Studies On Bio-Evaluation Of Anticonvulsant And Neuroprotective Activity Of Epiphyllum Oxypetalum And Tradescantia Spathacea Using Animal Models

Rohitkumar R. Jajoo[1*], Nitin I. Kochar[2], Anil V. Chandewar[3], Abhijit Shrirao[4]

[1,2,3, 4]P. Wadhwani College of Pharmacy, Yavatmal, Maharashtra - 422601*
**Corresponding Author: Rohitkumar R. Jajoo[1]*
**P. Wadhwani College of Pharmacy, Yavatmal, Maharashtra - 422601*

KEYWORDS

Neuroprotective, PTZ kindled, Morris maze, Light and Dark compartment, Oxidative parameters, GABA T, AchE cognitive effects.

Abstract

In recent times, many folks with convulsions not having proper seizure control though there is significant advancement in medications. A common issue observed with these medications is the adverse cognitive effect with other side effect like anxiety. That's why people still need anticonvulsant drugs without such side effects. For holistic approach, many of the natural resources can have antiepileptic action without such side effects which are scientifically not proven. In this study we established designed to check the neurobehavioral and neuro-protective results of Epiphyllum Oxypetalum and Tradescantia Spathacea evaluated in Pentylenetetrazol (PTZ) kindling model of epilepsy in rats. As carried out in our previous research to check anticonvulsant properties of EO and TS, to induce kindling a 35 mg/kg dose of PTZ was injected i.p. in every 48 hrs for 30 days. Spatial memory performance was tested using Morris water maze and black and light compartment model, following which brains were then decapitated for oxidative stress biomarker study, biochemical analysis (AchE and GABA T estimations) and histopathological investigations. EO and TS was able to minimize oxidative stress and enhance memory in Rats indicating these plants extracts have neuroprotective properties as well which is beneficial results to retain cognitive effects that usually happens in chronic epilepsy.

INTRODUCTION

Water maze

The Morris water maze model was used to investigate spatial learning and memory, and Cobas 6000 was used to assess serum sodium, calcium, potassium, and chloride levels. Trials enhanced spatial learning for all groups, but rats receiving Epiphyllum Oxypetalum 200 mg/kg ($p<0.001$), Epiphyllum Oxypetalum 400 mg/kg ($p<0.01$), Tradescantia Spathacea 200 mg/kg ($p<0.001$), Tradescantia Spathacea 400 mg/kg ($p<0.01$) and Sodium Valproate 200 mg/kg ($p<0.05$) had considerably higher average escape latency (s) than rats. Between rats that received 400 mg/kg of Epiphyllum Oxypetalum and vehicle, there was no discernible difference in latency ($p>0.05$). Rats that were given all dosages of Epiphyllum Oxypetalum & Tradescantia Spathacea extract exhibited significantly greater thigmotaxis ($p<0.001$). Rats receiving 400 mg/kg of Tradescantia Spathacea spent considerably less time in the target quadrant ($p<0.05$). In rats given Epiphyllum Oxypetalum & Tradescantia Spathacea, there was an inverse correlation between the serum calcium level and the escape delay ($R = -0.417$, $p<0.05$). When given sub chronically, but not sub acutely, Epiphyllum Oxypetalum & Tradescantia Spathacea extract, and juice was linked to a decrease in serum calcium levels and an impairment in learning and memory. Research ought to be done on the neural underpinnings of this modification.

PLANT UNDER INVETIGATION

1. EPIPHYLLUM OXYPETALUM

It is native to Mexico and Central America found cultivated and naturalized in Venezuela, Brazil, Galapagos Islands, the Caribbean, India, and China. [1,2,3,4,21] and dried leaves are used it has been traditionally using to treat heavy or painful menstrual periods, for heart conditions such as the crushing pain of angina and for

spasmodic pain and hemorrhage. The flowers mixed into soup are said to clear phlegm and strengthen the lungs. Plant juices have been used for bladder infections, shortness of breath and water retention. Applied externally, it has been used for rheumatism. It is also assumed to be an aphrodisiac. The flowers are edible. Pluck wilted buds in the morning, then rehydrate before use in vegetable soups. [5,6,7,22]. Its major chemical constituents: 3.042 % of Polyphenols contents-Methoxyphenol & methyl acetophenone (Phenolic products) , Hexadecenoic acid, Phytol, Beta-Stigmasterol, alpha- Tocopherol ,Kaempferol, Geranylgeraniol moieties Saponins, Phenolic compounds, steroids, glycosides, tannins, terpenoids, and resins
Polyphenols has shown beneficial effects in rodent models of epilepsy and anxiety, possibly mediated through GABAergic and regulation of BDNF pathways [7,8,9,10,11]
It has scientifically possessed antioxidant and anti-inflammatory properties, thus can be reduce oxidative stress caused by seizurogenic agents.

2. TRADESCANTIA SPATHACEA

It is Native to southern Mexico and Central America. Its Distribution is naturalized all over world. It has been introduced to many tropical regions including China, Japan, Africa, Southeast Asia, India, USA, West Indies, Australia, and the Pacific islands.[12,13,14,23-25]. Dried Leaves are used in the experiment
This has been using Traditionally in Mexico and Southeast Asia, where the flowers and leaves are used to treat cancer, superficial mycoses, coughs, colds, and dysentery, “nervios”, anxiety, and depression. Scientific papers on the medicinal value of Tradescantia Spathacea scientifically reported to possess anti-microbial, insecticidal, anti-inflammatory, anti-cancer, and anti-fertility activities [15,16,17,18,26]. It contains Chemical constituent are Alkaloids, Flavonoid contents– Rutin, Epigallocatechin, Peltatoside, luteolin, Quercetin, Phenolic mixes, Terpenoid, Anthocyanin’s, Carotenoid, waxes, Coumarins and Steroidal moieties. Phenolic mixes, Anthocyanins, Coumarins, flavonoids have shown beneficial effects in rodent models of epilepsy and anxiety, possibly mediated through GABAergic and BDNF pathways [19,20]

Material and Methods

Study design

76 Adult Wistar rats are used in PTZ kindled model and GABA involvement check study each as mentioned in observation tables. They were retained at a precise temperature mentioned room at 25±1 °C, were daily exposed to light and dark cycle of 12 hours duration and provided with food and water ad libitum. The experimental protocol was approved with IEAC Approval No.: 650/PO/Re/S-2002/2022/CPCSEA/26, P. Wadhwani College of Pharmacy, Yavatmal, and Maharashtra – 422601.

Soxhlet Extraction

The leaves of the plant were shade dried and were made into a coarse powder. Around 250 gm of finely powdered leaves were evenly packed in a large Soxhlet apparatus and extracted with alcohol (95 % v/v). Collected material was filtered through Whatman no. 1 filter paper.

HPLC studies

HPLC studies done to check on chemical constituents
Chemicals and reagents: analytical and HPLC grades are used. Flavonoids like Rutin, Quercetin , Kaempferol (Sigma) of the highest grade used as the external standards.

