

Exploration of Central Nervous System Activities of Caryota Urens ethanolic Leaf Extract on Neurological Disorders in Experimental Animal Models

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Abstract

Central nervous system plays a vital role in the physiological organization of the completely human body. In modern world, everyday people suffered by depression, anxiety, epilepsy and restlessness due to stress. According to WHO report, about 450 million people suffer from a mental or behavioural disorder. Use of herbal medicine has been increased due to its safety and plays vital role in CNS disorders. The urens is the principle phytoconstituent present in the Caryota urens, and others are flavonoids, phenolic compounds, amino acids, alkaloids, proteins and carbohydrate which are medicinally important and responsible for treating various illness including neurological disorders. The study was conducted with an aim, to assess the influence of Caryota urens leaf extract on CNS by evaluating its effect on various neurological disorders in experimental animal models. The dried leaves of Caryota urens was extracted by maceration process using ethanol as solvent. The CNS activities of dried ethanolic leaf extract of Caryota urens (200 and 400mg/kg) were evaluated using various models for Antidepressant activity, Anxiolytic activity, Locomotor activity, Muscle relaxant activity and Anti-convulsant activity. The ELECU dose dependently increase the number of head dips, which indicate, that the leaves of Caryota urens shows antidepressant activity. The elevated plus maze is considered to be an etiologically valid animal model of anxiety. The number of entries and time spent in the open arms have been found to be increased by anxiolytics and reduced by anxiogenic agents. The reference standard diazepam significantly increase both the number of entries and time spent in open arm. Similar effect was produced by the ELECU in the open arm that indicates the anxiolytic activity of ELECU. In the presence study we concluded that the ethanolic leaf extract of Caryota urens possess significant antidepressant, anxiolytic, locomotor, muscle relaxant and anticonvulsant activities in mice.

Keywords: Caryota urens, Ethanol Extract, Antidepressant activity and Anxiolytic activity.

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INTRODUCTION

In recent times the research deal with the effects of plants and their constituents on the Central Nervous System (CNS), relevant to behavioral aspects. It is not easy to know the baseline of human behavior without regular doses of plant extracts as tea, coffee, and chocolate and inhaled volatiles from tobacco, because these are an essential part of life for almost everyone in the developed world and as well in industrially developing communities. (1) Rational treatment of CNS disorders by plant constituents is in its childhood due to the complex chemistry, organization of the CNS, also to the complex between chemistry and pharmacology of a plant extract which may include an incomprehensible variety of chemical compounds. Ethno pharmacology provides clues of plants worthy of exploration which are used in traditional medicine, as poisons and in religious rituals. Earliest pharmacopoeias from various regions of the world comprise of herbal medicines that are supposed to have psychotropic potential; these propose a huge repository of prospective substances that can be developed into psychiatric pharmaceuticals. In fact, almost 25% of today's conventional drugs originated directly or indirectly from plants; several valuable psychoactive drugs, such as Yohimbine, Ephedrine and d-tubocurarine owe their origin to folklore medicines. At present a small number of plant-derived drugs are approved for clinical use. (2) This is mainly because nearly all herbal medicines are complex mixtures of chemical components and have different biological and pharmacological actions.

Considering the restrictions of the available conventional pharmacotherapeutic agents for psychiatric illnesses, high deterioration rates and various adverse side effects that happen through long-standing treatments, herbal remedies could offer a substitute for patients, particularly intended for individuals with enduring circumstances and intolerant to adverse effects. Actually, a number of clinical studies have established the beneficial effects of herbal remedies in the treatment of definite psychiatric conditions, most markedly depression, anxiety, insomnia, and dementia. *Caryota urens* L. is belongs to the family *Arecaceae* (*Palmaceae*). These are one of most useful flowering plants to after the grass mankind from time immemorial. The term *Caryota* from the Greek word *karyotes*, meaning "nutlike." *Urens* means "burning." *Caryota urens* (*Kitul Palm*) is also called as *Kundalpanai* or *Thippali* or *Choondapana* in south India. It is widely occurring across South Asia, i.e., India, Sri Lanka, Malaysia and Indonesia to Philippines from up to 2,000 MSL. Ayurveda recommends the use of *Caryota urens* for seminal weakness and urinary disorders, the juice is applied on the forehead for hemicranias. In traditional medicine porridge prepared from *Caryota urens* flower is used to treat malaria, gastric ulcer, migraine headaches, snake bite poisoning, as well as rheumatic swellings. (3) Palm heart is used locally as flour, especially for control of diabetes and in ayurvedic medicines. *Caryota urens* species are known as Sugar palm also, which is used in ancient medicine to treat

