

The hepatoprotective and antioxidant effects of *Momordica charantia* methanolic extract against paracetamol induced hepatotoxicity in rats

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Abstract

The fruits of *Momordica charantia* are commonly used in Asian countries for various medicinal purposes. In this study, the hepatoprotective and antioxidant effects of *Momordica charantia* fruits and leaves methanolic extracts were investigated in rats against paracetamol (PCM) induced liver injury. Pretreatment of rats with MCME of fruits and leaves parts (300 mg/kg for 7 days) significantly prevented the PCM (1500 mg/kg) induced hepatic damage as indicated by the decrease in serum marker enzymes (AST, ALT, and ALP). Parallel to these changes, MCME treatments also prevented PCM-induced oxidative stress in the rats' liver by restoring the levels of hepatic antioxidant enzymes (SOD, CAT, and GPx). Histologically, our results indicate that MCME of fruits and leaves conserved the hepatic tissue architecture and prevented the hepatic injuries associated with PCM intoxication. In conclusion, the

MCME of fruits and leaves proved to have hepatoprotective and antioxidant potentials and further studies should be performed to isolate the bioactive compounds in these extracts.

Key words

Momordica charantia, paracetamol, hepatoprotective, antioxidant.

Introduction

liver plays a crucial role in metabolism, detoxification and excretion of many endogenous and exogenous substances, so it is more predisposed to inflammation (1,2).

Acute liver failure happens suddenly and it is usually caused by poisoning or an overdose of drugs (3-5). In most cases, liver problems are associated with elevated enzyme markers including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (6)

Since liver is responsible for metabolizing drugs, there are many types of free radicals produced as a result of this process such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) lipid hydroperoxides, 4-hydroxynonenal, isoprostan, 8-hydroxyguanine, and ubiquinol-10 (7). However, low or moderate concentrations of ROS has beneficial effects on cellular response and immune function, but high levels may cause liver toxicity and disrupt the antioxidant defense system of the body which may lead to the oxidative stress (8).

Paracetamol (PCM) is a widely-used antipyretic and analgesic drug for people of all ages (9, 10). PCM undergoes biotransformation via cytochrome P 450s (CYPs) including CYP2E1, CYP3A4 and CYP1A2 into a highly reactive radical, N-acetyl-p-benzoquinoneimine (NAPQI), which can be detoxified with glutathione (GSH) conjugation when produced at low levels (11, 12). PCM at large doses can produce high levels of NAPQI

that exceed the amount of GSH needed to metabolize it, which can cause enhanced ROS generation (12).

In the last decades, there is a growing interest has been noticed in the study of the role of natural products including fruits, vegetables, and herbs in the treatment of liver diseases (13) because chemical drugs can cause a huge harm to the liver cells that can be even worse than the disease itself (14).

One of these herbs that are currently under investigation for its potential therapeutic effects is *Momordica Charantia* which is also known as bitter melon, balsam pear or Karela. *M.charantia* is a flowering vine in the family Cucurbitaceae and is considered as a common food in Indian cuisine and has been widely used in folk medicine as a treatment for diabetes(15).

Many researches revealed that *M.charantia* would possess anti-hyperglycemia, anti-cholesterol, immunosuppressive, anti-ulcerogenic, anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, anti-microbial and anti-tumor activities (16-18).

The therapeutic properties that *M. charantia* fruits possess are because of the active ingredient that it has. It contains glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil, free acids, charantin, and charine. While the leaves are nutritious sources of calcium, magnesium, potassium, phosphorus and iron; both the edible fruit and the leaves are great sources of the B vitamins (17). It is a well-documented fact that *M. charantia* are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant property (18). Flavonoids are a group of polyphenolic compounds that work as a free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. These compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants (19). Based on these advantageous

phytoconstituents, we intended to study the possible ameliorative effects exerted by MCME of fruits and leaves against the oxidative damage induced by PCM on liver cells of rats.

