INTERNATIONAL JOURNAL OF ADVANCED RESEARCH
ISSN: 3106-7980: 3106-7972
Volume 3, Issue 2, 2026

ASSESMENT OF THE NON-TOXIC BEHAVIOUR OF CHEMICALLY MODIFIED PLANT SEED MUCILAGE USING RATE AS MODEL RODENT SPECIE

Uzma Aslam [*1], Aiman Ishfaq[2], Saeed Rasheed[3]

[*1,2] *Faculty of Sciences, Superior University Lahore, Lahore 54000, Pakistan*
[3] *Faculty of CS&IT, The Superior University Lahore, Lahore 54000, Pakistan*

[*1]uzmamaken786@gmail.com

DOI: http://doi.org/10.5281/zenodo.21545951

Keywords
Mucilage, Copolymerization, Acute oral toxicity

Article History
Received: 28 April 2026
Accepted: 15 June 2026
Published: 30 June 2026

Corresponding Author: *
Uzma Aslam

Abstract

Active pharmaceutical ingredients (APIs) are vital to the drug development process. APIs can be authorized for use in pharmaceuticals, opening the door to novel treatments, after undergoing extensive testing to guarantee their safety and effectiveness. OECD 402 and 420 criteria were followed while evaluating the acute oral and dermal toxicity of a composite hydrogel made from acrylamide and Salvia spinosa seed mucilage. Four different groups of rats were used as experimental animals. Animals in categories B, C, and D received one dose of composite hydrogel, while animals in category A served as controls and received no treatment. All of the animals remained alive during the 14-day toxicity research period, and no notable negative effects were seen in the animals. The blood analysis and biochemical test were carried out after 14 days. Together, the results demonstrate that the produced composite hydrogel is a safe substance that shows promise as a non-toxic excipient in drug delivery systems.

1. Introduction

Natural polysaccharides are the ideal polymer for the creation of DDSs due to their high swelling, prolonged drug release capabilities, non-toxicity, biodegradability, and biocompatibility [1-9]. Furthermore, the properties of natural polysaccharides can be enhanced by chemical modifications such as crosslinking, acetylation, and polymerization, making them appropriate materials for cutting-edge applications. These changes make it possible to create smart materials that react to temperature, pH, ethanol, salt, and other stimuli. On the other hand, graft copolymerization with acidic monomer produces materials that are highly swellable and appropriate for applications requiring sustained release [10-27]. In order to evaluate the potential of naturally occurring polysaccharides and their modified forms as safe DDSs, toxicity studies have recently been carried out on them.

Previous studies have shown that the mucilage of *S. spinosa*. Here, we sought to investigate the safety evaluations of a composite hydrogel made of methacrylic acid and mucilage from M. puidca seeds. The goal of the study is to ascertain the composite hydrogel's acute toxicity and irritant characteristics in order to pave the road for its possible biomedical usage.Acute toxicity evaluations will be evaluated in accordance with OECD criteria 420 and 402 [28, 29]. The objective is to investigate the different hematological and biochemical parameters.

Additionally, acute dermal toxicity studies and the ocular irritation test will be carried out to identify any irritation on the skin or eyes.

2. Methodology

2.1. Materials

S. spinosa seeds were purchased from the Sargodha district of Pakistan's public market and thoroughly cleaned to guarantee their purity before being used. Acrylamide (AAm), *N, N'*-methylene-bis-acrylamide (MBA), potassium persulfate (KPS), and solvents such ethanol and n-hexane were among the reagents employed in this study. The solution or dispersion was created using distilled water (DW).

2.2. Mucilage Extraction and Composite Hydrogel Synthesis

Mucilage from *S. spinosa* seeds was extracted using the previously described method [5] and treated with acrylamide using the method described by Ali et al. [4] to create composite hydrogel.

2.3. Acute Toxicity Testing

Evaluations of the composite hydrogel's acute oral toxicity were carried out in accordance with OECD guidelines and protocol, respectively [28, 29]. Accurate testing was performed in compliance with Good Laboratory Practices (GLP). The Superior University Sargodha, Pakistan Animal Ethics Committee gave its approval to the study. A single dose of the composite hydrogel (0.05, 0.3, and 2 g/kg of bodyweight) was administered to animal models in groups B through D, whereas animals in group A were left untreated and marked as the control group. For 12 hours before to composite hydrogel dose, none of the animals were permitted to eat. Following a one-hour research period, food and water were given to the animals in each group, and they were monitored regularly for 14 days.

