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Original Research Article

Evaluation of Toxicity and Anticonvulsant Activities of *Solanum torvum Sw*., Fruits

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ABSTRACT

Solanum torvum Swartz , a member of the Solanaceae family, is a significant medicinal plant that is found all over the world and is used in traditional medical systems to treat conditions like diabetes, high blood pressure, tooth decay, and reproductive issues. The study aimed to evaluate the anticonvulsant and neuroprotective effects of *Solanum torvum* fruit extract (STF) in rodent models and to assess its acute and subacute toxicity. Acute toxicity was assessed in Wistar rats at doses up to 4000 mg/kg, while subacute toxicity was evaluated over 30 days at doses of 125, 250, and 500 mg/kg. The anticonvulsant activity was evaluated using Maximal Electroshock (MES) and Pentylenetetrazole (PTZ)-induced seizure models at 200 and 400 mg/kg. The neurotransmitter levels (acetylcholine and dopamine) and oxidative stress markers (superoxide dismutase [SOD], catalase [CAT], and malondialdehyde [MDA]) levels in the brain were measured to explore their neuroprotective effects. The phytochemical analysis revealed the presence of various bioactive compounds. Acute and subacute toxicity studies showed no adverse effects on body or organ weight, haematological parameters, or organ health, confirming safety. In the MES and PTZ models, STF demonstrated dose-dependent anticonvulsant effects, significantly reducing hind limb tonic extension (HLTE), hind limb tonic flexion (HLTF), jerking duration, and increased survival, achieving 100% protection at 400 mg/kg in the PTZ model. STF reduced Acetylcholine (Ach) levels and partially restored dopamine levels, counteracting neurochemical imbalances in the convulsion's pathophysiology. In addition, STF increased SOD and CAT levels and reduced MDA levels, proving its antioxidant potential. STF is observed as safe and exhibits significant anticonvulsant and neuroprotective properties, particularly at 400 mg/kg. Its ability to modulate neurotransmitters and reduce oxidative stress suggests its potential as an adjunctive therapy for convulsive disorders, warranting further exploration of its mechanisms and therapeutic applications.

Keywords: *Solanum torvum*, Anticonvulsant activity, MES, PTZ

Introduction

Medicinal plants have been used to alleviate ailments in human societies. Many contemporary Western medications have been developed from traditional knowledge by studying and identifying these plants.[1] The toxicity of numerous bioactive chemicals found in medicinal plants still needs to be better understood despite their potential for therapeutic use. Even though these plants offer a wealth of phytoconstituents that can be used to cure various illnesses, the application of herbal treatments has grown faster than the thorough scientific evaluation of their efficacy and safety. Concerns regarding possible toxicity and negative effects have increased. A thorough assessment of their safety and therapeutic efficacy becomes necessary to realize their benefits for humanity fully.[2]

The main cause of epilepsy, a chronic disorder that affects over 40 million people globally, is recurring spontaneous aberrant electrical discharges in a cluster of brain neurons. Despite the effectiveness of many commercially available antidepressants, they frequently have negative side effects, such as impaired vision, agitation, exhaustion, weight gain, nausea, dry mouth, and sexual dysfunction.

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Similar side effects include tiredness, memory loss, weight swings, and physical dependence on anti-anxiety drugs like diazepam and benzodiazepines.[3-4] Epilepsy is a prevalent neurological disorder affecting 65 million people worldwide, characterized by recurrent seizures.[5] It arises from diverse causes, including genetic mutations, structural abnormalities, infections, and autoimmune conditions, though its etiology often remains unknown. Despite the availability of over 20 anti-seizure drugs, one-third of patients experience drug-resistant epilepsy, highlighting the need for alternative treatments. The pathophysiology involves epileptogenesis, marked by molecular and cellular changes, including neuroinflammation, oxidative stress, and blood-brain barrier dysfunction, leading to neuronal hyperexcitability.[6] Advances in animal models have enhanced understanding, yet translation to human therapies remains limited. Accurate diagnosis through EEG, imaging, and clinical evaluations is crucial but challenging, particularly in resource-limited settings. The growing interest in plant-based therapies, offering diverse bioactive compounds with fewer side effects, presents promising adjunctive options.[7-8]

Solanum torvum Sw., commonly known as Turkey berry, belongs to the Solanaceae family and holds significant value in traditional medicine across the globe. Various parts of the plant—fruits, seeds, and leaves—treat conditions such as fever, cough, pain, hypertension, and liver disorders. Additionally, it is employed in managing tooth decay and reproductive issues and as an antidote for certain toxins.[9] Known for its rich nutritional profile, *S. torvum* is a dietary staple in some cultures, providing a natural source of vitamins and minerals and its therapeutic effects.[10]

Phytochemical studies have revealed that *S. torvum* contains a diverse array of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, glycosides, and notable levels of vitamins C, B, and E.

Compounds such as solasonine, solamargine, and polyphenols contribute to its antioxidant, anti-inflammatory, and antimicrobial properties.[11-12] Pharmacological investigations have further confirmed its cardioprotective and nephroprotective effects, primarily through antioxidant mechanisms that combat oxidative stress in cardiovascular and kidney diseases.[13] The plant's anti-inflammatory and analgesic actions are linked to the inhibition of prostaglandins and other inflammatory mediators, while its anti-ulcer activity is associated with enhanced gastric mucosal integrity.[14] Additionally, *S. torvum* exhibits promising antimicrobial and antiviral activities, including efficacy against herpes simplex virus, supporting its traditional uses and potential applications in modern natural medicine.[15-16]

The limitations of current AEDs, including seizure control failure and adverse side effects, have spurred interest in natural treatments for epilepsy. Plant-based therapies offer a promising alternative, leveraging diverse bioactive constituents with unique therapeutic mechanisms.[17] Herbs such as *Gastrodia elata*, *Uncaria rhynchophylla*, and *Curcuma longa* have demonstrated anticonvulsive, anti-inflammatory, and antioxidative properties, enhancing neurotransmitter regulation, neuroprotection, and mitochondrial function.[18] These bioactive compounds, including flavonoids, alkaloids, and terpenoids, modulate synaptic functions, neurotransmitter pathways (e.g., GABA, glutamate), ion channels, and oxidative stress, thereby reducing seizure frequency and severity. While preclinical studies are promising, the lack of robust clinical trials and standardization limits their mainstream adoption. Integrating herbal medicines with modern therapies may offer a safer, more accessible solution for epilepsy management, particularly in resource-limited settings.[19]

In this study, systematically evaluated the anticonvulsant potential of *S. torvum* through phytochemical profiling and toxicity testing using *in vitro* and *in vivo* models. Our findings aim to evaluate the possible mechanism of the antiepileptic activity of *S. torvum* and establish its potential as a safe and effective treatment for epilepsy, bridging traditional knowledge with modern scientific approaches to advance therapeutic strategies.

Materials and Methods

Plant material

The ripe fruits of *S. torvum* were collected in December 2023 from a fully grown tree in Hyderabad, Telangana State, India, and authenticated by the Botanica Survey of India, Deccan Regional Center, Hyderabad, Telangana, India (BSI/DRC/2022-23/identification/40). The shade-dried fruits were powdered and extracted with ethanol using the Soxhlet apparatus.[20]

Preliminary phytochemical analysis

Ethanol extraction of *S. torvum* fruit yielded 3.6% from 1000 g of dried fruit. Following conventional protocols, preliminary phytochemical analyses of the ethanol extract of matured fruits of *Solanum torvum* were conducted to identify the main phytochemical elements, including alkaloids, proteins, carbohydrates, tannins, sterols, triterpenoids, saponins, and flavonoids[21-22]

Acute toxicity studies

Acute toxicity studies were conducted on male and female Wistar rats following slightly modified OECD-423 guidelines. The animals were divided into five groups: males and females. A single oral dose was administered at 1000, 1500, 2000, and 4000 mg/kg body weight for the test groups. The extract was suspended in a 1% (v/v) Tween-20 solution and thoroughly mixed before administration. Conversely, the control group was administered 1% (v/v) Tween-20 as a vehicle. Animal ethical committee approval was taken for conducting these experiments (MLRIP/CPCSEA/IAEC/CL/2023/02).

Before the experiment began, all rats were weighed, labelled for identification, and subjected to overnight fasting, although they were given unrestricted access to water. After dosing, an additional 4-hour fasting period was observed. Continuous monitoring of mortality or abnormal changes was performed on each rat within their respective groups for the initial 4 h and then after a 24-hour interval post-drug administration.

For the subsequent 14 days, the rats were observed twice daily to identify any toxic effects, viz., alterations in eye, fur, and skin pigmentation, food and water consumption, presence of tremors, seizures, salivation, diarrhoea, lethargy, respiratory patterns, aggressive behavior, and motor activity levels.[23-25]

Sub-acute toxicity

The study was conducted on male and female Wistar rats according to OECD-407 guidelines, with slight modifications.[26] Approximately 40 healthy animals of both sexes were selected for 30 days. They were divided into four groups, where each group contained five male and five female rats, that is, Group-1 (control-normal saline), Group-2 (125 mg/kg dose), Group-3 (250 mg/kg dose), and Group-4 (500 mg/kg dose). Each animal was subjected to twice-daily assessments for signs of mortality and illness. Physical evaluations incorporate a multifaceted approach, focusing on the fur texture, skin condition, eye appearance, and mucus membrane health. Autonomic responses like piloerection, fluctuations in pupil diameter, tear production, and atypical respiratory rhythms were also closely scrutinized.[27]

Parameters tested

Body weight and Organ weight

Body weights were measured every five days, and organ weights were measured after 30 days. Before conducting the postmortem examination, all animals were subjected to fasted overnight. Anesthesia was induced using ketamine hydrochloride (intramuscularly administered at 100 mg/kg), and the animals were sacrificed. A comprehensive macroscopic examination focused on the external surfaces, all body orifices, and thoracic, abdominal, cranial, and pelvic cavities.

During the procedure, critical organs, namely, the liver, kidneys, pancreas, heart, lungs, and stomach, were harvested from male and female rats. Subsequently, these organs were rinsed and preserved in a 10% neutral buffered formalin solution. A thorough macroscopic examination of each organ was performed to identify any lesions or abnormal development.

