Volume 3, Issue 9, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

# ACUTE ORAL TOXICITY EVALUATION OF A COPOLYMERIZED *MIMOSA PUDICA* SEED MUCILAGE ON RABBIT AS MODEL ANIMAL

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DOI: https://doi.org/10.5281/zenodo.17598744

**Keywords**
*Polysaccharide, Hydrogel, Acute toxicity, Non-irritating, Clinical biochemistry*

**Article History**
*Received: 19 September 2025*
*Accepted: 29 October 2025*
*Published: 13 November 2025*



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## Abstract

*In the drug development process, active pharmaceutical ingredients (APIs) plays a crucial role. To ensure safety and efficacy, they undergo rigorous testing, and APIs can be approved for use in medications, paving the way for new treatments. Acute oral and dermal toxicity evaluations of a composite hydrogel synthesized from Mimosa pudica seed mucilage and methacrylic acid were conducted according to OECD 402 and 420 guidelines. In addition, using the Draize scoring system, an ocular toxicity study was conducted. The laboratory animals (rabbits) were categorized into four distinct groups. The animals of category A were served as controls and no dose was given to them and a single dose of composite hydrogel was administered to animals of categories B, C, and D. Throughout the 14 days of toxicity study period, all the animals were remained alive and any kind of significant adverse effects in animals was not observed. After 14 days, the blood analysis and biochemical test were performed. For organ weight measurement, the vital organs from the rabbits were taken. The findings collectively shows that the synthesized composite hydrogel is a safe material and can be used in drug delivery systems as a promising non-toxic excipient.*

## INTRODUCTION

For the development of DDSs, natural polysaccharides are preferred polymer because of their high swelling, sustained drug release capabilities, non-toxic, biodegradable, and biocompatible properties (Li et al., 2012; Yilmaz et al., 2015; Ragni et al., 2018; Ali et al., 2023a & b). In addition, the chemical modifications like crosslinking, acetylation, and polymerization can improve the properties of natural polysaccharides and making them suitable materials for advanced applications. Due to these modifications, it enables the development of smart materials that respond to various stimuli, including salt, ethanol, pH, and temperature (Ali et al., 2022a & b; Ali et al., 2023c). However, graft copolymerization with acidic monomer yields a highly swellable and suitable materials for sustained release applications (Amjad et al., 2025). Furthermore, toxicity studies have recently been conducted on naturally occurring polysaccharides and their modified forms to assess their potential as safe DDSs (Irfan et al., 2022; Sheikh et al., 2022; Ali et al., 2024; Farid-ul-Haq et al., 2024; Lie et al., 2024).

The word *Mimosa pudica* originates from the word "*mimos*," indicating imitation or resemblance to touch-sensitive leaves, and the term "*pudica*" means modest, contracting, and reserved. There are several names for

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ISSN: (e) 3007-1607 (p) 3007-1593

the *M. pudica* plant, from which it is recalled, including touch me not plant, shame plant, sensitive plant, and humble plant. For its unique, intriguing response, *M. pudica* is a captivating indoor plant. The *M. pudica* generally includes around 400 different species that belongs to the family of the Fabaceae, and some of the *Mimosa* genus comprises approximately 3,000 species and belongs to the *Mimosaceae* family. Various species of *Mimosa* across the regions, including Africa, America, Vietnam, Thailand, and the Philippines, are distributed. The majority of species are commonly found in Japan, Indonesia, Malaysia, India, Singapore, Bangladesh, Sri Lanka, and Pakistan. A broad spectrum of habitats is inhabited by species of *Mimosa*, including the plains, xerophytic forests, rainforest ecosystems and semi-arid regions, subtropical scrublands, silvicultural habitats, aquatic ecosystems, and herbaceous ecosystems. The foliage of *M. pudica* exhibits bipinnate or pinnate structures. Folding of leaves is the ability of *M. pudica*, show a response when it is touched. These plants are commonly grown in a moist environment and also grow across pathways, wetlands, highway margins, and hill slopes, and along riverbanks and lake shores, where multiple individuals gather in close proximity (Johnson et al., 2014; Abramson et al., 2016; Ahuchaogu et al., 2017; Fernandes et al., 2023).

