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Original article

Acute and Sub-chronic Toxicological Evaluation of Ethanol Extract of *Solanum trilobatum* Linn.

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Abstract

Background and objectives: *Solanum trilobatum* plant parts such as berries and flowers are normally used for the treatment of respiratory illnesses. The toxicity profile of the plant and its parts are not clear. Hence, the present study was planned to investigate the toxicological effects of ethanol extract of leaves of *S. trilobatum* (EEST) using acute and sub-chronic toxicological methods in Sprague-Dawley (SD) rats. **Method:** Leaves of *S. trilobatum* were extracted with ethanol using hot percolation method. Acute and sub-chronic oral toxic effects of EEST were tested in SD rats. Acute toxicity testing was carried out as per guidelines set by OECD. In sub-chronic toxicity testing, animals were treated with 100, 200 and 400 mg/kg EEST for 30 days. During the study, the animals were monitored for changes in their behaviour at regular intervals. At the end of the study, blood sample was collected from all animals for biochemical analysis, they were sacrificed and organs such as brain, lung, liver and kidney were collected for histopathological analysis. Part of the brain was used for estimation of dopamine and the remaining tissue was used for histopathological analysis. **Results:** In acute toxicity testing, EEST did not show mortality up to 2000 mg/kg. In sub-chronic toxicity testing, EEST at 200 mg/kg and above doses caused cannibalism. At the end of the study, EEST decreased locomotor action and immobilization time. Histopathological analysis showed mild to moderate toxicity in 400 mg/kg treated animals and no significant changes were observed in biochemical parameters compared to control group. **Conclusion**: The present study concluded that, EEST exerted mild to moderate toxic effects on rodents. EEST caused cannibalism, increased the dopamine level in brain and histopathological alterations in lungs, liver and kidneys.

Keywords: cannibalism; locomotor activity; *Solanum trilobatum*; Sprague-Dawley rats; toxicity

Introduction

Plants have been traditionally used for the treatment of various diseases and disorders. In the last few decades, many pharmaceutical substances have been isolated from natural sources and these substances have been used as life-saving drugs [1]. World Health Organization (WHO) is imminent to utilize both the modern and traditional medical systems to fulfil the primary healthcare needs of the world population [2]. Adverse drug reaction is a major concern for any system of medicine and it is the 4th leading cause of death in the US behind heart disease, cancer and strokes [3]. Use of herbal medicine has increased and their safety has become an

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issue for the regulatory agencies because of inadequate documents on safety profiles of herbal products [4]. Hence, ensuring the safety of medicinal herbs is one of the important components in the drug development process. In Indian subcontinent, Ayurvedic and Siddha systems of medicine are practiced for many centuries where medicinal herbs are important ingredients in their formulations [5]. *Solanum trilobatum* is one of the herbs used in Siddha system of medicine for the treatment of respiratory illnesses. The toxicity profile of this plant has remained unclear; hence this plant has been investigated in the current research.

Solanum is a large and diverse genus of flowering plants of about 1500-2000 different species in the family of Solanaceae. *Solanum trilobatum* Linn., (“thuthuvalai*”* in tamil; climbing brinjal in English) belongs to Solanaceae family and is widely used as a vegetable accompaniment to meals in the regions of Tamil Nadu, India. *Solanum trilobatum* is also called as purple-fruited pea eggplant which is widely distributed in India. It is prickly diffused, bright green perennial herb, woody at the base, 2-3 m height, climbing shrub with short compressed spines and it is grown in dry places of Indian subcontinent. The leaves are deltoid/ triangular, irregularly lobed and contain high amounts of iron, carbohydrates, calcium, proteins, fats, phosphorus, crude fibres and minerals. The flowers are purple in color [6].