Parts of Instruments	Model	Information
System	UHPLC	Ultra High Performance Liquid Chromatograph
Model no.	LC 20 AD	---------
Company	-----	Shimadzu Japan
Pump	LC-20 AD	Quaternary Gradient
Column	Make : Shimadzu	C-18 250 x 4.6 mm, 5µ
Detector	SPD-20 A	Dual Wavelength UV-Vis
Column Oven	CT0-10 AS VP	Max. Temp. 80°c
Autosampler	SIL-20AC HT	0.1 µL to 100 µL
Software	Version DB 6.110	Lab Solution

Chemicals/ Solvents used	Grade	Make
Acetonitrile	HPLC Gradient	Finar
Water	HPLC	Molychem
Ortho Phosphoric acid	SQ	Qualigens

Using these chromatographic conditions, we confirm the retention time of polyphenolic compounds by injection of each standard separately

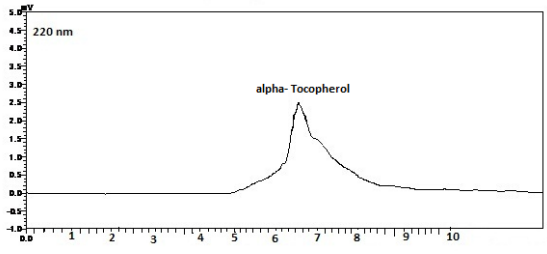
Sample preparation

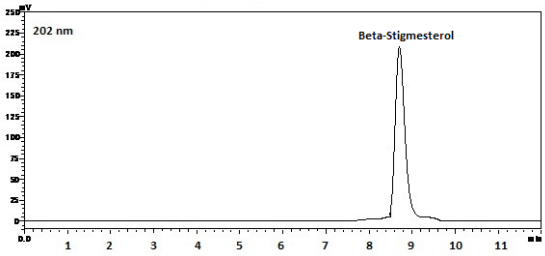
Accurately weighed 10 mg of plant extract transferred it to 10 ml volumetric flask and made-up volume with mobile phase (conc. 1000 ppm) Pipetted out 2.0 ml from above solution and diluted up to 10 ml with mobile phase (conc. 200 ppm)

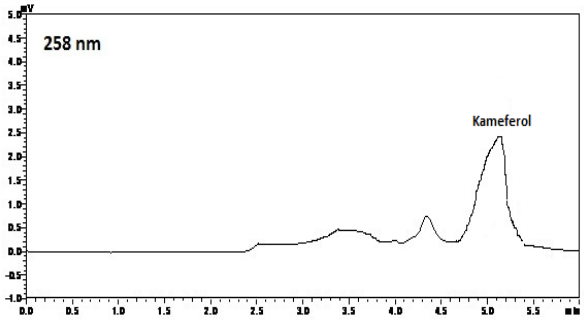
Preparation of standard solution

Accurately weighed appropriate amounts of the reference compounds (Rutin RUT; Quercetin QUE) were mixed and dissolved in methanol in a 200-mL volumetric flask, to obtain a stock solution.

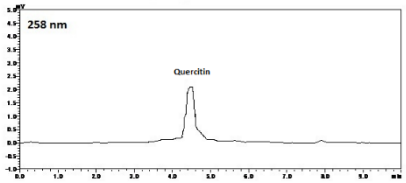
Graphs obtained for Epiphyllum Oxypetalum extract-

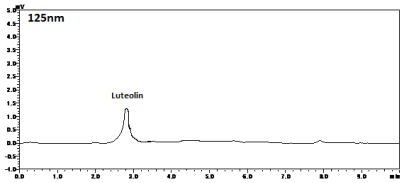


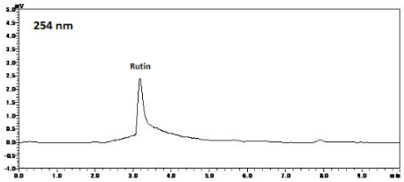




Tradescantia Spathacea extract







This analytical study was aimed at developing a chromatographic system, capable of eluting and resolving flavonoids compounds in a crude Methanolic extracts of EO and TS. The UV spectra were recorded from 125 to 260 nm. Flavonoids exhibited their maximum wavelengths that was recorded

GABA, Glutamate Involvement Check [(10)]

GABA and Glutamate antagonist check for receptor specificity and possible molecular level mechanism of action involved. Flumazenil competitively antagonizes at the $GABA_A$ receptor- GABAergic activity

Groups	Treatment (Dose)	Onset (s)	Duration (s)	Mortality Protection (%)
Group I	1% CMC (10 mg/kg)	57.2 ± 5.67	19.2 ± 1.58	0
Group II	Flumazenil (2 mg/kg)	48 ± 5.07	17 ± 1.08	0
Group III	Sodium Valproate (200 mg/kg)	405.6 ± 5.45	6 ± 2.5	100 ± 2.05
Group IV	Sodium valproate + Flumazenil	169.2 ± 5.87	17.8 ± 1.5	87 ± 3.69
Group Va	EO (400 mg/kg)	387 ± 5.48	10.4 ± 1.56	100 ± 3.05
Group Vb	TS (400 mg/kg)	398 ± 5.09	11.3 ± 1.09	100 ± 3.67
Group VIa	EO (400 mg/kg) + Flumazenil	156.2 ± 5.4	18 ±1.59	78 ± 3.48
Group VIb	TS (400 mg/kg) + Flumazenil	161.3 ± 5.06	22 ± 1.98	82 ± 3.7

Flumazenil (Group II): It antagonizes at the GABAA receptor, which is crucial for GABAergic activity. As a competitive antagonist, it binds to the receptor without activating it, preventing GABA from exerting its effects. This leads to reduced GABAergic activity, potentially altering neurophysiological responses.

Sodium Valproate (Group III & IV) [(20)]: Sodium valproate is often used as an anticonvulsant and mood-stabilizing drug. In this context, it may interact with GABAergic mechanisms by enhancing GABA synthesis and inhibiting GABA degradation. This can result in increased GABAergic activity. In combination with Flumazenil (Group IV), it likely shows some modulatory interaction between these compounds.

EO and TS (Groups Va-VIb) [17]: These experimental compounds are likely being tested for their effect on GABAergic or glutamatergic activity, given the context of your experiment. The data suggest they may have protective effects (similar to sodium valproate) on mortality, though the combination with Flumazenil (Groups VIa & VIb) reduces their efficacy somewhat, indicating possible interference with GABAergic signaling.

Mortality Protection: Groups III, Va, and Vb show 100% mortality protection, implying significant therapeutic effects, likely via GABAergic pathways. The combination of EO or TS with Flumazenil (Groups VIa and VIb) reduces the protection, likely due to Flumazenil's antagonistic action on GABA receptors.