For organ weight analysis, both absolute and relative weights were determined. The organs were momentarily placed on absorbent paper to remove excess moisture before weighing them. The organs examined included the liver, kidneys, pancreas, heart, lungs, and stomach. The absolute weight of each organ was recorded in grams (g).

Relative organ weight = (organ weight/ day 30 body weight) ×100

Blood was drawn through a retro-orbital puncture using capillary tubes. The samples were immediately transferred into sterilized tubes containing the anticoagulant EDTA for haematological assessment and tubes devoid of anticoagulants for biochemical evaluation. All rats were subjected to overnight fasting before blood collection and samples were obtained before the planned necropsy procedures.

The following parameters were estimated using an automatic haematological analyzer (Sysmex XS-1000i): Haemoglobin, WBC count, RBC count, Haematocrit percentage, Platelets count, mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Percentage of Mean corpuscular haemoglobin concentration (MCH%), and Differential cell counts such as Neutrophils, Lymphocytes, Eosinophils, and Monocytes. The biochemical parameters were evaluated using an automated biochemical analyser (ADVIA 2400, Siemens Healthcare) and standard diagnostic test kits (Span Divergent Ltd., Surat, India). Parameters such as Glucose level, Creatinine level, Urea level, Ions, Sodium, Potassium, Chloride, Total protein, Albumin, Bilirubin, SGPT, SGOT, ALP, Triglycerides, Total cholesterol, HDL, LDL, and VLDL.

Urine analysis

Urine samples from all the animals were collected overnight in metabolic cages. An automatic urine analyser and test strips were used to analyzed the following urine parameters: Volume, Colour,

Transparency, pH, Specific gravity, Urobilinogen, Bilirubin, Glucose, Ketone bodies, Blood (RBC), Protein, WBC, and Epithelial cells.

Histopathological studies
The study was conducted on the heart, kidney, liver, lungs, pancreas, stomach, and testis. The organs were placed in a 10% formalin solution. Thin sections of approximately 5µm thickness were obtained using a microtome. Staining was performed using hematoxylin and eosin staining. The sections were examined under a microscope to determine pathological conditions.[28]

Neurological parameters
Open field test
The rats were transferred to a sound-attenuated observation room and allowed to acclimate for one hour. After 125, 250, or 500 mg/kg administration, the rats were returned to their home cages and observed for 10 minutes. Observations in the home cages included posture, the presence or absence of tremors and convulsions, spontaneous vocalization, and biting. This was followed by observation of lacrimation, salivation, handling activity, piloerection, and ease of removal from the cage. The animals were then placed in an open field covered with a clean absorbent paper and allowed to explore freely for 2 min. The parameters explored during this time were arousal activity, gait, mobility, and unusual movements. After the allotted time, the number of fecal boluses and the presence or absence of diarrhoea on the absorbent paper were recorded. Sensorial responses were also monitored, such as response to various stimuli (click stimulus using a metal clicker, pinch on the tail using the metal tweezers, constriction of the pupil to a penlight stimulus, touch in the corner of the eye using fine cotton thread). The parameters of home cage assessment, Handheld observations, open field observations, and sensorial responses were measured. [29-30]

In-vitro AchE inhibition assay
The inhibition of AChE activity was measured using a 96-well microplate reader based on Ellman's method. The enzyme hydrolyzes the substrate acetyl thiocholine iodide (ATCI) to thiocholine and acetic acid. Thiocholine reacts with 5,5- dithiobis(2-nitro benzoin acid) (DTNB), resulting in the development of a yellow color that can be detected at 405 nm, which is proportional to enzyme activity. In 96-well plates, a reaction mixture of 25 µl of 15 mM ATCI in water, 125 µl of 3 mM DTNB in buffer, and 25 µl of STF (0.25, 0.5, 1, and 2µl) was added, and the absorbance was measured at 405 nm. Next, 25 µl of AChE solution (0.22 U/ml) was added to the wells, and the microplate was read at the same wavelength ten times at 1 min intervals. Galanthamine dissolved in methanol was used as a standard drug at a 1 mg/ml concentration, and a blank of methanol in 50 mM Tris-HCl (pH 8) was used. The percentage inhibition for each test solution was then calculated using the following equation.[31]
Inhibition (%) =1-($A_{sample}/A_{control}$) X 100

In- vivo Anticonvulsant activity
Maximum Electroshock (MES) induced convulsions
Electroshock was applied separately to each rat via ear-clip electrodes, administered saline or the respective drug was administered at appropriate times. The stimulus duration was 0.2 sec, and the current frequency was 50 Hz. The animals were observed for the occurrence of the Hind limb tonic extensor (HLTE) and Hind limb tonic flexion (HLTF) for 30 min. The endpoint of this model was defined as the protection against seizures. The animals that gained their normal exploratory behavior within 10 sec of stimulation were protected.[32]

Pentylenetetrazole (PTZ) induced convulsion
PTZ-induced seizures in rodents are an experimental model for myoclonic convulsions in humans. Clonic convulsions were induced in rats by the subcutaneous administration of PTZ at a dose of 100 mg/kg. Following PTZ injection, the rats were placed separately in a transparent plexiglass cage and observed for 30 min for clonic seizures. Clonic seizures were defined as clonus of the whole body lasting over 3 sec with an accompanying loss of righting reflex.[33]

Estimation of biochemical parameters in brain homogenate
Determination of superoxide dismutase
Superoxide dismutase (SOD) activity was estimated using the method described by Kakar et al., with slight modifications. 0.1 ml of phenazine methosulfate (186 µmol), 1.2 ml of sodium pyrophosphate buffer (0.052 mmol; pH 7.0), and 0.3 ml of the supernatant (0.1 mL) after centrifugation (1500 × g for 10 min followed by 10,000 × g for 15 min) of the rat brain homogenate were mixed. The enzyme reaction was initiated by adding 0.2 ml of nicotinamide adenine dinucleotide (NADH, 780 µmol) and stopped after 1 min by adding glacial acetic acid (1 mL). The amount of chromogen formed was measured by recording the color intensity at 560 nm. Results are expressed in units/mg tissue.[34]

Determination of catalase
A mixture of 2.5 ml of 50 mmol phosphate buffer (pH 5.0), 0.4 ml of 5.9 mmol hydrogen peroxide (H_2O_2), and 0.1 ml of rat brain homogenate was prepared. The absorbance of the reaction solution was measured at 240 nm after one minute. One unit of catalase activity was defined as a 0.01 unit/min change in absorbance.[35]

Determination of MDA
Lipid peroxidation was determined by measuring the rate of production of thiobarbituric acid-reactive substances (TBARS) (expressed as malondialdehyde (MDA) equivalents). Brain homogenate (1ml) was mixed with 25% trichloroacetic acid (TCA) and 1 ml of 0.67% thiobarbituric acid (TBA). The samples were thoroughly mixed, heated for 20 minutes in a boiling water bath, cooled, and centrifuged at 4000 × g for 20 minutes. The absorbance of the supernatant was measured at 535 nm against a blank containing all reagents except for the tissue homogenate. MDA concentration was calculated using a molar extinction coefficient of 1.56 × 105 $M^{-1}cm^{-1}$ and expressed in nmol/g wet tissue.[36]

Statistical analysis
The results of the experiments and observations were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism version 9.0, using appropriate tools to understand significance.

Results and Discussion

Phytochemical analysis
Phytochemical screening of the *S. torvum* fruit extract revealed high levels of alkaloids and flavonoids, moderate levels of terpenoids, saponins, and steroids, trace amounts of tannins, and an absence of cardiac glycosides. (Table 1).

Table 1: Phytochemical analysis of *S. torvum* ethanolic fruit extract

S. No	Phytochemical	Present or Absent
1	Alkaloids	+
2	Terpenoids	+
3	Saponins	+
4	Steroids	+
5	Flavonoids	+
6	Tannins	+
7	Cardiac Glycosides	-

'+' Present '-' Absent

Acute Toxicity studies
The initial body weights for male rats showed no significant changes on days 7 and 14. On Day 1, body weights ranged around 155 g across doses, and by Day 14, the weights showed only a slight increase, with

averages ranging from 172.2±0.86 g to 173±0.71 g. This consistent pattern across the days indicated that STF did not cause any adverse or significant effects on the body weight of male rats at any of the administered doses (Table 2).

A similar pattern was observed in the female rats. The initial weights on day 1 ranged around 134-135 g across all doses, with minor weight increases over time. By Day 14, the body weights across different STF doses showed only slight increases without significant variation between doses. This stability in body weight suggests that STF does not adversely affect the body weight of female rats. These results indicate that *S. torvum* fruit extract, even at a high dose of 4000 mg/kg, does not produce any toxic effects that influence body weight in male or female rats. These data support the safety of STF at these acute doses, with no notable impact on the growth or body weight of the test animals over the study period.

The Hodge and Sterner scale, which determines the toxicity level of any drug administered orally, divides the material's LD_{50} value into five classes. Class 1 is the most harmful (LD_{50}< 1 mg/kg); Class 2 is the most harmful (LD_{50}< 1–50 mg/k); Class 3 is the fairly harmful (LD_{50}< 50–500 mg/kg); Class 4 is the least harmful (LD_{50}< 500–5000 mg/kg); and Class 5 is the non-poisonous ($LD_{50}$is 5000–15000 mg/kg). STF may belong to classes 4 or 5; its LD_{50} is expected to be greater than 4000 mg/kg body weight because no toxicity is seen at the aforesaid dosage.

Table 2: Effect on the body weight of male and female rats

Sex	Day	STF mg/kg				
		Control	1000	1500	2000	4000
Male	1	154±1.3	155.6±0.68^{ns}	155±1.1^{ns}	153.6±1.63^{ns}	156±1.14^{ns}
	7	164±1.14	164±1^{ns}	164.2±1.66^{ns}	165.4±1.36^{ns}	164.8±1.02^{ns}
	14	172.6±0.93	173±0.71^{ns}	172.2±0.86^{ns}	173±0.71^{ns}	172.8±0.86^{ns}
Female	1	134.2±1.16	134±1.58^{ns}	135.4±1.21^{ns}	135.4±1.08^{ns}	133.8±1.07^{ns}
	7	144.2±1.16	145.6±1.03^{ns}	145±1.14^{ns}	145±1.26^{ns}	144±1.52^{ns}
	14	152.2±0.86	152±0.71^{ns}	153.4±0.51^{ns}	152.8±0.86^{ns}	153±0.71^{ns}

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. $^{***}p< 0.001$ $^{**}p< 0.01$ $^{*}p< 0.05$, ^{ns} non-significant with the control.

