https://dx.doi.org/10.21577/0103-5053.20230001

J. Braz. Chem. Soc., Vol. 34, No. 7, 906-917, 2023

Article

pH-Responsive, Hemocompatible, and Non-Toxic Polysaccharide-Based Hydrogel from Seeds of *Salvia spinosa* L. for Sustained Release of Febuxostat

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Herein, a polysaccharide-based hydrogel from seeds of *Salvia spinosa* (SSH) was evaluated as a pH-responsive, superporous, hemocompatible, non-toxic, and sustained release material. The SSH-based tablets showed pH-dependent swelling (pH 7.4 > 6.8 > 4.5 > 1.2) as well as swelling and de-swelling behavior at pH 7.4 and 1.2, respectively. Sustained release of febuxostat was achieved by mimicking the gastrointestinal tract conditions for 14 h and following the zero-order kinetics and super case-II transport mechanism. Scanning electron microscopy revealed the porous nature of SSH even after compression. SSH was found to be hemocompatible with antioxidant properties. Acute toxicity studies ensured the safety of SSH at a maximum dose level of 2.0 g kg^{-1} body weight of the animals. SSH was also found as non-irritant to the eye. The histopathology of vital organs did not show any lesions or inflammation. Conclusively, SSH can be considered a safe ingredient for oral, dermal, and ophthalmic formulations.

Keywords: acute oral toxicity, hemocompatible, pH-dependent swelling, *Salvia spinosa* hydrogel, sustained release

Introduction

The demand for naturally occurring pH-responsive hydrogels has been increasing day by day for their use in the development of pH-responsive drug delivery systems (DDS) mainly due to their easy availability, cost-effectiveness, biodegradability, biocompatibility, non-immunogenicity, and non-toxicity over synthetic ones.[1-3] These materials have various hydrophilic functional groups, i.e., OH, COOH, NH_2, etc., for possible chemical modifications as well as for exhibiting several advantageous attributes including pH-responsive dynamic swelling, on-off switching (swelling and de-swelling), targeted and sustained drug release, tissue engineering, wound healing dressing, etc.[4-6] These features make them ideal candidates as inactive pharmaceutical ingredients for different biomedical applications.

Recently, naturally occurring polysaccharides-based hydrogel-able materials, such as sodium alginate, guar gum, pectin, chitosan, carrageenan, etc. have been reported as promising candidates for sustained drug delivery applications mainly due to their pH-responsive swelling. These polysaccharides can absorb a large amount of the swelling media at the pH value of the small and large intestines. Therefore, such materials are considered ideal candidates for developing sustained and targeted drug delivery systems.[7-10] However, before relying on these hydrogels, it is necessary to investigate their safety profile. Therefore, toxicity studies (acute, sub-acute, sub-chronic, and chronic) of pharmaceutically important hydrogels isolated from *Araucaria heterophylla*,[11] *Curcuma longa*,[12]

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Editor handled this article: Fernando C. Giacomelli (Associate)

Mangifera indica,[13] *Plantago ovata*,[14] etc. have been performed.

Salvia spinosa (synonym Kanocha) is one of the most widely distributed plants of *Salvia* specie and is mostly found across the Mediterranean and phytogeographic regions of Saharo-Arabian. Seeds of *S. spinosa* are tasteless and contain a pertinacious mucilage layer that can be extracted through hot water extraction.[15] The mucilage and other parts of the plants are used for the treatment of inflammation and many infections.[16] *S. spinosa* hydrogel (SSH) is a thermally stable and chemically modifiable polysaccharide-based pH-responsive hydrogel isolated from its seeds. SSH has a considerably high swelling capacity in deionized water (DW) as compared to other swellable polysaccharide-based pH-responsive hydrogels of natural origin. The SSH is a polysaccharide-based material containing glucose, rhamnose, uronic acid, etc. The structure of this novel material is not fully explored yet. Therefore, it is not good to coin it with any name of sugar-based polymers. The seeds of this material are commercially available and economical. The yield of the SSH is comparable to the other structurally related polysaccharide materials isolated from flaxseeds and *Plantago ovata* husk, etc. Furthermore, different parts of the plant and their constituents have been used for treating gonorrhea, chest and stomach infections, pile disorder, and internal body inflammation. On comparing the equilibrium swelling capacity of SSH to the already reported swellable polysaccharide-based hydrogel, it was shown that the swelling capacity of SSH was 2.02, 1.76, and 1.55 times higher than *Linum usitatissimum* hydrogel, *Plantago ovata* husk hydrogel and *Mimosa pudica* hydrogel, respectively.[17] Therefore, due to having greater swelling capacity than other commercially available hydrogels, the SSH is a super-selected material for the development of a pH-responsive drug delivery system.[17,18] Moreover, due to the widespread potential applications of pH-responsive hydrogels, it is important to determine the toxicity profile and biocompatibility of the SSH. To the best of our knowledge, such studies have not been reported yet for SSH.

The present study aimed to isolate the SSH and evaluate its potential as a pH-responsive sustained release material after compression into tablet forms. The pH-responsive swelling as well as swelling and de-swelling properties of SSH were evaluated at acidic and basic pH. The morphology of the SSH-based tablet was examined through scanning electron microscopy (SEM). The evaluation of the hemocompatible nature and antioxidant potential of SSH is also the aim of the study. The non-toxic potential of SSH via acute oral toxicity studies in two animal species, i.e., rats and rabbits were monitored. Moreover, monitoring the toxic effects of SSH on eyes and skin through eye irritation tests and acute dermal toxicity studies were focused. Therefore, detailed *in vitro* and *in vivo* toxicity studies covering physical assessments, determination of hematological and biochemical parameters, and histopathology of vital organs were conducted to establish the safety profile of SSH for the possible applications in various DDS.

