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ORIGINAL PAPER

A comparative study of neuropharmacological properties of *Tabernaemontana divaricata* (Apocynaceae) leaves extracts in a Swiss albino mouse model

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ABSTRACT

Introduction and aim. Interest in natural products and nutraceuticals for the treatment of mental diseases such as anxiety, stress, sadness, and psychosis has increased due to their high safety index and cost. The primary objective of this work was to analyze the neuropharmacological attributes of leaf extracts of *Tabernaemontana divaricata* using models from Swiss albino mice.

Method and materials. Methanol, acetone, and ethyl acetate extracts were prepared from authenticated *T. divaricata* leaves. Experiments were conducted on 170 mice to evaluate the effects of thiopental sodium on sleeping time, hole cross, hole board, and open field behaviors. The duration of sleep caused by thiopental sodium was assessed at several doses, including 50 mg/kg, 100 mg/kg, and 200 mg/kg of body weight. Additionally, doses of 100 mg/kg and 200 mg/kg of body weight were used in the remaining tests.

Results. All extracts significantly increased thiopental-induced sleeping time in a dose-dependent manner, with maximum effects observed at 200 mg/kg (methanol: 684.77%, acetone: 655.63%, ethyl acetate: 666.89%). Locomotor and exploratory

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behaviors were reduced in all behavioral models, including a significant decrease in head dips and square crossings ($p<0.01$), supporting central nervous system depressant activity.

Conclusion. The study reveals that extracts of *T. divaricata* exhibit depressive and hypnotic effects on the central nervous system, indicating the need for further research.

Keywords. diazepam, GABA, locomotive behavior, neuropharmacological properties, *Tabernaemontana divaricata*, thiopental sodium

Introduction

In today's world, neuroprotective and neuropharmacological agents are crucial to improving attention and brain memory in people with depression and stress.[1] Depression is a prevalent medical or mental illness that affects 10%-30% of the global population.[2] Psychiatric conditions, also known as psychiatric diseases, are mental health issues affecting various aspects of life. Stress, a complex mental disorder, can lead to depression, anxiety, and cognitive dysfunction, affecting cognitive processes and slowing learning.[3–6] Sedative hypnotics, which suppress the central nervous system and affect gamma-aminobutyric acid (GABA) receptors, relieve anxiety and maintain relaxation and sleep by causing neurotransmitters in the brain.[7] Antidepressants are drugs that mitigate the effect of depression conditions by altering the chemical imbalanced mood and brain neurotransmitters. Common adverse drug reactions include dry throat, fatigue, nervous anxiety, drowsiness, vertigo, stomach problems, and cardiac arrhythmias (heart and apostasy disorders).[8] The most common antidepressants are selective serotonin reuptake inhibitors (SSRI). SSRIs reduce stress by increasing serotonin levels in the brain. As one chemical messenger, serotonine may transport impulses between brain cells. Synthetic medications, now widely available, can cause cognitive decline, physical dependence, and immunity issues due to serious side effects such as respiratory, digestive, and immune dysfunction.[8]

Plants serve as a fundamental reservoir of important medicinal compounds in worldwide healthcare. Throughout history, humans have employed many plant species for an extended period of time to address and alleviate a wide range of ailments.[9,10] The World Health Organization reports that over 75% of those usually use herbal medicines to meet their daily health needs.[11,12] *Tabernaemontana divaricata* is an arboreal plant that belongs to the family apocynaceae popularly known as "jasmine-vane," "dairy-two brothers," "jasmine", "forquilheira and snake bark".[13,14] This species is currently distributed in the north, north-east, southeast, central-west, and south areas.[15] The folk medicine used in this herb is antiophytic, vermifugal, dental antidota, anti-inflammatory, and analgesic extracts; injections and alcohol extracts of that plant.[14,16–18] A variety of chemical elements such as alkaloids, triterpenoids, hormones, flavonoids, phenylpropanoids, and phenolic acids have been thoroughly studied and isolated from leaves, roots, stems, and whole plant.[19,20]

Aim

This study evaluates the role of Indian plants in neuropharmacological mouse regulation, highlighting the benefits of sedative and hypnotic medications, which can be manufactured from prescriptions despite possible side effects and allergic reactions.

