

Appraisal of acute oral toxicity of glucuronoxylan hydrogel from *Mimosa pudica* seeds

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Glucuronoxylan hydrogel (GXH) isolated from *M. pudica* seeds was assessed for acute toxicology in albino mice that were alienated into four groups. Three groups, i.e., II, III and IV received GXH at a dose of 1, 2 and 5 g/kg, respectively while group I was retained untreated and provided routine diet. After administering GXH, mice were examined for vomiting, diarrhea, allergy and tremors for 8 h. All animals were carefully observed for food and water consumption at 1, 2, 3, 7 and 14 day after administering GXH. At the end of studies, blood samples were drawn for investigation of hematological and biochemical parameters. All animals were sacrificed, relative body weight of vital organs was calculated and their histopathology was studied. It was concluded that there was insignificant difference in body weight, behavioral pattern, food and water intake among treated and control groups. Haematology and biochemistry of blood samples from all groups were found analogous. Histopathological evaluation of vital body organs exhibited no lesions in all groups. Ocular, cardiac and dermal safety of GXH was also established on albino rabbits.

Keywords: Glucuronoxylan. Acute toxicity. Histopathology. Toxicology. *Mimosa pudica*.

INTRODUCTION

Mimosa pudica L. is a medicinal plant of family *Mimosaceae* which is cosmopolitan in nature and contains phytochemicals such as alkaloids, steroids, terpenoids, tannins and flavonoids (Muhammad *et al.*, 2016; Bum *et al.*, 2004; Dinda *et al.*, 2006). The plant is traditionally promised to cure stomach disorders, haemorrhoids, respiratory troubles, fever, snake bites, anxiety, dysentery and inflammatory problems (Molina, Contreras, Tellez-Alcantara, 1999; Khalid *et al.*, 2011). The seeds of *M. pudica* (MP) extrude glucuronoxylan hydrogel in water, which mainly consists of D-xylose and D-glucuronic acid (Muhammad *et al.*, 2015; Saraswat, Pokharkar, 2012).

Irrespective of low bioavailability and higher frequency of doses, drugs are frequently administered to patients via oral route (Zhang *et al.*, 2004; Gupta, Shivakumar, 2009). To overcome this problem, gastro-retentive drug delivery systems are developed (Maderuelo, Zarzuelo, Lanao, 2011) using muco-adhesive polymers (Ponchel, Irache, 1998), floating ion exchange resins (Atyabi *et al.*, 1996) and water swellable hydrogels (Chen *et al.*, 2000; Saiyed *et al.*, 2015).

Polysaccharide hydrogels hold sufficient quantity of water owing to the existence of hydrophilic functional groups (-OH, -COOH, -CONH₂). The water retention tendency also depends upon elasticity of network, degree of crosslinking and permeability of polymer (Hoffman, 2012). Hydrogels are smart, intelligent, biodegradable, biocompatible, abundant and non-toxic polymers which are used in cosmetics, agronomy, medicines, gardening, engineering, immobilization of enzymes and diapers (Hussain *et al.*, 2016; Good, Bowman, Davis, 2004; Liu *et al.*, 2007; Ashraf *et al.*, 2017). They are particularly potential candidates for developing targeted delivery

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because of pH responsive nature (Haseeb *et al.*, 2016; Zou, Zhao, Ye, 2015; Muhammad *et al.*, 2015).

Herein, we are aimed to establish oral safety of an innovative, super porous and intelligent polysaccharide, GXH extruded from MP seeds. The study of acute toxicity of GXH was carried out in agreement to United State Pharmacopeia-NF regulatory requirement of excipients. The study protocol approved by the research ethics committee of University of Sargodha, Sargodha Pakistan was strictly followed (Ref. No. 75-2014/PREC).

MATERIAL AND METHODS

Material

MP seeds procured from local market were cleaned and stored in air tight container after taxonomic authentication from Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan.

