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Acute Toxicity Evaluation of Methanolic Seed Extract of Bitter Melon (*Momordica charantia*) in Swiss Albino Mice

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Abstract

Bitter melon scientifically known as Momordica charantia, is an important vegetable with a variety of medical uses. This study aimed to analyze toxicity profiling of methanolic seed extract of M. charantia in Swiss albino mice. For this purpose, Swiss albino mice were provided with the methanolic extract of the seed, that was given orally in a single dose of 2000 mg/kg of body weight. Following this, the mice kept under observation for the eight consecutive days. At the end of the acute stage, mice were dissected and samples of blood were collected to analyze various biochemical (ALT, AST, ALP, uric acid, urea, creatinine, LDL, and HDL) and hematological parameters (RBCs, WBCs, MCV, hematocrit, platelets, and hemoglobin). Some major organs (liver, kidneys, spleen, lungs, and heart) were surgically removed for histopathological examination. It was found that the oral administration of the methanolic extract of M. charantia seed had no harmful effects on biochemical and hematological parameters. Moreover, the methanolic seed extract of M. charantia did not exert toxic effects on vital organs of the mice, suggesting its effective use in treatment of various disease.

1. INTRODUCTION

Traditional herbal treatments use plants to heal a variety of diseases. Herbal medicine uses plants or plant extracts for curative objective, either internally or externally (Sam *et al.*, 2019). Humans have long relied on medicinal herbs for antioxidant and anti-inflammatory actions of phytochemicals in vegetables, fruits, spices, and herbs (Zahn *et al.*, 2019). Numerous illnesses have been treated and prevented with therapeutic herbs. Studies have shown that curcumin, a chemical contained in turmeric, blocks important inflammation pathways and has potent anti-inflammatory properties (Alajeeli *et al.*, 2023).

Momordica charantia, commonly known as bitter gourd, bitter melon, balsam pear, karela, or pare, is an important medicinal plant. It has long been used as a food and medicine throughout tropical America, including the Amazon, East Africa, Asia, India, and the Caribbean. The fruits are long and thin. Unripe fruit, which can be green or white, becomes astringent and bitter as it matures (Das *et al.*, 2015). *M. charantia* is an important commercial source of human nutrition. (Talukder *et al.*, 2013). Bioactive phytochemicals in *M. charantia* include steroids, proteins, saponins, triterpenes, flavonoids, alkaloids, and acids (Chekka *et al.*, 2020).

The use of methanolic extracts in pharmacognosy can be explained by the fact that methanol has the ability to extract a wide variety of phytocompounds. *In vitro* research is essential in initial screening of biological activity (Abdullahi, 2024). The safety and efficacy of materials on living organisms can only be confirmed *in vivo*. The medicinal potential of the extracts may be assessed with the aid of animal models evaluating

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various parameters. Such studies are important towards the translation of *in vitro* results into viable therapeutic alternatives (Jia *et al.*, 2017). This combinatorial approach facilitates the identification of the median fatal dose (LD50) and safe dosage ranges via acute toxicity testing, while mechanistic insights into cellular-level cytotoxicity can be acquired from *in vitro* tests.

The acute toxicity regarding the methanolic seed extract of *M. charantia* indicated that the extract was safe by a margin in the Swiss albino mice model which supports the use of the extract in the control of atherosclerosis. In accumulated experiments, there are several indexes used to observe including the changes in weight, blood glucose, the activity of antioxidant enzymes, and other physiological changes that happened in the subjects to achieve the desired effects (Mele *et al.*, 2018). The therapeutic doses were tested for different hematological (RBC, WBC, Hb, and platelet counts) and biochemical parameters (Hussain *et al.*, 2019). The objective of this work is to elucidate the effects of the *M. charantia*'s methanolic seed extract on the biochemistry, hematology, and histopathology of Swiss albino mice, consequently enhancing the knowledge of its safety and therapeutic potential.