2.4. Mortality and Physical Observation

After administering composite hydrogel to the animals, a 14-day observation period was used to track any adverse events, such as salivation, diarrhea, tremors, allergic symptoms, convulsions, and behavioral changes, as well as to document any mortality. Additionally, if an animal died during the study period, it was noted in every group.

2.5. Assessment of Bodyweight, Food, and Water Intake

Composite hydrogel toxicity was evaluated based on the animals' overall health, the alternating bodyweights of the experimental and control animals, and their food consumption habits. Thus, for the first three days and then on days 7 and 14, the body size, hydration intake, and food consumption of laboratory animals in both the control and experimental groups were measured before and after composite hydrogel injection.

2.6. Blood Analysis and Biochemical Testing

TLC, RBCs, Hb, MCV, thrombocyte count, average hemoglobin per red blood cell, and biochemical indicators were among the hematological parameters for which blood samples were analyzed for the study. Animal blood samples from the control and treated groups were collected on the fifteenth day of the trial. Animals were put under anesthesia using chloroform. Ethylenediaminetetraacetic acid (EDTA)-coated tubes were filled with a blood sample drawn from the animals' cervical arteries. Blood samples, total white blood cell count, erythrocytes, hemoglobin, mean cell volume (MCV), platelet count, and average hemoglobin content were assessed. On the other hand, the blood serum was examined for urea, blood cholesterol, uric acid, blood creatinine, triglycerides, alanine transaminase, and aspartate transaminase.

3. Results and Discussions

3.1. Physical Condition Evaluation and Mortality Rates

No neurological or gastrointestinal problems, such as seizures, regurgitation, hypersalivation, or other negative reactions, were observed in the animals in the treated groups. Following the consumption of composite hydrogel in rats, none of the animals displayed any discernible negative

effects. According to the study, dosages up to 2 g/kg of bodyweight did not result in any fatalities. According to the globally harmonized system (GHS) criteria, substances with LD50 values greater than 2 g/kg fall under category 5.The composite hydrogel can thus be classified as a category 5 material. Furthermore, according to the classification, labeling, and packaging (CLP) regulation, composite hydrogel is a non-toxic material [30].

3.2. Assessment of Bodyweight and Dietary Consumption

Following the administration of composite hydrogel, all animals' food and water intake patterns were noted (Tables 1-3). The apparent and immediate decrease in body weight is indicative of toxicity. The weight of the rats decreased during the first three days, but this was soon rectified. The weight loss following the composite hydrogel dosage may have resulted from a decrease in food intake on the first day. During the first week, rats gained weight, indicating normal growth and physiological activities. Furthermore, for the duration of the investigation, the difference between the comparative control weights and treated samples was shown to be statistically insignificant. On day 1, group C and D of rats showed a slight decrease in food consumption (Tables 1-3), most likely as a result of feeling fuller after receiving a greater dose of composite hydrogel. By the end of days 7 and 14, the consumption of food and drink had returned to normal.

Table 1: Bodyweight (g) of the treated and control group of rats (mean ± SD)

Parameters	Group "A"	Group "B"	Group "C"	Group "D"
Pretreatment	187.42±2.42	182.13±4.23	174.17±4.17	192.34 ±4.45
Day 1	184.34±4.63	182.263±5.54	177.65±3.56	186.55 ±5.56
Day 2	183.61±2.76	181.32±7.56	173.45±5.45	184.34 ±2.34
Day 3	183.55±5.48	179.66±4.33	179.34±3.33	187.76 ±7.56
Day 5	184.34±6.33	182.47±2.23	175.23±5.54	185.56 ±4.76
Day 7	187.77± 7.85	185.53±6.54	186.76±4.25	191.45 ±3.91
Day 14	196.59±4.47	193.75±3.12	183.46±6.76	189.55 ±6.34

Table 2: Mean values of water consumption (mL) of control and treated groups of rats

