第 49 卷 第 9 期
2022 年 9 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)

Vol. 49 No. 9
September 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.9.8

Acute and Sub-Acute Toxicity of Spray-Dried Extract of *Tribulus Terrestris*

Sidra Yasmeen[1], Sadaf Yasmeen[2], Fatima Qamar[3]*, Saif Ur Rehman Khattak[4], Safila Naveed[3], Mahrukh Mughal[4], Halima Sadia[5]

[1] Department of Pharmaceutics, Faculty of Pharmacy, Jinnnah University for Women, Karachi, Pakistan

[2] Department of Pharmacology, Faculty of Pharmacy, University of Sargodha, Pakistan

[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jinnnah University for Women, Karachi, Pakistan

[4] Drug Regulatory Authority of Pakistan

[5] Department of Pharmacy Practice, Faculty of Pharmacy, Jinnnah University for Women, Karachi, Pakistan

Abstract: The use of herbal medicines has tremendously grown over the past few years. Owing to their safety, they are more preferred over conventional allopathic medicine. Plant-based therapies have significantly improved and protected human health. Additionally, dry powdered herbal medications are more effective than liquid extracts. The numerous claims are not backed up by any data that can be verified. It is the first time that the activity of *Tribulus Terrestris* spray-dried extract has been linked to acute and suba-cute oral toxicity. The objective of the current work was to investigate the acute and sub-acute toxicity of spray-dried extract (SDE) of *Tribulus terrestris*. For acute toxicity, two different doses, i.e., 2000 and 5000 mg/kg, were given to the female Wistar rats. Only one dose was administered and each animal was observed for any signs of mortality along with behavioral changes and physical symptoms for 2 weeks. Multiple doses of the SDE were given to the Wistar (both genders) rats for 28 days in the sub-acute toxicity. Animals of both the genders received 500, 1000, and 1500 mg/kg/day SDE doses for 28 days consecutively. Blood samples were obtained for biochemical and hematological analysis on the 29th day of the experiment, following which subjects were sacrificed for the histopathological examination. The results of acute toxicity revealed no signs of morbidity and mortality in rats. Similar results were recorded in the sub-acute toxicity. Mild variations were observed at 1000 and 1500 mg/kg/day, however results were non-significant. The SDE of *Tribulus terrestris* did not show any significant harmful effects in the treated animals after 14 and 28 days of treatment. Therefore, SDE of *Tribulus terrestris* can be safely employed in pharmaceutical formulations for therapeutic use, addressing further safety in chronic and sub-chronic study.

Keywords: *Tribulus terrestris*, spray-dried extract, acute toxicity, sub-acute toxicity, Wistar rats, histopathology.

刺蒺藜喷雾干燥提取物的急性和亚急性毒性

摘要：在过去的几年里，草药的使用有了极大的增长。由于它们的安全性，它们比传统的对抗疗法药物更受欢迎。基于植物的疗法已显着改善和保护人类健康。此外，干粉草药比液体提取物更有效。众多声明没有任何可以验证的数据支持。这是首次将刺蒺藜喷雾干燥提取物的活性与急性和亚急性口服毒性联系起来。当前工作的目的是研究刺蒺藜喷雾干燥提取物(SDE)的急性和亚急性毒性。对于急性毒性，给予雌性维斯塔大鼠两种不同的剂量，即 2000 和 5000 毫克/公斤。仅施用一剂，并且观察每只动物的任何死亡迹象以及行为变化和身体症状 2 周。在亚急性毒性下，给予维斯塔（两性）大鼠多剂量的 SDE，持续 28 天。两种

Received: June 25, 2022 / Revised: July 21, 2022 / Accepted: August 20, 2022 / Published: September 30, 2022

About the authors: Sidra Yasmeen, Department of Pharmaceutics, Faculty of Pharmacy, Jinnnah University for Women, Karachi, Pakistan; Sadaf Yasmeen, Department of Pharmacology, Faculty of Pharmacy, University of Sargodha, Pakistan; Fatima Qamar, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jinnnah University for Women, Karachi, Pakistan; Saif Ur Rehman Khattak, Drug Regulatory Authority of Pakistan; Safila Naveed, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jinnnah University for Women, Karachi, Pakistan; Mahrukh Mughal, Drug Regulatory Authority of Pakistan; Halima Sadia, Department of Pharmacy Practice, Faculty of Pharmacy, Jinnnah University for Women, Karachi, Pakistan

Corresponding author Fatima Qamar, fatimamudassar2009@hotmail.com

性别的动物连续 28 天接受 500、1000 和 1500 毫克/公斤/天的 SDE 剂量。实验第 29 天取血样进行生化和血液学分析，随后处死受试者进行组织病理学检查。急性毒性的结果显示大鼠没有发病和死亡的迹象。在亚急性毒性中记录了类似的结果。在 1000 和 1500 毫克/公斤/天观察到轻微的变化，但结果不显着。蒺藜的 SDE 在治疗 14 天和 28 天后在治疗动物中没有显示出任何显着的有害影响。因此，蒺藜的 SDE 可以安全地用于治疗用途的药物制剂，进一步解决慢性和亚慢性研究中的安全性问题。

关键词：刺蒺藜，喷雾干燥提取物，急性毒性，亚急性毒性，维斯塔大鼠，组织病理学.

1. Introduction

Globally, many people use plant-based medicines for managing their primary healthcare needs. Plant-based medicines are generally perceived as safe and non-toxic. Moreover, owing to their easy accessibility and affordability, they are sought as the most reliable medicines, and interest in them has increased considerably over the years [1].

Compared with conventional allopathic medicines, plant-based medicines are frequently administered as viscous products, liquid extracts and dry powders. Drying is generally achieved by directly drying the extract or drying and comminuting the plant material. Of all forms, dried powder form is the most preferred. It offers the advantage of microbial and physicochemical stability, higher concentration of active compound, easy to transform into other pharmaceutical forms, compared to the conventional liquid form [2].