Subacute Toxicity studies

During the study, there were no signs of mortality in animals treated with STF at doses of 125, 250, or 500 mg/kg body weight. Over the course of the 30-day experiment, none of the rats (males or females) exhibited overt morbidity or clinical symptoms of toxicity. Symptoms included changes in the skin and fur, eyes, respiratory rate, autonomic (salivation, perspiration, and piloerection), and stereotypical activities. No clinical symptoms of toxicity were observed in the control group.

Body weight

In the subacute toxicity study of *S. torvum* fruit extract (STF), male and female rats were administered doses of 125, 250, and 500 mg/kg, and their body weights were monitored (mentioned in Table 3) over a 30-day period to determine any potential impact on growth or health

For male rats, initial body weights on Day 0 ranged between 152.4±0.81 g and 154±0.71 g across all doses. Over the 30-day period, the body weight gradually increased in each group. By Day 30, weights had risen to 180.8±0.49 g and 181.8±0.8 g, showing a consistent and gradual increase. This steady weight gain, without notable differences among the various doses, suggests that STF does not negatively affect body weight in male rats, even with continuous exposure over 30 days. (Table 3).

The female rats followed a similar trend. Initial weights on Day 0 ranged between 133±0.63 g and 134.2±0.8 g across the doses. Throughout the 30-day study, weights steadily increased, reaching values between 161±0.63 g and 161.4±0.81 g by Day 30. The gradual weight gain observed across all STF doses indicated that the extract did not adversely affect the body weight of the female rats. *The S. torvum* fruit extract did not produce any toxic effects that influenced body weight in either male or female rats over a 30-day period, even at the highest dose of 500 mg/kg. The consistent weight gain in both sexes supports the safety of STF at these subacute doses, as it did not hinder growth or cause body weight loss in the test animals.

Table 3: Effect on the body weight of male and female rats

Sex	Day	STFE mg/kg			
		Control	**125**	**250**	**500**
Male	0	154.2±1.10	152.4±0.81^{ns}	153.8±0.66^{ns}	154±0.71^{ns}
	5	157.8±2.86	155.8±0.8^{ns}	155.6±0.68^{ns}	156±1.05^{ns}
	10	163.2±0.84	162.6±0.75^{ns}	163±0.45^{ns}	161.4±0.51^{ns}
	15	166.2±0.84	163.2±0.58**	164.4±0.51^{ns}	165±0.84^{ns}
	20	171.4±0.89	167.6±1.44^{ns}	169±0.55*	169.8±0.49
	25	177±1.00	174.6±0.4*	175.6±0.4^{ns}	176±0.71^{ns}
	30	183.2±0.84	180.8±0.49^{ns}	181.2±0.37*	181.8±0.8*
Female	0	135.2±0.84	133±0.63^{ns}	133.8±0.73^{ns}	134.2±0.8^{ns}
	5	139.4±0.89	136.8±0.58*	137.6±0.75^{ns}	138±0.84^{ns}
	10	143.2±0.84	140.6±0.4**	141.4±0.75^{ns}	141.4±0.68^{ns}
	15	146.2±0.84	143.6±0.4**	143.8±0.49*	144±0.32**
	20	152.2±0.84	150.2±0.58^{ns}	150.8±0.37^{ns}	151±0.55^{ns}
	25	157.6±0.55	154.2±0.49**	154.8±0.73*	155.2±0.8^{ns}
	30	162.4±0.89	161±0.63^{ns}	162±0.71v	161.4±0.81^{ns}

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. $^{***}p< 0.001$ $^{**}p< 0.01$ $^{*}p< 0.05$, ^{ns} non-significant with the control.

Organ weight

The weights of the vital organs, including the liver, kidney, pancreas, heart, stomach, and lungs, were measured to assess any potential impact. For male rats, liver weights remained consistent across doses, ranging from 3.44±0.1 g at 125 mg/kg to 3.56±0.07 g at 500 mg/kg, indicating no adverse effect on liver size. Kidney weights showed minimal variation, from 0.5±0.01 g at 125 and 250 mg/kg to 0.51±0.01 g at 500 mg/kg. Pancreas weights remained stable at around 0.29 g to 0.3 g across all doses. Heart weights also showed consistency, with values remaining at approximately 0.51 g across all STF doses. At the highest dose of 500 mg/kg, stomach weights were slightly reduced from the control at 2.62 ± 0.04 g at the lowest dose and showed a minor increase to 2.72 ± 0.08 g at the control. Lung weights varied slightly from 1.25±0.03 g at 125 mg/kg to 1.28±0.04 g at 500 mg/kg. These findings suggest that STF did not significantly affect the organ weights of male rats, and female rats showed a similar trend. Liver weights ranged from 3.44±0.1 g at 125 mg/kg to 3.56±0.07 g at 500 mg/kg. Kidney weights remained between 0.5±0.01 g and 0.51±0.01 g across doses. Pancreas weights were stable at approximately 0.29 g to 0.3 g. Heart weights were consistently around 0.51 g across all doses. The stomach weights ranged from 2.62±0.04 g at 125 mg/kg to 2.72±0.08 g at 500 mg/kg. Lung weights showed minimal variation, from 1.25±0.03 g at 125 mg/kg to 1.28±0.04 g at 500 mg/kg. (Table 4).

The organ weights of both male and female rats were stable across all doses of STF, with only minor variations observed. This suggests that *S. torvum* fruit extract does not produce significant toxic effects on organ weights in either sex, supporting its safety at subacute doses of up to 500 mg/kg.

Table 4: Effect on the organ weight of male and female rats

Sex	Organ	STF mg/kg			
		Control	**125**	**250**	**500**
Male	liver	3.44±0.26	3.44±0.1^{ns}	3.54±0.09^{ns}	3.56±0.07^{ns}
	kidney	0.55±0.05	0.5±0.01^{ns}	0.5±0.01	0.51±0.01^{ns}
	pancreas	0.34±0.03	0.29±0.01^{ns}	0.29±0.01^{ns}	0.3±0.01^{ns}
	heart	0.60±0.03	0.51±0.01^{ns}	0.51±0.02^{ns}	0.51±0.01^{ns}
	stomach	3.5±0.17	2.62±0.04^{ns}	2.68±0.06^{ns}	2.72±0.08^{ns}
	lungs	1.41±0.06	1.25±0.03^{ns}	1.27±0.04^{ns}	1.28±0.04^{ns}
Female	liver	3.34±0.35	3.44±0.1^{ns}	3.54±0.09^{ns}	3.56±0.07^{ns}
	kidney	0.48±0.02	0.5±0.01^{ns}	0.5±0.01^{ns}	0.51±0.01^{ns}
	pancreas	0.27±0.03	0.29±0.01^{ns}	0.29±0.01^{ns}	0.3±0.01^{ns}
	heart	0.49±0.03	0.51±0.01^{ns}	0.51±0.02^{ns}	0.51±0.01^{ns}
	stomach	2.60±0.12	2.62±0.04^{ns}	2.68±0.06^{ns}	2.72±0.08^{ns}
	lungs	1.19±0.10	1.25±0.03^{ns}	1.27±0.04^{ns}	1.28±0.04^{ns}

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. $^{***}p< 0.001$ $^{**}p< 0.01$ $^{*}p< 0.05$, ^{ns} non-significant with the control.

Urine analysis

The subacute toxicity study of *S. torvum* fruit extract (STF) included a urine analysis of male and female rats to evaluate any potential impact on urinary parameters at doses of 125, 250, and 500 mg/kg (mentioned in Table 5). For male rats, in 24 hrs, urine volume slightly increased across doses, from 20.6±2.07 mL in the control group to 23.8±1.48 mL at the highest dose of 500 mg/kg. The urine colour remained yellow across all doses, and the transparency was clear. Urinary pH ranged from 6 to 8 in all groups, with no deviations. Specific gravity remained stable between 1.015 and 1.020 across doses, and other biochemical parameters, including urobilinogen (0.2 mg/dL), bilirubin (0 mg/dL), glucose (0 mg/dL), ketone bodies (0 mg/dL), occult blood (0 RBC/µL), protein (0 mg/dL), leukocytes (0 WBC/µL), and epithelial cells (0 HPF), showed no abnormal findings across all doses. (Table 5).

In female rats, urine volume remained relatively stable, slightly increasing from 15.4±1.67 mL in the control to 17.2±0.84 mL at 500 mg/kg. As with males, the urine colour was yellow, transparency was clear, and pH ranged from 6 to 8 across all doses. Specific gravity remained within 1.015-1.020 across all groups, with urobilinogen at 0.2 mg/dL, and no bilirubin, glucose, ketone bodies, occult blood, protein, leukocytes, or epithelial cells were detected at any dose.

These findings indicate that *S. torvum* fruit extract did not produce any adverse effects on the urinary parameters of male or female rats at doses of up to 500 mg/kg over the study period. Urine analysis showed stability across all measured parameters, suggesting that STF lacks nephrotoxicity or other urinary-related toxic effects at subacute doses.

Haematological Analysis

The subacute toxicity study of *S. torvum* fruit extract (STF) included an assessment of haematological parameters in male and female rats to evaluate any potential impacts on blood components at doses of 125, 250, and 500 mg/kg over 30 days (Table 6).

In male rats, haemoglobin levels remained stable across doses, with slight fluctuations from 15.15±0.12 g/dL at 125 mg/kg to 14.08±0.21 g/dL at 500 mg/kg. White blood cell (WBC) counts ranged between 10.66±0.18 and 11.1±0.18 × 10^3/µL across doses, showing no marked deviations. Red blood cell (RBC) counts were consistent, with values from 9.34±0.05 9.46±0.05 × 10^6/µL. Haematocrit levels ranged slightly from 41.06±0.2% at 125 mg/kg to 42.62±0.09% at 500 mg/kg, while platelet counts remained stable between 7.74±0.06 and 8.6±0.05 × 10^5/mm³. Other parameters, such as the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), showed minor variations without significant differences. Neutrophil, lymphocyte, eosinophil, and monocyte percentages also remained within normal ranges, suggesting no adverse effects on immune cell distributions.

