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Hepatoprotective Effect of *Momordica charantia* Extract against CCl4 Induced Liver Damage in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author SBM designed the study, wrote the protocol and the first draft of the manuscript. Author HMI approved the study and reviewed the protocol. Author MMA performed the statistical and phytochemical analyses. Author HAM Managed the literature searches and sample collection. Author AA managed the laboratory work. All authors read and approved the final manuscript

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ABSTRACT

Aim: The study was aimed to evaluate *Momordica charantia* leaves extract for hepatoprotective effect against carbon tetrachloride (CCl4) induced hepatotoxicity in rats.
Methodology: A total of twenty five male rats were randomly divided into five groups of five rats each. The extract was administered orally for fifteen days at 200 and 400 mg/g body weight.
Results: The results obtained showed that, treatment with the extract significantly ($P<0.05$) restored liver weight to near normal. The result showed a significant ($P<0.05$) increase in Heamoglobin (Hb) and Packed Cell Volume (PCV) compared to toxin control group. Also treatment with the extract caused a significant ($P<0.05$) decrease in the activities of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatise (ALP) and the level of total bilirubin: and a significant ($P<0.05$) increase in total protein level compared to control group. Similarly, the extract caused a significant ($P<0.05$) decrease in the level of reduced Glutathione (GSH) and Malondialdehyde (MDA) and a significant ($P<0.05$) elevation in the activities of Superoxide Dismutase (SOD) and Catalase (CAT) compared to toxin control group.

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Conclusion: This study found that, administration of aqueous leaves extract of *M. charantia* ameliorated hepatotoxicity induced experimentally by CCl4.

Keywords: Oxidative stress; liver damage; Momordica charantia; protective effect; CCl4.

1. INTRODUCTION

Hepatic injury induced by different types of environmental toxins and hepatotoxic agents have been recognized as major health problems worldwide for decades. Liver is an abdominal organ which plays a vital role in metabolism, detoxification and excretion of many endogenous and exogenous substances. It neutralizes toxins and manufactures bile which aids fat digestion and removes toxins through the bowels [1]. Continuous exposure and intoxication of liver to toxic compounds on a daily basis may lead to hepatic dysfunction [2]. Liver disease is a major cause of death in many developing countries. In spite of tremendous advances in modern medicine, medical management is still insufficient and no effective drug has successfully prevented the progression of hepatic diseases, even though newly developed drugs have been used to treat chronic liver disorders, these drugs have often adverse effects [3]. Also some of the modern drugs, which are used for the treatment of liver diseases, may themselves cause liver damage [4]. Most of the liver diseases are mediated by reactive oxygen species (ROS) which play an important role in the development of tissue damage and pathological conditions in living organisms [5]. Administration of single or repeated dose of CCl4 is one of the common methods to investigate the possible mechanisms of hepatic injury in rats. This model has been implemented in various studies for the deposition of extracellular matrix in the cases of liver cirrhosis and fibrosis [6]. The biotransformation process of CCl4 causes the formation of haloalkane free radicals which can damage the hepatocytes making liver an important target for CCl4 [7]. Silymarin has been used for over 20 years in clinical practice for the treatment of toxic liver diseases [8]. It has been described to be an antioxidant and exhibits anticarcinogenic, antiinflammatory, hepatoprotection and growth modulatory effects [9]. In this study, silymarin was used as a positive control to against the carbon tetrachloride-induced liver damage in rats. Medicinal plants and folk medicine has been in practice by traditional healers for a long time in the treatment of diseases such as jaundice, diabetes, diarrheal, arthritis, skin ulcers and gastrointestinal disturbance [10]. Medicinal plants have been reported to contain antioxidants that could prevent the formation of free radicals [11]. Also plants contain non-nutritional constituents with beneficial health effects, such as anti-inflammatory, anti-carcinogenic and analgesic properties [12]. *Momordica charantia* (bitter melon) belongs to the family of Cucurbitaceae which is a food as well as medicine. The plant posses antimicrobial activity [13] and contains antioxidant compounds such as vitamin C, calcium, magnesium, sulphur and other trace elements [14]. Although *Momordica charantia* has been widely used as folk medicine and health food, no scientific investigation had been reported regarding its in-vivo antioxidant efficacy against carbon tetrachloride-induced liver damage. Therefore the present study was aimed to evaluate hepatoprotective effect and antioxidant potential of aqueous leaves extract of *Momordica charantia* against CCl4 induced liver damage.