Many techniques are employed for the drying of plant extracts. However, thermal stability and physico-chemical properties of the plant material are the critical factors that need to be undertaken before selecting any of the drying technique. In contrast to the other drying techniques, spray drying technique offers more advantages. Safety, efficacy and quality of the products is guaranteed and the standardization of the active pharmaceutical ingredient can be easily achieved. Literature has reported that dried powders achieved via this technique can be used as final products in solid dosage form and even have good reconstitution characteristics [3].

The target of the current investigation was to evaluate the toxicity profile of the spray-dried extract (SDE) of Tribulus terrestris (T.T); through single-dose acute toxicity and 28 days of repeated dosing in Wistar rats. *Tribulus terrestris*, commonly known as the yellow vine or gokharu, belongs to Zygophyllaceae. The plant is extensively found in the southern part of Europe, Africa, China, Japan, Korea, and Western Asia. Extracts of this plant have been reported to have numerous therapeutic effects in various ailments. The plant possesses anti-bacterial, anti-inflammatory, anti-urolithic, anti-hypertensive, anti-viral, and aphrodisiac properties. The therapeutic activity of the plant is contributed by the rich composition of flavonoids and steroidal saponins constituents. Owing to many steroidal saponins in the plant, the plant is of vital pharmaceutical interest [4, 5]. Kamenov et al. reported good outcomes with T.T in males with erectile dysfunction and reduced sexual desire [6]. Protodioscin is the main component of this plant. A study established that protodioscin enhanced the sperm development in distressed males, in addition to eliminating menopause libido problems in females [7]. Another study reported that T.T concentrate can be used as a substitute for treating hypoactive sexuality in females after menopause. It has also been reported to minimize menopausal symptoms and adverse effects by increasing serum levels of bioavailable, unbound testosterone TT to effectively treat kidney infections [8]. Indians used it as a herbal tonic for infectious diseases of urethra and a diuretic agent for renal problems. Sharma et al. separated TT extract and described that the n-butanol aliquots had a markable antiurolithiatic activity in vitro and ex vivo [9]. A Asadmobini et al. studied antioxidant effect of TT on cyropreserved sperm samples, which improves human sperm motility and viability [10]. A study examined protodioscin prohibited cell multiplication in leukemia HL-60 [11]. Data regarding the toxicity profile of T.T is very scarce. Although a study has reported the toxicity profile of the methanolic extract of T.T oral administration where no drastic changes were observed [12]. Moreover, there is insufficient information regarding the toxicity profile of the SDE in Wistar rats.

2. Methods and Materials

T.T was obtained from the local markets of Karachi and verified from the Pharmacognosy department of Faculty of Pharmacy, Jinnah University for Women. The herbs were dried and then subjected to crushing. The crushed herbs were mixed in the menstruum for extraction which, composed of purified water and ethyl alcohol (1:1.5). The mixture of crushed herbs and solvent mixture in the ratio of 1:5 was boiled for 120 ± 5 min with temperature kept up to 77 ± 5°C and

pressure up to 430 ± 30 mmHg. Following the decantation of the extract at the frequency of 41 ± 1 Hz, the extract was collected in the receiving chamber. Subsequently, the resultant extract was evaporated after being filtered through the sieve of 350 mesh size. The dense liquid was then introduced into the feeding container for spray drying. The drying operation was accomplished at 183 ± 2°C. The spray-dried extract was then subjected to blending done in a blender set at a speed of 20 ± 2 rpm and then gathered in an air lock polybag and double sealed. It was kept in another polybag with 40 gm silica gel. The end product was yellowish-brown fine powder.

2.1. Toxicity Studies

The study complies with the ARRIVE guidelines (https://www.nc3rs.org.uk/arrive-guidelines), the U.K. Animals (Scientific Procedures) Act 1986, and connected guidelines, EU Directive 2010/63/EU for animal experiments (https://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) or the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised in 1978). Healthy Wistar rats measuring about 150–250 g and 10–12 weeks grown were picked. For acute toxicity investigation, only females were used, while, for the sub-acute toxicity, both the genders were used. Rats were acquired from the Animal House of the Pharmacology Department, Faculty of Pharmacy, Jinnah University for Women. The animals were kept in propylene cages under specified environmental conditions and were provided free access to water *ad libidum*. Before starting the experiment animals were adapted to the laboratory environment.

2.2. Acute Toxicity

It was conducted according to directives provided by the OECD TN. 423. Nine Wistar female rats were splitted into three groups of three rats. Two groups were given unit doses of 2000 and 5000 mg/kg of the SDE of T.T respectively. The third group was the control. Subsequently, the rats were strictly monitored for the first 30 min and, subsequently, for the first 24 h. After that, they were daily observed for two weeks. Meanwhile, rats were also observed for any altered central and autonomic nervous system effects, changes in feed consumption, water drinking and variation in fur and body weight. Moreover, signs for any mortality were also noted down. After 14 days, animals of the higher dose treated group were humanely dissected under chloroform anesthesia and vital organs (heart, liver and kidney) were stored in formalin for the histo-pathological evaluations.

2.3. Sub-Acute Toxicity

It was also conducted following the OECD directives (OECD, Test No. 407). Forty Wistar rats of both the genders were divided in to 4 groups of 6 males and 4 females each. Three groups received oral experimental doses of SDE T.T 500, 1000 and 1500 mg/kg/day respectively. The last group was the control. The experimental doses were administered repeatedly for four weeks. Variations in the body weight of the animals were noted weekly in addition to the general morphological and behavioral changes. After four weeks, animals were humanely dissected under chloroform anesthesia. Blood specimens were obtained by heart puncture into gel and clot activator tubes for serum biochemical parameters evaluation and EDTA tubes for hematological analysis. Serum was obtained via centrifugation following the blood congealing for 30 min. Vital organs (liver, kidney, and heart) were then obtained from the animals after sacrificing them for the histopathological examination. Additionally, mortality was also recorded in each group during the study period.

2.4. Biochemical and Hematological Parameters

The biochemical profile includes Na, K, Cl, Cr, urea, uric acid, total protein, albumin, globulin, albumin-globulin ratio, S.total bilirubin, S. direct bilirubin, S. indirect bilirubin, ALP, Gamma GT, AST, ALPT, triglycerides, serum cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL- cholesterol and non-HDL cholesterol. They were evaluated after serum separation at Dow laboratory, Karachi. Hematological parameters include hemoglobin HB, (RBC), HCT, MCV, MCH and (MCHC), (WBC), neutrophils, lymphocyte, eosinophils, basophils, monocyte, and platelet count. Estimation was performed using Hematology Analyzer at Dow Laboratory, Karachi, Pakistan [13, 14].

