolic disorders, and exposure to certain chemicals, drugs or toxins. It can manifest as inflammation, fatty liver disease, cirrhosis, jaundice, necrosis, tumors or acute liver failure, depending on the cause and severity. The frequent cause of hepatic injury is drug and some certain chemicals but it also depends on its biochemical and physiological function” [2]. “Drug induced liver injury (DILI) and its active metabolite induced different appearance on liver at cellular and genetic level. Extensive use of drugs and some chemicals even at therapeutic level damage liver in susceptible individuals” [2].

“=The high prevalence of liver diseases has become a serious challenge and it seems a warning alarm globally as it is the major cause of morbidity, mortality and economic burden in Nigeria and world at large. There were an estimated of 1.5 billion cases of chronic liver disease worldwide in 2017 [3] including 10.6 million cases of decompensated cirrhosis and 112 million cases of compensated cirrhosis [4]. “In Nigeria, (35 million) 2-20% of the population, are infected with hepatitis B and C virus with a prevalence rate of 4.3%-23.3% and 0.5-15% been reported respectively from different part of the country depending on the geographical location. A prevalence rate of 4.3% was reported from Port Harcourt, 5.7% from Ilorin, 11.6% from Maiduguri, and 8.3% from Zaria, 6.78% from Ado-Ekiti among pregnant women, 13.50% from Lagos, 11.50% from Abuja Urban among HIV Patients with a seroprevalence of 23.3% been reported among patients attending all clinics in Kano” [5].

“Carbon tetra chloride (CCl₄) is one of the most extensively used toxicant for inducing liver injury for mutagenicity and DNA damage study in animals. Hepatic microsomal enzyme (CYP2E1) is an enzyme which metabolized this carbon tetra chloride in to two degraded metabolites namely, trichloromethyl (CCl₃) and tri-chloromethyl peroxy (CCl₃O₂) which are mainly responsible for hepatotoxicity” [6]. “These two metabolites are unstable radicals that strong binding affinity towards protein and lipids in the cell membrane or removing a hydrogen atom from an unsaturated lipid, there by triggering lipid peroxidation and causing liver damage” [7-8].

Momordica Balsamina L. (Cucurbitaceae) is a plant frequently called balsam apple, southern balsam pear or African pumpkin (English), Garahuni (Hausa), Akbon-ndewe (Igbo) and Ejirin (Yoruba) [9], that comprises of about 120 genera and around 965 species that is mainly disseminated in tropical and subtropical regions all over the world [9]. *M. balsamina* is also an important source of nutrient having 17 amino acid and adequate minerals composition such Na⁺, K⁺, Mg²⁺, Ca²⁺, Mn²⁺, Cu²⁺, and Fe²⁺.

“The extract of various part of this plant show a lot of properties which include anti-viral properties, anti-inflammatory, shigellocidal, anti-diarrhoeal, antiseptic, antibacterial, antimicrobial properties and hypoglycemic effects in rats. ‘Momordins’ present in the plant is capable of inhibiting the growth of HIV and other viruses” [10].

Treatment of liver injury and diseases it’s associated with often involves addressing the underlying cause, lifestyle changes (such as avoiding alcohol or certain medications), and sometimes medication to support liver function or manage specific conditions. Early detection and management are crucial for preventing further damage and promoting liver health.

2.0 MATERIALS AND METHODS

2.1 STUDY SITE

The study was carried out in Chemical Pathology Laboratory, School of Medical Laboratory Science, Usmanu Danfodiyo University, Sokoto and Department of Pharmacognosy and Ethnopharmacy laboratory, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto.