Parameters	Group "A"	Group "B"	Group "C"	Group "D"
Water consumption				
Pretreatment	6.43±1.36	5.45±1.34	5.34±1.23	6.55±1.34
Day 1	5.23±1.43	5.76±1.65	4.31±1.54	5.45±1.65

Day 2	5.65±1.53	6.34±1.45	4.32±1.66	6.76±1.71
Day 3	4.45±1.55	6.56±1.67	5.56±1.27	5.34±1.13
Day 7	5.76±1.67	5.66±1.45	6.43±1.65	6.65±1.43
Day 14	6.45±1.34	6.76±1.55	5.56±1.45	7.71±1.55

Table 3: Mean values of food consumption (mL) of control and treated groups of rats

Parameters	Group "A"	Group "B"	Group "C"	Group "D"
Food consumption				
Pretreatment	7.43±1.23	8.54±1.41	7.34±1.13	8.54±1.41
Day 1	7.14±1.24	7.34±1.45	8.76±1.54	7.23±1.45
Day 2	8.±76.41	6.76±1.65	7.33±1.34	8.44±1.67
Day 3	7.22±1.45	7.31±1.67	6.56±1.21	8.34±1.42
Day 7	8.45±1.65	7.37±1.72	7.56±1.54	7.65±1.51
Day 14	7.34±1.67	8.24±1.89	8.71±1.65	8.76±1.53

3.3. Hematology and Biochemical Analysis

The complete blood count (CBC) can be altered by drugs that impact the bone marrow, which is responsible for producing the blood cells. Serum enzyme like ALT, ALP, and total bilirubin are other critical health indicators in animals. The liver damage produced by hepatoxicity was revealed by the change in these enzyme biomarker levels. Correspondingly, the kidney functions can be measured by blood urea and creatinine levels [31]. Thus, animal hematology and biochemistry studies were conducted to ascertain the harmful impact of composite hydrogel on these essential organs. Tables 4 and 5 tabulate the results of the biochemical analysis and hematological. No variations were identified in liver enzyme levels, urea levels, and creatinine values, acting as confirmation that blood cells, liver, and kidneys are not severely affected by composite hydrogel. The composite hydrogel is recognized as safe and non-toxic, as all parameters were normal or comparable to the control group. Variations in electrolyte concentrations can result in serious health problems, especially cardiovascular problems. The electrolyte levels were determined to be unchanged from the control. Lipid profile values were also observed within normal norms.

Table 4: Hematological parameters of rats

Parameters	Group "A"	Group "B"	Group "C"	Group "D"
TLC (µL-1)	8.4	9.4	8.5	8.4
RBC (µL-1)	7.6	6.5	7.7	6.3
Hb (g/dL)	17.3	15.2	17.2	16.6
HCT (PCV) (%)	44.5	45.7	44.4	46.4
MCV (fL)	79.7	81.3	82.6	78.5
MCH (pg)	21.2	20.9	21.3	20.9
MCHC (g/dL)	35.5	33.5	34.7	33.4
Platelet count (µL-1)	252.4	274.7	287.8	283.6
Neutrophils (%)	42.23	44.4	39.4	41.3
Lymphocytes (%)	42.7	43.5	41.9	44.7
Monocytes (%)	5.9	5.8	5.6	4.5
Eosinophils (%)	3.5	4.9	5.4	4.9

Table 5: Clinical biochemistry parameters of rats

Parameters	Group "A"	Group "B"	Group "C"	Group "D"
Lipid Profile				
Cholesterol (mg/dL)	126.4	124.5	122.5	121.4
Triglyceride (mg/dL)	87.2	97.3	93.3	102.7
HDL (mg/dL)	43.7	47.7	49.9	44.8
LDL (mg/dL)	92.5	112.9	105.4	97.5
VLDL (mg/dL)	16.9	19.5	18.2	15.7
Liver function test				
Bilirubin (mg/dL)	1.5	0.7	0.8	0.91
SGPT (ALT) (IU/L)	23.2	25.3	22.6	21.5
SGOT (AST) (IU/L)	16.6	19.5	22.45	17.7