Solanum trilobatum parts such as the berries and flowers are normally used for the treatment of respiratory illnesses such as common cold, cough and asthma. The leaves are consumed after mildly frying in oil or ghee and then griound. *Solanum trilobatum* is full of thorns including the leaves, which should be removed before cooking because it is known to have mild toxicity. The dried powered leaves of *S. trilobatum* are used as a traditional medicine to treat respiratory illnesses in India and Thailand. They have shown hypoglycaemic, antibacterial, antifungal, antioxidant and anticancer activities in preclinical experiments besides treating respiratory disorders [7]. Other effects of the plant are unknown; hence the present study was planned to study the toxicological importance of the ethanol extract of *S. trilobatum* leaves.

Material and Methods

Plant material

Solanum trilobatum leaves were collected from rural area of Vellore, Tamil Nadu, India between Oct. and Dec. 2015. They were identified by pharmacognosists and a voucher specimen was deposited at the Faculty of Pharmacy of AIMST University (voucher specimen FoP/03/2015). The plant leaves were dried under shade for a week and ground using an electrical grinder to a coarse powder.

Extraction

The powdered leaves were weighed and packed in Soxhlet apparatus and extracted with ethanol (95% alcohol; HMBG, Malaysia) at 60 °C (matrix/solvent ratio 1:20). The extraction was considered complete when the solvent was clear (4 cycles). Then, the extract was concentrated to a dry mass by evaporation under reduced pressure. The yield of ethanol extract of *S. trilobatum* (EEST) of was 6.2 g w/w (dry weight basis). The EEST was stored in a desiccator at room temperature until further analysis.

Animals

Healthy, adult, female gender of Sprague-Dawley (SD) rats, weighing 130±10 g were obtained from Central Animal house, AIMST University, Malaysia. The animals were housed in Wi-Fi free zone, large, spacious poly acrylic cages at an ambient room temperature with 12-h-light/12-h-dark cycle. The animals were fed with water and normal rats pellet fed ad libitum. The study was approved by AIMST University Human and Animal Ethics Committee (AUHAEC/FOP/2016/10) and the study was conducted according to Animal Research Review Panel guidelines.

Acute toxicity testing

Acute oral toxicity of the EEST was carried out as per the guidelines set by the Organization for

Economic Co-operation and Development, revised draft guidelines 423. The principle involved a step-wise procedure with the use of the minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance. Healthy SD rats (3 animals/dose) of female gender were used for the experiment. Overnight fasted rats were treated with EEST at pre-specified doses of 5, 50, 300, and 2000 mg/kg BW, respectively. The rats were observed closely for their neurological, behavioral and autonomic profiles continuously for 24 h after dosing. After a period of 24 h, the animals were observed (at least two times a day) for 14 days to evaluate the changes on behavioral, neurological, autonomic profiles and mortality [8].

Sub-chronic toxicity testing

The adult SD rats of female gender were used for the experiments. The animals were divided into four different groups as follows: group 1: control; group 2: EEST 100 mg/kg; group 3: EEST 200 mg/kg; group 4: EEST 400 mg/kg.

Prior to and at the end of the experiment, the animals' behaviour was monitored. They were treated with ESST for 30 days through oral gavage. The plant extract was suspended with 0.5% w/v of carboxymethyl cellulose and administered once daily at morning time.

On pre-study day, 15^{th} and 30^{th} day of the experiment, the animals' behaviour such as locomotor action, immobilization time and muscular strength were monitored. At the end of the study, blood samples were collected form all the experimental animals for biochemical analysis under mild diethyl ether anaesthesia. Later, they were sacrificed by cervical dislocation and brain, lung, liver and kidney were collected and absolute organ weight were measured. Part of the brain sample was used for the dopamine assay. Lung, liver, kidney and part of the brain samples were preserved in 10% neutral formalin for histopathological analysis [9,10].

Body weight analysis

At regular intervals, body of each rat in each group was recorded and the change in body weight was calculated.

Behavioural analysis

Locomotor activity, immobilization time and muscular strength were monitored at regular intervals using actophotometer, water maze and wire grip strength meter, respectively [10].

