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Sedative, Anxiolytic and Anticonvulsant effects of different extracts from the leaves of *Ipomoea Carnea* in experimental Animals

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Abstract
The objective of this study is to investigate the sedative, anxiolytic and anticonvulsant activities of different leaf extracts of *Ipomoea carnea*.
Materials and methods: The sedative effect of the different leaf extracts at dose level 100, 200 and 400 mg/kg was evaluated in mice and rats using phenobarbitone induced sleeping time and hole board models. Its anxiolytic effect was evaluated using the Evaluated Plus Maze (EPM) and the Y maze (YM) methods. The anticonvulsant activity was evaluated in mice and rats using the strychnine, picrotoxin and MES-induced seizure models. The acute toxicity studies and phytochemical analysis of the extract were also carried out.
Results: The methanolic and aqueous extracts) produced significant ($P < 0.01$) reduction in the time of onset of sleep induced by phenobarbitone. The prolongation of phenobarbitone sleeping time by the extract (200 mg/kg) was comparable to that produced by diazepam (4 mg/kg). At doses of 100–400 mg/kg, the extract produced a dose dependent decrease in exploratory activity of the mice. The reduction in exploratory activity produced by the extract (400 mg/kg) was greater than that of chlorpromazine. The results obtained from the experiments indicate that the extract has central nervous system depressant and anxiolytic activities. In anxiolytic effect the extracts showed dose dependent prolongation of the cumulative time spent in the open arms of the elevated plus maze and Y maze compared with the control. It also produced a significant ($P < 0.01$) dose dependent increase in onset of convulsion compared to the control for strychnine, picrotoxin and MES -induced seizures. The LD50 obtained for the acute toxicity studies using oral route of administration was 3000 mg/kg respectively.
Conclusion: These findings justify the use of *Ipomoea carnea* in traditional medicine for the management of convulsion and psychosis.

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Key words:
Sedative, anxiolytic, epilepsy, *I.Carnea,* Phenobarbitone, PTZ, MES

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INTRODUCTION

Insomnia defined as persistent difficulty in falling or staying asleep that affects daytime function can induce significant psychological and physical disorder. According to clinic questionnaire, around 44% patients treated insomnia with the long-term use of benzodiazepines analogues. But certain drugs

in this class have limited benefits which shorten slow wave sleep (SWS) and rapid-eye-movement sleep (REM sleep) resulting in producing residual sedative effects, such as impaired cognitive function, memory and general daytime performance [1] . Anxiety disorder is increasingly recognized as a highly prevalent and a chronic disorder with onset during the teenage years, with an incidence of 18.1% and in lifetime prevalence of 28.8% [2] . The disorder is associated with significant disability (including educational and occupational) which has a negative impact on the quality of life [3]. Pharmacotherapeutic approaches for the management of anxiety disorders include Psychotropic drugs, but these agents are limited by their side effects profile, the need for dietary precautions, and drug interactions [4]. Regular use of benzodiazepines cuses deterioration of cognitive functioning, addiction, psychomotor impairment, confusion, aggression, excitement, anterograde amnesis, physical dependence and tolerance [5]. Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behaviour due to the disordered, synchronous and rhythmic firing of populations of brain neurons [6]. It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients [7, 8]. The conventional antiepileptic agents like phenytoin, carbamazeipine and sodium valporate carry with them several serious side effects notably neurotoxicity [9]. As majority of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. However, newer antiepileptics like gabapentin, vigabatrin, lamotrigine etc are used supplemental to the conventional agents. These are some of the factors that caused interest in many researcher to evaluate a new compounds from plant origin in the hope to identifying other anxiolytic drugs with fewer unwanted side effects. On the other hand, recent studies have shown herbal drugs exert good hypnotic and sedative effects with few side effects, Medicinal plants have been used to treat many psychotropic and behavioral conditions, such as anxiety, depression, seizures, poor memory, dementia, insomnia, and drug intoxication. Herbal therapy may well provide an alternative treatment for some psychiatric conditions. *Ipomoea carnea* (Fam. Convolvulaceae) is a plant of tropical American origin but is now widely distributed in the tropical regions of the world. This plant is erect, densely leafed, and almost unbranched, growing as shrubs to 2-3 m high [10] . Natural intoxication occurs in livestock that chronically ingest the plant, and the early poisoning reports were from Sudan and India [11, 12]. The toxicity has been confirmed in feeding experiments with goats and sheep [13, 14]. The nortropane alkaloids calystegines B2 (4) and C1 (6), together with swainsonine (1) have been detected in the leaves collected in Mozambique where goats were intoxicated [14]. Calystegines B2 and C1 are potent inhibitors of glycosidases [15]. Hence, whether the poisoning by this plant is due to a single effect of swainsonine or a combination of toxic effects by swainsonine and calystegines is a very important problem [16] . The preliminary phytochemical screening of different extracts were carried out presence of various phytoconstituents such as Alkaloids,Carbohydrates, Glycosides, Flavonoids, Tannins and Phenolic compound.It is used to have stimulatory allelopathic effect [17]. Roots are boiled to use as laxative and to provoke menstruation.The milky juice of plant has been used for the treatment of Lecucoderma and other related skin diseases. It has depressant effect on central nervous system [18]. Also shows muscle relaxant property [19]. The juice is collected and applied externally on affected parts, anti-inflammatory [20].The main objective of the study is to ascertain the central pharmacological actions of the different extract from the leaves of *I. Carnea*.

