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Toxicological Evaluation of Gum (Galactomannans) Isolated from *Senna tora* Seeds

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Abstract

Seed gums possess excellent emulsifying, suspending, binding, thickening stabilizing and water-holding properties. Therefore they are used in various pharmaceutical dosage forms like tablets, syrups, suspensions, lotions, ointments and for sustained drug release systems. Gum derived from the seeds of *Senna tora* L. is common herbaceous annual occurring weed throughout the India. The present investigation reports preliminary phytochemical screening and toxicological evaluation of the isolated seed gum from the *Senna tora*. The acute toxicity study was carried out in adult albino rats by "fixed dose" method. The sub-acute toxicity study was carried out for 28 days in wistar albino rats. All the Animals used were observed for clinical signs, physical abnormalities, changes in body weight and pre-terminal deaths. Laboratory investigations such as hematology and clinical chemistry were performed at sacrifice and the data were statistically analyzed. Based on the results of the acute oral toxicity on the polysaccharide in Wistar rats, it may be concluded that the LD50 of the *Senna tora* gum is greater than 2000 mg/kg. In the sub-acute toxicity study, the gum treated groups did not show any sign of toxicity after getting treated at dose levels of 500, 1000 and 1500 mg/kg daily for 28 days.

Keywords: Toxicity; Phytochemical screening; Seed gum; *Senna tora;* Galactomannans

Introduction

Seed gums are vital food hydrocolloids used globally in various food and pharmaceutical industries. The rising industrial applications of these gums in the area of paper, textile, petroleum, food and pharmaceutical industries has resulted in an impetus in India for intensified research on new sources of gum and their derived products [1]. These gums are normally stable at a wide pH range and have good interaction abilities with organic, inorganic and food constituents. They are biocompatible, cheap and easily available. Natural materials have advantages over synthetic ones since they are chemically inert, nontoxic, less expensive, biodegradable and widely available [2]. They possesses excellent binding, suspending, emulsifying, thickening stabilizing and water-holding properties and could be utilized for the preparation of pharmaceutical dosage forms like tablets, syrups, suspensions, lotions, ointments and for sustained drug release systems [3].

Gum isolated from the seeds of *Senna tora* L. is common herbaceous annual occurring weed throughout the India. It is also commonly known as 'Sickle Pod' [4]. Literature survey revealed presence of various phytoconstituents such as anthraquinone glycosides, naphthopyrone glycosides, flavanoids and phenolic compounds in different parts of *Senna tora* plant [5-7]. Several medicinal properties have been credited to *Senna tora* in Indian system of medicine. The seeds of *Senna tora* have been used in Chinese medicine as aperients, antiasthnic, diuretic agent and also improve the visual activity [8]. The *Senna tora* leaves extract has been found to exhibit significant hepatoprotective activity and anti-inflammatory activity [9,10].

It is important to study phytochemistry and toxicity of the galactomannans isolated from *Senna tora* seeds to find its utility, suitability and acceptability as a polymer/excipient in formulating various pharmaceutical dosage forms. The objective of acute oral toxicity study was to assess the toxicological profile of the *Senna tora* gum when administered to rats by a single oral gavage. This study aimed at providing a rational basis for risk assessment in human being. The purpose repeated dose (28-day) oral toxicity study was to assess the systemic toxic potential of the test item when administered by gavage to rats. This study provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The study was performed at Ultra College of Pharmacy in compliance with OECD (Organization for Economic Co-operation and Development) guidelines.

Materials and Method

Collection and authentication of plant material

The pods of *Senna tora* were collected in the month of September-October from Maharashtra region. The seeds were separated manually and dried under shade. Plant material was authenticated by Dr. Rajendra D. Shinde, Associate Professor, Blatter Herbarium; St. Xavier's College, Mumbai and was identified as *Senna tora* (L.) Roxb (Herbarium Specimen no.8361). The herbarium specimen of *Senna tora* was stored in Ultra College of Pharmacy, Madurai for future reference.