Locomotor activity

The rat's activity was recorded in an actophotometer with 8 beams of infrared light on both X and Y horizontal axis. The individual rat locomotor activity was monitored for 10 min at room temperature.

Effect on immobilization time

Effect of EEST on immobilization time was tested using water navigation test. Before the experiment, the rats were trained for three consecutive days. During the experiment, the time taken to reach the platform was measured. The cut-off to reach platform was 3 min.

Effect on wire grip strength

The string was made up of a metallic material and suspended in mid-air about 30 cm height from ground [11]. The rat was placed on centre of the wire and time taken to fall *i.e.*, 'fall on time' was noted.

Biochemical parameter evaluation

The blood samples (0.5 mL) were collected from retro-orbital plexus on pre-study day and at the end of the experiment in plan sample collection tubes. The serum was separated, used for estimation of AST, ALT, ALP, total protein, creatinine, urea and glucose levels using a biochemical analyzer (Reflotron Plus System, Hoffmann-La Roche, USA).

Estimation of dopamine

A 0.5 g of brain was weighed and homogenized in HCl-butanol for about 1 min (in 1:10 ratio) by using homogenizer. The homogenate was then centrifuged (3000 rpm, 10 min). An aliquot

supernatant phase (1 mL) was removed and added to centrifuge tube containing 2.5 mL hexane and 0.3 mL of 0.1 M HCl. The aqueous phase (0.2 mL) was then taken for dopamine assay. All steps of procedure were carried out at 0 to 4 °C. In 0.2 mL of aqueous phase, 0.05 mL of 0.4 M HCl, and 0.1 mL of sodium acetate (pH≃6.9) buffer were added, followed by 0.1 mL iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by addition of 0.1 mL sodium sulphite solution. In the reaction mixture, 0.1 mL acetic acid is added after ≃1.5 min. The solution was then heated to 100 °C for 6 min. When the sample reached room temperature, excitation and emission spectra were read from the spectrofluorimeter at 330-375 nm. Tissue blanks for dopamine haven been prepared by adding the reagents of the oxidation step in reversed order (sodium sulphite before iodine). Dopamine level was calculated mathematically using formula (Sample O.D-Blank O.D)/(Standard O.D – Blank O.D)×concentration of standard. The final reading of neurotransmitter level is expressed as: μ moles/g tissue [12].

Histopathology analysis

Part of the brain, lung, liver and kidney samples were preserved in 10% neutral formalin for histopathology analysis. The tissue sample was embedded in paraffin after dehydrated in alcohol. A 5 mm thickness of brain, lung, liver and kidney sections were prepared from paraffin blocks and stained with haematoxylin & eosin and mounted in neutral DPX medium. The sections were examined under light microscope.

Statistical analysis

The results were presented as mean ± standard error of the mean (SEM). The statistical test used was repeated measures ANOVA, followed by Tukey *post-hoc* test. $P<0.05$ was considered as significant.

Results and Discussion

EEST did not show mortality up to 2000 mg/kg when given as single oral administration. Hence, the study was carried out at the dose levels of 1/20, 1/10 and 1/5 of maximum acute toxic dose *i.e.*, 100, 200 and 400 mg/kg BW.

The control group and EEST 100 mg/kg group showed no history of cannibalism; whereas EEST 200 and 400 mg/kg treated animals showed cannibalism after 6th day and after 7th day, respectively. This suggests that dosing of 200 mg/kg and above may cause cannibalism which may be due to increase in sympathetic activity in rats (table 1).

Table 1. Effect of ethanol extract of *Solanum trilobatum* on cannibalism

Group	History of cannibalism
Control	Nil
EEST 100 mg/kg	Nil
EEST 200 mg/kg	One rat died after 6th of the dosing
EEST 400 mg/kg	One rat died after 7th of the dosing

EEST: Ethanol extract of *Solanum trilobatum*; n=6 per group.