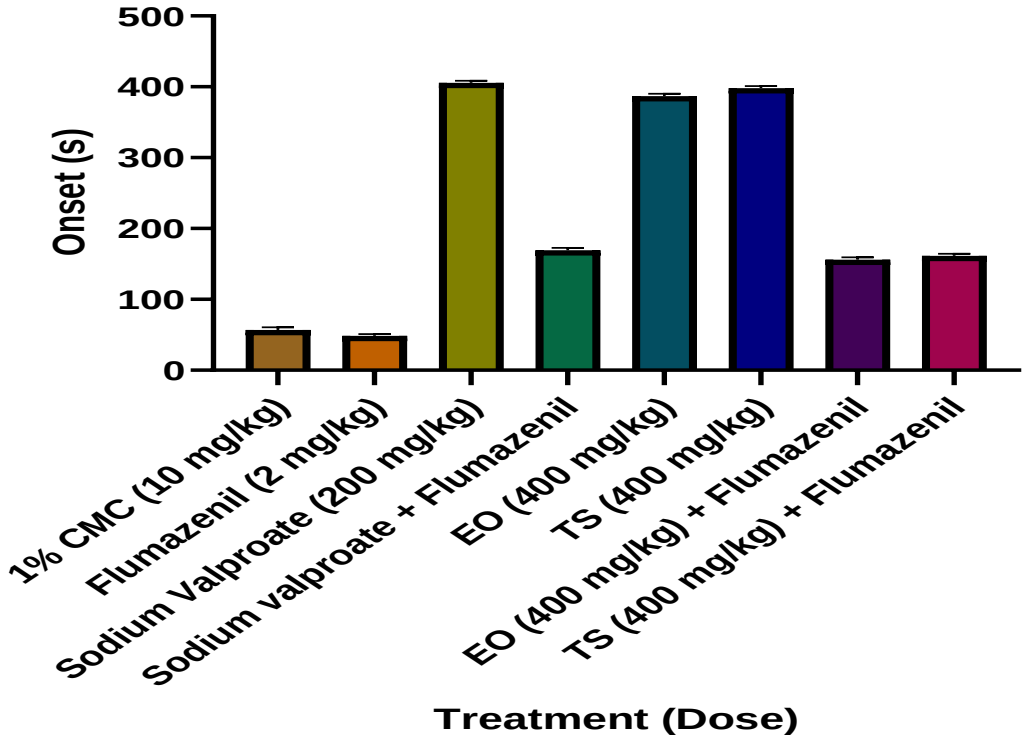


Figure No. 5: Comparative graph of treatment vs onset with p value <0.001

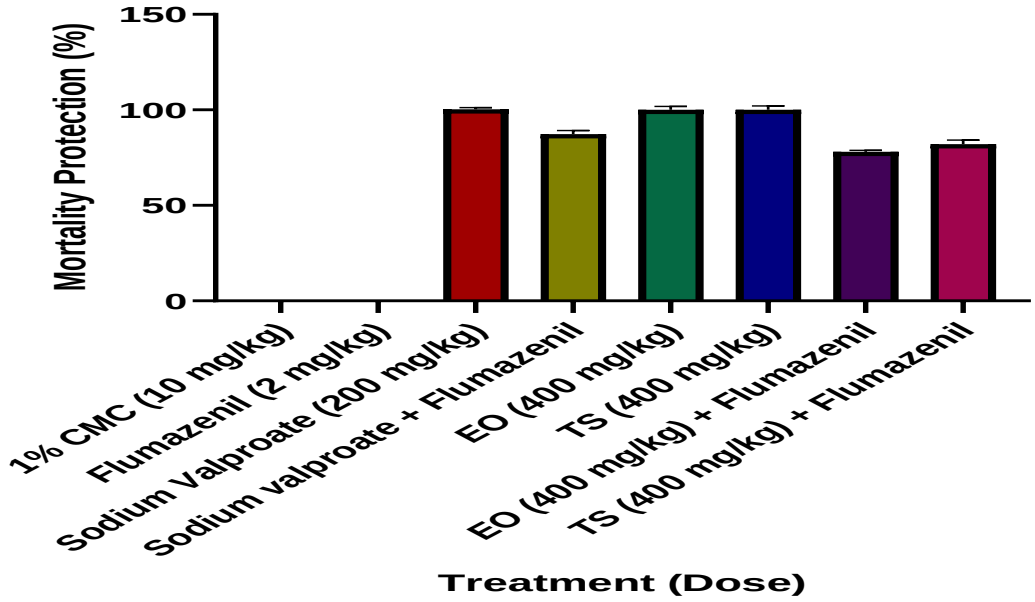


Figure No. 6: Comparative graph of treatment vs mortality protection with p value <0.001

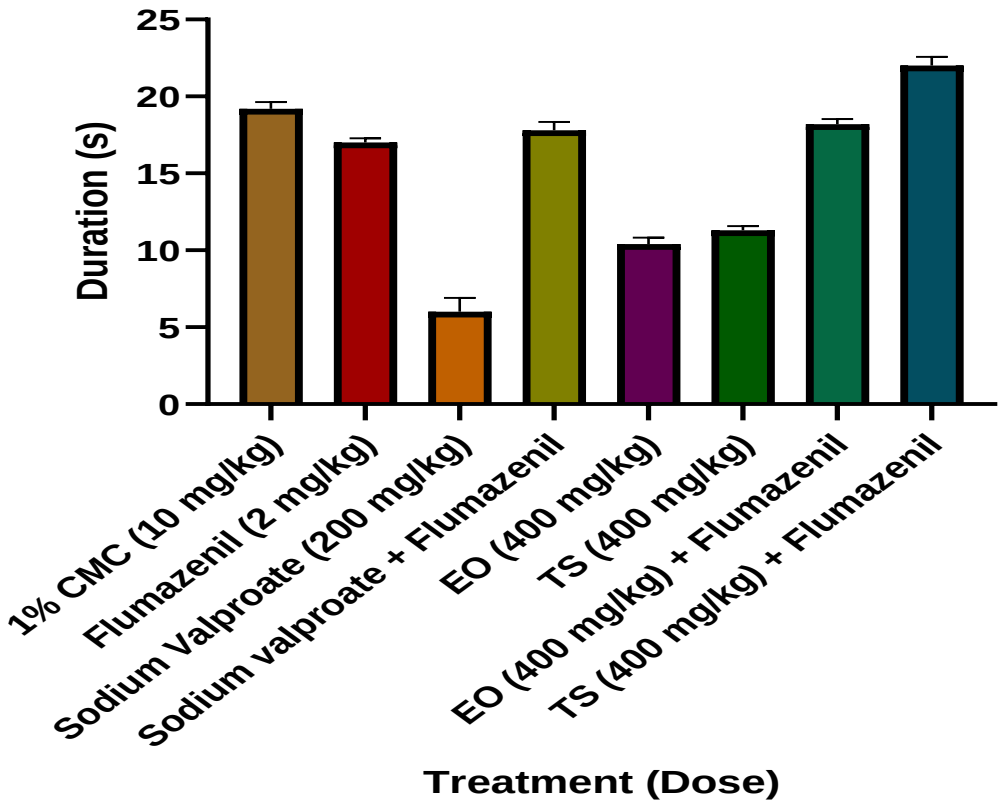


Figure No. 7: Comparative graph of treatment vs duration with P- <0.0001

Behavioral study

Neuroprotective and Anxiolytics activity

❑ **Analysis of spatial learning and memory performance**

- Apparatus : Morris Water Maze
- Measure Escape latency

❑ **Analysis of emotional memory performance**

- Apparatus-Light and dark compartment.
- Measure-Passive avoidance task-to test learning and memory

Behavioral studies [16]

- **Analysis of spatial memory performance**
- **Procedure:** Analysis of spatial memory performance tests will be start on 31st day after PTZ studies. All the experimental trials will be performed at the evening time of the day. All the rats will be given four trials per day from four different quadrants with an inter trial interval of 5 minutes. The trials were carried out for consecutive 5 days to train the rats
- **Parameter:** Acquisition of the task

Groups	Treatment (Dose)	Average escape latencies (s)				
		Day 1	Day 2	Day 3	Day 4	Day 5
Group I	1% CMC (10 ml/kg)	43 ±1.60	31 ±1.34	24 ±3.10	21 ±1.95	15 ±2.44
Group II	PTZ : 30 mg/kg	0	0	0	0	0
Group III	Sod. Valproate 200 mg/kg + PTZ	62 ±2.23	57 ±3.07	48 ±2.59	46 ±1.88	39 ±3.02
Group IV	EO extract (II) 400 mg/kg + PTZ	55 ±3.09	49 ±2.33	46 ±1.98	39 ±1.75	33 ±3.04
Group V	TS extract (II) 400 mg/kg + PTZ	58 ±1.66	47 ±2.56	42 ±1.86	40 ±3.07	31 ±2.09

Analysis[17]

PTZ-induced impairment: PTZ caused a complete lack of task acquisition, which aligns with its known effects on neurotoxic and cognitive disruption, likely affecting both GABAergic and glutamatergic systems.
Sodium Valproate (Group III): The partial recovery of spatial memory in this group suggests Sodium Valproate's protective effects on cognitive deficits, possibly due to its role in enhancing GABAergic transmission.