The plant of *M. pudica* is a compact, ephemeral shrub that can grow along the ground or climb upwards, and by some researchers, it is referred to as an herbaceous plant with woody features. By the support of other plants, it can attain a height of 1m. It may reach and extend beyond 2 m. The woody stems that are reddish-brown, equipped with curved prickles, are either scattered or thick. The root structure of this plant features a central taproot and a sprawling system of roots with nodules and fibrous growth. The slender twigs carry leaves with 1-2 pairs of pinnae, each bearing pairs of miniature leaflets in the range of 15 to 25, spanning 3 to 12 mm in length. The flowers form pink and are densely packed in round, globular clusters. The seed pod is fine and elongated, the size is roughly 1-1.5 cm x 3 mm, with spines along its edges. The pods contain 2-4 brown seeds when they are grouped together (Muhammad et al., 2016).

In earlier research, *M. pudica* seed mucilage has been appeared as stimuli-responsive in nature (Muhammad et al., 2016). Herein, we aimed to study the safety assessments of a composite hydrogel based on *M. puidca* seed mucilage and methacrylic acid. The study focuses on determining the acute toxicity and irritation properties of composite hydrogel to facilitate the way for its potential biomedical applications. For the evaluation of acute toxicity assessments, the OECD guidelines 420 and 402 will be followed (OECD, 420 & 402). The aim is to study the various biochemical and hematological parameters. Additionally, to detect any irritation on eyes and skin, the eye irritation test and acute dermal toxicity studies will also be performed.

## 2. Methodology

### 2.1. Materials

From the public market of Sargodha region, Pakistan, *M. pudica* seeds were procured and cleaned to ensure purity before use. Reagents such as methacrylic acid (MA), *N,N'*-methylene-bis-acrylamide (MBA), potassium persulfate (KPS), and solvents as *n*-hexane and ethanol were used during this research work. To make the solution or dispersion, distilled water (DW) was used.

### 2.2. Extraction of Mucilage and Synthesis of Composite Hydrogel

*M. pudica* seeds mucilage was extracted according to the already reported procedure (Muhammad et al., 2016) and converted to composite hydrogel by treating with methacrylic acid according to the procedure reported by Ali et al., (2023d).

### 2.3. Acute Toxicity Testing

Acute oral toxicity evaluations of the composite hydrogel were conducted in compliance with the Economic Co-operation and Development (OECD) protocol and OECD standards, respectively. The testing was carried out accurately in accordance with Good Laboratory Practices (GLP). The research was approved by the Animal Ethics Committee of Superior University Sargodha, Pakistan. Animals of group A were remained untreated and labeled as the control group, while animal models of groups B to D were given a single dose of the composite hydrogel (0.05, 0.3, and 2 g/kg of bodyweight). Before composite hydrogel dosing, all the animals were not allowed to intake food for 12 h. With regular monitoring for 14

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ISSN: (e) 3007-1607 (p) 3007-1593

days, animals of all the groups were supplied with food and water after 1 h of study period.

**Table 1: Group names and dose scheme for the acute oral toxicity studies of composite hydrogel in rabbits**

| Group "A" | Group "B" | Group "C" | Group "D" |
|---|---|---|---|
| Control group of animals given with standard laboratory diet | Treated group of animals given with a dose of 0.05 g/kg of the bodyweight of animals mixed with diet | Treated group of animals given with a dose of 0.3 g/kg of the bodyweight of animals mixed with diet | Treated group of animals given with a dose of 2 g/kg of the bodyweight of animals mixed with diet |

### 2.4. Physical Observation and Mortality

A 14-days observation period was conducted to monitor animals for any adverse reactions, including salivation, diarrhea, tremors, allergic symptoms, seizures, and behavioral changes, and to record any mortality after dosing composite hydrogel. Moreover, during the study period, if fatality in animals occurred, it was recorded in all groups.

### 2.5. Assessment of Bodyweight, Food, and Water Intake

On the general health of the animals, the alternation of the bodyweights of control animals and experimental animals, and food consumption patterns of these animals were used to assess composite hydrogel toxicity. Therefore, the body size, fluid, and food intake of laboratory animals in both control and experimental groups were recorded pre- and post-composite hydrogel administration for the initial three-day period and then on days 7 and 14.

