Journal of Young Pharmacists, 2026; 18(3):807-815.
https://www.jyoungpharm.org

Original Article

Preclinical Toxicological Evaluation of Sharbat-e-Toot Siyah, a Traditional *Morus nigra* L. Based Unani Syrup

Mohd Urooj[1,*], Gulam Mohammed Husain[1], Durgesh Kumar Dwivedi[1], Divya Kodavath[1], Enumula Vinay[1], Ghazala Javed[2]

[1]Department of Pharmacology and Toxicology, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, Telangana, INDIA.
[2]Department of Moalajat, National Research Institute of Unani Medicine for Skin Disorders, Hyderabad, Telangana, INDIA.

ABSTRACT

Background: Sharbat-e-Toot Siyah (STS) is a herbal Unani syrup that is traditionally used for the treatment of *Waram-e-Lauzatain* (Tonsilitis), *Warm-e-Hanjara* (Laryngitis), *Sual* (Cough) and *Nazla* (Catarrh). To investigate the toxicity of STS in rats after both acute and 28-day repeated oral doses. **Materials and Methods:** The acute toxicity was tested at a limit dose of 20 mL/kg bw using female rats. For repeated dose toxicity the rats were assigned into 04 groups, each including 05 animals of each sex. STS was given orally at dosages of 2, 4, and 10 mL/kg bw daily, whereas the control group was given a vehicle alone. **Results:** Administering oral STS for twenty eight days to both male and female rats did not cause any treatment-related adverse effects on survival. There were relatively slight, non-toxicologically significant differences from controls in terms of haematological, biochemical, body weight, food consumption, and proportional organ weights. With the exception of modest to moderate alterations in the histology for liver and lungs. **Conclusion:** The findings from the acute toxicity study showed that the oral LD_{50} of STS in female SD rats was higher than 20 mL/kg bw. The results of the repeated dosage trial confirmed that, as compared to controls, STS had no toxicologically significant effects on body weight, feed consumption, behaviour, haematological and biochemical markers, or relative organ weights at any tested dose. Under the conditions of this study, STS exhibited a favorable safety profile and did not produce any treatment-related adverse or toxic effects at the tested doses.

Keywords: *Morus nigra*, Sharbat, Toxicity, Unani.

Correspondence:
Dr. Mohd Urooj
Department of Pharmacology and Toxicology, National Research Institute of Unani Medicine for Skin Disorders, A.G Colony Road, Erragadda-500038, Hyderabad, Telangana, INDIA.
Email: urooj.ccrum@ccrum.res.in
ORCID: 0000-0003-3060-3251

Received: 06-05-2026;
Revised: 23-06-2026;
Accepted: 13-08-2026.

INTRODUCTION

Sharbat-e-Toot Siyah (STS) is a classical Unani polyherbal formulation (Syrup) traditionally prescribed for the management of various inflammatory and respiratory disorders. In Unani medicine, it is described as possessing *Mohalill-e-Waram* (anti-inflammatory) and *Mulattif* (demulcent) properties, which contribute to its therapeutic efficacy in alleviating inflammation and soothing irritated mucosal surfaces. STS has been extensively used for the treatment of *Waram-e-Lauzatain* (tonsillitis), *Waram-e-Hanjara* (laryngitis), *Nazla* (catarrh), and *Sual* (cough) (Anonymous, 2006). Owing to its longstanding clinical use and favorable traditional reputation, the formulation continues to be widely prescribed in Unani practice for upper respiratory tract ailments.

The genus *Morus* is believed to have originated in the Himalayan foothills of India and China and is now widely distributed across many arid and semi-arid regions. The leaves, fruits, roots, and stems of *Morus nigra* and *Morus alba* are rich in nutrients, including minerals, carbohydrates, proteins, fats, dietary fibers, vitamins, tannins, and various phytochemicals. Both species have been reported to exhibit a wide range of pharmacological activities, such as antimicrobial, antifungal, antioxidant, anti-obesity, hypocholesterolemic, and antidiabetic effects (Dhanhani *et al.*, 2025).

Although modern cough syrups provide symptomatic relief, their use may be associated with adverse effects such as sedation, dizziness, gastrointestinal disturbances, allergic reactions, and, in some cases, dependence, respiratory depression, and cardiovascular complications. These limitations underscore the need to evaluate and develop safer therapeutic alternatives, including traditional herbal formulations with a long history of clinical use.

Despite STS extensive traditional usage, there is a paucity of scientific evidence regarding its safety profile, particularly following repeated administration. Establishing the toxicological safety of traditional formulations is essential for their

OPEN ACCESS
Manuscript TECHNOMEDIA

DOI: 10.5530/jyp.20260007

Copyright Information :
Copyright Author (s) 2026 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

evidence-based utilization and wider acceptance in contemporary healthcare systems. Therefore, the present study was undertaken to evaluate the oral toxicity and safety profile of STS following repeated administration in rats for a period of 28 days in accordance with standard preclinical toxicological assessment protocols. The study aimed to generate scientific data on potential toxic effects through the evaluation of clinical observations, body weight, feed consumption, hematological and biochemical parameters, organ weights, and histopathological examination of major organs.