Experimental

Materials

S. spinosa seeds were obtained from the indigenous marketplace of District Sargodha, Pakistan. Seeds were sieved and stocked in an air-tight jar. NaOH and HCl were acquired from Merck Chemicals GmbH, Darmstadt, Germany. Potassium dihydrogen phosphate (KH_2PO_4) and KCl were purchased from Sigma-Aldrich, Hamburg, Germany. *n*-Hexane was provided by Riedel-de Haen, Seelze, Germany. Febuxostat (United States Pharmacopeia (USP) standard) was used to study the SSH as sustained-release drug delivery systems (DDS). All other solvents were of analytical grade and used without any further purification. Deionized water (DW) was used during this research work.

Isolation of SSH

The *Salvia spinosa* mucilage/hydrogel (SSH) was isolated as described in the literature.[17] Briefly, seeds of *S. spinosa* were soaked in deionized water DW (water/seed ratio, 25:1 m/v) for 2.5 h at 50 °C. Mucilage extruded from seeds was isolated by placing it in cotton cloth followed by rubbing with the spatula. The mucilage was separated and further purified and washed with *n*-hexane (three times) and DW to remove non-polar and polar impurities. After purification, mucilage was centrifuged at 4000 rpm for 1 h to isolate the sediment paste, i.e., SSH. This purified SSH was placed in a steel tray and put in a hot vacuum oven to dry at 50 °C. Finally, vacuum-dried SSH was homogenized to fine powder by passing through mesh No. 60 and stored in a desiccator until further use.

Preparation of tablets

The ability of SSH as sustained release material was evaluated by pressing it in tablet form with a standard drug febuxostat using the wet granulation method as described elsewhere with necessary modification (Table 1).[3] In a typical formulation, i.e., F3, SSH (250 mg) was first mixed with febuxostat (80 mg) and microcrystalline cellulose (20 mg)

in a pestle and mortar and then homogenized by passing through a sieve No. 40. The mixture was further processed by by adding a 10% (m/v) aqueous solution of tragacanth gum. The forming damp and granulated mass was passed through sieve No. 12 and dried at 50 °C in a vacuum oven. The final dried granules were appraised through different pre-compression parameters and stored in an air-tight jar in a vacuum desiccator until future use. A similar protocol was used to develop three more formulations, i.e., SF (without febuxostat), F1 and F2. Preliminary studies (data not shown here) were carried out to determine the swelling capacity, swelling de-swelling (on-off switching) properties, and sustained drug release potential of all three formulations, i.e., F1, F2, and F3. After the evaluation, formulation F3 exhibited a better profile of swelling, on-off switching, and sustained drug release compared to formulations F1 and F2. Therefore, formulation F3 was further utilized for all studies, and the results are being reported here. Moreover, formulation SF was only used to observe the morphology of SSH after compression.

Table 1. Composition of SSH and febuxostat-based oral tablet formulations

Ingredient	Composition / mg			
	SF	F1	F2	F3
SSH[a]	250	150	200	250
Febuxostat	–	80	80	80
Microcrystalline cellulose	100	120	70	20
Tragacanth gum	50	50	50	50
Total weight	400	400	400	400

[a]*Salvia spinosa* hydrogel.

pH-Responsive dynamic swelling

The swelling properties of the febuxostat-based oral formulation, i.e., F3 were studied using the tea bag method as reported in the literature.[19] Accurately weighed tablets of formulation F3 were taken in each of the four pre-weighed tea bags and hung in beakers (100 mL) containing buffers of pH 1.2, 4.5, 6.8, and 7.4. The beakers were then kept on a shaking incubator (JSSI-100C, JS Research Inc. Republic of Korea) at ambient temperature (25 °C). Tea bags were taken out from each swelling media periodically and blotted to remove excessive media before weighing to record swelling capacity using equation 1.

$$\text{Swelling capacity (g/g)} = \frac{W_s - W_o - W_e}{W_o} \quad (1)$$

where, W_s, W_o, and W_e are the weight (g) of the wet tea bag with a swollen tablet, the initial weight of the tablet in dry form, and the weight of the empty wet tea bag, respectively.

pH-Responsive swelling and de-swelling (on-off switching)

The swelling and de-swelling properties of the tablet formulation F3 were studied in the buffer of pH 7.4 and 1.2 using the tea bag method, as described in the previous section. A tablet of formulation F3 was placed in the pre-weighed tea bag and then allowed to dip in a beaker containing a buffer of pH 7.4 (a swelling media) for 30 min. After determining the swelling capacity, the same tea bag was transferred to another beaker containing a buffer of pH 1.2 (a de-swelling media) for 30 min. The swelling capacities were calculated using equation 1. The swelling and de-swelling studies for F3 were repeated over three consecutive cycles to monitor the reproducibility of the results and reported the mean value.

In vitro drug release studies

To evaluate the potential of SSH as a drug delivery carrier, the release of febuxostat from formulation F3 (because of having a high concentration of SSH) was studied at pH 1.2 and 6.8 (900 mL) for 12 h using USP Dissolution Apparatus II (Pharma test, Germany) at 37 ± 0.5 °C and 50 rpm. Aliquots of dissolution media (5 mL) were taken out with a pipette after pre-determined time intervals, such as 0.25, 0.50, 0.75, 1, 2, 4, 6, 8, 10, and 12 h. The sample to be taken was filtered through a 0.45 μm nylon filter, suitably diluted, and run on a UV-Vis spectrophotometer at 315 nm. The level of the dissolution media was maintained with the corresponding buffer. Additionally, the release studies of F3 were also conducted in a buffer of pH 1.2 for 2 h, pH 4.5 for 3 h, pH 6.8 for 5 h, and pH 7.4 for the next 4 h to study the febuxostat release behavior that mimicked the gastrointestinal tract (GIT) environment, i.e., pH and transit time through different segments. The percentage of cumulative drug release was calculated using the calibration curve method.

Drug release kinetic and mechanism

The drug release data acquired from F3 were subjected to the zero-order kinetic model and the Korsmeyer-Peppas model to determine the rate and mechanism of febuxostat release from tablets, respectively.

