ACUTE TOXICITY OF A POLYSACCHARIDE-BASED HYDROGEL FROM SEEDS OF *OCIMUM BASILICUM*

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The toxicity of a hydrogelable polysaccharide from the seeds of *Ocimum basilicum* (syn.: sweet basil) was evaluated by carrying out acute oral toxicity studies to prove it a safe excipient for oral drug delivery systems. Toxicity studies were conducted on white albino rats and white albino rabbits. The animals were divided into four groups (Groups A-D). The animals (rats and rabbits) of Group A were left untreated and marked as control, whereas Groups B-D were marked as treated groups. Basil seed hydrogel (BSH) was given to these treated groups (Groups B-D) in amounts of 50, 300 and 2000 mg/Kg body weight of the animals, respectively. The treated and control group animals were monitored to observe their general health, food and water consumption, body weight and behavioral pattern throughout the study period of 14 days. On day 15, blood samples of all the animals were collected for hematology and biochemical analyses. The gross necropsy of vital organs was carried out for histopathology. BSH was also evaluated for dermal and ocular irritation. No anomalies were noticed in the general health of the treated animals. The hematological and biochemical parameters of treated animals (rats and rabbits) were found to be comparable with those of the control group. The histopathology of vital organs unveiled normal cellular architecture without any lesions of the organs, reflecting the safety of BSH. The safety profiles of BSH for topical application onto skin and eyes were also established. The topical application of BSH to the skin of rabbits reflected no signs of irritation or allergy. Similarly, no signs of iritis were observed on instillation of BSH into the eyes of rabbits. LD_{50} for BSH for rats was estimated to be 9 g/Kg body weight, while that for rabbits was not calculated.

Keywords: sweet basil, histopathology, hematology, polysaccharide, glucomannan

INTRODUCTION

Relying on plants for the diagnosis, treatment and prevention of ailments is a very common practice in developed as well as in underdeveloped countries.[1,2] The flexibility in dosage form design, biocompatibility, biodegradability and wide availability are the major advantages offered by plant-derived materials.[3-5] Recognizing the widespread use of plants for medicinal purposes reveals the importance of the assessment of their safety profile in order to safeguard the public against their possible hazardous effects. For the appraisal of the safety profile of plant-based materials, acute toxicity studies are conducted on animals. Recently, the acute toxicity of natural materials, especially polysaccharides, isolated from ispaghula,[6] and seeds of *Linum usitatissimum*,[7] *Mimosa pudica*[8] and *Cydonia oblonga*[9] has been investigated.

Ocimum basilicum is commonly known as sweet basil in English, Babui tulsi in Hindi and jungle tulsi in Urdu. It is a popular ornamental and culinary herb, which is also used for various medicinal purposes.[10-12] The mucilage extruded from basil seeds (BS) is mainly composed of carbohydrates, *i.e*., D-glucose, D-mannose, D-rhamnose, D-mannose, hemicellulose and pectin, while minor components include minerals, fats and proteins.[13] BS mucilage isolated through cold water extraction, followed by precipitation with alcohol, is composed of two major fractions, *i.e.* the xylan fraction (24%) and the glucomannan fraction (43%). The xylan fraction possesses acidic side chains, while the glucomannan one contains glucose and mannose in a 10:2 ratio.[14]

The objective of this work has been to appraise the safety profile of hydrogelable mucilage/polysaccharide extracted from BS in order to establish it as a safe excipient for use in the

oral dosage form. The acute toxicity study of the BS hydrogel (BSH) will be performed in albino rats and rabbits. The toxicity effects of BSH will be observed through the oral, dermal and ocular routes of administration. Any harmful effect of BSH on vital organs will be assessed through the histopathology of these organs.

EXPERIMENTAL

Material

Ocimum basilicum seeds were procured from a local market from Sargodha, Pakistan. The taxonomic identification of *O. basilicum* seeds was done by a botanist from the Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan, and after sieving, they were stored in well-closed containers.

Isolation of basil seed hydrogel

The isolation of the basil seed hydrogel (BSH) was accomplished by the hot water extraction method.[15] Seeds (50 g) were soaked in deionized water for 4 h and kept in an oven at 40 °C to optimize the yield. The separation of the extruded hydrogel/mucilage was carried out by gently pressing the seeds in a nylon gauze. The extruded hydrogel was washed with *n*-hexane (200 mL) to defat. Purified BSH was dried in a vacuum oven at 60 °C for 48 h. The dried hydrogel was crushed, sieved through sieve no. 60 and stored in a vacuum desiccator for further use.