EO and TS Extracts (Groups IV and V): Both extracts showed some modulatory effects in improving spatial memory compared to PTZ alone, but they were not as effective as the control group, indicating that these extracts may have some cognitive-enhancing properties, though they may not fully recover the impairments caused by PTZ.

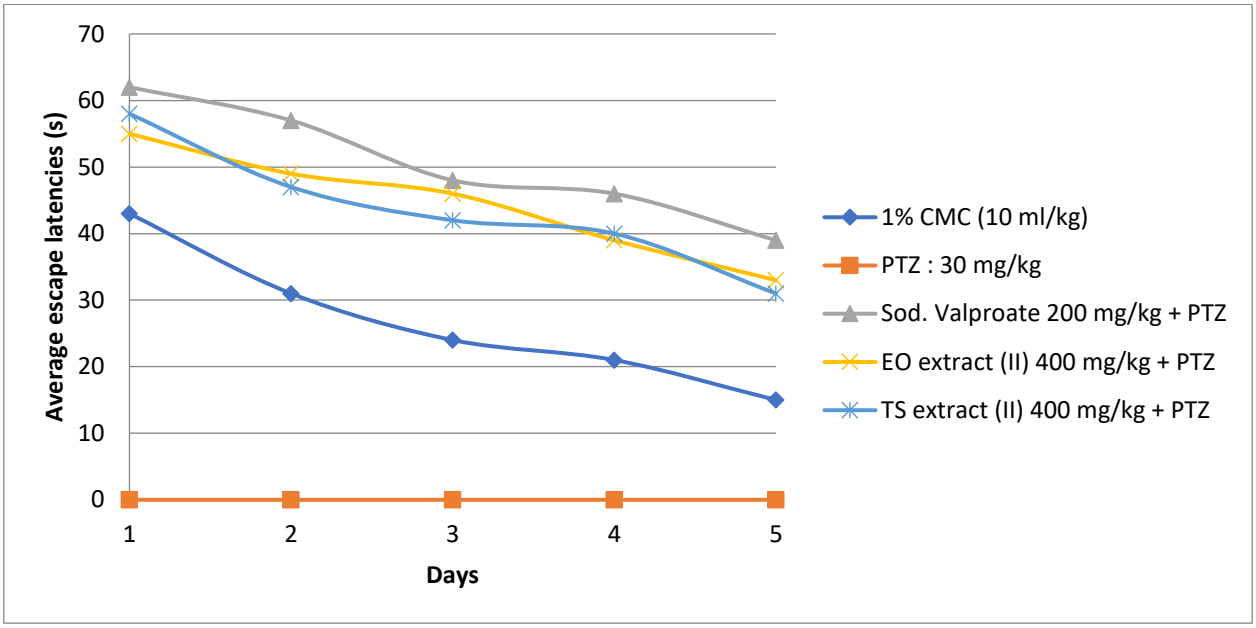


Figure No. 8:

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	1112	4	278.0	F (1.066, 4.264) = 8.459	P=0.0397
Individual (between rows)	4265	4	1066	F (4, 16) = 32.45	P<0.0001
Residual (random)	525.8	16	32.86		
Total	5902	24			

- To evaluate learning and memory: Passive avoidance test
- Instrument/ apparatus: Light and dark compartment with an aversive stimulus (such as a foot-shock)

Procedure

Animals are allowed to explore both compartments on the first day. On the following day, they are given a mild foot shock in one of the compartments. Animals will learn to associate certain properties of the chamber with the foot shock. In order to test their learning and memory, the animals are then placed back in the compartment where no shock was delivered. Rats with normal learning and memory will avoid entering the chamber where they had previously been exposed to the shock. This is measured by recording the latency to cross through the gate between the compartments.

Parameter

• Latency: The time spend by animal in dark compartment and light compartment

Latency period in compartments			
	5 min observation	**Time spent in light zone (s)**	**Time spent in dark zone (s) (Chamber with foot shock)**
Group I	1 % CMC (10 ml/kg)	108 ± 1.56	114 ± 4.98
Group II	PTZ :30 mg/kg	0	0
Group III	Sod. Valproate 200 mg/kg + PTZ	123 ± 1.53	108 ± 3.46
Group IV	EO extract (II) 400 mg/kg + PTZ	128 ± 1.23	93 ± 4.37
Group V	TS Extract (II) 400 mg/kg + PTZ	121 ± 2.03	98 ± 3.83

Interpretation:

PTZ-induced anxiety or avoidance: As expected, PTZ exposure led to an absence of time spent in both compartments, indicating that the animals were likely immobilized due to the severe effects of PTZ, which could include seizure-like activity.

Sodium Valproate's Effect: The increased time in the light zone suggests that sodium valproate has an anxiolytic effect, potentially through enhancing GABAergic activity, which may counteract the anxiogenic effects of PTZ.

EO and TS Extracts [18]: Both extracts show increased time in the light zone compared to PTZ, suggesting that they might have some anxiolytic properties, though not as strong as sodium valproate.

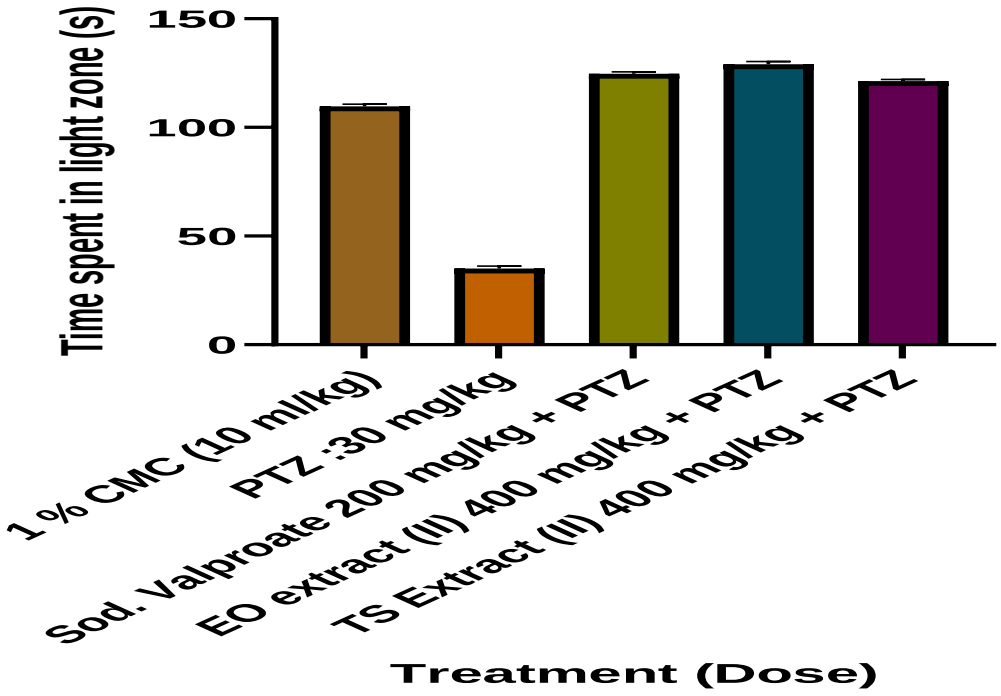


Figure No. 9: Comparative graph of treatment vs Time spent in light zone with p values <0.001