2. MATERIALS AND METHODS

2.1 Chemicals, Reagents and Drugs

Diagnostic kits for the serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), serum bilirubin were purchased from Randox Laboratories Ltd, (United Kingdom). Bovine serum albumin (BSA), trichloro acetic acid (TCA), thiobarbituric acid (TBA), reduced glutathione (GSH), pyrophosphate, ethylene diamine tetra acetic acid disodium salt (EDTA) 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), *β*-nicotinamide adenine dinucleotide hydrogen (NADH) were obtained from Sigma Chemical (St. Louis, MO, USA). Silymarin was purchased from Vellore, India. Pyridine (C_5H_5N), disodium hydrogen phosphate (Na_2HPO_4), hydrogen peroxide (H_2O_2), dihydrogen potassium phosphate anhydrous (KH_2PO_4), Potassium heptochromate (VI), Hydrogen peroxide (H_2O_2), dimethylsulfoxide (DMSO), carbontetrachloride (CCl4) were purchased from Merck India Ltd (Mumbai, India). All other chemicals and reagents were of analytical grade.

2.2 Experimental Animals

A total of twenty five apparently healthy male Wister albino rats of three months old, weighing between 160-180g were purchased from the animal house, Department of Pharmacology Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. The animals were kept in a clean plastic cage under 12 hr light and dark cycles and were allowed free access of water and standard pellet diets *ad libitum.* The animals were allowed to acclimatize to the laboratory environment for one week before the commencement of the experiment.

2.3 Plant Sample Collection and Identification

The leaves of *Momordica charantia* Linn were collected in polythene bags from Damba Village Gusau, Zamfara State, Nigeria in the month of May, 2012. The sample were identified and authenticated by Mr. U. S. Gallah of the herbarium unit, Department of Biological sciences, Ahmadu Bello University Zaria Nigeria, and a voucher number was given VN/528/2012.

2.4 Sample Processing and Preparation of Extract

The leaves of *Momordica charantia* Linn were cleaned, washed with tap water and dried under shade. The dried leaves were broken into small pieces using pestle and mortar and then pulverized using electric blender into fine powder. Five hundred grams (500g) of fine powder were weighed and soaked into two litre of distilled water. The mixture was shaken regularly at interval of 2 hours and kept at room temperature for 24 hours. After 24 hours the homogenate was filtered using muslin cloth and the filtrate obtained were re-filtered using Whatman No. 1 filter paper, the filtrate obtained was evaporated to dryness using water bath set at 45ºC.

2.5 Acute Toxicity Study

The median lethal dose (LD_{50}) of aqueous leaves extract of *Momordica charantia* was carried out according to the method of described by [15]. The method involved two phases of which nine rats were grouped into three groups of three rats each. They received single

dose of 10, 100 and 1000 mg/kg body weights of the extracts respectively. In the second phase also nine rats were grouped into three groups of three rats each and they received 1600, 2900 and 5000 mg/kg body weights. The rats were observed daily for any signs of toxicity including death for twenty four hours.

2.6 Phytochemical Analyses

Quantitative phytochemicals analyses of stem bark extract of *M. charantia* were carried out according to the following methods: Tannins [16], saponins and alkaloids [17] and flavonoids [18].

2.7 Experimental Design and Treatment

A total of twenty five male rats weighing 160-180 g were randomly divided into five groups of five rats each:

Group 1: (Served as normal control) were administered DMSO 1ml /kg bwt orally as vehicle for the 15^{th} days of the experimental period.

Group 2: (Served as toxin control) were administered (*i.p*) 1 ml/kg bwt CCl4 in DMSO (1:1) on the 7^{th} and 14^{th} days only.

