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Acute Oral and Acute Dermal Toxicity Evaluation of a Chemically Modified Salvia Hispanica Seed Mucilage in Rabbits as Model Animal

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Abstract

The development of safety assessment for active pharmaceutical ingredients and their excipient is vital to their regulatory clearance for human use. The acute oral and dermal toxicity studies of a copolymeric hydrogel synthesized from the polymerization of *Salvia hispanica* seed mucilage and acrylamide were established by following the protocols and guidelines 420 and 402 of the OECD. The ocular toxicity and eye irritation tests were performed and analyzed through the Draize scale. For toxicity studies, the rabbit was used as model animal and were categorized into four distinct groups. The animals (rabbits) in group A1 were not treated with copolymeric hydrogel and served as control. While the animals of other groups, i.e., A2, A3, and A4 were administered copolymeric hydrogel dose of 0.05, 0.3, and 2 g/kg of the bodyweight, and served as the treated groups. All animals were observed for their behavior, food consumption, water intake, allergic reactions, mortality rate, and any other side effects for 14 days. All the observed animals survived without any significant behavioral and physiological abnormalities. The biochemical analysis and hematological tests were assessed after 14 days in both animal groups, control and treated, which were consistent and comparable. The major internal organs of animals were exercised to determine the weight of the organs. The cumulative findings from acute oral and dermal toxicity studies indicated that the synthesized copolymeric hydrogel is safe and non-toxic when orally administered or directly applied to the skin surface. Consequently, it can be demonstrated as potential excipient for pharmaceutical drug delivery systems and wound dressing applications.

Keywords: *Salvia Hispanica Mucilage, Acrylamide; Acute Oral Toxicity, Clinical Biochemistry, Hematology*

Introduction

The toxicity study of any novel material is crucial for evaluating them as excipients, drug delivery carriers, and active pharmaceutical ingredients (APIs). They were thought to be inert and pharmacologically inactive; safety assessments of excipients and drug delivery systems were not given priority in the past (Osterberg et al., 2011). The necessity for further toxicity testing has been highlighted in recent years by reports of toxicological reactions, such as renal toxicity caused by *β*-cyclodextrin, diarrhea triggered by mannitol, dermal irritation by propylene glycol, and digestive disorders by lactose (Osterberg et al., 2003). Natural polysaccharides are polymers known for their swellability, pH-dependent drug release, biodegradability, and biocompatibility (Ali et al. 2023). These properties are enhanced through chemical modifications such as copolymerization and crosslinking and the results obtained in the development of materials that are responsive to pH, temperature, salt, and ethanol (Ali et al., 2022, Ali et al., 2023).

The *Salvia* genus is a member of the Lamiaceae family that contains around 1000 species and grows in arctic, tropical, subtropical, and temperate regions (Kahraman et al., 2010). *Salvia* species are well known for their antiseptic, analgesic, soothing, antimicrobial, antioxidant, antifungal, haemocompatible, non-toxic, and anti-inflammatory properties (Coisin et al., 2010). The term *Salvia* is derived from the Greek word salviare, which means "to restore" or "to secure" health, reflecting the medicinal properties (Salehi et al., 2014). *Salvia hispanica* is an herbaceous, biannually cultivated plant, derived from the mint family (Labiatae/Lamiaceae). It grows in an arid environment. *S. hispanica* seeds contain protein, fats, carbohydrates, dietary fibers, ash contents, minerals, vitamins, and dry matter (Olivos-Lugo et al., 2010, Reyes-Caudillo et al., 2010). *S. hispanica* seeds naturally contain antioxidants, fatty acids, fiber (soluble or insoluble), minerals, and vitamins (Ixtaina et al., 2008). The mucilage of *S. hispanica* seeds is polysaccharidal in nature and contain hydrophilic functional groups like COOH and OH that can be modified to produce materials with a wide range of biomedical applications. *S. hispanica* seeds mucilage is a natural polysaccharide, evaluated for medicinal uses because of its hypoallergenic, non-irritating, non-toxic, haemocompatible nature, has high water retention, swelling, and deswelling abilities, pH-responsive release of drug, and soothing properties (da Silva Marineli et al., 2014, Khatoon et al., 2024).