The drug release data were evaluated through the zero-order kinetic model (equation 2), and the Korsmeyer-Peppas model (equation 3) using DDSolver software.[20-22]

High regression coefficient values (R^2) predicted the best fit of the kinetic model to the release data.

$$Q_t = K_0 t \quad (2)$$

where, Q_t and K_0 are the amounts of the drug released at time t and the rate constant for the zero-order kinetic model, respectively.

$$\frac{M_t}{M_\infty} = k_p t^n \quad (3)$$

where, M_t/M_∞, k_p and n characterize the fraction of drug released after time t, rate constant for Korsmeyer-Peppas kinetic model, and diffusion exponent, respectively. The values of diffusion coefficient (n) help to understand the release mechanism. For $n \leq 0.45$, $0.45 < n < 0.89$, $n = 0.89$, and $n \geq 0.89$, the mechanism will be followed by Fickian diffusion, non-Fickian diffusion, case-II transport, and super case-II transport, respectively.[21,22]

SEM analysis

The surface morphology of SSH-based tablet formulation (SF) was evaluated by recording their scanning electron microscopic images using an SEM (FEI-NOVA, NanoSEM-450, USA) equipped with a low-energy Everhart-Thornley detector (ETD). The texture and cross-section area of the SF, broken surface of SF, and swollen then freeze-dried SF were captured. The samples were coated with gold using a sputter coater (Denton, Desk V HP, UK) and SEM images were recorded along transverse and longitudinal cross-sections at different magnifications.

Hemocompatibility and antioxidant studies of SSH

Hemocompatibility studies of SSH were performed following the protocol given by the International Standard Organization (ISO) (ISO 10993-4, 1999)[23] through the determination of thrombogenicity (formation of thrombus) and hemolytic potential.

Thrombogenicity

The thrombogenicity potential of SSH was determined using the gravimetric method.[3] Accurately weighed amount of SSH (500 mg) was placed in phosphate buffer saline (PBS) for 24 h at 37 °C ± 0.5. After decanting the excessive PBS, citrate blood (2 mL) and $CaCl_2$ (0.2 mL, 0.1 M) were added and kept aside for 45 min. After that, DW was added to stop the clotting of blood. The formed clots were then fixed with formaldehyde (36-38%, 5 mL), separated, dried, and weighed. A similar procedure was adopted for positive (without SSH) and negative control (without SSH and blood). The thrombose percentage was calculated using equation 4.

$$\text{Thrombose}(\%) = \frac{\text{mass of test sample} - \text{mass of (–) control}}{\text{mass of (+) control} - \text{mass of (–) control}} \times 100 \quad (4)$$

Hemolytic potential

The determination of the extent of hemolysis upon contacting a sample directly with the blood is called hemolytic potential. The protocol as explained by the American Society for Testing and Materials (ASTM) was opted to perform hemolytic potential.[24] SSH (500 mg) was placed in PBS for 24 h at 37 °C ± 0.5. After washing with PBS, the SSH was incubated with a known quantity of citrate blood and PBS for 3 h at 37 °C ± 0.5 followed by centrifugation at 10^4 rpm for 15 min. The supernatant solution was separated to determine the optical density (OD) on the UV-Vis spectrophotometer at 540 nm. A similar procedure was adopted for positive and negative control using a known concentration of citrate blood with DW, and citrate blood with PBS, respectively.[25] Hemolytic potential of SSH was determined using equation 5.

$$\text{Hemolytic index}(\%) = \frac{\text{OD of test sample} - \text{OD of (–) control}}{\text{OD of (+) control} - \text{OD of (–) control}} \times 100 \quad (5)$$

Antioxidant activities

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test as described by Zhang *et al*.,[26] was adopted with necessary modifications to assess the antioxidant efficacy of the SSH. Different concentrations of SSH (0.25, 0.50, 0.75, 1.0, 3.0, 5.0, 7.0, and 10.0 mg L^{-1}) were prepared. To the 2 mL (1.0 mM) freshly prepared DPPH-ethanol solution, a 2 mL sample of each concentration was dispersed. Later, each sample mixture was stirred and incubated for 30 min in the dark at 25 °C. Ultimately, each sample was run on a UV-Vis spectrophotometer at 517 nm and the scavenging affinity of the DPPH radical was recorded using equation 6.

$$\text{DPPH radical scavenging effect (\%)} = \left[1 - \frac{A1 - A2}{A3}\right] \times 100 \quad (6)$$

where, A1, A2, and A3 represented the absorbance of the sample in DPPH, dehydrated ethanol, and absorbance of DPPH in dehydrated ethanol at 517 nm, respectively. Experiments were conducted in triplicates and the mean of the values was recorded and reported.

Acute oral and dermal toxicity studies

Animal selection and study design

To evaluate the non-toxic nature of SSH, the Swiss albino rats (170-220 g) and rabbits (1520-1620 g) were chosen as model animals. The animals of both species were acquired from the animal house of the University of Sargodha, Sargodha. Animals were divided into four different groups (n = 5) (Table 2) and shifted to the laboratory for acclimatization two weeks before conducting the toxicity studies. All animals were housed in neat and clean cages of stainless steel maintaining the controlled conditions of humidity (50-60%) and temperature (22 ± 3 °C). Cages were alternately kept for 12 h each in light and dark. Animals were fed with a standard laboratory diet and provided free access to tap water. The protocol adopted to study the toxicity of SSH was approved by the Institutional Animal Ethics Committee of the University of Lahore, Pakistan No. IREC-2018-76-M. All tests were conducted by following the good laboratory practices as described by the United States Food and Drug Administration and OECD.[27]