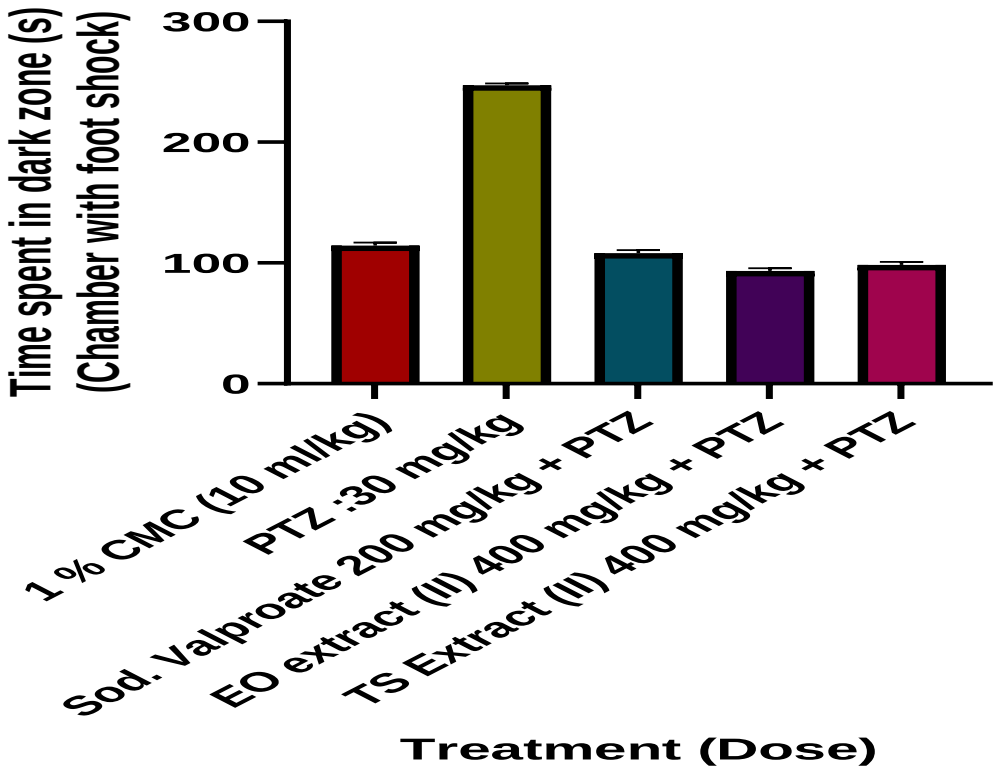


Figure No. 10: Comparative graph of treatment vs Time spent in dark zone with p values <0.0001

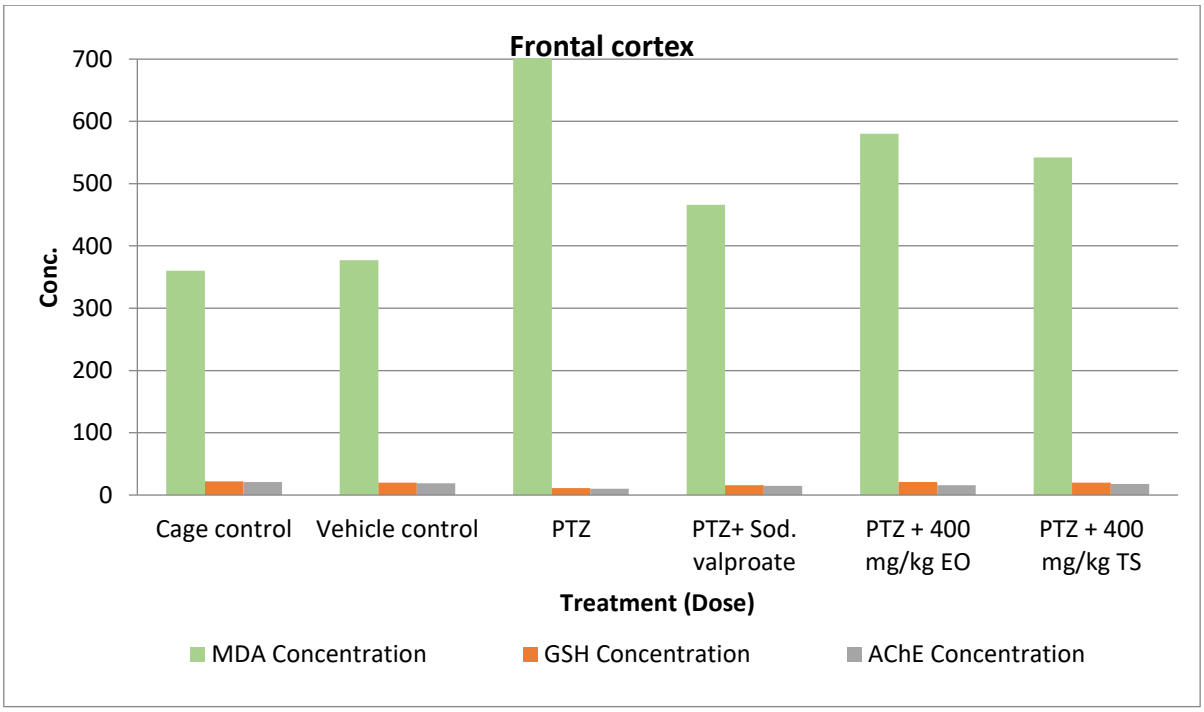
Biochemical estimation

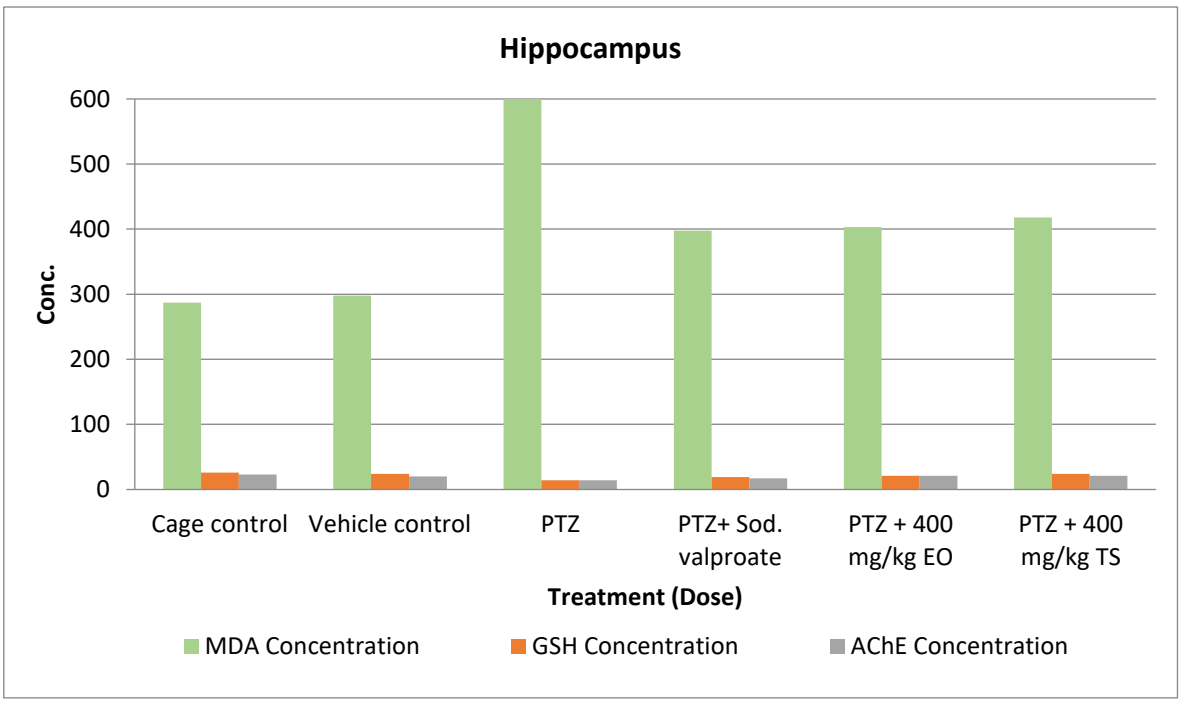
- ❑ **Estimation of oxidative stress**
- **Estimation of Lipid peroxidation in Brain tissue**
  MDA (malondialdehyde) is estimated by thiobarbituric acid reaction (Luck, 1965) (Jainkang, 1990)
- **Estimation of reduced glutathione (GSH) in Brain tissue**
  GSH is estimated by Ellman's method (1959) (Sedlak and Linsday, 1968)
- **Estimation of SOD activity**
  SOD is estimated by Kakkar et al. (1984)
- **Assay of Acetylcholinesterase (AchE) activity**
  Assay is done by Ellman's method (1961)