2.2 PLANT COLLECTION AND IDENTIFICATION

Fresh leaves of *Momordica balsamina* were purchased from Marina Market Sokoto, Nigeria. The plant was taken to the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto for identification and authentication and voucher number was given as **PCG/UDUS/CURC/0003**

2.3 PLANT PREPARATION AND EXTRACTION

The fresh leaves of *Momordica balsamina* were collected, washed with distilled water and air-dried at room temperature in a dust-free environment over 2 weeks. The dried leaves were blended using an electronic blender (Binatone BLG 450, London, United Kingdom) and sieved through 40-mesh (0.4 mm) to get a very fine powder. 400g of the powder was soaked into 4000 mL methanol and allowed to macerate at room temperature for 48 hours. Maceration method: maceration involves soaking a powdered plant material in a stopper container (Beaker) with a solvent and allowed to stand at room temperature with frequent agitation. The mixture was filtered using Whatman filter paper (No.4). The filtrate was evaporated to dryness in an electric blast oven set at 45 °C until a methanolic-free solid brown (crude extract) powder was obtained. It was weighed, stored in wide mouth labeled container and preserved in the refrigerator at 4°C until use. The percentage (%) yield of the extract was calculated based on the formula;

$$\% \text{ yield} = \frac{\text{Weight of final extract}}{\text{Weight of powdered plant material}} \times 100$$

The percentage yield was calculated and $154/400 \times 100 = 38.5\%$ was yielded

2.4 PHYTOCHEMICAL SCREENING

Phytochemical analysis was carried out using standard procedures to identify the phytochemical constituents as described by Harbone, [11]; Trease and Evans, [12]; Sofowora and Harbone [13] to detect the presence or absence of flavonoids, tannins, carbohydrates, and alkaloids, cardiac glycosides, saponins, steroids, protein and phenols in the plant’s extract.

2.5 EXPERIMENTAL ANIMALS

A total of forty two (42) Wistar rats ($170\pm 20\text{g}$) of either sex roughly of the same age (8-10 weeks) were purchased from the Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The rats were housed in conventional well-ventilated wire cages under standard laboratory conditions in the Animal house, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto ($\pm 30^\circ\text{C}$) and lighting period of about 12 hours daily. They were allowed to acclimatize for two weeks before use. They were fed standard commercial pelletized grower's feed and drinking water. Principles of Laboratory Animal Care' were followed as well as specific National laws where applicable. All the experimental protocols followed institutional animal ethics committee guidelines were applied.

2.6 EXPERIMENTAL INDUCTION OF LIVER INJURY

Induction of liver injury were done using CCl_4 chemical and was dissolved in olive oil in a ratio 1:2 v/v.

Liver injury were induced in Wistar Rats following subcutaneous injection of the mixture of CCl_4 and olive oil in the lower abdomen at a dose of 2 mL/kg daily for 4 days [14].

2.7 ACUTE TOXICITY STUDY

Acute toxicity testing was conducted using Lorke's Method, [15]. In phase I: nine (9) rats were used and randomly assigned into three (3) groups of three (3) rats each. The methanolic leaf extract of *Momordica balsamina* was dissolved in distilled water and administered at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. In Phase II, three (3) rats were used and randomly assigned into three (3) groups of one (1) rats each, the rats were administered with doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. The rats were observed for 24 hours for clinical signs of toxicity and mortality.

2.8 EXPERIMENTAL DESIGN

The rats were grouped in to six (6) groups of 7 rats each

Group I: Non-Liver injured group (Normal Control). The rats were orally administered with 1.0 mL of Normal Saline with vital feeds daily.

Group II: CCl_4 Liver injured group not on treatment (Negative Control). The rats were orally administered with 1.0 mL of Normal Saline and vital feeds.

Group III: CCl_4 Liver injured group on standard medication (Silymarin 50 mg/kg). The rats were fed with water and vital feeds.

Group IV: Pre-treatment group of 500 mg/kg of *Momordica balsamina* for 3weeks followed by 2 mL of CCl_4 administration for 4 days

Group V: Pre-treatment group of 1000 mg/kg of *Momordica balsamina* for 3weeks followed by 2 mL of CCl_4 administration for 4 days

Group VI: Pre-treatment group of 1500 mg/kg of *Momordica balsamina* for 3weeks followed by 2 mL of CCl_4 administration for 4 days

2.9 SAMPLE COLLECTION

At the end of the experiment, the rats were anaesthetized and blood samples were collected through cardiac puncture. About 3 mL of the blood samples were collected and were immediately transferred into plain vacutainer tube. Clear serum and plasma samples were obtained from the blood sample after centrifugation at 1200 rpm for 5 minutes and stored in appropriate vials for biochemical analysis. Following euthanasia, the liver was immediately and

carefully removed using surgical blade and dissecting forceps and transferred into specimen containers containing 10% formalin for proper fixation before processing for onward Histological investigation.