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh plant of *Ipomoea Carnea* was collected from the rural belt of Anandapur, Orissa, during Feb-2010. It was cleaned and dried at room temperature in shade and away from direct sunlight. The plant was authenticated in the Department of Biosciences, Sardar Patel University, Anand, Gujarat. The plant was collected in bulk and washed with tap water to remove adhering soil and dirt particles and then shade dried. A voucher specimen was deposited at the school of pharmaceutical science, SOA University, Bhubaneswar. 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), alcohol and water as solvent for 48 h each. The three extracts petroleum ether (PEE), alcohol (ALE) and aqueous (AQE) so obtained were dried using rota-evaporator and used for further studies. The extract was suspended in distilled water, using Tween 40, as suspending agent and administered to the animals in appropriate dose levels by oral route of administration.

Animals

Swiss albino mice and wistar albino rats of either sex weighing between 25-35g and 150-220g respectively obtained from the Animal house of School of Pharmaceutical Sciences, Bhubaneswar, Odisha were used. All animals were housed in groups of six under standard environmental conditions: 25º ± 2º C, 45-55% Relative Humidity and 12:12 hr light/dark cycle [21, 22]. All the animals were free access of food and water *ad libitum* under strict hygienic conditions. After obtaining permission from Institutional Animal Ethics Committee (IAEC) of School of Pharmaceutical Sciences, Bhubaneswar, animal studies were performed as per rules and regulations and in accordance to the guidelines of CPCSEA. All experiments were carried out during the light period (09.00:18.00 hours).

Phytochemical characterization

The different extracts were subjected to general phytochemical analysis for presence of carbohydrates, proteins, amino acids, tannins, phenolics, flavonoids, alkaloids, anthraquinones, glycosides, saponin and steroidal nucleus using the standard methods [23, 24].

Drugs and Chemicals

Chlorpromazine hydrochloride (Indus Pharmaceuticals Limited, India), diazepam (Ranbaxy Laboratories Ltd. India), Phenobarbitone sodium (Rhone-Poulenc India Limited, India), Diethyl ether and all other chemicals of highest available purity were obtained from Merck, Mumbai, India.

Acute toxicity studies (LD_{50})

The acute oral toxicity study was carried out as per guidelines set by Organisation for Economic Cooperation and Development (OECD) [25, 26]. The median lethal dose of the pet-ether alcohol and aqueous were determined by orally administering the extracts in increasing dose levels of 0.1, 0.2, 0.5, 1, 1.5 and 2 g/kg body weight to healthy adult albino mice of either sex. The animals will be observed continuously for 2 h under the following profiles:

I. Behavioural profile: Alertness, restlessness, irritability and fearfulness.
II. Neurological profile: Spontaneous activity, reactivity, touches response, pain response and gait.
III. Autonomic profile: Defecation and urination.