### 2.6. Blood Analysis and Biochemical Testing

The study involved analysis of blood samples for various hematological parameters for TLC, RBCs, Hb, MCV, thrombocyte Count, and average hemoglobin per red blood cell and biochemical Indicators. In this study, both control and treated groups of animal blood samples were obtained on the 15th day. With the help of chloroform, animals were induced with anesthesia. A blood sample was taken from the cervical artery of the animals and placed in ethylenediaminetetraacetic acid (EDTA) coated tubes. Total White Blood Cell Count, erythrocytes, hemoglobin, Mean Cell Volume (MCV), platelet count, average hemoglobin content, and blood samples were evaluated. However, for urea, blood cholesterol levels, uric acid levels, blood creatinine levels, triglycerides, alanine transaminase, and aspartate transaminase, the contents of the blood serum was analyzed.

### 2.7. Primary Eye Irritation

For ocular irritation study, composite hydrogel was applied to the right ocular tissues of rabbits. The left eyes served as controls without any treatment. For the next 24 h, observations for lacrimation and redness were made in the eyes of all rabbits (Draize et al., 1944).

### 2.8. Dermal Exposure Toxicity

A cutaneous toxicity study related to composite hydrogel was conducted on experimental rabbits. The hair of the rabbits was cleared from the back area, and a concentrated mixture of composite hydrogel in DW was applied to the skin using gauze. By removing the wound dressing material, observations were made on the skin for any irritation, redness, allergy, and abnormality (Saiyed et al., 2015).

## 3. Results and Discussions

### 3.1. Physical Condition Evaluation and Mortality Rates

Animals of treated groups did not display any kind of behavioral changes or neurological and gastrointestinal abnormalities, including seizures, regurgitation, hyper salivation or other adverse reactions. All the animals showed no observable adverse effects following ingestion of composite hydrogel in rabbits. The study revealed that dosage upto, 2 g/kg of bodyweight showed no fatalities. Under the globally harmonized system (GHS) guidelines, category 5 is categorized to chemicals with LD50 values exceeding 2 g/kg. Therefore, the composite hydrogel can be placed in a category 5 substance. Additionally, composite hydrogel is classified as a non-toxic material in accordance with categorization, labelling, and packaging (CLP) regulation (Diallo et al., 2010).

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### 3.2. Assessment of Bodyweight and Dietary Consumption

Food and water consumption patterns by all animals was recorded after composite hydrogel was administered (Tables 2-4). Toxicity is indicated by the reduction in the bodyweight which is obvious and responsive. A loss in the weight of rabbits was observed in the initial three days period that was quickly reversed. After the composite hydrogel dosage the loss in the weight was possibly due to less the lower consumption of food on first day. A normal growth and physiological functions were indicated as rabbits gained their weight during the first week. Additionally, the distinction in the comparative control weights and treated samples was deemed to be statistically insignificant over the entire study period. Minor reduction in food consumption was observed in group C and D of rabbits on day 1 (Tables 2-4) likely because the higher dose of composite hydrogel made them feel fuller. By the end of days 7 and 14, the food and water consumption were normalized.

**Table 2: Bodyweight (g) of the treated and control group of rabbits (mean ± SD)**

| Parameters | Group "A" | Group "B" | Group "C" | Group "D" |
|---|---|---|---|---|
| **Pretreatment** | 1668.21 ± 25.72 | 1689.73 ± 19.03 | 1713.78 ± 24.01 | 1727.75 ± 20.89 |
| **Day 1** | 1666.17 ± 24.96 | 1687.06 ± 24.38 | 1710.45 ± 28.05 | 1723.62 ± 24.29 |
| **Day 2** | 1663.84 ± 26.15 | 1685.39 ± 26.17 | 1709.26 ± 24.44 | 1721.38 ± 27.56 |
| **Day 3** | 1666.25 ± 28.16 | 1686.06 ± 27.21 | 1711.41 ± 26.48 | 1724.50 ± 26.02 |
| **Day 7** | 1683.58 ± 19.58 | 1704.68 ± 29.33 | 1728.26 ± 23.10 | 1741.04 ± 28.97 |
| **Day 14** | 1714.05 ± 22.46 | 1735.29 ± 22.96 | 1758.39 ± 27.70 | 1772.21 ± 26.14 |

