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ASSESSMENT OF NEUROPHARMACOLOGICAL ACTIVITIES OF *TAMARINDUS INDICA* L. IN EXPERIMENTAL ANIMALS

G. B. Alaka Kar[1], Dr. Susanta Kumar Rout*[2] and Debashisa Mishra[1]

[1]IMT Pharmacy College, Puri, New Nabakalabara Road, Sai Vihar, Gopalpur, Puri, Odisha.

[2]Patent Information Centre, Science and Technology Department, Secretariat, Odisha.

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***Corresponding Author**
Dr. Susanta Kumar Rout
IMT Pharmacy College, Puri, New Nabakalabara Road, Sai Vihar, Gopalpur, Puri, Odisha.

ABSTRACT

Tamarindus indica Linn is a plant that is used in traditional medicine for the treatment of cold, fever, stomach disorder, diarrhoea, jaundice and as skin cleanser. *Tamarindus indica* L. is a fruit tree (Magnoliophyta) is widely distributed in Asia. The available scientific information about Central nervous system (CNS) disorders of this species is scarce and there are no reports related to its possible effect on the CNS. The purpose of this study is to investigate the neuropharmacological activities like sedative effect (Phenobarbitone induced sleeping time, spontaneous motor activity, rotarod), anxiolytic effect (elevated plus-maze), antidepressant (forced swimming test), anticonvulsant (maximal electroshock and PTZ induced epilepsy) effects respectively, in laboratory animals by using hydroalcoholic leaf extract of *Tamarindus indica* L. The extract (250 mg and 500 mg) and standard drugs were administered orally. The extract at 500 mg/kg had an insignificant $p>0.05$ effect on shortened the onset time of sleep and prolonged the duration of sleep induced by phenobarbitone sodium. The extract also exhibited a significant ($P<0.05$) decrease of motor activity and exploratory behaviour in hole cross and open field tests. In the FST, the extract (250 and 500 mg/ kg) was as effective as fluoxetine (10 mg/kg) in reducing immobility, along with a significant increase in swimming and climbing, respectively. The extract (250-500 mg/kg), could not offered significant protection against MES and PTZ induced convulsion, but were found to delay significantly ($p<0.05$) the onset of tonic/clonic convulsion and also prolonged the time of death of the treated mice. These results suggest that some of the components of the hydroalcoholic extract of *Tamarindus indica* L. may have sedative, anxiolytic and antidepressant-like properties which deserve further investigation. In conclusion, the present work evidenced that sedative and anxiolytic

effects of the extract might involve an action on benzodiazepine-type receptors, and also an antidepressant effect where noradrenergic and serotoninergic mechanisms will probably play a role.

KEYWORDS: Sedative, anxiolytic, anticonvulsant activity, Central nervous system.

1. INTRODUCTION

Research on medicinal plant has increased recently all over the world. Medicinal plants have been used in various systems, as they have potential against numerous diseases. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacology.[1] *Tamarindus indica* Linn. (Caesalpiniaceae), is a medicinal plant, used in folk medicine for treating asthma, dysentery, vaginal and uterine complaints, inflammation and variety of other condition. It is cultivated throughout India, self sown in waste places and forest lands in central India, It is also planted along roadsides throughout India. According to Ayurveda, *Tamarindus indica* Linn is used in the treatment of biliousness, vaginal and uterine complaints, inflammations, burning sensation, asthma and other conditions.[2] The methanolic extract of leaves contain ascorbic acid and a-carotene is proven to be anti-lipoperoxidant and anti- hepatotoxic. Some studies have reported immunomodulatory effect of *Tamarindus indica* Linn.[3] However no scientific data are available regarding the effect of *Tamarindus indica* Linn in the treatment of CNS disorders. The present study is to evaluate the pharmacological screening of the *Tamarindus indica* Linn on various aspects of Central Nervous System Diseases like Insomnia, Anxiety, Epilepsy and Depression using various animal models.

2. MATERIALS AND METHODS

2.1 Drugs, chemicals and equipment

Diazepam (Lupin Laboratories Limited, India), phenobarbitone sodium (Rhone-Poulenc India Limited, India) was used as a standard CNS depressant and anticonvulsant. Pentylenetetrazol (Sigma Aldrich), ethanol (Lab Chemicals) China, petroleum ether (60 – 80 °C, Merck), rotary evaporator, actophotometer, rotarod, electroconvulsiometer were used in the study.