For female rats, haemoglobin levels ranged from 12.224±0.27 g/dL at 125 mg/kg to 13.3±0.19 g/dL at 250 mg/kg, showing minor fluctuations. WBC counts ranged from 8.06±0.22 8.64±0.18 × 10^3/µL across doses. RBC counts were also consistent, with values between 8.26±0.05 and 8.64±0.07 × 10^6/µL. The haematocrit levels showed slight variations, with values ranging from 36.66±0.09% at 125 mg/kg to 37.64±0.12% at 250 mg/kg. Platelet counts were stable, ranging from 6.68±0.22 7.1±0.05 × 10^5/mm³. Minor variations were observed in the MCV, MCH, and MCHC, but all remained within normal limits. The percentages of neutrophils, lymphocytes, eosinophils, and monocytes were stable, and showed no signs of immune compromise.

The haematological parameters in both male and female rats were stable across all STF doses, with no significant changes observed, even at the highest dose of 500 mg/kg. These findings suggest that the *S. torvum* fruit extract does not adversely affect blood health or immune parameters in either sex at subacute doses, supporting its safety profile.

Table 5: Urine analysis of male and Female rats

Sex	Parameters	units	STF mg/kg			
			Control	**125**	**250**	**500**
	Volume	mL	20.6±2.07	22.4±1.9^{ns}	23±1.23^{ns}	23.8±1.48^{ns}
	Colour		Yellow	Yellow	Yellow	Yellow
	Transparency		clear	Clear	clear	clear
	pH		6 to 8	6 to 8	6 to 8	6 to 8
	Specific gravity		1.015-1.020	1.015-1.020	1.015-1.020	1.015-1.020
	Urobilinogen	mg/dL	0.2	0.2	0.2	0.2
Male	Bilirubin	mg/dL	0	0	0	0
	Glucose	mg/dL	0	0	0	0
	ketone bodies	mg/dL	0	0	0	0
	Occult blood	RBC/µL	0	0	0	0
	Protein	mg/dL	0	0	0	0
	Leukocytes	WBC/µL	0	0	0	0
	Epithelial cells	HPF	0	0	0	0
	Volume	mL	15.4±1.67	15±0.7^{ns}	15.6±1.14^{ns}	17.2±0.84**
	Colour		Yellow	Yellow	Yellow	Yellow
	Transparency		clear	Clear	clear	clear
	pH		6 to 8	6 to 8	6 to 8	6 to 8
	Specific gravity		1.015-1.020	1.015-1.020	1.015-1.020	1.015-1.020
	Urobilinogen		0.2	0.2	0.2	0.2
Female	Bilirubin	mg/dL	0	0	0	0
	Glucose	mg/dL	0	0	0	0
	ketone bodies	mg/dL	0	0	0	0
	Occult blood	RBC/µL	0	0	0	0
	Protein	mg/dL	0	0	0	0
	Leukocytes	WBC/µL	0	0	0	0
	Epithelial cells	HPF	0	0	0	0

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. $^{***}p< 0.001$ $^{**}p< 0.01$ $^{*}p< 0.05$, ^{ns} non-significant with the control.

Table 6: Haematological parameters of male and female rats

Sex	Parameters	units	STF mg/kg			
			Control	**125**	**250**	**500**
	Haemoglobin	gms/dL	14.51±0.32	15.15±0.12*	14.98±0.11^{ns}	14.08±0.21^{ns}
	WBC	$10^3/\mu L$	10.71±0.22	10.666±0.25^{ns}	11.1±0.18^{ns}	10.66±0.18^{ns}
	RBC	$10^6/\mu L$	9.74±0.09	9.34±0.05***	9.48±0.05**	9.46±0.05**
	Haematocrit	%	41.70±1.00	41.06±0.2^{ns}	41.46±0.09^{ns}	42.62±0.09^{ns}
	Platelets	$10^5/mm^3$	8.44±0.11	8.6±0.05^{ns}	7.74±0.06****	7.74±0.06****
	Mean corpuscular volume	fL	55.20±1.30	52.8±0.37*	54.8±0.49^{ns}	56.4±0.68^{ns}
Male	Mean corpuscular haemoglobin	pg	16.40±2.07	16.8±0.58^{ns}	14.4±1.03^{ns}	16.8±0.58^{ns}
	Mean corpuscular hemoglobin concentration	%	34.00±2.0	37±0.71^{ns}	35.4±0.51^{ns}	34.8±0.73^{ns}
	Neutrophils	%	24.40±1.14	27.8±0.37**	24.2±0.58^{ns}	23.4±0.68^{ns}
	Lymphocytes	%	73.20±1.30	76±0.71*	77.6±0.93*	77.4±0.68**
	Eosinophils	%	1.74±0.18	1.78±0.04^{ns}	1.52±0.04^{ns}	1.5±0.03^{ns}
	Monocytes	%	2.28±0.11	2.76±0.05***	2.62±0.07*	2.36±0.07^{ns}
	Haemoglobin	gms/dL	12.46±0.13	12.224±0.27^{ns}	13.3±0.19*	11.246±0.17**
	WBC	$10^3/\mu L$	8.64±0.18	8.06±0.22^{ns}	8.16±0.14^{ns}	8.34±0.2^{ns}
	RBC	$10^6/\mu L$	8.38±0.16	8.26±0.05^{ns}	8.64±0.07^{ns}	8.32±0.09^{ns}
	Haemotocrit	%	38.94±0.55	36.66±0.09***	37.64±0.12**	36.44±0.1***
	Platelets	$10^5/mm^3$	6.68±0.22	7.1±0.05*	6.82±0.04^{ns}	6.72±0.07^{ns}
	Mean corpuscular volume	fL	55.20±2.59	57.8±0.37^{ns}	54.2±0.8^{ns}	53±0.32^{ns}
Female	Mean corpuscular haemoglobin	pg	18.00±0.71	13.6±0.51***	14.2±0.66**	16.8±0.37^{ns}
	Mean corpuscular hemoglobin concentration	%	33.60±1.82	32.8±0.86^{ns}	34.6±0.75^{ns}	33.6±0.93^{ns}
	Neutrophils	%	21.80±1.64	22.6±0.51^{ns}	23.2±0.58^{ns}	23.2±0.73^{ns}
	Lymphocytes	%	75.40±3.36	73.8±0.86^{ns}	75.8±0.66^{ns}	66.6±0.93**
	Eosinophils	%	1.44±0.18	1.28±0.04^{ns}	1.48±0.04^{ns}	1.76±0.05*
	Monocytes	%	2.26±0.11	2.24±0.06^{ns}	2.4±0.07^{ns}	2.64±0.05**

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. $^{***}p< 0.001$ $^{**}p< 0.01$ $^{*}p< 0.05$, ^{ns} non-significant with the control

Biochemical Analysis

In subacute toxicity studies, the biochemical parameters of male and female rats administered STFE at 125, 250, and 500 mg/kg were assessed to evaluate potential toxic effects (Table 7).

Significant changes were observed in several parameters in male rats. Glucose levels decreased slightly, but not significantly, across all the dose groups. Creatinine levels showed a significant decrease at all doses compared to the control (0.6±0.07 mg/dL), with the most pronounced reduction at 250 mg/kg (0.42±0.13 mg/dL, p<0.01) and 500 mg/kg (0.52±0.31 mg/dL, p<0.001). Total protein levels increased significantly at the highest dose (5.96±0.08 g/dL, p<0.01), while albumin levels decreased significantly at all doses, with the most marked decrease at 500 mg/kg (3.16±0.15 g/dL, p<0.001). Globulin levels decreased significantly at the highest dose (2.26±0.11 mg/dL, p<0.001). Bilirubin (D) levels showed a significant increase at the 250 mg/kg and 500 mg/kg doses (0.18±0.01 mg/dL, p<0.01). SGPT levels decreased significantly at 250 mg/kg (48.6±0.55 IU/L, p<0.01) and 500 mg/kg (47.2±0.84 IU/L, p<0.001), while SGOT levels increased slightly but significantly at the highest dose (43.8±0.84 IU/L, p<0.05). ALP levels were slightly decreased at 125 mg/kg (122±1.58 IU/L, p<0.05). Total cholesterol levels decreased significantly at the 250 mg/kg (82.4±1.14 mg/dL, p<0.05) and 500 mg/kg (82±0.70 mg/dL, p<0.001) doses. In contrast, VLDL levels showed a significant decrease at all doses, with the most significant reduction at 500 mg/kg (13.4±0.55 mg/dL, p<0.001).

In female rats, the glucose levels remained stable across all dose groups. Creatinine levels showed a slightly non-significant increase across doses. Urea levels significantly increased at the highest dose (50.36±0.58 mg/dL, p<0.05). Chloride levels increased significantly at 125 mg/kg (113.6±1.14 mmol/L, p<0.01) and 250 mg/kg (112.6±1.82 mmol/L, p<0.05). Total protein levels decreased significantly at 125 mg/kg (6.28±0.08 g/dL, p<0.05). Globulin levels increased significantly at 125 mg/kg (3.48±0.08 mg/dL, p<0.01) and 500 mg/kg (3.7±0.10 mg/dL, p<0.001). SGPT levels increased significantly at 250 mg/kg (58.21±1.76 IU/L, p<0.05). ALP levels decreased significantly at 125 mg/kg (123.84±1.50 IU/L, p<0.05) and 250 mg/kg (121.68±0.75 IU/L, p<0.01). VLDL levels decreased significantly at 125 mg/kg (17.37±1.11 mg/dL, p<0.05) and 250 mg/kg (16.97±1.11 mg/dL, p<0.05).

These data suggest that STFE administration affects several biochemical parameters in male and female rats. In male rats, significant changes were observed in creatinine, total protein, albumin, globulin, bilirubin (D), SGPT, SGOT, total cholesterol, and VLDL levels. In female rats, significant changes were observed in the urea, chloride, total protein, globulin, SGPT, ALP, and VLDL levels. These findings indicate the potential dose-dependent biochemical effects of STFE, warranting further investigation to understand the underlying mechanisms and potential health implications.