Group 3: Were administered orally 50 mg/kg bwt silymarin (standard drug) throughout the 15^{th} days of the experimental period and then 1 ml/kg bwt (*i.p*) of CCl4 in DMSO (1:1) on the 7^{th} and 14^{th} days only.

Group 4: Were administered orally 200 mg/kg bwt of the extract throughout the 15^{th} days and then 1 ml/kg of CCl4 in DMSO (*i.p*) on the 7^{th} and 14^{th} days only.

Group 5: Were administered orally 400 mg/kg bwt of the extract throughout the 15^{th} days and then 1 ml/kg bwt CCl4 in DMSO (*i.p*) on the 7^{th} and 14^{th} days only.

2.8 Evaluation of Body and Organ Weights

The initial and final body weights of all rats in each group were measured and recorded. Liver weights of all rats in each group were also measured after post treatment sacrifice.

2.9 Evaluation of Haemoglobin and Packed Cell Volume

The blood sample was transferred into properly labelled sample bottle and centrifuged at 4000g for 15 min. The plasma obtained was used for the determination of haemoglobin (Hb) packed cell volume (PCV) with the aid of an Auto Blood analyzer (Mindray Haematology analyzer, BC-2300).

2.10 Evaluation of Hepatic Biochemical Parameters

At the end of the experimental period, animals were fasted overnight for 12 hrs and sacrificed by cervical dislocation. Serum was harvested from the blood and was used for determination of biochemical parameters using commercial reagent kits (Randox

Laboratories, United Kingdom) by the following methods: AST and ALT [19], ALP [20], total bilirubin [21] and total protein [22].

2.11 Preparation of Liver for Evaluation of Antioxidant Parameters

The liver was immediately isolated and washed with normal saline, blotted with filter paper, weighed and homogenized with 10 times (w/v) using a homogenizer in ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 800g for 5 min at 4ºC to separate the nuclear debris. The supernatant so obtained was further centrifuged at 10,000g for 15 min at 4ºC to get the post mitochondrial supernatant which was used to assays the activities of superoxide dismutase (SOD) according to the method described by [23] and Catalase according to the method described by [24].The levels of reduced glutathione (GSH) was determined by the method of [25] and Thiobarbituric acid reactive substances (TBARS), assayed as malondialdehyde (MDA) was determined using the method described by [26].

2.12 Histopathological Study

Small pieces of liver tissues in each group were collected in 10% neutral buffered formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections were cut and stained with haematoxylin and eosin (H and E). The tissue sections were examined microscopically at x 100 magnification.

2.13 Statistical Analysis

The results were expressed as the Mean ± standard deviation using one-way analysis of variance (ANOVA), followed by Duncan Post hoc test and $P<0.05$ was considered as statistically significant**.**

3. RESULTS

The result for the acute toxicity study indicated that, the LD_{50} of aqueous leaves extract of *Momordica charantia* linn was greater than 5000 mg/kg (Data not showed). The extract did not exhibit any sign of toxicity and no death was recorded throughout the time period of this study. This is a clear indication that, the leaves extract of *Momordica charantia* was relatively safe under our study conditions. The result of phytochemical studies presented in (Table 1) indicated that the presence of certain amount of metabolites. However polyphenolics were the highest with 0.98g/100g and tannins was having the least amongst of 0.25g/100g of all the phytochemicals analysed.

Table 1. Quantitative phytochemical of leaves of *Momordica charantia*

Sample	Tannins (g/100g)	Saponins (g/100g)	Alkaloids (g/100g)	Flavonoids (g/100 g)	Polyphenolics (g/100 g)
Stem bark	0.25±0.02	0.43±0.01	0.78±0.02	0.62±0.03	0.98±0.05

Values are Mean ± SD of triplicates determinations.

Similarly there was significant ($P<0.05$) increase in liver weight of rats in the entire treated groups compared to normal control group (Table 2). However, the treatments with 200 and 400 mg/kg of leaves aqueous leaves extract of *M. charantia,* significantly ($P<0.05$) ameliorated the increase in liver weight in a dose-related manner. For instance, the highest

percentage increase of liver weight of 67.7% was recorded in group 2 (toxin control) compared to group 1 (normal control) and the lowest percentage increase of 13.4% was observed in group 3 (silymarin-treated group). However, there was slight but significant reduction in liver weight of extracted treated groups, although extracts activity was lower than that of standard drug (silymarin).