The composition of the study formulation is mentioned in Table 1A.

MATERIALS AND METHODS

Experimental Animals

It was confirmed that the female rats were nulliparous and non-pregnant prior to study initiation. The animals were housed in polycarbonate cages under standard laboratory conditions in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA). The study was conducted following both national (CCSEA) and international (OECD) guidelines for animal experimentation. The study protocol (IAEC/17/2022/01/P03) was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) on 19 March 2022 before commencement of the experiment. Prior to initiation of the study, the animals were acclimatized to the laboratory environment for one week.

Drug/Formulation

Sharbat Toot Siyah (STS) was prepared in the Department of Pharmacy at National Research Institute of Unani Medicine for Skin Disorders, Hyderabad following the standard method for sharbat preparation described in classical Unani literature. Fresh fruits of *Morus nigra* L. were procured from the local market, thoroughly cleaned, and processed to obtain the juice. The extracted juice was filtered to remove impurities and collected until the desired volume of 1 litre was obtained. Subsequently, 1.5 kg of honey was added to the filtered juice, and the mixture was heated under controlled conditions with continuous stirring. Heating was continued until the preparation attained the characteristic consistency and organoleptic properties prescribed for sharbat formulations in the classical texts. The finished formulation was then allowed to cool and stored in suitable containers until further use (Anonymous 2006).

Dose Selection

The recommended clinical dose of STS described in traditional literature is 20-40 mL/day for adults (Anonymous, 2006). For toxicity assessment, an acute oral toxicity study was conducted in accordance with OECD Guideline 425, employing a limit dose of 20 mL/kg body weight, which represented the maximum feasible volume that could be administered orally to experimental animals.

For the repeated-dose toxicity study, dose levels were selected based on the lower therapeutic human dose of 20 mL/day. Assuming an average adult body weight of 60 kg, the human dose corresponds to approximately 0.33 mL/kg body weight. The equivalent rat dose was calculated using the Body Surface Area (BSA) conversion method described by (Reagan-Shaw *et al.*, 2008), according to the following equation:

$$\text{Animal dose} = \text{Human dose} \times \text{Km(Human)/Km(Rat)}$$

where the Km factor is 37 for humans and 6 for rats. Based on this conversion, the therapeutic equivalent dose in rats was approximately 2 mL/kg body weight. This dose was selected as the low-dose level. To evaluate any dose-dependent toxicological effects and to establish a sufficient safety margin, two additional doses corresponding to 2-fold (4 mL/kg bw) and 5-fold (10 mL/kg bw) were included as the mid- and high-dose levels, respectively.

Thus, the repeated-dose toxicity study was conducted at 2, 4, and 10 mL/kg body weight, representing the therapeutic equivalent dose and its multiples, thereby enabling assessment of the safety profile of STS over a range of exposure levels relevant to and exceeding the anticipated human therapeutic use.

Drug Administration

Control animals were given an equivalent volume of water, while STS was given orally as a water-based solution, with a maximum volume of 2 mL per 100 g of body weight. To maintain uniformity, doses were given orally by gavage once a day for 28 days in a row at the same time every day.

Duration of Treatment

The repeated-dose oral toxicity study lasted 28 days, while the acute toxicity phase lasted 14 days.

Experimental Design

In accordance with OECD test guideline 407, a 28-day repeated dosage oral toxicity study was conducted (OECD 2025). Male and female SD rats were divided into four groups at random, with five males and five females in each group.

For 28 days, the control group was given the same amount of water as the vehicle. For 28 days, the remaining three groups received STS orally at doses of 2, 4, and 10 mL/kg bw daily.

Rats were monitored twice daily throughout the study for any abnormal signs. Periodic detailed clinical assessments, including functional observation parameters, were conducted 1 hr after dosing to detect potential toxicological effects, immediately after dosing. Body weights and feed consumption were measured weekly.

At the conclusion of the study, all the animals were fasted overnight with free access to water. On the day of necropsy, after measuring the final body weight, the blood samples for hematological and biochemical test, withdrawn through the puncturing of the retro-orbital sinus of animals under isoflurane anaesthesia (EZ Anaesthesia-1339), as reported.

Statistical Analysis

Statistical analyses were conducted separately for male and female animals to assess potential sex-related differences in the measured parameters. Results were expressed as mean ± Standard Error of the Mean (SEM). Comparisons among groups were performed using one-way Analysis of Variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was considered at a *p*-value of less than 0.05.

It is acknowledged that sample size (n=5 animals per sex per group) provides limited statistical power, particularly for detecting small effect sizes. Therefore, the findings were interpreted with due consideration of this limitation, and the study was primarily designed in accordance with regulatory toxicological guidelines rather than to maximize statistical power.

RESULTS

Survival and Clinical Examination

After 28 days of oral STS delivery, neither male nor female rats showed any post-dose deleterious effects on survival. At every studied dose level, there was no mortality. When STS-treated animals were compared to the control group, clinical examinations performed at predetermined intervals showed no aberrant toxic symptoms.