Animals

Swiss albino rats (150-175 g) and albino rabbits (1400-1600 g) were used to conducting acute toxicity studies of BSH. Animals (male and female) were acquired from the University of Sargodha, Sargodha, Pakistan. All the animals were examined for their general health before being taken to the laboratory. The animals (rats and rabbits) were divided into four groups (A-D) (n=5) and shifted to neat and clean cages, where they were fed with the standard pellet diet and were provided free access to tap water. All the animals were kept at 25 °C and 40-60% humidity, and were provided with 12 h photoperiod. Weight variations among the animals were within ±20% of the mean weight.[16] All the tests were performed in compliance with the Good Laboratory Practices (GLP) provided by the United States Food and Drug Administration (USFAD). All the procedures adopted in the toxicity studies were in accordance with the guidelines of the Organization for Economic Co-operation and Development (OECD).[17] The study protocols were approved by the Pharmacy Research Ethics Committee of the University of Lahore.

Acute oral toxicity studies

Acute oral toxicity of BSH was investigated in albino rats and rabbits as per guidelines of OECD 420.[17] All the animals (rats and rabbits) were kept on fasting for 12 h, prior to administration of BSH. A single-dose of BSH (50, 300 and 2000 mg/Kg of body weight of the animal) was mixed with food and administered orally to Group B, C and D of test animals, respectively, while Group A of the rats and rabbits was marked as control group (Table 1).

Free access to food and water was provided to the animals 3 h after the administration of BSH. The dose of BSH for toxicity testing was kept higher than that of the daily intake of the excipients.[18] After administration of BSH, close monitoring of all the animals was carried out for behavioral changes, allergic reactions, diarrhea, salivation and tremors for 8 h. All the animals were monitored for general health on a daily basis for a period of 14 days.

Evaluation of physical parameters

Physical parameters, such as behavioral pattern, salivation, tremors, diarrhea, lacrimation and skin rashes, of both albino rats and rabbits were observed throughout the toxicity studies of BSH.

Table 1
Distribution of animals in groups and dose of BSH administered to each group

Group A	Group B	Group C	Group D
Control group of animals fed with standard diet	Treated group of animals fed with BSH (50 mg/Kg body weight) mixed with diet	Treated group of animals fed with BSH (300 mg/Kg body weight) mixed with diet	Treated group of animals fed with BSH (2000 mg/Kg body weight) mixed with diet

Assessment of body weight

To appraise the impact of the intake of BSH on the general health of the treated groups of rats and rabbits, the body weight of the animals from the treated group was recorded on days 1, 2, 3, 5, 7, 10 and 14, and compared with that of the animals from the control group. The body weights of all the groups were taken as means and compared with that of the control group animals.

Food and water intake

The food and water consumed by the control and the treated group animals were recorded as mean values. Food and water intake by both control and treated animals was recorded on days 1, 2, 3, 5, 7, 10 and 14.

Eye irritation testing

To ascertain the potential risk of eye irritation induced by BSH, five albino rabbits were obtained from the University of Sargodha. The eyes of each animal were thoroughly monitored for eye lesions as per the Draize scale.[19]

BSH (3 mg/10 mL) was prepared in distilled water and instilled into the conjunctival sac of the left eye of each rabbit with the help of dropper, while the right eye was used as control. The lower and upper lids of the eyes of the rabbits were kept close with the help of fingers for a short time to avoid the loss of the instilled sample. The eyes of the rabbits were monitored for allergic reaction, the color of the corneal surface, lacrimation and swelling for 72 h after instillation. Irritation was observed by dividing the sum of erythema and edema scores by the number of times of observations.[20]

Acute dermal toxicity

For assessing the dermal toxicity of BSH, a thick paste of sample (200 mg BSH/5 mL water) was applied to the gauze pad (4×4), which was applied to the rabbit's skin. The animals were examined earlier to ensure the absence of rashes, redness and allergy. Micropore™ adhesive tape was used to prevent the dislocation of the patch from the skin. Patches were removed from the skin after 24 h and the skin was observed for allergy, redness, color and rashes, and compared to that of the animals from the control group (OECD 402).[21]

Estimation of absolute organ weight

Absolute organ weight of all the animals, both rats and rabbits, was recorded upon completion of the toxicity studies. On day 15, all the animals were slaughtered and their vital organs, *i.e.*, kidneys, heart, liver, spleen, intestine, and lungs were removed, observed macroscopically for lesions and weighed on a weighing balance for determining absolute organ weight.

