

Research Article



Acute and Subacute Toxicity Studies of the Saponin Rich Butanol Extracts of *Tribulus terrestris* Fruits in Wistar Rats

S.Hemalatha*, Rajeswari Hari

Department of Biotechnology, Dr. M.G.R Educational and Research Institute University, Chennai, Tamilnadu, India.

*Corresponding author's E-mail: rajihar@gmail.com

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ABSTRACT

Acute and Sub acute toxicity study of the saponin rich butanol extracts of *Tribulus terrestris* fruit was investigated. The purpose of acute toxicity study is to determine the LD₅₀ value which helps in determining the safe dose range for the drug to be used and to ensure that there is no harmful or lethal effects on the animal. There is no mortality occurred during acute toxicity studies which shows that SFTT did not produce death even at high dose Of 2.0g/kg body weight. So this drug is found to be very safe. In sub acute toxicity study SFTT was administered orally at a dose of 100mg/kg, 200mg/kg, 400 mg/kg for 28 days. There were no drastic changes in the normal ranges of hematological, biochemical and histopathology studies.

Keywords: Acute toxicity, Sub acute toxicity, *Tribulus terrestris*.

INTRODUCTION

The use of traditional medicines and medicinal plants in most developing countries for the prevention and treatment of diseases, has been widely observed.¹ The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly the plant drugs, for their primary healthcare.² Herbal drugs have gained importance in recent years for the management of variety of ailments due to their easy access, better compatibility and for economic reasons. They are used as either single plant extracts or its fractions or mixtures of extracts from different plants. People have a strong belief that herbal remedies are derived from nature, they are devoid of adverse or toxic side effects.³ Although, many medicinal preparations have been reported to be non-toxic, many others have shown to be toxic.⁴ Hence experimental screening method is important in order to ascertain the safety and efficacy of traditional and herbal products.⁵

Tribulus terrestris L. belongs to the family Zygophyllaceae and the fruit is commonly known as caltrops or devil's thorn, found throughout the world. In several countries *Tribulus terrestris* is used as folk medicine such as tonic, aphrodisiac, astringent, analgesic, stomachic, anti hypertensive, diuretic and urinary anti-septic.⁶ Historically, *Tribulus terrestris* was used by the cultures of India and Greece as a rejuvenation tonic.⁷ The plant has been extensively investigated by a number of workers, for the presence of steroidal glycosides, steroidal Saponins, flavonoids and alkaloids and many people have reported that the Saponins present in the plant is responsible for the broad range of bioactivities and their health-promoting properties. Many pharmaceutical preparations and food supplements with these Saponins as the active compound have been commercially available. Examples of these are "Tribestane" and "Vitanone", which have been used to treat impotency, as well as "tribusaponins"

and "Xin-nao-shutong", which have been used for the treatment of cardiovascular disorders.⁸ In spite of the world wide use of *Tribulus terrestris* fruits mainly as aphrodisiac in traditional medicine, no systematic evaluation of its toxic effects has been carried out till date. Therefore the aim of the present study is under taken to investigate the acute and sub acute toxic effects of the saponin rich butanol fraction of *Tribulus terrestris*, (SFTT) fruits in Wistar albinorats, to provide preliminary safety information regarding its traditional usage.

MATERIALS AND METHODS

Plant material

The ripe fruits of *Tribulus terrestris* were collected from the Sairam siddha Medical College and research center India and were authenticated by Dr. Sankaranarayanan, Assistant Director, Dept of Research and Development, of the same college. The voucher specimen is also available in herbarium file of the same center.

Preparation of plant extract

The Saponins isolation was carried out according to the method of Kostova et al., 2002.⁹ The fruits of *Tribulus terrestris* (500 g) were shade-dried and pulverized to a coarse powder and was extracted with 70% ethanol (3 × 24 h) at room temperature. The combined ethanol solutions were concentrated to a small volume and extracted in succession with chloroform (3 × 24 h) and n-BuOH (3 × 24 h). The n-BuOH layer was concentrated to dryness to give crude saponin extract. The presences of Saponins were confirmed by frothing test (0.5 ml filtrate + 5 ml distilled water). The Saponins content was calculated as, a percentage of total Saponins = weight of residue × 100 / weight of sample taken. The yield of the Saponins prepared by the above procedure was found to be 0.34 % (w/w). All the extracts obtained were stored at 4°C in air tight containers until assay.