Material and methods

Chemicals and drugs

Thiopental sodium and Diazepam are utilized as standard medications. All required components were purchased from the Lab Trading Laboratory, Aurangabad, India.

Plant materials

The leaves of *T. divaricata* were bought from Rise N' Shine Botanical Boutique, Pune, Maharashtra, India. Dr. K. Madhava Chetty, Director of the Botanical Department of Sri Venkateswara University, Tirupati, 517502, Andhra Pradesh, India, authenticated the herbarium. A voucher specimen no. 0972 was deposited. After collecting the authentication certificate, the extraction procedure was performed.

Drying and grinding

The leaves of *T. divaricata* were separated, dried, and pulverized into a coarse powder to prevent decomposition and photochemical debasement, ensuring that the dynamic constituents remained intact. Before the study began, the powder was meticulously enclosed within a container and stored in an environment that included low temperature, lack of light, and dry conditions.

Preparation of methanol, acetone and ethyl acetate extracts

300, 350 and 350 g of granulated leaves of *T. divaricata* were doused in 1000 mL, 1200 mL and 1500 mL of 95% methanol, acetone, and ethyl acetate, respectively, in separate glass compartments for 10 days going with standard shaking and mixing. The entire blend experienced rough filtration through some fine white cotton material. It is separated by Whatman filter paper and the extracts are obtained. The extracts of methanol, acetone, and ethyl acetate obtained from the leaves exhibited yield values of 2.07% w/w, 2.01% w/w, and 2.23% w/w, respectively.[21]

Experimental animals

170 Swiss albino mice (22–25 g) were used in the present study and these were obtained from Flair Labs, Gujrat, In-

dia. Both sexes of mice were between six and seven weeks old. They were kept in animal cages, adhering to standard environmental parameters, including a temperature range of 22–25°C, a humidity level of 60–70%, and a light-dark cycle of 12 hours each. The mice were provided with a standard pellet diet. The study was conducted following internationally recognized guidelines for the use and welfare of laboratory animals. Flair Labs' research ethics committee, Surat-394315, Gujarat, India, authorized our research procedures and *in vivo* studies (ethical approval number: 1250/PO/RcBi/S/23/CPCSEA).

Phytochemical screening

Identify functional groups as described; phytochemical screening of *T. divaricata* was performed.[20]

Sleeping time test

The study by Ali et al. utilized a specific methodology to investigate the effects of three extracts on the thiopental sodium-produced sleep time test produced by thiopental sodium.[22] In this case, the mice were divided into eleven distinct groups, each consisting of five. The control group, group I, received distilled water and diazepam (0.5 mg/kg, body weight, p.o.). The standard was utilized and acquired by group II. Groups III, IV, V, VI, VII, VIII, IX, X, and XI have individually administered all extracts at 50, 100 and 200 mg/kg body weight doses. After thirty minutes, thiopental sodium (20 mg/kg body weight) was administered intraperitoneally to all experimental groups to induce sleep. As a result of their lack of coordination, individual mice placed an object on a table and recorded it. The mice were seen to promptly suppress their right reflex after administering thiopental sodium, resulting in a sleep duration characterized by the period between the initial inhibition. The proportion of effect was obtained by employing the following equation:

$$\text{Effect (\%)} = \frac{\text{Average duration of loss of right reflex in the test group}}{\text{Average duration of loss of right reflex in the control group}} \times 100$$

