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ACUTE ORAL TOXICITY EVALUATION OF A CITRIC ACID-MEDIATED HYDROGEL ON RAT AS MODEL ANIMAL

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Abstract

APIs plays a pivotal role in pharmaceutical development and must undergo comprehensive toxicological evaluation to establish their safety profile. In this study acute oral toxicity assessment of citric acid cross-linked hydrogel composed of chia seed mucilage were carried out in compliance with OECD guidelines 402 and 420, respectively. Rats were categorized into four groups, with one untreated control group, while the remaining groups received a single dose of composite hydrogel. Over 14 days of observation period, all animals survived without exhibiting any significant toxic effects. Hematological, biochemical and organs weight assessments confirmed the non-toxic nature of the hydrogel, supporting its potential use as a safe excipient in drug delivery system.

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INTRODUCTION

Because of their high swelling, prolonged drug released capabilities, non-toxicity, biodegradability, and biocompatibility natural polysaccharides are chosen for the creation of DDSs [1-4]. Furthermore, chemical changes such as polymerization, acetylation, and crosslinking can enhance the characteristics of natural polysaccharides and make them appropriate materials for cutting edge applications. These changes make it possible to create smart materials that react to different stimuli, like temperature, salt, ethanol and pH [4-9]. Crosslinking using acidic and hydrophilic crosslinking agent leads to the formation of highly swellable material suitable materials for sustained release applications [10-16]. In order to verify their toxicity and biocompatibility, recent studies have evaluated the potential of naturally occurring polysaccharide and their modified form as safe DDSs [17-26].

Chia (Salvia hispanica) seed mucilage has been shown to be stimuli-responsive in previous research [3]. Here, our goal was to investigate the safety evaluations of a crosslinked hydrogel made of citric acid and the mucilage from chia seeds. In order to pave the road for its possible biomedical applications, the study focuses on identifying the composite hydrogels acute toxicity and irritant characteristics. The OECD guidelines 420 and 402 shall be adhered to in [27, 28]. Studying the different hematological and biochemical markers is the goal. Acute dermal toxicity investigations and the ocular irritation test will also be carried out in order to identify any skin and eye discomfort.

2. Methodology

2.1. Materials

Before being used chia seeds were washed and obtained from the Sargodha region's public market in Pakistan. During this study, reagents and solvents used were of analytical

grade. To make the solution or dispersion was made with distilled water (DW).

2.2. Mucilage Extraction and Hydrogel Synthesis

The mucilage from chia seeds was extracted using the previously described method [3], and it was then transformed into cross-linked hydrogel by treating it with citric acid in accordance with the method described by [9].

2.3. Testing for Acute Toxicity

The cross-linked hydrogel was tested for acute oral toxicity in accordance with OECD guidelines and the Economic Co-operation and Development (OECD), procedure [23-28]. In compliance with the Good Laboratory Practices (GLP), the testing was performed precisely. The Superior University Sargodha, Pakistan, Animals Ethics Committee gave its approval to the study. Group animals animal models in groups B through D received a single dosage of the composite hydrogel (0.05, 0.3, and 2 g/kg of bodyweight), whereas group A was left untreated and designated as the control group. All the animals were denied food for 12 h before to hydrogel dose. All group's animals received food and water during the 14 days of routine observation.

Table 1: Group names and dose scheme for the acute oral toxicity studies of cross-linked hydrogel in rats

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	Treated group of animals given with a dose of 0.3 g/kg of the bodyweight of	Treated group of animals given with a dose of 2 g/kg of the bodyweight of

	animals mixed with diet	animals mixed with diet	animals mixed with diet

2.4. Death and Physical Observation

Animals were observed for 14-days to record any death following composite hydrogel dose and to watch for any negative responses, such as was conducted to monitor animals' salivation, diarrhea, tremors, allergic symptoms, seizures, and behavioral abnormalities. In addition to, any animal deaths that happened throughout the research time period were documented for each group.