2.5. Organ Weights and Histopathology

All major organs, namely heart, liver, left and right kidneys, were separated, observed, washed with normal saline, weighed, and stored in 10% buffered formalin. Then, relative organ weight (ROW) was determined by the following formula (14):

$$\text{ROW} = \frac{Organ\ weig\ ht}{body\ weig\ ht\ before\ \ dissection} \times 100$$

The preserved organs were inserted in paraffin, sliced into slender sections and stained with blue dye hematoxylin and pink dye eosin and final glass slides were visualized under a picture microscope for any pathology and photographs were taken.

2.6. Statistical Data Analysis

Statistical inference was conducted using SPSS version 20.0. Comparisons between sample and control groups were executed by one-way analysis of variance (ANOVA) succeeded by Tukey's and Dunnet's multiple comparison. A p-value less than 0.05 was recognized to be significant.

3. Results and Discussion

3.1. Acute Toxicity

All the selected subjects were carefully monitored for any indications of toxicity. The animals were monitored at the start of the experiment and during different intervals throughout the study period, with special emphasis on the first 4 h of the experiment. The clinical parameters do not show any indication of toxicity at either of the doses. Moreover, no mortality was found, indicating that the toxic dose of SDE of T.T was higher than 5000 mg/kg. The histopathological evaluation of the liver, kidneys and heart further revealed that the LD50 of the SDE of T.T is far greater than 5000 mg/kg. The histopathological sections showed no significant pathological changes with normal appearance and preserved architecture (Fig. 1).

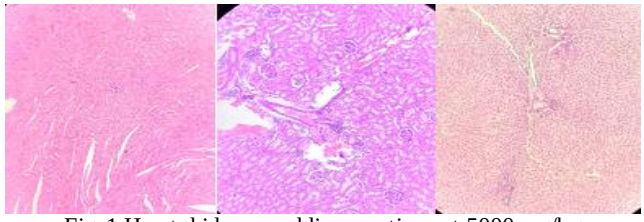

Fig. 1 Heart, kidney, and liver sections at 5000 mg/kg

3.2. Sub-Acute Toxicity

It was conducted using three different doses of the SDE of T.T (500, 1000, and 1500 mg/kg/day) for 4 weeks. On the 29th day of the experiment, biochemical, hematological, and histopathological assessments were done. The body weights of all rats showed a significant change. A rise in the weights of rats was observed throughout the study. Compared to the control, all the three treated groups showed a steady raise in the body weight. Contrary to the body weights, no significant variation was noted in the relative organ weight in the treated groups in contrast to the control (Table 1).

Table 1 Results of various doses of SDE T.T on body weight and relative organ weight of rats

Dose mg/kg	Gender	Body weight Day 1	Body weight Day 28	Heart	R. Kidney	L. kidney	Liver
Control	M	234 ± 12	302 ± 22	0.95 ± 0.9	0.89 ± 0.7	0.86 ± 0.9	9.1 ± 1.2
	F	170 ± 5.6	210 ± 12	0.86 ± 0.11	0.61 ± 0.05	0.56 ± 0.05	5.8 ± 0.3
500	M	243 ± 6.7	293 ± 27	0.98 ± 0.09	0.83 ± 0.08	0.85 ± 0.07	8.9 ± 0.8
	F	171 ± 3.8	215 ± 4.8	0.84 ± 0.05	0.64 ± 0.05	0.59 ± 0.09	5.6 ± 0.3
1000	M	203 ± 8.9	279 ± 28.8	0.93 ± 0.02	0.80 ± 0.1	0.86 ± 0.11	8.2 ± 1.2
	F	183 ± 3.4	224 ± 16	1.04 ± 0.33	0.66 ± 0.1	0.61 ± 0.1	6.3 ± 1.3
1500	M	253 ± 3.33	336 ± 20	1.33 ± 0.1	0.96 ± 0.1	1.04 ± 0.03	10.9 ± 1.9
	F	170 ± 5.0	192 ± 2.2	0.71 ± 0.04	0.52 ± 0.04	0.48 ± 0.03	5.6 ± 0.2

Notes: R. - right, L. - left, M - male, F - female. The values are stated as mean and standard error of the mean (n = 5). For statistical inference one-way ANOVA followed by Tukey's and Dunnet's multiple comparison was applied. p-value higher than 0.05 is not statistically significant.

3.3. Biochemical Parameters

Uric acid, urea, creatinine, sodium and potassium levels were observed for determining the effect of different doses of SDE T.T on renal parameters. The results reveal that a raise was observed in the uric acid levels at all the doses, urea and creatinine were lowered. However, this change was not statistically significant. Similarly, no significant variation was noted in the sodium and potassium levels (Table 2).

Table 2 Consequences of various doses of SDE T.T on renal profile

Dose mg/kg	Gender	Body weight Day 1	Body weight Day 28	Heart	R. kidney	L. kidney	Liver
Control	M	234 ± 12	302 ± 22	0.95 ± 0.9	0.89 ± 0.7	0.86 ± 0.9	9.1 ± 1.2
	F	170 ± 5.6	210 ± 12	0.86 ± 0.11	0.61 ± 0.05	0.56 ± 0.05	5.8 ± 0.3
500	M	243 ± 6.7	293 ± 27	0.98 ± 0.09	0.83 ± 0.08	0.85 ± 0.07	8.9 ± 0.8
	F	171 ± 3.8	215 ± 4.8	0.84 ± 0.05	0.64 ± 0.05	0.59 ± 0.09	5.6 ± 0.3
1000	M	203 ± 8.9	279 ± 28.8	0.93 ± 0.02	0.80 ± 0.1	0.86 ± 0.11	8.2 ± 1.2
	F	183 ± 3.4	224 ± 16	1.04 ± 0.33	0.66 ± 0.1	0.61 ± 0.1	6.3 ± 1.3
1500	M	253 ± 3.3	336 ± 20	1.33 ± 0.1	0.96 ± 0.1	1.04 ± 0.03	10.9 ± 1.9
	F	170 ± 5.0	192 ± 2.2	0.71 ± 0.04	0.52 ± 0.04	0.48 ± 0.03	5.6 ± 0.2

Notes: M - male, F - female. Values are stated as mean and standard error of the mean (n = 5). For statistical inference one-way ANOVA followed by Tukey's and Dunnet's multiple comparison was applied. p-value higher than 0.05 is not statistically significant.

