ACUTE TOXICITY STUDIES OF METHACRYLIC ACID BASED COMPOSITE HYDROGEL OF *SALVIA SPINOSA* SEED MUCILAGE: A POTENTIAL NON-TOXIC CANDIDATE FOR DRUG DELIVERY

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Safety evaluation of a newly designed polymeric drug delivery system (DDS), with/without the addition of active pharmaceutical ingredients (APIs), is now mandatory for their regulatory approval for human use. Hence, *Salvia spinosa* seed mucilage/hydrogel (SSH) was treated with methacrylic acid (MAA) to synthesize a composite hydrogel (SSH-*co*-MAA). Acute oral and acute dermal toxicity studies of the SSH-*co*-MAA for API delivery were ascertained following OECD guidelines 420 and 402, respectively. Moreover, an ocular toxicity study was also performed and analyzed through Draize scale. Animals of two species, rodent (rat) and non-rodent (rabbit), were divided into four groups. Group A of both rats and rabbits was assigned as control and remained untreated. Meanwhile, groups B, C, and D were labelled as treated groups and received a single dose of SSH-*co*-MAA, *i.e*., 0.05, 0.3 and 2 g/kg body weight of the animal. During 14 days after the treatment, animal monitoring was done for behavioral changes, food and water intake, adverse effects, and mortality. All animals remained alive, with no statistically significant abnormality. Hematological and biochemical parameters of control and treated animals were analyzed after the completion of 14 days and found in harmonization. The vital organs of animal models were removed to determine absolute organ weights. Histopathology of the vital organs of animal models revealed normal cellular architecture, without any lesions. SSH-*co*-MAA was also free from dermal and ocular toxicity. The overall results of acute oral and dermal toxicity studies prove that SSH-*co*-MAA is safe, especially after oral administration. Hence, SSH-*co*-MAA can be used as a non-toxic excipient for drug delivery systems.

Keywords: polysaccharide, *Salvia spinosa* mucilage, copolymerization, acute dermal toxicity, hematology, histopathology

INTRODUCTION

Toxicity testing is mandatory in the screening of not only active pharmaceutical ingredients (APIs), but also excipients and drug delivery systems (DDSs) before human use. In the past, the safety evaluation of excipients or DDSs was not given so much importance, as they were considered inert and pharmacologically inactive.[1] In last few years, several toxicological reactions have been reported due to excipients, including renal toxicity from intravascular administration of β-cyclodextrin, dermatitis caused by propylene glycol, diarrhea associated with mannitol, and indigestion caused by lactose.[2] To overcome such toxic effects of excipients and the DDS prepared from these excipients, it is recommended to evaluate any new excipient and relevant DDS through toxicity studies. Such evaluation is now considered as an integral part of the research. The requirement of safety evaluation for APIs or DDSs depends upon the route of administration, duration of the treatment, dose level and frequency, clinical conditions, *etc*. Therefore, the toxicity testing may include acute and chronic toxicity, reproductive, mutagenicity, carcinogenicity, biocompatibility, skin sensitization, and eye irritation studies.[3]

Natural polysaccharides are polymers of choice for researchers in screening novel DDSs due to their high swelling and sustained drug release ability, non-toxic nature, biodegradability, and biocompatibility.[4,5] Moreover, the above-mentioned properties of these natural polysaccharides can be improved through chemical modifications, *i.e*., crosslinking, acetylation, copolymerization, *etc*.[6-8] Such modifications lead to the preparation of smart materials, which are pH, salt, ethanol and temperature responsive.[9] Among these modifications, graft copolymerization using methacrylic acid resulted in a highly swellable sustained-release material with sufficient mechanical strength.[10,11] For a drug labile to gastric fluid or irritating to gastric mucosa, a pH-responsive drug delivery system has been developed to target the delivery of the drug only in the intestinal tract, not in the stomach. Methacrylic acid (MAA) has been extensively used in the fabrication of pH-sensitive hydrogels. MAA is a pH-sensitive material and demonstrates extreme variations in swelling behavior when there is a change in pH and ionic strength. Its pH-sensitive behavior makes it a very significant material in temporal or spatial delivery. Recently, the swellable naturally occurring polysaccharides and their modified forms have been evaluated through toxicity studies to explore their potential as non-toxic DDSs.[12-15]