2.5. Evaluation of Water Intake, Food and Body Weight

General health of the animals and the toxicity of the composite hydrogel were evaluated by assessing changes in the body weight and monitoring food and water intake in both control and experimental groups. Thus, before and after the composite hydrogel was administrated for the first three days, and then on days seven and fourteen, the body size, hydration consumption, and food intake of the laboratory animals in both the control the body size, fluid, and food intake of laboratory animals in both control and experimental groups were noted.

2.6. Biochemical Testing and Blood analysis

Blood samples were analyzed under several hematological parameters like TLC, RBCs, Hb, MCV, thrombocyte count, average hemoglobin per red blood cell and biochemical indicators were analyzed in blood samples as part of the study. On the fifteenth day of the trial, blood samples from the control and treatment groups of animals were collected. Chloroform was used to produce anesthesia in the animals. The animals' cervical arteries were used to draw blood, which was then put into tubes coated with

ethylenediaminetetraacetic acid (EDTA). Blood samples, erythrocytes, hemoglobin, mean cell volume (MCV), platelet count, total white blood cell count and average hemoglobin content were assessed. The blood's components, however, include urea, blood cholesterol levels, uric acid levels, blood creatinine, triglycerides, alanine transaminase, and aspartate transaminase.

3. Results and Discussions

3.1. Assessment of Physical Condition and Death Rates

No behavioral alterations or neurological or gastrointestinal problems such as, seizures, regurgitation, hypersalivation or other negative responses, were seen in the animals in the treated groups. After consuming composite hydrogel, none of the animals had a discernible negative effects. According to the study, there were no deaths at dosage up to 2 g/kg of bodyweight. Chemicals having LD50 values greater than 2 g/kg are classified as category 5 under the globally harmonized system (GHS) criteria. As a result, the composite hydrogel falls within substance category 5. Furthermore, according to the classification, labelling, and packaging (CLP) standards, composite hydrogel is categorized as non-toxic substance [29, 30].

3.2. Evaluation of Dietary Consumption and Body Weight

Food and water intake of all animals were recorded following composite hydrogel administration (Tables 2-4). A temporary reduction in body weight, often associated with toxic response, was noted during the first three days. which was recovered very quickly. This initial and temporary weight loss was due to low food intake on the first day. The rats gained weight throughout the first week, indicating normal development and physiological activities. Overall, the difference between the treated samples and comparison control weights was statistically negligible throughout the whole

investigation. 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 rats gained their weight. 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 [31, 32].

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

Parameters	Group "A"	Group B"	Group "C"	Group "D"
Pretreatment	161.48±4.43	203.59±3.88	185.72±6.05	217.91±5.20
Day 1	158.32±2.67	201.15±4.38	181.77±5.70	215.28±3.55
Day 2	163.73±4.58	205.54±3.29	186.78±3.09	221.64±2.78
Day 3	167.94±5.89	209.79±4.71	191.74±4.48	226.01±5.71
Day 7	181.39±4.64	225.19±5.97	207.28±5.01	239.41±2.42
Day 14	206.38±3.48	249.95±4.81	232.46±3.06	261.95±4.70

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

Parameters	Group "A"	Group "B"	Group "C"	Group "D"
Pretreatment	6.18±1.56	5.71±1.84	7.50±1.97	6.53±1.46
Day 1	6.54±1.47	6.12±1.33	7.63±2.20	5.66±1.68
Day 2	7.61±1.71	6.41±1.93	6.58±2.29	5.83±1.79

Day 3	6.56±2.28	7.65±2.32	7.89±1.98	6.71±1.84
Day 7	5.82±1.97	7.19±1.59	8.27±2.56	7.40±2.29
Day 14	6.63±1.35	6.19±1.18	8.16±2.04	6.34±1.60

3.3. Analysis of Hematology and Biochemistry

The complete blood count (CBC) can be altered by chemicals that impact the bone marrow, which creates the blood cells. Other important markers of animal include serum enzymes such as ALT, ALP and total bilirubin. The alteration in these enzymes' biomarker levels demonstrated the hepatotoxic liver injury. Accordingly, blood urea and creatinine levels can be used to evaluate renal function [29-32]. Therefore, animal hematology and biochemistry studies were conducted to ascertain the harmful effect of composite hydrogel on these essential organs. The results of the biochemical and hematological analyses are given in Tables 4 and 5. The fact that there no changes in the levels of liver enzymes, urea, or creatinine indicates that composite hydrogel has no discernible effect on the liver, kidney, or blood cells. Since all parameters were normal or close to those of the control group, the composite hydrogel is considered safe and non-toxic. Changes in electrolyte concentrations can led to serious health problems, especially cardiovascular problems. Electrolyte levels did not significantly differ from the control. Lipid profile levels were also found to be within normal limits. Composite hydrogel has been found to be a safe substance for oral intake.