Isolation and purification of gum

The endosperms of the *Senna tora* seed were separated mechanically followed by milling. The powder of the endosperm obtained was soaked in benzene–ethanol solution (1:1) overnight to remove lipids and then

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it was dried in vacuum oven. The gum (galactomannan) was isolated from dried and defatted endosperm powder using solvent precipitation method reported in literature [11]. The obtained crude gum was dissolved in warm water, re-precipitated using ethanol (1:1), dried at 40°C, powdered and stored in airtight container at room temperature. The process of dissolution in water and precipitation with alcohol was repeated until an almost white precipitate was obtained. The dried polysaccharide was milled and sifted with a 60 mesh for further use.

Preliminary phytochemical evaluation

The purity of the isolated polysaccharide was determined by performing preliminary phytochemical tests. The purified *Senna tora* gum was analyzed for the presence of various phytoconstituents such as carbohydrates, reducing sugars, tannins, saponins, flavonoids, terpenes/steroids, alkaloids, glycosides (Anthraquinones and Cardiac glycosides) and proteins. The chemical tests were performed as per the procedure mentioned in standard reference book/literature [12-14].

Acute oral toxicity study of isolated gum in Wistar rats

The acute toxicity study was carried out in adult albino rats by "fixed dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No. 423. The toxicity study protocol was approved by the Institute animal Ethics Committee (Protocol No. UCP/IAEC/2013/067) of Ultra College of Pharmacy, Madurai. Fixed dose method as in annexure 2d: Test procedure with a starting dose of 2000 mg/kg body weight was adopted.

Animal species: Three wistar female rats (Rat No. UCP01, UCP02 and UCP03), 8-12 weeks-old, were used for study (Obtained from Ultra College of Pharmacy Animal House).

Housing and feeding conditions: The temperature and relative humidity in the experimental animal room were maintained at 22 ± 2°C and 50-60% respectively. Lighting was artificial (12 hour's light and 12 hours dark cycle).For feeding, conventional laboratory diets were used with an unlimited supply of drinking water.

Preparation of animals: The animals were uniquely identified and kept in their cages for five days prior to dosing for acclimatization to the laboratory conditions. During acclimatization the animals were observed for ill health.

Preparation of doses: The gum powder was dispersed in the distilled water. For the limit test the dose of 2000 mg/kg was used.

Administration of dose: The animals were kept on fasting prior to dosing by withholding food overnight. Fasted body weight of each rat was determined and then dose was calculated according to the body weight. The gum powder was administered in a single dose by gavage, using an oral dosing needle. Individual animals were dosed in sequence at 24 hour intervals, one at a time, and then observed for a minimum of 24 hours.

Observations

Toxic signs and pre-terminal deaths: Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days. Changes in skin and fur, eyes and mucous membranes, and behavior pattern were also observed.

Food consumption: Measurement of food consumption was made daily.

Body weight: Individual weights of animals were determined shortly before the test substance was administered and weekly thereafter. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and then humanely killed.

Pathology: All animals were subjected to gross necropsy. All gross pathological changes were recorded for each animal.

Repeated dose (28 Day) oral toxicity study by gavage with polysaccharide in wistar rats

The sub-acute toxicity study was carried out according to OECD (Organization for Economic Co-operation and Development) Guideline. Wistar albino rats of either sex weighing 100-200 g were assigned to each group (4 groups). The test group 2, 3 and 4 received the *Senna tora* gum at dose of 500, 1000 and 1500 mg/kg p. o. respectively, once daily for 28 days. The control group-1 received water without the gum sample. Animals from all the groups were observed for clinical signs, physical abnormalities, changes in body weight and pre-terminal deaths. Laboratory investigations such as hematology and clinical chemistry were performed at sacrifice and the data were statistically analyzed. The rats were subjected to detailed necropsy at terminal sacrifice.

Animals used: Animals: Wistar rats, random breed (in-house); Source: Ultra college of Pharmacy, Madurai; Age of animals: 8 to 12 weeks; No. of groups: Four: Vehicle control, low, mid and high dose groups; No. of animals per group: 03; Identification: Rat accession number, cage card and corresponding Picric acid body markings; Acclimatization: Five days under experimental conditions.

Animal selection and grouping: The animals were weighed and grouped into body weight ranges (Example: 150-159, 160-169, 170-179, etc.). These body weight stratified rats were distributed to all the study groups in equal numbers and the animals with extreme body weights were discarded. The details of the animals used in the study are summarized in Table 1.