Table 2. Effect of aqueous leaves extract of *M. charantia* on liver weights in normal and CCl4 intoxicated rats

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Liver weight (g/100g b.w)	3.59±0.01[a]	6.02±0.02[b] (I = 67.7%)	4.07±0.06[a] (I = 13.4%)	5.66±0.05[b] (I = 57.7%)	5.27±0.06[b] (I = 46.8%)

Values are Mean ± SD (n=5). Values with different superscripts in a row are statistically different compared to control at P<0.05, I: Percentage increase compared to normal control. D: Percentage decrease compared to control,

The level of Haemoglobin (Hb) and Packed Cell Volume (PCV) of all, the treated groups showed a significant (P<0.05) decreased compared to group 1 (Normal control). However, treatments with 200 and 400 mg/kg extract caused a significant (P<0.05) increase in a dose related manner (Table 3).

Table 3. Effect of aqueous leaves extract of *M. charantia* on the level of haemoglobin and packed cell volume in CCl4 intoxicated rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
HB (g/dl)	15.07±0.21[e]	10.06±0.76[a] (D = 33.2)	14.15±0.26[d] (D = 6.1)	12.11±1.01[b] (D = 19.6)	13.00±0.45[c] (D = 13.7)
PCV (%)	40.17±0.80[e]	26.33±1.53[a] (D = 34.5)	38.36±1.65[d] (D = 4.5)	31.01±2.91[b] (D = 22.8)	37.10±1.61[c] (D = 8.9)

Values are Mean ± SD (n=5). Values with different superscripts in a row are statistically different compared to control at P <0.05, D: Percentage decrease compared to control, I: Percentage increase compared to normal control.

The results for markers of liver damage (Table 4) indicated a significant (P<0.05) alterations compared to normal control. For instance in the entire treated group, a percentage increase in the activity of serum ALT, AST and ALP were obtained with group 2 (Toxin control) having the highest increase of 175.8%, 118.7% and 67.8% for ALT, AST and ALP respectively. However the highest decrease in the activities of ALT, AST, and ALP were found in group 3 (silymarin-treated group) compared to normal control. Also the entire treated groups showed a significant (P<0.05) increase in total bilirubin level compared to normal control. The highest percentage increase of 122.9% was recorded in group 2 (Toxin control). While the total protein content of the entire treated groups were significantly (P<0.05) decreased with toxin control (Group 2) having the highest percentage decrease of 60.4%.

The antioxidant parameters were significantly (P<0.05) altered (Table 5). The result obtained showed an elevation of MDA level in all treated groups compared to normal control group. However, the treatments with the aqueous leaves extract of *M. charantia* caused a significant (P<0.05) reduction of MDA level compared to normal control. The highest percentage increase of 104.4% was recorded in group 2 (toxin control) and the lowest percentage increase of 2.3% was recorded in group 3 (Silymarin-treated group) compared to normal control group. Similarly there was significant (P<0.05) decrease in reduced

glutathione (GSH) level in the entire treated groups compared to normal control. The highest percentage decrease of 69.6% was recorded in group 2 and the lowest percentage decrease of 3.8% was recorded in group 3 compared to normal control group. Also, the SOD and CAT were decrease in the entire treated groups. However, treatment with the extract significantly ($P<0.05$) increases the activities of SOD and CAT compared to normal control. For instance the highest percentage decrease of 52.9% and 52.0% for SOD and CAT were recorded in group 2 (toxin control) and the lowest percentage decrease of 1.1% and 5.2% were recorded in group 3 (silymarin treated group).