Acute oral toxicity

Acute oral toxicity studies were performed according to the OECD Test Guidelines 420[27] using Swiss albino rats and rabbits. Group A was marked as a control group and fed with standard laboratory food, while rats and rabbits of the rest of the groups (groups B, C, and D) were labeled as sample groups and different doses of SSH, i.e., 0.05, 0.30, and 2.0 g kg^{-1} were administered after mixing with standard laboratory food (Table 2). Afterward, rats and rabbits of sample groups were carefully examined for any symptoms of toxic reactions, e.g., allergy, diarrhea, reflexes, salivation, tremors, sleep disorder, and any abnormal behavior for 8 h after administration of the dose. Other parameters of toxicity studies like body weight and amount of water and food consumed by the rats and rabbits were also noted before as well as after the administration of SSH on days 1, 2, 3, 7, and 14. After 14 days (on day 15), animals were anesthetized and blood samples of rats were drawn by cardiac puncture from the posterior vena cave with the help of a 22 G needle while blood samples of rabbits were collected from the jugular vein. After that, all animals were sacrificed and vital body organs, i.e., kidney, heart, lungs, liver, small intestine, and colon were confiscated and macroscopically examined for lesions. The weight of each organ of both control and tested animals was also recorded with great accuracy.

Primary eye irritation testing

For primary eye irritation testing, five white albino rabbits were selected. Well ground and moistened SSH (0.03 g/10 mL) were drop-casted into the conjunctival sac of the right eye of each rabbit (sample eye) keeping the left eye of each rabbit as a control for comparison. To reduce the risk of material loss, both eyelids (upper and lower) of the sample eyes were kept close with fingers for a few minutes. Eyes were examined after a pre-defined time interval, i.e., 1, 2, 4, 24, 48, and 72 h for redness, allergic symptoms, lacrimation, and corneal opacity, and scored according to the Draize scale.[28] The sum of erythema and edema score was taken and divided by the number of eye irritation observations as described by the Draize scale.

Acute dermal toxicity

Acute dermal toxicity of SSH was also assessed using rabbits as model animals following the guidelines of OECD 402.[29] A thick paste of SSH was prepared by suspending 0.2 g in 5 mL DW. The prepared paste was then applied to the shaved skin of each rabbit through a gauze pad (4 × 4 inches). The skin of the animals was further wrapped with Micropore™ adhesive tape (3 inches wide) to make it safe from displacement. The pads from sample rabbits were carefully removed after 24 h and the skin of rabbits was observed for redness, rashes, and color change (if any), and a comparison was made with the skin of control animals.

Hematology and clinical biochemistry

On the 15th day, blood samples from anesthetized rats and rabbits of each group were collected in tubes lined with ethylenediaminetetraacetic acid (EDTA) and determined the hemoglobin, white blood cells count (WBC), red blood cells count (RBC), platelets, erythrocyte sedimentation rate (ESR), monocytes, neutrophils, lymphocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Liver function was assessed through estimation

Table 2. Group scheme for acute oral toxicity studies of SSH

Group A	Group B	Group C	Group D
control given only standard laboratory diet and water	fed with a single dose of SSH[a] (0.05 g kg^{-1}) ground and mixed with diet	fed with a single dose of SSH[a] (0.30 g kg^{-1}) ground and mixed with diet	fed with a single dose of SSH[a] (2.0 g kg^{-1}) ground and mixed with diet

[a]*Salvia spinosa* hydrogel.

of the plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein content, albumin, and globulin. Renal function was ascertained through the estimation of creatinine, urea, and uric acid. Lipid profile was also assessed by measuring blood cholesterol level, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). Serum electrolytes of both rats and rabbits were also counted.

Gross necropsy and histopathology of vital organs

At the end of the toxicity studies (after 14 days), all the control and sample group animals were kept fasting for the next 12 h and then sacrificed to obtain vital organs, i.e., heart, liver, kidney, small intestine, colon, and lungs, and preserved them in 10% formalin. To check the inflammation and any pathophysiological changes in the normal architecture of these organs, tissues of these organs were sliced into 4-5 μm thick pieces using a sharp surgical blade, mounted on a glass slide, and stained with hematoxylin-eosin dye. Microscopic evaluation of these prepared slides was carried out using the microscope (XSZ 107 BN).

Statistical analysis

The results obtained after performing different parameters of toxicity studies of SSH were expressed as their mean values along with the standard deviation (± SD). The numeric data of body weights and food and water intake of both rats and rabbits were further analyzed through two-way analysis of variance (ANOVA) followed by the Bonferroni model, while organ weight of rats and rabbits were compared through one-way ANOVA followed by the Bonferroni model using Prism software.[30] For statistical significance, both one-way and two-way ANOVA (Bonferroni test) were applied using a *p*-value of 0.05.

Results and Discussion

pH-responsive dynamic swelling

The pH-responsive dynamic swelling properties of the *Salvia spinosa* mucilage/hydrogel (SSH)-based oral tablet formulation (F3) were evaluated at pH 1.2, 4.5, 6.8, and 7.4, and the obtained results are incorporated in Figure 1a. A considerably high dynamic swelling of F3 at pH 7.4 and 6.8 as compared to a buffer of pH 4.5 and 1.2 was found. The overall sequence at which F3 swelled appeared as pH 7.4 > pH 6.8 > pH 4.5 > pH 1.2. A possible reason behind this trend is that the carboxylic acid (COOH) groups present on the polymeric (SSH) backbone undergo ionization at pH 7.4 and pH 6.8, and are converted to carboxylate anion (COO^-). Consequently, electrostatic repulsions, i.e., anion-anion repulsions would appear due to which the polymeric chains allowed the swelling media to get into its polymeric network and hence, swell.[14] At pH 4.5, considerably low swelling as compared to pH 6.8 and 7.4 and high swelling as compared to pH 1.2 is due to the presence of less ionized COOH that results in less electrostatic repulsion of ionized COO^-. As a result, swelling media face hindrance while penetrating the chains of SSH and leads to the decrease of swelling. The least swelling of F3 was found at pH 1.2 because the acidic environment of the swelling media retained the COOH of SSH in unionized form (protonated) due to which the attractive forces among the polymeric chains dominated.

pH-Responsive swelling and de-swelling (on-off switching)

The formulation F3 swelled rapidly at pH 7.4 and when transferring the swollen F3 to the buffer of pH 1.2, it de-swelled. At pH 7.4, the COOH groups of SSH get started to convert into COO^- form, resulting in electrostatic repulsion between the similar COO^- groups. Consequently, hydrogen bonding (inter and intramolecular) present in the polymeric chains of SSH is lost and abrupt swelling is observed. Conversely, at pH 1.2, the COO^- groups re-converted into COOH, i.e., protonated form, and SSH re-gained its hydrogen bonding (inter and intramolecular) and hence de-swelled. Figure 1b shows the findings of the F3 swelling and de-swelling experiment (3 cycles), which described the reproducibility of swelling and de-swelling at pH 7.4 and pH 1.2, respectively.