2.10 LABORATORY ANALYSIS

2.10.1 Biochemical Analysis: Serum Malondialdehyde was determined using the colorimetric method. This method was estimated using thiobarbituric acid reactive substance assay (TBARS) as described by Shah and Walker's, [16]. The liver function test such as ALT, AST and ALP were analyzed by kinetic methods using Randox kits while TB, DB, TP and Alb were analyzed using kits from Agappe Diagnostics Ltd, India. iChm 535 semi-auto analyzer was used.

2.10.2 Histopathological Examination: Histopathological slides of the liver tissue were prepared at the Department of Histopathology Laboratory, Usmanu Danfodiyo University Teaching Hospital (UDTH), Sokoto State. The tissues were subjected to standard routine histological procedures as described by Kiernan, [17].

2.11 DATA ANALYSIS

Data generated from this study were analyzed using Statistical Package for Social Sciences (SPSS) version 25.0 (SPSS Inc., Chicago, IL, USA). The results were compared using One way ANOVA followed by Tuckey Post hoc analysis to determine the statistical significance across all the groups. While values were expressed as mean \pm standard deviation of the mean (mean \pm SD). A P-value of less than 0.05 ($P < 0.05$) was considered as significant.

3.0 RESULTS

The phytochemical screening of the methanolic extract of *Momordica balsamina* was conducted qualitatively and the phytochemical constituent detected were shown in the table below.

Table 1. Phytochemicals Constituents of methanolic leaf extract of *Momordica balsamina*.

Compounds	Phytochemical test	Inference
Carbohydrates	Molich's test	+
Saponins	Frothing test	++
Flavonoids	Shinoda's test	+
Tannins	Lead Acetate test	+
Phenols	FeCl ₂ test	+
Protein/Amino Acids	Xanthoproteic test	++
Alkaloids	Mayer's test	+
Cardiac Glycosides	Killer-Killiani test	+
Steroids/Terpenoids	Salkowki's test	+
	Liebermann-Burchard's	+

test

Key: - + Trace, ++ moderate.

Acute Toxicity Study of methanolic leaf extract of *Momordica balsamina*.

The result of the acute toxicity study is presented in the Table below. The acute toxicity result showed that there was no toxicity/death observed for the methanolic leaf extract of *Momordica balsamina* at doses less than or equal to 5000 mg/kg body weight after 24 hours. This implies that the extract is safe for consumption and is non-toxic up to 5000 mg/kg body weight, hence it's relatively safe

Table 2. Acute Toxicity Study of methanolic leaf extract of *Momordica balsamina*

S/N	DOSE (mg)	OBSERVATION	
		First Phase	Second Phase
1	10	0/3	-
2	100	0/3	-
3	1000	0/3	-
4	1600	-	0/1
5	2900	-	0/1
6	5000	-	0/1

Acute toxicity study after 24 hours was ≤ 5000 mg/kg.

Key: 0/3 and 0/1; 0 indicated no death, 1 and 3 indicated number of rats per group.