This investigation focuses on the synthesis of copolymeric hydrogel based on *S. hispanica* seed mucilage and acrylamide to evaluate its acute oral and acute dermal toxicity studies, and eye irritation effects to broaden its potential for biomedical applications. For the evaluation of acute oral and dermal toxicity, the guidelines 420 and 402 by OECD will be employed, respectively OECD, 402 and 420). The impacts of copolymeric hydrogel on the biochemical and hematological indicators of rabbits will be analyzed. The aim is to evaluate the non-irritating nature of the copolymeric hydrogel by conducting eye irritation tests.

Methodology

Materials

From the retail market, seeds of *S. hispanica* were obtained. This research utilized different chemicals and reagents that include *N, N'*-methylene-bis-acrylamide (MBA), acrylamide (AM), ammonium persulfate (APS), *n*-hexane, and ethanol. Such highly purified pharmaceutical-grade chemical reagents were used without any additional purification. For the preparation of solutions, distilled water (DW) was used throughout the experimental work.

Extraction Mucilage from *S. hispanica* Seeds and Synthesis of Copolymeric Hydrogel

The mucilage from chia seeds was extracted according to the procedure reported by Khatoon et al. (2024). The copolymeric hydrogel was synthesized by treating *S. spinosa* seed mucilage with

acrylamide using APS as initiator and MBA as cross-linker. Already reported method with slight modification in the procedure was opted for the synthesis of copolymeric hydrogel (Mishra et al., 2008).

Testing of Acute Oral Toxicity

To evaluate the acute toxicity of copolymeric hydrogel, Swiss albino rabbits were used as model animals. These model animals were provided by the laboratory animal unit. The model animals were kept in clean, well-organized enclosures with a favorable environment including a 12 h photoperiod, 40% humidity, and a temperature of about 25°C. The experiment was conducted by following the rules of good laboratory practices (GLP) and Organization for Economic Co-operation and Development (OECD) (OECD, 402 and 420). A dose of copolymeric hydrogel was administered to the model animals (rabbits) of groups A2 (0.05g), A3 (0.3g) and A4 (2g) as per kg of their bodyweight and the model animals (rabbits) of group A1 were not treated with any dose and they worked as control group of model animals (Table 1). All the model animals were kept starving for 12 h before the administration of copolymeric hydrogel. The water and food were supplied uniformly to the model animals of each group after 1 h of copolymeric hydrogel administration and they were regularly monitored for 14 days.

Table 1. Group and dose scheme for acute oral toxicity in rabbits

Group "A1"	**Group "A2"**	**Group "A3"**	**Group "A4"**
Control group of animals fed with standard laboratory diet	Treated group of animals fed with a dose of 0.05 g/kg of the bodyweight of animals mixed with diet	Treated group of animals fed with a dose of 0.3 g/kg of the bodyweight of animals mixed with diet	Treated group of animals fed with a dose of 2 g/kg of the bodyweight of animals mixed with diet

Mortality Rate and Physical Observation

Any negative effects or odd symptoms, such as tremors, salivation, diarrhea, seizures, allergic reactions, or behavioral changes, were monitored in all the animals for 14 days. Additionally, if any animal died in any of the groups of model animals throughout the experimental work of 14 days was noted.

Evaluation of Bodyweight, Food and Water Intake

Any change in the weights of both treated and control groups of model animals (rabbits) and the consumption of food by these model animals (rabbits) was used as an indicator for measuring any harsh effects of the copolymeric hydrogel on their physical state. Hence, the weight, food, and water intake of both treated and control groups of model animals (rabbits) were documented before and after the administration of copolymeric hydrogel on the 1^{st}, 2^{nd}, 3^{rd}, 7^{th}, and 14^{th} days.

Biochemical and Hematological Analysis

The blood samples of model animals (rabbits) from treated and control groups were collected after the completion of 14 days of study and transferred to the test tubes that were already coated by using ethylenediaminetetraacetic acid (EDTA). The collected blood samples from model animals (rabbits) were analyzed for white blood cell count, red blood cell count, hemoglobin content, platelet count, and average cell volume. Additionally, the serum samples were assessed for levels of different metabolic, kidney, and liver function indicators.