Animals

Adult albino male rats of Wister strain weighing 120-250 g were used for the toxicological studies. The inbred animals were taken from animal house in Vel's College of pharmacy Chennai, India. The animals were maintained in well ventilated room temperature with natural 12 ± 1 h day–night cycle in the propylene cages. They were fed balanced rodent pellet diet from Poultry Research Station, Nandanam, Chennai, India and tap water *ad libitum* was provided throughout the experimental period. The animals were sheltered for one week and prior to the experiment they were acclimatized to laboratory temperature. The protocol was approved by Animal Ethics Committee constituted for the purpose as per CPCSEA Guideline.

Acute toxicity

Acute oral toxicity study was conducted with SFTT extracts in Wister albino rats according to the guidelines of Organization for Economic Co-operation and Development (OECD, 425).¹⁰ The Saponin fraction of *Tribulus terrestris* (SFTT) extract was suspended in 2% (v/v) CMC solution. Healthy rats of either sex, weighing between 120-250g were divided in groups of 6 (3male + 3female). The extract was administered by gavages at doses of 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0, g/kg body weight in single doses to both female and male rats. The animals were observed for general behavioral changes, signs of toxicity and mortality continuously for 1h after treatment, then intermittently for 4h, and there after over a period of 24 h. The rats were further observed for up to 14 days following treatment for behavioral changes and signs of toxicity /death and the latency of death. Mortality rate was recorded after 24 hours.

Sub acute toxicity study

The sub-acute oral toxicity study was conducted according to the guidelines of the Organization for Economic Co-operation and Development (OECD, 407).¹¹ Twenty four animals were randomly divided into four groups of six animals each. Group I served as control and received a suspension of 2ml/Kg CMC *p.o.* (2% v/v of CMC) Group II- Animals received 100mg/Kg body weight of SFTT in 2ml/Kg of 2% CMC *p.o.* daily for 28 days. Group III: Animals received 200mg/Kg body weight of SFTT in 2ml/Kg of 2% CMC *p.o.* daily for 28 days. Group -IV: Animals received 400mg/Kg body weight of SFTT in 2ml/Kg of 2% CMC *p.o.* daily for 28 days. Food and water intake was recorded daily; body weight was recorded once in a week throughout the study period.¹¹ At the end of experimental period, the animals were euthanized, blood samples collected through orbital sinus and taken into heparinized tube for hematological studies and non-heparinized tube from which serum was isolated by centrifugation at 3000 rpm for 10 minutes and used for biochemical estimations.

Estimation of hematological and biochemical parameters

Hematological parameters namely, White blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb) levels, percentage of lymphocytes (LY), Monocytes (MO), Granulocytes (GR) neutrophils, Eosinophils, Platelet Count (PLT), ESR and packed cell volume (PCV) was recorded using Hematology Analyzer. To analyze the liver and kidney functions estimations of enzymes Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were carried out. The Biochemical parameters like Total Bilirubin Total Protein Albumin Globulin Urea Creatinine Uric acid were estimated. The serum lipid profile such as total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL-C)] were assessed and low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) calculated by Friedewald's formula.¹² All the enzymatic and biochemical assays were analyzed using commercially available kit and were read at specific wavelength using Shimadzu spectrophotometer, UV-1601 model.

Organ weight and histopathology

Animals were sacrificed under mild ether anesthesia. After sacrifice, organ weights (liver, kidney, heart, lung, brain, spleen and stomach) were recorded. The vital organs like liver and kidney were dissected out and cleaned well with cold physiological saline to remove blood and adhering tissues. The samples were then fixed in 10% formalin- saline and embedded in paraffin. Serial sections were cut at 5mm and stained with haematoxylin and eosin. The sections were examined under light microscope for possible histopathological damages and photographs were taken.