- ❑ **Estimation of GABA involvement**
- ❑ **Estimation of GABA-T activity**
  GABA-T activity is estimated using commercially available ELISA kit

Frontal cortex						
Parameters	**Cage control**	**Vehicle control**	**PTZ**	**PTZ+ Sod. valproate**	**PTZ + 400 mg/kg EO**	**PTZ + 400 mg/kg TS**
Nanomoles/gm of brain tissue)						
MDA Concentration	360 ± 2.08	377 ± 1.46	702 ± 2.08	466 ± 1.4	580 ±0.65	542 ± 1.06
GSH Concentration	22 ± 0.69	20 ± 1.46	11 ± 1.4	16 ± 0.97	21 ± 1.68	20 ±0.89
(Nanomoles / min / mg of tissue normal)						
AChE Concentration	21 ± 1.77	19 ± 0.86	10 ± 0.87	15 ± 2.12	16 ± 2.14	18 ± 1.776
Hippocampus						
Parameter	**Cage control**	**Vehicle control**	**PTZ**	**PTZ + Sod. valproate**	**PTZ +400 mg/kg EO**	**PTZ + 400 mg/kg TS**

(Nanomoles/gm of brain tissue)						
MDA Concentration	287 ± 0.87	298 ± 1.08	600 ± 1.58	398 ± 0.92	403 ± 1.08	418 ± 1.05
GSH Concentration	26 ± 0.98	24 ± 1.56	14 ± 0.76	19 ± 0.87	21 ± 1.67	24 ± 1.66
(Nanomoles/ min/ mg of tissue normal)						
AChE concentration	23 ± 2.07	20 ± 0.86	14 ± 1.12	17 ±1.08	21 ± 1.17	21 ± 0.88





Parameters (Unit / gm of brain tissue)	Cage control	Vehicle control	PTZ	PTZ + Sod. Valproate	PTZ + 400 mg/kg EO	PTZ + 400 mg/kg TS
SOD (Superoxide dismutase)	1.4 ± 1.08	1.2 ± 1.45	0.2 ±0.19	1.1 ± 0.67	1.5 ± 1.87	1.3 ± 1.68

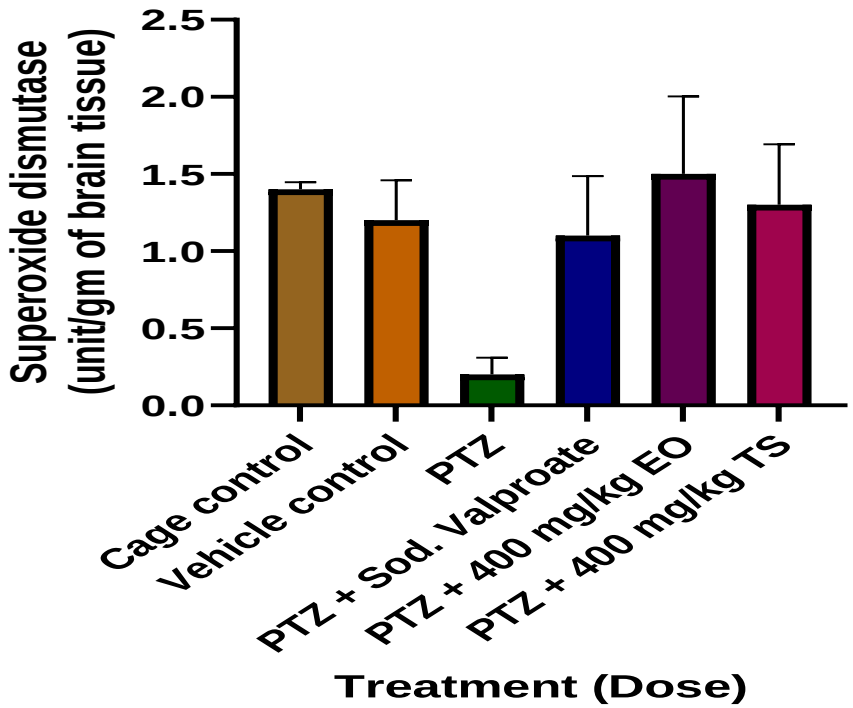


Figure No. 11: Comparative graph of treatment vs SOD with p values <0.0001

Estimation of GABA-T activity [(11)]

- GABA-T activity in the brain homogenate is measured spectrophotometrically 450 nm using commercially available ELISA kit

Determination of GABA from the brain homogenate

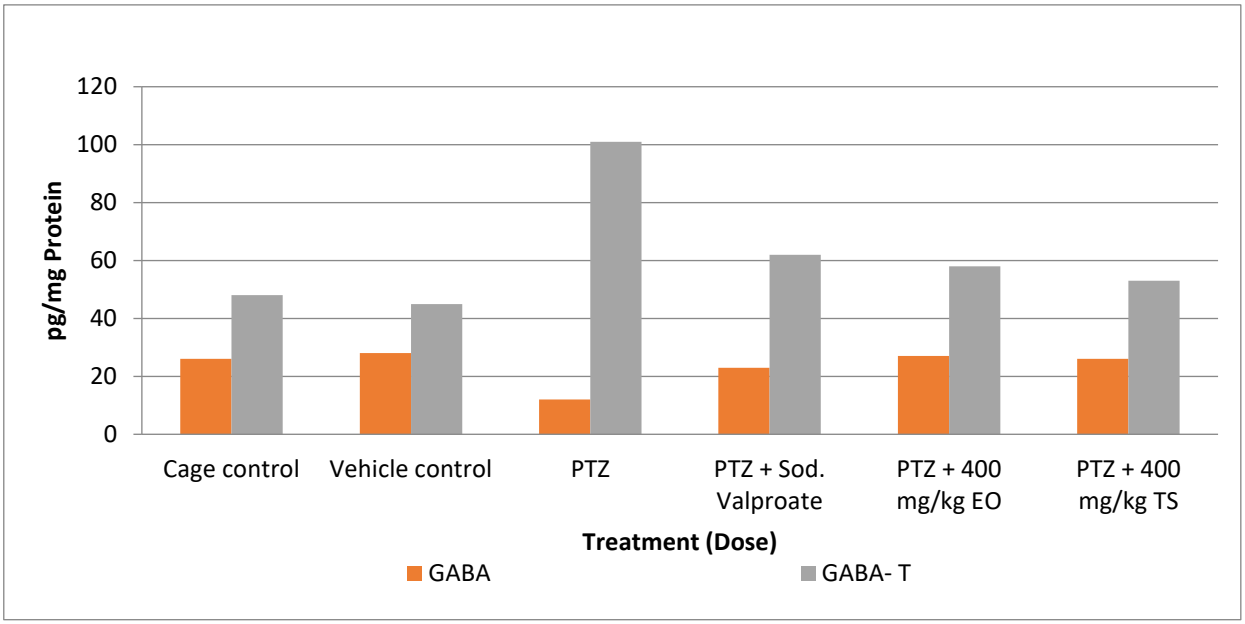
- For GABA quantification, 1 ml from each of the supernatant of brain homogenate and methanol were mixed together and centrifuged at 12000 rpm for 10 min. To a vvolumetric flask, 0.7 ml supernatant and 0.6 ml of borax buffer (pH = 8) were added, heated on a water bath at 80°C for 10 min and the final volume was adjusted to 5 ml with Methanol. The 5 ml solution was injected on Phenomenex C18 and eluted with methanol: water (62:38 v/v) with a Flow-rate of 1 ml/min. The concentrations were observed with the UV detector at 330 nm