The genus *Salvia* is a member of the Lamiaceae family and contains more than 1000 species. *Salvia spinosa* has folklore claims for the treatment of diarrhea, urinary disorders, stomach pain, and piles.[16] The SSH is a polysaccharide, mainly composed of glucose, rhamnose, uronic acid, polyglucan *etc*., like other polysaccharides of similar origin. The SSH has been explored for its pharmaceutical applications due to its porous nature, high swelling, pH-responsive drug release, and swelling deswelling ability.[12] In a previous study, the methacrylic acid based composite hydrogel of mucilage of *S. spinosa* seeds has been investigated as pH-responsive material for sustained and targeted delivery of venlafaxine HCl,[9] and the present work is an extension of that study.[9]

Moreover, naturally occurring swellable materials have been shown to possess a relatively low mechanical strength, which limits their use in drug delivery applications. Therefore, to improve the mechanical strength of SSH, the graft copolymerization with methacrylic acid was performed in our previous study. SSH organizes in a rather intricate supramolecular structure formed by the intermolecular cohesion of polysaccharide molecules, which is an extended intra/intermolecular network of hydrogen bonds, making it easy to combine with other natural or synthetic monomers by reconstructing hydrogen bonds. SSH-*co*-MAA has demonstrated pH-responsive sustained and targeted drug delivery potential. However, as SSH-*co*-MAA is a chemically synthesized material and such materials can be toxic due to the presence of any potential intermediate compound, it is important to evaluate the toxicity of this new material before considering its administration to humans. Therefore, it is necessary to perform the toxicity study according to the standard guidelines. Such a study will be helpful for the establishment of SSH-*co*-MAA as a safe material for other biomedical applications. Therefore, in this work, we aim to investigate the acute oral toxicity, acute dermal toxicity, and eye irritation of the SSH-*co*-MAA. These tests will be helpful for potential use of SSH-*co*-MAA in oral sustained release DDSs, dermal and transdermal semisolid dosage forms, and as soothing and lubricating agent for dry eyes, respectively. For this purpose, OECD guidelines 420 and 402 will be used for acute oral and acute dermal toxicity studies, respectively.[17,18] Moreover, the effect of SSH-*co*-MAA on the biochemical and hematological parameters of rats and rabbits will be assessed. Histopathological studies of the vital organs will also be carried out to assess any adverse effect on the tissue structure.

EXPERIMENTAL

Materials

S. spinosa seeds were purchased from a local market of District Sargodha, Pakistan. *N*-methylene bisacrylamide (MBA), methacrylic acid (MAA), potassium persulfate (KPS) (Sigma-Aldrich, Germany), and *n*-hexane and ethanol (Riedel-de Haen, Germany) were used during this research work. All reagents were of analytical grade and used as such, without any further purification. Distilled water (DW) was used as such or to prepare solutions/dispersions.

Methods

Formulation of SSH-co-MAA

S. spinosa mucilage was extracted from its seeds using a hot water extraction method.[6] The methacrylic acid-based composite hydrogel of *S. spinosa* mucilage (SSH-*co*-MAA) was prepared by a previously reported method.[9] Briefly, SSH-*co*-MAA was synthesized

through the free radical copolymerization method. *S. spinosa* mucilage and MAA were used as polymers, MBA was used as a cross-linker, and KPS was used as the initiator.