Effects of methanolic leaf extract of *Momordica balsamina* on MDA

The result of the effect of methanolic leaf extract of *Momordica balsamina* on MDA in CCl₄-induced liver injury in Wistar Rats was determined and presented in Table 3 below. The effect of methanolic leaf extract of *Momordica balsamina* on Malondialdehyde (MDA) concentration was estimated as indices for the oxidative stress status of the rats. Subcutaneous injection of carbon tetrachloride (CCl₄) significantly increased the lipid peroxidation marker (MDA) of the group that received CCl₄ alone. The mean serum level of MDA (nmol/L) in normal control group was (85.35±17.38) and was significantly low ($p < 0.001$) compared to the level of MDA (nmol/L) in the negative control group (133.98±2.91) with the standard control group (109.88±4.83). Exposure of CCl₄ after pre-treatment of rats with the extract for 3weeks mildly increased in mean serum level of MDA (nmol/L) at 500 mg/kg+CCl₄ (121.32±1.21) and at 1000 mg/kg+CCl₄ (110.55±2.44) but at 1500 mg/kg+CCl₄, the mean MDA level was near to normal (96.24±3.58) compared to normal control group.

Table 3. Effects of methanolic leaf extract of *Momordica balsamina* on MDA in CCl₄ induced liver injury

Group (n =7)	MDA (nmol/L)
Group I	85.35±17.38
Group II	133.98±2.91 ^a

Group III	109.88±4.83 ^{ab}
Group IV	121.32±1.21 ^{abc}
Group V	110.55±2.44 ^{abcd}
Group VI	96.24±3.58 ^{abcde}

Data were presented as Mean ± Standard Deviation. Mean values with different superscripts on the row differs significantly. Where **Group I** normal control; **Group II** negative control; **Group III** standard control (50 mg/kg); **Group IV** pre-treated with 500 mg/kg + CCl₄; **Group V** pre-treated with 1000 mg/kg + CCl₄, **Group VI** pre-treated with 1500 mg/kg + CCl₄. **MDA** Malondialdehyde; The Result was statistically at $P < 0.05$

Effects of methanolic leaf extract of *Momordica balsamina* on Liver Function Tests

The prophylactic effects of methanolic leaf extract of *Momordica balsamina* on liver function test was show in table below.

The mean serum liver enzymes activity of AST (U/L), ALT (U/L) and ALP (U/L) in normal control group was significantly lower ($p < 0.001$) compared to negative control group (14.86±1.06, 24.14±3.62 and 121.86±7.26) with standard control (8.71±1.60, 11.00±1.91 and 89.57±5.31) respectively. There was a significant increase in serum TB and DB (mg/dL) concentration (1.68±0.25 and 0.65±0.09) in negative control group as compared to normal control group (0.56±0.35 and 0.17±0.09) with reference control group (0.85±0.13 and 0.18±0.09). Equally, there was a significant decrease in serum Alb (g/L) and TP (g/L) concentration in negative control group (2.91±0.25 and 5.50±0.21) as compared to normal control group (3.94±0.20 and 6.42±0.23) with the reference control group (3.80±0.15 and 7.38±0.42).

Exposure of CCl₄ after pre-treatment of rats with the extract for 3weeks ameliorates the serum liver enzyme activity at dose dependent manner. The serum liver enzymes activity of AST (U/L), ALT (U/L) and ALP (U/L) in prophylactic groups was (11.00±0.81, 8.00±1.00 and 6.71±0.95); (12.71±0.95, 11.14±0.90 and 9.29±1.38) and (92.57±2.63, 80.86±3.93 and 76.14±4.41) respectively when compared to control. In pre-treatment group at 500 mg/kg+CCl₄, the serum concentration of TB (mg/dL) was (0.88±0.06) while it was (0.82±0.11) at 1000 mg/kg+CCl₄ as compared in pre-treatment group at 1500 mg/kg+CCl₄ (0.78±0.09) and the mean serum DB (mg/dL) concentration at 500 mg/kg+CCl₄ was significantly high (0.25±0.07) than in pre-treatment group at 1000 mg/kg+CCl₄ (0.22±0.09) and (0.21±0.10) at 1500 mg/kg+CCl₄ respectively. There was a significant similar mean Alb (g/L) concentration (4.11±0.09, 4.31±0.12 and 4.41±0.28) in both pre-treatment at 500 mg/kg+CCl₄, 1000 mg/kg and 1500 mg/kg+CCl₄ respectively. Equally, the mean serum TP concentration (g/L) was (7.50±0.37) at 500 mg/kg+CCl₄, (6.68±0.45) and (7.02±0.22) at 1500 mg/kg+CCl₄ and 1000 mg/kg+CCl₄ respectively.

