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Impact of *Momordica charantia* extract on kidney function and structure in mice

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ABSTRACT

Background: Bitter Melon (BM) is known for its hypoglycemic effect and is commonly used in populations.

Objectives: This study examined the effects and safety of bitter melon fruit in laboratory mice.

Materials and Methods: In this experimental study 70 male mice (25-30 gr) were randomly divided into 7 groups. The mice were injected intraperitoneally with single doses of 0, 100, 500, 1000, 2000 and 4000 mg/kg and multiple doses 500 mg/kg daily for 7 days. The mice were then observed for 72 hours before sacrificing. Immediately kidneys were taken out for histological examinations. Tubular cell vacuolization and flattening as well as hyaline casts, debris and dilatation of tubular lumen were the morphologic lesions which were assessed with scores from 0 to 4, while zero score addressed normal renal tissue. Serum samples were assayed for kidney function (creatinine; Cr and Blood Urea Nitrogen; BUN). Blood and bitter melon antioxidant activities were measured, too. Data were analyzed with Stata software (Stata Corp. 2011. Stata Statistical Software: Release 12. College Station, TX: Stata Corp LP) using ANOVA and Bonferroni tests.

Results: All single dose groups showed normal behavior after the dosing and no statistical changes were observed in blood parameters ($p>0.05$). Histological examinations revealed normal organ structures, however, the group treated for 7 days showed statistically a significant change in BUN ($p=0.002$) and a borderline significance in Cr ($p=0.051$).

Conclusions: Administration of up to 4000 mg/kg did not have any effect on the mice kidney function and histology, however chronic administration were nephrotoxic. More studies with different dosage regimens are suggested.

Original Article

Implication for health policy/practice/research/medical education:
Bitter melon is known for its hypoglycemic effect and is commonly used in populations. This study examined the effects and safety of bitter melon fruit in laboratory mice. The results of this study showed that 4000 mg/kg of *M. charantia* fruit extract (Bitter melon) as single dose has not any significant adverse effects on renal function and structure. Longer-term consumption for 7 days can cause some complications in kidney tissue and its function. We therefore can conclude that the consumption of this drug for short time and low dose may have not toxicity effects on kidney tissues. However, long term treatment should be checked regularly and if it has disrupting effects, the drug should be discontinued.

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1. Background

Momordica charantia is a member of Cucurbitaceae family known as bitter melon. It is grown in tropical and subtropical countries (1). This plant has traditionally been used as herbal medicine (2). The fruit contains charantin, momordium, carbohydrates, mineral matters, ascorbic acid, alkaloids and glucosides. The ethanolic extract of the fruit showed the presence of proteins, alkaloids, tannins, steroids, glycosides and carbohydrates and two classes of saponins known as cleanane and oleanane (3,4). Bitter melon has positive effect on diabetes, blood pressure, immune system, pneumonia, cancer and infection (5-7). The extract of fruits has protective effect on diabetic kidney disease due to its antioxidant properties (8). Also it can stimulate insulin secretion and induce glucose uptake in liver in diabetic rats (9). In some experimental studies, it has shown low toxicity following oral intake (7-10). There are lots of reports published on beneficial effects of bitter melon, however side effects of this plant have not been proven, yet (11,12). The aim of this study was to examine the renal toxicity of bitter melon with different concentrations of this herb by evaluating serum creatinine, BUN and examining histological changes in kidneys of mice.

2. Materials and Methods

2.1. Extraction procedure

Momordica charantia fruits were purchased from India and confirmed by expert botanist. Hydroalcoholic extract was prepared by percolation method (13) with 95% ethanol, followed by steam evaporation (14). Five concentrations of the extract were prepared.

2.2. Phytochemical analysis

Total flavonoid content was estimated by aluminium chloride colorimetric method. Rutin was used as standard and different concentrations of 25, 50, 100, 250 and 500 ppm in methanol 60% was prepared, then 1 ml of each solution was transferred to a test tube and 1 ml solution of aluminum chloride 2% was added. Then 6 ml of potassium acetate 5% was added to the solution and after 40 minutes the absorbance was measured at 415 nm (15).

2.3. Measurement of total phenol content

Total phenol content was determined by Folin-Ciocalteu reagent and the absorbance was measured at 760 nm. Total phenol content in terms of mg/g of dried extract was calculated (13).

