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Research Article

Acute Toxicity Study of Ethanolic Extracts of Leaf and Fruit of Two Different Varieties of *M. Charantia* in *Danio Rerio*

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ABSTRACT
The present study was to elucidate the oral toxicity level of leaf and fruit extracts of two varieties of *M. charantia*, i.e., green-fruited variety (BGG-Bitter gourd green variety) and white fruited varieties (BGW- Bitter gourd white). 5 g of Finely ground powders of leaves and fruits of *M. charantia* were extracted with ethanol using soxhlet apparatus. Using these extracts of *M. charania* oral Acute toxicity was tested with *Danio rerio* as per the OECD guidelines 203. Leaf extracts of Green Fruited varieties of *M. charantia* showed very high toxicity when compared to the other plant extracts. At test dose of 200mg/L >70% mortality was observed in *Danio rerio* at the 24 hrs of the observation period. Mortality of 70% and 100% was observed at 50 mg/L of leaf extract at the 48 hrs of the observation period. A lethal dose to kill 50% of test fishes were recorded as 200 mg/L and 50 mg/L at 24 hrs and 96 hrs exposure period respectively. The results indicated that the ethanolic extract of *M. charantia* leaf does not induce toxicity when used at dose of ≤ 25 mg/L

KEYWORDS: Bitter gourd; antioxidant; toxicity; mortality

INTRODUCTION

Momordica charantia, a widely used vegetable in many parts of the world is very famous for its medicinal properties. M. charantia, belonging to the family Cucurbitaceae, is a vine growing abundantly in tropical regions and eaten as a raw vegetable or cooked along with other dishes [1]. This plant has exhibited many medicinal properties and proved very useful in the treatments of diabetes. All parts of the plant are used in the preparation of Ayurvedic medicine. Even though there are reports regarding its medicinal properties, no toxicity studies have not been conducted in this plant. There are some unauthorized reports related to its toxicity but not scientifically proved data is available. The present study was to elucidate the oral toxicity level of leaf and fruit extracts of two varieties of *M. charantia*, i.e., green-fruited variety (BGG-Bitter gourd green variety) and white fruited varieties (BGW- Bitter gourd white variety).

M. charantia is safe in humans at a dose of 2000 mg/kg body weight. However, toxicity and even death have been reported in laboratory animals when high doses of the extracts were administered intravenously or intraperitoneally [2]. Leaves have demonstrated greater toxicity than the fruits of the plant. Some documented adverse effects of *M. charantia* like

hypoglycemic coma and convulsions in children, reduced fertility in mice, a favism-like syndrome, increases in gamma-glutamyltransferase and alkaline phosphatase levels in animals, and headaches [3]. *M. charantia* generally causes few adverse reactions. Gastrointestinal effects like abdominal pain, diarrhea, etc. and headache has been reported in clinical trials [2]. Case reports exist of hypoglycemic coma and atrial fibrillation associated with *M. charantia* intake[4]. Increases in liver enzymes have been observed experimentally but without histological changes. *M. charantia* should be used with caution in patients with impaired hepatic function. *M. charantia*, if taken in excessive quantities can turn out to be a nightmare for pregnant women [5].

The present study was mainly focused on the effect of *Momordica charantia* on the growth and development of Danio rerio (Zebrafish). *M. charantia*, one of the commonly used vegetable in our food, is not studied for its toxic compounds and background data on the probable effect of this plant upon continuous consumption is rare. The present study is an attempt to look into the possible toxic effects the plant may produce because of the continuous use of the *M. charantia* juice and raw vegetable such as fruits and leaves. The primary aim of work was to identify adverse effects and to determine the limits of exposure level at which such effects might occur. Toxicity testing could reveal some of the risks that are associated with the use of herbs especially in sensitive populations.

MATERIALS AND METHODS

Materials

Oxygen meter, equipment for determination of hardness of water, adequate apparatus for temperature control, fish tank, experimental setup tanks, and anti-chlorine solution were used in the conduct of the experiment. Leaves and fruits of green fruited varieties (BGG-Bitter gourd green variety) and white fruited varieties (BGW- Bitter gourd white variety) of *Momordica charantia* were used as experimental plants. *Danio rerio* was used as the experimental animal. The fish selected was of two-month-old having 1.0 g body weight and 2.0 cm length. They were tested at the rage of 21-25 °C temperature. A total of 7 fish was selected for the experiment.