Materials and Methods

Chemicals

Paracetamol (PCM) was purchased from Almaya co. (Libya), and N-Acetylcysteine (NAC) from Laboratoires Galpharma (Tunisia). Kits for the determination of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT), and Total Bilirubin were purchased from Biomaghreb Laboratoires. GPx, CAT, SOD and MDA kits were purchased from Biodiagnostics Co. (Cairo, Egypt). All other chemicals were purchased from standard commercial suppliers and were of analytical grade. All solutions were prepared immediately before use.

Animal care and monitoring:

Male and female wistar rats aged 90-110 days' weight 200-300g were used in the experiments. These rats were divided into 5 groups and kept in plastic cages (47*34*18) under a 12 h dark cycle at room temperature (22 ° C), with free access to Purina rations and water. Animal care and experimental protocol followed the principles and guidelines suggested by faculty of Pharmacy - Misurata university, and were approved by the local ethical committee.

Preparation of plant crude Extracts:

The bitter melon was collected from a farm in Iron and Steel company group - Libya from October to November. Fruits and leaves were cut into small pieces and dried at room temperature (25 C ± 2C) for 3 days. The air-dried plant (1000g) were successively extracted with a solvent of increased polarity; methanol, by soxhlet extractor at 70C°. Extracts were

concentrated by Rotary evaporation to dryness at 45C under reduced pressure for 15-30 min. (figure 1).



Figure (1): Preparation of plant crude Extracts

Qualitative phytochemical screening:

The tested extracts were subjected to preliminary phytochemical investigation for detection of following compounds; alkaloids, carbohydrates, steroids, terpenoids, saponins, tannins & polyphenolic compounds and flavonoids (20).

Experimental design: Experimental animals were randomly divided into 5 groups, five rats in each group. These rats were treated for 8 days as following:

Group I: control: received 0.5 ml saline P.O. daily

Group II: leaves extract (300 mg/kg, p.o daily)

Group III: fruit extract (300 mg/kg, p.o daily)

Group IV: Acetylcysteine (50 mg/kg p.o daily)

Group V: Paracetamol (1500 mg/kg) I.P.

All the treatments except PCM were given for 7 days and 24 hours after the last dose PCM 1500 mg/kg injected I.P. On the 9th day, animals were euthanized and blood was collected for the estimation of serum biomarkers. On the same day, a part of liver is removed and stored in 10% formalin solution for the histopathological studies; the other part is homogenized for antioxidant assay.

Liver function tests:

Blood was collected and allowed to stand for 20min, and then centrifuged for 15-20 minutes at 2000 rpm to separate the serum that was used for biochemical estimations of liver enzymes including aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (21-23) .

Assay of antioxidant enzymes:**Sample preparation for the measurement of Antioxidant enzymes activity:**

To perform this test, each liver was homogenized in 4-8 volumes1 (per weight tissue) of cold buffer (e.g., 50mM EDETA and 1 Mm 2 mercaptoethanol). Then the samples were centrifuged to 4000 rpm for 10-20 minutes at 2-8°C. After centrifugation, the supernatant fluid was collected, frozen at 70°C before use.

Determination of Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD), and Catalase (CAT):

The three enzymes are assayed using Kits obtained from Biodiagnostic, Egypt. The GPx is indirectly assayed according the rate of conversion of NADPH into NADP⁺ which is accompanied by a decrease in absorbance at 340 nm in order to recycle the oxidized into

reduced glutathion; GSSG to GSH⁽²⁴⁾. According to⁽²⁵⁾ the SOD assay is based on the ability of the enzyme to hinder the reducing power for phenazine methosulphate of nitroblue tetrazolium dye, while the CAT reacts with a known quantity of H₂O₂, the reaction is stopped after exactly one minutes with catalase inhibitor. 0.1 ml of H₂O₂ was added to 0.5 ml of the sample to be hydrolyzed with catalase enzyme and this reaction is stopped after incubation for exactly 1 min by supplementing a chromogen inhibitor. A chromophore results from the reaction between the remaining H₂O₂ with 3,5-Dichloro -2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) after addition peroxidase (HRP). This measurement was according to⁽²⁶⁾.