No significant change was noted in the liver enzymes in the tested groups. Mild variation was observed but, it was found to be statistically non-significant (Table 3).

Table 3 Consequences of various doses of SDE T.T on liver profile

Liver parameters	Group 1 (Control)		Group 2 (500 mg/kg)		Group 3 (1000 mg/kg)		Group 4 (1500 mg/kg)		p-value
	M	F	M	F	M	F	M	F	
Total Bilirubin (mg/dL)	0.2 ± 0.05	0.14 ± 0.01	0.17 ± 0.03	0.13 ± 0.01	0.155 ± 0.01	0.13 ± 0.03	0.18 ± 0.01	0.15 ± 0.02	0.655
Bilirubin direct (mg/dL)	0. ± 0	0.09 ± 0.01	0.1 ± 0	0.1 ± 0	0.08 ± 0.04	0.1 ± 0	0.1 ± 0.01	0.12 ± 0.02	0.233

Continuation of Table 3									
Bilirubin Indirect (mg/dL)	0.1 ± 0.01	0.08 ± 0.01	0.07 ± 0.03	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.9 ± 0.02	0.09 ± 0.01	0.168
ALP (U/L)	315.32 ± 10.16	286 ± 10	250 ± 76	48 ± 1	96.8 ± 13.8	139 ± 95.7	142 ± 18	94.7 ± 4.9	0.696
SGOT (U/L)	168.24 ± 6.21	157 ± 12	180 ± 0	202 ± 1.5	199 ± 21	222 ± 16.7	206 ± 9.11	235 ± 10	0.304
SGPT (U/L)	26 ± 2	25 ± 1	32 ± 2	31.5 ± 2.5	34.8 ± 2.3	35.33 ± 6.8	40 ± 2.22	34.7 ± 1.6	0.280
Gamma GT	< 4 ± 0	< 4 ± 0	< 4 ± 0	< 4 ± 0	< 4 ± 0	< 4 ± 0	< 4 ± 0	< 4 ± 0	-

Notes: M - male, F - female. Values are expressed as mean and standard error of the mean (n = 5). For statistical inference one-way ANOVA followed by Tukey's and Dunnet's multiple comparison was applied. p-value higher than 0.05 is not statistically significant.

Similarly, no statistically significant change was observed in the albumin/globulin ratio in the treated groups compared to the control (Table 4).

Table 4 Consequences of various doses of SDE T.T on albumin/globulin ratio

	Group 1 (Control)		Group 2 (500 mg/kg)		Group 3 (1000 mg/kg)		Group 4 (1500 mg/kg)		p-value
	M	F	M	F	M	F	M	F	
Total Protein (g/dl)	6.7 ± 0.11	7.06 ± 0.3	6.3 ± 0.67	7.2 ± 0.1	6.9 ± 0.4	7.0 ± 0.25	6.16 ± 0.12	6.5 ± 0.2	0.502
Globulin (g/dl)	2.6 ± 0.13	3.3 ± 0.2	3.95 ± 0.3	3.8 ± 0.1	4.6 ± 0.5	4.5 ± 0.3	3.6 ± 0.13	4.1 ± 0.4	0.218
Albumin (g/dl)	4.03 ± 1.1	3.8 ± 0.1	2.38 ± 0.4	3.3 ± 0.1	2.4 ± 0.2	2.5 ± 0.6	2.6 ± 0.17	2.9 ± 0.2	0.062
Albumin/Globulin ratio	1.6 ± 0.1	1.1 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.070

Notes: Values are stated as mean and standard error of the mean (n = 5). For statistical inference one-way ANOVA followed by Tukey's and Dunnet's multiple comparison. p-value higher than 0.05 is not statistically significant.

Lipid profile revealed that all results were found to be within the specific range and were not significantly varying from the control ($p > 0.05$) except for serum cholesterol. There was a gradual reduction in serum cholesterol levels with the increase in extract dose (p value = 0.023) (Table 5).

Table 5 Results of various doses of SDE T.T on lipid profile

	Group 1 (Control)		Group 2 (500 mg/kg)		Group 3 (1000 mg/kg)		Group 4 (1500 mg/kg)		p-value
	M	F	M	F	M	F	M	F	
Triglycerides (mg/dl)	88 ± 10	68 ± 6	66 ± 17.3	47.5 ± 2.5	54.5 ± 12	63 ± 11.1	48.3 ± 3.1	49.3 ± 4.4	0.746
Serum cholesterol (mg/dL)	71 ± 4.8	59 ± 2	67.7 ± 3.6	67.5 ± 0.5	68.5 ± 7.8	60.3 ± 6.4	60.3* ± 3.7	53.6* ± 3.8	0.023
HDL-cholesterol(mg/dL)	13 ± 2	14 ± 3.3	16.7 ± 3.1	19 ± 0	15.8 ± 1.3	14.7 ± 1.8	14.3 ± 1.5	13 ± 2	0.117
LDL- cholesterol (mg/dL)	20 ± 3	16 ± 2.2	11 ± 0.7	6 ± 0	9.8 ± 0.8	8.3 ± 1.8	7.6 ± 1.6	7.5 ± 1	0.710
VLDL-cholesterol (mg/dl)	16 ± 3	15 ± 1.5	13.3 ± 3.1	9.5 ± 0.5	11 ± 2.5	12.7 ± 2.2	10.6 ± 1.8	10.1 ± 1.9	0.952
Non-HDL-cholesterol (mg/dl)	55 ± 3.3	51 ± 4.6	50.1 ± 1.1	49 ± 0	51.5 ± 5.2	46 ± 7.3	48 ± 4	45 ± 3	0.093

Notes: Values are stated as mean and standard error of the mean (n = 5). For statistical inference one-way ANOVA followed by Tukey's and Dunnet's multiple comparison was applied. p-value higher than 0.05 is not statistically significant. * declare significance of $p < 0.05$.