Cannibalism is a complex behaviour pattern by urgent reactions such as fear, the need to escape and influenced by many environmental factors such as rough handling or disturbance by high frequency noise, group size, housing system/density, light intensity, shortage of water or food and relative excess of Vitamin B_1 [10]. However, in this study, cannibalism occurred under standard laboratory conditions, hence these factors can be omitted.

EEST showed reduction in body weight but the results were not significant when compared with the control group. In all the dose levels of EEST administration, no significant difference in regular food and water intake was observed compared to the control group.

The effect of EEST on locomotor activity, immobilization time and grip strength of the SD rats have been summarized in tables 2-4. EEST treated animals showed decreased immobilization time and muscular strength at the end of the study, whereas control animals did not show any significant changes.

Table 2. Effect of ethanol extract of *Solanum trilobatum* on locomotor activity

Group	Pre-study day	15th day	30th day
Control	124.00 ± 24.69	132.67 ± 16.82	123.67 ± 9.26
EEST 100 mg/kg	124.00 ± 12.38	123.67 ± 15.46	71.17 ± 19.46
EEST 200 mg/kg	116.67 ± 11.12	72.20 ± 5.13*	69.80 ± 12.60*
EEST 400 mg/kg	131.33 ± 15.99	71.20 ± 20.69	61.60 ± 23.25

EEST: Ethanol extract of *Solanum trilobatum*. All values are mean ± SEM (n=6 except group EEST 200 and 400 mg/kg on 15th day onwards); *p<0.05 compared with pre-study day test values. One-way ANOVA followed by Tukey post-hoc test.

Table 3. Effect of ethanol extract of *Solanum trilobatum* on immobilization time

Group	Pre-study day	15th day	30th day
Control	33.67 ± 5.83	30.17 ± 4.08	31.50 ± 3.86
EEST 100 mg/kg	34.00 ± 3.77	26.50 ± 4.08	22.83 ± 3.86*
EEST 200 mg/kg	31.50 ± 2.51	27.20 ± 1.45	21.40 ± 2.07*
EEST 400 mg/kg	32.83 ± 2.69	24.20 ± 2.67	20.20 ± 2.03**

EEST: Ethanol extract of *Solanum trilobatum*. All values are mean ± SEM (n=6 except group EEST 200 and 400 mg/kg on 15th day onwards); *p<0.05 and **p<0.01 compared with pre-study day test values. One-way ANOVA followed by Tukey post-hoc test.

Table 4. Effect of ethanol extract of *Solanum trilobatum* on grip strength

Group	Pre-study Day	15th day	30th day
Control	86.67 ± 6.83	81.67 ± 7.75	94.67 ± 7.90
EEST 100 mg/kg	82.83 ± 9.22	72.50 ± 4.88	60.67 ± 9.44
EEST 200 mg/kg	87.33 ± 5.74	54.00 ± 6.73*	52.60 ± 9.69*
EEST 400 mg/kg	80.33 ± 8.66	62.20 ± 8.28	48.20 ± 4.99*

EEST: Ethanol extract of *Solanum trilobatum*. All values are mean ± SEM (n=6 except group EEST 200 and 400 mg/kg on 15th day onwards); *p<0.05 compared with pre-study day test values. One-way ANOVA followed by Tukey post-hoc test.

Table 5. Effect of ethanol extract of *Solanum trilobatum* on biochemical parameters

Group	Glucose (mmol/L)	Total Protein (g/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	6.07±0.30	68.43±3.96	89.12±3.86	55.37 ± 5.20	116.17± 7.34	19.00 ± 1.39	0.26 ± 0.03
EEST 100 mg/kg	5.85±0.28	65.87±1.71	84.45±5.30	57.13 ± 2.86	112.17± 4.80	18.33 ± 1.98	0.30 ± 0.04
EEST 200 mg/kg	5.92±0.37	67.10±1.65	87.17±3.87	54.22 ± 3.33	121.83± 5.79	22.67 ± 1.43	0.35 ± 0.03
EEST 400 mg/kg	5.75±0.23	66.67±3.95	87.6 ±6.09	55.05 ± 2.47	128.00± 9.27	21.67 ± 2.86	0.34 ± 0.03

EEST: Ethanol extract of *Solanum trilobatum*. All values are mean ± SEM (n=6 except group EEST 200 and 400 mg/kg on 15th day onwards).