Acute toxicity testing

Toxicity studies were carried out in compliance with the regulations of the Organization for Economic Co-operation and Development (OECD) 420.[17] Swiss albino rats and albino rabbits were used to evaluate the acute toxicity of SSH-*co*-MAA. Animals were received from the animal house of the University of Sargodha. All animals were retained in neat and clean cages and under controlled conditions of temperature, humidity, and light, *i.e.*, 25 °C, 40%, and 12 h photoperiod, respectively. Good laboratory practices (GLP) were strictly followed during the testing. The study protocol was approved by the Institutional Animal Ethics Committee of the University of Lahore, Pakistan, through letter no. IREC-2018-76-M on May 17, 2018.

A single dose of SSH-*co*-MAA (0.05, 0.3, and 2 g/kg of the body weight) was administered to the rats and rabbits of groups B, C, and D, respectively, whereas, animals of group A were left untreated and labeled as the control group. All animals were kept fasting for 12 h before SSH-*co*-MAA administration. After 1 h of the administration of SSH-*co*-MAA, food, and water were provided to the animals of all groups with regular monitoring for 14 days.

Physical observation and mortality

Monitoring of animals for any adverse effects or abnormal symptoms of salivation, diarrhea, tremor, allergic symptoms, seizure, and behavioral changes were monitored for the next 14 days. Moreover, the death of any animal, if happened, in all groups, was noted during the study period, *i.e.*, 14 days.

Estimation of body weight, food, and water consumption

The variation of the weights of control and treated animals, and of the food consumed by these animals was used as an indicator of the adverse effects of the SSH-*co*-MAA on the general health of the animals. Therefore, the documentation of body weight, water, and food consumed by rats and rabbits of both control and treated groups was accomplished before and after the administration of SSH-*co*-MAA for the first three consecutive days and then on days 7 and 14.

Hematology and clinical biochemical analysis

Blood samples of animals of both control and treated groups were collected on day 15 before the necropsy. Animals were anesthetized with chloroform and blood was collected by cardiac puncture from rats, while blood was drawn from the jugular artery of the rabbits and transferred in the tubes lined with ethylenediaminetetraacetic acid (EDTA). Blood samples were analyzed for total leucocyte count (TLC), red blood cells (RBCs), hemoglobin (Hb), mean corpuscular volume (MCV), platelet count, and mean corpuscular hemoglobin (MCH). Blood serum was analyzed for urea, cholesterol, uric acid, creatinine, triglycerides, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) contents.

Absolute organ weight and gross necropsy

After taking the blood sample on day 15, the animals were sacrificed and vital organs, *i.e.*, liver, heart, intestine, kidneys, spleen, and lungs of the animals of both groups were removed. A macroscopic examination of the organs was carried out to examine each organ for lesions. The absolute organ weight was recorded and compared with the control group animals.

Histopathology evaluation

The vital organs of animal models were investigated for any possible tissue damage from SSH-*co*-MAA. Therefore, tissues (4-5 mm) of vital organs were sliced and stained with hematoxylin-eosin dye. The stained tissues were observed under a microscope to evaluate the cellular architecture.

Primary eye irritation

SSH-*co*-MAA was placed in the right eye of six rabbits. The left eyes of these rabbits were kept untreated and considered as a negative control. The eyes of all rabbits were observed for any lacrimation and redness for the next 24 h.[19]

Acute dermal toxicity

SSH-*co*-MAA was tested for dermal toxicity in six white albino rabbits. The hair from the back of rabbits was shaved and a thick paste of SSH-*co*-MAA (500 mg) in DW was applied on the skin with gauze for 24 h. The gauze was removed and skin was observed for any irritation, redness, allergy or abnormality.

Statistical analysis

Mean values of all parameters were recorded and reported with the standard deviation (SD). A paired *t*-test was used to calculate the *p*-value. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The safety evaluation of a newly designed DDS is an essential step for the establishment of the safety profile and for human use. Therefore, SSH-*co*-MAA was evaluated through acute oral and dermal toxicity and eye irritation studies.