Histological procedures:

Liver tissues were excised from the sacrificed animals, weighed, and fixed in 10% formalin for 48 h and were sequentially embedded in paraffin wax blocks according to the standard procedure, and sectioned at 5 μ thickness. Then, the sections were further deparaffined with xylol, and the histological observations were performed by using the light microscope after staining for functional liver tissue by H & E method described by Stevens (1982).

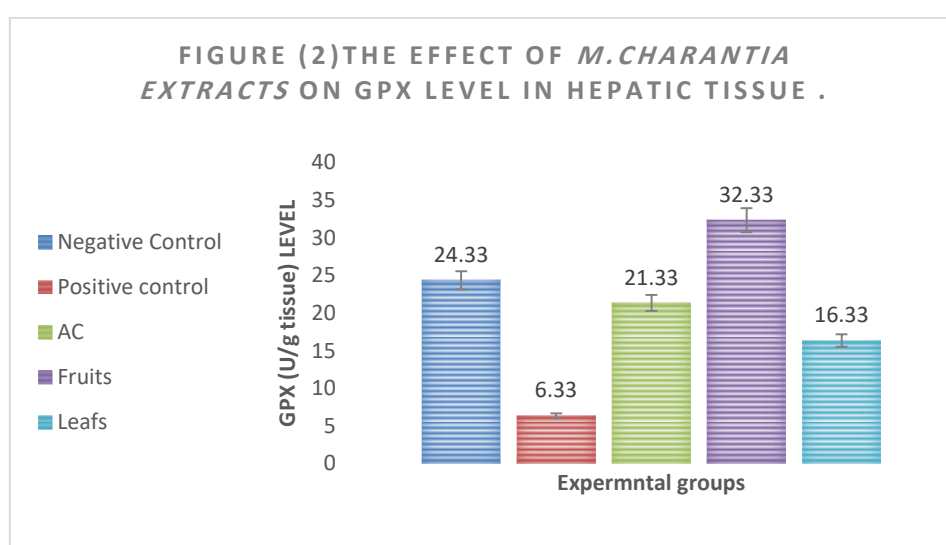
Statistical Analysis:

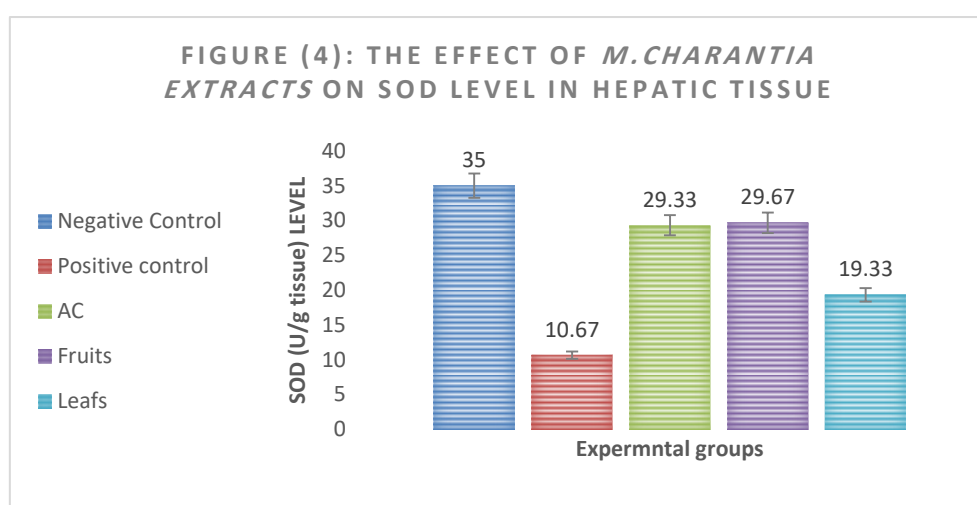
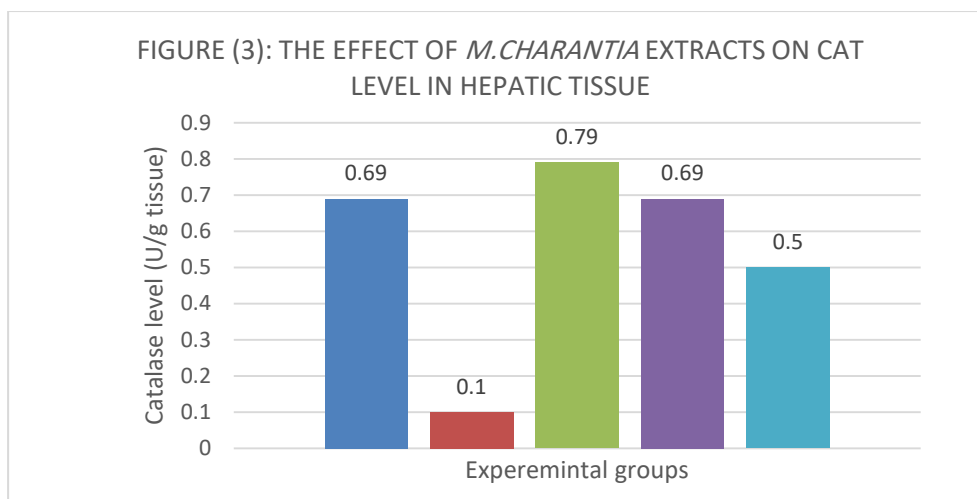
The data obtained were expressed as means (\pm SDM), and analyzed using repeated measures of variance. The differences between the means were analyzed statistically with one-way analysis of variance (ANOVA) and LSD as a Post Hoc test using PSPP program (Linux operating system). Values of $p < 0.05$ were taken to imply statistical significance.