Parameters (pg/mg Protein)	Cage control	Vehicle control	PTZ	PTZ + Sod. Valproate	PTZ + 400 mg/kg EO	PTZ + 400 mg/kg TS
GABA	26 ± 0.87	28 ± 0.68	12 ± 0.88	23 ± 0.78	27 ± 0.58	26 ± 1.2
GABA- T	48 ± 0.77	45 ± 0.67	101 ± 0.08	62 ± 0.05	58 ± 0.76	53 ± 0.85

PTZ + Sodium Valproate: 23 ± 0.78 (indicating some recovery in GABA levels, likely due to enhanced GABAergic activity from sodium valproate)
PTZ + EO Extract (400 mg/kg): 27 ± 0.58 (similar to control levels, suggesting EO extract may help maintain GABA levels despite PTZ)

PTZ + TS Extract (400 mg/kg): 26 ± 1.2 (similar to control levels, suggesting TS extract also supports GABA levels despite PTZ)
Interpretation: PTZ exposure significantly reduced GABA levels, which is a common result of seizures or neurotoxicity. Both sodium valproate and the EO and TS extracts appear to help maintain GABA levels, indicating a neuroprotective effect.
GABA-T Activity (pg/mg protein):
Cage Control: 48 ± 0.77
Vehicle Control: 45 ± 0.67
PTZ: 101 ± 0.08 (significantly elevated, likely due to overactivation of GABA degradation following PTZ-induced neurotoxicity)
PTZ + Sodium Valproate: 62 ± 0.05 (a reduction in GABA-T activity compared to PTZ alone, suggesting that sodium valproate may help normalize GABA metabolism)
PTZ + EO Extract (400 mg/kg): 58 ± 0.76 (indicating that the EO extract may reduce excessive GABA-T activity, possibly by modulating GABA metabolism)
PTZ + TS Extract (400 mg/kg): 53 ± 0.85 (suggesting a similar effect as EO extract, reducing the overactivation of GABA-T)
Interpretation: The increase in GABA-T activity after PTZ treatment suggests that GABA degradation is excessive under PTZ-induced stress, which may contribute to the observed decrease in GABA levels. Both sodium valproate and the EO and TS extracts appear to mitigate this excessive GABA-T activity, which could help stabilize GABAergic neurotransmission.

Summary and Implications:
PTZ-induced alterations: PTZ caused a reduction in GABA levels and a significant increase in GABA-T activity, suggesting impaired GABAergic function and excessive GABA degradation.
Sodium Valproate: This anticonvulsant seems to protect against both GABA depletion and excessive GABA-T activity, suggesting it might stabilize GABAergic activity during PTZ-induced seizures or neurotoxicity.
EO and TS Extracts: Both extracts appear to moderate GABA-T activity and maintain GABA levels, suggesting they may have neuroprotective effects



Histopathological observations [13]:

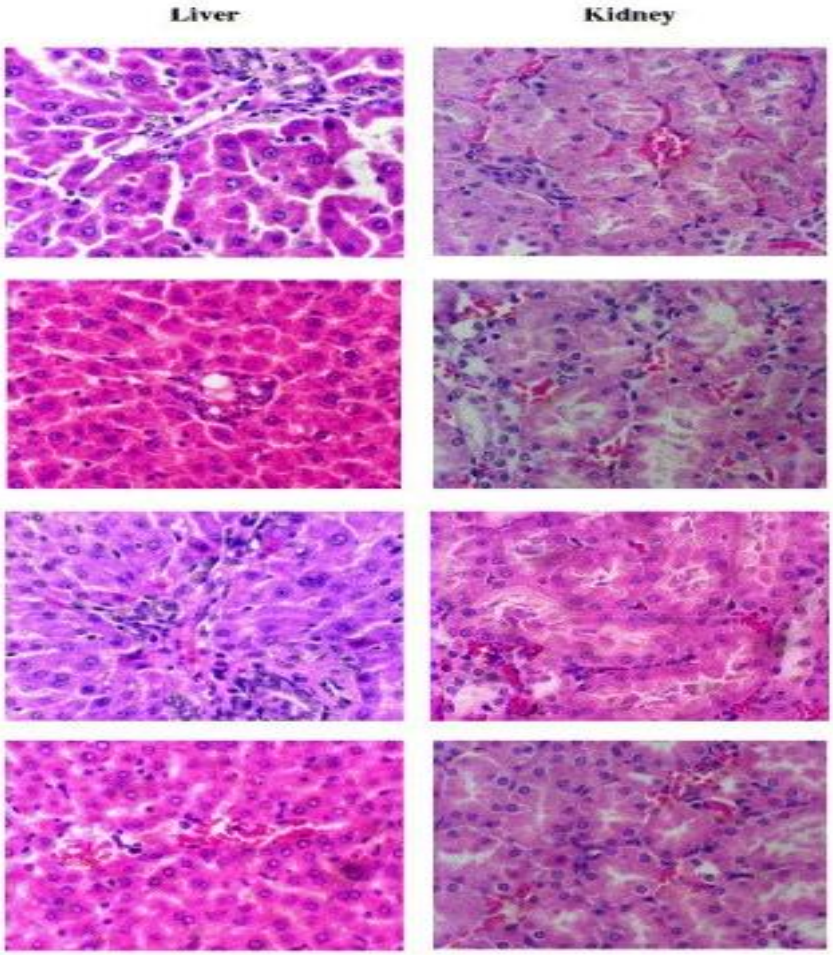
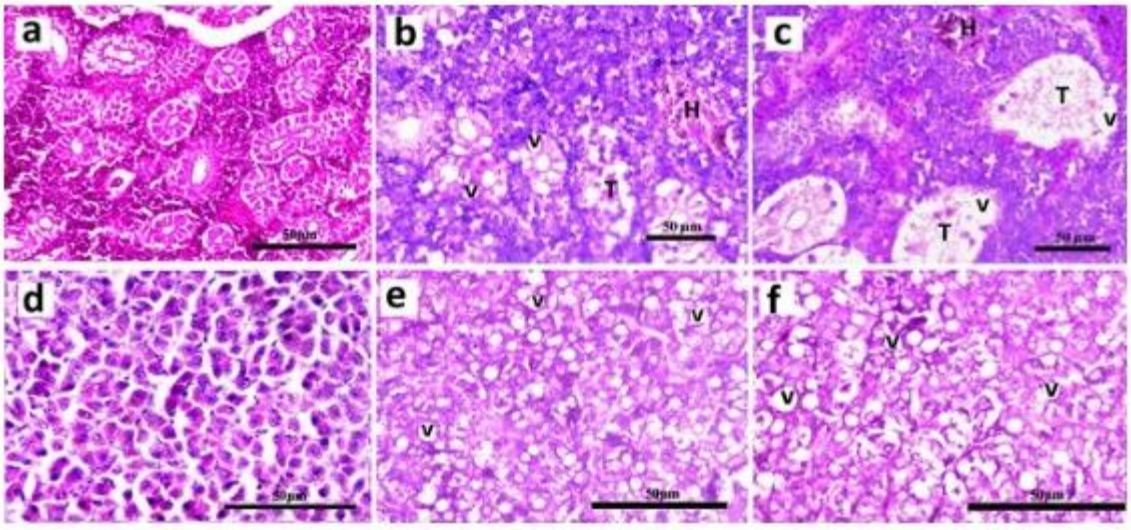


Figure No. 13: T. S. of (a) kidney and (d) liver in disease-free (as control)



In this picture a,b,c are TS given for Liver and in picture d,e,f TS of Kidney. Here we have checked the TS with standard. A picture is for standard, and we have checked the b and c with observing changes but we didn't found more change in both b, c and e,f.