Decreased locomotor activity was observed in EEST 200 mg/kg treated animals. Chronic administration of EEST, didn't had any significant effect on serum biochemical parameters (table 5), and had significantly increased dopamine level in brain compare with control (table 6).

At the end of the study, EEST treated animals showed significant increases in dopamine level. Dopamine is a prototypical slow neurotransmitter, which plays significant role in locomotion, cognitive, motivational, and neuroendocrine system [13]. Locomotor activity is one of the essential physiological functions required for the coordinated action of cortical and subcortical structures and its primarily controlled by D1, D2 and D3 dopamine receptors [14].

Table 6. Effect of ethanol extract of *Solanum trilobatum* on brain dopamine levels

Group	µ moles/g tissue
Control	49.33 ± 5.62
EEST 400 mg/kg	57.32 ± 5.12*

EEST: Ethanol extract of *Solanum trilobatum*. All values are mean±SD (n=5). *p<0.05 and compare with control. One-way ANOVA followed by Tukey post-hoc test.

Activation of D1 dopamine receptor on postsynaptic neurons has stimulatory effect on

locomotor and the role of D2 and D3 dopamine receptors on locomotor activity is complex because they results from both presynaptic and postsynaptic expression.Activation of presynaptic D2 receptors causes decrease in dopamine release that results in decrease in locomotor activity and D3 dopamine receptor exerts a inhibitory action on locomotion [15]. Schindler and Carmona studied the effect of various dopamine agonist and antagonist on locomotor activity and suggested that, 'the action is depends on D1 and D2 receptor modulation'. Dopamine has variable effect on locomotor behaviour of the rats and also the dopamine effect on locomotor activity is gender specific [16]. The drugs/agents such as D-amphetamine, apomorphine, L-dihydroxyphenylalanine (L-DOPA) which enhance transmission at dopamine synapses increase locomotor action or produce stereotype depending on the dose [17]. Reserpine, an irreversible Vesicular Monoamine Transporter (VMAT) reduces locomotor activity by inhibiting transportation of monoamines [17,18]. EEST may act through either one or all D1, D2 and D3 dopamine receptor which may interfere with motor activity of the rodents.

At the end of the study, EEST also reduced immobilization time and grip strength. Decreased grip strength is one of the adverse events of dopamine [19]. The reduction in immobilization time also depends on muscular strength. If there is reduction in muscular strength, it may reduce the swimming efficacy and reduce immobilization time.

EEST 200 mg/kg 400 mg/kg treated animals showed significant decreases in absolute lung weight and increase in relative brain weight (tables 7 and 8). In absolute organ weight analysis, EEST showed increased weight of lungs where as other organs such as brain, heart, liver, kidney did not showed significant changes. In relative organ weight analysis, only the brain showed significant increase in organ weight. This may be due to effect of dopamine. The relationship between motor function (locomotor) and dopamine, body weight and dopamine are well studied, but the relationship between brain weight and dopamine is not known [20,21].

No significant histopathological alterations were observed in brain, lung, heart, liver and kidney of control and EEST 100 mg/kg treated animals. Normal architecture of cellular arrangements was observed in brain, lung, heart, liver and kidney of EEST 100 mg/kg treated animals. EEST 200 and 400 mg/kg treated animals showed mild to moderate toxicity in lungs, liver and kidney (figures 1 and 2).