Table 4. Effect of aqueous leaves extract of *M. charantia* on the activity of serum ALT, AST and ALP, total bilirubin and protein levels in CCl4 intoxicated rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
ALT (U/l)	29.66±0.59^{a}	81.81±0.31^{e} (I = 175.8)	32.99±0.01^{b} (I = 11.2)	45.08±0.47^{d} (I = 51.9)	39.07±0.14^{c} (I = 31.7)
AST (U/l)	64.77±0.69^{a}	141.67±0.58^{e} (I = 118.7)	68.15±0.26^{b} (I = 5.2)	105.18±0.32^{d} (I = 62.4)	86.28±0.24^{c} (I = 33.2)
ALP (U/l)	70.33±0.58a	118.00±0.00e (I = 67.8)	75.50±0.50^{b} (I = 7.4)	97.53±0.50^{d} (I = 38.7)	80.33±0.58^{c} (I = 14.2)
TBIL (mg/dl)	6.85±0.05a	15.27±0.25^{e} (I = 122.9)	7.35±0.09^{b} (I = 6.80)	10.35±0.31^{d} (I = 51.1)	8.07±0.12^{c} (I = 17.8)
Protein (mg/dl)	11.58±0.50^{e}	4.59±0.09^{a} (D = 60.4)	9.63±0.08^{d} (D = 16.8)	6.52±0.09^{b} (D = 43.7)	8.07±0.22^{c} (D = 30.3)

Values are Mean ± SD (n=5). Values with different superscripts in a row are statistically different compared to control at P<0.05, D: Percentage decrease compared to control, I: Percentage increase compared to normal control.

Table 5. Effect of aqueous leave extract of *M. charantia* on the activity of CAT and SOD and the levels of MDA and GSH in CCl4 intoxicated rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
TBARS (MDA) (µmol/mg tissue)	6.83±0.04^{a}	13.96±0.06^{b} (I = 104.4)	6.99±0.01^{c} (I = 2.3)	10.33±0.57^{d} (I =51.2)	8.63±0.33^{e} (I = 26.4)
GSH (µmol/mg tissue)	90.37±0.32^{a}	27.50±0.44^{b} (D = 69.6)	86.92±0.12^{c} (D = 3.8)	66.55±0.51^{d} (D = 26.4)	72.45±0.77^{e} (D = 19.8)
SOD (U/mg tissue)	78.35±0.31^{a}	36.88±0.20^{b} (D = 52.9)	77.48±0.05^{b} (D = 1.1)	62.67±0.73^{c} (D = 20.0)	69.13±0.22^{d} (D = 11.8)
CAT (U/mg tissue)	35.15±0.25^{a}	16.86±0.24^{b} (D = 52.0)	33.33±0.56^{c} (D = 5.2)	22.52±0.58^{d} (D = 35.9)	28.33±0.58^{e} (D = 19.4)

Values are Mean ± SD (n=5). Values with different superscripts in a row are statistically different compared to control at P <0.05, D: Percentage decrease compared to control, I: Percentage increase compared to normal control.

The histopathological examination of liver section revealed that CCl4 caused marked damage of rat hepatocytes in the form of fatty degeneration and hepatocellular necrosis. However, treatments with 200 and 400 mg/kg aqueous extract of *Momordica Charantia* leaves and silymarin (standard drug), markedly attenuated the CCl4 induced histopathological changes in rat liver (Fig. 1(a-e)).