Husbandry

Conditions: Animals were housed and maintained under laboratory conditions of temperature 22 ± 2°C, relative humidity 50-60% and with light cycle of 12 hours light and 12 hours dark.

Housing: The rats were housed in groups of 3 per cage in standard rat cages (size Approximately: L 410 x W 220 x H 140 mm) with stainless steel top grill having facilities for holding pellet food and drinking water in bottles fitted with stainless steel sipper tubes.

Diet ad libitum: Pelleted rat feed was provided.

Water ad libitum: Water filtered through 'Aquaguard' on-line

Group No.	Group Dose (mg/kg)	Dose (mg/kg)	No. of rats	Rat numbers
I	Vehicle Control	0	03	UCP04 UCP05 UCP06
II	Low Dose	500	03	UCP07 UCP08 UCP09
III	Mid Dose	1000	03	UCP10 UCP11 UCP12
IV	High Dose	1500	03	UCP13 UCP14 UCP15

Table 1: Details of animal used in sub-acute toxicity study.

water filter cum purifier manufactured by M/s. Eureka Forbes Ltd., was provided to animals in water bottles with stainless steel sipper tubes.

Administration of dose: The animals were dosed with the test substance daily seven days each week for a period of 28 days. The test substance was administered in a single dose by gavage, using an oral dosing needle. Body weight of each rat was determined and then dose was calculated according to the body weight. Control group received distilled water.

Observations and measurements

Toxic signs and pre-terminal deaths: Toxic signs were observed once a day. All animals were observed for pre-terminal deaths twice daily.

Body weight and food intake: The body weight of each rat was recorded at the beginning (first day of treatment) and at the beginning of each week of the experimental period. Three days food intake was carried out once the study period. The input and output were recorded. Food consumption was expressed as g/animal/day.

Clinical laboratory investigations: The following hematological and clinical chemistry parameters were analyzed for all rats at the end of the treatment period.

Hematology

Blood collection: At the end of the study all the animals were fasted overnight (water allowed) and blood was collected in heparin tubes from the retro-orbital plexus under light ether anesthesia for clinical chemistry.

At the end of the treatment period, blood samples for hematology and clinical chemistry were taken at necropsy from all rats. The following hematological parameters were measured on samples collected using EDTA-2K as an anticoagulant: Hb: Hemoglobin, RBC: Red blood cells, WBC: White blood cells, N: Neutrophils, L: Lymphocytes, E: Eosinophils, M: Monocytes, PCV: Packed cell volume, PLT: Platelet count.

Clinical biochemistry: The following clinical chemistry measurements were made on sera obtained by centrifugation of the aforementioned blood samples: Cholesterol, SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamic pyruvic transaminase), BUN (Blood urea nitrogen), RBS (Random blood sugar), TG (Triglycerides) and CRN (Creatinine).

Pathology: All animals were subjected to gross necropsy. All gross pathological changes were recorded for each animal. The main organs such as brain, pituitary, heart, kidney, liver, thymus and spleen from each rat were weighed and organ to body weight ratios determined. Histopathology was carried out on the liver, kidney and spleen of all animals in the treatment group and control group.

Gross necropsy: All surviving animals in the study are subjected to gross necropsy after proper bleeding immediately after blood collection. The gross findings are recorded. The animals sacrificed at term were fasted overnight (water allowed), weighed and exsanguinated under ether anesthesia.

Tissue collection: The following organs and tissues are collected, trimmed of any adherent tissue, as appropriate and their weights were taken as soon as possible after dissection to avoid drying and autolytic changes.

The collected tissues/organs were preserved in 10% buffered neutral formalin and processed through paraffin embedding technique and 5 µ sections are examined for histopathological evaluation.

Histopathology: Histopathological evaluations were carried out on the preserved organs and tissues of all animals in the control and high dose groups. Tissues specimens were collected from animals belonging to different treatment groups. After collection the tissues were immediately preserved in the 10% neutral buffered formalin, processed by routine method for histological observation. Processed tissue were sectioned (at 5 µm) and taken on the clean glass slides and stained by hematoxylin and eosin and observed under microscopes at different magnifications.

Results and Discussion

The gum obtained from *Senna tora* seeds was an amorphous free flowing powder with dull brown colour. The results of the preliminary phytochemical evaluation are summarized in Table 2.