Table 4. Prophylactic effects of methanolic leaf extract of *Momordica balsamina* in CCl₄ induced liver injury in wistar rats

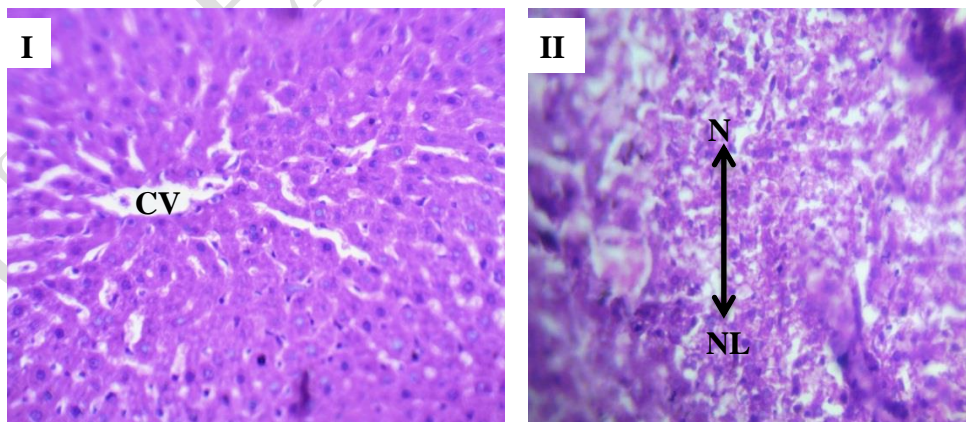
Group (n = 7)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (mg/dL)	DB (mg/dL)	Alb (g/L)	TP (g/L)
Group I	5.43±0.97	8.43±1.27	75.43±6.37	0.56±0.35	0.17±0.09	3.94±0.20	6.42±0.23

Group II	14.86±1.06 ^a	24.14±3.62 ^a	121.86±7.26 ^a	1.68±0.25 ^a	0.65±0.09 ^a	2.91±0.25 ^a	5.50±0.21 ^a
Group III	8.71±1.60 ^{ab}	11.00±1.91 ^b	89.57±5.31 ^{ab}	0.85±0.13 ^b	0.18±0.90 ^b	3.80±0.15 ^b	7.38±0.42 ^{ab}
Group IV	11.00±0.81 ^{abc}	12.71±0.95 ^{ab}	92.57±2.63 ^{ab}	0.88±0.06 ^b	0.25±0.07 ^b	4.11±0.09 ^b	7.50±0.37 ^{ab}
Group V	8.00±1.00 ^{abd}	11.14±0.90 ^b	80.86±3.93 ^{bcd}	0.82±0.11 ^b	0.22±0.09 ^b	4.31±0.12 ^{abc}	7.02±0.22 ^{ab}
Group VI	6.71±0.95 ^{bcd}	9.29±1.38 ^{bd}	76.14±4.41 ^{bcd}	0.78±0.09 ^b	0.21±0.10 ^b	4.41±0.28 ^{abc}	6.68±0.45 ^{bcd}

Data were presented as Mean ± Standard Deviation. Mean values with different superscripts on the row differs significantly. Where **Group I** normal control; **Group II** negative control; **Group III** standard control (50 mg/kg); **Group IV** pre-treated with 500 mg/kg + CCl₄; **Group V** pre-treated with 1000 mg/kg + CCl₄; **Group VI** pre-treated with 1500 mg/kg + CCl₄. **AST** Aspartate Transaminase; **ALT** Alanine Aminotransferase; **ALP** Alkaline Phosphatase; **TB** Total Bilirubin; **DB** Direct Bilirubin; **Alb** Albumin; **TP** Total Protein. Result was statistically at $P < 0.05$.