2.4. Antioxidant activity assays

β-Carotene/Linoleic Acid-Coupled Oxidation Reaction

A solution of β-carotene was prepared by dissolving 2 mg in 10 mL of chloroform. An amount of 0.02 mL of linoleic acid and 0.2 mL of tween 40 was afterward added, and the mixture was left at 20°C for 15 min. After evaporation 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 min) to form an emulsion (β-carotene/linoleic acid emulsion). The necessary wells of a 96-well microtiter plate were charged with different volumes of sample and 100 µL of emulsion per well. A control sample was also prepared the same. Absorbance measurements (470 nm) were made at t=0 min and after incubation at 50°C for 120 min. All experiments were performed in triplicate. Antioxidant activity was expressed as the percent of inhibition with respect to the control sample and calculated as follows:

$\{AA\% = [1 - (S_{A0} - S_{A1})/ (C_{A0} - C_{At})] \times 100\}$

where S_{A0} and C_{A0} are the absorbance values of the sample and the control determined at 0 min; the S_{At} and C_{At} are the absorbance values of test

sample and control measured after 120 min. BHT was used as positive control (16).

2.5. Animals and treatments

In this experimental study 70 male db/db mice weighing 25-30 g were divided into seven groups with ten animals in each group as follows:

Group 1: control group (without drug) (sham group)

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

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

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

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

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

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

All the mice were maintained on a 12-h light/dark cycle. All protocols for animal experiments 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 (17).

At the beginning of the experiment blood samples were collected in test tubes without anticoagulant (EDTA) for estimation of biochemical parameters (18). After one week on the 8th day various doses of extract were injected intraperitoneally to mice. The mice were observed for 72 hours and then they were anesthesia and blood samples were collected in tubes for biochemical measurements (19). Then blood samples were centrifuged at 3500 rpm for 15 min to detect serum creatinine and Blood Urea Nitrogen (BUN) (20). Serum Cr and BUN were measured by enzymatic method using standard kits, using Automatic Analyzer 902 Hitachi (Germany) (21).

2.6. Ferric reducing ability

Ferric Reducing Ability of Plasma (FRAP) reagent was mixed with 90 µl of distilled water and 30 µl 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 (22).

2.7. Histological study

After animals were euthanized by ether, livers and kidneys were collected and put into 10% buffered formalin for 48 h. Then, organs were embedded within paraffin. Solid sections of 5 µm thickness were made using a microtome. The sections were stained with hematoxylin and eosin and then observed by light microscopy for histopathological examinations (20). In this study morphology of tubular cells of kidney were observed. Renal tubular damages of tissue samples were assessed using damage scores from 0 to 4. The ratings were as follows:

Score 0 = normal tubular cells, score 1= 0-19 % tubular cell injury, score 2= 20-49 % tubular cell injury, score 3= 50-69% tubular cell injury and score 4 was considered 70-100 tubular cell injury (21-23). Tissue damages including cell loss and degeneration, hyaline casts, intra-tubular debris, tubular cell vacuolization, flattening and dilatation were scored (22,23).

2.8. Statistical analysis

Data were analyzed with Stata software (Stata Corp. 2011. Stata Statistical Software: Release 12. College Station, TX: Stata Corp LP) using ANOVA and Bonferroni tests. $P<0.01$ was considered

significant for all data.

3. Results

Antioxidant capacity of bitter melon (percent inhibition of peroxidation in linoleic acid production) was calculated to be 68%. The amount of the extract flavonoid was 54 µg/g, flavonol was 45 µg/g and the amount of phenolic content was 413 µg/g.

3.1. Antioxidant capacity of the blood

Antioxidant capacity of blood before intervention was 568 µmol/lit and at the end of the experiment in different groups was as follows:
Group 1: (control group) 564 µmol/lit, Group 2: 1108 µmol/lit, Group 3: 741 µmol/lit, Group 4: 553 µmol/lit, Group5: 703 µmol/lit, Group 6: 624 µmol/lit and Group 7: 436 µmol/lit.

3.2. Plasma creatinine and BUN levels

According to Table 1, between groups there are no significant differences in terms of creatinine levels (p=0.0517). Comparison of BUN between groups showed that there were significant differences between groups 7 and 1 (p=0.026), 7 and 4 (p=0.025) and 7 and 6 (p=0.001).