Results and Discussion

Antioxidant enzymes are the main line of defense against free radicals in animal and plant cells. In this experiment, we investigated the antioxidant enzyme levels including (GPX, CAT, and SOD). in liver tissue. The positive control showed significant ($P \leq 0.05$) depression of these enzymes due to production of reactive oxygen radicals where the paracetamol is biotransformed into NAPQI (27), a reactive radical, which could cause damage of hepatocyte and reduce the level of antioxidant enzyme levels specially GPx.

On contrary to that methanolic fruit extract significantly ($P \leq 0.05$) increased the level of antioxidant enzymes GPX (32.33 ± 1.45), CAT (0.69 ± 0.08), and SOD (29.67 ± 1.43) compared with the paracetamol group (6.33 ± 0.88 , 0.10 ± 0.03 , 10.67 ± 1.20) respectively, and there was a non-significant difference with AC group. However, leaf extract showed a moderate increase in the antioxidant enzymes compared with fruit extract as presented in figures (2, 3 and 4). These findings are consistent with Semiz and Sin who showed that there was a significant increase in the hepatic antioxidant enzymes including SOD, CAT and GPx activities in *M.charantia* treated groups against CCL4 induced hepatic toxicity (28).





Since hepatocytes are the main component that regulates various metabolic activities of liver, they are more susceptible to necrosis which causes the releases of liver enzymes such as AST, ALT, and ALK in the circulation (29). Our results revealed that the methanolic fruit and leaf extracts of *M. charantia* have a remarkable effects on AST, ALT and ALP. This treatment reduced the level of liver enzymes in comparison with positive control group as shown in figure (5). It was likely that the reduced levels of ALT, AST, and ALP in the serum by the effect of MC fruit and leaf extract was an indication of alleviation of plasma membrane damage produced by PCM. Moreover, the hepatoprotective role of MC extracts in our findings seems to be due to the presence of flavonoids, and other components such as saponins, tannins, and alkaloids (Table 1) which have the ability to scavenge free radicals

and so increase the antioxidant enzymes (30-32) These results are in the same line with other studies which confirmed the antioxidant and hepatoprotective potential of *Momordica charantia* fruit extract in ammonium chloride-induced toxicity in rats (33).