2.2. Plant material

Tamarindus indica Linn leaves were collected from Anandapur, Keonjhar district of Odisha in India. The plant was authenticated in the Department of Biosciences, Sardar Patel

University, Anand, Gujarat. The leaves were collected in bulk and washed with running tap water to remove adhering soil and dirt particles and then shade dried. The dried plant materials were coarsely powered and stored in airtight, non-toxic polyethylene bags until used. Powdered leaves of the plant were extracted successively using soxhlet extractor with petroleum ether (60-80°C).

2.3. Preparation of extracts

The leaves were shade-dried and coarsely powdered with a grinding mill. The coarse powder of plant leaves was de-fatted using petroleum ether (60 – 80ºC) and then macerated with chloroform, methanol and aqueous with constant stirring. The solvent incorporating the extractives was filtered and the marc pressed to squeeze out residual extractives. This process was repeated thrice to achieve complete extraction. The extracts obtained during the three cycles were combined and reduced to 1/8th of its original volume in a rotary evaporator at 45°C and then lyophilized in a freeze dryer to obtain a yield.

2.4. Preliminary Phytochemical Analysis

The preliminary phytochemical group tests of the plant extract/fractions were done by standard methods for the presence of alkaloids, terpenoids, steroids, amino acids, flavonoids, gums, reducing sugars, tannins and saponins.[4,5,6]

2.5. Acute toxicity study

Determination of maximum tolerable dose was performed according to OECD (Organization for Economic Corporation and Development) guideline 423.[7] The study was performed at graded doses level of 5, 50, 300 and 3000 mg/kg P.O. of extract/fractions by suspending in 1% tween solution, using female rats (160 – 180 g). The rats were deprived of food 3-4 h prior to the experiment and thereafter individually administered the extract/fractions. Each animal was continuously monitored during the first 30 min, then on hourly basis for the next 4 h, and subsequently, at four hourly interval. Finally, they were placed under observation for 14 days to monitor any abnormal signs and symptoms depicting toxicity. The animals were then humanely killed by a high inhalation dose of diethyl ether and observed for any changes in skin, eyes, mucous membrane (ear), respiratory, circulatory, autonomic and central nervous systems, as well as somato-sensory activity and behavioral pattern. Attention was given to phenomena such as tremors, convulsions, salivation, diarrhoea, lethargy, sedation, hypnosis and coma.[8]

2.6. Behavioural evaluation

Evaluation of general behavioural profile was performed by the method described by Irwin et al, (1968).[18] Sixty healthy adult albino mice were divided into ten groups. The first eight groups of animals were administered with the extracts each at 250 and 500 mg/kg dose level by oral route. The last two groups receive chlorpromazine (5mg/kg) as standard drug or 1%w/v tween solution (5ml/kg) as solvent control. The animals were under observation for behavioural changes if any, at 30 minutes interval in the first hour and at one hour intervals for next 4 h for different parameters.[9]

2.6.1. Awareness, alertness and spontaneous activity

The awareness and alertness were recorded by visual measure of the animal's response when placed in different positions and its ability to orient itself without bumps or falls.[10] The normal behaviour at resting position was scored as 0. Similarly little activity (+), moderate flexibility (++), strong response (+++) and abnormal restlessness (++++) were recorded. The spontaneous activity of mice was recorded by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behaviour. Less or moderate activity was scored as ++ and strong activity as +++. If there is slight or little motion, the score was + while the animal sleeps, the score was -. Excessive or very strong inquisitive activity like constant walking or running was scored as ++++.A similar test was performed with the same scoring, when the animal are removed from the jar and placed on a table.[11]

Touch, pain and sound responses

The touch response was recorded by touching the mice with a pencil or forceps at a various parts of the body (i.e. on the side of the neck, abdomen and groin). The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted. Albino mice normally utter no sound, so that vocalization may indicate noxious stimulus.