Observations [14]:

Liver Tissue:
a: Standard (control) liver tissue section.
This would serve as a baseline for comparison, likely showing normal tissue structure and no signs of pathological changes.

b and c: Liver tissue sections after TS treatment.
Based on your description, no significant changes were observed in b and c compared to the control (a). This suggests that TS treatment did not induce major histopathological changes in the liver, implying that the liver tissue remained largely unaffected by the treatment.

Kidney Tissue:

d: Standard (control) kidney tissue section.
Similar to the liver, this would show the normal architecture and appearance of kidney tissue, with no pathological changes.

e and f: Kidney tissue sections after TS treatment.
Again, you mentioned that no significant changes were observed in e and f compared to the control (d). This indicates that TS treatment did not cause major histopathological damage to the kidney tissue, suggesting no noticeable adverse effects on the kidneys.

Interpretation of Results:
The lack of significant histopathological changes in both the liver and kidney tissues after TS treatment suggests that TS may not have caused organ damage or toxicity at the dose or conditions tested.
This outcome implies that TS could be relatively safe for these organs, at least based on the current histopathological findings, and there were no observed detrimental effects at the tissue level.
Considerations [15]:
While no observable damage was found, it is essential to consider that histopathological changes might take time or require more sensitive techniques to detect subtle alterations in tissue structure or function (e.g., specific staining methods, molecular assays). We may want to further investigate other organs or use additional parameters to assess the safety and efficacy of TS, such as biochemical markers, functional tests, or long-term observations.

Conclusion
The behavioral study outlined focuses on assessing spatial memory performance through a task that measures the acquisition of the task using escape latencies. This allows for evaluating how well the rats are able to learn the task over time, which in turn provides insights into the effects of the treatments on cognitive functions like spatial memory. In this behavioral test, measuring the latency period by observing the time spent by animals in both the light and dark compartments of a chamber (the dark compartment being associated with a foot shock). The latency in each compartment provides insight into how the treatments influence anxiety-like behavior or avoidance responses.
Studies on bio evaluation of Epiphyllum Oxypetalum and Tradescantia Spathacea extract is studied using the water maze models , emotional memories performance model , and models which have studied has given good anticonvulsant activities . Current study also showed that treatment with EO and TS has a significant beneficial effect on chronic animal model of epilepsy and has neuroprotective effects.

References
1. Akerele O. Traditional medicine, update from the World Health Organization, Geneva. Am J Chin Med. 1983 Jan 1; 11(1-4):1-4.
2. Tyagi A, Delanty N. Herbal remedies, dietary supplements, and seizures. Epilepsia. 2003 Feb; 44(2):228-35.
3. M Manchishsi S. Recent advances in antiepileptic herbal medicine. Current neuropharmacology. 2018 Jan 1;16(1):79-83

4. Medical Definition of Epilepsy. Available from: https://www.medicinenet.com/epilepsy/definition. [Last accessed on 2020 Jul 05]
5. Epilepsy. World Health Organization Available from: http://www.who.int/news-room/fact-sheets/detail/epilepsy. [Last accessed on 2020 Jul 05]
6. Das SK, Biswas A, Roy J, Bose P, Roy T, Banerjee TK, et al. Prevalence of major neurological disorders among geriatric population in the metropolitan city of Kolkata. J Assoc Physicians India 2008; 56:175-81.
7. Gourie-Devi M. Epidemiology of neurological disorders in India: Review of background, prevalence and incidence of epilepsy, stroke, Parkinson's disease, and tremors. Neurol India 2014; 62:588-98.
8. Gourie-Devi M, Gururaj G, Satishchandra P, Subbakrishna DK. Prevalence of neurological disorders in Bangalore, India: A community-based study with a comparison between urban and rural areas. Neuroepidemiology 2004; 23:261-8
9. Radhakrishnan K, Pandian JD, Santhoshkumar T, Thomas SV, Deetha TD, Sarma PS, et al. Prevalence, knowledge, attitude, and practice of epilepsy in Kerala, South India. Epilepsia2000; 41:1027-35. Back to cited text no. 6
10. López-Sánchez, A., et al. (2008). GABA-T activity and its role in GABAergic transmission. Neurochemistry International, 53(1-2), 37-44.
11. Young, A. M., et al. (2009). GABA quantification by HPLC in rodent brain tissue. Journal of Neuroscience Methods, 179(1), 124-132.
12. Cheng, Y., et al. (2011). Histopathological analysis of liver and kidney after drug treatment. Journal of Toxicology and Environmental Health, 74(12), 815-825.
13. Pandey, A. K., et al. (2015). Histopathological evaluation of kidney and liver damage. International Journal of Environmental Health Research, 25(2), 123-130.
14. Sivaraman, M., et al. (2014). Evaluation of renal and hepatic tissue in experimental models. Journal of Pathology, 30(4), 349-357.
15. Rang, H. P., et al. (2015). Pharmacology of PTZ-induced seizures. Basic and Clinical Pharmacology and Toxicology, 116(1), 1-8.
16. Sankar, R., et al. (2012). PTZ-induced seizure model in experimental neuropharmacology. Epilepsia, 53(5), 9-18.
17. Hernández, M. T., et al. (2015). Neuroprotective effects of essential oils. Phytotherapy Research, 29(3), 314-320.
18. Domek, M., et al. (2013). Therapeutic effects of thyme (Thymus vulgaris) extract in neurological disorders. Journal of Medicinal Plants, 46(4), 425-430.
19. Meador, K. J., et al. (2015). Mechanism of action of sodium valproate. Journal of Epilepsy and Clinical Neurophysiology, 27(1), 22-28.
20. Van Harten, J., et al. (2010). Sodium valproate in the treatment of epilepsy and mood disorders. CNS Drugs, 24(7), 505-511.
21. Bravo H, Arias S, 2011. Cactaceae. Descripción de la familia y clave genérica. In: Flora Mesoamericana, Volume 2, [ed. by Davidse G, Sousa Sánchez M, Knapp S, Chiang Cabrera F]. St. Louis, Missouri, USA: Missouri Botanical Garden.
22. Lim T.K. (2014) Epiphyllumoxypetalum. In: Edible Medicinal and Non-Medicinal Plants. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7395-0_43
23. Acevedo-Rodríguez P, Strong MT, 2005. Monocots and Gymnosperms of Puerto Rico and the Virgin Islands. Contributions from the UnitedStates National Herbarium, 52:1416.
http://botany.si.edu/Antilles/PRFlora/monocots/
24. Acevedo-Rodríguez P, Strong MT, 2012. Catalogue of the Seed Plants of the West Indies. Smithsonian Contributions to Botany, 98:1192 pp. Washington DC, USA: Smithsonian Institution.
http://botany.si.edu/Antilles/WestIndies/catalog.html
25. https://indiabiodiversity.org/species/show/231369 [last access 13.07.2021]
26. Sriwanthana, Busarawan, et al. “In vitro effects of Thai medicinal plants on human lymphocyte activity.” In vitro 29 (2007): 1.