hemicranias and rheumatic swelling. Ancient medicine technologies recommend these flowers of the trees are used as a home remedy and improve the hair growth. The roots of the trees are used as the tooth ailments. The sap of fishtail palm is sweet in nature. So it is used to produce sugar which is known as jiggery. (4) So far there is no scientific evidence for its use in central nervous functions. Current study is aim to assess the influence of *Caryota urens* leaf extract on CNS by evaluating its effect on various neurological disorders in experimental animal models.

Materials and Methods

Plant Materials

Collection & Identification

The leaves of *Caryota urens* was collected from IRTT Perundururai Medical College Campus, Perundururai, Tamilnadu. Prof. R. Duraisamy, Pharmacognosist and voucher specimen (NCP/Phcog/2016/0202) has been retained, for future reference in the herbarium of Pharmacognosy department, Nandha College of Pharmacy, Erode, India and department of pharmacology, KIMS.

Extraction of Plant Material

The collected *Caryota urens* leaves were, shade dried and grounded using mechanical blender to get coarse powder. The 200gm of coarsely powdered leaves of *Caryota urens* was soaked in one litre of ethanol (90%) in a tightly sealed flat bottom flask at room temperature, protected from sun light for 72 hrs with occasional shaking. After 72 hrs the mixture was filtered through muslin cloth and the solvent was evaporated by rotary evaporator at 40°C to get dry mass. The dried ethanolic leaf extract of *Caryota urens* was stored in desiccators and used for further pharmacological studies.

Animals

The Swiss albino mice (18-22 g) of either sex were used for the study. The animals were obtained from king's institute, Guindy and was housed in central animal house, Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Kanchipuram. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted mice chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (1818/GO/Ere/S/15/CPCSEA) and were in accordance with the Institutional ethical guidelines.

Experimental Protocol

The animals are divided into four groups of six animals each. Group 1, control animals are administer orally with 10ml/kg

of 0.1% Carboxymethylcellulose (CMC) solution and group 2 are reference control animals, receives diazepam (4 mg/kg, i.p). Group 3 and 4 are treated with ethanolic leaf extract of Caryota urens (200 & 400mg/kg) respectively through oral route. All the test drugs are administer 30 minutes before the commencement of the study.

Antidepressant Activity

Hole-board Test:

The poking of the nose into a hole is the usual behavior of mice indicating the definite degree of curiosity. Twenty four Swiss albino mice were divided into four groups of six animals in each group. Group 1, control animals were administered orally with 10ml/kg of 0.1% Carboxymethylcellulose (CMC) solution and group 2 animals served as reference control, received diazepam (1 mg/kg, i.p). The animals of group 3 and 4 were treated with ethanolic leaf extract of Caryota urens (200 & 400mg/kg) respectively through oral route. After the test drug administration animals were placed in the center of the hole-board and allow freely to explore the equipment for 5 min. The number of heads dipping recorded by visual examination. Head dip scored if both eyes disappeared keen on the hole.(5)

Anxiolytic Activity

Elevated Plus Maze:

The elevated plus maze test is the most extensively used to access the anxiety that depend upon the study of spontaneous behavior. Twenty four Swiss albino mice were divided into four groups of six animals in each group. Group 1, control animals were administered orally with 10ml/kg of 0.1% Carboxymethylcellulose (CMC) solution and group 2 animals served as reference control, received diazepam (1 mg/kg, i.p). The animals of group 3 and 4 were treated with ethanolic leaf extract of Caryota urens (200 & 400mg/kg) respectively through oral route. After treatment with extract, the animals were individually placed in the centre of the elevated plus maze and observed the number of open and closed arm entries and time spent on open and closed arm by the mice.(6)