Oral Acute toxicity of ethanolic extract of *M. charania* leaf was tested with *Danio rerio* as per the OECD guidelines 203 (OECD, 1992)[6]. Fish were kept in 100 L tanks with a water circulation system and 16/8 hour light and dark cycle and were fed pellet feed at 1% of their body weight. After seven days of adaptation, fish were transferred to separate test vessels with a volume of 10 L and allowed to adapt for a further 24 hrs before starting the toxicity experiments. Dechlorinated tap water was used for all experiments (dechlorination performed by vigorous aeration for at least 48 hrs). [6] To avoid overestimating the toxicity, the feeding of the fish was stopped 48 hrs before starting the experiments to minimize the risk of sample absorption in the fecal material or food and minimize the dissolved organic carbon (DOC) in the exposure tanks. The fish were inspected at 24, 48 and 96 hours. Concentrations of 12.5, 25, 50, 100, 200, and 400mg/L [6] of leaf and fruit extracts of two varieties of *M. charantia* (BGG and BGW varieties) were selected as effective concentrations for performing the main toxicity tests of the extract. The fish were exposed to the sample based on a static exposure regime. For every experiment, seven healthy fishes were directly transferred into each prepared concentration. Control groups (7 fishes) were also maintained for each treatment. The mortalities were recorded at 24, 48 and 96 hours post exposure and the LD50 values were calculated [6].

RESULTS

The study conducted on acute toxicity revealed that the leaf extracts of Green fruited (BGG) varieties of *M. charantia* were more toxic to *Danio rerio* than other extracts used in the experiment. Toxic effects of *M. charantia* leaf extract of green fruited variety (BGG) were observed at ≥50mg/L. At the observation time of 24hrs, the leaf extracts of BGG started to induce toxicity at 200mg/L. At the observation time of 48hrs, the leaf extracts started to induce toxicity at 50mg/L and the toxic level was very high in other concentrations of 100mg/L, 200 mg/L and 400 mg/L where we observed 100% mortality of *Danio rerio* (Table1; Fig. 1-8).

Table 1: Concentration dependent change in the mortality during 24, 48 and 96hrs exposure to M. charantia Green leaf extract (BGG)

Group (mg\kg bw)	Number of fishes/group	Number of dead fish	Erratic Behavior	Loss of Equilibrium	Mortality ratio (%)	LD_{50} (mg\kg bw)
Observation period:	24hrs					
Control	7	0	0	0	0	200
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	0	0	0	0	
100mg/L	7	1	0	0	14.29	
200mg/L	7	5	0	0	71.43	
400mg/L	7	6	0	0	85.71	
Observation period:	48hrs					
Control	7	0	0	0	0	50
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	5	0	0	71.43	
100mg/L	7	7	0	0	100	
200mg/L	7	7	0	0	100	
400mg/L	7	7	0	0	100	
Observation period:	96hrs					
Control	7	0	0	0	0	50mg/L
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	7	0	0	100	
100mg/L	7	7	0	0	100	
200mg/L	7	7	0	0	100	
400mg/L	7	7	0	0	100	

Fig 1: Control

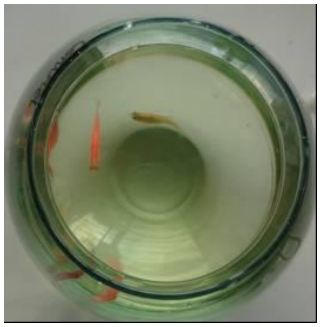

Fig 2: 12.5mg/L

Fig 3: 12.5mg/L

Fig 4: 50mg/L

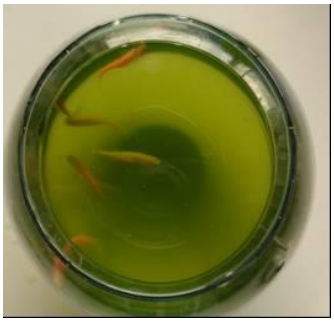

Fig 5: 100mg/L

Fig 6: 200mg/L

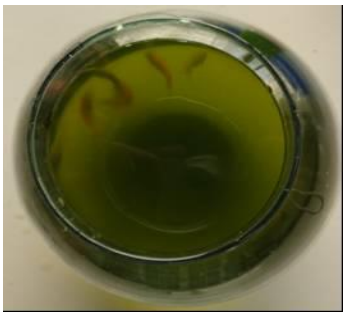

Fig 7: 400mg/L

Figures 1-7: Experimental setup for determination of acute toxicity of Bittergourd green (BGG) leaf extract.

