

The effects of *Momordica charantia* on liver function and histological structure

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Abstract

Introduction: The application of herbal plants because of their lower side-effects has been increasing in recent years.

Objectives: we aimed to study the effects of hydro-alcoholic extract of *Momordica charantia* (bitter melon) on liver function and tissue structure in mice.

Materials and Methods: This study was conducted on 70 male mice which were randomly designated into 7 groups of 10 and were injected with single doses of 0, 100, 500, 1000, 2000 and 4000 mg/kg and multiple daily dose of 500 mg/kg for 7 days, intraperitoneally. Finally, liver tissues were taken out for histological examinations. Serum samples were assayed for liver enzymes activities of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase. Also the antioxidant activity of blood and bitter melon were measured. Subsequently, data were analyzed by analysis of variance (ANOVA) and Bonferroni tests using Stata software.

Results: The results of the present study showed that a single dose of *Momordica charantia* fruit extract at doses up to 4000 mg/kg extract does not significant adverse effects on the liver enzymes and tissue structure.

Conclusion: Using this drug for short time and low dose has not toxicity effects on liver enzymes activity and tissue structure, hence, it is safe in this range of doses.

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Introduction

Since ancient times the use of medicinal plants has been common in the treatment of many diseases (1). But, some constituents of these herbs may lead to severe and on occasion fatal poisoning. Hence we are today faced with case reports of toxic herbs, constantly (2). A lot of herbal products are used to reduce blood sugar in different cultures, among them *Momordica charantia* is the most common medical plant (3).

Bitter melon belongs to the *Cucurbitaceae* family, *Momordica* genus and it is also known as Bitter ground in England, Karela in India, Paharkia in Tamil, Pinyin in China and in Malaysia (4).

It has various effects such as anti-diabetic, anti-viral, anti-tumor, anti-ulcer, anti-inflammatory, lowering cholesterol, lowering triglycerides, blood pressure, immune stimulant, insect repellent, prevention of pregnancy, antimalarial, anti-eczema, property menstrual stimulant, anti-pneumonia,

Core tip

Administration of *Momordica charantia* for short time and low dose has not toxicity effects on liver enzymes activity and tissue structure, hence, it is safe in this range of doses.

anti-rheumatoid arthritis, anti-scabies, anti-psoriasis, and cytotoxic properties (5). Hypoglycemic activity is one of the most obvious medicinal properties of this herb so that all parts of the plant such as fruits, seeds, leaves and roots have this feature and because of the long-term history of use, this plant is known as herbal insulin. Over the years several mechanisms have been introduced to explain the hypoglycemic activity of this drug. For example, one of these proposed mechanisms is inhibition of glucose absorption in intestinal tract by drugs (6). Other evidences suggest that *Momordica charantia* reduces gluconeogenesis, increases glycogen synthesis in the liver and also rises glucose

oxidation in peripheral erythrocytes and fat cells (7). Some studies have reported that *Momordica charantia* increases the secretion of insulin from the pancreas. Hence, this hypothesis was created that *Momordica charantia* extract increases the production of beta cells in the pancreas, although this mechanism has not been confirmed by studies (8).

Objectives

There are some unofficial reports denoting the possible liver toxicity of this plant. While, there are not sufficient published reports about side effects of bitter melon, we intended to investigate the effects of this herbal medicine on liver enzymes activity and histological changes.

Materials and Methods

Momordica charantia fruits were obtained from India and approved by expert botanist in Medical Plants Research Center of Shahrekord University of Medical Science, Shahrekord, Iran.

Extraction process

Hydro-alcoholic extract of *Momordica charantia* was prepared by percolation method with 95% ethanol, followed by steam evaporation, after that, five different concentrations of extract were prepared.

Phytochemical analysis

Total flavonoids content was estimated by aluminum chloride colorimetric method. The standard solutions of routine with concentrations of 25, 50, 100, 250 and 500 ppm from 60% methanol was prepared, then 1 mL of each solution was transferred to test tubes and 1 mL solution of aluminum chloride 2% and then 6 mL of potassium acetate 5% was added to the solution and after 40 minutes the absorbance was measured at 415 nm (9).