Effects of methanolic leaf extract of *Momordica balsamina* on histology of the liver in CCl₄ induced liver injury in Wistar rats

The result of the effect of methanolic leaf extract of *Momordica balsamina* on histology of the liver in CCl₄-induced liver injury in wistar rats is presented in Plate 1 below. The H and E staining showed that extract of *Momordica balsamina* treatment significantly improved the histological appearance of this organ in contrast to the negative control. Similarly, the reference control groups also improved the histopathology of the liver. The hepatocytes of rat liver treated with CCl₄ (negative control) showed centrilobular hepatocyte necrosis, micro vesicular fatty changes with lymphocytic aggregate were observed on the mid-zonal (zone 1 necrosis) or entire lobe. Liver tissue of rat treated with CCl₄ and Silymarin showed good recovery with the absence of necrosis, fatty depositions and recovery to normal histological appearance of the hepatocytes, the central vein has minimal portal inflammation. Equally, the section of liver treated with 500 mg/kg+CCl₄, 1000 mg/kg+CCl₄ and 1500 mg/kg+CCl₄ (group IV, V and VI) in prophylactic groups show significant recovery with the disappearance of fatty deposition and necrosis at the highest dose. The portal triaditis appear almost clear with normal shape of central vein indicating a potent therapeutic and prophylactic activity of the extract.



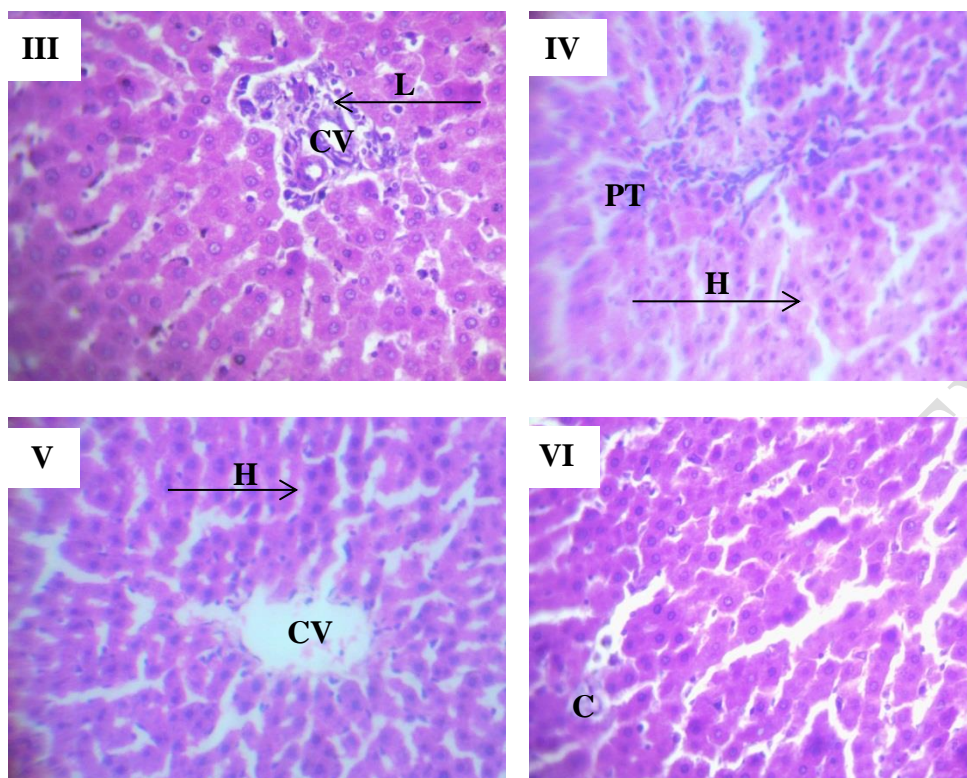


Plate 1: Photomicrograph of the liver section showing effects of methanolic leaf extract of *Momordica balsamina* on CCl₄ induced liver injury in Wistar rats (H & Eosin X400). **Group I:** Control; **Group II:** Negative control; **Group III:** Standard control (50 mg/kg Silymarin); **Group IV:** 500 mg/kg MB extract+CCl₄; **Group V:** 1000 mg/kg MB extract+CCl₄; **Group VI:** 1500 mg/kg MB extract+CCl₄; **CV:** Central vein; **NL:** Necrosis of a Liver cell; **LA** Lymphocytic Aggregates; **PT:** Portal triaditis; **H:** Hepatocyte.