2.7. Phenobarbital-induced hypnosis

In order to evaluate the potentiation of hypnosis, Phenobarbitone sodium (45 mg/kg) was injected to male mice 1h after the oral administration of the *Tamarindus indica* Linn extracts at 250 and 500 mg/kg dose levels. The healthy albino rats of 150-180g body weight were divided into nine groups of six animals each. The solvent (15w/v of tween with water) and test extracts were administered at 250 and 500 mg/kg dose levels by oral route. The latency

and the duration of hypnosis were recorded. Hypnosis time was measured by the loss of the righting reflex, being the recovery of this reflex considered as the hypnosis endpoint.[12]

2.8. Assessment of spontaneous motor activity

For this study, the animals were divided into ten groups of five animals each. Group I served as control (received 1% Tween solution), group II served as standard (received Diazepam 4 mg/ Kg p.o.). Remaining groups received the test solutions and served as test groups. The locomotor activity was then assessed by recording the scores after every 30 min. using actophotometer.[10]

2.9. Exploratory behaviour

Exploratory behaviour of the animals was evaluated using Y-maze and head dip tests.

2.9.1. Y-maze test

The test was performed in 4 groups of 6 albino rats (weighing 150-180gm) at 30, 60, 90 and 120 min after administration of 1% Tween solution, extract (250 and 500 mg/kg) and diazepam(4 mg/kg) respectively. The rats were placed individually in a symmetrical Y-shaped runway (33 × 38 × 13cm) for 3 min and the number of times a rat entered in the arm of the maze with all 4ft (an 'entry') were counted.[13,14]

2.9.2. Head dip test

Five groups of female albino mice (n=6) were placed on the top of a wooden box with 16evenly spaced holes, 45 min after administration of the extracts (250 and 500 mg/kg), vehicle (1% Tween solution) and diazepam (4 mg/kg) respectively. The number of times that each animal dipped the head into the hole was counted for a period of 3 min.[15]

2.9.3. Elevated plus maze

Each mouse was placed at the centre of the elevated plus maze with its head facing the open arm. During the 5 minutes experiment, the behaviour of the mouse was recorded as: i) preference of the mouse for its first entry into the open or closed arms, ii) the number of entries into the open or closed arms, and iii) time spent by the mouse in each of the arms.[16]

2.10. Muscle relaxant Activity

The effect of extract on muscle relaxant activity was studied by using traction and rotarod tests.

2.10.1. Rota-rod test

The motor coordination and performance of each male mouse was evaluated 45 minutes after the treatment of the extracts in a rota-rod apparatus with 2.5-cm diameter bar divided in six parts and it is placed at a height of 25 cm, rotating at 25 rpm. Latency to fall from the rotating bar and number of falls in a period of 1 min test was registered.[17]

2.10.2. Traction test

The screening of the animals was done by placing the forepaws of the male mice in a small twisted wire rigidly supported above a bench top. Normally the mice grasp the wire with the forepaws, and place at least one hind foot on the wire within the 5sec when allowed to hang free. The test was conducted on ten group of animals (n=6) which were previously screened, 30 min after administration of the extracts at 250 and 500mg/kg dose levels, vehicle (5 ml/kg, 1% tween solution) and diazepam (4 mg/kg) respectively. The inability to put at least one hind foot was considered as failure in the traction test.[17]

2.11. Experimental convulsions

2.11.1. Pentylenetetrazol induced seizure

Pentylenetetrazol (PTZ) 60 mg/kg i.p. was used to induce generalized clonic-tonic convulsions.[28] Ten groups of mice (n = 6) were used. Group I was administered the vehicle, i.e., 1% w/v Tween solution (1 ml/150g body weight) and served as control, Group II received reference standard (diazepam, 4 mg/kg, p.o.) while Groups III to X were administered different doses of extracts, p.o., respectively, Two hours later, PTZ was administered (60 mg/kg, i.p.) to all four groups. The animals were observed for 30 min and the onset and duration of convulsion noted.[18]

2.11.2. Maximal electroshock induced seizure

MES model was used to evaluate the anticonvulsant activity of the extract. The electrical stimulus (50mA; 60 Hz; 0.2 sec duration) was applied through ear clip electrodes using an electroconvulsiometer. Animals were grouped into ten (n=6). Eight groups were treated with test doses, one group was treated with standard phenytoin (25 mg/kg,Po.) and last group was kept as control. Electroshock was given by ear electrodes 30 minutes after the administration of standard drug and test extract/fractions. Here hind limb Tonic Extension and mortality was considered as a protective measure against MES induced seizures.[19]

3. Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis of difference between groups was evaluated by ANOVA followed by Dunnett's t test. p-value less than 0.05 was considered significant.