Hole cross test

As stated, the research was carried out by Uddin et al.[23] A cage measuring 0.30×0.20×0.14 m was utilized. A divider was affixed to the central location of the enclosure. A circular aperture measuring 0.03 m in diameter was precisely positioned at a height of 0.075 m in the frame's center. The experimental subjects were split into three groups: control, standard and extract. They were then placed on one side of the frame. After administering the control, standard, and test samples, subsequent quantification of the mouse's passage through the hole connecting the two chambers was conducted. This quantification was performed in 3 minutes. The mice were divided into eight groups, and each group had five. Group I was designated as the control group and administered distilled water, while group II was administered diazepam, which served as standard treatment. Methanol leaf extracts were obtained for groups III and IV at doses of 100 and 200 mg/kg body weight, respectively. The experimental study involved administering acetone leaf extract to two groups, group V and group VI, at the same dosages. The experimental subjects in groups VII and VIII received leaf extract of ethyl acetate at the same doasges.

$$\text{Movements Inhibition (\%)} = \frac{\text{Mean No. of movements (control)} - \text{Mean No. of movements (test)}}{\text{Mean No. of movements (control)}} \times 100$$

Hole board test

The technique mentioned above was employed in a study by Sheikh et al.[24] The present investigation employed a level base of 0.9×0.9 m in diameter, featuring 16 evenly spaced holes. Furthermore, the height of this stage was 0.05 m. The mice were divided into eight groups: control, standard, and test. Each group consists of five mice (n=5). Group 1 was assigned to the control condition and administered distilled water. Diazepam was administered orally at a dose of 1 mg/kg body weight and served as the standard in group II. Groups III, IV, V, VI, VII, and VIII have individually administered all extracts at 100 and 200 mg/kg body weight doses. The study recorded the frequency of head dips made by individual mice into monitoring holes for 10 minutes.

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of head dips (control)} - \text{Mean No. of head dips (test)}}{\text{Mean No. of head dips (control)}} \times 100$$

Open field test

The investigation described in this study was conducted by Anisuzzman et al.[25] The test apparatus consisted of a flat field measuring 0.5 m^2 with a square pattern. One side of the field had squares painted in alternating black and white, resembling a chessboard. The mechanical system used in the experiment had a compartment height of 0.1 m. The mice were divided into eight groups, each containing five (n=5) mice. Group I served as the control and received distilled water. Diazepam (1 mg / kg, bw, po) was administered to group II as standard treatment. Groups III, IV, V, VI, VII and VIII received different extracts (methanol, acetone, and ethyl acetate) at doses of 100 and 200 mg/kg body weight doses. The number of squares moved by the animals at various intervals after oral administration of the test substances was recorded.

$$\text{Movements inhibition (\%)} = \frac{\text{Mean No.of movements (control)}-\text{Mean No.of movements (test)}}{\text{Mean No.of movements (control)}} \times 100$$

Statistical analysis

Data were analyzed using SPSS statistical tools, version 20, IBM, Chicago, IL, USA. Findings were expressed as mean±standard error of the mean (SEM). Additionally, a single-way ANOVA accompanied by a post hoc Dunnett test for sleep time, hole board, hole cross and open field tests were used to compare groups.

Results

Phytochemical screening

The phytochemical composition of the extracts, as depicted in Table 1, revealed the existence of various distinct groups of chemicals such as alkaloids, flavonoids, saponins, tannins, steroids, gums, glycosides, and terpenoids. The methanol extract (ME) did not include steroids, glycosides, or terpenoids, while the acetone extract (AE) lacked saponins or glycosides, and the ethyl acetate extract (ETAE) lacked tannins or gums.