Assessment of physical conditions and mortality

Throughout the study period, no behavioral changes, vomiting, seizures, diarrhea, increased salivation, or allergic reactions appeared in any animal of the treated groups (Tables 1 and 2),

which indicated the absence of neurological or gastrointestinal abnormalities.[20] After oral administration of SSH-*co*-MAA to the rats and rabbits, all the animals were observed to be healthy and active. Also, not a single case of mortality was observed, even at the highest tested dose, *i.e*., 2 g/kg body weight. According to the globally harmonized system (GHS), any tested chemical having an LD_{50} value greater than 2 g/kg is classified as belonging to Category 5. Hence, SSH-*co*-MAA can be classified as a Category 5 material. Moreover, as per the specifications of the classification, labeling, and packaging (CLP) regulation (EC No 1272/2008), SSH-*co*-MAA is categorized as non-toxic.[21]

Assessment of body weight, food, and water consumption

Following the administration of SSH-co-MAA, the quantity of food and water consumed by all the animals of both species was assessed (Tables 1 and 2). A decrease in body weight is a very simple and sensitive indicator of toxicity. During the first three days, a slight decrease in the weights of rats was observed, which was soon recovered. The weight loss was possibly due to less food intake on day 1, following oral administration of SSH-*co*-MAA. The weight of rats and rabbits started increasing during the week, which indicated normal physiological function and growth in the animals. Moreover, the change in weight of the control and treated groups during the whole study period was considered to be statistically insignificant. There is no statistically significant variation in the food and water intake of treated and control groups, which indicates normal body functions, especially gastrointestinal tract function in treated animals. Such results are in accordance with the OECD guidelines 420, as the results are statistically insignificant and comparable with those of the control group animals. Rats of groups C and D consumed slightly less food on day 1 (Table 1), which might be caused by a feeling of fullness after taking a high dose of SSH-*co*-MAA. The food and water intake were normalized afterward on days 7 and 14.

Table 1
Assessment of clinical observations, body weight, and food and water consumption in rats

	Group A	Group B	Group C	Group D
Signs of illness				
Vomiting, diarrhea, seizure, increased salivation, allergic reactions	Nil	Nil	Nil	Nil
Body weight (g)				
Pretreatment	159.02 ± 3.09	169.89 ± 3.32	153.86 ± 3.77	166.19 ± 3.23
Day 1	159.23 ± 3.63	167.42 ± 2.14	151.89 ± 2.30	164.24 ± 2.41
Day 2	159.44 ± 2.30	167.55 ± 1.34	150.82 ± 1.52	164.49 ± 2.85
Day 3	160.86 ± 4.27	168.53 ± 3.70	152.86 ± 2.11	165.38 ± 2.59
Day 7	162.87 ± 2.60	169.36 ± 3.52	155.67 ± 2.35	167.45 ± 1.90
Day 14	165.76 ± 3.88	171.93 ± 2.10	157.95 ± 2.71	169.01 ± 8.04
Water consumption (mL/day/animal)				
Pretreatment	5.64 ± 1.56	6.23 ± 2.09	6.52 ± 1.94	6.42 ± 2.53
Day 1	6.23 ± 1.80	5.78 ± 1.39	6.03 ± 1.76	6.17 ± 1.51
Day 2	6.59 ± 1.44	5.93 ± 1.65	6.67 ± 1.85	6.53 ± 1.06
Day 3	5.76 ± 2.67	6.31 ± 2.06	6.45 ± 1.29	6.46 ± 1.96
Day 7	5.91 ± 1.76	6.16 ± 1.54	6.93 ± 1.92*	6.63 ± 1.82
Day 14	6.36 ± 2.12	6.43 ± 1.86	6.81 ± 1.63	6.68 ± 1.97
Food consumption (g/day/animal)				
Pretreatment	6.43 ± 1.50	6.21 ± 1.88	6.18 ± 2.17	6.53 ± 2.64
Day 1	6.54 ± 1.65	6.13 ± 1.76	6.04 ± 1.53	6.25 ± 1.76
Day 2	6.57 ± 1.46	6.68 ± 1.39	6.27 ± 1.66	6.19 ± 1.87
Day 3	6.02 ± 1.16	6.78 ± 2.13	6.36 ± 1.52	6.30 ± 2.27
Day 7	6.57 ± 1.51	6.93 ± 1.66	6.48 ± 2.19	6.66 ± 1.96
Day 14	6.07 ± 1.38	7.06 ± 2.17*	6.77 ± 1.87	6.64 ± 1.83*