Appraisal of hematological and biochemical parameters

Blood samples of all the animals (rats and rabbits) were collected before anesthetization with chloroform. Blood from rats was withdrawn by cardiac puncture, while from rabbits it was collected by puncturing the jugular vein. Blood samples were kept in EDTA lined tubes to prevent coagulation and analyzed for platelets, neutrophils, monocytes, lymphocytes, eosinophils, red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb) and mean corpuscular volume (MCH). Plasma was also removed by centrifugation of blood samples at 4000 rpm for 30 min, and analyzed for different biochemical parameters, such as cholesterol, triglycerides, urea, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Gross necropsy and histopathology studies

On completion of toxicity studies (day 15), all the animals were anesthetized with chloroform, slaughtered and their vital organs, *e.g.*, kidneys, liver, intestine, lungs, spleen and heart, were removed and preserved in 10% v/v formalin. The histopathological appraisal of vital organs was carried out to evaluate the effect of BSH on their cellular architecture. This was accomplished by slicing the tissues (4-5 µm thick) with a sharp surgical blade. The tissues were stained with hematoxylin-eosin dye and cellular architecture was observed under a microscope.

Statistical analysis

The values of different parameters for both control and treated group animals were expressed as mean ± standard deviation (SD). Statistical analysis of the numeric values of the control and treated groups of both rats and rabbits was carried out through one-way analysis of variance (ANOVA), using graph pad prism software. One-way ANOVA was performed considering the p-value <0.05 as statistically significant.

RESULTS AND DISCUSSION

Assessment of physical parameters

After the administration of a single dose of BSH to Groups B, C and D of rats and rabbits, no signs of toxicity were observed in the animals throughout 14 days of toxicity studies. The behavioral pattern of all the animals was observed to be normal, and no signs of tremor or diarrhea were noticed during this study. However, some rats of Group D showed salivation and lacrimation, which diminished after 24 h. In the case of rabbits, only Group D rabbits developed skin irritation and a few animals showed lacrimation, which diminished within 72 h. Therefore, these abnormalities are considered minor and supposed to be caused by careless handling/accidental. Overall, the results of the toxicity studies elicit no sign of toxicity of BSH in treated animals. The LD_{50} of BSH for rats was calculated to be 9 g/Kg of body weight, while the LD_{50} for rabbits was not estimated.

Assessment of body weight

The body weight of albino rats and rabbits were recorded on days 1, 2, 3, 5, 7, 10 and 14 (Tables 2 and 3). It was noticed that the weight of the animals (rats and rabbits) slightly decreased on days 1-3, but it was recovered steadily. This weight loss might be attributed to less food consumption during these days. Because of the highly swellable nature of BSH, the animals may have felt fullness in the abdomen, therefore, refrained from food intake, which ultimately resulted in the loss of body weight. A slight weight gain was observed on days 10-14, as more food and water were consumed by the animals (see Tables 2 and 3).

Food and water consumption

Monitoring the food and water consumed by the treated and the control group animals revealed no significant difference in the consumption of food on days 2-14. However, food consumption was observed to be significantly less in groups B and C of rats and rabbits on days 1, 2 and 3, which might be ascribed to the fullness of the stomach caused by the intake of BSH. The dietary intake was normalized as the study proceeded (Tables 4 and 5).