Body Weight

STS treatment did not produce any significant changes in body weight compared to control group (Figure 1).

Feed Consumption

Male and female rats receiving STS at all tested doses showed comparable weekly feed intake to their respective control groups, with no noticeable differences (Figure 2).

Haematology

Haematological parameters are summarized in Table 1B. STS-treated male and female rats showed no significant changes in haematological parameters compared to control animals, with all values remaining within normal physiological ranges.

Biochemistry

The impact of STS on biochemical parameters is presented in Tables 2A and 2B. In female rats, STS caused no significant changes except for a significant (p<0.05) reduction in AST level at tested dose levels 4 and 10 mL/kg bw compared to controls. In male rats, biochemical parameters remained similar to those of the control group.

Organ Weights

Oral treatment with STS for 28 days at doses of 2, 4 and 10 mL/kg bw did not cause any changes in relative organ weights of

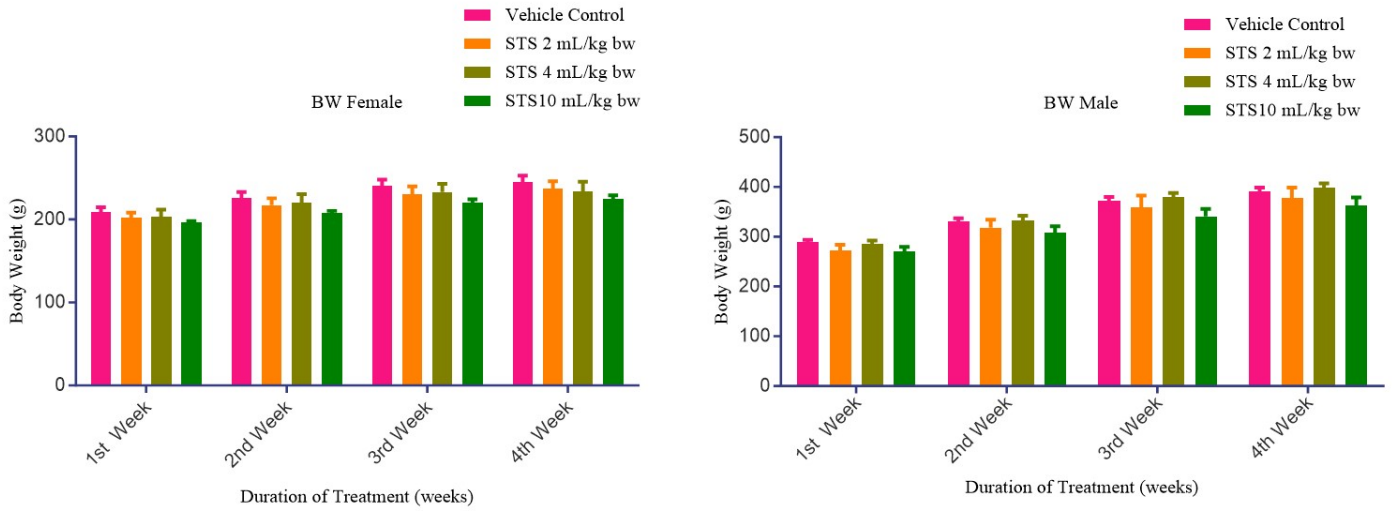


Figure 1: Mean body weight (±SEM) of male and female rats in (n=5) control and STS-treated groups (one-way ANOVA followed by Tukey's test).

Table 1A: Composition of STS.

Sl. No.	Ingredient	Scientific/Common Name	Quantity
1.	Aab-e-Toot Siyah	*Morus nigra* L.	1 L
2.	Qand Safaid	Honey or White Sugar	1.5 kg

Table 1B: Impact of STS on blood parameters in rats.

Sl. No.	Parameter	Vehicle Control	STS 2 mL/kg bw	STS 4 mL/kg bw	STS 10 mL/kg bw
			Female		
1.	Haemoglobin (g/dL)	15.36± 0.21	15.26± 0.24	15.62± 0.43	15.68± 0.33
2.	RBC (10^6/µL)	8.09± 0.08	8.11± 0.21	8.49± 0.29	8.66± 0.39
3.	WBC (10^3/µL)	11.82± 0.84	11.48± 0.92	14.78± 1.91	12.86± 1.05
4.	Platelets (10^3/µL)	1045± 43.70	1150± 46.80	1020± 76.69	929.4± 55.97
5.	HCT (%)	44.52± 0.57	44.38± 0.78	46.20± 1.37	47.20± 1.34
6.	MCV (fL)	55.04± 0.68	54.76± 0.58	54.44± 0.76	54.66± 1.08
7.	MCH (pg)	18.98± 0.21	18.84± 0.22	18.40± 0.15	18.20± 0.45
8.	MCHC (g/dL)	34.48± 0.17	34.40± 0.13	33.80± 0.25	34.00± 0.17
			Male		
1.	Haemoglobin (g/dL)	15.98± 0.60	15.84± 0.18	15.90± 0.21	15.72± 0.09
2.	RBC (10^6/µL)	8.48± 0.30	8.30± 0.22	8.35± 0.20	8.54± 0.09
3.	WBC (10^3/µL)	11.30± 1.78	13.50± 1.01	13.10± 0.96	14.06± 0.77
4.	PLT (10^3/µL)	1093± 44.15	924.8± 48.18	944.0± 47.93	971.6± 30.76
5.	HCT (%)	47.98± 1.58	47.18± 0.53	47.56± 0.63	47.50± 0.40
6.	MCV (fL)	56.58±0.55	56.98±1.32	57.00±0.78	55.62±0.50
7.	MCH (pg)	18.94±0.12	19.14±0.55	19.06±0.28	18.42±0.24
8.	MCHC (g/dL)	33.26±0.26	33.54±0.23	33.44±0.13	33.12±0.19