2. Materials and Methods

2.1. Collection of plant material

Momordica charantia vegetable was purchased from the local market and its seeds were collected using a sharp knife.

2.2. Preparation of methanolic extract of *M. charantia* seeds

Momordica charantia's seeds were collected and dried in shade at room temperature after a thorough tap water wash. After drying, the seeds were ground into a coarse powder in electric grinder. Sieved particles were placed in a zip-top bag to eliminate big particles. 50 grams of leaf powder and 75% methanol were mixed in 1 gram to 10 milliliters on an orbital shaker and stirred for 72 hours at 24 °C. Following this, the mixture was filtered by passing it through a Whatman filter paper No. 1. For later use, the extract was kept at 4 °C after drying (Nauroze *et al.*, 2023).

2.3. Toxicity profiling in mice model

2.3.1. Maintenance of animals

We obtained male and female Swiss albino mice from the University of Veterinary and Animal Sciences, Lahore, Pakistan. The mice weighed 20-30 grams and were 5-6 weeks old. The mice had free access to water, standard pelleted feed, a 23-25 °C temperature range, and a 12-hour light/dark cycle. They had a week to adjust to the lab atmosphere, before starting the experiment.

2.3.2. Ethical approval

To initiate this study, ethical approval was obtained under letter No. UO/ERC/2023/Z16, dated: 23-12-2023 from the Ethical Research Committee, University of Okara, Punjab, Pakistan.

2.3.3. Measurement of body weight

The body weights of all mice were recorded using a digital weighing balance at the beginning of the experiment, and on a daily basis throughout the experimental period. The mice's average body weight was used to prepare all doses.

2.4. Acute toxicity study

Swiss albino mice were divided into two groups of five to start the experiment. Experimental mice provided with one oral dose of 2000 mg/kg body weight of methanolic seed extract of *M. charantia*. Control and treated mice received food and water regularly. For the first 24 hours after the dosing, the mice were monitored for toxicity or unusual behavior. Close observation continued for eight days after oral administration. All mice had to fast for one night before being dissected at the end of trial. Blood was collected from heart after the mice was anaesthetized with chloroform. Blood was collected for biochemical and hematological analyses (Kanwal *et al.*, 2022).

2.4.1. Biochemical analysis

Blood samples were cooled at room temperature for 5-10 minutes and then centrifuged at 3,000

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rpm for 10 minutes in standard evacuated biochemical tubes to collect plasma and serum. Commercially available diagnostic kits were used to measure some biochemical parameters such as ALT, AST, ALP, uric acid, urea, creatinine, HDL and LDL in mice serum (Nauroze *et al.,* 2023).

2.4.2. Hematological analysis

Blood samples were analysed for complete blood count (CBC) includes RBCs, WBCs, hematocrit, PLTs, MCV, and hemoglobin (Hb) using a hematological analyzer (Coulter counter S-plus) (Nauroze *et al.,* 2023).

2.4.3. Histopathological examination

After dissection, the liver, kidneys, spleen, lungs, and heart were surgically removed and cleaned debris with phosphate buffer saline. Afterward, the analytical balance weighed each organ in grams. These organs were then immersed in a pH 7.4 and 10% formalin solution for 24 hours. Next, all the vital organs were dehydrated by increasing alcohol exposure (70%, 90%, and 100%). After xylene extraction, the alcohol was fixed in paraffin wax at 58-60 °C. The blocks were sliced into 5-millimeter slices using a microtome. We stained all tissue sections with H&E. After staining, photomicroscopes were used at various magnifications to evaluate the sections (Kanwal *et al.*, 2022).

2.5. Statistical analysis

The data was tabulated as means and SEM. Statistics were calculated using GraphPad Prism 5 with unpaired t-test. Statistical significance was assessed using the P value of 0.05.