Statistical analysis

Values reported are mean ± S.E. The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's 't' test. P values <0.05 were considered as significant.¹³

RESULTS AND DISCUSSION

Acute oral toxicity

Acute oral toxicity studies were carried out for SFTT starting from 50mg/kg up to a dose level of 2000mg/kg (Table 1). The animals survived throughout the experimental period and did not show any sign of toxic symptoms or any kind of behavioral changes immediately after dosing and during the period of 14 days. From this study it is revealed that the LD₅₀ dose of the SFTT is above 2.0g/kg body weight indicating the low oral toxic nature of the extract. Ramesh et al., in his studies have reported that substances with an LD₅₀ value of 1000 mg kg by the oral route are regarded as being safe.¹⁴



Table 1: Incremental dose and its Signs of Toxicity after oral administration of SFTT in rats

Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
50	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
250	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
500	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
1000	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
2000	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality

Table 2: Effect of SFTT extracts sub acute oral administration on Body Weight in Rats

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Group-I (control)	113.42±5.00	115.52±6.00	116.24±5.25	118.05±5.21	121.10±5.26
Group-II (SFTT 100mg/kg b.w treated)	115.17±1.15 a ^{ns}	116.41±5.11 a ^{ns}	120.12±5.72 a ^{ns}	122.61±6.00 a ^{ns}	124.51±5.28 a ^{ns}
Group-III (SFTT 200mg/kg b.w treated)	116.20±5.47 b ^{ns}	118.10±6.00 b ^{ns}	121.18±6.12 b ^{ns}	123.12±5.10 b ^{ns}	125.30±6.14 b ^{ns}
Group-IV (SFTT (400mg/kg b.w treated)	114.10±6.22 c ^{ns}	116.21±5.32 c ^{ns}	118.10±6.00 c ^{ns}	120.64±4.32 c ^{ns}	123.25±5.32 c ^{ns}

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. Comparison between a - Group I vs Group II, b - Group I vs Group III and c-Group I vs Group IV. P Values: ns – non significant.

Table 3: Effect of SFTT extracts on Hematological parameters after 28 days treatment in rats

Parameter	Group-I (control)	Group-II (SFTT 100mg/kg b.w treated)	Group-III (SFTT 200mg/kg b.w treated)	Group-IV (SFTT (400mg/kg b.w treated)
Red blood cell (x10 ⁶ /mm ³)	8.2±0.72	7.91±0.64a ^{ns}	8.12±0.54b ^{ns}	9.00±0.50c ^{ns}
Hemoglobin (%)	14.11±0.30	15.24±0.32a ^{ns}	14.86±0.40b ^{ns}	15.10±0.42c ^{ns}
Leukocyte (x10 ³ /mm ³)	10124±110.92	11245±106.24a ^{ns}	11255±124.12b ^{ns}	11268±112.43c ^{ns}
Platelets (x10 ³ /mm ³)	1382±40.12	1399±38.42a ^{ns}	1234±31.02b ^{ns}	1295±32.40c ^{ns}
Differential leukocyte count (%)				
Neutrophils	5.61±1.30	5.34±1.22a ^{ns}	5.28±0.98b ^{ns}	5.10±3.74c ^{ns}
Lymphocytes	94.34±2.29	91.85±3.25a ^{ns}	93.82±3.22b ^{ns}	94.20±3.28c ^{ns}
Monocytes	2.0±0.31	2.0±0.34a ^{ns}	2.22±0.38b ^{ns}	2.30±0.42c ^{ns}
Eosinophils	1.00±0.00	1.0±0.22a ^{ns}	1.0±0.11b ^{ns}	1.00±0.11c ^{ns}
ESR(mm)	1±00	1±00a ^{ns}	1±00b ^{ns}	1±00c ^{ns}
PCV	46.12±2.51	45.21±2.12	45.10±3.20b ^{ns}	45.61±2.28c ^{ns}

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. Comparison between a - Group I vs Group II, b - Group I vs Group III and c- Group I vs Group IV. P Values: ns – non significant.

Sub acute oral toxicity

Body weight gain, food and water intake

Acute toxicity studies have limited clinical application because the cumulative toxic effects at very low doses occur in only multiple dose administration. Hence the Sub acute toxicity or repeat-dose study is performed to evaluate the safety profile of phytomedicines which usually ranges from weeks to a few months. A sub-acute toxicity study was carried out by administering the SFTT

extract at three doses of 100, 200 and 400mg/ kg body weight to the wister rats (Table 2). No significant differences in body weights were recorded in the 28 day treatment period. Further there was no alteration in food and water intake in all the treated groups as compared to control. Generally body weight changes are an indicator of adverse side effects, after exposure to toxic substances. The animals that survive cannot lose more than 10 % of the initial body weight.¹⁵⁻¹⁷ In sub acute toxicity study rats treated with 100, 200 and 400 mg/kg

doses of SFTT extract had a progressive gain in the body weight during the study period of 28 days and this increase in weight was not significantly different from

that of the control. This gradual weight increase may be due to the improvement in the nutritional state of the animals due to the increased food and water intake.