Table 2
Body weight (g) of rabbits before and after treatment (mean ± SD)

Time duration	Group A	Group B	Group C	Group D
Pretreatment	1534 ± 38.1	1419 ± 29.4	1521 ± 32.8	1460 ± 25.8
Day 1	1536 ± 31.8	1398 ± 30.5	1502 ± 21.3	1445 ± 24.3
Day2	1536 ± 33.5	1380 ± 29.5	1488 ± 24.2	1430 ± 21.7
Day3	1539 ± 27.8	1378 ± 29.1	1484 ± 27.1	1434 ± 20.6
Day5	1541 ± 31.2	1396 ± 30.2	1497 ± 20.7	1449 ±26.4
Day 7	1546 ± 29.2	1410 ± 25.9	1512 ± 23.1	1466 ± 24.1
Day10	1558 ± 28.7	1426 ± 24.6	1528 ± 27.2	1471 ± 21.4
Day 14	1567 ± 26.4	1436 ± 27.4	1536 ± 26.3	1475 ± 22.7

Table 3
Body weight (g) of rats before and after treatment (mean ± SD)

Time duration	Group A	Group B	Group C	Group D
Pretreatment	152.67 ± 2.9	159.48 ± 1.6	161.33 ± 2.1	158.04 ± 2.1
Day 1	152.11 ± 3.0	155.34 ± 2.0	159.01 ± 2.8*	155.63 ± 1.7*
Day2	153.15 ± 2.4	155.57 ± 1.5	157.33 ± 1.4	154.77 ± 2.2**
Day3	154.47 ± 2.6	153.03 ± 2.7	158.03 ± 1.9	151.01 ± 3.5**
Day5	156.33 ± 2.9	157.19 ± 1.8	160.01 ± 2.0	153.14 ± 2.8
Day 7	157.35 ± 2.9	159.39 ± 2.7	161.41 ± 2.2	154.72 ± 2.3
Day10	159.22 ± 3.1	160.88 ± 2.2	162.04 ± 2.1	157.2 ± 1.6
Day 14	163.33 ± 2.5	161.20 ± 1.2	163.12 ± 2.7	158.71 ± 2.3

*$P < 0.05$ represents a significant difference as compared to the control, **$P < 0.05$ is a significant difference within the group

Table 4
Food and water intake of treated and untreated groups of rabbits (mean ± SD)

Parameters	Group A	Group B	Group C	Group D
Water intake (mL)				
Pretreatment	23.4 ± 0.6	20.5 ± 0.6	19.9 ± 0.2	20.7 ± 0.7
Day 1	21.9 ± 0.6	18.9 ± 0.6	18.7 ± 0.8	20.3 ± 0.4
Day 2	22.4 ± 0.5	20.9 ± 0.5	20.3 ± 0.7	20.5 ± 0.5
Day 3	21.0 ± 1.0	22.4 ± 0.6	20.7 ± 0.3	20.2 ± 0.4
Day 5	22.3 ± 0.5	21.7 ± 0.7	21.3 ± 0.5	21.0 ± 0.7
Day 7	22.3 ± 0.5	21.5 ± 0.4	19.8 ± 0.7	20.1 ± 0.6
Day 10	21.8 ± 0.5	22.3 ± 0.6	20.8 ± 0.4	20.6 ± 0.3
Day 14	22.2 ± 0.5	22.1 ± 0.8	20.6 ± 0.6	20.7 ± 0.5
Food intake (g)				
Pretreatment	24.7 ± 1.4	23.4 ± 0.8	22.6 ± 0.7	23.8 ± 0.7
Day 1	22.4 ± 1.0	21.6 ± 0.8	18.9 ± 0.6**	19.2 ± 0.8**
Day 2	23.1 ± 0.8	21.5 ± 1.0	20.1 ± 0.8	21.4 ± 0.8
Day 3	21.5 ± 0.8	22.2 ± 1.1	23.1 ± 0.9	22.7 ± 0.9
Day 5	22.3 ± 0.9	21.9 ± 0.6	22.9 ± 0.7	22.5 ± 0.8
Day 7	23.3 ± 0.9	21.7 ± 0.6	22.7 ± 0.7	22.0 ± 0.8
Day 10	22.6 ± 0.9	22.5 ± 0.8	22.9 ± 0.7	22.7 ± 0.6
Day 14	22.6 ± 0.7	22.6 ± 0.0	23.0 ± 0.8	23.2 ± 1.0

*$P < 0.05$ represents a significant difference as compared to the control, **$P < 0.05$ is a significant difference within the group

Table 5
Food and water intake of treated and control group of rats (mean ± SD)