All values are expressed as (mean ± SD), *$p < 0.05$ is a significant difference as compared to the control, n = 5

Hematology and biochemical analysis

Blood cells are synthesized in the bone marrow and any tested substance that adversely affects the bone marrow results in a change in CBC (complete blood count). Other important biochemical parameters to check the health status of animals include serum enzyme biomarkers (ALT, ALP, and total bilirubin). Alteration in these enzyme biomarkers levels point out liver injury due to hepatotoxicity. Similarly, blood urea and creatinine levels are used to check the renal status.[22]

Hence, hematology and serum biochemistry tests of the animals were conducted to ascertain the potential toxicity of SSH-*co*-MAA to these vital organs.

Table 2
Assessment of clinical observations, body weight, and food and water consumption in rabbits

	Group A	Group B	Group C	Group D
Signs of illness				
Vomiting, diarrhea, seizure, increased salivation, allergic reactions	Nil	Nil	Nil	Nil
Body weight (g)				
Pretreatment	1720.19 ± 10.21	1659.97 ± 8.12	1670.71 ± 6.19	1637.41 ± 7.23
Day 1	1719.23 ± 6.74	1650.75 ± 4.54	1662.46 ± 9.72	1626.23 ± 7.39
Day 2	1726.53 ± 6.65	1647.44 ± 4.05	1660.49 ± 4.79	1625.41 ± 5.81
Day 3	1728.91 ± 7.58	1656.13 ± 8.27	1665.55 ± 5.73	1633.26 ± 6.71
Day 7	1759.88 ± 8.04	1661.21 ± 6.70	1672.95 ± 4.96	1648.51 ± 21.25
Day 14	1764.88 ± 9.48	1672.36 ± 5.63	1677.21 ± 6.85	1660.74 ± 25.79
Water consumption (mL/day/animal)				
Pretreatment	21.50 ± 2.04	22.31 ± 3.85	21.14 ± 1.89	21.75 ± 1.60
Day 1	21.35 ± 1.90	22.52 ± 2.96	20.83 ± 1.17	20.45 ± 2.64
Day 2	23.48 ± 2.31	22.64 ± 2.45	21.69 ± 1.46	20.86 ± 1.58
Day 3	22.17 ± 3.19	23.42 ± 2.52	22.47 ± 2.89	22.16 ± 2.04
Day 7	23.66 ± 2.42	21.93 ± 1.78	21.32 ± 3.25	21.65 ± 1.31
Day 14	23.37 ± 3.16	22.71 ± 2.13	22.83 ± 2.54	20.48 ± 2.97
Food consumption (g/day/animal)				
Pretreatment	20.56 ± 1.79	22.25 ± 2.83	21.16 ± 2.95	21.89 ± 3.57
Day 1	21.69 ± 2.14	20.84 ± 3.79	20.45 ± 2.67	20.32 ± 2.29
Day 2	20.50 ± 2.66	21.55 ± 1.60	22.32 ± 2.18	21.24 ± 2.32
Day 3	22.39 ± 1.73	23.10 ± 2.67	21.27 ± 1.72	22.45 ± 2.99
Day 7	23.21 ± 3.25	22.14 ± 2.73	22.38 ± 1.69	21.59 ± 1.06
Day 14	22.37 ± 1.18	23.67 ± 2.46	22.50 ± 2.91	20.89 ± 2.71