Table 7: Biochemical parameters of male and female rats

Sex	Parameters	units	STFE mg/kg			
			Control	125	250	500
Male	Glucose	mg/dL	75±1.00	85±0.71^{ns}	83.8±0.73*	83±0.45***
	Creatinine	mg/dL	0.6±0.07	0.66±0.01^{ns}	0.58±0.01^{ns}	0.55±0.01^{ns}
	Urea	mg/dL	42.6±1.51	49.48±0.24*	50.15±0.67*	51.26±0.22^{ns}
	Sodium	mmol/L	139.92±1.76	145±0.84*	145.42±1.27**	146.96±0.56^{ns}
	Potassium	mmol/L	6.52±0.21	6.92±0.05^{ns}	6.97±0.09^{ns}	7.15±0.09^{ns}
	Chloride	mmol/L	106.4±1.51	113±0.45^{ns}	111.6±0.51^{ns}	104.2±0.37^{ns}
	Total protein	g/dL	5.42±0.21	6.4±0.04^{ns}	6.28±0.04^{ns}	6.56±0.09^{ns}
	Albumin	g/dL	3.72±0.14	3.74±0.05*	3.3±0.04^{ns}	3.52±0.04**
	Globulin	mg/dL	2.82±0.08	3.16±0.02**	3.44±0.05***	3.48±0.06***
	Bilirubin (T)	mg/dL	0.52±0.08	0.74±0.06^{ns}	0.72±0.04^{ns}	0.54±0.04^{ns}
	Bilirubin (D)	mg/dL	0.14±0.02	0.15±0.01^{ns}	0.16±0.01*	0.2±0.01*
	SGPT	IU/L	53±1.58	60.58±2.08^{ns}	57.78±1.77**	62.69±1.91**
	SGOT	IU/L	41±1.58	43.82±0.57^{ns}	44.1±0.31^{ns}	44.52±0.36*
	ALP	IU/L	125.2±0.84	127.8±0.28^{ns}	125.46±0.46^{ns}	124.02±0.34^{ns}
	Triglyceride (TG)	mg/dL	84.8±0.84^{ns}	86.68±0.54^{ns}	85.56±0.29^{ns}	84.63±0.66^{ns}
	Total cholesterol	mg/dL	84.6±0.55	87.87±0.55^{ns}	86.05±0.38^{ns}	84.64±0.67**
	HDL	mg/dL	43.5±2.21	36.36±0.55^{ns}	36.97±0.94^{ns}	37.17±0.98^{ns}
	LDL	mg/dL	38.52±1.12	45.65±0.67*	43.43±0.71**	42.22±0.59*
	VLDL	mg/dL	17±1.00	18.58±0.4^{ns}	15.76±0.52^{ns}	14.14±0.32^{ns}
Female	Glucose	mg/dL	84.8±2.78	85±0.71^{ns}	83.8±0.73^{ns}	83±0.45^{ns}
	Creatinine	mg/dL	0.62±0.05	0.66±0.01^{ns}	0.58±0.01^{ns}	0.55±0.01^{ns}
	Urea	mg/dL	47.48±1.39	49.48±0.24^{ns}	50.15±0.67^{ns}	51.26±0.22^{ns}
	Sodium	mmol/L	146.1±1.83	145±0.84^{ns}	145.42±1.27^{ns}	146.96±0.56^{ns}
	Potassium	mmol/L	7.2±0.31	6.92±0.05^{ns}	6.97±0.09^{ns}	7.15±0.09^{ns}
	Chloride	mmol/L	109.2±1.30	113±0.45^{ns}	111.6±0.51^{ns}	104.2±0.37^{ns}
	Total protein	g/dL	6.44±0.09	6.4±0.04^{ns}	6.28±0.04^{ns}	6.56±0.09^{ns}
	Albumin	g/dL	3.32±0.19	3.74±0.05^{ns}	3.3±0.04^{ns}	3.52±0.04^{ns}
	Globulin	mg/dL	3.18±0.08	3.16±0.02^{ns}	3.44±0.05^{ns}	3.48±0.06^{ns}
	Bilirubin (T)	mg/dL	0.62±0.08	0.74±0.06^{ns}	0.72±0.04^{ns}	0.54±0.04^{ns}
	Bilirubin (D)	mg/dL	0.13±0.02	0.15±0.01^{ns}	0.16±0.01^{ns}	0.2±0.01^{ns}
	SGPT	IU/L	55.15±1.49	60.58±2.08^{ns}	57.78±1.77^{ns}	62.69±1.91^{ns}
	SGOT	IU/L	44.1±1.10	43.82±0.57^{ns}	44.1±0.31^{ns}	44.52±0.36^{ns}
	ALP	IU/L	127.62±2.33	127.8±0.28^{ns}	125.46±0.46^{ns}	124.02±0.34^{ns}
	Triglyceride (TG)	mg/dL	86.68±2.02	86.68±0.54^{ns}	85.56±0.29^{ns}	84.63±0.66^{ns}
	Total cholesterol	mg/dL	88.07±3.14	87.87±0.55^{ns}	86.05±0.38^{ns}	84.64±0.67^{ns}
	HDL	mg/dL	35.05±2.07	36.36±0.55^{ns}	36.97±0.94^{ns}	37.17±0.98^{ns}
	LDL	mg/dL	44.1±1.18	45.65±0.67***	43.43±0.71***	42.22±0.59***
	VLDL	mg/dL	19.17±0.71	18.58±0.4^{ns}	15.76±0.52**	14.14±0.32***

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. ***p< 0.001 **p< 0.01 *p< 0.05, ^{ns} non-significant with the control.

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Histopathological studies

Vital organs such as the liver, pancreas, kidney, heart, stomach, and lungs from both sexes, testes from male rats, and ovaries from female rats showed no major visible microscopic changes compared with the control group. The liver and kidney sections appeared normal with no abnormalities, and the cells were normal in size and shape in both males and females. Normal cellular structures were observed in both sexes' hearts, lungs, and stomachs. In the case of reproductive parts, both males and females appeared normal, with no major observable changes in tissue structure (Figures 1 and 2).

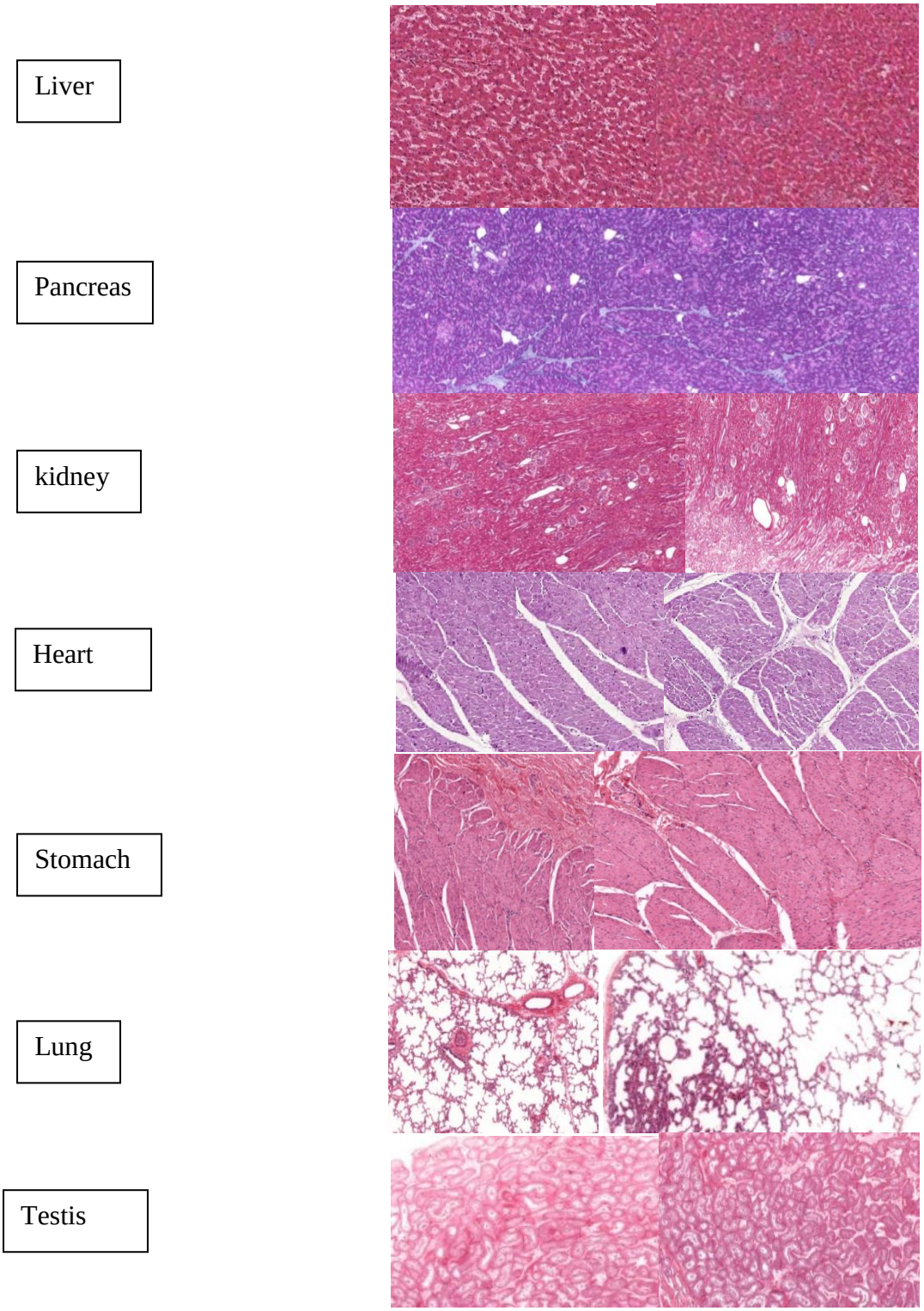

Figure 1: Histopathological analysis in male rats comparing the greatest dose levels at 500 mg/kg, body weight, with the control.