Animals

For the evaluation of toxicological studies, Swiss albino mice (24-29 g) of either sex and albino rabbits (1110-1180 g) were taken from the animal house of University of Sargodha, Sargodha, Pakistan. After careful inspection of animals, they were placed in uncontaminated cages at room temperature. The animals were regularly brought to sunlight and administered with routine diet and water. All tests and procedures were according to the Good Laboratory Practice (GLP) regulations as described by United States Food and Drug Administration (USFDA) and Organization for Economic Co-operation and Development (OECD) Test Guidelines 425 (Up and Down Procedure) (Chen *et al.*, 2006). Before starting experiment, approval was taken from research ethics committee of University of Sargodha, Sargodha, Pakistan (Ref. No. 75-2014/PREC). Four groups of animals (with three animals each) were chosen for toxicological evaluation. Group I was fed with routine diet while other three groups (II-IV) were administered with 1, 2 and 5 g/kg dose of GXH, respectively.

Isolation of hydrogel

MP seeds extrude hydrogel when soaked in water for 12 h. Mild heating (50 °C) was carried out for 30 min to improve the yield of hydrogel. The GXH was detached from seeds using cotton cloth and then washed with *n*-hexane to get rid of lipophilic substances. GXH was dried at 50 °C in oven and then kept in container provided with screwed lid.

Evaluation of toxicology

Before toxicological assessment, animals (7-8 weeks) were retained in laboratory for one week for adaptation of environment. After fasting of 12 h, groups II-IV were administered with single oral dose of GXH in pellets, dosing 1, 2 and 5 g/kg body weight per oral route, respectively. After 1 h of administration of GXH, mice were given access to tap water and normal diet. The study was carried out for 14 days. Group I animals were left untreated and were given access to normal diet and water.

Parameters to be assessed

During 14 days study, animals were routinely and cautiously observed for any toxicity, death, sickness, diarrhea, dizziness, salivation and tremors. Furthermore, mice were also witnessed for any change in fur, skin, mucus membranes, eyes and behavior.

Estimation of body weight, consumption of food and water

On 1st, 2nd, 3rd, 7th and 14th days, weight of animals of all groups was noted with great care. Additionally, food and water intake of animals of all groups (I-IV) were also observed.

Ocular irritation

Six albino rabbits of either sex were chosen for eye irritation test. For assessment of ocular irritation, fine suspension of GXH in water was placed in conjunctiva of right eye of each albino rabbit. Eye lids were gently closed with fingers to keep GXH in eyes for a while. Ocular irritation was observed after 1, 24, 48 and 72 h after administering GXH in eyes and results were compared with untreated left eye of each rabbit.

Acute dermal toxicity

Before acute dermal toxicity evaluation, six healthy white albino rabbits were carefully observed for any sign of ailment. After shaving hairs of rabbits, thick paste of GXH applied on a 4 ply gauze pad (4"×4") and affixed it on the rabbit's skin with Micropore™ tape. The pad was detached after 12, 24, 36 and 48 h to observe any variation in skin colour of treated animals from control.

Relative organ body weight

To observe any lesion in vital organs, mice were sacrificed on day 15th, dissected, and heart, kidney, lungs, spleen, liver and gastro intestinal tract (GIT) were removed. Absolute weight of organs of both control and test animals was determined and relative organ weight (ROW) was computed using formula (Sahgal *et al.*, 2010) given in Eq. (1). ROW of both treated and control group animals was also compared.

$$\text{ROW} = \frac{\text{Absolute weight of organ}}{\text{Total body weight of mice at the end of study}} \quad (1)$$

Histopathology of vital organs

Animals were sacrificed to separate their vital organs for histopathological evaluation. The body organs such as heart, liver, kidney, intestinal mucosa and spleen were kept in formalin (10% v/v). To examine cellular structures, small part of each organ was cut with rotary microtome. Slides were prepared by staining with eosin and heamatoxylin dyes.

Hematological and biochemical analysis of blood

At the end of study, blood samples were taken from animals before they were sacrificed to study hematology and biochemistry of blood. Posterior vena cava was selected for collection of sample using 22 G syringe by cardiac puncture. Sahli heamometer was used to determine hemoglobin in the blood. Likewise, counting of RBCs, WBCs and platelets was commenced. The liver profile, renal function and lipid profile was judged by biolyser.