4. RESULTS

The preliminary phytochemical screening of crude methanolic extracts was performed by standard methods and the results indicate the presence of tannins, terpenoids, flavonoids, steroids and reducing sugars.

4.1. Toxicity Study

The crude methanolic extract of *T indica* Linn and different fractions of *T indica* Linn were found to be non-toxic up to doses of 5000 mg/kg and did not cause any death of the tested animals. This indicates that the LD50 value of the extracts were more than 5000 mg/kg.

4.2. Effect on behavioural profiles

The results obtained from the experiments are presented in Table 1. The extract and the extracts affected spontaneous activity, sound and touch responses at higher doses and in low dose produced moderate or slight depression relating to awareness and alertness. However, the standard drug chlorpromazine hydrochloride caused significant depression of all these responses compared with methanol extracts. The results indicate that the extract influences general behavioural profiles, as evidence in the spontaneous activity, touch, sound and pain responses.

4.3. Effect on phenobarbitone sodium-induced sleeping time

The extract significantly potentiated the phenobarbitone sodium-induced sleeping time at the doses studied, with respect to the control (Table 2). The onset and duration of sleep increases in a dose dependent manner in the potency order of methanol extract followed by aqueous extract and chloroform extract. The maximum sleep duration found in the experiment is significant ($p<0.5$ to $p<0.01$) when compared with solvent treated group.

4.4. Effect on muscle relaxant activity

The muscle relaxant study was performed by traction and rota-rod test and depicted in Table 3 and 4. The mice treated with the crude methanolic extract and aqueous extract showed a significant failure (decrease in time of holding) in traction at higher doses level. The result of

the rota-rod test report showed that the time of fall in second decreases in all tested extract in the order of crud methanol extract, followed by aqueous and Chloroform and the fall is significant when compared with solvent control group.

4.5. Exploratory behaviour potentials

In the Y-maze test (Table 5), the animals treated with the crude methanolic extract and other extracts in tested dose levels showed a marked decrease in exploratory behaviour compared with controls. In head dip test (Table 6), there was a significant ($p<0.05$) reduction in the number of head dip in mice treated with the extracts, compared with the control. In elevated plus maze (Table 7) the crude methanolic extracts at 500 mg/kg produces increase in permanence in the open arms of the maze.

4.6. Anticonvulsant activity

The crude methanolic extract/fraction of *T indica* Linn and diazepam significantly prevent PTZ induced seizures (Table 8). The test crude methanolic extracts of *T indica* Linn showed a dose dependent seizure protection in the order of methanolic extract followed by aqueous extract. It showed significant ($P< 0.001$) delayed in the onset of jerks and clonic convulsion. In MES induced convulsions, (Table 9), high doses level 300 mg/kg of Crude methanolic extract produced significant ($p<0.001$) activity but not shown by lower dose level. The tested extracts showed delayed on the onset of clonus, the duration of tonic extensor phase and offered a good percentage protection of the animals, when compared to the control group of animals.

Table 1: Effect of methanolic extract/ fractions of *T indica* Linn on general behavioural profiles in rats.

Behaviour	Chloroform Extract (mg/kg)		Methanolic Extract (mg/kg)		Aqueous Extract (mg/kg)		CPZ	Solvent
	250	500	250	500	250	500	5mg/kg	5ml/kg
Spontaneous activity	-	-	+	++	+	++	+++	-
Alertness	-	+	++	++	++	+++	++++	-
Awareness	-	-	+	++	+	++	+++	-
Sound response	-	-	+	++	+	++	+++	-
Touch response	-	-	++	++	++	+++	++++	-
Pain response	-	-	+	++	+	++	+++	-

- No effect, + Slight depression, ++ moderate depression, +++ Strong depression, ++++ Very strong depression, n= 10

Table 2: Effect of fractions and extract of *T indica Linn* on Phenobarbitone induced sleeping time in rats.

Group	Treatment	Dose (mg/kg)	Onset of sleep (min)	Duration of sleep (min)
Gr. I	Solvent	10 ml/kg	3.8 ± 0.46	50.12 ± 1.28
Gr. II	CETI	250	3.3± 0.24	52.3 ± 2.4
Gr. III		500	3.4 ± 0.33	53.6 ± 2.6
Gr. IV	METI	250	2.8 ± 0.46[b]	96.3 ± 3.9[c]
Gr. V		500	2.1 ± 0.40[c]	123.6 ±0.66[c]
Gr. VI	AQTI	250	2.8 ± 0.31	96.6 ± 3.3[c]
Gr. VII		500	2.6 ± 0.30[a]	98 ± 2.5[c]

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at ap<0.05, bp<0.01 and cp<0.001 respectively, in comcomparison to group-I).