ALP (IU/L)	74.3	82.3	80.7	64.8
Total protein (g/dL)	9.7	8.7	9.8	9.5
Albumin (g/dL)	5.3	5.6	6.5	5.7
Globulin (g/dL)	4.72	4.45	4.45	4.72
A/G Ratio	3.45	3.76	3.32	3.45
Renal function test				
Urea (mg/dL)	21.5	24.56	26.6	23.6
Creatinine (mg/dL)	0.78	0.8	0.7	1.8
Hematology				
ESR (mm/h)	8.4	7.4	7.9	8.9
Serum electrolyte				
Potassium (mmol/L)	7.3	6.6	7.1	7.45
Sodium (mmol/L)	136.6	143.5	150.5	141.6
Uric acid (mg/dL)	5.5	5.8	5.45	5.7

4. Conclusions

There were no negative results from the composite hydrogel toxicity studies in rats. It is determined to be a safe material for contact with the skin and eyes. Therefore, the research showed that the composite hydrogel may be a good option for oral medication delivery. Further toxicological evaluations, including cytotoxicity, mutagenicity, and chronic toxicity, must be conducted in order to examine its overall safety behavior.

REFERENCES

[1] Abramson, C.I. and Chicas-Mosier, A.M., 2016. Learning in plants: lessons from Mimosa pudica. Frontiers in psychology, 7, p.417.

[2] Ahuchaogu, A.A., Chukwu, O.J. and Echeme, J.O., 2017. Secondary metabolites from Mimosa pudica: isolation, purification and NMR characterization. IOSR J. Appl. Chem, 10(3), pp.15-20.

[3] Abbas Bukhari, S.N., Fatima, M., Ali, A., Fatima, T., Hussain, M.A., Alenzi, H.M., Abdelgawad, M.A., Al-Sanea, M.M. and Ahmad, N., 2026. Citric acid crosslinking of arabinoxylan engenders a stimuli-responsive hydrogel as a smart material for intelligent drug delivery. RSC Advances, 16(13), pp.12055-12067.

[4] Bhatti, M.A., Ahmad, N., Ali, A., Bukhari, S.N.A., Haseeb, M.T., Elkomy, M.H., Hussain, S.Z., Alsaidan, O.A. and Zafar, A., 2026. Polysaccharide-based smart copolymeric hydrogel from chia seeds: Fabrication, pH-responsiveness, sustained drug release, and acute toxicity studies. International Journal of Biological Macromolecules, p.151609.

[5] Ali, A., Bakhsh, A., Qasim, S., Sheikh, F.A., Ali, H., Khaliq, N.U., Haseeb, M.T., Dar, M. and Shaaban, I.A., 2026. Citric acid–mediated crosslinking of chia seed glucoxylan and hydroxypropyl cellulose: ph responsiveness, haemocompatibility and sustained drug release. Chemistry & Biodiversity, 23(2), p.e03389.

[6] Babar, S., Sheikh, F.A., Bukhari, S.N.A., Haseeb, M.T., Ali, A., Ul Khaliq, N., Alafar, R.A., Abdelgawad, M.A., Zafar, A. and Ahmad, N., 2026. Formulation design of glucuronoxylan-based stimuli-responsive microparticles for zero-order drug delivery. Journal of Pharmaceutical Innovation, 21(2), p.171.

[7] Amjad, F., Ali, A., Hussain, M.A., Haseeb, M.T., Farid-ul-Haq, M., Ajaz, I., Sher, M. and Imran, M., 2026. Fabrication of a superabsorbent and pH-responsive glucomannan-based hydrogel: crosslinking, characterization, toxicological evaluation, and sustained-release of itopride. Materials Advances, 7(3), pp.1495-1507.

[8] Hussain, M.A., Raees, N., Ali, A., Tayyab, M., Muhammd, G., Uroos, M. and Batool, M., 2025. Optimization of rhamnogalacturonan extraction from linseed using RSM and designing a pH-responsive tablet formulation for sustained release of ciprofloxacin. Cellulose Chemistry and Technology, 59, pp.547-558.