Safety pharmacology study

As per guidelines of International Conference of Harmonization, pharmacological attributes of each excipient are obligatory either with toxicology or alone (ICH, 2005). Modified Langendorff technique was employed to evaluate toxicology of GXH on heart of albino rabbits. Rabbits were made unconscious by injecting heparin (1000 IU). The heart with aorta (1 cm) separated by surgery was washed with oxygenated Ringers solution (NaCl, 45.0 g; NaHCO₃, 1.0 g; D-glucose, 5.0 g; KCl, 2.1 g; CaCl₂.2H₂O, 1.6 g; in 5 L of distilled water). The squeezing of heart was done repetitively to remove the left over blood from heart before they were transferred to the perfusion apparatus (Radnoti Isolated Heart System,

AD Instrument, Australia). The bubbling of mixture of oxygen (95%) and carbon dioxide (5%) through heart was carried out after fixing heart with glass cannula. Before administration of GXH (0.001, 0.01, 0.1 and 1 mg/mL), hearts were stabilized for 30 min. The force of contraction, heart rate and perfusion pressure were explored before and after the drug administration.

With the help of threads, heart was connected to Palmer clip on one end and force displacement transducer (MLT 844; AD Instruments, Australia) on the other end for the investigation of mechanical force of contraction of heart. The heart rate was noted using Lab Chartpro 5 software (AD Instruments, Australia). The intensity of signals from force transducer increased before sending to an analog-to-digital converter (Power Lab Data Acquisition and Analysis system of AD Instruments, Australia).

Statistical analysis

The experimental data obtained was shown as mean \pm SEM and discrepancies in experimental data among control and treated groups were expressed using one way ANOVA (Turkeys multiple comparison test) and probability value (< 0.05) was considered significant.

RESULTS AND DISCUSSION

Assessment of Corporeal factors

Careful observations revealed that health of the mice was satisfactory throughout the study and no mortality was noticed. The eyes, skin and mucosa were also normal. Moreover, no alteration in behaviour, sleep, tremors, diarrhea and salivation was discerned.

Appraisal of body weight, food and water intake

No significant variations in weight of mice of treated and control group animals were observed. A slight decrease during first three days of study was noted. This is because of low food and water intake during first three days. After 3rd day, increase in average weight of animals was noticed. No significant difference was witnessed in food and water intake and body weight among the animals of control and treated groups after first day (Table I).

Irritation to eyes and skin

According to guidelines of United States Pharmacopoeia, safety of an excipient should be evaluated

TABLE I - Results of body weight, and food and water consumption by control and treated groups of albino mice

S/No	Clinical Observations	Group-I control	Group-II Treated (1 g/kg)	Group-III Treated (2 g/kg)	Group-IV Treated (5 g/kg)
1	Body weight				
	Before treatment	27.5±0.63	26.4±0.61	27.4±0.51	28.56±0.81
	Day 1	26.96±0.67	26.0±0.69	26.5±0.57	27.97±0.83
	Day 2	27.07±0.57	26.1±0.68	26.7±0.51	28.03±0.83
	Day 3	27.23±0.71	26.3±0.68	26.73±0.70	28.22±0.91
	Day 7	27.8±0.68	26.53±0.73	27.03±0.90	28.53±0.91
	Day 14	28.1±0.76	26.9±0.81	27.44±0.87	28.84±0.92
2	Food intake				
	Before treatment	2.10±0.13	2.20±0.12	2.40±0.18	2.74±0.12
	Day 1	1.63*±0.12	1.70*±0.13	1.83*±0.17	2.22*±0.14
	Day 2	1.87±0.18	2.06±0.14	2.21±0.13	2.52±0.14
	Day 3	1.96±0.13	2.30±0.14	2.28±0.14	2.71±0.13
	Day 7	2.10±0.16	2.41±0.13	2.45±0.15	2.78±0.10
	Day 14	2.13±0.13	2.45±0.13	2.50±0.11	2.83±0.13
3	Water intake				
	Before treatment	11.3±0.23	11.56±0.81	12.1±0.63	12.16±0.70
	Day 1	10.7±0.35	10.96±0.82	11.66±0.61	10.96*±0.61
	Day 2	11.5±0.38	11.7±0.79	12.06±0.47	11.93±0.73
	Day 3	11.6±0.42	11.93±0.81	11.9±0.78	12.43±0.55
	Day 7	11.6±0.58	12.16±0.58	12.4±0.73	12.8±0.78
	Day 14	12.0±0.34	12.30±0.84	12.53±0.80	12.6±0.78

Consumption of water and food, and body weight are depicted as mean ± SEM

before its use in oral or dermal formulations. The results displayed that GXH caused no irritation to eyes and skin of rabbits. It was also noted that color of eyes of rabbits of all groups were same and exhibited no redness, corneal opacity and iritis for 72 h (Table II). For grading, scores were calculated using Kay and Calandra eye irritation scale (Kay, Calandra, 1962).