Table 1. Comparison (mean ±SD) of serum creatinine and blood urea nitrogen levels in the study groups

Groups	BUN (mg/dl)	Cr (mg/dl)
1(Control)	31.6±2.6	0.34±0.07
2(100 mg/kg)	32.2±2.5	0.39±0.11
500 mg/kg))3	32.7±4.3	0.45±0.15
4(1000 mg/kg)	31.8±3.6	0.42±0.09
5(2000 mg/kg)	32.6±2.8	0.38±0.03
6(4000 mg/kg)	28.6±2.9	0.41±0.07
7(500 mg/kg/day)	37±3.6*	0.48±0.1
P Value	0.0002*	0.0517

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

3.3. Effects of different doses of Momordica charantia on renal histology

There were no significant differences for morphologic variables of cell loss and cell debris between groups (p=0.064). However, there were significant differences between the scores of group 7 and other groups in other variables as follows:
Variable Score Between group 7 and groups 4, 5 and 6 was significant (p=0.01), vacuoles between group 7 and groups 4, 5 (p=0.004), and cell damage between group 7 and group 4 (p=0.047). There was a borderline significant difference between group 7 and groups 1 and 6 for hyaline (p=0.054) vacuoles between groups 7 and 4 & 5 (p=0.004), and cell damage between groups 7 and 4 (p=0.047) were significant respectively.

4. Discussion

In this study, 70 mice in 7 groups were treated with different doses of *Momordica charantia* extract. The lowest dose was 100 mg/kg and the maximum dose was 4000 mg/kg. Also, a group was treated with 500 mg/kg/day for one week. In this study the kidney function test was assessed measuring BUN and Cr. Also morphologic lesions of renal histology were examined. BUN and Cr levels of the group which was treated for one week was not significantly different compared to the control and other groups.
The results in our study were in accord with the results of another study which was carried out on 30 male Wistar rats to evaluate the effect of polypeptide isolated from seeds of *Momordica charantia* in which the animals were divided into three groups and treated with single dose of the extract. The animal groups were treated with doses of 0, 500 or 1000 mg/kg and were monitored for 72 hours, then BUN and Cr and renal morphologic lesions were assessed. The results showed that there was no significant difference

Table 2. Comparison of changes in the scores of histology variable in the cells and tissues of the studied groups.

Groups	Score	Tubular dilatation	Cell damage	Vacuoles	Cell debris	Hyaline casts	Cell loss
(Control)1	2.7±1	0	1.3±1	1.4±1	0.1±0.3	0	0
2(100 mg/kg)	1.8±1	0	1.1±1	0.7±0.9	0	0	0
500 mg/kg))3	2.4±2	0.1±0.3	1.1±1	1.1±1	0.2±0.4	0.1±0.3	0
4(1000 mg/kg)	1±1	0.1±0.3	0.6±0.9	0.2±0.6	0.1±0.3	0.1±0.3	0
5(2000 mg/kg)	1.7±2	0.2±0.6	0.8±1	0.5±1	0.3±0.6	0.1±0.3	0.1±0.3
6(4000 mg/kg)	2±3	0.4±0.8	0.8±1	0.8±1	0.1±0.3	0	0
7(500 mg/kg/d)	6.3±5	0.2±0.4	2.5±1*	3±3*	0	0.6±0.9	0
F Ratio(ANOVA)	3.35	0.92	2.24	3.56	0.86	2.48	1
P- value	0.0064*	0.48	0.05	0.006*	0.064	0.0326*	0.43

Data are shown as the Mean ±SEM, n=7 (*$p<0.05$ was significant).

for different parameters between groups which received the extract and control group (12).

The results of the present study showed that kidney tissue treated with 4000 mg/kg as single dose of *Momordica charantia* did not cause any damage in the kidneys tissues; however a week-long drug treatment led to some pathological changes in the kidneys of mice. In the study of Nazrul-Hakim et al, histological examination showed that till 1000 mg/kg dose of bitter melon for 72 hours would not cause any change in renal structure of mice (26). Furthermore, tubular cell destruction, necrosis and hemorrhage in kidneys of mice after treatment with Bitter melon fruit juice was observed for two weeks (26). The results of this study are endorsing the results of our research.

5.Conclusions

The results of this study show that 4000 mg/kg of *Momordica charantia* fruit extract as single dose has not any significant adverse effects on renal function and structure. Longer-term consumption for 7 days can cause some complications in kidney tissue and its function. We therefore can conclude that the consumption of this drug for short time and low dose may have not toxicity effects on kidney tissues. However, long term treatment should be checked regularly and if it has disrupting effects, the drug should be discontinued.

Authors' contributions

SM, MRK and RK conducted the research. AA analyzed the data. HN and SH prepared the primary draft. MRK edited the manuscript.

Conflict of interests

The author declared no competing interests.

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