[9] Hussain, M.A., Taj, T., Ali, A., Haseeb, M.T., Hussain, S.Z., Muhammad, G. and Bukhari, S.N.A., 2025. Cross-linking of hydroxypropylcellulose with flaxseed rhamnogalacturonans using citric acid produces a hemocompatible biocomposite for PH-responsive rifaximin delivery. Journal of Applied Polymer Science, 142(38), p.e57486.

[10] Hussain, M.A., Shahzad, K., Ali, A., Haseeb, M.T. and Hussain, S.Z., 2025. Development of a novel smart bio-composite hydrogel based on dextran, citric acid, and glucoxylan for pH-dependent drug delivery and stimuli-responsive swelling and release. Polymers and Polymer Composites, 33, p.09673911251350240.

[11] Ali, A., Haseeb, M.T., Hussain, M.A., Tayyab, MUHAMMAD., Muhammad, G., Ahmad, N., Alotaibi, N.F., Hussain, S.Z. and Hussain, I.R.S.H.A.D., 2022. Extraction optimization of a superporous polysaccharide-based mucilage from Salvia spinosa L. Cell. Chem. Technol, 56, pp.957-969.

[12] Hussain, M.A., Ali, A., Alsahli, T.G., Khan, N., Sharif, A., Haseeb, M.T., Alsaidan, O.A., Tayyab, M. and Bukhari, S.N.A., 2023. Polysaccharide-based hydrogel from seeds of Artemisia vulgaris: extraction optimization by Box–Behnken design, pH-responsiveness, and sustained drug release. Gels, 9(7), p.525.

[13] Ali, A., Hussain, M.A., Haseeb, M.T., Farid-Ul-Haq, M., Erum, A. and Hussain, M., 2024. Acute toxicity studies of methacrylic acid based composite hydrogel of Salvia spinosa seed mucilage: a potential non-toxic candidate for drug delivery. Cellulose Chemistry & Technology, 58.

[14] Farid-ul-Haq, M., Hussain, M.A., Ali, A., Haseeb, M.T., Muhammad, G., Tabassum, T., Ashraf, M.U., Tulain, U.R. and Erum, A., 2024. A pH-responsive Artemisia vulgaris mucilage-co-acrylic acid hydrogel: Synthesis, characterization, controlled drug release, toxicity studies, and MRI. Journal of Drug Delivery Science and Technology, 93, p.105468.

[15] Irfan, J., Ali, A., Hussain, M.A., Haseeb, M.T., Naeem-ul-Hassan, M. and Hussain, S.Z., 2024. Citric acid cross-linking of a hydrogel from Aloe vera (Aloe barbadensis M.) engenders a pH-responsive, superporous, and smart material for drug delivery. RSC advances, 14(12), pp.8018-8027.

[16] Ali, A., Hussain, M.A., Abbas, A., Khan, T.A., Muhammad, G., Haseeb, M.T. and Azhar, I., 2022. Comparative isoconversional thermal analysis and degradation kinetics of Salvia spinosa (Kanocha) seed hydrogel and its acetates: a potential matrix for sustained drug release. Cellul. Chem. Technol., 56(3–4), pp.239-250.

[17] Amjad, F., Ali, A., Hussain, M.A., Haseeb, M.T., Ajaz, I., Farid-ul-Haq, M., Hussain, S.Z. and Hussain, I., 2025. A superabsorbent and pH-responsive copolymer-hydrogel based on glucomannans from Ocimum basilicum (sweet basil): A smart and non-toxic material for intelligent drug delivery. International Journal of Biological Macromolecules, 315, p.144452.

[18] Khatoon, M., Ali, A., Hussain, M.A., Haseeb, M.T., Muhammad, G., Sher, M., Hussain, S.Z., Hussain, I. and Iqbal, M., 2025. A chia (Salvia hispanica L.) seed mucilage-based glucoxylan-grafted-acrylic acid hydrogel: a smart material for pH-responsive drug delivery systems. Materials Advances, 6(8), pp.2636-2647.

[19] Ali, A., Hussain, M.A., Abbas, A., Haseeb, M.T., Azhar, I., Muhammad, G., Hussain, S.Z., Hussain, I. and Alotaibi, N.F., 2023. Succinylated Salvia spinosa hydrogel: Modification, characterization, cadmium-uptake from spiked high-hardness groundwater and statistical analysis of sorption data. Journal of Molecular Liquids, 376, p.121438.