Table 3: Effect of fractions and extracts of *T indica Linn* on muscle relaxant activity by Traction test in mice.

Group	Treatment	Dose (mg/kg)	Time of holding (sec)
Gr. I	Solvent	10 ml/kg	7.6 ± 1.260
Gr. II	Diazepam	4	3.4 ± 0.58[c]
Gr. III	CETI	250	7.2 ± 0.67
Gr. IV		500	6.4 ± 1.22
Gr. V	METI	250	5.4 ± 0.76[a]
Gr. VI		500	3.8 ± 0.54[b]
Gr. VII	AQTI	250	5.6 ± 0.76[a]
Gr. VIII		500	4.4 ± 0.68[b]

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to group-I).

Table 4: Effect of fractions and extracts of T indica Linn on muscle relaxant activity by using Rota-rod test in rats.

Group	Treatment	Dose(mg/kg)	Fall off time (sec)		
			30 min	60 min	120 min
Gr I	Solvent	10 ml/kg	116.4 ± 5.74	114.6 ± 6.34	112.6 ± 4.66
Gr II	Diazepam	4	32.2 ± 3.28[c]	26.4 ± 3.77	24.88 ± 2.46
Gr III	CETI	250	113.5 ± 5.46	112.3 ± 4.23	111.4 ± 3.64
Gr IV		500	115.6 ± 4.32	113.5 ± 7.26	110 ± 4.54
Gr V	METI	250	108.6 ± 6.37[a]	105.8 ± 2.7[b]	96.9 ± 2.24[b]
Gr VI		500	96.8 ± 5.34[b]	85 ± 5.68[c]	76.3 ± 4.68[c]
Gr VII	AQTI	250	110.4 ± 6.4[a]	107.3 ± 6.32[b]	109.8 ± 5.36[b]
Gr VIII		500	98.6 ± 6.54[a]	97.2 ± 4.34[c]	88.4 ± 5.39[c]

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at ap<0.05, bp<0.01 and cp<0.001 respectively, in com

Table 5: Effect of extracts/fractions and of *T indica Linn* on exploratory behavior by Y-maze test in rats.

Group	Treatment	Dose(mg/kg)	Number of entries after treatment in 5 min		
			30 min	60 min	90 min
Gr. I	Solvent	10 ml/kg	12.3 ± 1.45	11.1 ± 1.13	10.5 ± 0.84
Gr. II	Diazepam	4	5.1 ± 1.07b	4.6 ± 0.80c	4 ± 0.68c
Gr. III	CETI	250	11 ± 0.57	8.8 ± 0.70	9.3 ± 0.66
Gr. IV		500	10.16 ± 1.13	7.5 ± 0.84	8.5 ± 0.42
Gr. V	METI	250	7.5 ±0.76[a]	7.1 ± 0.6[b]	6.5 ± 0.76[b]
Gr. VI		500	6.8 ± 1.22[b]	6.3 ± 0.95[b]	5.1 ± 1.01[c]
Gr. VII	AQTI	250	8.5 ± 1.05[a]	7 ± 1.12[b]	6.6 ± 0.61[b]
Gr. VIII		500	7 ± 0.93[b]	6.5 ± 0.84[b]	6.3 ± 0.88[b]

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to group-I).

Table 6: Effect of fractions and extract of *T indica Linn* on exploratory behaviour by Head dip test in mice.

Group	Treatment	Dose (mg/kg)	Number of head dip		
			30 min	60 min	90 min
Gr.I	NS + Tween	10 ml/kg	90.4 ± 4.46	86.4 ± 4.67	82.6 ± 4.95
Gr.II	Diazepam	4	30.7 ± 2.91	27 ± 2.25	23.7 ± 3.59
Gr.III	CETI	250	89 ± 6.08	84 ± 6.63	79.3 ± 6.33
Gr.IV		500	78.6 ± 3.38	75.3 ± 3.92	77.1 ± 4.80
Gr.V	METI	250	55.7 ± 4.65^{c}	52.4 ± 3.69^{c}	49.8 ± 2.95^{c}
Gr.VI		500	37.5 ± 4.09^{c}	33.16 ± 1.86^{c}	29.5 ± 2.57^{c}
Gr.VII	AQTI	250	59.2 ± 7.47^{b}	63.6 ± 7.61^{a}	62.1 ±4.98^{b}
Gr.VIII		500	40.9 ± 4.04^{c}	35.9 ± 2.12^{c}	33 ± 3.30^{c}

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at ^{a}p<0.05, ^{b}p<0.01 and ^{c}p<0.001 respectively, in comparison to group-I).