All values are expressed as (mean ± SD), n =5

Table 3
Hematological parameters of rats

Parameters	Group A	Group B	Group C	Group D
TLC ($\times 10^3$ μL^{-1})	5.1	6.3	6.8	4.9
Neutrophils (%)	47.3	49.8	51.2	53.7
Lymphocytes (%)	21.4	31.5	25.9	24.3
Monocytes (%)	3.1	2.6	2.0	3.8
Eosinophils (%)	2.0	3.7	3.5	2.2
RBC ($\times 10^6$ μL^{-1})	4.9	4.5	4.1	5.2
Hb (g/dL)	13.7	13.3	14.0	12.6
HCT (PCV) (%)	42.5	40.6	43.2	41.3
MCV (fL)	89.3	80.9	92.6	77.1
MCH (pg)	29.0	27.4	23..3	28.5
MCHC (g/dL)	32.6	30.9	31.8	34.0
ESR (mm/h)	3.9	4.1	4.5	5.4
Platelet count ($\times 10^3$ μL^{-1})	171.2	224.0	159.1	168.4

Table 4
Hematological parameters of rabbits

Parameters	Group A	Group B	Group C	Group D
TLC ($\times 10^3$ μL^{-1})	5.6	6.1	4.3	4.7
Neutrophils (%)	46.4	51.6	49.0	53.9
Lymphocytes (%)	29.0	22.8	20.6	24.1
Monocytes (%)	4.5	5.3	3.2	2.2
Eosinophils (%)	2.0	3.7	2.1	1.3
RBC ($\times 10^6$ μL^{-1})	4.5	4.8	5.0	5.2
Hb (g/dL)	13.7	12.9	12.6	13.4
HCT (PCV) (%)	42.3	47.1	41.9	43.5
MCV (fL)	78.0	82.5	80.0	76.3
MCH (pg)	21.6	30.7	27.1	29.0
MCHC (g/dL)	32.1	29.0	31.4	30.5
ESR (mm/h)	3.1	7.2	9.0	6.5
Platelet count ($\times 10^3$ μL^{-1})	175.3	156.1	204.6	193.7

Table 5
Clinical biochemistry parameters of rats

Parameters	Group A	Group B	Group C	Group D
Lipid profile				
Cholesterol (mg/dL)	126.0	121.7	104.5	119.6
Triglyceride (mg/dL)	97.5	77.3	71.9	103.0
HDL (mg/dL)	40.1	43.0	49.4	39.2
LDL (mg/dL)	92.0	87.1	111.3	81.9
Liver function test				
Bilirubin (mg/dL)	0.2	0.9	0.7	0.4
SGPT (ALT) (U/L)	11.8	25.3	24.9	21.6
SGOT (AST) (U/L)	13.7	19.1	10.8	11.2
ALP (U/L)	21.5	23.6	28.4	31.7
Total protein (g/dL)	6.6	7.0	7.2	7.5
Albumin (g/dL)	3.9	3.5	4.3	3.6
Globulin (g/dL)	2.8	3.0	2.4	2.9
A/G Ratio	1.39	1.17	1.79	1.24
Renal function test				
Urea (mg/dL)	25.6	17.9	28.7	21.3
Creatinine (mg/dL)	0.9	1.0	0.6	0.7
Uric acid (mg/ dL)	3.87	4.02	3.99	4.77
Serum electrolyte				
Potassium (mmol/L)	4.3	4.0	3.9	3.5
Sodium (mmol/L)	140.2	137.1	135.8	142.3

Table 6
Clinical biochemistry parameters of rabbits

Parameters	Group A	Group B	Group C	Group D
Lipid profile				
Cholesterol (mg/dL)	115.3	162.1	112.2	110.7
Triglyceride (mg/dL)	73.6	95.5	104.1	75.9
HDL (mg/dL)	49.1	55.8	41.2	40.0
LDL (mg/dL)	86.7	94.2	101.0	109.8
Liver function test				
Bilirubin (mg/dL)	0.7	1.1	0.6	0.4
SGPT (ALT) (IU/L)	18.5	24.7	29.1	21.0
SGOT (AST) (IU/L)	27.9	19.6	13.7	15.8
ALP (IU/L)	22.8	25.1	30.7	26.3
Total protein (g/dL)	7.0	6.5	6.8	7.1