Table 4: Effect of SFTT extracts on liver and Kidney function test parameters after 28days treatment in rats

Parameter	Group-I (control)	Group-II (SFTT 100mg/kg b.w treated)	Group-III (SFTT 200mg/kg b.w treated)	Group-IV (SFTT (400mg/kg b.w treated)
Total Bilirubin (mg/dL)	0.218 ± 0.05	0.210 ± 0.05a ^{ns}	0.218 ± 0.06b ^{ns}	0.214 ± 0.06c ^{ns}
Bilirubin direct (mg/dL)	0.1 ± 0.04	0.1 ± 0.05a ^{ns}	0.1 ± 0.04b ^{ns}	0.1 ± 0.05c ^{ns}
Bilirubin indirect(mg/dL)	0.1 ± 00	0.1 ± 00a ^{ns}	0.1 ± 00b ^{ns}	0.1 ± 00c ^{ns}
ALP (U/L)	381.32 ± 10.16	374.21 ± 12.32a ^{ns}	286.38 ± 10.30b ^{**}	294.1 ± 12.32c ^{**}
SGOT (U/L)	168.24 ± 6.21	160.67 ± 6.58a ^{ns}	156.38 ± 5.80b ^{ns}	154.01 ± 6.57c ^{ns}
SGPT(U/L)	46.4 ± 2.34	44.8 ± 3.20a ^{ns}	45.08 ± 2.58b ^{ns}	46.62 ± 4.19c ^{ns}
Total Protein(g/dl)	10.02 ± 1.30	9.17 ± 0.30a ^{ns}	8.52 ± 0.27b ^{ns}	9.12 ± 0.46c ^{ns}
Albumin(g/dl)	3.21 ± 0.25	3.19 ± 0.24a ^{ns}	3.46 ± 0.33b ^{ns}	3.22 ± 0.12c ^{ns}
Globulin(g/dl)	6.02 ± 0.18	5.18 ± 0.26a ^{ns}	4.98 ± 0.21b [*]	4.86 ± 0.30c ^{ns}
Urea(mg/dL)	55.42 ± 1.63	54.34 ± 3.56 a ^{ns}	55.2 ± 2.14 b ^{ns}	53.88 ± 1.36 c ^{ns}
Creatinine (mg/dL)	0.78 ± 0.05	0.76 ± 0.05 a ^{ns}	0.78 ± 0.06 b ^{ns}	0.79 ± 0.05 c ^{ns}
Uric acid (mg/dL)	1.6 ± 0.12	1.6 ± 0.18 a ^{ns}	1.6 ± 0.16 b ^{ns}	1.6 ± 0.14 c ^{ns}
Sodium(m.eq/l)	140.78 ± 5.26	141.2 ± 5.00 a ^{ns}	141.12 ± 5.22 b ^{ns}	141.10 ± 5.00 c ^{ns}
Potassium (m.eq/l)	20.20 ± 2.88	19.45 ± 1.68 a ^{ns}	20.0 ± 1.46 b ^{ns}	20.15 ± 2.02 c ^{ns}
Chloride(m.eq/l)	100.05 ± 4.24	101.20 ± 5.21 a ^{ns}	99.88 ± 4.82 b ^{ns}	101.04 ± 5.16 c ^{ns}

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. Comparison between a - Group I vs Group II, b – Group I vs Group III and c- Group I vs Group IV. P Values: * < 0.05, ** < 0.01, *** < 0.001, ns – non significant.