After a period of 24 h they will be observed for any lethality or death (% of mortality).

Animal grouping and treatment

For the following activities the animals divided into eleven groups, each group containing six animals except general behavioural study. Group I for control, group II for standard, group III, IV and V for PEE (100,200 and 400 mg/kg), group VI, VII and VIII for ALE (100,200 and 400 mg/kg), group IX, X and XI for AQE (100,200 and 400 mg/kg) respectively.

General behavioural profile

Evaluation of general behaviour profiles was performed by the method of Dixit and Verma et al (1976) [27], in this method albino male mice were divided into eleven groups (n=6). The PEE, ALE and AQE extract each (100,200 and 400 mg/kg,) were administered to groups III -XI, where as Groups I and II were treated with solvent (10ml/kg) and diazepam (4 mg/kg) and respectively in a similar manner. The animals were under observation for their behavioural changes, if any, at 30 min intervals in the first one hour and at 1hr intervals for the next 4hr for the following parameters.

Awareness, alertness and spontaneous activity

The awareness and alertness was recorded by visual measure of the animals' response when placed in a different position and its ability to orient itself without bumps or falls The normal behaviour at resting position was scored as (-), little activity (+), moderate flexibility (+ +), strong response (+ + +) and abnormal restlessness as (+ + + +).These responses were tested by placing the animal in a ball jar. It usually shows a moderate degree of inquisitive behaviour.

Spontaneous Activity-Moderate activity was scores as (+ +) and strong activity as (+ + +). If there is little motion, the score was (+), while if the animal sleeps, the score was (–). Excessive or very strong inquisitive activity like constant walking or running was scores as (+ + + +). A similar test was performed with the same scoring, when the animals are removed from the jar and placed on a table [28].

Touch response- This was noted when the mice were touched with a pencil or forceps (i.e. on the side of the neck, abdomen and groin) [29].

Sound response-Albino mice normally utter no sound; vocalization may indicate a noxious stimulus.

Pain response-This was graded when a small artery clamp was attached to the base of the tail.

Assessment of Phenobarbitone sleeping time

Healthy albino mice weighing between 20-30 gms were fasted for 24 hrs before the experiment and were divided into eleven groups of 6 animals each. Group I was maintained as normal control which was given solvent (10 ml/kg, P.O.), group II was treated with standard drug chlorpromazine (CPZ) (5 mg/kg, P.O.) , group III, IV and V were treated with different doses of ALE (100,200 and 400 mg/kg, P.O.), group VI, VII and VIII were treated with AQE (100,200 and 400 mg/kg, P.O.) and group IX, X, XI were treated with PEE (100,200 and 400 mg/kg, P.O.) in a similar manner. After 30minutes of administration of test drugs, Phenobarbitone sodium was administered intra-peritoneal to all groups of animals at a dose of 40mg/kg. The time between the loss and recovery of the righting reflex was taken as the sleeping time [30].

Assessment of locomotor activity:

The spontaneous locomotor activity of each mouse was recorded individually for 10 min using actophotometer, which enables movement of the animal across a light beam to be recorded as a locomotion count. This test can demonstrate a CNS depressant or stimulant activity profile. The animals were allowed to adapt to the new environment for at least 5 min and then the locomotor activity was counted. The plant extract (100,200 and 400 mg/kg, P.O.) or the standard drug Diazepam (4 mg/kg P.O.) was administered 30 min before the assessment of locomotor activity. Counts were then taken after 30, 60, 90 and 120 min [31, 32].

Assessment of Exploratory behaviour (Head dip test & Y-maze test):

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

Head dip test

Eleven groups of albino mice (n=6) were placed on the top of a wooden box with 16evenly spaced holes, 30 min after received of the extract (100,200 and 400 mg/kg P.O., vehicle (5ml/kg,) and diazepam (4mg/kg) respectively. The number of times that each animal dipped the head into the hole was counted for a period of 3 min [33].

Y-maze test-:

The test was performed in different groups of 6 albino rats at 30, 60, 90 and 120 min after treatment of either Normal saline (10ml/kg), extracts (100,200 and 400 mg/kg) and diazepam(10 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 [34].