Locomotor Activity:

Actophotometer is used to assess CNS property and motor coordination. Twenty four Wistar albino rats were divided into four groups of six animals in each group. Group 1, control animals were administered orally with 1ml/kg of 0.1% Carboxymethylcellulose (CMC) solution and group 2 animals served as reference control, received diazepam (1 mg/kg, i.p). The animals of group 3 and 4 were treated with ethanolic leaf extract of Caryota urens (200 & 400mg/kg) respectively through oral route. After drug administration the animals were kept in actophotometer for 5 min. To prevent any turbulence in the reading of an animal, by the odor of the previous animal, ethanol 5% solution was used to wipe out the surface of activity cage after taking readings from each animal.(7)

Muscle Relaxant Activity

Rota-rod Method:

The test used to evaluate the activity of extracts interferes with motor coordination. Twenty four Swiss albino mice were divided into four groups of six animals in each group. Group 1, control animals were administered orally with 10ml/kg of 0.1% Carboxymethylcellulose (CMC) solution and group 2 animals served as reference control, received diazepam (1 mg/kg, i.p). The animals of group 3 and 4 were treated with ethanolic leaf extract of Caryota urens (200 & 400mg/kg) respectively through oral route. After drug administration the animals were individually placed in the rotating rod and the time taken for fall from the rotating rod is counted.(8)

Anticonvulsant Activity

Maximal Electro Shock Induced Convulsions:

The mice were considered a model of human absence epilepsy and myoclonic seizure.

Thirty two Swiss albino mice were divided into four groups of six animals in each group. Group 1, control animals were administered orally with 10ml/kg of 0.1% Carboxymethylcellulose (CMC) solution and group 2 animals served as reference control, received diazepam (1 mg/kg, i.p). The animals of group 3 and 4 were treated with ethanolic leaf extract of Caryota urens (200 & 400mg/kg) respectively through oral route. All the test drugs were administered 30 minutes before the commencement of the study.

The electroshock induced in animals through passing a current of 45 mA for 0.2 sec duration through electro convulsion meter (Techno, India) using ear electrodes. Hind limb tonic extension of mice was noticed. Mice that did not show Hind limb tonic extension within 5 mins were considered protected. The number of mice protected was determined in each dose group. The latency and duration of seizures were also determined.(9)

Statistical Analysis

Results are expressed as mean \pm SEM. The datas are analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's 't' test using GraphPad version 3. P values < 0.05 was considered as significant.

Results

Table 1. Effect of Ethanolic Leaf Extract of Caryota urens on number of head dips in Hole Board Test in mice

Groups	Drug Treatment	Number of Head Dips in 5 Minutes
I	Control 0.1% CMC (10ml/kg, p.o)	15.50 \pm 2.60
II	Diazepam (4mg/kg, i.p)	51.00 \pm 3.39***

III	ELECU (200mg/kg, p.o)	39.00±1.51**
IV	ELECU (400mg/kg, p.o)	48.50±1.34***

Values are in mean ± SEM (n=6),
*P<0.05 , **P<0.01, ***P<0.001 Vs Control

The effects of ethanolic leaf extract of *Caryota urens* at two dose levels of 200mg and 400mg/kg on Hole Board test were studied in mice and the results were given in table 1. The number of head dips observed with the ethanolic leaf extract of *Caryota urens* at 200mg and 400mg/kg were 39.00±1.51 and 48.50±1.34 respectively. The study showed that 400 mg of ethanolic leaf extract of *Caryota urens* produced more significant (P<0.001) increase in head dip responses whereas 200mg ethanolic leaf extract of *Caryota urens* showed moderately significant (P<0.01) increase in head dip response compared to control. The standard drug diazepam produced 51.00±3.39 head dips and showed significant (p<0.001) increase in head dip response compared to control. The high dose of ethanolic leaf extract of *Caryota urens* showed equipotent activity as that of Diazepam treated groups.

Table 2. Effect of Ethanolic Leaf Extract of *Caryota urens* on Locomotor Activity using Actophotometer in rats.