Parameters	Group A	Group B	Group C	Group D
Water intake (mL)				
Pretreatment	8.6 ± 0.7	8.6 ± 1.21	8.9 ± 0.6	9.5 ± 1.1
Day 1	8.8 ± 0.5	6.3 ± 0.7	5.6 ± 1.1	6.1 ± 1.2
Day 2	8.0 ± 0.6	7.3 ± 0.8	8.1 ± 1.1	7.3 ± 0.8
Day 3	9.2 ± 0.6	8.5 ± 0.8	8.6 ± 1.2	8.3 ± 1.0
Day 5	10.1 ± 1.2	9.1 ± 1.3	9.2 ± 1.0	8.6 ± 1.0
Day 7	9.5 ± 0.7	9.3 ± 0.9	9.0 ± 0.9	9.0 ± 1.0
Day 10	8.7 ± 1.2	10.1 ± 1.2	8.7 ± 1.1	8.9 ± 0.9
Day 14	10.5 ± 1.2	9.3 ± 1.0	9.4 ± 1.1	9.4 ± 1.0
Food intake (g)				
Pretreatment	6.3 ± 0.7	7.2 ± 0.6	6.8 ± 0.4	7.1 ± 0.5
Day 1	6.6 ± 0.7	6.6 ± 0.5	6.0 ± 0.6	6.1 ± 0.7
Day 2	6.1 ± 0.8	7.0 ± 0.6	6.4 ± 0.4	6.5 ± 0.5
Day 3	6.1 ± 0.6	6.9 ± 1.0	6.9 ± 0.5	6.8 ± 0.6
Day 5	6.2 ± 0.6	7.0 ± 0.4	6.6 ± 0.4	7.0 ± 0.4
Day 7	6.3 ± 0.7	7.1 ± 0.5	7.0 ± 0.5	7.1 ± 0.6
Day 10	6.0 ± 0.6	7.0 ± 0.4	6.9 ± 0.4	7.0 ± 0.5
Day 14	6.4 ± 0.7	7.2 ± 0.8	7.2 ± 0.6	6.9 ± 0.6

Eye irritation test

No signs of eye irritation were noticed after instillation of BSH into the rabbits' eyes. The treated eyes of the rabbits were clear and there were no signs of conjunctivitis and iritis in the treated eyes. All the animals were assigned 0 score according to the Draize scale.[19] However, in a few animals from group D, lacrimation was noticed during the first hour of instillation of BSH, which stopped afterwards.

Acute dermal toxicity

Overall, the outcome of the studies revealed no sign of skin irritation, allergy and erythema on the skin. However, rabbits of Group D developed rashes on the skin, which disappeared within 72 h.

These findings suggest BSH is a safe excipient for skin products and is suitable for designing skin patches.

Absolute organ body weight

The absolute organ weight of the treated rats and rabbits was determined and compared with that of the control group animals. No significant differences in absolute organ weight were recorded between the treated animals and those of the control group (Tables 6 and 7).

Hematology and clinical biochemistry

The hematological parameters of the treated group animals were almost similar to those of the control group animals, and, statistically, no significant differences were observed. Hematological parameters, such as RBCs, platelet count, WBCs and hemoglobin (Hb), of the treated rats and rabbits were in normal ranges (Tables 8 and 9). The biochemical parameters of blood, such as serum triglycerides, liver profile and renal profile, of the treated rats and rabbits and of the untreated animals were alike, suggesting that oral administration of BSH is non-toxic and it slightly affects hepatic function, renal function, biochemical parameters of blood and hematology of blood (Tables 10 and 11).

Table 6
Absolute organ weight of rabbits (mean ± SD)

Organs	Group A	Group B	Group C	Group D
Heart	0.251 ± 0.03	0.287 ± 0.06	0.259 ± 0.03	0.276 ± 0.02
Liver	3.423 ± 0.21	3.304 ± 0.25	3.671 ± 0.40	3.806 ± 0.08
Kidney	0.406 ± 0.03	0.461 ± 0.05	0.459 ± 0.02	0.467 ± 0.03
Stomach	1.269 ± 0.15	1.141 ± 0.09	1.181 ± 0.03	1.259 ± 0.05
Intestine	6.125 ± 0.70	6.086 ± 0.8	6.261 ± 0.92	6.336 ± 0.35

Table 7
Absolute organ weight of rats (mean ± SD)