Assessment of skeletal muscle relaxant activity (Motor Coordination):

Motor coordination of the mice was evaluated by using a rotarod apparatus consisting of a bar with a diameter of 3 .0 cm, subdivided into five compartments by a disk 24 cm in diameter. Mice were placed on a horizontal steel rod (32mm diameter) rotating at the speed of 25 rpm. The mice capable of remaining on the top for 3 min or more, in three successive trails were selected for the study. The selected animals were divided into eleven groups (n=6). Groups were received the extracts at 50, 100 and 200mg/kg, orally, solvent (10ml/kg) and diazepam (4 mg/kg) respectively in a similar manner as above. Each group of animals was then placed on the rod at an interval of 30, 60, 90 and 120 min. The difference in the fall off time from the rotating rod between the control and the treated mice (standard-Diazepam/extract) was taken as an index of muscle relaxation [35, 36].

Assessment of Anticonvulsant activity (MES and PTZ induced Convulsion)

(i) Maximal electroshock (MES) induced convulsion

Protection against electroshock induced seizures in mice or rats is used as an indication for compounds which may prove effective in 'grand mal epilepsy'. Electric stimuli evoke tonic hind limb extensions, which are suppressed by anti-epileptic drugs. The animals were given maximal electroshocks of 150 mA for 0.2 seconds to the cornea by using electroconvulsometer [8, 9]. fifty healthy and convulsion free Swiss albino rats (120-150 gm) were randomly divided into 10 groups (n = 5). The plant aqueous extract and it's fractions at the dose of 100 and 200 mg/kg, standard drug phenytoin and vehicle control were administered 30 min prior to MES. The vehicle treated animals exhibit the characteristic maximal electroshock convulsions which can be divided into 5 phases- (a) tonic flexion, (b) tonic extensor, (c) clonic convulsions, (d) stupor and (e) recovery or death. The animals are observed for 2 min after the shock. Disappearance of the hind limb extensor tonic convulsions is taken as the criterion of protection [37].

(ii) Pentylenetetrazole (PTZ)-induced seizures

This test is considered as indicative of anticonvulsant activity of drugs against 'petit mal seizures'. PTZ produces generalized asynchronized clonic movements which are superceded by tonic convulsion characterized by flexion of limbs followed by extension. The excitatory effects of PTZ may be due to decrease in neuronal recovery time in the postsynaptic pathway of the spinal cord [38,39]. The plant aqueous extract and it's fractions at the dose of 100 and 200 mg/kg, standard drug phenobarbitone sodium and vehicle control were administered 30 min prior to PTZ (80 mg/kg). The onset and number of death after showing tonic hindlimb extension were also recorded. Mice that did not convulse 30 min after pentylenetetrazole administration were considered protected [40].

Statistical analysis

Results are presented as mean ± S.E.M. Statistical significance between the groups was analyzed by means of an analysis of variance followed by Dennett's multiple comparison tests. *P* values less than 0.05 were considered significant.

RESULTS

Phytochemical Screening

The preliminary phytochemical analysis of the leaf extracts of *I.arnea* showed the presence of carbohydrates, phenols, saponins, tannins and alkaloids but devoid of steroids. All the extracts were stored in a clean glass bottles for further pharmacological studies.

Toxicity study

A preliminary acute oral toxicity study, the plant extracts has not produced death up to dose level 2000 mg/kg and did not cause any toxic effect up to dose of 3000 mg/kg in mice.

Effect on behavioural profiles

The results obtained from the experiments are presented in (Table 1). The extracts affected spontaneous activity, sound and touch responses at a dose of 200 and 400mg/kg and 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 ethanol extract. The results indicate that the extract influences general behavioural profiles, as evidence in the spontaneous activity, touch, sound and pain responses.

Effect on phenobarbitone sodium-induced sleeping time

The test extracts except 100 mg/kg, significantly potentiated the Phenobarbitone sodium-induced sleeping time, with respect to the control (Table 2). The duration of sleeping time in phenobarbitone induced experimental model increases in a dose dependent manner. The Potentiation of phenobarbitone sodium-induced sleeping time is possibly through a CNS depressant action or a tranquilizing action.