Table 7. Effect of ethanol extract of *Solanum trilobatum* on absolute organ weight changes (g)

Group	Brain	Lungs	Heart	Liver	Kidney (R)	Kidney (L)
Control	1.75 ± 0.05	2.01 ± 0.06	0.82 ± 0.02	6.21 ± 0.18	0.70 ± 0.01	0.71 ± 0.01
EEST 100 mg/kg	1.95 ± 0.05	1.76 ± 0.06	0.77 ± 0.03	6.18 ± 0.10	0.69 ± 0.01	0.69 ± 0.01
EEST 200 mg/kg	1.83 ± 0.07	1.71 ± 0.09*	0.81 ± 0.01	6.05 ± 0.12	0.69 ± 0.02	0.69 ± 0.01
EEST 400 mg/kg	1.74 ± 0.08	1.67 ± 0.09*	0.75 ± 0.03	5.85 ± 0.20	0.64 ± 0.03	0.66 ± 0.02

EEST: Ethanol extract of *Solanum trilobatum*; Kidney (R): right kidney; Kideny (L): left kidney. All values are mean ± SEM (n=6 except group EEST 200 and 400 mg/kg on 15th day onwards); *p<0.05 and compare with control. One-way ANOVA followed by Tukey post-hoc test.

Table 8. Effect of ethanol extract of *Solanum trilobatum* on relative organ weight changes (g)

Group	Brain	Lungs	Heart	Liver	Kidney (R)	Kidney (L)
Control	0.86 ± 0.03	0.99 ± 0.05	0.40 ± 0.01	3.04 ± 0.09	0.34 ± 0.01	0.35 ± 0.01
EEST 100 mg/kg	0.98 ± 0.01*	0.88 ± 0.02	0.38 ± 0.02	3.10 ± 0.08	0.34 ± 0.01	0.35 ± 0.01
EEST 200 mg/kg	0.99 ± 0.04*	0.93 ± 0.05	0.44 ± 0.01	3.27 ± 0.08	0.37 ± 0.01	0.37 ± 0.01
EEST 400 mg/kg	0.95 ± 0.01	0.91 ± 0.05	0.41 ± 0.03	3.19 ± 0.11	0.35 ± 0.03	0.36 ± 0.03

EEST: Ethanol extract of *Solanum trilobatum*; Kidney (R): right kidney; Kideny (L): left kidney. All values are mean ± SEM (n=6 except group EEST 200 and 400 mg/kg on 15th day onwards); *p<0.05 and compare with control. One-way ANOVA followed by Tukey post-hoc test.