In vitro drug release studies

The release of febuxostat from F3 at pH 1.2 was negligible (9.9% after 12 h), whereas at pH 6.8 a sustained and extended-release pattern was observed (Figure 1c). The cumulative drug release from F3 was determined to be 88.19% after a 12 h study at pH 6.8. The drug release kinetics was determined by applying the data of cumulative drug release in a zero-order equation and found the value of R^2 as 0.9823 approaching near 1, hence considering that the release of febuxostat from F3 followed the zero-order kinetics (Figure 1e). To find the mechanism of drug release from the prepared formulation, the Korsmeyer-Peppas model was applied. The values of R^2 and n calculated from the Korsmeyer-Peppas model were 0.9914 and 1.212, respectively, which indicated the super case-II transport mechanism of febuxostat release from F3 (Figure 1f).

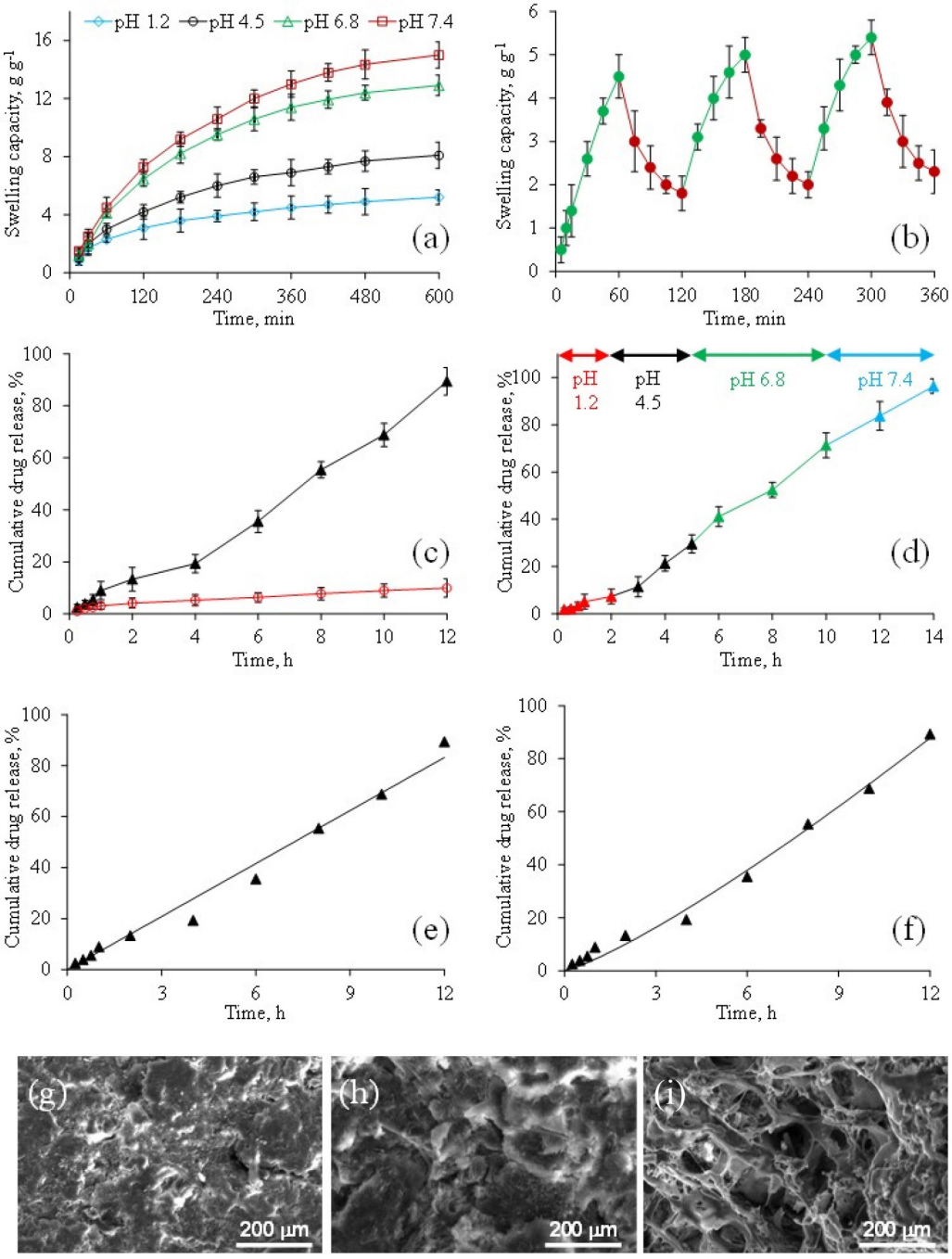

Figure 1. pH-responsive swelling properties of F3 at a buffer of pH 1.2, 4.5, 6.8, and 7.4 (a), on-off switching (swelling and de-swelling) studies of F3 in a buffer of pH 7.4 and 1.2 (b), cumulative release of febuxostat from F3 at pH 1.2 (red line) and 6.8 (black line) (c), febuxostat release mimicking the transit time and GIT pH (d), zero-order kinetic model of febuxostat release from F3 at pH 6.8 (e), Korsmeyer-Peppas model of febuxostat release from F3 at pH 6.8 (f), and SEM images of the surface (g), broken surface (h), and swollen then a freeze-dried tablet of the formulation SF (i).