Measurement of total phenolic content

Total phenolic content was determined by folin-ciocalteu reagent method and the absorbance was measured at 760 nm. Total phenolic content in terms mg/g of extract was calculated (10).

Antioxidant activity assays

B-carotene/linoleic acid-coupled oxidation reaction

A solution of β -carotene was prepared by liquefying 2 mg in 10 mL of chloroform. Then 0.02 mL of linoleic acid and 0.2 mL of tween 40 was added, and the mixture was remained at 20°C for 15 minutes. After dehydration of the chloroform in a rotary evaporator at 40°C, 50 mL of oxygen-saturated distilled water at 25°C was added and the mixture was vortexed strongly (1 minute) to form an emulsion (β -carotene/linoleic acid emulsion). The required wells were charged (of a 96-well microtiter plate) with each different amount of samples and 100 μ L of emulsion per well. The microplate was located on a horizontal shaker and shaken at 100 rpm (during 1 minute). A control sample was also arranged similarly. Absorbance mea-

surements (470 nm) at $t = 0$ minute were made after incubation at 50°C during 120 minutes. All experiments were done in triplicate. Antioxidant activity was mentioned as the percent of inhibition with regard to the control sample and calculated as follows:

$$AA=100[1-A_0-A_{t1}]/[A_0-A_{t0}]$$

where S_{A0} and C_{A0} are the absorbance values of the sample and the control defined at 0 minute; the S_{At} and C_{At} were the absorbance values of test sample and control measured after 120 minutes. BHT was used as positive control (11).

Animal and treatment

Seventy male *db/db* mice weighing 25-30 g and 3 weeks old were distributed into seven groups with 10 animals randomly in each group as follows:

Group 1: control group (without drug)

Group 2: obtained at 100 mg/kg extract as a single dose.

Group 3: received at 500 mg/kg extract as a single dose.

Group 4: gained 1000 mg/kg extract as a single dose.

Group5: received 2000 mg/kg extract as a single dose.

Group 6: obtained 4000 mg/kg extract as a single dose.

Group 7: received 500 mg/kg/day extract for one week.

All of the mice were maintained on a 12-hour light/dark cycle. All protocols for animal use were approved by the animal care committee of Shahrekord University of Medical Sciences animal care. All the experiments were done after one week in order for the mice to become accustomed to the new environment and they could freely access water and food (12). After one week on the eighth day various doses of the extract was injected intraperitoneally to mice. The mice were cared for 72 hours and then they were anesthetized and blood samples were stored in tubes for biochemical parameters estimation (13). Then blood samples were centrifuged at 3500 rpm for 15 minutes to evaluate serum liver enzymes. Liver enzymes were measured with enzymatic method (Pars Azmoon kit) by Automatic Analyzer 902 (Hitachi, Germany).

Ferric reducing ability

Ferric reducing ability of plasma (FRAP) reagent was mixed with 90 μ L of distilled water and 30 μ L of test sample solutions. The reaction mixture was then incubated at 37°C for 10 minutes and absorbance was recorded at 593 nm, using a spectrophotometer (uv3100 Shimadzu, Japan). The concentrations of $FeSO_4$ were in turn plotted against concentrations of the standard antioxidants (14).

Histological study

After animals were euthanized by ether, liver tissues were collected and put into 10% buffered formalin for 48 hours. Subsequently, organs were embedded within paraffin. Solid sections of 5 μ m thickness were made using a microtome. The sections were stained with hematoxylin and eosin (H&E) and then observed by light microscopy for histopathological examination (15).

Ethical issues

The research followed the tenets of the Declaration of Hel-

sinki. The research was approved by ethical committee of Shahrekord University of Medical Sciences. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Shahrekord University of Medical Sciences.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) and chi-square tests using SPSS software. $P < 0.05$ was considered significant for all data.