Albumin (g/dL)	3.6	4.0	3.9	4.3
Globulin (g/dL)	2.8	3.0	3.2	3.4
A/G Ratio	1.29	1.33	1.22	1.26
Renal function test				
Urea (mg/dL)	11.7	17.4	19.1	23.6
Creatinine (mg/dL)	0.6	0.1	0.8	0.9
Uric acid (mg/ dL)	6.01	5.23	5.55	6.34
Serum electrolytes				
Potassium (mmol/L)	4.0	5.1	4.4	4.3
Sodium (mmol/L)	141	139.2	148.3	137.0

The results of hematology and biochemical analysis are presented in Tables 3-6. There was no change observed in enzyme levels, urea, and creatinine levels, providing the evidence that SSH-*co*-MAA does not significantly affect the blood cells, liver, and kidney. As the values of all tested parameters were within normal ranges or comparable with the control, the SSH-*co*-MAA can be considered a non-toxic material.

Fluctuation in the serum electrolyte concentrations may result in serious health issues, especially, cardiovascular emergencies.[23] No change in electrolyte levels was observed when compared with the control (Tables 5 and 6). The lipid profile was also observed to be in the acceptable range. According to the OECD guidelines, the hematological and biochemical parameters of the sample group animals should be comparable with those of the control group animals, and there should not be any statistically significant difference among the values of these essential parameters. These studies suggested that the SSH-*co*-MAA is safe to use for oral administration.

Absolute organ body weight

The absolute organ body weight of the animal is one of the parameters for the assessment of acute oral toxicity, as devised by OECD guidelines. Any change (increase or decrease) in the weight of vital organs may be associated with the abnormality of the cells/tissues of those organs. Such abnormalities can be linked to the toxicity of the sample material given to the animals. The weights of the vital body organs of both treated and control group animals were noted and compared. The results are shown in Tables 7 and 8. As noted from the results, there is no significant variation between the absolute organ weights of the treated and control group animals.

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

Organs	Group A	Group B	Group C	Group D
Heart	0.249 ± 0.02	0.220 ± 0.02	0.255 ± 0.01	0.235 ± 0.01
Kidney	0.416 ± 0.02	0.409 ± 0.02	0.448 ± 0.01	0.424 ± 0.02
Stomach	1.208 ± 0.06	1.110 ± 0.04	1.154 ± 0.02	1.060 ± 0.05
Intestine	6.001 ± 0.13	5.898 ± 0.04	5.825 ± 0.13	6.042 ± 0.17
Liver	3.401 ± 0.10	3.452 ± 0.02	3.767 ± 0.07	3.472 ± 0.08

Table 8
Absolute organ weight (g) of control and treated group of rabbits (mean ± SD)

Organs	Group A	Group B	Group C	Group D
Heart	0.398 ± 0.01	0.353 ± 0.01	0.365 ± 0.01	0.407 ± 0.02
Kidney	0.785 ± 0.01	0.746 ± 0.03	0.788 ± 0.01	0.792 ± 0.02
Stomach	2.414 ± 0.05	2.248 ± 0.02	2.580 ± 0.06	2.385 ± 0.03
Intestine	7.577 ± 0.22	7.255 ± 0.05	7.156 ± 0.12	7.791 ± 0.13
Liver	5.145 ± 0.07	4.998 ± 0.03	5.631 ± 0.09	5.275 ± 0.04

