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Evaluation of antiepileptic activity of *Lantana camara* (Linn.) flowers in Swiss albino mice

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ABSTRACT

Introduction: Globally, approximately 50 million people are being affected by epilepsy and nearly 80% occurring in the developing countries. The plant *Lantana camara* Linn. (Family: *Lamiaceae*) is native to the tropical regions of the America, Africa, and Asia. It is found in Kumaon and Garhwal region of Uttarakhand, India. The plant has been used for various ailments in traditional systems of medicines for the treatment of epilepsy, wounds, tumor, stomach ache, and inflammation. **Objective:** The present study was planned with an objective to evaluate antiepileptic activity of *L. camara* flowers in mice. **Methods:** Maximum electric shock (MES) and pentylenetetrazole-induced (PTZ) model of epilepsy were employed in the present study. **Results:** Among, the various extracts prepared, namely, petroleum ether, chloroform, ethanol and aqueous, pentylenetetrazole-induced only ethanol and chloroform extract by successive solvent extraction method showed significant antiepileptic activity in MES and PTZ model of epilepsy. Highest antiepileptic activity ($P < 0.05$) was shown by ethanol extract at a dose of 200 mg/kg whereas chloroform extract showed moderate anticonvulsant effect at 200 mg/kg dose. **Conclusion:** The findings of present study lead to conclusion that ethanol extract of *L. camara* flowers showed potent antiepileptic activity which scientifically, validates the traditional claim of *L. camara* for the treatment of epilepsy.

Keywords: Antiepileptic activity, *Lantana camara*, maximum electric shock, pentylenetetrazole model

INTRODUCTION

Epilepsy is a chronic neurological disorder of the central nervous system (CNS) categorized by recurring seizures with seizures being a brief lapse of attention or muscle jerks to serve and prolonged convulsions.[1] According to the World Health Organization, about 450 million people are suffering from neurological problems, mental problems or behavioral problems at sometime in their life in the entire world.[2] Globally, approximately 50 million people are being affected by epilepsy and nearly 80% occurring in the developing countries and most of them do not get adequate medical treatment.[3] Therefore, research should continue to develop newer, more effective and safer neuroprotective agents for the treatment of epilepsy. About 80% of the world's population and especially of the developing countries uses medicinal plants for the treatment of epilepsy because of better compatibility with the human body and lesser side effects.[4] Medicinal plants are proven/considered an important source of traditional medicines to treat epilepsy. Various plants have scientifically demonstrated that they possess promising anticonvulsant activities in animal models for the detection of anticonvulsant activity and various plants are yet to be scientifically investigated.[5]

The plant *Lantana camara* Linn. [Family: *Lamiaceae*], commonly known as shrub Verbenas, is native to the tropical regions of the America, Africa, and Asia. It is found in Kumaon and Garhwal region of Uttarakhand, India. Conventionally, the plant has been used as diaphoretic, carminative, tonic, and antispasmodic. It is useful in the treatment of tetanus, malaria, epilepsy, and gastropathy. Powdered leaves are used for cuts, wounds, ulcers, and swelling. An infusion of leaves is good for bilious fever, eczema, and eruptions. Fruits are useful in tumors and rheumatism.[6] Flowers are used to cure epilepsy, skin inflammation, rheumatism and used to stimulate vomiting for food poisoning.[7] *L. camara* possesses various bioactive agents having therapeutic potential such

as alkaloids, carbohydrates, tannins, flavonoids, terpenoids, glycosides, and phenols.[8-10] The antiepileptic activity has not been evaluated on flowers of this plant so far. Therefore, it was considered worthwhile to evaluate antiepileptic activity of *L. camara* flowers.

MATERIALS AND METHODS

Plant Material

The flowers of the plant *L. camara* were collected from the region of Balawala, Dehradun, Uttarakhand, India. The plant was identified and authenticated by Scientist-D/HOO Kumar Ambrish, Department of Botany, Botanical Survey of India, Northern Region Center, 192, Dehradun, India, vide reference no. 118094. The voucher specimen is maintained in Botanical Survey of India laboratory for the further reference.