(Values are expressed as Mean ± SEM; sample size *n*=5; analyzed using one-way ANOVA).

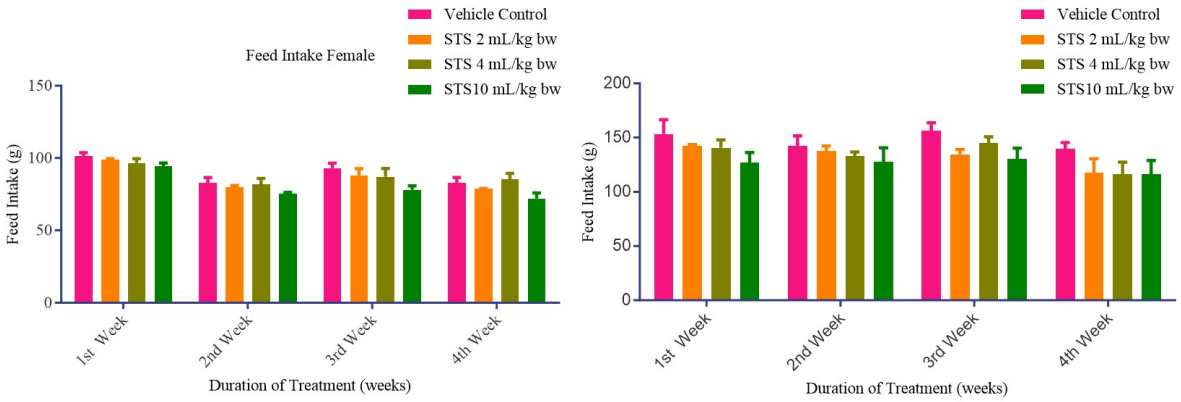


Figure 2: Mean feed consumption (±SEM) of male and female rats in (*n*=5) control and STS-treated groups (one-way ANOVA followed by Tukey's test).

the Testis, Epididymis, Uterus and Ovaries, Brain, Heart, Liver, Spleen, Adrenals and Kidney. No significant difference were between STS treated male and female rats and the control group (Table 3).

Histopathology

Histopathological evalution of organs (thyroid, thymus, brain, spleen, heart, adrenals, kidney, stomach, small intestine, testes, ovaries and uterus) collected from control and high dose (10 mL/kg bw) groups revealed no abnormalities, except for lung and liver described in Tables 4 and 5, respectively.

Histopathological findings were limited to mild changes in the lungs and liver, which were observed in both control and STS-treated groups. The pulmonary lesions were consistent with spontaneous background findings, while the hepatic alterations were mild, non-dose-dependent, and unaccompanied by biochemical evidence of liver dysfunction. These observations were considered incidental and unrelated to treatment. Overall, STS did not produce any treatment-related pulmonary or hepatic toxicity at the tested dose levels. Representative images of tissues with changes are presented in Figure 3.

DISCUSSION

Herbal-based formulations are being widely considered as safer alternatives to conventional allopathic medicines, leading to their increasing use globally for health promotion, disease prevention, and therapeutic purposes. Despite their long back history of traditional use and generally favorable safety profiles, growing evidence suggests that certain medicinal plants and herbal formulations may be associated with toxicity and Adverse Drug Reactions (ADRs). Such adverse effects may arise from inappropriate use, excessive dosage, prolonged administration, improper processing, contamination, adulteration, herb-drug interactions, or use without adequate professional guidance. Therefore, systematic safety and toxicological evaluations are essential to establish the safe use of herbal medicines and support their evidence-based integration into healthcare systems (Choudhary *et al.*, 2023).

Phytotherapy is used by over 80% of indigenous people in underdeveloped nations, while about 25% of medications sold in western pharmacies are made from plants. Herbal medicines have undoubtedly returned to the mainstream of primary health care due to their extensive availability in a variety of retail establishments, their widespread promotion in the media, and the growing involvement of large pharmaceutical companies in the production of phytomedicinal formulations (Afolabi *et al.*, 2012).