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Table 4: Hematological parameters of rats

Parameters	Group "A"	Group "B"	Group "C"	Group "D"
TLC (μL^{-1})	8.0	9.6	8.7	9.1
RBC (μL^{-1})	4.2	4.6	5.0	4.3
Hb (g/dL)	12.5	15.2	13.6	12.8
HCT (PCV) (%)	46.3	49.1	44.9	51.0
MCV (fL)	78.5	82.7	90.6	85.2
MCH (pg)	29.5	26.2	30.1	31.0
MCHC (g/dL)	30.9	31.5	31.7	30.2
Platelet count (μL^{-1})	380.4	367.1	371.2	298.0
Neutrophils (%)	47.5	54.6	44.3	49.7
Lymphocytes (%)	27.1	25.4	29.1	22.5
Monocytes (%)	6.9	7.6	8.3	7.8
Eosinophils (%)	3.8	4.5	3.5	4.3

Table 5: Clinical biochemistry parameters of rats

Parameters	Group "A"	Group "B"	Group "C"	Group "D"
Lipid Profile				
Cholesterol (mg/dL)	116.9	105.1	113.4	121.0
Triglyceride (mg/dL)	74.2	78.9	70.1	72.3
HDL (mg/dL)	41.7	39.1	40.6	41.3
LDL (mg/dL)	85.2	90.5	87.4	88.9

Liver function test				
Bilirubin (mg/dL)	0.5	0.3	0.4	0.2
SGPT (ALT) (U/L)	10.4	10.7	11.3	10.9
SGOT (AST) (U/L)	11.7	10.2	10.9	11.1
ALP (U/L)	72.3	71.8	81.2	83.5
Total protein (g/dL)	6.8	6.1	7.0	6.2
Albumin (g/dL)	3.4	3.0	3.2	3.5
Globulin (g/dL)	2.5	2.3	2.4	2.6
A/G Ratio	1.36	1.30	1.33	1.35
Renal function test				
Urea (mg/dL)	14.1	17.6	16.9	12.7
Creatinine (mg/dL)	0.6	0.5	0.7	0.6
Hematology				
ESR (mm/h)	2.0	2.4	1.6	1.9
Uric acid (mg/dL)	3.7	3.9	4.0	3.4

3.4. Total Weight of Organs

Table 6 shows, comparison of absolute organ weight with no significant changes upon comparison with body organ. The comparison we

made between the experimental and control groups of body weight of major organs.

Table 6: Absolute organ weight (g) of control and treated group of rats(mean ± SD)

Parameters	Group "A"	Group "B"	Group "C"	Group "D"
Heart	.354±.01	.292±.01	.313±.01	.282±.01
Kidney	.748±.01	.703±.02	.641±.01	.628±.02
Stomach	2.143±.06	1.648±.02	1.875±.03	1.608±.03
Pancreas	.260±.01	.225±.01	.223±.01	.216±.01
Lungs	.549±.02	.422±.02	.504±.01	.405±.01
Intestine	6.729±.05	5.514±.17	5.979±.21	5.278±.06
Liver	4.783±.06	3.904±.08	3.918±.06	3.591±.04

4. Conclusions

In rabbits the composite hydrogel toxicity tests revealed no negative side effects. It is considered as a safe substance into contact with the skin and eyes. As a result, the research showed that composite hydrogel would be a good option for oral medication administration. For the investigation of its overall safety behavior, additional toxicological evaluations, including mutagenicity, cytotoxicity and chronic toxicity, must be performed.

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