Effect on Locomotor activity on mice

A significant decrease in locomotor activity was observed in case of petroleum ether, aqueous and ethanolic extracts at different doses High dose of ALE, PEE and AQE extracts (200 and 400 mg/kg p.o) and diazepam (4 mg/kg P.O.) decreased the locomotor activity significantly ($P<0.01$) whereas, low dose of (100 mg/kg P.O.) did not show a significant reduction in the locomotor activity (Table 3).

Effect of extracts on exploratory behavioural potential:

Head dip test

The head dip test revealed that ALE and AQE at 200 and 400 mg/kg dose level significantly reduced the number of head dips on a wooden board with 16 evenly spaced holes within 3 minutes, while standard drug diazepam also showed a significant reduction in the head dips responses occurred in mice treated with the extracts compared with the control (Table 4).

Y-Maze Model

A significant decrease in the number of visits in the arms of the Y-maze was observed in the Diazepam treated animals as compared to the control animals. Both the doses (100 and 200 mg/kg of ALE and AQE) of *I.carnea* showed a significant decrease in the number of visits in the arms of the Y-maze which was comparable with the standard Diazepam (Table 5).

Effect on Motor co-ordination on mice:

The result from the rotarod test showed (Table 6) that the extracts significantly reduced the motor co-ordination of the tested animals. This test,ALE and AQE (200 and 400 mg/kg, P.O.) significantly reduced the time spent by the animals on revolving rod when compared to control ($P<0.01$). The standard drug (diazepam) also showed significant effect when compared to control ($P<0.01$) Low dose of drug (100 mg/kg) did not show any significant effect.

Effect on MES induced Convulsion

The result of MES induced convulsion depicted in (Table 7). The duration of extensor phase was recorded in control and drug treated animals before the electroshock. A significant ($P<0.001$) reduction in the extensor phase and a significant reduction in stupor phase was observed with Phenytoin when compared with control. In the test, the ALE and AQE at the dose of 200 and 400 mg/kg significantly ($p<0.01$) failure in extensor phase**,** when compared with the solvent control group and the result is comparable to that produced by phenytoin. In case of stupor phase, similarly the the dose of 400 mg/kg 99% significantly ($p<0.01$) decrease**,** when compared

to the solvent control group. There was no death observed for all the fractions and the test drug.

PTZ induced convulsion

The result of PTZ induced convulsion depicted in (Table 8) the test extract with standard drug delayed the onset of time and increased the duration of convulsion except PEE at the dose of 200 and 400 mg/kg. The ALE and AQE at the dose of 200 mg/kg significantly ($p<0.05$) delayed the onset of convulsion and the dose of 200 mg/kg 95% significantly ($p<0.05$) increased the duration of convulsion except PEE against PTZ induced convulsion when compared to the solvent control group, the effect also comparable with those result produced by standard drug.

DISCUSSION

In the present study different extracts (PEE, ALE, AQE) of *I.carnea* leave were studied for CNS activity using several animal models such as locomotor activity, muscle relaxant activity, exploratory behaviour and Phenobarbital induced sleeping time. The preliminary phytochemical analysis of the extracts of *I.carnea*, revealed the presence of carbohydrates, tannins, flavonoids and saponin. The results indicate that, the ALE, PEE and AQE influences general behavioural profiles, as evidenced by decrease in the alertness, reactivity to touch and auditory stimuli indicative of depressant profile. The PEE, ALE and AQE significantly dose-independently reduced the onset and prolonged the duration of sleep induced by phenobarbitone. By potentiating the phenobarbitone -induced sleep, the extracts seems to possess sleep inducing properties [41].potentiated the phenobarbitone induced sleeping time in a dose dependent manner, possibly through CNS depressant action or tranquilizing action [42, 43] . Locomotor activity is considered as an index of alertness and a decrease in it is indicative of sedative activity [44] . *I.carnea* extracts decreased locomotor activity at all the tested doses reinforcing the CNS depressant effect. Sedative hypnotic agents act to increase gamma amino butyric acid (GABA) mediated synaptic inhibition either by directly activating GABA receptors or, more usually, by enhancing the action of GABA on GABAA receptors. Benzodiazepines and barbiturates are examples of widely used therapeutic agents that act as positive allosteric modulators at GABAA receptors [45] .The ability of the extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission. The reduction in exploratory behavioural study pertaining to head dip revealed the CNS activity of the test extracts. The possible CNS activity of the ethanolic, aqueous and petroleum ether extract was further tested against other common psychological tests (i.e. the rotarod test). A significant lack in motor coordination and muscle relaxant activity were noted in animals treated with the test extracts. The results of the present studies reveal that, the ALE, AQE and PEE may have CNS depressant activity like psychopharmacological agents. The drug(s) showing positive response in gross behavioural parameters, muscle in-coordination, exploratory behaviour must have effect on the region of brain [46]. The effectiveness of the extracts in the experimental convulsion paradigm used probably suggests that the herb could be used in both the petitmal and grand mal types of epilepsy. Pentyltetrazole (PTZ) destabilizes nervous cell membrane to produce convulsion [47]. GABA is the predominant inhibitory neurotransmitter in the mammalian CNS, and is widely implicated in epilepsy, mediating inhibition of neuronal responsiveness (excitability) and activity by increasing the chloride ion conductance through opening of the chloride-ion channel [48]. It may be suggested that the extracts have effect on the region of brain, which is responsible for the behavioural and other tested parameters.