A significant constraint of traditional medical systems is the difficulty in systematically identifying and evaluating the potential adverse effects and toxicological risks associated with their therapeutic interventions. Therefore, scientific safety assessments are essential to ensure their safe and effective use (Sharwan *et al.*, 2015). Despite the fact that these products have been utilised in clinical settings for centuries, little is known about their active ingredients or potentially harmful substances. In order to evaluate the safety of STS Unani formulation a 28-day repeated oral dose toxicity study in SD rats was conducted. Throughout the course of the research, there were no deaths or clinical indications of systemic toxicity. Monitoring changes in body weight and feed intake is a crucial component of toxicological studies since these variations can reveal possible harmful effects of the test drug.

Table 2A: Impact of STS on blood biochemical markers in female rats.

Sl. No.	Parameter	Vehicle Control	STS 2 mL/kg bw	STS 4 mL/kg bw	STS 10 mL/kg bw
1.	Glucose (mg/dL)	73.20± 4.44	83.00± 4.70	91.60± 7.94	80.60± 2.76
2.	AST (IU/L)	128.4± 4.45	123.6± 1.50	100.2± 7.41*	101.2± 9.89*
3.	ALT (IU/L)	47.00± 2.88	40.20± 2.55	37.20± 3.83	42.80± 2.63
5.	ALP (IU/L)	122.8± 12.14	107.4± 15.24	126.4± 15.99	140.8± 19.06
6.	Blood Urea (mg/dL)	40.20± 2.92	42.60± 2.83	44.60± 4.38	42.80± 1.88
7.	Creatinine (mg/dL)	0.41± 0.02	0.42± 0.02	0.42± 0.02	0.44± 0.02
8.	Uric Acid (mg/dL)	1.86± 0.16	1.84± 0.19	1.68± 0.18	1.26± 0.06
9.	Total Cholesterol (mg/dL)	78.00±4.64	79.60±5.25	69.20±3.33	72.60±4.52
10.	Triglyceride (mg/dL)	55.60±7.80	74.20±8.42	68.40±5.52	58.40±6.43

(Values are expressed as Mean ± SEM; sample size of 5 per sex; analyzed by ANOVA; * indicates $p<0.05$ compared to Vehicle Control).

Table 2B: Impact of STS on blood biochemical parameters in male rats.

Sl. No.	Parameter	Vehicle Control	STS 2 mL/kg bw	STS 4 mL/kg bw	STS 10 mL/kg bw
1.	Glucose (mg/dL)	77.60±6.43	91.60±6.54	101.6±7.11	100.6±2.48
2.	AST (IU/L)	95.80±4.56	123.0±10.97	122.0±6.87	108.2±7.95
3.	ALT (IU/L)	43.60±2.31	52.80±3.44	55.40±2.56	40.80±3.48
5.	ALP (IU/L)	281.2±29.90	281.8±25.24	343.6±24.04	274.4±33.00
6.	Blood Urea (mg/dL)	42.80±0.86	38.20±1.59	38.60±3.4	40.40±2.31
7.	Creatinine (mg/dL)	0.44±0.01	0.44±0.02	0.58±0.03	0.58±0.03
8.	Uric Acid (mg/dL)	1.54± 0.15	1.32± 0.09	1.36± 0.02	1.34± 0.04
9.	Total Cholestrol (mg/dL)	56.00±4.42	55.80±3.91	50.20±2.7	55.40±1.07
10.	Triglyceride (mg/dL)	96.40±11.3	116.6±20.7	102.2±8.9	137.8±6.37

(Values are expressed as Mean ± SEM; sample size = 5 per sex; analyzed using ANOVA).

Table 3: Rats treated with STS and control groups' relative organ weight.

Organ	Vehicle Control	STS 2 mL/kg bw	STS 4 mL/kg bw	STS 10 mL/kg bw
Female				
Brain	0.86±0.01	0.88±0.03	0.88±0.02	0.91±0.03
Heart	0.35±0.02	0.34±0.00	0.41±0.02	0.40±0.03
Liver	3.72±0.04	3.61±0.18	3.56±0.13	3.40±0.14
Spleen	0.26±0.01	0.24±0.01	0.27±0.02	0.29±0.01
Adrenals	0.03±0.00	0.11±0.08	0.04±0.00	0.03±0.00
Kidneys	0.79±0.01	0.77±0.02	0.76±0.03	0.76±0.03
Uterus and Ovaries	0.39±0.05	0.34±0.02	0.33±0.03	0.31±0.02
Male				
Brain	0.57±0.03	0.51±0.12	0.59±0.01	0.61±0.02
Heart	0.31±0.011	0.34±0.01	0.34±0.01	0.37±0.02
Liver	3.62±0.26	3.87±0.07	3.72±0.10	3.97±0.14
Spleen	0.24±0.02	0.25±0.02	0.24±0.01	0.20±0.01
Adrenals	0.01±0.00	0.018±0.00	0.018±0.00	0.021±0.00
Kidneys	0.80±0.01	0.81±0.04	0.80±0.01	0.82±0.02
Testes and Epididymis	1.01±0.14	1.38±0.09	1.20±0.10	1.25±0.09

(Values are expressed as Mean ± SEM; sample size = 5 per sex; analyzed using ANOVA.).