Furthermore, dermal application of GXH instigated no rashes, inflammation, redness and swelling among rabbits of control and treated groups.

Histopathology of vital organs and their relative weights

On 15th day, vital organs like heart, liver, kidneys, spleen and stomach were separated and weighed cautiously. The weights of all vital body organs were calculated relative to the body weight of mice. The relative weights of organs showed insignificant differences among control and treated mice (Table III). Histopathological studies exhibited no lacerations, indicating the potential

utilization of GXH in oral formulations is non-toxic (Figure 1a-e).

Blood biochemistry and hematology

Hematological study of blood samples taken from mice of different groups revealed that number of white blood cells (WBCs), red blood cells (RBCs), platelets and amount of hemoglobin were observed to be in normal ranges. Insignificant difference in hematological parameters was observed among different groups of mice. Biochemistry of blood depicted normal functioning of liver and kidney. Amounts of serum triglycerides were also normal (Table IV). Hematology and biochemistry of blood samples suggested non-toxic nature of GXH in oral formulations.

Safety pharmacology

Cardiac safety of GXH excipients was evaluated using standard procedure reported in International

TABLE II - Scores calculated using Kay and Calandra eye irritation scale

Studied groups	Ocular observations	Time duration (h)			
		01	24	48	72
Group I	Corneal opacity	0	0	0	0
	Iritis	0	0	0	0
	Conjunctivitis	0	0	0	0
	Severity (MMTS)	0	0	0	0
Group II	Corneal opacity	0	0	0	0
	Iritis	0	0	0	0
	Conjunctivitis	0	0	0	0
	Severity (MMTS)	0	0	0	0
Group III	Corneal opacity	0	0	0	0
	Iritis	0	0	0	0
	Conjunctivitis	0	0	0	0
	Severity (MMTS)	0	0	0	0
Group IV	Corneal opacity	0	0	0	0
	Iritis	0	0	0	0
	Conjunctivitis	0	0	0	0
	Severity (MMTS)	0	0	0	0