Organ Weight Measurement
The model animals (rabbits) were euthanized, and major internal organs, including heart, kidneys, stomach, liver, and intestine were excised from model animals of both the groups. A detailed macroscopic analysis was conducted to assess individual organs to check for any abnormalities or damage. The precise weight of the organs was noted, and this weight was evaluated against the model animal (rabbits) control group's weight.

Eye Irritation
Copolymeric hydrogel was applied to the right eyes of the treated animals (rabbits), and the untreated left eyes. The eyes of all of the model animals (rabbits) were monitored for any sign of lacrimation and redness for about 24h (Draize, 1944).

Acute Dermal Toxicity
Copolymeric hydrogel was examined for acute dermal toxicity assessment in albino rabbits. The fur/hair was removed from the dorsal side of model animals (rabbits), and a creamy layer of copolymeric hydrogel (550 mg) prepared in DW was spread on the surface of the clear, sanitized skin surface with the help of a sterile bandage for about 24 h. After removing the sterile bandage, the skin surface was inspected for inflammation, discomfort, and allergy (Saiyed et al., 2015).

Results and Discussion

Synthesis of Copolymeric Hydrogel and Toxicity Studies
The copolymeric hydrogel was synthesized by free radical polymerization reaction between *S. hispanica* seed mucilage and acrylamide and then its non-toxic nature was assessed by conducting acute oral, acute dermal toxicity, and eye irritation tests using rabbits as model animal.

Testing of Acute Oral Toxicity Studies
To evaluate the acute toxicity of copolymeric hydrogel, the tests were performed on Swiss albino rabbits. The animals were kept in clean, well-organized enclosures with a favorable environment including a 12 h photoperiod, 40% humidity, and a temperature of about 25°C. The experiment was conducted by following the rules of good laboratory practices (GLP) to make sure that the tests were accurate. A dose of copolymeric hydrogel was given to the rabbits of treated groups, A2 (0.05g), A3 (0.3g), and A4 (2g), per kg of their bodyweights, and the rabbits of group A1 were not treated with any dose and they worked as control group of model animals. All the animals were kept starving for 12 h before the administration of copolymeric hydrogel. The water and food were supplied uniformly to animals of each group after 1 h of copolymeric hydrogel administration, and they were regularly monitored for 14 days.

Mortality Rate and Physical Observation
After the administration of the dose of copolymeric hydrogel, no noticeable signs were observed on the skin, eyes, hair, nails, fur, or in the behavior of treated group animals. Any negative effects or odd symptoms, such as tremors, salivation, diarrhea, seizures, allergic reactions, or behavioral changes, were monitored in all animals and no indication of these abnormal activities was observed. All the observed animals remained healthy and energetic throughout the 14 day observation, and no mortality was recorded. Hence, the copolymeric hydrogel can be categorized as safe, non-irritant, and non-toxic for intended applications.

Evaluation of Bodyweight, Food, and Water Intake

The bodyweight, food, and water intake of both animals of all groups, i.e., treated and control rabbits, were documented after pre- and post-dose of copolymeric hydrogel on the 1st, 2nd, 3rd, 7th, and 14th day (Tables 2, 3, and 4). A modest drop in weight was noted in rabbits within the first 3 days of study due to the starvation of 1st day and copolymeric hydrogel administration. During the first week of study, the weight of rabbits was gradually increased, which indicated the normal growth and physiological function in animals. The difference in the weight of animals of both groups, treated and control, was not statistically significant during the whole study period. As results followed the protocols and regulations 402 and 420 in accordance with OECD and comparable with the animals of the control group.