Table 4: Summary of histopathological findings in the lungs of control and STS-treated rats.

Sl. No.	Histological findings	Vehicle control *n*=10	STS 10 mL/kg bw *n*=10
1.	Normal	0 (0%)	2/10=20%
2.	Chronic Interstitial Pneumonitis (CIPn) - I	8/10=80%	6/10=60%
3.	CIPn - II	1/10=10%	1/10=10%
4.	CIPn - III	1/10=10%	0 (0%)
5.	Not Included (NI)	0 (0%)	1/10=10%

Lungs of 20% of animals in the STS group were normal histologically. Chronic interstitial pneumonitis of different grades was observed in lungs of the control (CIPn - I- 80%, CIPn - II-10%, and CIPn - III-10%), STS group (CIPn - I- 60%, CIPn - II-10%).

Table 5: Summary of histopathological findings in the liver of control and STS treated rats.

Sl. No.	Histological findings	Vehicle control *n*=10	STS 10 mL/kg bw *n*=10
1.	Normal	1/10=10%	3/10=30%
2.	Focal inflammation <2 Foci/HPF (Score 1)	0 (0%)	0 (0%)
3.	Microvesicular steatosis (Mivs) 5-33% (Score 1); Total Score = 1	0 (0%)	0 (0%)
4.	Lobular inflammation (Score 1); Total Score = 1	4/10=40%	1/10=10%
5.	Mivs 5-33% (Score 1), Lobular inflammation (Score 1); Total Score = 2	5/10=50%	4/10=40%
6.	Mivs 33- 66% (Score 2); Total Score = 2	0 (0%)	0 (0%)
7.	Lobular Inflammation (Score 2); Total Score = 2	0 (0%)	0 (0%)
8.	Mivs 33-66% (Score 2); Total Score = 2	0 (0%)	2 (20%)

10% livers of the control group and 30% in STS treated group were normal histologically. 40% animals of the control group exhibited lobular inflammation while STS group showed 10%. 50% liver of control and 40% of STS treated animals showed combined steatosis and lobular inflammation. 20% of STS treated animal showed microvascular steatosis.