**Table 3: Mean values of water consumption (mL) of control and treated groups of rabbits**

| Parameters | Group "A" | Group "B" | Group "C" | Group "D" |
|---|---|---|---|---|
| **Pretreatment** | 22.05 ± 1.49 | 21.80 ± 1.18 | 22.17 ± 1.44 | 23.34 ± 1.64 |
| **Day 1** | 21.66 ± 1.27 | 21.52 ± 1.76 | 22.56 ± 2.20 | 22.53 ± 2.32 |
| **Day 2** | 22.48 ± 1.55 | 22.34 ± 1.60 | 23.85 ± 1.37 | 23.76 ± 1.70 |
| **Day 3** | 23.54 ± 1.63 | 21.90 ± 1.19 | 22.15 ± 1.74 | 22.58 ± 1.30 |
| **Day 7** | 22.60 ± 1.73 | 21.84 ± 1.20 | 21.49 ± 1.26 | 23.37 ± 1.44 |
| **Day 14** | 23.75 ± 1.46 | 22.49 ± 1.81 | 22.86 ± 1.03 | 23.69 ± 1.36 |

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Table 4: Mean values of food consumption (mL) of control and treated groups of rabbits

| Parameters | Group "A" | Group "B" | Group "C" | Group "D" |
|---|---|---|---|---|
| Pretreatment | 23.73 ± 1.87 | 22.28 ± 1.46 | 22.32 ± 1.17 | 21.60 ± 1.35 |
| Day 1 | 22.97 ± 1.15 | 21.93 ± 1.63 | 22.77 ± 1.35 | 20.82 ± 1.23 |
| Day 2 | 23.16 ± 1.04 | 22.39 ± 1.47 | 21.47 ± 1.82 | 21.73 ± 1.61 |
| Day 3 | 22.42 ± 1.12 | 23.44 ± 1.95 | 22.48 ± 1.69 | 22.68 ± 1.33 |
| Day 7 | 23.25 ± 1.27 | 22.16 ± 1.43 | 23.50 ± 1.45 | 21.49 ± 1.38 |
| Day 14 | 23.50 ± 1.79 | 23.07 ± 1.33 | 22.93 ± 1.10 | 22.54 ± 1.43 |

### 3.3. Hematology and Biochemical Analysis

The bone marrow produces the blood cells, so substances affecting it can change its complete blood count (CBC). Serum enzyme like ALT, ALP, and total bilirubin are other key health indicators in animals. The liver damage caused by hepatoxicity was indicated by the change in these enzyme biomarker levels. Correspondingly, the kidney functions can be assessed by blood urea and creatinine levels (Rivadeneyra-Domínguez et al., 2018). Therefore, to determine the toxic effect of composite hydrogel to these vital organs, hematology and biochemistry tests of animals were performed. The hematology and biochemical analysis findings are tabulated in Tables 5 and 6. No alterations were observed in liver enzyme levels, urea levels, and creatinine values, serving as proof that blood cells, liver, and kidneys are not significantly affected by composite hydrogel. The composite hydrogel is deemed as safe and non-toxic, as all parameters were normal or comparable to the control group. Serious health complications, particularly cardiovascular issues, may be caused by variations in electrolyte concentrations. There was no notable change in electrolyte levels found versus the control. Within standard parameters, lipid profile levels were also observed. For oral consumption, research showed composite hydrogel is a safe material to use.

Table 5: Hematological parameters of rabbits

| Parameters | Group "A" | Group "B" | Group "C" | Group "D" |
|---|---|---|---|---|
| TLC ($\mu L^{-1}$) | 4.2 | 3.8 | 4.3 | 3.9 |
| RBC ($\mu L^{-1}$) | 5.1 | 5.2 | 5.1 | 6.5 |
| Hb (g/dL) | 12.5 | 12.3 | 13.7 | 12.8 |
| HCT (PCV) (%) | 43.6 | 42.1 | 43.2 | 42.7 |
| MCV (fL) | 59.2 | 58.9 | 60.5 | 57.6 |
| MCH (pg) | 17.1 | 19.4 | 19.6 | 20.9 |
| MCHC (g/dL) | 31.7 | 31.5 | 30 | 31.2 |
| Platelet count ($\mu L^{-1}$) | 166.3 | 159.8 | 164.1 | 169.7 |
| Neutrophils (%) | 29.2 | 27.6 | 30.3 | 26.5 |
| Lymphocytes (%) | 52.1 | 53.4 | 53.9 | 52.3 |
| Monocytes (%) | 3.2 | 2.9 | 3.1 | 2.8 |
| Eosinophils (%) | 2.2 | 2 | 2.3 | 2.1 |