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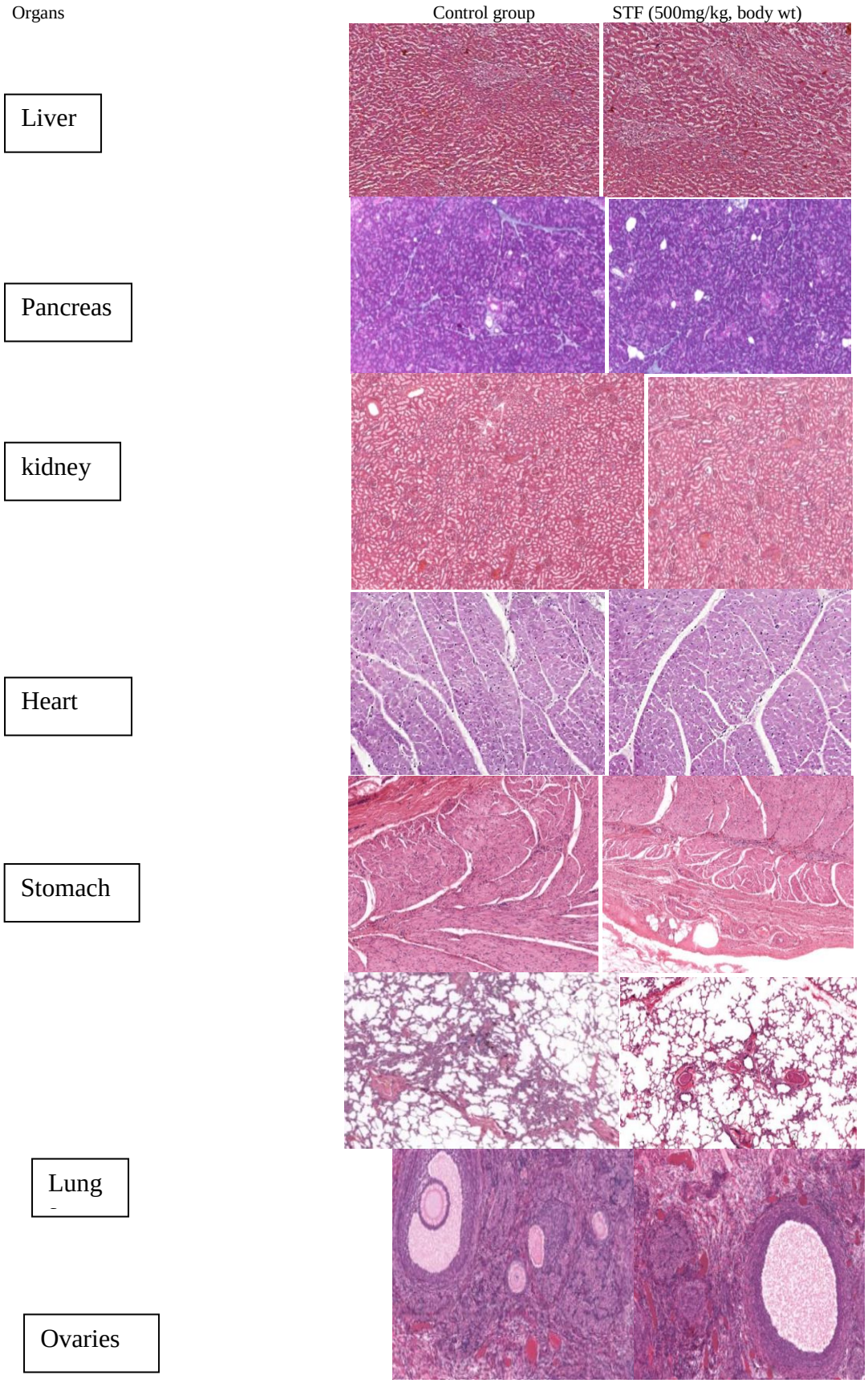

Figure 2: Histopathological analysis in female rats comparing the greatest dose levels at 500 mg/kg, body weight, with the control.

Neurological Parameters

A detailed study of neurological aspects is presented (Table 8). In the test group, the body posture appeared normal, whereas there were no signs of convulsions, biting, or vocalization. The above condition reflects the home cage assessment, which appears normal in both male and female rats. Hand-held observations, such as ease of removal of animals from cages, were similar to those of the test animals, and animal reactions during handling appeared normal in the control group. There were no signs of lacrimation, salivation, or piloerection during handling in either the male or female rats. Animal Arousal, gait, mobility and stereotypical behaviour appear normal, and no diarrhoea conditions were noticed during open-field observation in both sexes. Sensory responses such as tail pinch, touch, click, pupil responses and righting reflex were normal in male and female rodents.

Table 8: Neurological parameters of male and female rat

Sex	Parameters	Observations	STF mg/kg			
			Control	125	250	500
Male	Home cage assessment	Body posture	Normal	Normal	Normal	Normal
		Convulsions/tremors	No	No	No	No
		Biting	No	No	No	No
		Vocalization	No	No	No	No
		Ease of removal from cage	Easy	Easy	Easy	Easy
	Hand held observations	Reactivity to Handling	Normal	Normal	Normal	Normal
		Lacrimation	No	No	No	No
		Salivation	No	No	No	No
		Piloerection	No	No	No	No
	Open field observations	Arousal	Normal	Normal	Normal	Normal
		Gait score	Normal	Normal	Normal	Normal
		Mobility	Normal	Normal	Normal	Normal
		Stereotypical behavior	Normal	Normal	Normal	Normal
		Diarrhea	No	No	No	No
	Sensorial responses	Tail pinch response	Normal	Normal	Normal	Normal
		Touch response	Normal	Normal	Normal	Normal
		Click response	Normal	Normal	Normal	Normal
		Righting reflex	Normal	Normal	Normal	Normal
		Pupil response	Normal	Normal	Normal	Normal
Female	Home cage assessment	Body posture	Normal	Normal	Normal	Normal
		Convulsions/tremors	No	No	No	No
		Biting	No	No	No	No
		Vocalization	No	No	No	No
		Ease of removal from cage	Easy	Easy	Easy	Easy
	Hand held observations	Reactivity to Handling	Normal	Normal	Normal	Normal
		Lacrimation	No	No	No	No
		Salivation	No	No	No	No
		Piloerection	No	No	No	No
	Open field observations	Arousal	Normal	Normal	Normal	Normal
		Gait score	Normal	Normal	Normal	Normal
		Mobility	Normal	Normal	Normal	Normal
		Stereotypical behavior	Normal	Normal	Normal	Normal
		Diarrhea	No	No	No	No
	Sensorial responses	Tail pinch response	Normal	Normal	Normal	Normal
		Touch response	Normal	Normal	Normal	Normal
		Click response	Normal	Normal	Normal	Normal
		Righting reflex	Normal	Normal	Normal	Normal
		Pupil response	Normal	Normal	Normal	Normal

Anticonvulsant activity

In-vitro AchE inhibition assay

The *in-vitro* acetylcholinesterase (AchE) inhibition assay of *S. torvum* fruit extract was conducted using Ellman's colorimetric method to evaluate its potential inhibitory effects at various concentrations. Galantamine, a known AchE inhibitor, was used as a positive control for comparison. At a 0.25 mg/mL concentration, STF inhibited AchE by 19.79±1.71%, whereas galantamine exhibited a higher inhibition of 25.01±0.17%. As the concentration increased to 0.5 mg/mL, STF achieved 26.13±2.81% inhibition, whereas galantamine reached 32.85±0.62%. At 1 mg/mL, STF inhibition rose to 36.96±1.12%, with galantamine showing a notably higher inhibition of 51.89±0.87%. At the highest tested concentration of 2 mg/mL, STF showed 51.52±2.96% inhibition, while galantamine exhibited a strong inhibition of 76.91±0.64% (Table 9).

The IC_{50} values, representing the concentration required to inhibit AchE activity by 50 %, were calculated for both substances. STF had an IC_{50} of 1.85±1.85 mg/mL, indicating a moderate inhibitory potency. These results suggest that the *S. torvum* fruit extract exhibits dose-dependent AChE inhibitory activity, although it is less potent than galantamine.

Table 9: AchE inhibition assay

Conc.	STF
0.25	19.79±1.71
0.5	26.13±2.81
1	36.96±1.12
2	51.52±2.96
IC_{50} values	1.85±1.85

All values are expressed as Mean±SEM

In-vivo anticonvulsant activity

Maximal Electro-shock (MES) induced convulsions model

The experimental results demonstrated the effects of *S. torvum* fruit extract (STF) on Maximal Electroshock (MES)-induced convulsions in rats. The study was performed across four groups: a control group

(Group I) that received saline, a positive control group (Group II) that received standard diazepam, and two STF-treated groups at doses of 200 mg/kg (Group III) and 400 mg/kg (Group IV) (Figure 3).

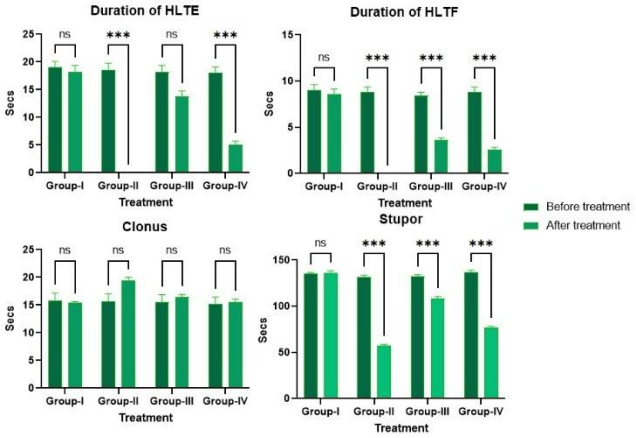

Figure 3: Effect of *S. torvum* on MES-induced convulsions.

Table 10: Effect of *S. torvum* fruit (STF) on the duration of HLTE in MES-induced convulsion

Treatment	Duration of HLTE	
	Before treatment	After treatment
Group I: Normal control	18.86±1.14	18.31±1.13
Group II: Standard diazepam (1mg/kg per oral)	18.57±1.14	0***
Group III: (STF -200 mg/kg, per oral)	18.13±1.14	13.80±0.97
Group IV: (STF -400 mg/kg, per oral)	17.87±1.14	5.00±0.71***

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. ***p< 0.001 **p< 0.01 *p< 0.05, ^{ns} non-significant with the standard.