3.4. Hematological Parameters

Mild variation was observed in the hematological parameters in the treated groups, which, however, was not statistically significant. Nonetheless, platelet count at the dose of 1500 mg/kg/day significantly decreased from that of the control, but, no signs of bleeding were noted in the animals (Table 6).

Table 6 Hematological parameters

Hematological Parameters	Group1 (Control)	Group 2 (500 mg/kg)		Group 3 (1000 mg/kg)		Group 4 (1500 mg/kg)		p-value
		M	F	M	F	M	F	
Hb (g/dL)	12.2 ± 0.3	14.2 ± 0.65	14.8 ± 0.5	13.2 ± 0.5	13.2 ± 1.9	13.6 ± 0.3	13.2 ± 0.1	0.267
RBC (10^{12}/L)	6.8 ± 0.6	7.45 ± 0.15	7.5 ± 0.1	7.16 ± 0.24	7.0 ± 1.1	7.2 ± 0.2	6.5 ± 0.5	0.425
HCT %	40 ± 5	47.5 ± 2.5	50 ± 1.0	45.3 ± 1.11	44 ± 6.0	46.5 ± 1.5	43.5 ± 1.5	0.094
MCV (fL)	58.7 ± 1.7	63.5 ± 1.5	66.5 ± 1.5	63 ± 2.0	63.5 ± 0.75	64.5 ± 0.5	62 ± 2	0.416
MCH (pg)	18.3 ± 0.5	19.5 ± 0.5	20 ± 1.0	18.7 ± 0.44	19 ± 0	19 ± 0	17 ± 2	0.240
MCHC (g/dL)	31.4 ± 0.4	29.5 ± 0.5	29.5 ± 0.5	29.3 ± 0.89	29.5 ± 0.5	29.5 ± 0.5	28.5 ± 1.5	0.690
WBC (10^9/L)	5.8 ± 0.3	5.25 ± 1.25	4.7 ± 0.6	9.13 ± 2.9	4.95 ± 1.75	12.8 ± 4.6	5.1 ± 1.5	0.566
Platelets (10^9/L)	541 ± 25	497 ± 130	696 ± 41.5	560 ± 107	425 ± 50	*351 ± 77	*287 ± 13	0.018
Neutrophils %	39 ± 5	36 ± 5	40.5 ± 5.5	30 ± 9.3	43 ± 10	27 ± 7	19 ± 1	0.496
Lymphocytes%	59 ± 7	60.5 ± 5.5	53 ± 5.0	66 ± 10	53.5 ± 9.5	66.5 ± 9.6	73.5 ± 2.5	0.526
Monocytes%	1.3 ± 0.6	0.35	1.5 ± 0.5	2.33 ± 0.44	0.5 ± 0.5	3 ± 1	3 ± 1	0.280
Eosinophils%	1.9 ± 3.5	3.5 ± 1.3	3.35 ± 1.5	1.33 ± 1.11	3 ± 1	3.5 ± 3.5	0.5 ± 0.5	0.132
Basophils	1 ± 0.6	0 ± 0	1.5 ± 0.5	0.33 ± 0.44	0 ± 0	0 ± 0	1 ± 0	0.352
ESR (Mm/hrs)	1.5 ± 1	1.5 ± 1	1.5 ± 1	1.5 ± 1	1.5 ± 1	1.5 ± 1	1.5 ± 1	-

Notes: Values are stated as mean and standard error of the mean (n = 5). For statistical inference one-way ANOVA followed by Tukey's and Dunnet's multiple comparison was applied. p-value higher than 0.05 is not statistically significant. * declare significance of $p < 0.05$.

3.5. Histopathology

The histopathological sections of the kidney at different doses of SDE T.T exhibited no significant variation in contrast to the control. The renal architecture was preserved, and the same symmetry of cells was found in all sections compared to the control. No signs of tubular atrophy and interstitial inflammation were observed (Fig. 2).

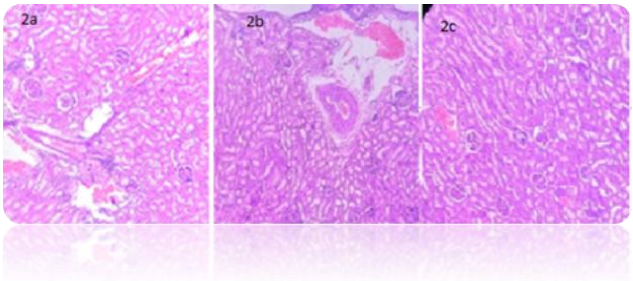

Fig. 2 a - Effect of 500 mg/kg/day of SDE T.T; b - Effect of 1000 mg/kg/day of SDE T.T; c - Effect of 1500 mg/kg/day of SDE T.T on rat's renal histology

Similar to the kidney sections, the liver sections of the treated rats showed no signs of any fatty changes or periportal or focal necrosis. There was no significant difference in the hepatocytes arrangement with respect to the control. Moreover, no evident signs of cirrhosis, fibrosis and inflammation were found (Fig. 3a-c).

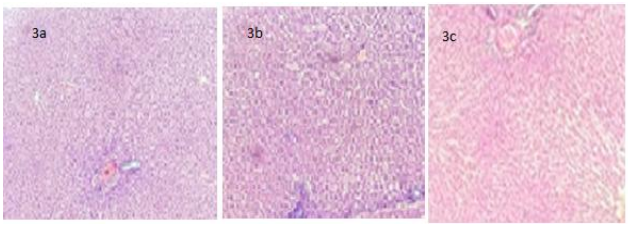

Fig. 3 a - Effect of 500 mg/kg/day of SDE T.T; b - Effect of 1000 mg/kg/day of SDE T.T; c - Effect of 1500 mg/kg/day of SDE T.T on rat's hepatic histology

The histopathological examination of the heart showed no significant variation compared to the control was found at 500, 1000, and 1500 mg/kg/day of the SDE T.T. Normal appearance and architecture of cardiomyocytes was seen. No significant variations were noted in capillary beds with no evidence of cell death (Fig. 4a-c).