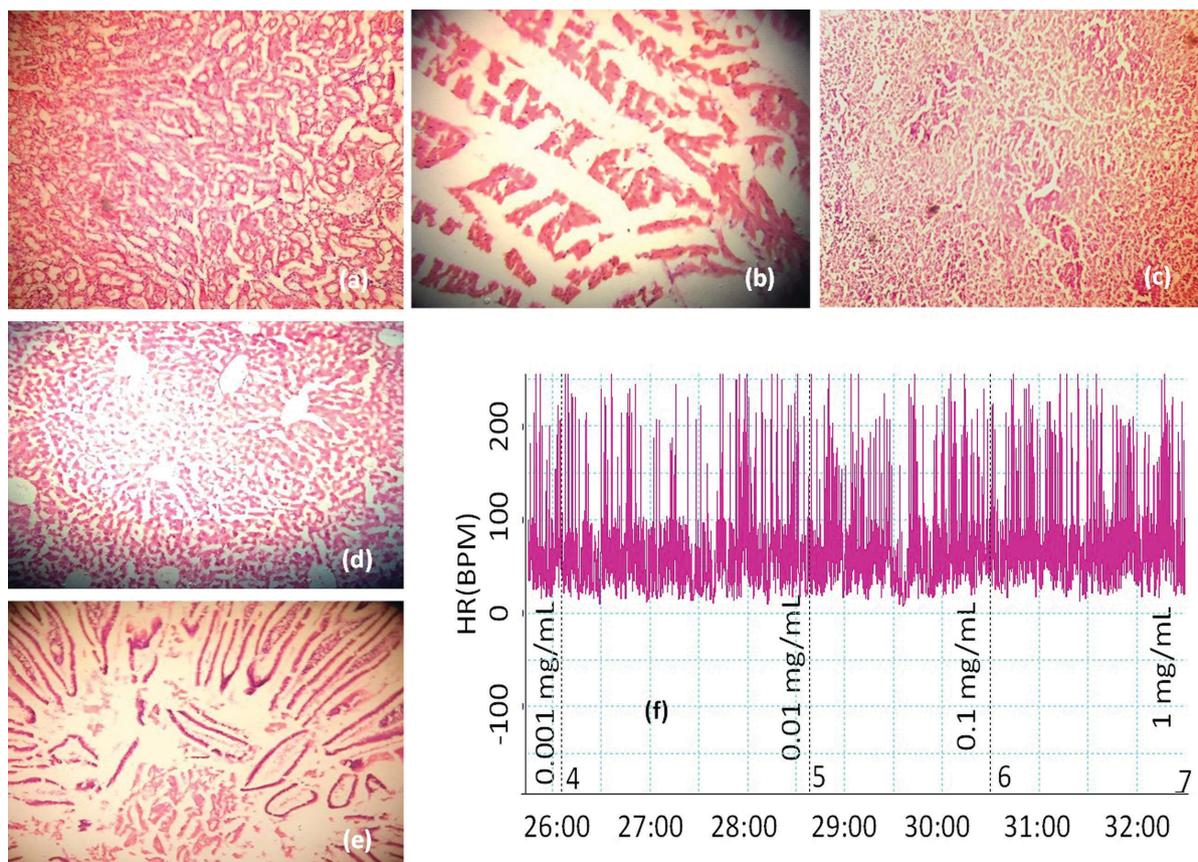


FIGURE 1 - Histopathology of various vital body organs of mice (a) intestine (b) heart (c) spleen (d) liver (e) kidney and (f) effect of various GXH concentrations on heart rate.

TABLE III - Relative weight of vital body organs of mice

Groups	Dose	Heart	Liver	Kidney	Spleen	Stomach
Group-I		0.47±0.01	1.67±0.11	0.80±0.02	0.57±0.13	1.24±0.03
Group-II	1g/kg	0.49±0.02	1.71±0.03	0.83±0.01	0.64±0.05	1.27±0.13
Group-III	2g/kg	0.45±0.01	1.61±0.04	0.77±0.03	0.54±0.10	1.15±0.05
Group-IV	5g/kg	0.52±0.03	1.78±0.13	0.89±0.04	0.70±0.04	1.33±0.06

Organ weight is expressed as relative organ body weight ± SEM

TABLE IV - Hematology and blood biochemistry of mice

Test	Group-I control	Group-II Treated (1 g/kg)	Group-III Treated (2 g/kg)	Group-IV Treated (5 g/kg)
Hematology				
Total WBC _s (×10 ³ /μL)	7.97±0.13	8.03±0.15	7.35±0.23	8.51±0.27
RBC _s (×10 ⁶ /μL)	7.79±0.21	7.91±0.11	7.60±0.20	8.44±0.17
Platelets (×10 ³ /μL)	603±1.16	656±2.09	584±2.61	764±3.17
Hb (g/dL)	11.21±0.12	11.39±0.23	11.10±0.19	12.10±0.29
Normal range (10-16 g/dL)				
Blood chemistry				
ALT (U/L)	43.1±1.31	51.4±2.02	42.33±2.44	58.34±2.08
Normal range (17-77)				
AST (U/L)	157.1±1.33	164.2±2.15	145.31±3.14	187.42±2.13
Normal range (54-298)				
Renal profile				
Creatinine (mg/dL)	0.37±0.02	0.39±0.01	0.34±0.03	0.49±0.01
Normal range (0.2-0.9 mg/dL)				
Urea (mg/dL)	54±1	61±2	51±1	63±2
Uric acid (mg/dL)	4.8±0.01	5.1±0.02	4.2±0.01	5.4±0.01
Lipide profile				
Serum cholesterol	133.54±2.31	144.23±2.14	128.22±2.13	162.61±2.15
Normal range (150-220 mg/dL)				
Serum triglycerides	112.21±2.43	114.22±2.17	109.12±1.92	119.28±2.25

conference of harmonization (ICH, 2005). The rate of heart beat was noted after administration of 0.001, 0.01, 0.1 and 1 mg/mL GXH on rabbit heart. The study demonstrated that GXH did not affect rate of heart beat (Figure 1f).

CONCLUSION

The parameters studied during acute toxicological evaluation of GXH led to conclusion that GXH can be used as safe oral drug excipient. It caused no toxicity

signs in albino mice and rabbits during acute toxicology studies. Nevertheless, long term toxicological studies are demanding to estimate LD₅₀ of GXH.

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DISCLOSURE STATEMENT

The authors report no conflicts of interest

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