Table 1. The phytochemical components present in leaf extracts of *T. divaricata*

Compounds	ME	AE	ETAE
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	-	+
Tannins	+	+	-
Steroids	-	+	+
Gums	+	+	-
Cardiac glycosides	-	-	+
Terpenoids	-	+	+

Table 2. Sleeping time in mice was induced by the effect of. *divaricata* leaves extracts on thiopental-Naa

Group	Dose (mg/kg)	Latent period	Sleeping time	Effect (%)
Control	10 mL/kg	11.8±0.37	30.2±4.71	0
Standard	0.5	2.5±0.316	199.4±7.44	660.26**
ME	50	7.0±0.71	72.6±4.01	240.4*
ME	100	4.3±0.49	147.8±6.74	489.4**
ME	200	2.2±0.25	206.8±5.30	684.77**
AE	50	6.8±0.73	66.8±3.94	221.19*
AE	100	4.0±0.32	136.4±4.02	451.66z**
AE	200	3.4±0.37	198.0±5.94	655.63**
ETAE	50	6.6±0.87	76.2±4.42	252.32*
ETAE	100	5.0±0.32	142.8±6.94	472.85**
ETAE	200	3.1±0.19	201.4±6.19	666.89**

[a] The results are presented as the mean value with the SEM for a sample size of 5, the statistical significance was determined using a one-way analysis of variance (ANOVA) followed by a Dunnett's test, the obtained were * – p<0.05, ** – p<0.01, indicating significant differences compared to the control group

Sleeping time test

The extracts at doses of 50, 100, and 200 mg/kg demonstrated a substantial dose-dependent reduction in the time to start sleep in the thiopental-induced hypnosis procedure, mainly in the case of leaf extracts of *T. divaricata***.** The results of the extracts at the beginning of sleep were equivalent to those of the standard drug diazepam. The study found that the leaf extracts of methanol, acetone and ethyl acetate leaf extracts had a maximum dose-dependent effect of 684.77%, 655.63%, and 666.89% during loss of right reflex, respectively (Table 2).

Hole cross test

The hole cross test of the treated *T. divaricata* groups indicated a reduction in activity from its rudimentary value of 0 to 120 minutes. At doses of 200 mg/kg (p<0.01), the maximum inhibition of locomotor activity was observed, which was similar to the standard diazepam (Table 3).

Table 3. Neuropharmacological potential test of *T.divaricata* leaf extracts by hole cross methoda

Group	Dose	Number of movement (% of movememts imhibition)				
		0 min	30 min	60 min	90 min	120 min
Control	10 mL/kg	4.8±0.58	5.4±1.21	4.2±0.58	4.8±0.80	4.4±0.51
Standard	1	2.0±0.55**	1.8±0.58**	2.6±0.24**	2.2±0.49**	1.2±0.37**
ME	100	3.8±0.20	3.6±0.60*	4.2±0.73	3.6±1.83*	3.8±0.37
ME	200	3.0±1.22**	3.6±0.68*	2.8±0.86**	3.2±0.86*	2.6±0.68**
AE	100	3.4±0.98*	2.8±0.37**	4.8±1.24	3.8±1.77	3.0±0.63**
AE	200	4.0±0.77	3.8±0.73*	2.6±1.03**	2.4±0.40**	2.0±0.45**
ETAE	100	6.4±0.68	4.2±1.02	4.8±1.11	6.2±1.59	4.2±0.86*
ETAE	200	3.6±0.40*	3.8±0.66	3.8±0.97	3.0±0.63**	2.6±0.24**

[a] the results are presented as the mean value with the SEM for a sample size of 5, the statistical significance was determined using a one-way analysis of variance (ANOVA) followed by a Dunnett test, the obtained p values were * – p<0.05, * – p<0.01, indicating significant differences compared to the control group

Hole board test

At the dosage amount of 100 mg/kg and 200 mg/kg body weight (p<0.01) of extracts from *T. divaricata* leaves, the number of holes transported by mice was substantially reduced from its original amount at 0 to 120 min. The outcome demonstrated that the leaf extracts of methanol, acetone, and ethyl acetate showed 31.19, 36.14, and 30.70% inhibition at the given doses, and the 67.34% inhibition was higher for the standard diazepam (Table 4).