It indicated the presence of Carbohydrates, reducing sugars, protein in isolated gum sample. The gum was found to contain galactomannans. The preliminary phytochemical evaluation indicated absence of glycosides and other phytoconstituents in the isolated polysaccharide indicating the purity of gum.

Acute toxicity study

There were no toxic signs and pre-terminal deaths. All the rats gained weight through the observation period (Table 3). At the end of the observation period the rats were sacrificed using diethyl ether anaesthesia and subjected to detailed necropsy and no abnormality was detected.

Based on the results of the acute oral toxicity of the test item Polysaccharide in Wistar rats, it can be concluded that the LD 50 of the test item is greater than 2000 mg/kg.

Sub-acute toxicity study

No treatment related clinical and/or toxic signs were observed in any of the doses tested and there were no pre-terminal deaths. There were no significant changes in the body weight in all the treatment groups compared to control group during the treatment period (Table 4).

There were no significant changes in the feed consumption in all the treatment groups compared to control group during the treatment period (Table 5).

Laboratory investigations

Hematology: There were no significant changes in all the hematology parameters analyzed in all the treatment groups compared to control group (Table 6).

Clinical chemistry: There were no significant changes in all the clinical chemistry parameters analyzed in all the treatment groups compared to control group (Table 7).

Organ weights and organ weight ratios: There were no significant changes in the absolute organ weight and organ weight ratio observed in all the treatment groups compared to control group (Tables 8 and 9).

Gross pathology: There were no incidences of gross pathological changes observed in all the groups.

S. No.	Test	Result
1	**Carbohydrate** *(Molisch Test)*	Positive
2	**Reducing sugar** *(Fehling's solution Test)* a) Crude sample b) Hydrolysate	 Negative Positive
3	**Protein** *(Biuret Test)*	Negative
4	**Alkaloids**	Negative
5	**Glycosides**	Negative
6	**Flavanoids**	Negative
7	**Tannins**	Negative
8	**Saponins**	Negative
9	**Sterols**	Negative
10	**Triterpenes**	Negative
11	**Galactomannans** a) Gum solution+Iodine solution b) Gum solution+Borax	 Positive Positive

Table 2: Phytochemical screening of isolated *S. tora* seed polysaccharide.

Dose (mg/kg b.wt.)	Rat No.	Sex	Body weight (g)					No. dead/ No. tested
			Initial	Day 8	Weight change (day 8 – Initial)	Day 15	Weight change (day 15 – Initial)	
2000	UCP01	Female	145	154	9	167	22	0/3
	UCP02	Female	151	160	11	176	25	
	UCP03	Female	153	164	11	178	27	

Table 3: Body weight changes and pre-terminal deaths.

Dose group (mg/kg b. wt./day)	Absolute weight (g)*				
	Day 0	Day 8	Day 15	Day 22	Day 28
0	161 ± 2.1	176 ± 3.2	190 ± 2.9	204 ± 2.5	224 ± 3.1
500	153 ± 3.2	180 ± 4.1	196 ± 3.6	208 ± 4.3	228 ± 2.3
1000	155 ± 2.7	171 ± 2.3	191 ± 1.8	201 ± 2.2	231 ± 2.9
1500	159 ± 2.4	176 ± 2.6	193 ± 3.4	205 ± 1.7	226 ± 2.2

**Values are mean ± S.D*

Table 4: Body weights of rats treated with polysaccharide.

Dose group (mg/kg b. wt./day)	Food consumption in g/animal/day*				
	Day 0	Day 8	Day 15	Day 22	Day 28
0	16 ± 1.26	18 ± 0.96	19 ± 0.50	23 ± 0.39	25 ± 0.33
500	14 ± 0.67	16 ± 0.41	18 ± 0.88	22 ± 0.60	23 ± 0.74
1000	15 ± 0.64	17 ± 0.04	20 ± 0.82	23 ± 0.58	26 ± 0.53
1500	14 ± 1.60	17 ± 0.47	19 ± 0.74	22 ± 0.40	25 ± 1.04

**Values are mean ± S.D*

Table 5: Feed consumption of rats treated with polysaccharide.