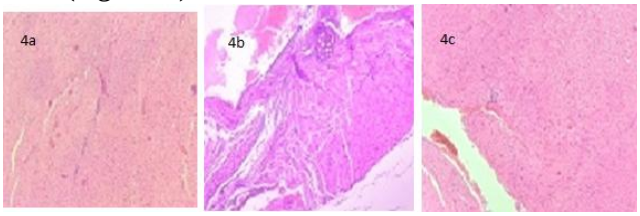

Fig. 4 a - Effect of 500 mg/kg/day of SDE T.T; b - Effect of 1000 mg/kg/day of SDE T.T; c - Effect of 1500 mg/kg/day of SDE T.T on rat's heart histology

The use of phytomedicines has tremendously enhanced over the past few years. Owing to their safety, they are more preferred over conventional allopathic medicine. Moreover, herbal medicines in the dried powder form exert more efficacy than liquid extracts. Spray drying is the most recommended technique for drying plant extracts. This study was designed to assess the safety profile and toxicity index of the SDE T.T in acute and sub-acute setting. Acute toxicity was performed at two different single oral doses; 2000 and 5000 mg/kg/day for 14 days. No behavioral and physical changes were noted along with no signs of mortality in any treated rat. The results were similar to the study by Sagar et al., where acute toxicological study of water-based extract of the T.T was performed [15]. Doses ranging from 2000 to 10000 mg/kg/day were tested, and no toxic symptoms appeared at any of the test dose. Repeated dose sub-acute toxicity revealed no significant variations in hematological and biochemical profiles except for body weight and serum cholesterol. Rats treated with 500, 1000, and 1500 mg/kg/day of SDE T.T showed a gradual reduction in body weight of rats.

Various studies have described the consequences of T.T on the lipid profile. Istiak et al. reported reduction in total cholestrol and triglyceride levels following a combined formulation of TT and *Andrographis paniculata* in mice, where as no changes in the LDL-cholesterol, HDL-cholesterol levels were observed [16]. Similarly, another study suggested that dietary intake of T.T decreases serum lipid profiles [10]. The results of these studies were very much alike to our study. Adequate scientific data on the hepatoprotective effect of T.T are available [17-19]. However, few studies have also reported the hepatotoxic potential of T.T. A recent study reported a hepatotoxicity in young women in Iran following a herbal tea of T.T for two to three months [17]. Another study has also reported the toxicity of T. T infusion in humans. Predominant toxic effects were observed on the liver and kidney [20]. Our study demonstrated that no toxic effects were observed on the liver of the treated rats. Many of the studies have reported the renoprotective effect of T.T [21]. Our results suggest that SDE T.T has no toxic effect on the kidneys when repeatedly administered at a high dose. Although T.T is effective in many diseases, there are very scarce data available for long-term toxicity studies. Our study revealed that repeated dose administration of SDE T.T produced no toxic signs and mortality in the experimental animals over a period of 28 days.

4. Conclusion

Standardized plant preparations are typically sold as liquid extracts, powders made from dried plant material, or extracts dried. In this situation, dried extracts are becoming more popular in the pharmaceutical industry because they have many advantages over traditional liquid forms, including chemical, physiochemical, and microbiological stability, ease of standardization, higher concentrations of active compounds, ease of transportation, need for less storage space, and reduced risk of microbial contamination. It is also common practice to turn a plant extract into a dry extract when creating herbal medicines. Spray drying is the most popular drying technique used to create dried extracts. The processing conditions should also be considered to protect the bioactive moieties associated with its pharmacological activity.

The acute toxicity findings revealed that the LD50 of the SDE T.T is greater than the unit dose of 5000

mg/kg. Repeated dose sub-acute toxicity for the 28 days revealed that the SDE T.T is relatively safe and non-toxic at 500, 1000, and 1500 mg/kg/day with no significant effect on hematological, biochemical parameters, histology and structure. Therefore, to conclude, SDE T.T can be safely employed in pharmaceutical formulations for therapeutic use after further addressing safety in chronic and sub-chronic study.

Acknowledgments

The authors acknowledge that the Faculty of Pharmacy, Jinnnah University for Women, Karachi, Pakistan, has fully supported the research work.

References

[1] ISHTIAQ S., AKRAM M., KAMRAN S. H., HANIF U., AFRIDI M. S. K., SAJID-UR-REHMAN, AFZAL A., ASIF A., YOUNUS M., and AKBAR S. Acute and sub-acute toxicity study of a Pakistani polyherbal formulation. *BMC Complementary and Alternative Medicine*, 2017, 17(1): 387. https://doi.org/10.1186/s12906-017-1889-7

[2] CORTÉS-ROJAS D. F., SOUZA C. R., and OLIVEIRA W. P. Assessment of stability of a spray dried extract from the medicinal plant Bidens pilosa L. *Journal of King Saud University - Engineering Sciences*, 2016, 28(2): 141-146. http://dx.doi.org/10.1016/j.jksues.2014.04.004

[3] GUNJAL S. D., & SHIROLKAR S. V. An Overview of Process Parameters and Spray Drying Agents Involved in Spray Drying of Herbal Extracts. *Paideuma Journal*, 2020, 13(7): 102-118. https://paideumajournal.com/gallery/8-july2020.pdf

[4] AZAM F., MUNIER S., BATOO M., AHMAD B., and ABBAS G. A review on advancements in ethnomedicine and phytochemistry of Tribulus terrestris – a plant with multiple health benefits. *International Journal of Biosciences*, 2019, 14(1): 21-37. http://dx.doi.org/10.12692/ijb/14.1.21-37

[5] GUNARATHNE R., NADEESHANI H., LU A., LI J., ZHANG B., YING T., and LU J. Potential Nutraceutical Use of Tribulus terrestris L. in Human Health. *Food Reviews International*, 2022. https://doi.org/10.1080/87559129.2022.2067172