Open field test

At administered dosages of 100 mg/kg and 200 mg/kg of body weight, leaf extracts significantly reduced the number of squares moved by mice compared to the initial count at 0 to 120 minutes (p<0.01). The study found that the methanol extract of the *T. divaricata* plant inhibited locomotive activity in mice, with a maximum inhibition of 40.8%, similar to the 45.1% inhibition of the acetone extract, and for the ethyl acetate extract 39.1% inhibition. This suggests leaf neuro-modulatory properties (Table 5).

Table 4. Neuropharmacological potential test of extracts from *T. divaricata* leaves using the hole board method[a]

Group	Dose (mg/kg)	Number of head dips	Inhibition (%)
Control	10 mL/kg	44.4±1.86	0
Standard	1	17.2±1.24	67.34**
ME	100	33.8±3.43	23.87*
ME	200	27.8±2.29	31.19**
AE	100	29.8±2.08	36.14**
AE	200	29.2±1.59	27.72**
ETAE	100	32.6±1.60	19.31*
ETAE	200	28.0±1.64	30.70**

[a] the results are presented as the mean value with the SEM for a sample size of 5, the statistical significance was determined using a one-way analysis of variance (ANOVA) followed by a Dunnett test, the obtained p-values were * – p<0.05, ** – p<0.01, indicating significant differences compared to the control group

Table 5. Neuropharmacological potential test of *T. divaricata* leaf extracts by open field methoda

Group	Dose	Number of movement (% of movememts imhibition)				
		0 min	30 min	60 min	90 min	120 min
Control	10 mL/kg	44.4±2.99	41.2±2.06	45.6±1.89	43.0±2.59	43.6±1.6
Standard	1	15.4±3.85**	17.2±4.29**	19.6±3.23**	19.6±3.74**	20.6±2.66**
ME	100	33.4±2.58	30.6±2.62*	32.4±2.18*	29.8±1.39**	31.6±2.71*
ME	200	26.6±1.94**	28.6±1.97*	22.2±1.24**	25.4±1.33**	25.8±1.66**
AE	100	35.8±1.77	34.6±1.44	33.6±2.25	31.0±1.70	32.6±2.11
AE	200	28.6±0.93*	29.2±1.69*	25.0±1.14**	27.8±1.07**	28.2±1.85*
ETAE	100	35.2±3.77	30.4±3.17*	24.2±3.31**	23.0±2.61**	28.4±1.83*
ETAE	200	29.0±1.38*	28.8±1.07**	27.4±3.04**	29.2±2.08*	28.4±1.70**

[a] the results are presented as the mean value with the SEM for a sample size of 5. The statistical significance was determined using a one-way analysis of variance (ANOVA) followed by a Dunnett test, the obtained p-values were * – p<0.05, ** – p< 0.01, indicating significant differences compared to the control group

Discussion

Plant-derived natural medicines have historically shown therapeutic potential, with natural chemicals commonly used in herbal medicines, minerals, nutritional supplements, and therapeutic interventions in various sectors. The study tested the sedative effects of *T. divaricata* on mice's spontaneous locomotor activity. The results showed that the extracts reduced the duration and frequency, suggesting a soothing effect. The study also found that head tilt is correlated with cognitive condition. The findings show a statistically significant reduction in hole crossings (p<0.05, p<0.01) following the oral administration of leaf extracts, including experimental methanol, acetone and ethyl acetate at 200 mg/kg of body weight (Table 3). When administering leaf extracts, two dose amounts were used: 100 mg/kg and 200 mg/kg body weight. Repressive activity was observed 120 minutes before extract administration was extended for 120 minutes. However, the experimental extracts caused significant inhibition (p<0.05, p<0.01,), which was expanded in the observation period from 0 minutes to 120 minutes in the doses measured (Table 5). Tables 3 and 5 show that the locomotive operation condensed with the extract supports the CNS-depressant results (Table 3 and 5). Both experiments significantly decreased mouse locomotion. GABA is the most important inhibitory intravenous neurotransmitter of the central nervous system implicated in physiological and emotional processes.[26] By modifying the alteration of the GABA receptor in the synthesis, eclectic medicine could modify the GABA system by potentiating post-synaptic induced GABA inhibition.[27–29] The conductivity of chloride or GABA performance can be improved by simultaneous voltage depression of the Ca^{2+} channel.[29] The study reveals that CNS GABAergic neurons can be inhibited or activated by brain neurons, enhancing GABA affinity and potentially increasing head dip in animals, indicating anxiety activity. However, the frequency of head dips related to depressing properties was reduced.[31,32]