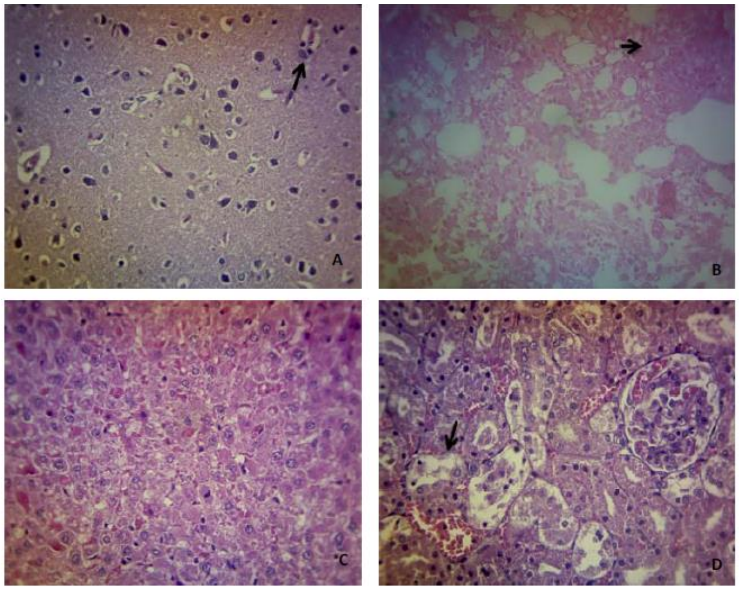

Figure 1. Histopathology of organs of ethanol extract of *Solanum trilobatum* (EEST) 200 m/kg treated animals. (A) Section from brain of EEST 200 mg/kg treated animals showing features of reactive gliosis with hyperaemic congestion (H & E ×400), (B) Section from lungs of EEST 200 mg/kg treated animals showing interstitial pneumonitis (H & E ×400), (C) Section from liver of EEST 200 mg/kg treated animals showing normal histology (H & E ×400), (D) Section from kidney of EEST 400 mg/kg treated animals showing fine granular degenerative changes in the tubules and acute hyperaemic congestion (H & E ×400)

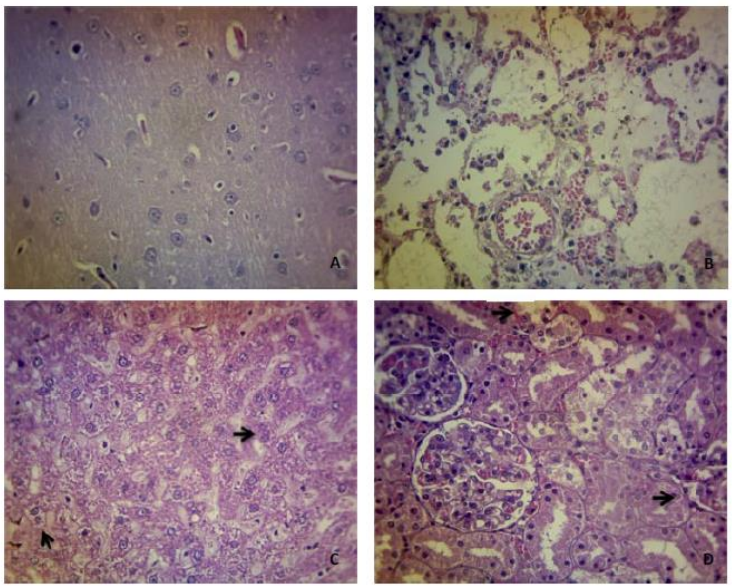

Figure 2. Histopathology of organs of ethanol extract of *Solanum trilobatum* (EEST) 400 m/kg treated animals. (A) Section from brain of EEST 400 mg/kg treated animals showing features of reactive gliosis with hyperaemic congestion (H & E ×400), (B) Section from lungs of EEST 400 mg/kg treated animals showing patch ill-defined occasionally nodular areas of necrotic material within the lung parenchyma with diffuse infiltration of inflammatory cells composed of both acute and chronic inflammatory cells (H & E ×400), (C) Section from liver of EEST 400 mg/kg treated animals showing more florid areas of feathery degeneration with marked sinusoidal widening and congestion (H & E ×400), (D) Section from kidney of EEST 400 mg/kg treated animals showing congested glomeruli, fine granular degenerative changes in the tubules with focal vacuolation along with interstitial congestion (H & E ×400).

Solanum trilobatum is known for its medicinal value. From the results of the present study, it is clearly understood that the EEST (400 mg/kg) had mild to moderate toxic effects on microscopic examinations of organs such as brain, lung, heart, liver and kidney but it did not caused any significant changes in biochemical parameters. EEST also enhanced dopamine levels in brain, which may be beneficial to improve the dopaminergic functions. Further studies are required to know the relationship between dopamine and brain weight and the effect of *S. trilobatum* on Parkinson's disease.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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List of abbreviations

ALP: Alkaine phosphatase
ALT: Alkaine phosphatase
AST: Aspartate aminotransferase
ANOVA: Analysis of variance
BW: Body weight
D: Dopamine
EEST: Ethanol extract of *S. trilobatum*
HCl: Hydrochloric acid
O.D: Optical density
SD rats: Sprague-Dawley rats
SEM: Standard Error of the Mean
WHO: World Health Organization