Table 11: Effect of *S. torvum* fruit on the duration of HLTF in MES induced convulsion

Treatment	Duration of HLTF	
	Before treatment	After treatment
Group I: Normal control	8.90±0.56	8.62±0.51
Group II: Standard diazepam (1mg/kg per oral)	8.82±0.55	0***
Group III: (STF -200 mg/kg, per oral)	8.64±0.54	3.60±0.24***
Group IV: (STF -400 mg/kg, per oral)	8.82±0.55	2.60±0.24***

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. ***p< 0.001 **p< 0.01 *p< 0.05, ^{ns} non-significant with the standard.

Clonus: The duration of clonus, however, showed a slight increase following STF treatment in both groups. In the STF 200 mg/kg group, clonus duration increased from 15.41±1.29 seconds to 16.40±0.51 seconds, while in the STF 400 mg/kg group, it increased from 15.10±1.27 seconds to 15.60±0.51 seconds. This minor increase in clonus duration suggests a potential limitation of STF's anticonvulsant activity of STF, possibly exacerbating certain clonic phases of convulsions, even as it reduces others Table 12).

Table 12: Effect of *S. torvum* fruit on the Clonus in MES induced convulsion

Treatment	Clonus	
	Before treatment	After treatment
Group I: Normal control	15.88±1.33	15.40±0.24^{ns}
Group II: Standard diazepam (1mg/kg per oral)	15.73±1.32	19.40±0.6^{ns}
Group III: (STF -200 mg/kg, per oral)	15.41±1.29	16.40±0.51^{ns}
Group IV: (STF -400 mg/kg, per oral)	15.10±1.27	15.60±0.51^{ns}

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. ***p< 0.001 **p< 0.01 *p< 0.05, ^{ns} non-significant with the standard.

Stupor: Regarding stupor, the STF 200 mg/kg group demonstrated a reduction in duration from 132.47±1.77 seconds before treatment to 108.58±1.89 seconds after treatment. In the STF 400 mg/kg group, stupor duration decreased from 137.11±1.99 seconds to 77.07±1.34 seconds post-treatment. This dose-dependent reduction in stupor duration suggests that STF may effectively mitigate the severity of the postictal symptoms associated with MES-induced convulsions (Table 13).

n=5, All values are expressed as Mean±SEM. Paired 't' test was used and compared before and after the treatment. ***p< 0.001 **p< 0.01 *p< 0.05, ^{ns} non-significant with the standard

Duration of Hind Limb Tonic Extension (HLTE): Before treatment, the duration of HLTE was 18.13±1.14 seconds in the STF 200 mg/kg group and 17.87±1.14 seconds in the STF 400 mg/kg group. After treatment, these durations decreased to 13.80±0.97 seconds and 5.00±0.71 seconds, respectively. This dose-dependent reduction, especially notable at a dose of 400 mg/kg, indicated STF's potential of STF to reduce the severity of HLTE, a primary indicator of convulsive activity in the MES model (Table 10).

Duration of Hind Limb Tonic Flexion (HLTF): For HLTF, the STF 200 mg/kg group had a duration of 8.64±0.54 seconds before treatment, which reduced to 3.60±0.24 seconds after treatment. In the STF 400 mg/kg group, the duration dropped from 8.82±0.55 seconds pre-treatment to 2.60±0.24 seconds post-treatment. These results suggest that STF reduces HLTF levels dose-dependently, indicating its ability to diminish certain tonic components of MES-induced convulsions (Table 11).

These results indicate that *S. torvum* fruit extract possesses anticonvulsant properties, with a higher efficacy observed at a dose of 400 mg/kg. STF demonstrated notable reductions in HLTE, HLTF, and stupor, which are important parameters for assessing anticonvulsant activity. However, its effects varied across different aspects of convulsions; while it reduced HLTE, HLTF, and stupor durations, it showed a slight increase in clonus duration. This selective action suggests that STF may be more effective against certain convulsive symptoms and less effective against others such as clonus. Compared

with diazepam, which eliminated HLTE and HLTF, STF exhibited a more moderate effect, reducing their duration without complete elimination. These findings suggest that STF may be useful in scenarios requiring a moderate anticonvulsant effect or as a component of combination therapies, particularly where selective control over specific convulsive symptoms is beneficial.

Table 13: Effect of *S. torvum* fruit on the Stupor in MES induced convulsion

Treatment	Stupor	
	Before treatment	After treatment
Group I: Normal control	135.02±1.58	136.31±2.15
Group II: Standard diazepam (1mg/kg per oral)	131.87±1.77	57.40±1.44***
Group III: (STF -200 mg/kg, per oral)	132.47±1.77	108.58±1.89***
Group IV: (STF -400 mg/kg, per oral)	137.11±1.99	77.07±1.34***

n=5, All values are expressed as Mean±SEM. Two-way analysis of variance (ANOVA) was used for the treatment groups with the positive control. ***p< 0.001 **p< 0.01 *p< 0.05, ^{ns} non-significant with the standard.

Pentylenetetrazole-induced (PTZ) induced seizure model

This study investigated the anticonvulsant activity of *S. torvum* fruit extract (STF) in a rat pentylenetetrazole (PTZ)-induced seizure model (Table 14). This model was used to assess the efficacy of STF in delaying seizure onset, reducing seizure duration, and increasing survival rates. The experimental groups included a normal control (Group I), a disease control (Group II), a diazepam-treated standard (Group III), and two STF-treated groups at doses of 200 mg/kg (Group IV) and 400 mg/kg (Group V) (Figure 4).

Table 14: Effect of *S. torvum* fruit on PTZ-induced convulsions.

Treatment	Onset of Seizures (Sec)	Duration of jerks (Sec)	Animals survived	Percentage of protection
Group I: Normal group	353.20±0.37	407.20±1.98	5/5	100
Group II: Disease Control	200.60±0.93$^{\#}$	783.20±3.06$^{\#}$	0/5	0
Group III: Standard diazepam (1mg/kg per oral)	331.00±1.82***	416.20±2.63***	5/5	100
Group IV: (STF -200 mg/kg, per oral)	309.20±2.4***	531.80±2.6***	4/5	80
Group V: (STF -400 mg/kg, per oral)	319.30±2.07***	458.00±2.88***	5/5	100

n=5, All values are expressed as Mean±SEM, statistical analysis by One-way ANOVA followed by Dunnett's test, ***p< 0.001 **p< 0.01 *p< 0.05, ^{ns} non-significant with indicates statistical significance, comparison with disease control, $^{\#}$P<0.001 indicates the comparison with the normal control.

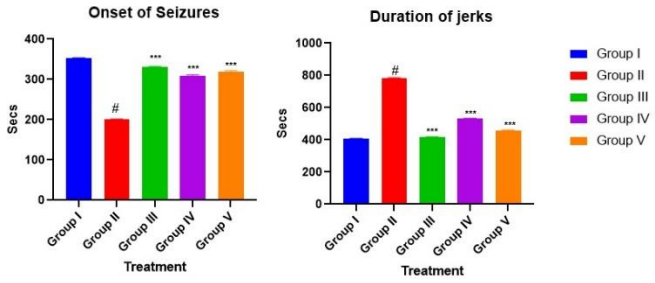

Figure 4: Effect of *S. torvum* in PTZ induced convulsion
n=5, All values are expressed as Mean±SEM, statistical analysis by One-way ANOVA followed by Dunnett's test, ***p< 0.001 **p< 0.01 *p< 0.05, ^{ns} non-significant with indicates statistical significance, comparison with disease control, $^{\#}$P<0.001 indicates the comparison with the normal control.

Onset of Seizures

In the normal control group, seizures occurred at 353.20±0.37 seconds, while the disease control group experienced a significantly earlier onset at 200.60±0.93 seconds, indicating the effect of PTZ in inducing convulsions. Treatment with diazepam delayed the onset of the seizures to 331.00±1.82 seconds. STF also showed a delay in seizure onset in a dose-dependent manner, with the 200 mg/kg dose showing an onset time of 309.20±2.4 seconds and the 400 mg/kg dose further delaying it to 319.30±2.07 seconds. This indicated that STF possesses anticonvulsant properties that reduce seizure susceptibility in PTZ-induced convulsions.

Duration of Jerks:

The duration of jerks was significantly longer in the disease control group at 783.20±3.06 seconds compared to 407.20±1.98 seconds in the normal control, showing the exacerbated convulsive activity induced by PTZ. Diazepam reduced the duration of jerks to 416.20±2.63 seconds. STF demonstrated a dose-dependent reduction in jerk duration, with the 200 mg/kg dose showing a duration of 531.80±2.6 seconds and the 400 mg/kg dose further reducing it to 458.00±2.88 seconds. This reduction in seizure duration highlights the effectiveness of STF in reducing PTZ-induced seizure severity.

Survival and Protection Percentage:

In the disease control group, none of the animals survived the seizures, resulting in a 0% protection rate. Both the diazepam-treated and STF 400 mg/kg groups showed a 100% survival rate, with all animals surviving convulsions. The STF 200 mg/kg group had an 80% survival rate, with four out of five surviving animals. These survival outcomes indicate that STF provides substantial protection against PTZ-induced mortality, particularly at high doses.

These results suggest that *S. torvum* fruit extract exhibits significant anticonvulsant activity in the PTZ-induced seizure model, particularly at a dose of 400 mg/kg, where it effectively delayed seizure onset, reduced the duration of convulsive jerks, and provided full protection from seizure-induced mortality. This dose-dependent response shows the potential of STF as an anticonvulsant, comparable to diazepam in some respects, although the effectiveness of STF appears to be moderate.

Biochemical parameters

Superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) are major antioxidant enzymes in the brain that play a vital role in the management of oxidative stress and associated diseases.

SOD is an important enzyme that helps to reduce oxidative stress. The normal group (Group I) had SOD levels of 66.30±1.09 U/mg tissue. In contrast, the PTZ group (Group II) showed a significant reduction in SOD levels to 18.82±0.74 U/mg tissue, indicating increased oxidative stress due to PTZ. Diazepam (Group III) moderately restored SOD

levels to 51.97±0.91 U/mg tissue. STF at 200 mg/kg (Group IV) and 400 mg/kg (Group V) resulted in SOD levels of 24.80±0.37 U/mg tissue and 34.60±1.36 U/mg tissue, respectively (Table 15).