Table 7: Effect of fractions and extracts of *T indica Linn* in Elevated plus maze (EPM) test in mice.

Group	Treatment	Dose(mg/kg)	Number of entries in open arm in 5 min	Time spent in openarm in 5 min (Sec)
Gr I	Solvent	10 ml/kg	4.25 ± 0.54	36.83 ± 3.15
Gr II	Diazepam	4	13.5 ± 1.1^{c}	119.1 ± 6.72^{c}
Gr III	CETI	250	4.5 ± 0.76	37.3 ± 1.08
Gr IV		500	4 ± 0.73	38 ± 2.7
Gr V	METI	250	6 ± 0.96^{b}	51 ± 4.28^{b}
Gr VI		500	10.5 ± 0.76^{c}	68 ± 10.25^{c}
Gr VII	AQTI	250	6.6 ± 0.66	39.3 ± 3.49
Gr VIII		500	11.16 ± 0.60^{c}	52 ± 4.44^{c}

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at ^{a}p<0.05, ^{b}p<0.01 and ^{c}p<0.001 respectively, in comparison to group-I).

Table 8: Effect of fractions and extracts of *T indica Linn* on Pentylenetetrazole (PTZ)-induced seizure in mice.

Group	Treatment	Dose (mg/kg)	Onset of convulsion (sec.)	Duration of convulsion (Sec.)
Gr I	NS + Tween	10 ml/kg	113.6 ± 5.35	124.3 ± 5.2
Gr II	Diazepam	4	335.8 ± 11.13[c]	630.3 ± 10.4[c]
Gr III	CETI	250	104.6 ± 5.69	135.6 ± 8.9
Gr IV		500	132.6 ± 7.35	163.3 ± 7.18
Gr V	METI	250	156.8 ± 3.56[b]	179.8 ± 8.74[b]
Gr VI		500	206.6 ± 6.8[c]	267.6 ± 12.2[c]
Gr VII	AQTI	250	148.5 ± 6.76[a]	155.8 ± 8.75[a]
Gr VIII		500	205.5 ± 13.81[c]	237.1 ± 8.83[c]

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to group-I).

Table 9: Effect of fractions and extracts of *T indica Linn* on MES induced convulsion in rats.

Group	Treatment	Dose (mg/kg)	Time in various phases of convulsion (sec)				
			Flexion	Extensor	Clonus	Stupor	R/D
Gr I	Solvent	10 ml/kg	5.3 ± 0.80	12.6 ± 0.76	6.3 ± 1.14	192.6 ± 4.5	R
Gr II	Phenytoin	25	3.6 ± 0.66[b]	3.3 ± 0.53	4.4 ± 0.59	83.3 ± 3.94[c]	R
Gr III	CETI	250	5.1 ± 0.47	11.3 ± 0.76[c]	5.3 ± 0.71	184.6 ± 6.9	R
Gr IV		500	5 ± 0.57	10.6 ± 0.80	5 ± 0.73	169.6 ± 11	R
Gr V	METI	250	4.2 ± 0.39	9.06 ± 0.59[b]	5.5 ± 0.88	122.3 ± 6.2[c]	R
Gr VI		500	3.5 ± 0.28[a]	6.4 ± 0.49[c]	4.6 ± 0.56	108.5 ± 7.26[c]	R
Gr VII	AQTI	250	4 ± 0.32	9.5 ± 0.45b	5.1 ± 0.4	132.6 ± 6.4[c]	R
Gr VIII		500	3.5 ± 0.43[a]	6.7 ± 0.62[c]	4.5 ± 0.42	112 ± 5.24[c]	R

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at [a]p<0.05, [b]p<0.01 and [c]p<0.001 respectively, in comparison to group-I).