CONCLUSION

Based on the results of the present study of different extracts on psychopharmacological tests, we

conclude that the extracts at 200 and 400mg/kg possess strong CNS depressant activity. Aqueous and alcoholic extracts showed the CNS depressant activity in dose dependent manner. The reduction in exploratory behaviour in animals is similar with the action of other CNS depressant agents. A significant lack in motor coordination and muscle relaxant activity was also noted in animals treated with crude extract. In small to moderate doses(200-400mg/kg) enhances the anticonvulsant effects in different animal models. This effect may be attributed to different mechanisms including the brain glutamate and increasing brain GABA levels The results altogether indicates that the extract shows CNS depressant activity. However, further studies are necessary to examine underlying mechanisms of CNS depressant effects of different fractions of the potent extract and to isolate the active compound (s) responsible for these pharmacological activities.

Table 1: Effect of extracts of *I.Carnea* on general behavioural profiles mice

Group No	Treatment	Dose (mg/kg)	Spontaneous activity	Alertness	Awareness	Sound response	Touch response	Pain response
I	Saline	10 (mg/kg)	-	-	-	-	-	-
II	CPZ	5 (mg/kg)	++++	++	+	+++	+++	++++
III	PEE	100 (mg/kg)	+	++	+	+	-	-
IV	PEE	200 (mg/kg)	+	++	+	+	-	+
V	PEE	400 (mg/kg)	+	++	+	+	-	+
VI	ALE	100 (mg/kg)	+	+	+	+	+	+
VII	ALE	200 (mg/kg)	+++	++	++	+++	++	+
VIII	ALE	400 (mg/kg)	++++	++	++	+++	+++	+++
V	AQE	100 (mg/kg)	+	+	+	+	+	+
VI	AQE	200 (mg/kg)	+++	++	++	++	++	++
VI	AQE	400 (mg/kg)	+++	++	++	+++	+++	+++

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

Table 2: Effect of various extracts of *I.Carnea* at different doses on Phenobarbitone sodium induced sleeping time

Groups	Treatment	Dose(mg/kg P.O. route)	Sleeping time mean
I	Normal Saline	10ml	30 ± 0.58
II	Chlorpromazine (CPZ)	5	62.36 ± 2.8**
III	PEE	100	31.16 ± 1.7
IV	PEE	200	36.6 ± 2.8
V	PEE	400	43.8 ± 2.67*
VI	ALE	100	29.4 ± 1.68
VII	ALE	200	42.8 ± 1.3**
VIII	ALE	400	58.54 ± 2.4**
IX	AQE	100	30.6 ±2.87
X	AQE	200	43.3 ± 2.9**
XI	AQE	400	56.6 ± 3.4**

One way ANOVA Followed by Dunnet's Test values are mean±SEM n=6 in each group *P<0.05 and **P<0.01 when compared to control.