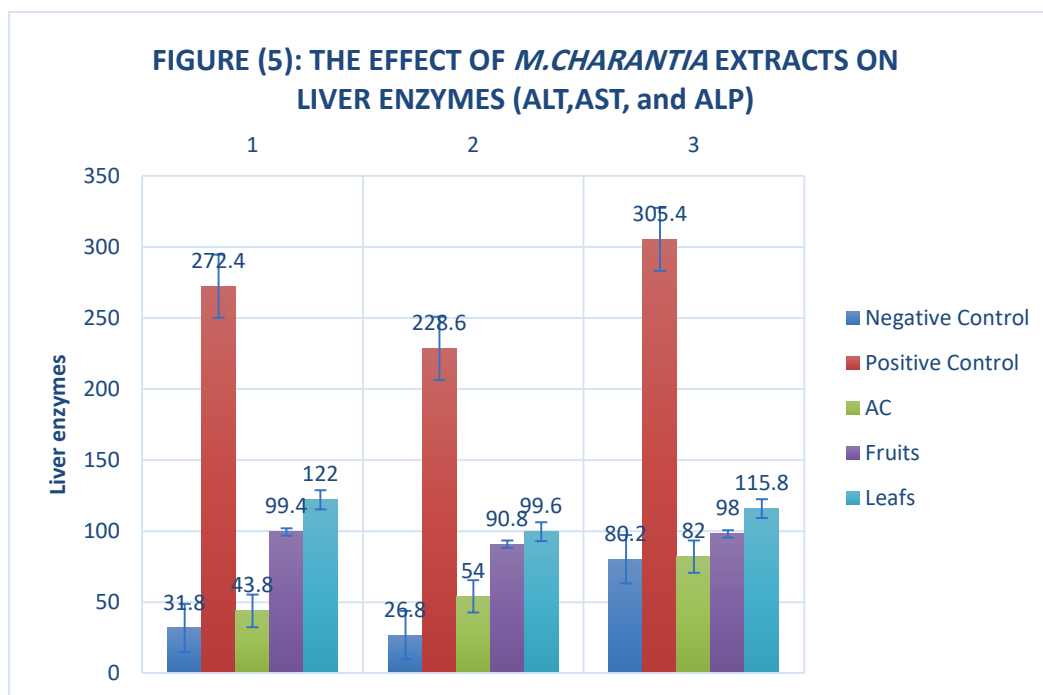


Table (1) The phytochemical screening of *Momordica charantia* methanolic extracts

Phytoconstituents	Fruit extract	Leaves extract
Poly phenolics	+ve	+ve
Carbohydrate	-ve	-ve
Alkaloid	+ve	+ve
Glycoside	+ve	-ve
Flavonoid	+ve	+ve
Saponin	+ve	+ve

Sections of liver from rats treated with acetaminophen showing extensive centrilobular necrosis, hydropic degeneration, congestion of centralveins(CV), karyolysis,

pyknosis and karyorrhexis of nuclei. Acetyl cystein treated liver tissue showed mild necrosis and mild vacuolar degeneration. Compared to the paracetamol control, the extent of damage was significantly less in the plant leaves-treated group: there was no confluent or spotty necrosis, mild ballooning degeneration and steatosis were visible, whereas in the plant fruits group mild improvement was noted (Table 2).

Table (2) The effect of MCME of fruit and leave extracts (300 mg/kg) and N-Acetyl cysteine (50 mg/kg) on liver histological structure against PCM intoxication in rats.

Histopathological findings	Positive control	Acetyl cysteine	Leaves extract group	Fruits extract group
Confluent necrosis	3+	1+	-	2+
Spotty necrosis	3+	1+	-	2+
Ballooning degeneration	3+	1+	1+	2+
Steatosis	3+	1+	1+	2+
congestion	3+	1+	1+	2+

(-) none; (1+) mild; (2+) moderate; (3+) severe

Thus, from the entire study it can be concluded that *M. charantia* can be used as potent natural antioxidant and hepato-protective agent.

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