Groups	Drug Treatment	Activity Score in Actophotometer
I	Control 0.1% CMC (10ml/kg, p.o)	205.50±2.22
II	Diazepam (4mg/kg, i.p)	93.50±2.57***
III	ELECU (200mg/kg, p.o)	170.83±3.55*
IV	ELECU (400mg/kg, p.o)	155.67±2.60**

Values are in mean ± SEM (n=6),
*P<0.05 , **P<0.01, ***P<0.001 Vs Control

The locomotor activity of ethanolic leaf extract of *Caryota urens* was observed by placing the animals individually in actophotometer and the results were given in Table 2. The control animals showed 205.50±2.22 as activity score, where as the reference control diazepam showed 93.50±2.57, which significantly decreased (P<0.001) the locomotor activity. The ethanolic leaf extract of *Caryota urens* showed mild to moderate CNS depressant activity in dose dependent manner. The activity score of ethanolic leaf extract of *Caryota urens* at 200mg and 400mg was 170.83±3.55 and 155.67±2.60 respectively.

Table 3. Effect of Ethanolic Leaf Extract of *Caryota urens* on Elevated Plus Maze using mice

Groups	Drug Treatment	Number of Entries		Time Spent	
		Open Arm	Closed Arm	Open Arm	Closed Arm
I	Control 0.1% CMC (10ml/kg, p.o)	2.83±0.40	8.17±0.48	93.33±3.64	179.50±1.06
II	Diazepam (4mg/kg, i.p)	9.67±0.67***	4.00±0.77**	202.50±4.66***	68.83±1.56***
III	ELECU (200mg/kg, p.o)	7.33±0.49***	5.17±0.83*	158.33±4.66**	120.50±5.21*
IV	ELECU (400mg/kg, p.o)	8.50±0.62***	4.50±0.43**	190.33±6.26***	85.33±2.42***

Values are in mean ± SEM (n=6), *P<0.05 , **P<0.01, ***P<0.001 Vs Control

The effect of ethanolic leaf extract of *Caryota urens* was studied on Elevated plus maze and the results were given in table 3. In Control group animals, number of entries in open and closed arm was 2.83±0.40 and 8.17±0.48 seconds respectively and time spent in open arm and closed arm was 93.33±3.64 and 179.50±1.06 seconds. The animals treated with diazepam, it significantly increase (P<0.001) the number of entries in open and reduces (P<0.01) the number of entries in closed arm compared to control. It also significantly increases (P<0.001) the time spent in open arm and reduces (P<0.001) the time spent in closed arm compared to control. The animals treated with ethanolic leaf extract of *Caryota urens* at 200mg/kg, showed significantly increase (P<0.001) the number of entries in open and reduces (P<0.05) the number of entries in closed arm compared to control. It also significantly increases (P<0.01) the time spent in open arm and reduces (P<0.05) the time spent in closed arm compared to control. The higher dose of ethanolic leaf extract of *Caryota urens*, 400mg/kg produced, significantly increase (P<0.001) the number of entries in open and reduces (P<0.01) the number of entries in closed arm compared to control. It also significantly increases (P<0.001) the time spent in open arm and reduces (P<0.001) the time spent in closed arm compared to control. Higher dose of ethanolic leaf extract of *Caryota urens* similar activity as that of the reference control, diazepam.

Table 4. Effect of Ethanolic Leaf Extract of Caryota urens on Muscle Grip Strength in mice using Rotarod.

Groups	Drug Treatment	Time taken to fall from rotating rod (secs)
I	Control 0.1% CMC (10ml/kg, p.o)	141.00±3.98
II	Diazepam (4mg/kg, i.p)	24.17±1.35***
III	ELECU (200mg/kg, p.o)	55.83±2.63***
IV	ELECU (400mg/kg, p.o)	36.00±2.65***

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Control

Skeletal Muscle Relaxant activity of ethanolic leaf extract of Caryota urens was studied in mice using Rota Rod and the results were shown in table 5. In muscle relaxation study, ethanolic leaf extract of Caryota urens at both the doses (200mg and 400mg/kg) showed highly significant (P<0.001) reduction in time spent by the animals on the revolving rod when compared to the control. The time taken to fall from the rotating rod after administration of 200mg and 400mg/kg of ethanolic leaf extract of Caryota urens was 55.83±2.63 and 36.00±2.65 seconds respectively. The standard drug (diazepam) also showed a highly significant effect when compared to the control (P < 0.001) and time spend by the mice was 24.17±1.35 seconds. The result from the Rotarod test showed that the extract significantly reduced the motor coordination of the tested animals.