Organs	Group A	Group B	Group C	Group D
Heart	0.271 ± 0.05	0.281 ± 0.06	0.271 ± 0.02	0.270 ± 0.04
Liver	3.623 ± 0.30	3.464 ± 0.25	3.511 ± 0.60	3.626 ± 0.08
Kidney	0.436 ± 0.02	0.461 ± 0.05	0.412 ± 0.02	0.407 ± 0.05
Stomach	1.319 ± 0.2	1.441 ± 0.10	1.281 ± 0.07	1.259 ± 0.25
Intestine	6.235 ± 0.78	6.126 ± 0.6	6.311 ± 0.79	6.236 ± 1.05

Table 8
Hematology parameters of rabbits

Parameters	Group A	Group B	Group C	Group D
TLC (μL^{-1})	10.35	13.71	9.2	10.1
RBC (μL^{-1})	4.7	5.06	6.2	5.2
Hb (g/dL)	12.11	12.26	13.3	11.7
HCT (PCV) (%)	36.41	36.57	39.2	36.1
MCV (fl)	57.21	60.13	62.0	56.7
MCH (pg)	20.11	21.87	20.1	22.4
MCHC (g/dL)	31.05	32.13	30.1	28.9
Platelets (μL^{-1})	375	304	321	403
Neutrophils (%)	43	41	48	51
Lymphocytes (%)	52	40	44	48
Monocytes (%)	1.27	2	2.0	21
Eosinophils (%)	0.5	1	0.5	1.0

Table 9
Hematological parameters of rats

Blood parameter	Group A	Group B	Group C	Group D
TLC (μL^{-1})	7.7	7.0	7.6	8.9
RBC (μL^{-1})	5.85	6.2	5.3	4.72
Hb (g/dL)	12.1	11.6	11.9	10.3
HCT (PCV) (%)	37.4	37.4	40.3	37
MCV (fL)	60.7	59.5	63.2	57
MCH (pg)	19.6	20.7	22.1	22
MCHC (g/dL)	30.4	32.5	29.1	30.7
Platelet count (μL^{-1})	409	325	253	281
Neutrophils (%)	51	39	33	49
Lymphocytes (%)	45	56	65	47
Monocytes (%)	2	2	2	3
Eosinophils (%)	1	1	1	1

Table 10
Clinical biochemistry of rabbits from treated and untreated groups

	Group A	Group B	Group C	Group D
Lipid profile				
Cholesterol (mg/dL)	45	52	68	61
Triglyceride (mg/dL)	83	64	101	97
HDL (mg/dL)	31	30	27	29
LDL (mg/dL)	24	26	22	23
Liver profile				
Bilirubin (mg/dL)	0.4	0.3	0.5	0.3
SGPT (ALT) (U/I)	60	58	70	62
SGOT (AST) (U/I)	81	71	69	55
ALP (U/I)	41	51	31	61
Total protein (g/dL)	6.0	5.7	5.5	7.1
Albumin (g/dL)	2.7	2.9	3.9	3.6
Globulin (g/dL)	1.9	2.1	2.4	2.8
A/G Ratio	1.1	0.9	0.8	1.1
Renal profile				
Urea (mg/dL)	26	22	29	27
Creatinine (mg/dL)	1.3	1.6	0.8	1.1
Hematology				
ESR (mm/h)	2	3	2	2
Serum electrolytes				
Potassium (mmol/L)	3.8	3.5	4.0	4.4
Sodium (mmol/L)	141	144	132	140

Histopathology and necropsy studies

The histopathology of the vital organs of the treated rats and rabbits after oral administration of BSH revealed the normal cellular architecture of all vital organs, including liver, heart, kidney, spleen, stomach and intestine. No lesions or abnormalities in the cellular architecture were noticed, demonstrating the safety of oral administration of BSH (Figs. 1-3).