Table 10: Effect of extract/ fractions of *T indica Linn* on Picrotoxin-induced seizure in rats.

Group	Treatment	Dose (mg/kg)	Onset of convulsion (sec)	Time of death (sec)
Gr I	Solvent	10 ml/kg	131.6 ± 5.82	1260.6 ± 16.03
Gr II	Phenytoin	25	1506.3 ± 9.68c	Recovery
Gr III	CETI	250	133.6 ± 8.56	1136 ± 29.75
Gr IV		500	155.8 ± 5.52	1280 ± 24.08
Gr V	METI	250	161 ± 10.15a	1287.1 ± 16.53
Gr VI		500	277 ± 8.13c	1394.3 ± 24.84c
Gr VII	AQTI	250	150.6 ± 8.26	1302.6 ± 21.6
Gr VIII		500	250.8 ± 7.3c	1387.3 ± 18.4b

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test. (F-value denotes statistical significance at *p<0.05, **p<0.01) (t-value denotes statistical significance at ap<0.05, bp<0.01 and cp<0.001 respectively, in comparison to group-I).

5. DISCUSSION

The ability of the crude methanol and aqueous extract at 500 mg/kg, reduce the mean onset of sleep and increase the duration of sleep indicate that it potentiates Phenobarbital sodium-induced sleep in mice. The potentiation of phenobarbitone sodium induced sleeping time is possibly through a CNS depressant action or a tranquilizing action.[20] Sedative-hypnotic agents act to increase GABA mediated synaptic inhibition either by directly activating GABA receptors or, more usually, by enhancing the action of GABA on GABA-A receptors.[21, 22] The ability of the extract to potentiate the sedative property of pentobarbital sodium suggests that it may possibly act by affecting GABA-mediated synaptic transmission. The inability of the crude methanol and aqueous extract (500 mg/kg) increase the number of foot slips suggests that it does not induce significant motor coordination deficit, and by implication, its depressant action is centrally and not peripherally mediated.[23,24] The hole-board test measures the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety and response to stress and a decrease in the number of head dips is reported to be a measure of CNS depressant activity.[25] As the tested extracts in hole-board test showed less number of head dip, hence it is suggested that the extracts have CNS depressant activity. The decrease in exploratory behaviour by the methanol and aqueous extract further supports its sedative potential. The elevated plus maze is a commonly used test in the search of anxiolytic agents in which the rodent typically avoids the open arms of the maze due to fear or anxiety induced by the open space.[26] However, the experimental protocol adopted was validated by the use of a standard anxiolytic agent, diazepam which

significantly increased both the number of open arm entries and the total time spent in the open arm.[23] The study showed that crude methanol and aqueous extracts (500 mg/kg) decrease the frequency and the amplitude of movements. The reduction of the locomotor activity of the tested could be attributed to the sedative effect of the extract/fractions that may be due to some GABA-ergic effect.

In MES induced convulsion animals are represent grandmal type of epilepsy. It has often been suggested stated that antiepileptic drugs that block MES induced tonic extension phase act by blocking seizure spread. The crude methanolic extract/fractions showed anticonvulsant activity against MES induced convulsion, it abolish tonic extension phase which might be attributed either by inhibiting voltage dependent Na+ channels or act as a NMDA antagonist.

The crude methanolic extract / fractions exhibited anticonvulsant activity as a result of increased time taken for onset of convulsion and tonic convulsion induced by PTZ. The anticonvulsant effect of the extract against PTZ induced convulsion might be due to GABAA agonist. Picrotoxin is a noncompetitive antagonist at GABAA receptors and it blocks the GABA activated chloride ionophore.[27,28] However, the extract was counteracting the action of picrotoxin, that effect might be the extract modified the function of GABAA receptor mediated chloride channel. In the present study the tested extract/fractions in all tested models are in support of CNS depressant activity and demonstrate significant anti-convulsant property in experimental animal models. Alkaloids, saponins and flavonoids have been variously reported to possess sedative activities and since similar phytoconstituents are also present in the tested extract/fractions of *T indica* Linn which may be responsible singly or in combination for the observed activity.

6. CONCLUSION

In conclusion, our result showed that the possible CNS activity of crude methanolic extract/fractions of leaves of *T indica* Linn have CNS depressant activity which contributes towards suppression of convulsion in different animal experimental models.

Conflict of interest statement

We declare that we have no conflict of interest.

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