[6] KAMENOV Z., FILEVA S., KALINOV K., and JANNINI E. A. Evaluation of the efficacy and safety of Tribulus terrestris in male sexual dysfunction—a prospective, randomized, double-blind, placebo-controlled clinical trial. *Maturitas*, 2017, 99: 20-26. https://doi.org/10.1016/j.maturitas.2017.01.011

[7] SALGADO R. M., MARQUES-SILVA M. H., GONÇALVES E., MATHIAS A. C., AGUIAR J. G., and WOLFF P. Effect of oral administration of Tribulus terrestris extract on semen quality and body fat index of infertile men. *Andrologia*, 2017, 49(5): e12655. https://doi.org/10.1111/and.12655

[8] DE SOUZA K. Z. D., VALE F. B. C., and GEBER S. Efficacy of Tribulus terrestris for the treatment of hypoactive sexual desire disorder in postmenopausal women: a randomized, double-blinded, placebo-controlled trial. Menopause, 2016, 23(11): 1252-1256. https://doi.org/10.1097/gme.0000000000000766

[9] SHARMA I., KHAN W., and AHMAD S. In vitro and ex vivo approach for anti-urolithiatic potential of bioactive fractions of gokhru with simultaneous HPLC analysis of six major metabolites and their exploration in rat plasma. *Pharmaceutical Biology*, 2017, 55(1): 701-711. https://doi.org/10.1080/13880209.2016.1266671

[10] ASADMOBINI A., BAKHTIARI M., KHALEGHI S., ESMAEILI F., and MOSTAFAEI A. The effect of Tribulus terrestris extract on motility and viability of human sperms after cryopreservation. *Cryobiology*, 2017, 75: 154-159. https://doi.org/10.1016/j.cryobiol.2017.02.005

[11] EL BAIRI K., OUZIR M., AGNIESZKA N., and KHALKI L. Anticancer potential of Trigonella foenum graecum: cellular and molecular targets. *Biomedicine & Pharmacotherapy*, 2017, 90: 479-491. https://doi.org/10.1016/j.biopha.2017.03.071

[12] ABBAS M. W., HUSSAIN M., AKHTAR S., ISMAIL T., QAMAR M., SHAFIQ Z., and ESATBEYOGLU T. Bioactive compounds, antioxidant, anti-inflammatory, anti-cancer, and toxicity assessment of Tribulus terrestris—In vitro and in vivo studies. *Antioxidants*, 2022, 11(6): 1160. https://doi.org/10.3390/antiox11061160

[13] BOSE S., DATTA R., and KIRLIN W. G. Toxicity Studies Related to Medicinal Plants. In: MANDAL S. C., CHAKRABORTY R., and SEN S. (eds.) *Evidence Based Validation of Traditional Medicines*. Springer, Singapore, 2021: 621–647. https://doi.org/10.1007/978-981-15-8127-4_30

[14] ȘTEFĂNESCU R., TERO-VESCAN A., NEGROIU A., AURICĂ E., and VARI C. E. A comprehensive review of the phytochemical, pharmacological, and toxicological properties of Tribulus terrestris L. *Biomolecules*, 2020, 10(5): 752. https://doi.org/10.3390/biom10050752

[15] NALAWADE S. A., PINGALE S. S., and CHASKAR M. G. Study of Acute Toxicity of Tribulus Terristris. *Journal of Current Pharma Research*, 2019, 9(3): 2947-2954. https://jcpr.humanjournals.com/study-of-acute-toxicity-of-tribulus-terristris/

[16] ISTIAK A., HAZRA P., DAS S. R., HOSSAIN M. I., AMINATU A. S., and RAFIQ K. Hypoglycemic, hypolipidemic and kidney protective potential of combined formulation of Tribulus terrestris and Andrographis paniculata in alloxan induced mice. *African Journal of Pharmacy and Pharmacology*, 2018, 12(21): 269-277. https://doi.org/10.5897/AJPP2018.4928

[17] ATAEE Z., & DADPOUR B. Hepatic failure in a young woman following ingestion of tribulus terrestris. *Asia Pacific Journal of Medical Toxicology*, 2020, 9(1): 33-34. https://dx.doi.org/10.22038/apjmt.2020.15318

[18] ALMASI F., KHAZAEI M., and GHANBARI A. Hepatoprotective Effects of Tribulus terrestris Hydro-Alcholic Extract on Non-Alcoholic Fatty Liver-Induced Rats. *International Journal of Morphology*, 2017, 35(1): 345-350. http://dx.doi.org/10.4067/S0717-95022017000100054

[19] ABDEL-KADER M. S., AL-QUTAYM A., SAEEDAN A. S. B., HAMAD A. M., and ALKHARFY K. M. Nephroprotective and hepatoprotective effects of Tribulus terrestris L. growing in Saudi Arabia. *Journal of Pharmacy & Pharmacognosy Research*, 2016, 4(4): 144-152. https://www.redalyc.org/pdf/4960/496053936002.pdf

[20] BROWN A. C. Kidney toxicity related to herbs and dietary supplements: Online table of case reports. Part 3 of 5 series. *Food and Chemical Toxicology*, 2017, 107: 502-519. https://doi.org/10.1016/j.fct.2016.07.024

[21] KILANY O. E., ABDOU R. H., EL-BELTAGY M. A.,

and MOHAMMAD H. M. Protective effects of Tribulus terrestris against gentamicin mediated nephrotoxicity, oxidative damage and apoptosis in male rats. *Egyptian Academic Journal of Biological Sciences, B. Zoology*, 2020, 12(1): 41-58. https://dx.doi.org/10.21608/eajbsz.2020.85736

参考文:

[1] ISHTIAQ S.、AKRAM M.、KAMRAN S.H.、HANIF U.、AFRIDI M.S.K.、SAJID-UR-REHMAN、AFZAL A.、ASIF A.、YOUNUS M. 和 AKBAR S. 急性和亚急性毒性研究巴基斯坦多草本配方。BMC 补充和替代医学, 2017, 17(1): 387. https://doi.org/10.1186/s12906-017-1889-7

[2] CORTÉS-ROJAS D. F.、SOUZA C. R. 和 OLIVEIRA W. P. 药用植物大叶鬼针草喷雾干燥提取物稳定性的评估。沙特国王大学学报 - 工程科学，2016 年，28(2)：141-146。http://dx.doi.org/10.1016/j.jksues.2014.04.004