Table 3: Effect of different extracts of *I.Carnea* on locomotor activity of mice

Groups	Treatment	Dose (mg/kg,P.O.)	Locomotor activity observed for 10 min	
			Before	After
I	N.S.	10ml	219 ± 8.42	222.3 ± 6.82
II	Diazepam	4	222.6 ± 11.25	135.16 ± 7.88**
III	PEE	100	207.66 ± 6.77	207.16 ± 6.98
IV	PEE	200	211.6 ± 6.2	209.5 ± 9.95
V	PEE	400	211 ± 8.48	154.1 ± 17.64*
VI	ALE	100	217.6 ± 9.01	211.6 ± 9.58
VII	ALE	200	214.5 ± 7.48	155.3 ± 9.81**
VIII	ALE	400	212.1 ± 7.83	111.5 ± 5.18**
IX	AQE	100	216.5 ± 8.67	215 ± 9.64
X	AQE	200	224.6 ± 6.06	208.1 ± 7.67*
XI	AQE	400	210.5 ± 5.09	170.5 ± 8.9**

n=6. Data were analysed by one-way ANOVA followed by Dunnet's Test . *P<0.05 and **P<0.01 when compared to control.

Table 4: Effect of different extracts of *I.Carnea* on exploratory behaviour (Head dip test) in mice.

Groups	Treatment	Dose(mg/kg P.O.)	Head dip test
I	Vehicle (N.S.)	10ml/kg	99.83 ± 5.57
II	CPZ	5	30 ± 2.3**
III	PEE	100	97.33 ± 2.91
IV	PEE	200	93 ± 6.21
V	PEE	400	87.66 ± 3.62*
VI	ALE	100	94.66 ± 5.2
VII	ALE	200	37 ± 4.21**
VIII	ALE	400	24.33 ± 3.04**
IX	AQE	100	95.83 ± 4.99
X	AQE	200	84.66 ± 4.19*
XI	AQE	400	29.16 ± 2.48**

One way ANOVA Followed by Dunnet's Test values are mean±SEM, n=6 in each group
*P<0.05 and **P<0.01 when compared to control.

Table 5: Effect of *I.Carnea* on exploratory behaviour (Y-maze test) in rats

Groups	Treatment	Dose (mg/kg)	Number of entries after treatment (Min)			
			30	60	90	120
I	N.S.	10ml	10.33±1.08	11±1.15	10±1.06	10.83±1.24
II	Diazepam	4	7.5±0.74*	5±0.63**	4.16±0.6**	3.5±0.99**
III	PEE	100	12.33±1.05	11.83±1.3	9.3±0.8	10.66±1.11
IV	PEE	200	9.66±1.2	9.5±0.99	9.33±0.88	9±0.81
V	PEE	400	5.5±1.08**	5.33±0.66**	4.66±0.76**	3.83±0.91**
VI	ALE	100	10±1.06	9.5±1.17	11.5±1.25	11.33±1.4
VII	ALE	200	6.5±0.92**	5.66±0.55**	4.16±0.6**	4.16±0.79**
VIII	ALE	400	4.83±1.07**	4.16±0.7**	3.66±0.49**	3.5±0.92**
IX	AQE	100	9.33±0.95	9.83±0.79	10±1.06	9±0.87
X	AQE	200	9±0.73	8.5±0.76*	8.16±0.6*	8.33±1.08*
XI	AQE	400	5.33±1.2**	4.66±0.66**	5.5±0.95**	4.6±0.88**

One way ANOVA Followed by Dunnet's Test values are mean±SEM, n=6 in each group
*P<0.05 and **P<0.01 when compared to control.