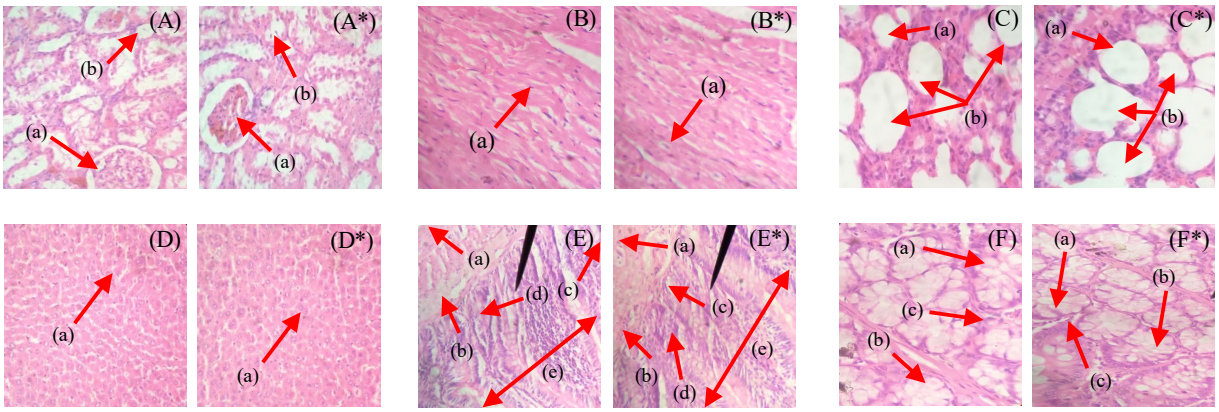

Figure 1: Histopathology of kidney (A, A*), glomerulus (a), and renal tubules (b); heart (B, B*), cardiac muscle fibers (a); lungs (C, C*): alveolus (a), and alveoli (b); liver (D, D*), plates of hepatocytes (a); small intestine (E, E*): lamina propria (a), muscularis mucosae (b), acinous lumen (c), columnar epithelial cell with basal nuclei (d), and small intestinal villi (e); colon (F, F*), lumen of crypt (a), colon crypt (b), and lamina propria (c) before (upper case letters) and after (upper case letters with an asterisk) administration of SSH-*co*-MAA

Histopathology and gross necropsy

As an essential part of the acute oral toxicity study according to OECD 420 guidelines, the histopathology studies were performed. According to these guidelines, any abnormalities observed during microscopic examinations of the organs should be reported. However, the histopathology of vital organs, such as the lungs, liver, heart, kidneys, and intestine of rabbits showed no changes in the cellular structure after oral administration of SSH-*co*-MAA. There were no signs of inflammation, degeneration, and necrosis in the tissues of the vital organs. Therefore, according to OECD 420 guidelines, the absence of such abnormalities (inflammatory cell infiltration, steatosis, lesion, *etc*.) indicated that the SSH-*co*-MAA is a non-toxic material that can be used for designing oral DDSs (Fig. 1).

Ocular and dermal toxicity testing

For safety evaluation of any excipient used either in dosage forms, *i.e*., oral administration, inhalation, or for dermal application, it is necessary to determine its potential to damage skin and eyes.[24] Therefore, eye irritation studies were performed after dosing SSH-*co*-MAA in the eyes of rabbits. All the tested animals were free from any kind of inflammation, irritation, or conjunctivitis, therefore, the material was reached "0" score according to the Draize scale.[25]

During acute dermal toxicity studies of SSH-*co*-MAA, no symptoms, such as lesions, abrasion, allergy, erythema and infection, were observed. As per the guidelines of OECD 402, absence of such adverse symptoms to the tested material confirmed its non-irritating behavior. Thus, this study indicated the absence of any ocular and dermal toxicity of SSH-*co*-MAA.

CONCLUSION

The *in vivo* toxicity studies of SSH-*co*-MAA, following OECD 420 and 402 guidelines, on experimental animals, *i.e.*, rats and rabbits, showed no significant changes in various hematological, biochemical, and histological parameters. Ocular and dermal testing of SSH-*co*-MAA further confirmed its ocular and dermal safety. Hence, all these evaluations concluded that graft-copolymerized *S. spinosa* seed mucilage with MAA is non-toxic in nature, and can be suggested as a safe carrier for oral administration of drugs. However, further toxicological evaluation in terms of chronic toxicity, cytotoxicity, and mutagenic testing is still necessary.

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