Parameter	Daily dosage of Polysaccharide in mg/kg body weight (n=3/group)*			
	0	500	1000	1500
Hb (g/dl)	12.2 ± 0.07	11.9 ± 0.08	12.4 ± 0.11	13.1 ± 0.09
RBC X 10^3/cmm	6.50 ± 0.44	6.12 ± 0.30	6.28 ± 0.32	6.62 ± 0.39
WBC X 10^3/cmm	8.8 ± 0.74	9.2 ± 0.85	10.1 ± 0.59	8.4 ± 0.41
PLT X 10^5/cmm	338 ± 0.56	329 ± 0.64	317 ± 0.47	328 ± 0.72
N%	47 ± 0.41	48 ± 0.32	47 ± 0.53	43 ± 0.49
E%	01 ± 0.00	01 ± 0.00	01 ± 0.00	01 ± 0.00
L%	51 ± 0.77	49 ± 0.82	51 ± 0.48	54 ± 0.55
M%	01 ± 0.00	01 ± 0.00	01 ± 0.00	01 ± 0.00
PCV%	34.6 ± 0.97	33.2 ± 0.51	35.2 ± 0.75	36.1 ± 0.88

**Values are mean ± S.D*

Hb: Hemoglobin, RBC: Red blood cells, WBC: White blood cells, N: Neutrophils, L: Lymphocytes, E: Eosinophils, M: Monocytes, PCV: Packed cell volume, PLT: Platelet count

Table 6: Hematological analyses of rats treated with polysaccharide.

Parameter	Daily dosage of Polysaccharide in mg/kg body weight (n=3/group)*			
	0	500	1000	1500
SGOT IU/L	113 ± 1.42	112 ± 1.82	108 ± 1.43	117 ± 1.56
SGPT IU/N	339 ± 1.11	332 ± 1.61	331 ± 1.44	341 ± 1.71
BUN mg/dl	14.2 ± 1.32	13.8 ± 1.53	14.3 ± 1.44	13.3 ± 1.91
CRN mg/dl	0.9 ± 0.03	1.0 ± 0.02	0.8 ± 0.05	0.8 ± 0.07
Cholesterol mg/dl	86 ± 0.84	92 ± 0.91	91 ± 0.77	89 ± 0.82
TG mg/dl	103 ± 1.22	106 ± 1.48	109 ± 1.90	105 ± 1.32
RBS mg/dl	98 ± 0.29	92 ± 0.34	96 ± 0.42	91 ± 0.56

**Values are mean ± S.D*

Cholesterol, SGOT: Serum Glutamic Oxaloacetic Transaminase, SGPT: Serum Glutamic Pyruvic Transaminase, BUN: Blood Urea Nitrogen, RBS: Random Blood Sugar, TG: Triglycerides, CRN: Creatinine

Table 7: Biochemical analyses of rats treated with polysaccharide.

Organ	Daily dosage of Polysaccharide in mg/kg body weight (n=3/group)*			
	0	500	1000	1500
Brain	1.716 ± 0.03	1.822 ± 0.07	1.779 ± 0.05	1.926 ± 0.05
Pituitary	0.0052 ± 0.0001	0.0059 ± 0.0004	0.0061 ± 0.0002	0.0055 ± 0.0005
Heart	0.819 ± 0.02	0.874 ± 0.05	0.846 ± 0.03	0.867 ± 0.02
Liver	10.167 ± 0.84	9.655 ± 0.56	9.821 ± 0.73	9.910 ± 0.66
Thymus	0.488 ± 0.03	0.543 ± 0.06	0.512 ± 0.03	0.497 ± 0.05
Kidney(left)	0.781 ± 0.02	0.834 ± 0.02	0.822 ± 0.05	0.817 ± 0.03
Kidney (right)	0.932 ± 0.04	0.961 ± 0.01	0.954 ± 0.03	0.971 ± 0.03
Spleen	0.554 ± 0.03	0.578 ± 0.02	0.533 ± 0.02	0.569 ± 0.04

**Values are mean ± S.D*

Table 8: Absolute organ weights of rats treated with polysaccharide.