Table 2. Bodyweight (g) of treated and control group of rabbits (mean ± SD)

Parameters	Group "A1"	Group "A2"	Group "A3"	Group "A4"
Pretreatment	1704.55 ± 24.93	1732.26 ± 22.51	1746.75 ± 25.88	1775.64 ± 23.49
Day 1	1702.23 ± 25.73	1729.53 ± 28.03	1743.15 ± 23.33	1773.56 ± 20.96
Day 2	1699.67 ± 31.09	1727.18 ± 29.56	1741.42 ± 27.05	1771.42 ± 24.56
Day 3	1702.11 ± 25.96	1731.62 ± 20.66	1744.57 ± 27.30	1775.44 ± 28.02
Day 7	1713.18 ± 26.53	1741.30 ± 28.86	1756.73 ± 23.86	1784.91 ± 23.77
Day 14	1734.28 ± 23.79	1761.31 ± 24.84	1775.24 ± 22.76	1805.39± 28.93

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

Parameters	Group "A1"	Group "A2"	Group "A3"	Group "A4"
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

Table 4. Mean values of water consumption (mL) of control and treated groups of rabbits

Parameters	Group "A1"	Group "A2"	Group "A3"	Group "A4"
Pretreatment	22.28 ± 1.82	22.18 ± 1.59	21.13 ± 1.10	22.76 ± 1.50
Day 1	21.73 ± 1.20	22.70 ± 1.20	20.79 ± 1.18	22.68 ± 1.21
Day 2	21.31 ± 1.68	23.44 ± 1.56	21.48 ± 1.82	23.19 ± 1.65
Day 3	22.29 ± 1.07	23.81 ± 1.14	22.87 ± 1.34	23.01 ± 1.28
Day 7	23.10 ± 1.50	22.43 ± 1.38	21.14 ± 1.45	22.63 ± 1.90
Day 14	22.78 ± 1.26	23.02 ± 1.53	22.52 ± 1.75	23.71 ± 1.19

Hematology and Biochemical Analysis

The bone marrow generates blood cells, and any compound influencing its function causes variations in CBC (complete blood count). Among the biochemical tests, serum enzyme biomarkers like alanine aminotransferase, alkaline phosphatase, and total bilirubin are commonly assessed; any fluctuations in their levels lead to hepatotoxic liver damage. Similarly, to check renal status, levels of urea and creatinine in blood are indicated (Rivadeneyra-Domínguez et al., 2018). The hematology and serum biochemistry analysis were performed to evaluate the toxicity of copolymeric hydrogel to the major internal organs of rabbits. The blood samples of rabbits from

treated and control groups were collected for CBC and serum biochemistry analysis on the completion of 14 days of study. The blood sample was collected and transferred to the test tubes that were already coated by using ethylenediaminetetraacetic acid (EDTA). The collected blood samples from rabbits were analyzed for white blood cell count, red blood cell count, hemoglobin content, platelet count, and average cell volume. Additionally, the serum samples were assessed for levels of different metabolic, kidney, and liver function indicators. Hematological results in both treated and control groups of rabbits were comparable and remained within the normal limits, supporting the safety profile of the copolymeric hydrogel. The values of hemoglobin, serum biochemistry, renal profile, liver profile, and lipid profile provided evidence that copolymeric hydrogel does not significantly affect blood cells, kidney, and liver. These studies demonstrated that copolymeric hydrogel is a biocompatible and non-toxic material, which makes it appropriate for oral use (Tables 5 and 6).

Table 5. Hematological parameters of rabbits

Parameters	Group "A1"	Group "A2"	Group "A3"	Group "A4"
TLC (μL^{-1})	4.6	3.8	4	4.2
RBC (μL^{-1})	4	4.5	4.3	4.1
Hb (g/dL)	12.8	13	13.2	13.4
HCT (PCV) (%)	40.1	42.7	40.5	45.6
MCV (fL)	62.3	65.6	69.9	71.2
MCH (pg)	19	17.8	20.6	21.9
MCHC (g/dL)	30.7	31.9	32	30.5
Platelet count (μL^{1})	246.3	256.1	272	261.3
Neutrophils (%)	52.1	47.4	50.3	45.2
Lymphocytes (%)	24.6	22.5	23.7	22.9
Monocytes (%)	2.9	3.2	3	3.3
Eosinophils (%)	2.4	1.9	2	2.2