Fig 8: Mortality observed in fish treated with bittergourd leaf extract at 200mg/L - 24hrs

In all other experiments carried out with leaf and fruit extracts of white fruited (BGW) variety and the fruit extracts of Green fruited (BGG) varieties, no mortality was observed and was found to be safe to consume. In the experiments used with leaf extracts of white fruited (BGW) variety and the fruit extracts of Green fruited (BGG) varieties of *M. charantia* showed mortality at 100 mg/L, and L50 was observed only at the concentration of 400 mg/L. No 100% mortality was observed in any of the concentrations used in those plant extract. At the observation time of 96hrs in the leaf extracts of Green fruited (BGG) varieties of *M. charantia* the toxic level was very high in the concentrations of 50mg/L, 100mg/L, 200 mg/L and 400 mg/L where we observed 100% mortality of *Danio rerio*. In other experiments used with leaf extracts of white fruited (BGW) variety and the fruit extracts of Green fruited (BGG) varieties, mortality was observed at 100 mg/L, and L50 was observed only at the concentration of 400 mg/L. No 100% mortality was observed in any of the concentrations used in those plant extracts. There was no lethality observed in the fruit extracts of white-fruited variety (Table 2-4).

Table 2: Concentration dependent change in the mortality during 24, 48 and 96hrs exposure to *M. charantia* Green leaf extract from a white variety (BGW)

Group	Number of fishes/group	Number of dead fish	Erratic Behavior	Loss of Equilibrium	Mortality ratio (%)	LD50 (mg\kg bw)
Observation period: 24hrs						
Control	7	0	0	0	0	0
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	0	0	0	0	
100mg/L	7	0	0	0	0	
200mg/L	7	0	0	0	0	
400mg/L	7	0	0	0	0	
Observation period: 48hrs						
Control	7	0	0	0	0	100
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	0	0	0	0	
100mg/L	7	4	0	0	57.14	
200mg/L	7	5	0	0	71.42	
400mg/L	7	7	0	0	100	
Observation period: 96hrs						
Control	7	0	0	0	0	100
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	0	0	0	0	
100mg/L	7	5	0	0	71.42	
200mg/L	7	5	0	0	71.42	
400mg/L	7	7	0	0	100	

Table 3: Concentration dependent change in the mortality during 24, 48 and 96hrs exposure to M. charantia fruit extract (BGG)

Group	Number of fishes/group	Number of dead fish	Erratic Behavior	Loss of Equilibrium	Mortality ratio (%)	LD50 (mg\kg bw)
Observation period: 24hrs						
Control	7	0	0	0	0	0
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	0	0	0	0	
100mg/L	7	0	0	0	0	
200mg/L	7	0	0	0	0	
400mg/L	7	0	0	0	0	
Observation period: 48hrs						
Control	7	0	0	0	0	400
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	

50mg/L	7	0	0	0	0	
100mg/L	7	1	0	0	14.28	
200mg/L	7	3	0	0	42.85	
400mg/L	7	4	0	0	57.14	
Observation	period: 96hrs					
Control	7	0	0	0	0	400
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	0	0	0	0	
100mg/L	7	2	0	0	28.57	
200mg/L	7	3	0	0	42.85	
400mg/L	7	5	0	0	71.42	

Table 4: Concentration dependent change in the mortality during 24, 48 and 96hrs exposure to M. charantia fruit extract (BGW)

Group	Number of fishes/group	Number of dead fish	Erratic Behavior	Loss of Equilibrium	Mortality ratio (%)	LD_{50} (mg\kg bw)
Observation	period: 24hrs					
Control	7	0	0	0	0	0
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	0	0	0	0	
100mg/L	7	0	0	0	0	
200mg/L	7	0	0	0	0	
400mg/L	7	0	0	0	0	
Observation	period: 48hrs					
Control	7	0	0	0	0	0
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	0	0	0	0	
100mg/L	7	0	0	0	0	
200mg/L	7	1	0	0	14.28	
400mg/L	7	2	0	0	28.57	
Observation	period: 96hrs					
Control	7	0	0	0	0	0
12.5mg/L	7	0	0	0	0	
25mg/L	7	0	0	0	0	
50mg/L	7	0	0	0	0	
100mg/L	7	0	0	0	0	
200mg/L	7	2	0	0	28.57	
400mg/L	7	3	0	0	42.85	