Organ	Daily dosage of Polysaccharide in mg/kg body weight (n=3/group)*			
	0	500	1000	1500
Brain	0.766 ± 0.013	0.799 ± 0.042	0.770 ± 0.033	0.852 ± 0.063
Pituitary	0.002 ± 0.0002	0.003 ± 0.0004	0.003 ± 0.0003	0.002 ± 0.0006
Heart	0.366 ± 0.011	0.383 ± 0.025	0.366 ± 0.042	0.384 ± 0.025
Liver	4.539 ± 0.201	4.235 ± 0.321	4.252 ± 0.143	4.385 ± 0.221
Thymus	0.218 ± 0.037	0.238 ± 0.031	0.222 ± 0.027	0.220 ± 0.046
Kidney(left)	0.349 ± 0.057	0.366 ± 0.039	0.356 ± 0.042	0.362 ± 0.028
Kidney (right)	0.416 ± 0.041	0.421 ± 0.035	0.413 ± 0.040	0.430 ± 0.017
Spleen	0.247 ± 0.047	0.254 ± 0.026	0.231 ± 0.022	0.252 ± 0.028

**Values are mean ± S.D*

Table 9: Relative organ weights of rats treated with polysaccharide (Relative organ weight: (absolute organ weight × 100%) / body weight of mice on the day of sacrifice).

Histopathology: All microscopic changes noticed in this study appeared to be incidental as their frequency and severity were nearly equal or less in high dose treatment group as compared to control and hence considered incidental. The histopathological sections (100X and 400X) of liver, kidney and spleen of a high dose treated animal are shown in Figure 1-3 respectively. No abnormalities were detected in both control as well as treated group animals.

Conclusion

The results of the study indicated that the *Senna tora* seed gum had no adverse effect on general health, growth, hematological and clinical chemistry parameters and organ weights of Wistar rats at a dose of 1500 mg/kg body weight. No dose related changes were observed in any of the parameters evaluated. From the above findings, the evaluated No Observed Adverse Effect Level (NOAEL) of polysaccharide in Wistar rats was 1500 mg/kg b.wt. under the testing conditions and doses

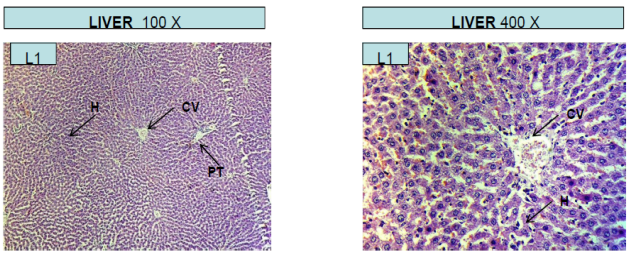

Figure 1: Histopathological section (100X and 400X) of liver (H: Hepatocytes; PT: Portal Triad; CV: Central Vein).

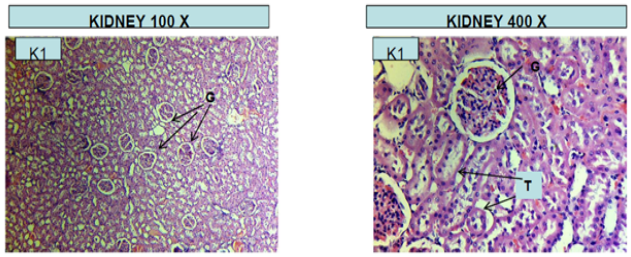

Figure 2: Histopathological section (100X and 400X) of kidney (G: Glomerular; T: Tubule).

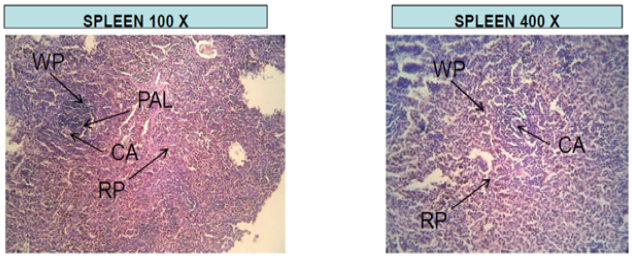

Figure 3: Histopathological section (100X and 400X) of spleen (RP: Red Pulp; WP: White pulp; CA: Central arteriole; PAL: Periarteriolar Lymphoid Sheath).

employed. Now a day Pharmaceutical industries are striving for safe and non-toxic polymers. *Senna tora* gum is one of the natural polymers which can be used for the preparation of various pharmaceutical dosage forms.

Conflict of Interest Statement

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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