Table 5. Effect of Ethanolic Leaf Extract of Caryota urens on MES induced Convulsion in mice.

Groups	Drug Treatment	No. of Animals Survived / Total Animals Used	Clonus Seizure (secs)		% Protection
			Onset	Duration	
I	Control 0.1% CMC (10ml/kg, p.o)	0/6	3.14±0.11	15.67±0.67	Nil
II	Diazepam (4mg/kg, i.p)	6/6	10.42±0.03***	1.24±0.02***	100%
III	ELECU (200mg/kg, p.o)	4/6	6.52±0.24**	15.67±0.67	62.5%
IV	ELECU (400mg/kg, p.o)	0/6	8.33±0.05***	1.56±0.02***	100%

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Control

The anticonvulsant activity of ELECU on MES induced seizure was studied in mice and the results were shown in the table 5. In control group, the onset and durations of was 3.14±0.11 and 15.67±0.67 seconds respectively. All the animals showed convulsion and no animals survived in control group. In the reference control, all the animals survived and only in 2 animals the initial symptoms of clonus seizure was noticed with the duration of 1.24±0.02 seconds hind limb tonic extension. The diazepam significantly (P<0.001) decreases the both onset and duration of clonus seizure as compared to non treated groups. The Animals treated with ELECU 200mg/kg, four animals were survived out of 6 animals and the % protection was 62.5%. In the 200mg/kg of ELECU, The onset of clonus seizure was 6.52±0.24 and the duration of hind limb tonic extension was found to be 3.18±0.19 seconds. ELECU 200mg/kg, also significantly decreased onset (P<0.01) and duration (P<0.001) of seizure as compared to control group. In the animals treated with 400mg/kg of ELECU, all the 8

animals were survived, and the % protections was 100%. In this group, the onset of clonus seizure was significantly increased (P<0.001) to 8.33±0.05 and it significantly reduced (P<0.001) the duration of hind limb tonic extension to 1.56±0.02 seconds. The effect produced by the ELECU 400mg/kg was comparable with the effect of reference control diazepam.

Discussion

Plant-derived substances have recently become of great interest owing to their resourceful applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.(10)

Medicinal plants, due to their natural origin, were used to be considered safe for human use.(11) Earlier reports on the

chemical constituents of plants and their pharmacology suggest that plants containing flavanoids, alkaloids, phenolic acids, essential oil, saponins and tannins possess activity against many CNS disorder.(12) Medicinal plants are a good source to find new remedies for these disorders. In the search for an alternative, more specific, less adverse effects and perhaps cost-free therapy, the present study was conducted to investigate the CNS activities of natural anxiolytic, anticonvulsant, muscle relaxant, as well as new antidepressant principles.

The dried leaves of *Caryota urens* were macerated in ethanol and the menstruum collected was concentrated by vacuum distillation to get a semisolid mass which was used for various CNS activities.(13) The literature review reveals the presence of various phyto-constituents like carbohydrates, tannins, flavonoids, terpenoids, saponins, polyphenols etc.(14) The ethanolic leaf extract of *Caryota urens* was studied for CNS activity using Hole Board test, elevated plus maze test, locomotor activity, muscle relaxant activity and anticonvulsant activity.

The Hole board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, depression, anxiety, and/or responses to stress in animals. (15) It has been shown that head dipping behaviour reflects the sensitivity towards the changes in emotional state of the animal and provides information that a fearless state in animals may be reflected by the increase in head dipping behaviour (16), while a decrease in the number of head dips was found to be correlated with the depressant effect.(17) ELECU increased the number of head dipping responses in dose dependent manner, which showed that it possess antidepressant as well as anti-anxiety activity in hole board test.