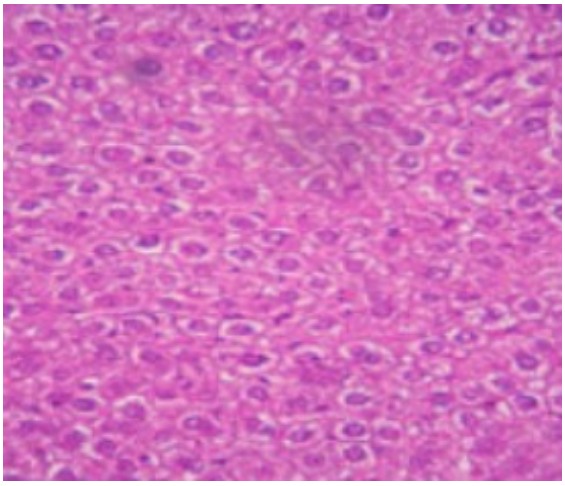

Fig. 1. (a) Normal control group

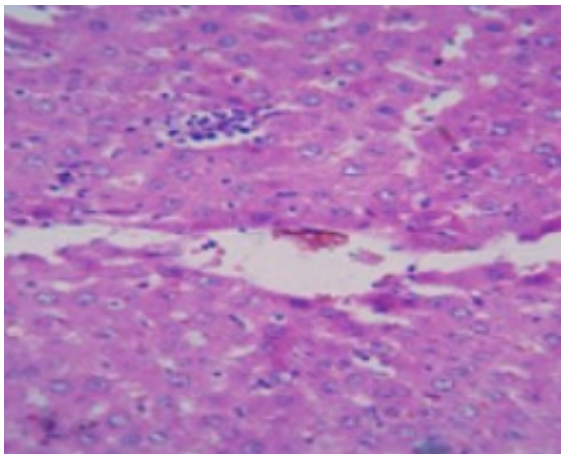

Fig. 1. (b) CCl4 (1ml/Kg) treated group (Positive control)