In GIT mimicking conditions, i.e., pH and transit time, febuxostat release was recorded to be 7.3% at pH 1.2 after 2 h, 29.6% at pH 4.5 after 3 h, 71.3% at pH 6.8 after 5 h, and 96.3% at pH 7.4 after 2 h (Figure 1d). These results indicated that at GIT mimicking conditions, the F3 tablet formulation maintained the sustained release behavior of febuxostat primarily at the intestinal pH. Hence, SSH could be used for developing sustained and targeted DDSs.

SEM analysis of the tablet

The SEM images of the surface, broken surface, and swollen then freeze-dried SSH-based tablet formulation SF are presented in Figures 1g-1i. These SEM images indicated that SF has microscopic pores and channels even after compression. Such channels are responsible for swelling.

Table 3. Results of hemocompatibility studies of SSH

Parameter	Observation		Inference
Thrombogenicity	Weight of blood clot / g	Thrombose / %	non-thrombogenic
	0.32 ± 0.05	91.75 ± 4.02	
Hemolysis	OD at λ_{max} = 540 nm	Hemolytic index / %	non-hemolytic
	0.37 ± 0.09	3.95 ± 0.64	

OD: optical density.

Hemocompatibility studies of SSH

The thrombogenicity potential of SSH was determined using the weight of clots formed during the interaction between SSH and blood, and compared with the positive control. Results depicted in Table 3 showed that the weight of blood clot and thrombose were 0.32 ± 0.05 g and 91.75 ± 4.02%, respectively. Whereas, in the case of positive control, the weight of the blood clot was comparatively greater. Therefore, these results indicate that SSH is non-thrombogenic. If the value of the hemolytic index of any material is less than 5%, then the material is considered a safe material for biomedical applications as mentioned in the safety standards of ISO 10993-4.[23] The value of the hemolytic index of SSH was recorded as 3.95%, which is in accordance to the safety standards of ISO 10993-4. Hence, SSH is considered a safe material and can be used for various biomedical applications.

Antioxidant activities of SSH

An excessive amount of reactive oxygen produced as a result of metabolic activities occurring in the human body under pathological conditions can harm human body cells. To overcome this problem, the materials possessing antioxidant activities are proved more effective as they scavenge free radicals by donating their protons.[31] Therefore, for the description of the antioxidant ability of any material, the DPPH radical scavenging test is one of the most widely used tests. Through this test, the scavenging effect of antioxidants on DPPH radical can be observed by reduction phenomenon, i.e., reduction of stable DPPH to a yellow color organic compound diphenylpicryl hydrazine by antioxidants.[32] It was observed that at a lower concentration of the SSH, the scavenging effect was lower. However, by increasing concentration, the ability to scavenge DDPH radical increased because SSH removed nearly 57.13 ± 3.1% at 5.0 mg mL^{-1} (Figure 2). After that, no more significant removal was observed. SSH have these activities because of its excellent redox characteristics. Therefore, SSH has antioxidant activities and deserves further investigation to expand its spectrum in biological applications.

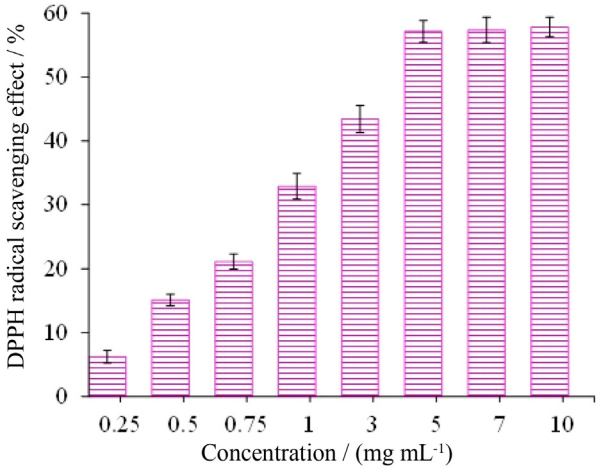

Figure 2. Antioxidant activities (percentage of DPPH radical scavenging effect) of SSH at different concentrations.

Acute oral and dermal toxicity studies of SSH

Assessment of general conditions

All the animals, i.e., rats and rabbits in the sample and control groups did not manifest any toxicity or mortality during the study period. None of the sample group animals exhibited abnormal behavior, allergic reactions, changes in fur, mucous membrane irritation, salivation, vomiting, lacrimation, tremors, or convulsions. However, just two rats from group C showed lacrimation in the eyes and vomiting within the first 24 h of the study, but diminished these symptoms after 48 h. Therefore, these abnormalities might be attributed to careless handling and also supposed to any irrelevant physiological change. Moreover, such abnormalities are statistically insignificant.

Assessment of body weight

The body weight of the animals of both control and sample groups was accurately weighed and recorded on days 1, 2, 3, 7, and 14 (Tables 4 and 5). It is evident that rats and rabbits of both the control and sample groups overall gained a little weight during 14 days. However, a slight decrease in the weight of rats and rabbits in all sample groups was noted on day 1. The feeling of fullness of the stomach due to the swollen nature of SSH might be considered for the slight weight loss on day 1 as well as

the less consumption of food. A statistically significant difference between the weight of rats in the control and sample groups was observed on day 1, while the difference in the weight of animals in sample and control groups was found insignificant on all other days. In the case of rabbits, no significant differences were observed in the weights of the control and sample group rabbits.