Table 6: Effect of different extracts of *I.Carnea* on motor coordination in mice

Groups	Treatment	Dose(mg/kg P.O. route)	Fall of time (sec)			
			30min	60min	90min	120min
I	Vehicle (N.S.)	10ml/kg	317.3 ±7.5	306.6 ± 3.9	314.1 ± 4.1	311.6 ± 7.2
II	Diazepam	4	148.3 ± 4.7 **	143.6 ± 6.4**	87.83 ± 5.1**	61.33 ± 6.1**
III	PEE	100	318 ± 6.4	312 ±7.46	311 ±7.58	307 ± 5.4
IV	PEE	200	308 ± 8.2	314.3 ± 8.7	303.5 ± 3.8	297.3 ± 7.4
V	PEE	400	280.3 ± 5.1*	290.6 ± 3.2*	274.66 ± 4.6*	287.33 ± 5.6*
VI	ALE	100	310.8 ± 3.4	313.3 ± 5.6	311 ±7.4	310 ±9.3
VII	ALE	200	278 ± 6.7**	193.6 ± 7.5**	182 ± 8.1**	171.3 ± 7.2**
VIII	ALE	400	128 ± 6.2**	90.33 ± 4.1**	85 ± 2.9**	78 ± 2.1**
IX	AQE	100	316.3 ± 3.6	311.5 ± 2.3	313.3 ± 6.8	315.3 ± 4.5
X	AQE	200	296 ± 5.3*	277.6 ± 7.1*	270.3 ± 8.2*	257.5 ± 6.2**
XI	AQE	400	138.1 ± 8.9**	121.1 ± 9.8**	98.66 ± 7.1**	86.33 ±7.8**

One way ANOVA Followed by Dunnet's Test values are mean±SEM, n=6 in each group
*P<0.05 and **P<0.01 when compared to control.

Table 7: Effect of leaves of *I.Carnea* against MES induced convulsions

Sl. No.	Treatment		Time (Sec) in various phases of convulsions (MEAN±SEM)				
	Drug	Dose (mg/kg)	Flexon	extensor	Clonus	Stuper	Recovery/Death
1	Normal Saline	5 ml/kg	8.66 ± 0.8	14.83 ± 1.7	19.33 ± 2.6	107.3 ± 5.6	Recovery
2	Phenytoin	25	4.33 ± 1.1**	0	32.3 ± 4.1**	63.8 ± 5.9**	Recovery
3	PEE	100	13.66 ± 2.01	20.6 v 3.3	24 ± 4.2	120 ± 7.1	Recovery
		200	13 ± 2.3	21 ± 3.6	33.3 ± 4.3	104.5 ± 5.7	Recovery
		400	6.1 ± 1.2*	20.6 ± 2.2	20 ± 2.3	94.3 ± 4.8*	Recovery
4	ALE	100	16.6 ± 2.9	23 ± 3.3	31.5 ± 2.6	106.6 ± 4.8	Recovery
		200	5.3 ± 1.02**	12.66 ± 1.3	19.5 ± 1.3	75.3 ± 5.4**	Recovery
		400	5.1 ±1.06*	12.5 ± 1.4	27.3 ± 1.3**	67.5 ± 5.4**	Recovery
5	AQE	100	10.3 ± 1.05	23.16 ± 2.5	36.16 ± 2.7	113.8 ± 4.7	Recovery
		200	7 ± 1.92*	23 ± 1.86**	33.8 ± 2.3**	77.8 ± 4.6**	Recovery
		400	6 ± 1.82*	12.83 ± 1.3	24.5 ± 2.1*	73.6 ± 6.3**	Recovery

Values are expressed as MEAN±SEM one way Anova followed by Dunnets 't' test
Note: n=6 in each group, *P<0.05,**P<0.01,***P<0.001

Table 8: Effect of leaves of *I.Carnea* against PTZ induced convulsions

Sl. No	Treatment		Onset of clonus	Onset of tonic	% Protection
	Drug	Dose (mg/kg)			
1	Normal Saline	5 ml/kg	84.16±3.6	381.6±7.36	00
2	Diazepam	5	Ab	Ab	100
3	PEE	200	89.6 ± 4.9	383.3 ± 10.9	40
		400	104.3 ± 4.7**	310.6 ± 9.2**	60
4	ALE	200	119.3 ± 7.4**	306.6 ± 11.7	60
		400	149.3 ± 10.2***	401.6 ± 12.6**	100
5	AQE	200	88.66 ± 7.6	264.5 ± 14.5	40
		400	101.3 ± 4.4	333.5 ± 15.7	84

Values are expressed as MEAN±SEM one way Anova followed by Dunnets 't' test
Note: n=6 in each group, *P<0.05,**P<0.01,***P<0.001

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