3. Results

All the mice were carefully monitored, and provided with the significant amount of food and water on a daily basis. All the mice survived for a duration of up to eight days from the time of administration of a single dose (2000 mg/kg of body weight) of the *Momordica charantia* seed extract. A close observation was kept on all mice in both the control and treated groups, and no significant effects were found in all the behavioural patterns, such as food and water consumption, mortality, body weight, etc. The extract did not induce any abnormal behavior in the treated mice (Table 1). Furthermore, there was no statistically significant variation in the body weight (Table 2) and in the weight of vital organs (Kidney, Spleen, Lungs, Heart, and Liver) (Table 3) between the control and treatment groups. For acute toxicity study, the mice's blood samples were collected for biochemical (AST, ALP, ALT, Urea, Uric acid, Creatinine, HDL, and LDL) and hematological parameters (RBCs, WBCs, PLT, MCV, Hb, and Hematocrit). There was no significant difference in the level of biochemical (Figure 1), and hematological parameters (Figure 2) of both control and treated groups of mice. Furthermore, histopathological analysis (Figure 3) revealed that, the *M. charantia* seed extract did not induce toxicological harm to the major organs of the treated mice compared to the control group, suggesting that the extract is safe at the tested dose.

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Table 1. The behavioral pattern of extract-treated mice observed during acute toxicity study.

Sr. No.	Parameters	Swiss albino mice	
		Control Group	Treated Group
1.	Lethargy	Not Found	Not Found
2.	Drowsiness	Not Found	Not Found
3.	Mortality	Not Found	Not Found
4.	Nasal bleeding	Not Found	Not Found
5.	Convulsions	Not Found	Not Found
6.	Salivation	Not Found	Not Found
7.	Lacrimation	Not Found	Not Found
8.	Water consumption	Normal	Normal
9.	Food consumption	Normal	Normal
10.	Body weight	Normal	Normal

Table 2. Body weight of control and treated group of mice.

Days	Control Group Body Weight (g)	Treated Group Body Weight (g)
Before Dose	24.7 ± 1.6	24.3 ±1.3
After Dose		
Day-1	24.8 ± 1.4	24.0 ± 1.2
Day-2	24.6 ± 1.5	24.1 ± 1.3
Day-3	25.5 ± 1.6	24.2 ± 1.3
Day-4	24.5 ± 1.4	24.6 ± 1.5
Day-5	25.0 ± 1.0	24.0 ± 1.0
Day-6	24.8 ± 1.0	24.6 ± 1.5
Day-7	24.8 ± 1.0	24.5 ± 1.4

Values are articulated as Mean ± SEM. Values are the mean of five replicates.

Table 3. Body weight of control and treated group of mice.

Sr. No.	Organs	Control Group	Treated Group
1.	Kidney	0.3 ± 0.2	0.3 ± 0.0
2.	Liver	1.5 ± 0.1	1.9 ± 0.0
3.	Spleen	0.2 ± 0.0	0.2 ± 0.0
4.	Heart	0.1 ± 0.0	0.2 ± 0.0
5.	Lungs	0.2 ± 0.0	0.2 ± 0.0

Values are articulated as Mean ± SEM. Values are the mean of five replicates.