Table 4. Body weight of control and sample group of rats (mean ± SD)

Parameter	Body weight / g			
	Group A	Group B	Group C	Group D
Pretreatment	213 ± 3	186 ± 5	175 ± 4	201 ± 6
Day 1	211 ± 5[a]	183 ± 5	173 ± 7	198 ± 3
Day 2	212 ± 4	186 ± 3	175 ± 3	197 ± 5
Day 3	215 ± 5	184 ± 5	176 ± 5	199 ± 4
Day 7	221 ± 3	194 ± 4	182 ± 4	208 ± 4
Day 14	229 ± 5	202 ± 4	189 ± 5	215 ± 3

[a]$p < 0.05$ shows a significant difference within the same group; SD: standard deviation.

Table 5. Body weight of control and sample group of rabbits (mean ± SD)

Parameter	Body weight / g			
	Group A	Group B	Group C	Group D
Pretreatment	1567 ± 6	1594 ± 6	1566 ± 7	1585 ± 6
Day 1	1552 ± 4	1560 ± 7	1546 ± 8	1565 ± 4
Day 2	1559 ± 6	1569 ± 8	1552 ± 6	1580 ± 6
Day 3	1565 ± 7	1561 ± 7	1561 ± 7	1582 ± 9
Day 7	1585 ± 7	1575 ± 7	1596 ± 8	1594 ± 8
Day 14	1618 ± 8	1594 ± 8	1627 ± 7	1613 ± 4

SD: standard deviation.

Assessment of food and water consumption

Assessment of food and water consumption by rats of control and sample group animals illustrated a statistically significant difference in the first two days. Afterward, there was no significant difference in food and water consumption throughout the study period (Tables 6 and 7). In the case of rabbits, only a statistically significant difference was depicted in food consumption in groups A and D on day 1, while no significant variations were documented in food consumption among the sample and control groups of rabbits during the study period.

Primary eye irritation testing

During the eye irritation study, all eyes of the sample animals were free from any inflammation, irritation, conjunctivitis, or iritis. According to the Draize scale, all animals scored "0", which showed the non-irritant, smooth and soft nature of the SSH. However, two animals each from groups C and D showed lacrimation in one eye within the first 2 h, which subsided afterward. Hence, such a condition was considered accidental and statistically insignificant.

Table 6. Food and water consumption of control and sample groups of rats (mean ± SD)

Parameter	Group A	Group B	Group C	Group D
Water consumption / mL				
Pretreatment	6.4 ± 0.5	6.6 ± 0.4	6.6 ± 0.6	6.6 ± 0.6
Day 1	6.4 ± 0.4	5.7 ± 0.4[a]	5.3 ± 0.2[a]	5.8 ± 0.4[a]
Day 2	6.5 ± 0.2	5.8 ± 0.3[b]	5.7 ± 0.1[a]	5.9 ± 0.3[a]
Day 3	6.6 ± 0.2	6.6 ± 0.3	6.3 ± 0.1	6.0 ± 0.1
Day 7	6.8 ± 0.2	6.5 ± 0.2	6.7 ± 0.6	6.5 ± 0.1
Day 14	7.1 ± 0.5	7.0 ± 0.2	6.7 ± 0.2	6.8 ± 0.5
Food consumption / g				
Pretreatment	5.8 ± 0.2	6.1 ± 0.2	6.2 ± 0.4	6.4 ± 0.4
Day 1	6.1 ± 0.3	5.5 ± 0.2[b]	5.6 ± 0.1[b]	5.3 ± 0.2[a]
Day 2	6.0 ± 0.4	5.8 ± 0.3	5.8 ± 0.3	5.7 ± 0.3[b]
Day 3	6.4 ± 0.4	6.2 ± 0.3	6.3 ± 0.2	6.0 ± 0.1
Day 7	6.2 ± 0.2	6.5 ± 0.3	6.8 ± 0.2	6.2 ± 0.3
Day 14	6.2 ± 0.4	6.5 ± 0.2	6.6 ± 0.2	6.4 ± 0.2

[a]$p < 0.05$ shows a significant difference within the same group; [b]$p < 0.05$ is a significant difference as compared to the control. SD: standard deviation.

Table 7. Mean values of food and water consumption of control and sample groups of rabbits

Parameter	Group A	Group B	Group C	Group D
Water consumption / mL				
Pretreatment	19.7 ± 0.4	19.4 ± 0.2	19.7 ± 0.5	19.9 ±0.3
Day 1	19.1 ± 0.2	19.2 ± 0.2	19.5 ± 0.4	19.2 ± 0.5
Day 2	19.1 ± 0.3	19.5 ± 0.3	20.2 ± 1.0	19.7 ± 0.3
Day 3	20.0 ± 1.0	19.7 ± 0.2	20.1 ± 0.8	19.5 ± 0.4
Day 7	19.4 ± 0.3	20.0 ± 0.2	19.9 ± 0.2	20.5 ± 0.6
Day 14	20.2 ± 1.0	20.1 ± 0.2	19.6 ± 0.4	20.1 ± 0.5
Food consumption / g				
Pretreatment	20.6 ± 0.9	20.0 ± 0.5	20.1 ± 1.5	19.6 ± 2.0
Day 1	20.8 ± 0.5	19.6 ± 0.6	19.8 ± 1.0	19.4 ± 1.9[a]
Day 2	21.1 ± 0.6	20.4 ± 0.4	19.4 ± 1.7	20.3 ± 1.6
Day 3	20.7 ± 0.7	20.8 ± 0.2	20.0 ± 2.1	21.5 ± 1.8
Day 7	21.1 ± 0.8	20.4 ± 0.4	21.0 ± 1.8	19.7 ± 2.1
Day 14	20.4 ± 0.4	20.9 ± 0.6	21.2 ± 2.6	20.5 ± 1.4

[a]$p < 0.05$ is a significant difference as compared to the control.

Acute dermal toxicity

Acute dermal toxicity of SSH revealed the non-irritant nature of the SSH as no sign of lesion, abrasion, allergy, erythema, or infection was observed. Therefore, SSH could

be used as an excipient in dermal and transdermal drug delivery systems.