Results

Antioxidant capacity of bitter melon (percent inhibition of peroxidation in linoleic acid production) was 68%. The amount of flavonoids was 54 mL/μg, flavonols 45 mL/μg and phenolic content 413 mL/μg in the extract.

Antioxidant capacity of blood

Antioxidant capacity of blood was 568 μmol/L before the study. After the intervention the blood antioxidant capacities in groups were as follows:

Group 1: (control group) 564 μmol/L; group 2: 1108 μmol/L; group 3: 741 μmol/L; group 4: 553 μmol/L; group 5: 703 μmol/L; group 6: 624 μmol/L; group 7: 436 μmol/L.

Effect of different doses of Momordica charantia on liver enzymes activity

There were no significant difference of alkaline phosphatase (ALP), aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT) activity between groups ($P > 0.05$; Table 1).

Effects of different doses of Momordica charantia on liver histology

Histological examination of the liver showed no pathologic findings and there was not significant different between liver tissue in control group and other treatment groups ($P > 0.05$).

Discussion

In this study, 70 mice were divided into 7 groups and were treated with different doses of *Momordica charantia* extract. The smallest dose was 100 mg/kg, and the greatest was 4000 mg/kg. Also, a group treated for one week 500 mg/kg extract. In this study, liver enzymes activity including ALP, SGPT and SGOT was measured. During the in-

tervention, liver enzymes were not significantly different between the control group and groups treated with extract. As well, there were not changes in histology of liver tissue in our study and there was not difference between treated groups and control group.

The results in our study were in agreement with the results of another study which was carried out on the effects of *Momordica charantia* on key hepatic enzymes and indicated that this valuable plant contain hepatotoxin capable of causing cellular damage but at molecular level without any significant histopathological change. While in many other investigations effects of *Momordica charantia* on kidney were confirmed because not only kidney is one of the most affected organs by diabetes but also in many researches the anti-diabetic properties of this worthy herbal drug has been proved currently like lots of other medicinal plant.

A report about the effect of *Momordica charantia* on diabetic mice showed that due to diabetes extra space between hepatic sinusoids of liver disappeared after treating with extract. The extract of bitter melon can protect damage tissue liver (16). Also in the study conducted by Nazrul-Hakim et al liver pathology was examined and they observed a small amount of congestion and also found that there were no significant differences between control and other groups (17). Subsequently, the results of the present study remarkably showed that a single dose of *Momordica charantia* fruit extract at doses up to 4000 mg/kg extract does have not significant adverse effects on the liver contrary to effect on kidney structure and tissue. Because of antioxidant activity of this medicinal plant and also its anti-diabetic characteristics we strongly recommend more research about its impact on all organs and tissues involved in diabetes.

Conclusion

Momordica charantia extract and low dose had not toxicity effects on liver enzymes activity and tissue for short time so long term treatment for patients with liver abnormalities should be checked regularly and if it has disrupting drug should be discontinued.

Authors' contribution

SM, EK and MRK conducted the research. AA conducted the statistical analysis. SK Prepared the primary draft. MRK edited the final manuscript. All authors read and sign the final paper.

Conflicts of interest

The authors declared no competing interests.

Table 1. Comparison mean of liver enzyme activity in the study groups

Groups	SGOT (mg/dL)	SGPT (mg/dL)	ALP (mg/dL)
(Control)	165.5 ± 51	90 ± 70	200 ± 95
(100 mg/kg)	196 ± 91	97.8 ± 68	206 ± 71
(500 mg/kg)	143 ± 57	67.7 ± 45	243 ± 108
(1000 mg/kg)	199 ± 92	77 ± 40	190 ± 110
(2000 mg/kg)	173 ± 130	49.5 ± 28	159 ± 62
(4000 mg/kg)	206 ± 38	88 ± 67	192 ± 99
(500 mg/kg)	180 ± 63	85.7 ± 40	223 ± 49
Total mean	180.8 ± 76	80.6 ± 54	204.7 ± 88

Data are shown as the Mean ± SEM, (n = 7, $P < 0.05$).

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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