CAT is another enzyme critical for combating oxidative stress. The normal group exhibited CAT levels of 216.16±1.91 U/mg tissue. The PTZ group experienced a drastic reduction to 38.98±1.56 U/mg tissue. Diazepam significantly improved CAT levels to 203.80±1.69 U/mg tissue. STF's impact was dose-dependent; the 200 mg/kg dosage increased CAT levels to 59.60±2.38 U/mg tissue, while the 400 mg/kg dose more effectively raised them to 94.40±1.60 U/mg tissue (Figure 5).

Table 15: Effect of *S. torvum* fruit on Biochemical parameters

Treatment	SOD (U/mg tissue)	CAT (U/mg tissue)	MDA (nmol/gm tissue)
Group I: Normal group	66.30±1.09	216.16±1.91	1.88±0.07
Group II: PTZ group (100 mg/kg)	18.82±0.74$^{\#}$	38.98±1.56$^{\#}$	4.58±0.14$^{\#}$
Group III: Standard diazepam (1mg/kg per oral)	51.97±0.91***	203.80±1.69***	2.16±0.04***
Group IV: (STF -200 mg/kg, per oral)	32.80±0.86***	161.00±1.14***	3.36±0.1
Group V: (STF -400 mg/kg, per oral)	42.60±0.68***	185.20±3.18***	2.90±0.04***

n=5, All values are expressed as Mean±SEM, statistical analysis by One-way ANOVA followed by Dunnett's multiple comparison test
***P<0.001 when compared to the PTZ group
$^{\#}$P<0.001when compared to the Normal group
^{ns} represents non significance

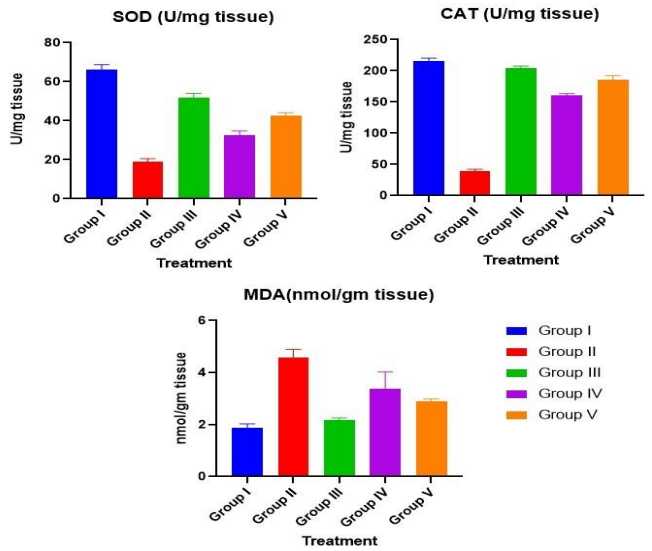

Figure 5: Effect of *S. torvum* on Biochemical parameters in PTZ induced convulsion
n=5, All values are expressed as Mean±SEM, statistical analysis by One -way ANOVA followed by Dunnett's multiple comparison tests *P<0.001 when compared to the PTZ group $^{\#}$P<0.001when compared to the Normal group, ^{ns} represents non-significance.

MDA is a marker for lipid peroxidation and oxidative stress. Lower MDA levels indicate reduced cellular damage. The normal group had MDA levels of 1.88±0.07 nmol/gm tissue, while the PTZ group showed significantly elevated levels at 4.58±0.14 nmol/gm tissue. Diazepam effectively reduced MDA levels to 2.16±0.04 nmol/gm tissue. STF at 200 mg/kg didn't significantly alter MDA levels (4.29±0.12 nmol/gm tissue), but at 400 mg/kg, it reduced them to 3.39±0.12 nmol/gm tissue, suggesting some degree of protection against lipid peroxidation at higher doses.

From the perspective of STF, these results indicate its potential in mitigating PTZ-induced oxidative stress, although its efficacy is dose-dependent and generally less potent than diazepam. STF showed the capability to enhance antioxidant enzymes, such as SOD and CAT, especially at the higher 400 mg/kg dosage, and to reduce oxidative damage, as indicated by lower MDA levels at this higher dose. These findings suggest that STF may have neuroprotective effects by combating oxidative stress, a common component of seizure pathophysiology.

Neurotransmitter Levels

This study examined the effects of *S. torvum* fruit extract (STF) on neurotransmitter levels in a seizure model and assessed acetylcholine (Ach) and dopamine levels to understand the potential impact of the extract on neurochemical balance. There were five groups: saline-treated control (Group I), disease control (Group II), diazepam-treated standard (Group III), and two STF-treated groups treated with 200 mg/kg (Group IV) and 400 mg/kg (Group V) (Table 16).

Table 16: Effect of *S. torvum* fruit on Neurotransmitter levels

Treatment	Ach levels (µg/ml)	Dopamine levels (µg/ml)
Group I: Normal group	4.2±0.49	0.3±0.01
Group II: PTZ group (100 mg/kg)	10.8±0.37**	0.14±0.01**
Group III: Standard diazepam (1mg/kg per oral)	4.2±0.37**	0.3±0.03**
Group IV: (STF -200 mg/kg, per oral)	4.8±0.58**	0.23±0.01*
Group V: (STF -400 mg/kg, per oral)	3.4±0.93**	0.27±0**

n=5, All values are expressed as Mean±SEM, statistical analysis by One-way ANOVA followed by Dunnett's multiple comparison test
**P<0.001, *P<0.01 when compared to the PTZ group
$^{\#}$P<0.001when compared to the Normal group^{ns} represents non significance

Acetylcholine (Ach) Levels: The control group had Ach levels of 4.2±0.49 µg/ml, while the disease control group showed a significant increase in Ach levels to 10.8±0.37 µg/ml, suggesting heightened cholinergic activity associated with seizure induction. Treatment with diazepam normalized Ach levels to 4.2±0.37 µg/ml. STF treatment also showed a dose-dependent effect on Ach levels; the 200 mg/kg dose reduced Ach to 4.8±0.58 µg/ml, while the 400 mg/kg dose brought Ach levels down further to 3.4±0.93 µg/ml. These results suggest that STF effectively reduced elevated Ach levels associated with seizures, comparable to diazepam, particularly at a dose of 400 mg/kg (Figure 6).

Dopamine Levels: In the control group, dopamine levels were measured at 0.3±0.01 µg/ml, while the disease control group showed a reduction to 0.14±0.01 µg/ml, indicating a depletion of dopamine associated with seizure activity. Diazepam treatment restored dopamine levels to 0.3±0.03 µg/ml. In the STF-treated groups, dopamine levels increased in a dose-dependent manner, with the 200 mg/kg dose increasing

dopamine to 0.23±0.01 µg/ml, and the 400 mg/kg dose increasing it further to 0.27±0. These findings suggest that STF helps restore dopamine levels to normal, with a more pronounced effect at higher doses, indicating its potential role in supporting dopaminergic balance in seizure conditions.

The results indicated that the *S. torvum* fruit extract exhibited neurochemical modulating effects in a dose-dependent manner. At 400 mg/kg, STF effectively reduced elevated Ach levels and partially restored dopamine levels, suggesting potential benefits in managing seizures by modulating neurotransmitter imbalances. These effects highlight the potential of STF as an adjunct in therapies aimed at restoring neurochemical balance in seizure-related disorders.

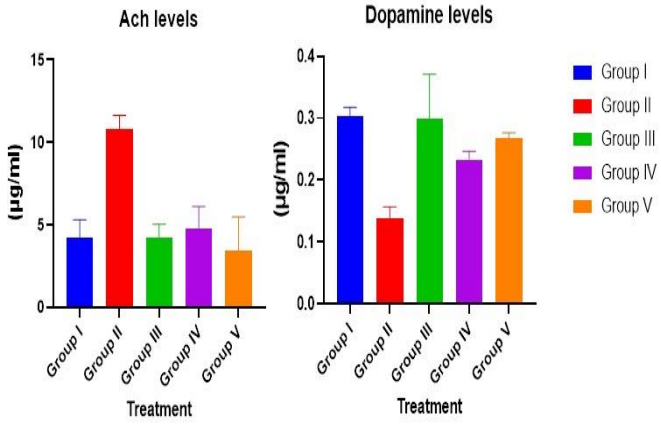

Figure 6: Effect of *S. torvum* on Neurotransmitter levels in PTZ induced convulsion
n=5, All values are expressed as Mean±SEM, statistical analysis by One-way ANOVA followed by Dunnett's multiple comparison test, **P<0.001, *P<0.01 when compared to the PTZ group, $^{\#}$P<0.001when compared to the Normal group.

Conclusion

This study evaluated the anticonvulsant properties of *S. torvum* fruit extract in convulsion models. Acute and subacute studies demonstrated the safety of various doses of STF, with no adverse effects.

In convulsion studies, *S. torvum* fruit extract showed a dose-dependent efficacy in reducing MES- and PTZ-induced convulsive symptoms. At 400 mg/kg, it significantly reduced the duration of hind limb tonic extension (HLTE) and hind limb tonic flexion (HLTF), and decreased seizure onset and duration in the PTZ model. This higher dose also resulted in 100% survival in PTZ-induced convulsions, showing neuroprotective potential. However, STF moderately increased the duration of clonus in MES, suggesting selective efficacy across the seizure phases.

It also demonstrated positive effects on neurotransmitter levels, reduced elevated acetylcholine (Ach) levels, and partially restored dopamine levels, addressing neurochemical imbalances related to seizures. Additionally, STF's antioxidant action of STF, as evidenced by increased SOD and CAT and decreased MDA levels, suggests that it could mitigate oxidative stress in seizure-related conditions.

The *S. torvum* fruit extract showed significant anticonvulsant and neuroprotective effects, with a strong safety profile. It reduces seizure severity, restores neurotransmitter balance, and mitigates oxidative stress, particularly at high doses. Although less potent than standard treatments such as diazepam, its selective efficacy suggests its potential as an adjunctive therapy for convulsive disorders. Further research is required to optimize its therapeutic use.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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