The Elevated plus Maze is etiologically authenticated and most widely accepted model for screening novel anxiolytic agents in rodents. (18) Increase in the number of open arm entries and consequent increase in time spend in open arm are the two parameters generally considered as index of anxiolytic activity of a drug.(19) However, the interpretation of parameters in EPM is always complex. Studies also report increase in time spent in open area, open arm entries and no change in closed arm entries as criteria for drugs to have anxiolytic activity. (20) The present investigation showed the dose dependent anxiolytic effect as mark from the significant increase in the frequency and time spent in open arms by the animals treated with 200 and 400 mg/kg of ELECU.

Locomotor activity is considered as an index of alertness and a decrease in it is indicative of sedative activity.(21) Diazepam which is a known sedative, significantly reduces the spontaneous movement. ELECU has moderately reduces the spontaneous movement in actophotometer which may be advantageous in demonstrating the alertness.(22)

The skeletal muscle relaxant activity of ELECU was also

explored. Rota rod was originally developed for testing curare-like agents. Later on, it has been used by many authors for testing compounds for muscle relaxing activity of both centrally acting and peripheral acting muscle relaxants.(23) On administration of ELECU, the animals were unable to stay on and also reduced the time spent on the revolving rod by mice. (24)

This proved that ELECU may have muscle relaxant activity. From the result, ELECU possess a significant skeletal muscle relaxant activity in experimental rats; the possible mechanism might be interaction with benzodiazepine receptor located adjacent to the GABA receptor.(25) Phyto-constituents like flavonoids, phenolic compounds, terpenoids etc which are the major content in the leaves of *Caryota urens*.(26) The medicinal plants which were already reported to have influence on the CNS activities, has rich source of the above mentioned phytochemicals. Free radicals have been recommended to be the largest part responsible for producing the neuronal changes mediating the behavioural deficits in CNS disorders. From the present study on ELECU in CNS might also be due to the presence of similar phytochemicals as well as the free radical scavenging property of *Caryota urens*. (27)

Convulsion is one of the chronic and most common neurological disorders, affecting approximately 50 million people worldwide.(28) The basic and major mechanisms associated with epilepsy are increased synaptic connectivity of excitatory neurons, channelopathies (weekening of potassium channels and/or more persistent sodium channels, changes in voltage-gated ion channels), perturbation in synaptic receptors (suppressed GABAergic receptors, altered nicotinic receptors), decrease in inhibitory neurotransmission (decreased GABA levels), enhanced excitatory neurotransmission (enhanced glutamate levels). (29)

ELECU was evaluated against MES induced convulsions in rodents. The electroshock delivered in MES model is well known to potentiate the sodium influx through opening of sodium channels, and also increases glutamate levels, glutamate is an excitatory neurotransmitter, which binds with NMDA receptors and induces the symptoms that exactly mimic the petit mal epilepsy in humans.(30) Based on the underlying mechanism of MES convulsions, it can be understood that the agents which could block the voltage-dependent sodium channels and/or the agents could that decrease the levels of excitatory amino acids and/or antagonize their actions are proved to be effective in MES-induced epilepsy model.(31) In this model, ELECU has showed promising anticonvulsant effect, that indicates it may enhance the inhibitory neurotransmitters, reduced the excitatory neurotransmitters as well as by closing the sodium channel.

Hence the present study with ELECU has showed significant Antidepressant Activity, Anxiolytic Activity, Locomotor Activity, Muscle Relaxant Activity and Anti convulsant activity as compared with the standard drug.

Conclusion

Several factors underlie the growing popularity of herbal treatments for a variety of chronic conditions. Interestingly, people who utilize alternative therapies for many medical illness and got benefited but the successful outcome of the treatment has not been revealed and proved. Many people using herbal medicines find the health care alternatives are more congruent with their own values, beliefs and philosophical orientations toward health and life. It is concluded in the current study that the ethanolic leaf extract of *Caryota urens* possess significant antidepressant, anxiolytic, locomotor, muscle relaxant and anticonvulsant activities in mice. However, further studies are necessary to explore the underlying mechanisms of CNS effects and to isolate the active compounds responsible for these activities.

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