4.0 Discussion

Over many years it has been known that liver injury/diseases and has deferred all control measures and has remained a major infectious disease threatening or affecting the individuals as it has become a serious challenge and it seems a warning alarm globally as it is the major cause of morbidity, mortality and economic burden in Nigeria and world at large. The present study was aimed at determining the prophylactic effects (potency) of methanolic leaf extract of *Momordica balsamina* against CCl₄ induced liver injury in Wistar rats.

“The diversity of herbal remedies and their uses between different countries makes scientifically evaluating and regulating them a very challenging” [18]. Hence, in some countries, herbal medicines are subjected to rigorous manufacturing standards, while in others, they are regarded as food supplements for which therapeutic claims are prohibited. Herbal products are considered safe for human consumption due to their natural origin. It is possible that some people preferred unconventional herbal medicine because they believe that herbal medicine is more natural than modern conventional drugs.

In this present study, some active phytochemical compounds (table 1) such as flavonoids, steroids, alkaloids, glycosides, tannins, saponins, carbohydrates, proteins, and phenols was detected. The phytochemical analysis of methanolic leaf extract *Momordica balsamina* indicated

that carbohydrates component had the highest concentration with ++ while other components of the compound such as flavonoids, saponins, tannins, phenols, alkaloids, glycosides, steroids and protein content possess +. It was therefore suggested that *M. balsamina* leaf extracts may be considered a good source of natural antioxidants, anti-inflammatory, anti-viral, anti-cancer, anti-bacterial, anti-fungal, hypoglycaemic, anti-proliferation, analgesic, anti-parasitic, anti-metastatic effect and immune modulatory etc. all this is because of the phytochemicals that were detected in the extract. This finding is in agreement with Thakur *et al.* [19] and Agrawal *et al.* [20]. Who reported that *Momordica balsamina* possesses an important source of nutrient for medicinal (phytochemicals) use such as anti-aging and other ailments relating to radical mechanisms.

The acute oral toxicity study of methanolic leaf extract of *Momordica balsamina* was determined to evaluate its safety, Table 2 shows no death or sign of toxicity in the experimental animals at a dose of 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight, and hence it is relatively safe. This indicates that the LD₅₀ of *Momordica balsamina* leaf extract is higher than 5000 mg/kg body weight, hence it is relatively safe, these finding is in agreement with Sunday *et al.* [21] who reported that the LD₅₀ of the leaf extract of *M. balsamina* was greater than 4.15 g/kg (4150 mg/kg).

The present study revealed decreased in serum concentration of Malondialdehyde (MDA) in prophylactic at dose-dependent manner when compared to negative control group and the highest dose is near to normal in control group which is statistically significant ($P < 0.001$) when compared to standard control.

The significant reduction of MDA level seen in prophylactic group could be due to improvement in liver injury, since *Momordica balsamina* extract have been shown to have anti-oxidant properties due to their phytochemicals detected such as saponins, steroids, phenols, tannins, alkaloids and flavonoids. All this have a protective role against oxidative damage, thus preventing lipid peroxidation and protecting the kidneys from severe increase of reactive oxygen species while increase in MDA level seen in negative group was as a result of the toxic effect and oxidative stress caused by CCl₄ been administer. This finding is in agreement with Siboto *et al.* [22] who reported that *Momordica balsamina* has anti-oxidant effects on oxidative stress caused by STZ-induced diabetic rats and in a similar research conducted by Deng *et al.* [23] reported that *Momordica charantia* significantly reduced the level of MDA in liver injury in restraint-stressed mice.