Assessment of hematology and clinical biochemistry

Hematological parameters of both sample and control groups of rats and rabbits were scrutinized after administration of SSH to investigate whether SSH caused abnormalities in blood cell generation or not. Results are depicted in Tables S1-S4 (Supplementary Information section). The findings of hematological parameters of the rats and rabbits in the sample group were in the normal range and comparable with the animals of the control group. Appraisal of biochemical parameters, i.e., lipid profile, liver function test, and renal functions unveiled normal pictures within the normal ranges as well as comparable to both control and sample groups. In light of the outcomes of the clinical parameter, SSH could be considered a safe and inert biomaterial for drug delivery applications.

Absolute organ body weight

Absolute organ weight of the heart, kidney, stomach, intestine, and liver was appraised, and a comparison of absolute organ body weight of sample and control group rats and rabbits revealed statistically insignificant differences.

Histopathology and gross necropsy

The histopathology of vital organs illustrated no sign of toxicity after the oral administration of SSH. The cellular architecture of vital organs was normal without any lesions, inflammation, hemorrhage, or necrosis (Figure 3).

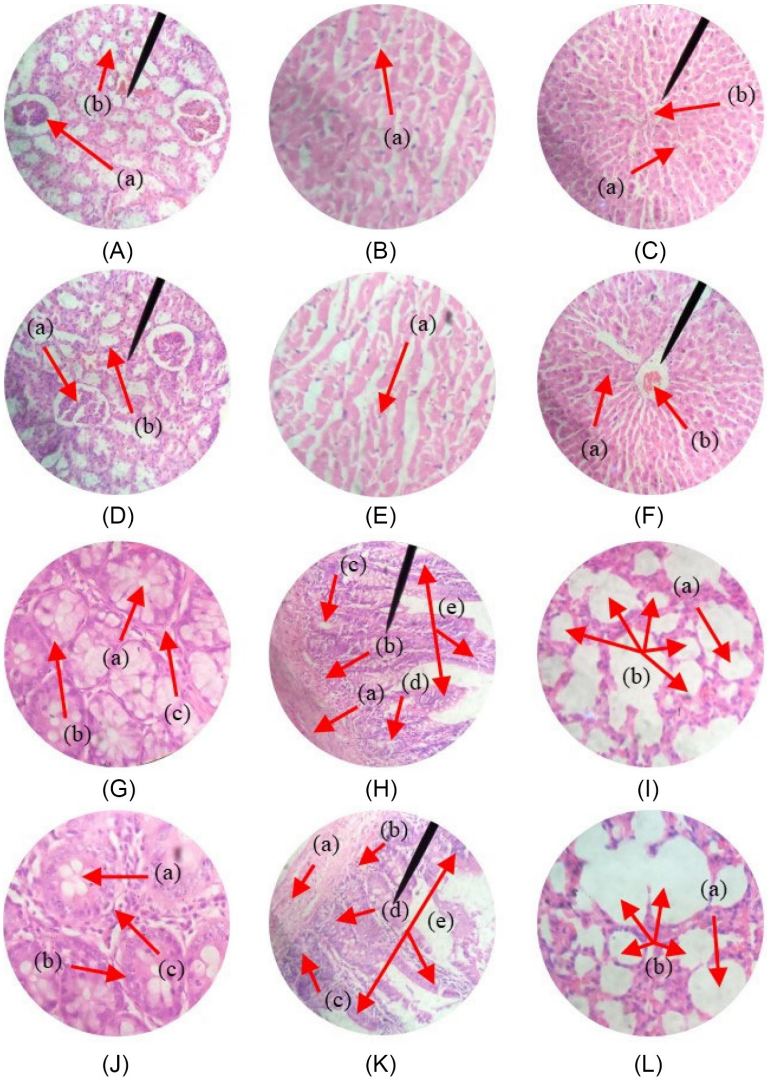

Figure 3. Histopathology of vital organs of the control (A, B, C, G, H, I) and sample (D, E, F, J, K, L) groups animals depicting the kidney (A, D): glomerulus (a), and renal tubules (b); heart (B, E): cardiac muscle fibers (a); liver (C, F): plates of hepatocytes (a) and blood vessels (b); colon (G, J): lumen of crypt (a), colon crypt (b), and lamina propria (c); small intestine (H, K): lamina propria (a), muscularis mucosae (b), acinous lumen (c), columnar epithelial cell with basal nuclei (d), and small intestinal villi (e) and lungs (I, L): alveolus (a) and alveoli (b).

Conclusions

The potential of SSH as a pH-responsive, hemocompatible, antioxidant, non-toxic, and sustained-release material for oral tablet formulation was evaluated. The pH-dependent swelling and sustained drug release behavior at intestinal pH proved the SSH-based tablet formulation as an effective DDS for target and site-specific delivery in the colon. Hemocompatible, antioxidant activities, acute oral, and dermal toxicity studies profiles of SSH were confirmed through standard procedures. The hemocompatible and non-toxic nature of SSH could broaden the horizon of SSH for tissue engineering, skin patches, dermal gels or lotions, and other biomedical applications. Therefore, from these studies, it can be concluded that SSH can be incorporated as an excipient in oral dosage forms to tailor drug release. The results obtained after conducting acute oral toxicity studies of hydrogel from *S. spinosa* have evoked that this novel biomaterial is a safe excipient to use for the development of different drug delivery systems and other therapeutic and biomedical applications.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

Irshad Hussain, LUMS, Lahore is acknowledged for the provision of SEM analysis.

Author Contributions

Arshad Ali was responsible for formal analysis, investigation, writing original draft; Muhammad A. Hussain for conceptualization, methodology, supervision, resources; Muhammad T. Haseeb for review, editing; Muhammad U. Ashraf for validation; Muhammad Farid-ul-Haq for formal analysis, investigation; Tahira Tabassum for investigation, resources; Gulzar Muhammad for data curation, resources; Azhar Abbas for data curation, investigation.

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Submitted: September 3, 2022
Published online: January 5, 2023