Table 5: Effect of SFTT extracts on Lipid profile after 28 days treatment in rats

Parameter	Group-I (control)	Group-II (SFTT 100mg/kg b.w treated)	Group-III (SFTT 200mg/kg b.w treated)	Group-IV (SFTT (400mg/kg b.w treated)
Total cholestrol(mg/dL)	71.45±2.57	71.12±2.46a ^{ns}	61.12±3.20b [*]	59.54±3.00c [*]
HDL-cholesterol(mg/dL)	13.02±1.44	13.25±1.77a ^{ns}	14.00±1.45b ^{ns}	14.20±2.31c ^{ns}
LDL- cholesterol (mg/dL)	42.00±2.83	41.05±3.61a ^{ns}	31.31±3.90 b ^{**}	30.28±3.32c ^{ns}
VLDL- cholesterol (mg/dl)	16.38±2.62	15.82±2.34a ^{ns}	16.00±1.64b ^{ns}	15.06±1.24c ^{ns}
Triglycerides (mg/dl)	88.24±3.00	85.16±2.22a ^{ns}	86.23±3.24b ^{ns}	68.44±2.78c [*]

Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. Comparison between a - Group I vs Group II, b – Group I vs Group III and c- Group I vs Group IV. P Values: * < 0.05, ** < 0.01, *** < 0.001, ns – non significant.

Hematology

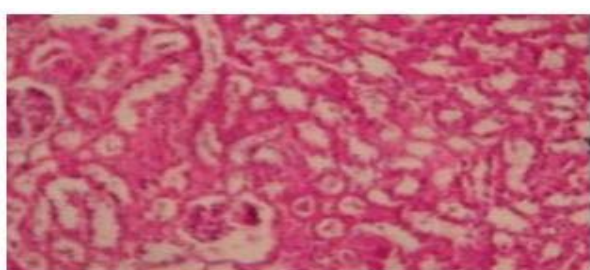
Table 3 depicts the hematological parameters of control and different concentrations of SFTT treated rats. All the drug treated animals did not show any significant variations in the hematological parameters such as White blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb) levels, percentage of lymphocytes (LY), Monocytes (MO), Granulocytes (GR) neutrophils, Eosinophils, Platelet Count (PLT), ESR and packed cell volume (PCV). However a slight increase in total RBC count values was obtained for animals in the dose group of 200 mg/kg and 400mg/kg. Decreased values of platelets were observed for animals in dose groups administered 100mg/ kg body weight of SFTT. According to Olson et al.¹⁸ blood parameters analysis is relevant to

risk evaluation as the hematological system has a higher predictive value for toxicity in humans (91%). Being a transport medium the blood carries many drugs and xenobiotics due to which the components of the blood such as red blood cells, white blood cells, haemoglobin and platelets are forced to initially exposed to significant concentrations of toxic compound. Any damage and destruction of the blood components will definitely reflects the normal functioning of the body. In the present investigation SFTT did not have any significant effect on the hematological parameters measured, suggesting the non toxic nature of the drug.

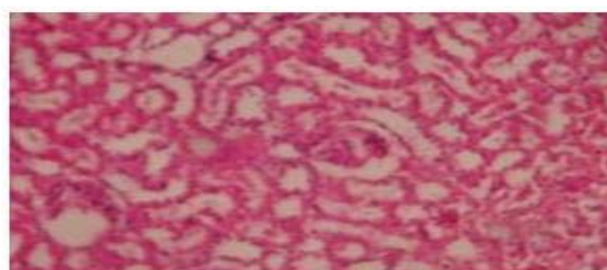
Table 6: Effect of SFTT extracts on organ weight after 28 days treatment in rats

Parameter	Group-I (control)	Group-II (SFTT 100mg/kg b.w treated)	Group-III (SFTT 200mg/kg b.w treated)	Group-IV (SFTT (400mg/kg b.w treated)
Liver (g)	5.20±0.17	5.32±0.15 a ^{ns}	4.92±0.12 b ^{ns}	5.15±0.18 c ^{ns}
Heart (g)	0.62±0.04	0.62±0.05 a ^{ns}	0.59±0.04 b ^{ns}	0.58±0.04 c ^{ns}
Lung (g)	1.49±0.06	1.44±0.14 a ^{ns}	1.36±0.24 b ^{ns}	1.52±0.15 c ^{ns}
Spleen (g)	0.65±0.05	0.68±0.04 a ^{ns}	0.66±0.04 b ^{ns}	0.65±0.05 c ^{ns}
Ovary (g)	1.69±0.14	1.78±0.15a*	1.78±0.18b*	1.76±0.15c*
Testes (g)	1.48±0.10	1.55±0.12a*	1.66±0.15b*	1.69±0.15c*
Brain (g)	1.56±0.15	1.58±0.13 a ^{ns}	1.56±0.14 b ^{ns}	1.53±0.14 c ^{ns}
Kidney (g)	0.73±0.04	0.71±0.04 a ^{ns}	0.70±0.04 b ^{ns}	0.72±0.05 c ^{ns}
Stomach (g)	1.36±0.12	1.34±0.10	1.38±0.11	1.35±0.12