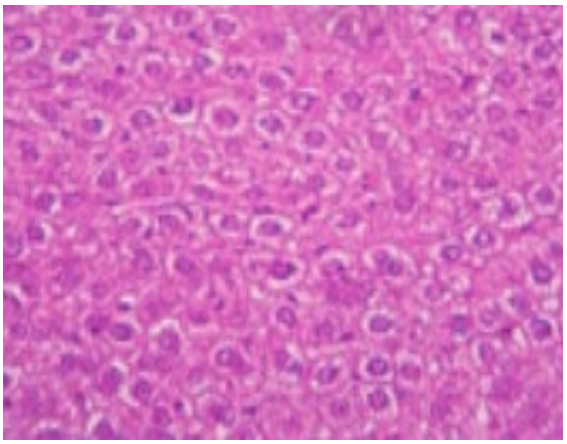

Fig. 1. (c) CCl4 (1ml/Kg) + Silymarin (50 mg/Kg) treated group

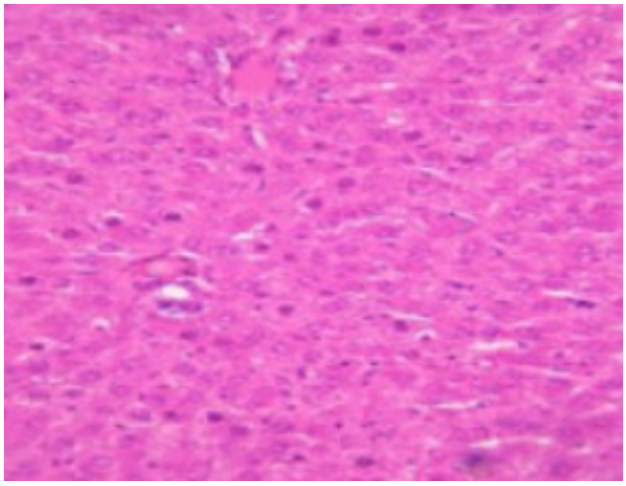

Fig. 1. (d) Aqueous extract of *M. charantia* (200 mg/Kg) treated group

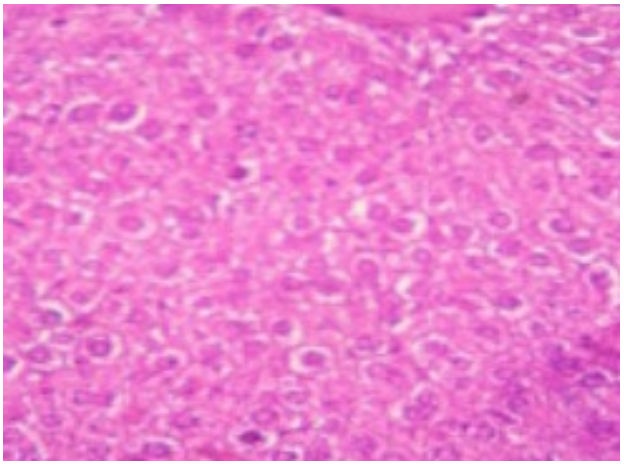

Fig. 1. (e) Aqueous extract of *M. charantia* (400 mg/Kg) treated group

4. DISCUSSION

The present research study was designed to evaluate hepatoprotective effect of the extract of *Momordica charantia* against CCl4 induced liver damage in rats. The hepatotoxicity induced by CCl4 is due to its metabolite CCl_3•, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on lipids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage [27]. The result of this study revealed that oral acute toxicity study (LD_{50}) of the aqueous leaves extract of *Momordica charantia* was greater than 5000 mg/Kg, implying that the plant was relatively safe under our study conditions. Also quantitative phytochemical analyses of the plant revealed the presence of tannins, saponins, alkaloids, flavonoids and polyphenolics. Again metabolites, particularly flavonoids and polyphenolics have been attributed for the antioxidant and hepatoprotective activity observed [28]. Administration of CCl4 in experimental animals induced a significant ($P<0.05$) increase in liver weight, impaired animal growth and organ function and thus

blocked secretion of hepatic triacylglycerols (TAGs) into plasma. However, treatment with *M. charantia* extract ameliorated the increase in liver weight to near normal [29]. Evaluation of haematological parameters especially haemoglobin (Hb) and packed cell volume (PCV) are relevant and vital indices to toxicity assessment. The result of our study indicated significant ($P<0.05$) decrease in the level of Hb and PCV in toxin control group compared to normal control group which could be due to excessive destruction of erythrocytes, disturbed haematopoiesis and reduction in the rate their formation. Treatment with *M. charantia* extract stimulated haematopoiesis and restored Hb and PCV towards normal values [30]. Aminotransferases are present in high concentration in liver, an important class of enzymes linking carbohydrate and amino acid metabolism. Alanine amino transferase and aspartate amino transferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream. Alkaline phosphatase is a membrane bound enzyme and its elevations in plasma indicate membrane disruption in the organ. Alkaline phosphatases, although not a liver specific enzymes, the liver is the major source of this enzyme. The level of this enzyme increases in cholestasis [31]. Hepatotoxicity is characterized by cirrhotic liver condition which in turn increased the bilirubin release [32]. In the present study, the activities of these enzymes, total billirubin were found to increase in the hepatotoxic animals and were significantly reduced in groups treated with aqueous extract of leaves of *Momordica charantia* administered rats as compared to that of toxin control group. This might be due to the higher contents of flavonoids. It probably did so by reducing the accumulation of toxic CCl_3 derived metabolites, which may contribute to the changes in the rough endoplasmic reticulum and the disturbance of protein metabolism in liver [33]. Malondialdehyde (MDA) is a breakdown product that is frequently quantified as a measure of lipid peroxidation. In the present study, it was observed that, there was significant ($P<0.05$) increase in the level of TBARS and significant decrease in GSH level, SOD and CAT activities in toxin control group compared to normal control group, indicating the development of oxidative stress in the experimental animals. However administration of aqueous leaves extract of *M. charantia* stimulated the antioxidant protective mechanisms against CCl_4 derived free radicals by reducing MDA level and elevating the level of GSH. Also, SOD and CAT activities were also elevated in liver homogenate [34]. Comparative histopathological study of the liver from different groups of rats corroborated the hepatoprotective efficacy of aqueous extract of leaves of *Momordica charantia* (Fig. 1a-e). Pathological changes like steatosis, centrilobular necrosis and vacuolization seen in toxin control group. These pathological changes were ameliorated in both treated groups. This might be due to presence of flavonoids and ascorbic acid. Antioxidant property is claimed to be one of the mechanism of hepatoprotective drugs.

5. CONCLUSION

The results of the present study found that, aqueous leaves extract of *Momordica charantia* posses hepatoprotective effect against CCl4 experimentally-induced liver damage in rats. The possible mechanism of the hepatoprotective effect observed could be due to the potential antioxidant activity of this plant proven experimentally.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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