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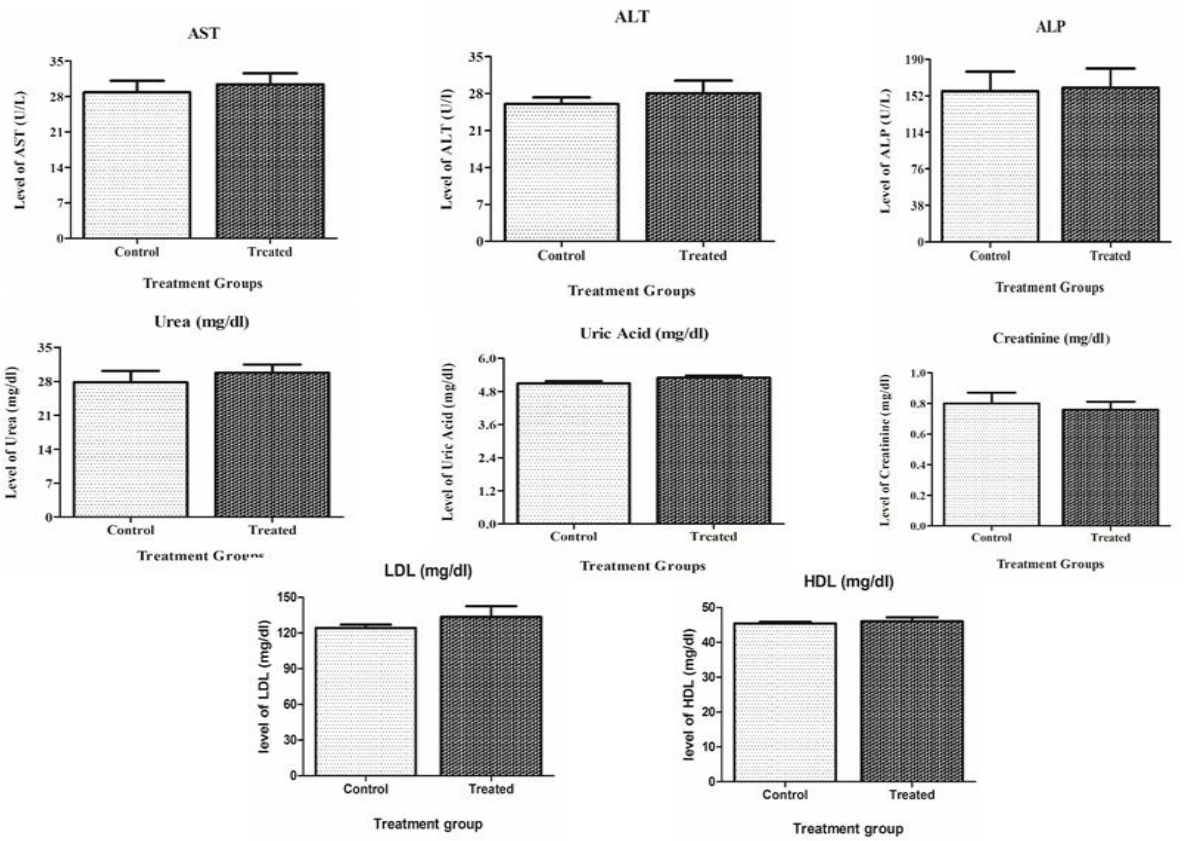


Figure 1. Effect of methanolic seed extract of *Momordica charantia* on the level of AST, ALP, ALT, Urea, Uric acid, creatinine, LDL, and HDL of Swiss albino mice in acute toxicity study.