The serum activity of AST (SGOT), ALT (SGPT) and ALP of the CCl₄ treated group (negative control) showed a significant increase ($p < 0.001$) compared to the normal control group, this significant increase was due to the toxic effect of CCl₄ been administer and cause hepatocyte damage, hence synthetic and metabolic function of the liver was impaired. Severe jaundice was reflected by an increased level of serum total bilirubin, direct bilirubin and decrease in level of serum albumin and total protein in CCl₄ treated group.

The prophylactic potency of methanolic leaf extract of *Momordica balsamina* at dose-dependent manner and the use of hepato-curative agent Silymarin as reference standard group all resulted in a significant decrease in the elevated level of serum AST, ALT, ALP, total bilirubin, direct bilirubin as well as significant increase in decreased level of serum albumin and total protein ($p < 0.001$). Decreased in these liver biomarkers and increase in serum albumin and total protein seen in these groups was as a result of phytochemicals detected in the extract specifically the flavonoids, tannins, phenols, alkaloids, glycosides and terpenoids. This finding is in agreement with a study conducted by Sahel *et al.* [24] and Moharir *et al.* [14] who reported that *Momordica balsamina* has protective effect of liver injury against CCl₄ in rats, also this finding agrees with

similar work conducted by Ampitan et al. [25] who reported on the Prophylactic potency of methanolic extract of *Momordica balsamina* against avian paramyxovirus-1 infection in broiler chickens.

The histopathological appearances of the hepatocytes reflect their condition (plate 1). Based on the histological examination, exposure of hepatocytes to toxic agents CCl₄ leads to histopathological changes of the structure from the normal cell appearance. The hepatocytes of rat liver treated with CCl₄ (Negative control) showed centrilobular hepatocyte necrosis, micro vesicular fatty changes with lymphocytic aggregate were observed on the mid-zonal (zone 1 necrosis) or entire lobe (plate 1 II). Liver tissue of rat treated with CCl₄ and Silymarin (Standard control) showed good recovery with absence of necrosis, no fatty depositions and recovery to normal histological appearance of the hepatocytes (plate 1 III), the central vein has minimal portal inflammation. Histological appearance of rat liver treated with 500 mg/kg+CCl₄, 1000 mg/kg+CCl₄ and 1500 mg/kg+CCl₄ of the methanolic leaf extract of *Momordica balsamina* show significant recovery with disappearance of fatty deposition and necrosis. The portal triaditis appear almost clear with normal shape of central vein indicating a potent prophylactic activity of the extract (plate 1 IV, V and VI). This finding is in agreement with Saleh et al. [24]. Who reported that *Momordica balsamina* have significant protective effects on hepatocyte from CCl₄ hepatotoxicity.

5.0 Conclusion

From the study carried out, the present findings have shown that the LD₅₀ of methanolic leaf extract of *Momordica balsamina* is higher than 5000 mg/kg body weight in wistar rats, treatment with various doses of the extract was well tolerated by all animals, as there were no toxic effects observed by gross visual observation of the animals throughout the experiment. There was no death and apparent behavioral changes recorded during the course of the experiment. This is an indication that it is relatively safe for human consumption. The finding also revealed that the extract of *Momordica balsamina* has an important phytochemical compound and nutritional value that make it medicinal important in treatment of various diseases. The results described in this study demonstrated that *Momordica balsamina* has potency of prophylactic effect on liver injury in wistar rats by significant decrease on serum liver enzymes, total bilirubin and direct bilirubin similar to Silymarin, probably because it contains flavonoids and other phytochemical agents as well as a gene that is responsible for its prophylactic effect.

Equally, the methanolic leaf extract of *Momordica balsamina* also improve the histological architecture of liver cell in a dose-dependent manner with a notable recovery and appearance of the histological characters of the hepatocyte at the highest dose. The finding also revealed that, the extract of *Momordica balsamina* has potential of prophylactic activity against CCl₄ induced liver injury in wistar rats.

ETHICAL APPROVAL

Ethical approval was obtained from the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto with ethical number PTAC/mb/(Me)/OT/74-24 assigned for the use and management of Animals for the research.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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