Dose-dependent sleep extends the sleep cycle, suggesting a deep sedating effect in sleep induced by Thiopental Sodium. Thiobarbiturate sodium is part of a thiopental pathway that contributes to sleep in humans and mice. It has an affinity for the GABA receptor complex and induces hyperpolarization of the post-synaptic neuron through GABA-mediated mechanisms.[33–35] It promotes GABA activity and can also hinder glutamate excitatory receptors. This molecular action leads to a reduction in neuronal function. A mixture of components could depend on the therapeutic benefits of conventional remedies. Several studies have reported the anxiolytic and sedative properties of alkaloids, glycosides, terpenoids, and flavonoids. Additionally, tannin can also be attributed to nonspecific CNS depression.[36–41] By activating protein kinase C and inducing cell survival genes to produce transcriptional factors, flavonoids and steroids are psychocinomatic.[42] The phytochemical investigations conducted in the extracts of *T. divaricata* indicated the presence of alkaloids, flavonoids, saponins, tannins, steroids, gums and glycosides. Bioactive compounds from nature and human nutrition are potential pharmaceutical candidates for the prevention of chronic diseases, the mitigation of stress-induced depression and neuro-pharmacological properties in antidepressant and anxiolytic medicines.[43,44]

Study limitations

The study on the neuropharmacological action of the *T. divaricata* plant extract in a mice model has limitations, as its results may not accurately represent human neurological systems. This investigation faces limitations in dosage optimization, species-specific metabolic pathways, and detailed mechanistic investigations. The neurochemical and behavioral results are encouraging,

but the precise molecular processes are not fully understood. The study may not consider potential toxicity or long-term effects, and its wider use may be restricted by a lack of research on extract standardization and interactions.

Conclusion

The study found a significant correlation between the dose administered and observed results, indicating that the crude extracts of *T. divaricata* have significant neuropharmacological activity. *The extracts of T. divaricata* leaves show potential for sedative, anxiolytic, and anticonvulsant properties, potentially reducing mouse locomotive function and avoiding the tranquilizing side effects of non-selective GABA agonists. Sedative components in methanol, acetone, and ethyl acetate extracts could be used to create insomnia treatments, but more research is needed to understand their neuropharmacological activity.

Declarations

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Authors' contributions

Conceptualization, K.T.K.D. and P.D.R.; Methodology, P.P.T.; Software, A.B.; Validation, M.M., J.K.G. and K.T.K.D.; Formal Analysis, P.D.P.; Investigation, P.P.; Resources, S.H.S.; Data Curation, K.D.; Writing – Original Draft Preparation, K.T.K.D. and P.D.R.; Writing – Review & Editing, J.K.G.; Visualization, P.D.P.; Supervision, K.T.K.D.; Project Administration, K.T.K.D.; Funding Acquisition, K.T.K.D.

Conflicts of interest

There are no conflicts of interest involved in the study.

Data availability

Due to privacy concerns, the data are not publicly available, but can be accessed upon reasonable request from the corresponding author with a signed data access agreement.

Ethics approval

Flair Labs' research ethics committee, Surat-394315, Gujarat, India, authorized our research procedures and the *in vivo* studies (ethical approval number: 1250/PO/RcBi/S/23/CPCSEA).

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