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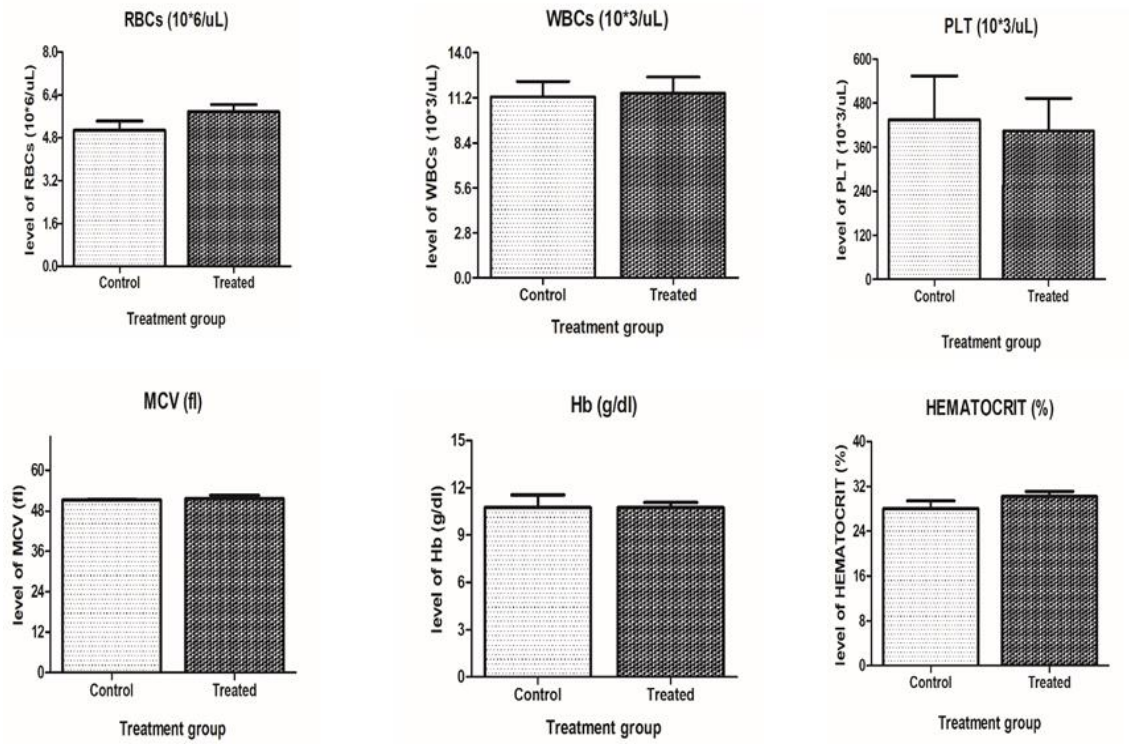


Figure 2. Effect of methanolic seed extract of *Momordica charantia* on the level of RBCs, WBCs, PLT, MCV, Hemoglobin, and Hematocrit of Swiss albino mice in acute toxicity study.