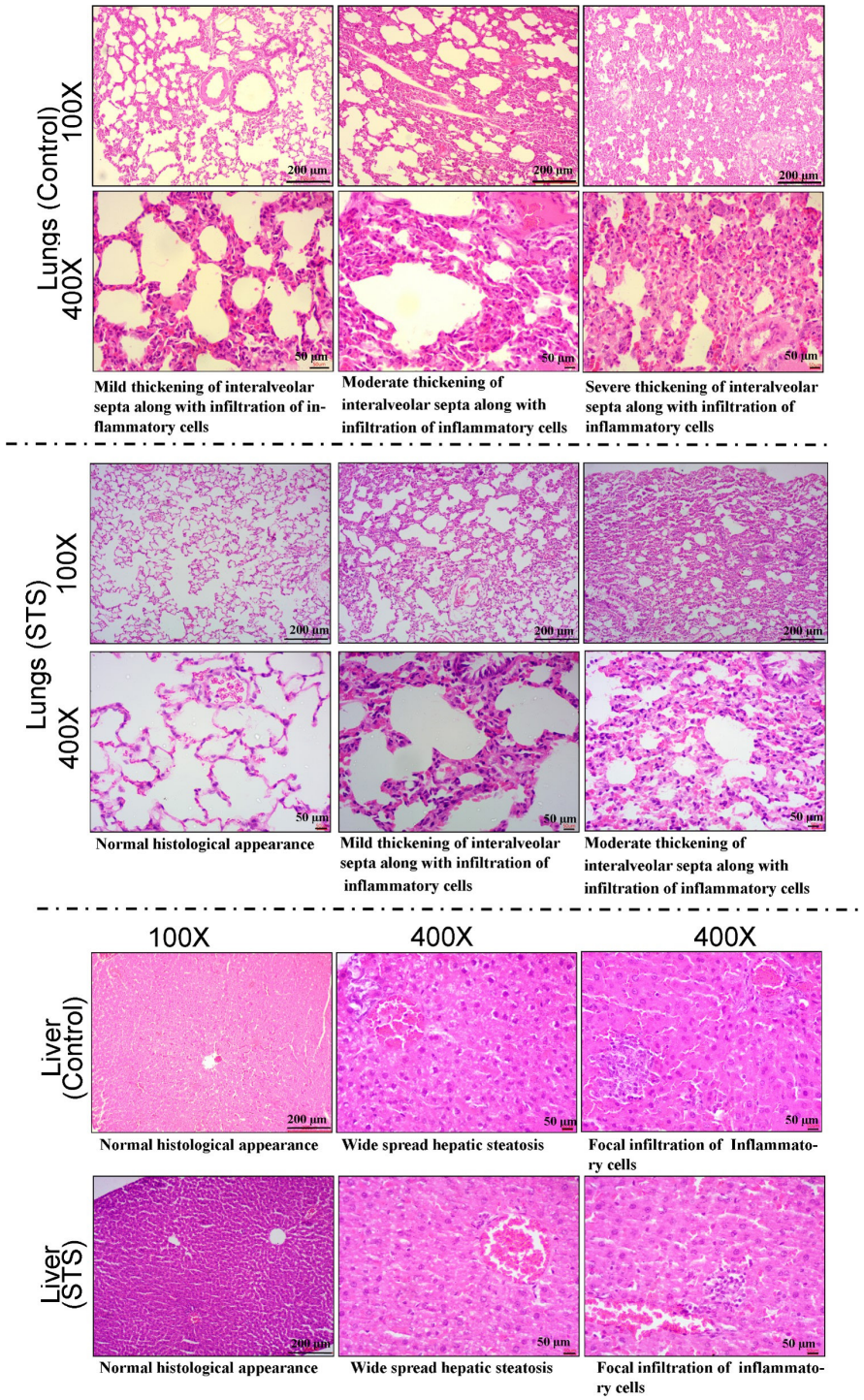


Figure 3: Representative photomicrographs (100 x & 400 x) of lung and liver from control and STS-treated rats. Lungs sections showing normal histoarchitecture, mild, moderate and severe thickening of interalvelolar septa, with inflammatory cells in different panels. Liver sections showing normal histoarchitecture, hepatic steatosis and inflammatory changes of different grades.

In experimental research, measurements of feed and water intake offer reliable indications of the animals' growth rate and overall physiological health (Todic *et al.*, 2003). There were no differences between the STS-treated and control groups' weekly body weights and feed consumption, suggesting that STS does not impair growth or result in systemic toxicity in rats.

STS did not produce significant changes in haematological parameters at any dose. Biochemical parameters were largely unaffected, except for a slight decrease ($p<0.05$) in AST level in females at STS 4 and 10 mL/kg bw, which remained within normal physiological limits and is not considered toxicologically relevant. Male rats showed no biochemical alterations.

High cholesterol and triglyceride levels are linked to atherosclerosis and a higher risk of cardiovascular disease (Todic *et al.*, 2003). In the present study, these lipid levels remained unchanged in both sexes at all tested doses.

No significant changes were observed in the measured biochemical parameters in the present study, corroborating the results of the acute and subacute oral toxicity study conducted by Figueredo *et al.*, which also reported the absence of treatment-related biochemical alterations following administration of the ethanolic extract of *Morus nigra* leaves (Figueredo *et al.*, 2018).

Organ-to-body weight ratios should be assessed in toxicity studies lasting seven days to a year, according to the Society of Toxicologic Pathology (Shen, 2007; Sellers *et al.*, 2007). In comparison to controls, the relative organ weights of male and female rats in this study did not significantly change at any tested dose of STS. No obvious anomalies in any organs or tissues were found during necropsy.

The most reliable way to diagnose a variety of pathological diseases is by histological testing (Urooj *et al.*, 2018). Histopathological evaluation revealed that treatment-related microscopic changes were not observed in any of the examined organs following repeated oral administration of STS. The notable histological findings were confined to the lungs and liver and were detected in both vehicle control and treatment groups. In the lungs, the observed lesions consisted of mild to moderate inflammatory and structural alterations that are commonly encountered as spontaneous background findings in laboratory rodents. The incidence, severity, and distribution of these pulmonary changes did not demonstrate any treatment-related pattern and were comparable between control and STS-treated animals.

Similarly, hepatic examination revealed only mild histological alterations, which were observed sporadically across groups without any clear dose-response relationship. Importantly, these microscopic findings were not associated with corresponding significant changes in serum biochemical parameters indicative of liver injury, such as hepatic enzyme levels, suggesting that liver function remained unaffected. The absence of dose dependency, together with the lack of supporting clinical, biochemical, or gross pathological evidence, indicates that the hepatic observations were incidental in nature and not attributable to STS administration.

Overall, the histopathological findings observed in the present study were considered either spontaneous background lesions or incidental changes commonly encountered in laboratory animals (McInnes and Scudamore 2014). No treatment-related adverse effects were identified in the lungs, liver, or any other examined organs. Therefore, repeated oral administration of STS at the tested dose levels was not associated with pulmonary or hepatic toxicity and demonstrated a favorable safety profile under the conditions of this study.

CONCLUSION

The results of the study demonstrated no toxicologically significant effects on body weight, feed consumption, behavioural observations, haematological parameters, biochemical markers, relative organ weights, or gross necropsy findings in either the STS-treated groups or the control group. Histopathological examination of all major organs revealed normal architecture, with only minimal to moderate changes observed in the lungs and liver of animals in both the vehicle control and STS-treated (10 mL/kg) groups. As similar findings were present in the control animals, these alterations were considered unrelated to STS administration. The study results demonstrate that STS was well tolerated, with no evidence of treatment-related toxicity at the evaluated dose levels.