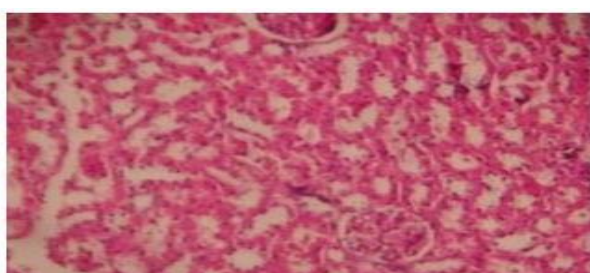
Values are mean ± SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. Comparison between a - Group I vs Group II, b – Group I vs Group III and c- Group I vs Group IV. P Values: * <0.05, ** <0.01, *** <0.001, ns – non significant.



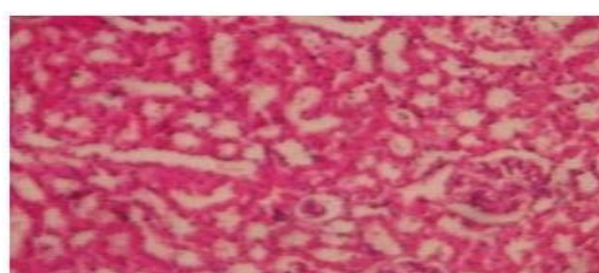
(A)



(B)



(C)



(D)

Figure 1 (A-D): Photomicrograph showing normal architecture of kidney in control (A), SFTT 100mg/kg (B), SFTT 200mg/kg (C) and SFTT 400mg/kg (D) administered rats for 28 days

Effect of SFTT extracts on liver and kidney

The saponin rich butanol fraction of *Tribulus terrestris* (SFTT) extract treatment for 28 days did not cause any disturbance in hepatic functions as compared to control rats (Table-4). In the entire drug treated animals the biochemical parameters and the liver marker enzymes were within normal biological and laboratory limits. However the Protein level is found to be elevated in animals group which received 100mg/kg and 200 mg/kg dose and the enzyme Aspartate Amino transferase levels is slightly decreased in animals group that received 400 mg/kg dose. The enzymes AST, ALT, ALP and the biochemical parameter bilirubin are considered as the markers of liver function.¹⁹ Hepatocellular damage is characterized by the rise in serum levels of both AST and ALT. But since ALT is located primarily in the cytosol of

hepatocytes, this enzyme is considered a more sensitive marker of hepatocellular damage than AST and within limits can provide a quantitative assessment of the degree of damage sustained by the liver.²⁰ ALP is most often measured to indicate bile duct obstruction.²¹ There were no significant changes in the serum levels of AST, ALT and ALP and bilirubin demonstrating that liver function was preserved in animals exposed to SFTT extract over the 28 days period. Kidney function was evaluated by means of serum urea, creatinine and blood electrolyte concentrations. In preclinical toxicity studies, renal changes are particularly liable to occur because of the high doses given and the fact that the kidneys eliminate many drugs and their metabolites.^{22,23} Repeated oral administration of SFTT extract for 90 days did not cause significant changes in renal function. From biochemical parameters it is evident that the kidney

function was well maintained as there was no increase in the values of urea creatinine and electrolytes levels in serum when compared to the normal rats. (Table 4)

Effect of SFTT extracts on lipid profile

The liver produces most of the plasma lipo proteins in the body such as total cholesterol, LDL and HDL cholesterol. Table 6 depicts the Effect of SFTT extracts on Lipid profile after 28days treatment in rats. The level of total and LDL

Cholesterol was found to be decreased in the group III and Group IV rats which received the dugs at the dose level of 200mg/kg and 400mg/kg bodyweight .However there was no significant changes observed in the HDL and VLDL fractions of the cholesterol as comparable to that of the control rats. However significant decrease in the triglycerides level is observed in group IV animals when comparable to the control, group II and group III animals.