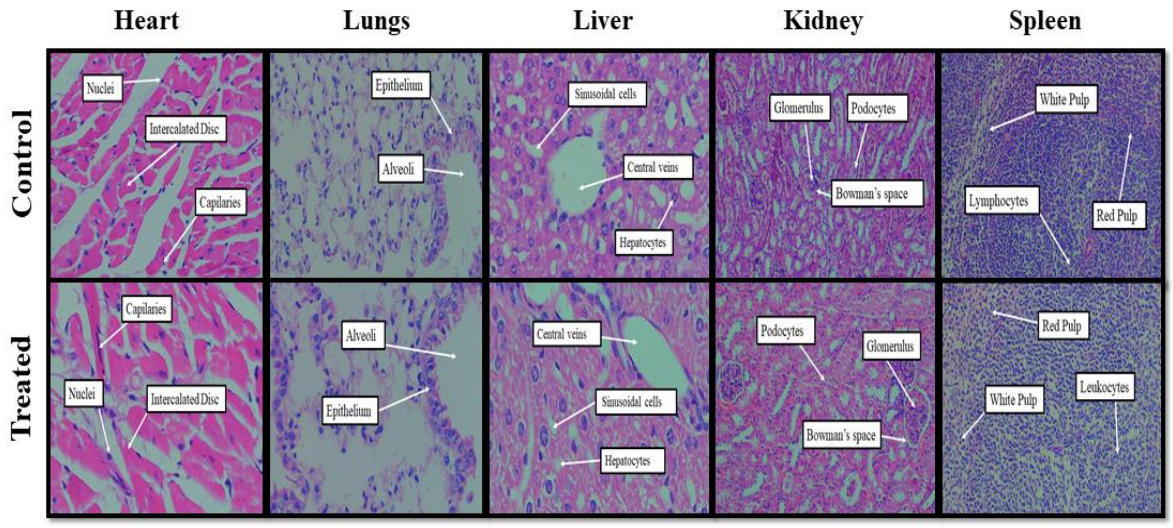


Figure 3. Histopathological comparison of vital organs in control versus *Momordica charantia* seed-extract treated mice. Hematoxylin–eosin stained sections were examined at 10 x and 40 x. Abbreviations: 'C' for Capillary, 'N' for Nuclei, 'ID' for Intercalated disc 'H' for Hepatocytes,'S' for Sinusoidal cells, 'CV' for Central vein 'G' for Glomerulus, 'B' for Bowman's space, 'P' for Podocytes 'WP' for White pulp, 'RP' for Red pulp, 'L' for Lymphocytes 'E' for Epithelium, 'A' for Alveoli.

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4. Discussion

The previous study conducted by Hassan (2020), suggested that the use of medicinal plants is an ancient form of treatment dating back to the origin of man. The use of plants to make medicine has been achieved by the means of trial and error in the discovery, or hit and trial, and over time man was able to meet his demands through the environment. Our work also explains the medicinal significance of herbal plants as these medicinal plants are a valuable source of much of the modern medicine.

According to Anilakumar *et al*., (2015), *Momordica charantia* (bitter gourd) has immunomodulatory, analgesic, anti-viral, antioxidant, anti-tumor properties, anti-ulcerogenic, and anti-mutagenic activities. They also stated that bitter gourd proteins, α-monorcharin, and β-monorcharin, can reduce HIV development *in vitro*. Several *in vivo* studies have shown that bitter gourd leaves, stems, and fruits are safe to eat. Following previous studies, our research suggests that the methanolic extract of M. *charantia* could be a safe and effective medicinal treatment. This is in addition to its nutritional value and historic application.

Ofuegbe *et al*., (2017), investigated toxicity profiling in Swiss albino mice. All the mice were given increasing doses over a predetermined duration to determine the extract's toxicity. Phytochemical studies revealed saponins, alkaloids, tannins, and flavonoids. Despite receiving greater doses of M. *charantia* methanolic seed extract, clinical observation, biochemical tests, and histological examination showed no adverse effects. Our findings also shows that the seed extract can be used in pharmacological research without side effects, which could lead to its use as a natural treatment. It needs more investigation to determine its long-term safety and efficacy.

In the phytochemical study of bitter melon methanolic seed extract, toxicity, and safety were considered by Mabasa *et al*., (2021). After giving mice different doses of the extract, blood was collected for different hematological and biochemical parameters. A series of different parameters were examined to detect blood composition changes. Creatinine, SGOT, urea, and SGPT were also included. The vital organs were collected for histopathological examination. Their study found no detrimental effects from M. *charantia* methanolic seed extract at various doses. Additional research may be needed to discover what happens over time, especially if conditions are changed. In agreement to this, our work determine M. *charantia's* traditional and therapeutic uses. We also took the same biochemical and hematological parameters to study and found no significant variations during acute toxicity period. Furthermore, the histopathological examination of vital organs revealed no signs of necrosis and inflammation that support the safety of methanolic seed extract of M. *charantia*.

5. Conclusion

Current study concluded that the methanolic seed extract of *Momordica charantia* exhibits a favorable safety profile in the Swiss albino mice model. Acute administration of the extract did not cause any significant alterations to the hematological or serum biochemical indices compared to the control group, indicating an absence of systemic toxicity. Furthermore, histopathological examination revealed no treatment-related morphological aberrations in the major vital organs (Kidney, spleen, liver, heart, and lungs) confirming its non-toxic nature at the tested dosage regimen. These findings provide critical preclinical evidence supporting the safe medicinal use of this seed extract. Given its established biocompatibility, it holds promising potential for future therapeutic applications, particularly as a nutraceutical or phytopharmaceutical agent in the management of metabolic disorders, where it could be leveraged for its bioactive constituents without the risk of any toxicity.

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