[20] Farid-ul-Haq, M., Ali, A., Hussain, M.A., Abbas, A., Kausar, F., Amin, H.M.A., Sher, M., Hussain, S.Z. and Hussain, I., 2021. Chemical modification of a polysaccharide from Artemisia vulgaris engenders a supersorbent for the removal of Cd2+ from spiked high-hardness groundwater. Desalin Water Treat, 212, 129–142.

[21] Ali, A., Akram, A., Siddique, A.B., Amin, H.M., Zohra, L., Abbas, A., Sher, M., Hussain, M.A., Haseeb, M.T. and Imran, M., 2025. A model batch and column study for Cd (II) uptake using citric acid cross-linked Salvia spinosa hydrogel: Optimization through Box-Behnken design. Journal of Industrial and Engineering Chemistry, 151, 746-761.

[22] Irfan, J., Ali, A., Hussain, M.A., Abbas, A., Haseeb, M.T., Naeem-ul-Hassan, M., Azhar, I., Hussain, S.Z. and Hussain, I., 2024. Chemical modification of Aloe vera leaf hydrogel for efficient cadmium-removal from spiked high-hardness groundwater. International Journal of Biological Macromolecules, 259, p.128879.

[23] Hussain, M.A., Abbas, A., Habib, M.G., Ali, A., Farid-ul-Haq, M., Hussain, M., Shafiq, Z. and Irfan, M.I., 2021. Adsorptive removal of Ni (II) and Co (II) from aqueous solution using succinate-bonded polysaccharide isolated from Artemisia vulgaris seed mucilage. Desalination and Water Treatment, 231, pp.182-195.

[24] Hussain, M.A., Abbas, A., Yameen, E., Ali, A., Muhammad, G., Hussain, M. and Shafiq, Z., 2022. Adsorptive removal of Pb2+ and Cu2+ from aqueous solution using an acid modified glucuronoxylan-based adsorbent. Desalination and Water Treatment, 248, pp.163-175.

[25] Hussain, A., Fatima, S., Abbas, A., Ali, A., Amin, M., Muhammad, G. and Sher, M., 2023. Removal of Cr (III) and Ni (II) from aqueous solution using a mixed cellulose ether-ester hydroxyethylcellulose adipate. Desalination and Water Treatment, 283, pp.153-163.

[26] Shehzad, M.K., Ali, A., Qasim, S., Mumtaz, A., Hussain, M.A., Fawy, K.F., Nishan, U., Azhar, I., Abbas, M.A. and Abba, A., 2025. Sustainable remediation of cadmium using succinate-functionalized glucoxylan from chia (Salvia hispanica) seeds hydrogel. Journal of Industrial and Engineering Chemistry.157, 591-608.

[27] Ali, A., Ali, M., Qasim, S., Ali, H., Mumtaz, A., Fawy, K.F., Nishan, U., Shaheen, M.A. and Abbas, A., 2025. Maleate-bonded acemannan (Aloe vera leaf mucilage): Synthesis, characterization, efficient removal of Pb (II) from groundwater, kinetics, mechanism, and regeneration studies. Polymer, p.129498.

[28] OECD (Organization for Economic Co-operation and Development) Guidelines for testing of chemicals. acute oral toxicity-fixed dose procedure No. 420 (2001), https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl420.pdf.

[29] OECD (Organization for Economic Co-operation and Development) guidelines for testing of chemicals. acute dermal toxicity: fixed dose procedure No. 402 (2017).

[30] Diallo, A., Eklu-Gadegkeku, K., Agbonon, A., Aklikokou, K., Creppy, E.E. and Gbeassor, M., 2011. Acute and sub-chronic (28-day) oral toxicity studies of hydroalcohol leaf extract of Ageratum conyzoides L (Asteraceae).

[31] Rivadeneyra-Domínguez, E., Becerra-Contreras, Y., Vázquez-Luna, A., Díaz-Sobac, R. and Rodríguez-Landa, J.F., 2018. Alterations of blood chemistry, hepatic and renal function, and blood cytometry in acrylamide-treated rats. Toxicology reports, 5, pp.1124-1128.