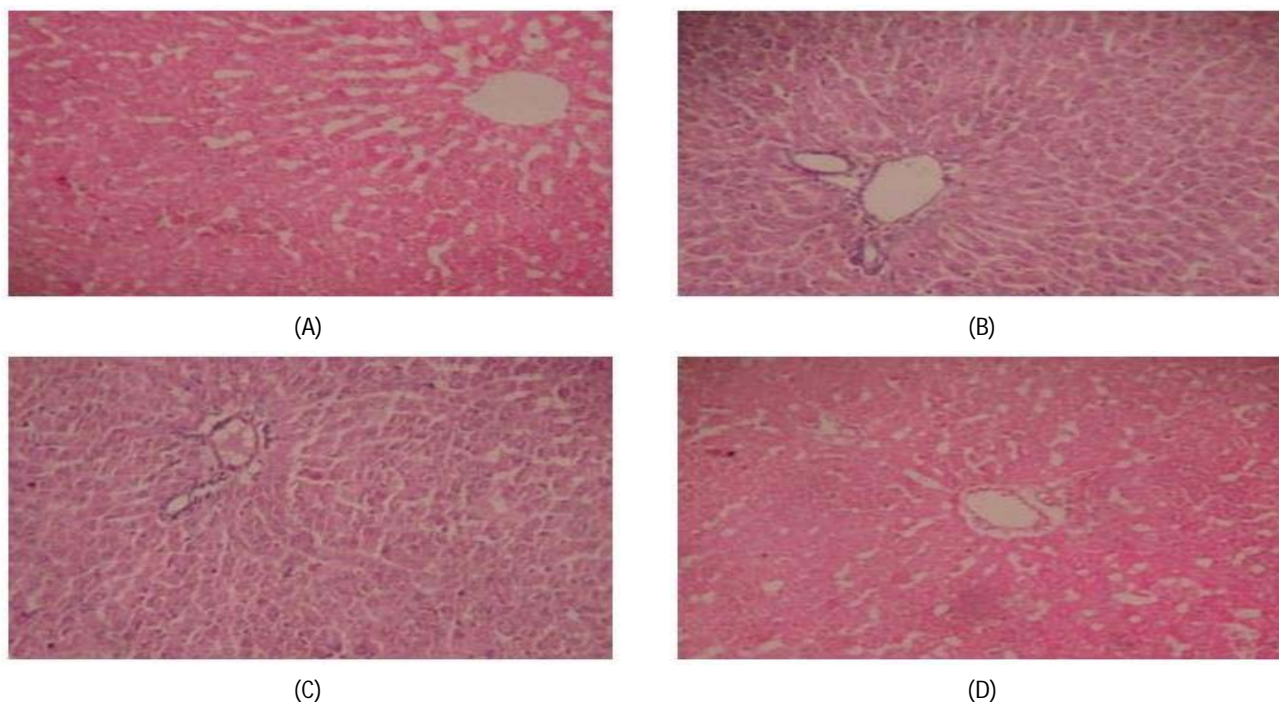


Figure 2 (A-D): Photomicrograph showing normal architecture of Liver in control (A), SFTT 100mg/kg (B), SFTT 200mg/kg (C) and SFTT 400mg/kg (D) administered rats for 28 days.

Effect of SFTT extracts on organ weight and Histopathology

Changes in organ weights are also an indicator of toxicity in the preclinical toxicity studies. There is a very high possibility that some of the herbal drugs may cause severe toxicity to the vital organs such as kidney, liver, spleen, brain, heart, stomach, and lungs because of their diverse roles in the human body. In the present investigation single administration of SFTT extract for 28 days did not record any change in the weight of the isolated organ such as liver, heart, lung, spleen, ovary, testis, Brain, kidney and stomach when comparable to the control animals. (Table 6) Similarly the detailed histopathological examination of the liver and Kidney did not reveal any observable cellular damage. Liver and kidney cells appear normal architecture on comparison with the control group. The evaluation of histopathological changes in organs remains a cornerstone in safety assessment of medicines.²⁴

CONCLUSION

The current study provides evidence that the saponin richbutanol fraction of *Tribulus terrestris*, (SFTT) fruit extract have no acute toxicity up to 2000 mg/kg. Oral

administration of SFTT extract to rats at doses of 100,200 and 400 mg/kg/day for 28 consecutive days caused no significant toxicological effects. Acute and sub acute oral toxicity studies of SFTT extract clearly demonstrate the non-toxic nature of the drug. The combined history of human use of *Tribulus terrestris*, fruits and the data from the current study support the safe use of the *Tribulus terrestris*, fruits.

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