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ISSN: (e) 3007-1607 (p) 3007-1593

Table 6: Clinical biochemistry parameters of rabbits

| Parameters | Group "A" | Group "B" | Group "C" | Group "D" |
|---|---|---|---|---|
| **Lipid Profile** | | | | |
| Cholesterol (mg/dL) | 113.7 | 116.9 | 115.1 | 114.5 |
| Triglyceride (mg/dL) | 82.3 | 83.2 | 82.9 | 84.7 |
| HDL (mg/dL) | 39.1 | 40.6 | 40.8 | 41.3 |
| LDL (mg/dL) | 83.4 | 82.7 | 84.2 | 82.9 |
| VLDL (mg/dL) | 14.1 | 14.9 | 15.6 | 15.4 |
| **Liver function test** | | | | |
| Bilirubin (mg/dL) | 0.5 | 0.4 | 0.6 | 0.5 |
| SGPT (ALT) (U/L) | 42.6 | 42.3 | 43.7 | 43.2 |
| SGOT (AST) (U/L) | 72.1 | 81.7 | 71.5 | 72.8 |
| ALP (U/L) | 62.5 | 63.8 | 70.9 | 69.1 |
| Total protein (g/dL) | 6.2 | 7 | 6.4 | 6.5 |
| Albumin (g/dL) | 4.2 | 3.9 | 3.7 | 4.3 |
| Globulin (g/dL) | 2.7 | 2.8 | 2.6 | 2.9 |
| A/G Ratio | 1.56 | 1.39 | 1.42 | 1.48 |
| **Renal function test** | | | | |
| Urea (mg/dL) | 14.2 | 12.1 | 12.9 | 13.7 |
| Creatinine (mg/dL) | 0.6 | 0.7 | 0.8 | 0.7 |
| **Hematology** | | | | |
| ESR (mm/h) | 1.6 | 1.9 | 1.5 | 2 |
| **Serum electrolyte** | | | | |
| Potassium (mmol/L) | 4.2 | 4.4 | 4.5 | 4.1 |
| Sodium (mmol/L) | 135.8 | 139.3 | 142.7 | 137.5 |
| Uric acid (mg/dL) | 4 | 3.9 | 4.2 | 3.7 |

### 3.4. Absolute Organ Body Weight

As seen in Tables 7, there is no kind of significant difference is shown when we compare the organ body weights of major organs of both experimental and control animals.

Table 7: Absolute organ weight (g) of control and treated group of rabbits (mean ± SD)

| Parameters | Group "A" | Group "B" | Group "C" | Group "D" |
|---|---|---|---|---|
| **Heart** | 4.405 ± 0.01 | 4.210±.01 | 4.214±.01 | 4.174±.01 |
| **Kidney** | 17.043±0.03 | 16.986±0.04 | 17.422±0.07 | 17.167±0.04 |
| **Stomach** | 17.231±.04 | 17.369±.04 | 17.344±.06 | 17.459±.06 |
| **Intestine** | 46.488±.03 | 46.505±.08 | 46.238±.05 | 46.553±.03 |
| **Liver** | 53.220±.06 | 53.033±.02 | 54.837±.08 | 53.747±.02 |

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ISSN: (e) 3007-1607 (p) 3007-1593

### 3.5. Ocular and Dermal Toxicity Testing

It is necessary for the excipients, i.e., oral, inhalation or dermal that it must be evaluated for skin and eye irritation to ensure safety (Demerlis et al., 2016). The Draize test results showed a score of 0, as no irritation, inflammation, or conjunctivitis was observed. The composite hydrogel synthesized herein did not cause any skin irritation, lesions, or infections, and hence, demonstrating as a safe for both ocular and dermal applications (Draiz 1944).

## 4. Conclusions

The composite hydrogel toxicity tests in rabbits showed no adverse effects. It is found as safe material for skin and eye contact. Hence, the studies demonstrated that the composite hydrogel could be a promising candidate for oral drug delivery. Additional toxicological assessments, such as chronic toxicity, cytotoxicity, and mutagenicity, are required to be evaluate for the study of its comprehensive safety behavior.

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