Table 6. Clinical biochemistry of rabbits

Parameters	Group "A1"	Group "A2"	Group "A3"	Group "A4"
Lipid Profile				
Cholesterol (mg/dL)	79.1	72.5	73.7	70.9
Triglyceride (mg/dL)	81.3	78.2	83.6	85.6
HDL (mg/dL)	42.5	39	40.9	44.1
LDL (mg/dL)	27.6	32.7	35	30.5
VLDL (mg/dL)	15.2	13.6	17.1	12
Liver function test				
Bilirubin (mg/dL)	0.7	0.5	0.7	0.6
SGPT (ALT) (U/L)	45	50.9	62.3	63.2
SGOT (AST) (U/L)	40.8	41.7	43.5	42.9
ALP (U/L)	91.4	67.1	60.2	74.7
Total protein (g/dL)	6.1	6.9	7.4	6.5
Albumin (g/dL)	3.2	2.8	2.5	3.4
Globulin (g/dL)	2.7	2.5	2.6	2.8
A/G Ratio	1.56	1.52	1.35	1.57

Renal function test				
Urea (mg/dL)	13.4	15.1	15.9	11.6
Creatinine (mg/dL)	0.9	0.6	0.6	0.8
Hematology				
ESR (mm/h)	2.1	1.8	2.2	1.5
Serum electrolyte				
Potassium (mmol/L)	4.3	4	3.7	4.9
Sodium (mmol/L)	120.5	129.3	135.1	132.4
Uric acid (mg/dL)	3.9	3.7	4	4.5

Absolute Organ Bodyweight

The weight of major internal organs was assessed and analyzed for both the treated and control groups of rabbits. It was indicated from the results that no significant difference was found in the weights of both animal groups treated and control (Table 7).

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

Parameters	**Group "A1"**	**Group "A2"**	**Group "A3"**	**Group "A4"**
Heart	4.830 ± 0.02	4.674 ± 0.02	4.626 ± 0.01	4.460 ± 0.01
Kidney	18.127 ± 0.01	18.432 ± 0.01	17.907 ± 0.02	18.178 ± 0.02
Stomach	18.230 ± 0.01	18.273 ± 0.06	17.631 ± 0.03	17.895 ± 0.03
Intestine	47.405 ± 0.07	46.632 ± 0.07	46.274 ± 0.07	46.254 ± 0.08
Liver	53.241 ± 0.07	53.628 ± 0.03	55.124 ± 0.06	54.127 ± 0.02

Eye Irritation and Dermal Toxicity

Evaluating the toxicological impacts of a copolymeric hydrogel carrier for used for dermal, inhaled, or orally administered is obligatory. Therefore, to check the harms of copolymeric hydrogel, ocular tests were conducted by applying copolymeric hydrogel in rabbit's right eyes. The eyes of all treated rabbits were monitored for any sign of lacrimation and redness for about 24 h. All the tested rabbits were found free from any signs of irritation, inflammation, or conjunctivitis, so they were graded as having no sign of irritation and other adverse symptoms observed during the experiment, and assigned the score of zero according to the Draize scale of ocular irritation (Draize, 1944).

Acute Dermal Toxicity Studies

The copolymeric hydrogel was examined for acute dermal toxicity assessment in albino rabbits. After removing the sterile bandage, the skin surface was inspected for inflammation, discomfort, and allergy. The acute dermal toxicity evaluation confirmed the non-irritating nature of the copolymeric hydrogel as no symptoms like allergy, lesion, abrasion, erythema, or infection were observed.

Conclusion

The toxicological evaluations of copolymeric hydrogel were conducted with the protocol and regulations (420 and 402) of OECD in model animals (rabbits) that indicated the stability of hematological profile, biochemical assessment, and histological parameters. The subsequent ocular and dermal assessments reaffirmed the non-irritant nature and safety profile of copolymeric hydrogel. The overall experimental results suggested that the synthesized copolymeric hydrogel is

a non-toxic, safe, and non-irritating material and is suitable to as a carrier in oral drug delivery systems and for the development of dressing of wounds. However, there is still a need for further toxicological evaluation, like chronic toxicity studies, cytotoxicity, and mutagenic testing to broaden its spectrum of applications.

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Availability of Data and Material
Corresponding authors will provide data on request.

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