In the present experiment, fish were considered dead if there were no visible movement and if touching of the caudal peduncle produced no reaction. Dead fishes were removed when mortalities were recorded. Records were kept of visible abnormalities like loss of equilibrium, swimming behaviour, respiratory function, and pigmentation. Measurement of pH and temperature were carried out daily. The

concentration of the test substance in water which killed 50 percent of a test batch of fish within a particular period of exposure was considered to arrive at LD_{50}. The lethal dose of the toxic level of the different plants extracts tested was calculated in gram per kilogram body weight and given in the following table (Table 5).

Table 5: LD50 showed for leaf and fruit extract of BGG and BGW varieties of M. charantia

	LD_{50} at 24 hrs (mg\kg bw)	**LD_{50} at 48 hrs (mg\kg bw)**	**LD_{50} at 96 hrs (mg\kg bw)**
Leaf extract of BGG	200	50	50
Leaf extract of BGW	Nil	100	100
Fruit extract of BGG	Nil	400	400
Fruit extract of BGW	Nil	Nil	Nil

DISCUSSION

Toxicological studies have revealed the fact that *M. charantia* leave extracts were toxic to the tested fish. The leaf extracts of BGG varieties induced the toxic effects at the dose of 50 mg/L and found the high mortality rate at the dose above 200 mg/L. The average weight of the fish tested was 1gm, and the IC_{50} value was 50mg/L at the exposure to 24 hours. The toxic level given was found to be very alarming in the consumption of leaves. Toxicity and even death have been reported in laboratory animals when high doses of the extracts were administered intravenously or intraperitoneally[3]. The leaves and seeds have demonstrated greater toxicity than the fruits or aerial parts of the plant. Some documented adverse effects of *Momordica charantia* are hypoglycemic coma and convulsions in children, reduced fertility in mice, a favism-like syndrome, increases in gamma-glutamyltransferase and alkaline phosphatase levels in animals, and headaches [3]. All these studies supported the present study conducted in zebrafish with respect to the toxicity studies. Many studies have revealed that momordin is acting as ribosome-inactivating proteins (RIP). It has been proved that momordin is one of the major phytochemical compounds present in the M. charantia [7]. Toxicity lesions caused by several ribosome-inactivating proteins like momordin were studied in animals [8]. Severe necrotic liver damage was observed in the fish treated with the extracts of *M. charantia* and considered to be the cause of damage which happens due to the lethal of doses of momordin, and ribosome-inactivating proteins, present in the extracts of M. charantia[8]. The toxicity of ribsome-inactivating proteins increased in the concentration dependent manner. Studies have proved that the momordin concentration is very high in leaves of green fruited varieties of *M. charantia* [7]. the present study revealed that the leaves of the green fruited variety were more toxic to the fish tested. It was observed that the leaves were less consumed by the people in their daily life. However, the leaves were used in the preparation of medicines in the Ayurveda. It is high time to establish the toxicity studies on natural products we use on different preparations like medicines, food, etc. It was observed that leaf extracts of both the varieties of *M. charantia* were found to be toxic, but the fruits were of less toxic.

CONCLUSION

The present study revealed the fact that the leaf extracts of Green fruited (BGG) varieties of *M. charantia* were more toxic to *Danio rerio* than other extracts used in the experiment. Experiments carried out with leaf and fruit extracts of white fruited (BGW) variety and the fruit extracts of Green fruited (BGG) varieties, no mortality was observed and was found to be safe to consume. In the experiments used with leaf extracts of white fruited (BGW) variety and the fruit extracts of Green fruited (BGG) varieties of *M. charantia* showed mortality at 100 mg/L, and L50 was observed only at the concentration of 400 mg/L. These studies have paved the way to exploit more into the toxicity of the bitter gourd plants which are widely used by the people as their food. More studies have to be conducted to prove more scientifically regarding

the toxicity level of the leaves and fruits of *M. charantia* in human beings.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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