ACKNOWLEDGEMENT

The authors acknowledge the Director General, Central Council for Research in Unani Medicine, Ministry of Ayush, Government of India, New Delhi for providing financial resources and infrastructure for the study. We also thanks Dr. Tasleem Ahmad and Dr. Syeda Hajra Fatima for providing service for hematological and biochemical analysis of blood samples. We also thanks National Institute of Nutrition, Hyderabad for providing services for histopathological examination.

ABBREVIATIONS

STS: Sharbat-e-Toot Siyah; **LD_{50}:** Lethal Dose 50; **SD:** Sprague Dawley; **bw:** Body Weight; **CCSEA:** Committee for Control and Supervision of Experiments on Animals; **OECD:** Organisation for Economic Co-operation and Development; **IAEC:** Institutional Animal Ethics Committee; **BSA:** Body Surface Area; **SEM:** Standard Error of the Mean; **ANOVA:** Analysis of Variance; **RBC:** Red Blood Cells; **WBC:** White Blood Cells; **PLT:** Platelets; **HCT:** Hematocrit; **MCV:** Mean Corpuscular Volume; **MCH:** Mean Corpuscular Hemoglobin; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **AST:** Aspartate Aminotransferase; **ALT:** Alanine Aminotransferase; **ALP:** Alkaline Phosphatase; **CIPn:** Chronic Interstitial Pneumonitis; **NI:** Not Included; **Mivs:** Microvesicular Steatosis; **HPF:** High Power Field; **ADRs:** Adverse Drug Reactions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Al Dhanhani, A., Tsombou, F., Mirza, S., Shahin, S., & Ridouane, F. (2025). Phytochemical and antimicrobial properties of Morus nigra and Morus alba grown in Fujairah, United Arab Emirates. Scientific Reports, 15(1), 34986. https://doi.org/10.1038/s41598-025-18872

Anonymous. (2006). National formulary of Unani medicine (Part I, p. 224). Central Council for Research in Unani Medicine, Ministry of Health & Family *Welfare (*Department *of AYU*SH), Government of India.

Anonymous. (2006). National formulary of Unani medicine (Part I, p. 333). Central Council for Research in Unani Medicine, Ministry of Health & Family Welfare (Department of AYUSH), Government of India.

Afolabi, S. O., Akindele, A. J., Awodele, O., Anunobi, C. C., Adeyemi, O. O. (2012). A 90-day chronic toxicity study of Nigerian herbal preparation DAS-77 in rats. BMC Complementary and Alternative Medicine, 12, 79.

Choudhury, A., Singh, P. A., Bajwa, N., Dash, S., Bisht, P. (2023). Pharmacovigilance of herbal medicines: Concerns and future prospects. Journal of Ethnopharmacology, 309, 116383.

Figueredo, K. C., Guex, C. G., Reginato, F. Z., Haas da Silva, A. R., Cassanego, G. B., Lhamas, C. L., Boligon, A. A., Lopes, G. H. H., de Freitas Bauermann, L. (2018). Safety assessment of Morus nigra L. leaves: Acute and subacute oral toxicity studies in Wistar rats. Journal of Ethnopharmacology, 224, 290-296.

McInnes, E. F., Scudamore, C. L. (2014). Review of approaches to the recording of background lesions in toxicologic pathology studies in rats. Toxicology Letters, 229(1), 134-143.

Organisation for Economic Co-operation and Development. (2025). Test No. 407: Repeated dose 28-day oral toxicity study in rodents (OECD Guidelines for the Testing of Chemicals, Section 4)..

Reagan-Shaw, S., Nihal, M., Ahmad, N. (2008). Dose translation from animal to human studies revisited. The FASEB Journal, 22(3), 659-661.

Sellers, R. S., Morton, D., Michael, B., Roome, N., Johnson, J. K., Yano, B. L., Perry, R., Schafer, K. (2007). Society of Toxicologic Pathology position paper: Organ weight recommendations for toxicology studies. Toxicologic Pathology, 35(5), 751-755.

Sharwan, G., Jain, P., Pandey, R., Shukla, S. S. (2015). Toxicity profile of traditional herbal medicine. Journal of Ayurveda and Herbal Medicine, 1(3), 81-90.

Shen, G. X. (2007). Lipid disorders in diabetes mellitus and current management. Current Pharmaceutical Analysis, 3(1), 17-24.

Todic, M., Bakić, S., Begović, B., Krosnjar, S., Zulić, I. (2003). Food and water consumption in assessment of acute oral toxicity of HEPALIP FORTE in rats. Bosnian Journal of Basic Medical Sciences, 3(4), 47-53.

Urooj, M., Khan, M. A., Thejaswini, G., Kazmi, M. H., Husain, G. M. (2018). Toxicity evaluation of a traditional polyherbal Unani formulation Jawarish Shahi in rats. The Journal of Phytopharmacology, 7(5), 412-418.

Cite this article: Urooj M, Husain GM, Dwivedi DK, Kodavath D, Vinay E, *et al*. Preclinical Toxicological Evaluation of Sharbat-e-Toot Siyah, a Traditional *Morus nigra* L. Based Unani Syrup. J Young Pharm. 2026;18(3):807-15.