[3] GUNJAL S. D. 和 SHIROLKAR S. V. 草药提取物喷雾干燥中涉及的工艺参数和喷雾干燥剂概述。派德玛杂志，2020 年，13（7）：102-118。https://paideumajournal.com/gallery/8-july2020.pdf

[4] AZAM F.、MUNIER S.、BATOO M.、AHMAD B. 和 ABBAS G. 蒺藜的民族医学和植物化学进展综述——一种具有多种健康益处的植物。国际生物科学杂志, 2019, 14(1): 21-37. http://dx.doi.org/10.12692/ijb/14.1.21-37

[5] GUNARATHNE R., NADEESHANI H., LU A., LI J., ZHANG B., YING T., 和 LU J. 蒺藜在人体健康中的潜在营养用途。国际食品评论，2022。https://doi.org/10.1080/87559129.2022.2067172

[6] KAMENOV Z.、FILEVA S.、KALINOV K. 和 JANNINI E. A. 蒺藜在男性性功能障碍中的疗效和安全性评估——一项前瞻性、随机、双盲、安慰剂对照的临床试验。成熟，2017，99：20-26。https://doi.org/10.1016/j.maturitas.2017.01.011

[7] SALGADO R. M.、MARQUES-SILVA M. H.、GONÇALVES E.、MATHIAS A. C.、AGUIAR J. G. 和 WOLFF P. 口服刺蒺藜提取物对不育男性精液质量和体脂指数的影响。男科书，2017, 49(5): e12655。https://doi.org/10.1111/and.12655

[8] DE SOUZA K. Z. D.、VALE F. B. C. 和 GEBER S. 蒺藜治疗绝经后妇女性欲减退症的疗效：一项随机、双盲、安慰剂对照试验。更年期, 2016, 23(11): 1252-1256。https://doi.org/10.1097/gme.0000000000000766

[9] SHARMA I.、KHAN W. 和 AHMAD S. 格鲁生物活性部分的抗尿石症潜力的体外和离体方法，同时高效液相色谱分析六种主要代谢物及其在大鼠血浆中的探索。药物生物学，2017, 55(1): 701-711. https://doi.org/10.1080/13880209.2016.1266671

[10] ASADMOBINI A.、BAKHTIARI M.、KHALEGHI S.、ESMAEILI F. 和 MOSTAFAEI A. 刺蒺藜提取物对冷冻保存后人类精子活力和活力的影响。低温生物学，2017，75：154-159。https://doi.org/10.1016/j.cryobiol.2017.02.005

[11] EL BAIRI K.、OUZIR M.、AGNIESZKA N. 和 KHALKI L. 胡芦巴的抗癌潜力：细胞和分子靶点。生物医学与药物治疗，2017，90：479-491。https://doi.org/10.1016/j.biopha.2017.03.071

[12] ABBAS M. W.、HUSSAIN M.、AKHTAR S.、ISMAIL T.、QAMAR M.、SHAFIQ Z. 和 ESATBEYOGLU T. 蒺藜的生物活性化合物、抗氧化剂、抗炎、抗癌和毒性评估—体外和体内研究。抗氧化剂, 2022, 11(6): 1160. https://doi.org/10.3390/antiox11061160

[13] BOSE S.、DATTA R. 和 KIRLIN W. G. 与药用植物相关的毒性研究。在：MANDAL S. C.、CHAKRABORTY R. 和 SEN S. (编辑。) 传统药物的循证验证。施普林格，新加坡，2021：621–647。https://doi.org/10.1007/978-981-15-8127-4_30

[14] ŞTEFĂNESCU R., TERO-VESCAN A., NEGROIU A., AURICĂ E., 和 VARI C. E. 蒺藜生物分子的植物化学、药理学和毒理学特性的综合综述, 2020, 10(5): 752. https://doi.org/10.3390/biom10050752

[15] NALAWADE S. A.、PINGALE S. S. 和 CHASKAR M. G. 刺蒺藜急性毒性研究。当代医药研究杂志，2019，9(3)：2947-2954。https://jcpr.humanjournals.com/study-of-acute-toxicity-of-tribulus-terristris/

[16] ISTIAK A.、HAZRA P.、DAS S.R.、HOSSAIN M.I.、AMINATU A.S. 和 RAFIQ K. 蒺藜和穿心莲联合制剂在四氧嘧啶诱导的小鼠中的降血糖、降血脂和肾脏保护潜力。非洲药学与药理学杂志, 2018, 12(21): 269-277. https://doi.org/10.5897/AJPP2018.4928

[17] ATAEE Z., & DADPOUR B. 一名年轻女性在摄入刺蒺藜后出现肝功能衰竭。亚太医学毒理学杂志, 2020, 9(1): 33-34. https://dx.doi.org/10.22038/apjmt.2020.15318

[18] ALMASI F.、KHAZAEI M. 和 GHANBARI A. 刺蒺藜水醇提取物对非酒精性脂肪肝诱导的大鼠的保肝作用。国际形态学杂志，2017，35（1）：345-350。http://dx.doi.org/10.4067/S0717-95022017000100054

[19] ABDEL-KADER M. S.、AL-QUTAYM A.、SAEEDAN A. S. B.、HAMAD A. M. 和 ALKHARFY K. M. 生长在沙特阿拉伯的刺蒺藜的肾脏保护和肝脏保护作用。药学与生药学研究杂志, 2016, 4(4): 144-152. https://www.redalyc.org/pdf/4960/496053936002.pdf

[20] BROWN A. C. 与草药和膳食补充剂相关的肾毒性：病例报告在线表。5 系列的第 3 部分。食品与化学毒理

学，2017，107：502-519。https://doi.org/10.1016/j.fct.2016.07.024

[21] KILANY O. E.、ABDOU R. H.、EL-BELTAGY M. A. 和 MOHAMMAD H. M. 刺蒺藜对雄性大鼠庆大霉素介导的肾毒性、氧化损伤和细胞凋亡的保护作用。埃及生物科学学术期刊，乙. 动物学，2020, 12(1): 41-58. https://dx.doi.org/10.21608/eajbsz.2020.85736