Table 11
Biochemical parameters of rats of treated and untreated groups

	Group A	Group B	Group C	Group D
Lipid profile				
Cholesterol (mg/dL)	90	101	102	98
Triglyceride (µmol/L)	35	45	36	44
HDL (mg/dL)	32	28	29	28
LDL (mg/dL)	41	44	46	51
Liver profile				
Bilirubin (mg/dL)	0.9	0.7	1.3	1.2
SGPT (ALT) (U/I)	39	35	41	42
SGOT (AST) (U/I)	96	121	110	124
ALP (U/I)	106	122	133	141
Total protein (g/dL)	5.0	5.1	5.3	5.7
Albumin (g/L)	2.3	2.1	2.6	3.0
Globulin (g/L)	3.4	3.1	3.7	3.5
A/G Ratio	0.67	0.68	0.7	0.86
Renal profile				
Urea (mg/dL)	84	71	90	78
Creatinine (mg/dL)	0.6	0.7	1.0	0.8
Hematology				
ESR (mm/h)	1.8	2.0	2.3	2.1
Serum electrolyte				
Potassium (mmol/L)	2.4	2.4	2.6	3.0
Sodium (mmol/L)	138	146	148	159

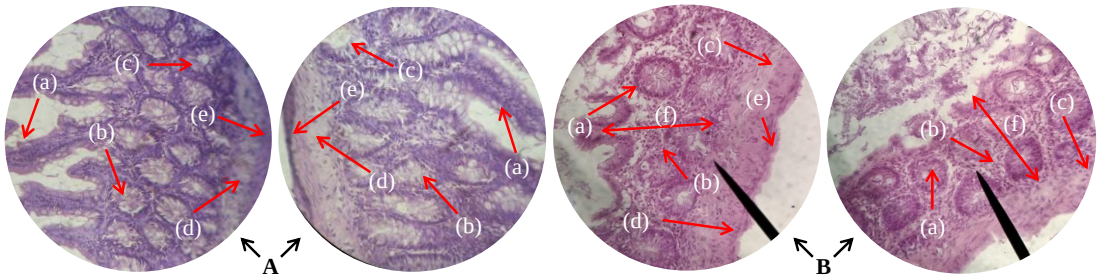

Figure 1: Histopathology of A) small intestine showing small intestinal villi (a), columnar epithelial cell with basal nuclei (b), acinous lumen (c), muscularis mucosae (d), lamina propria (e), and B) colon showing serosa (a), muscularis externa (b), submucosa (c), lamina propria (d), lumen of crypt (e), mucosa (f), colonic crypt (g)

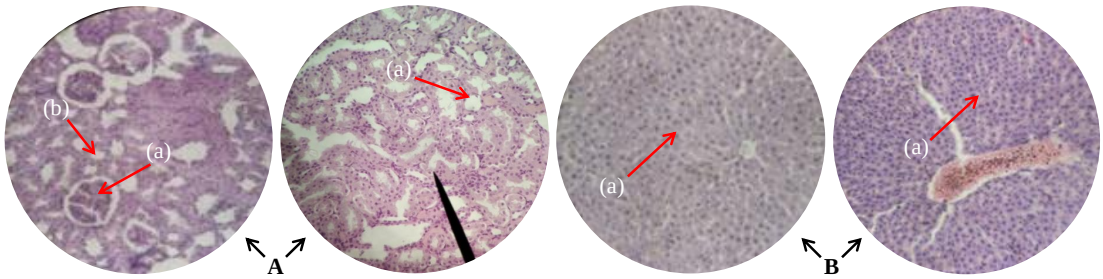

Figure 2: Histopathology of A) kidney showing glomerulus (a), renal tubules (b), and B) liver showing plates of hepatocytes (a)

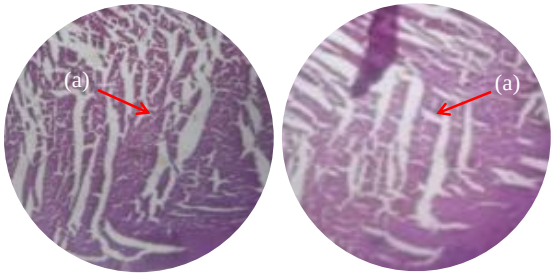

Figure 3: Histopathology of the heart indicating striated heart muscles (a)

CONCLUSION

The findings of the acute toxicity studies of BSH clearly reflect its safety for oral administration. The results for different physical parameters, such as food and water consumption, as well as body weight of the animals, their hematological parameters and the biochemical assay of blood reflect the safety of the polysaccharide. Therefore, from these studies, it can be concluded that BSH can be incorporated as an excipient in oral dosage forms to tailor drug release. Furthermore, skin irritation tests uncovered no irritation or allergy on the skin of rabbits, which demonstrates the safety of BSH as an excipient for skin patches, dermal gels or lotions.

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