Preparation of Extract

Various extracts were successive prepared using solvents in the increasing polarity, i.e., petroleum ether, chloroform, ethanol, and water. Dried flowers were crushed using a mechanical grinder. Powdered material (#60) was subjected to successive Soxhlet extraction using solvents in the increasing polarity order, i.e., petroleum ether, chloroform, and ethanol. Prior each extraction the powdered material was dried in hot air-oven below 50°C. Finally, marc was digested at 50°C with distilled water for 4 h to obtain the aqueous extract. All extracts were concentrated in rotary vacuum evaporator (40°C), freeze-dried and stored in a well-closed container and kept at 4°C. Extracts were weighed, and percentage was calculated in terms of the air-dried weight of the plant material. The extracts obtained were further used for performing preliminary phytochemical screening[11,12] and for determining the antiepileptic activity of the flower of *L. camara*.[13]

Animal

Swiss albino male mice of 20–25 g weight were used for the evaluation of pharmacological studies. Animals were maintained on standard environmental conditions and fed with standard rodent diet and tap water *ad libitum*. They were housed in the institutional animal house and were exposed to natural photoperiod. The experimental protocol was approved by Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Animal Ethics Committee and care of the animals (CPSEA/IAEC/SBS/2017-18/012) was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forest, Government of India.

Acute Toxicity Studies

The acute toxicity studies were carried out as per the Organization for Economic Co-operation and Development guideline (OECD) no. 423 (Acute Toxic Class Method). The study was carried out in Swiss albino mice. This study was revealed the non-toxic nature of the extracts at the highest starting dose of 2000 mg/kg body weight of the animal.[14]

Pharmacological Evaluation (Antiepileptic Activity)

Antiepileptic activity was evaluated using following models:

- Maximum electric shock method (MES method)
- Pentylenetetrazole (PTZ)-induced method.

MES method

Swiss albino male mice 20–25 g weighed were randomized and divided into six groups of six animals each ($n = 6$).

- Group I: Control: Vehicle treated group (Distilled water and 5% Tween 80, 10 mg/kg, p.o.)
- Group II: Standard drug-treated group (Phenytoin, 20 mg/kg., i.p.)
- Groups III and IV: Ethanol extract of *L. camara* (EELC) 100 mg/kg and 200 mg/kg, p.o., respectively
- Groups V and VI: Chloroform extract of *L. camara* (CELC) 100 mg/kg and 200 mg/kg, p.o., respectively.

The standard drug (phenytoin) was administered once daily intraperitoneally for 14 days. The standard and test (extracts) drugs were given orally 60 min before the experiment. On the 14th day, after 1 h of treatment of standard and test drugs seizures are induced to all the groups by using an electro convulsiometer. Maximal electroshock seizures were elicited by applying electric current of 45 mA intensity for 0.2 s and were applied through the corneal electrodes. The duration of various phases of epilepsy (flexion, extensor, clonus, and stupor) was observed. The percentage protection was estimated by observing the number of animals showing abolition of hind leg tonic extension (or) extension not >90°.[15]

PTZ-induced method

Swiss albino male mice 20–25 g weighed were randomized and divided into six groups of six animals each ($n = 6$).

Drugs
PTZ (Dose: 80 mg/kg, i.p; prepare a stock solution containing 8 mg/ml of the drug and inject 1 ml/100 g of body weight of mice).

- Group I: Control: Vehicle treated group (distilled water and 5% Tween 80, 10 mg/kg, p.o.)
- Group II: Standard drug-treated group (Diazepam, 4 mg/kg, i.p.)
- Groups III and IV: EELC 100 mg/kg and 200 mg/kg, p.o., respectively
- Groups V and VI: Chloroform extract of *L. camara* (CELC) 100 mg/kg and 200 mg/kg, p.o., respectively.

The standard drug (diazepam) was administered once daily intraperitoneally for 14 days. On the 14th day, after 1 h of treatment of standard and test drugs, PTZ (80 mg/kg body weight, s.c.) was administered to all the groups to induce clonic convulsions. The animals were observed for a period of 30 min post-PTZ administration. The parameters noted were the onset and duration of clonic convulsions. The anticonvulsant potential was assessed by the ability to reduce the duration of clonic convulsions and increase the latency of seizures.[16,17]

Statistical Analysis

The data obtained were expressed as mean ± standard error of the mean. The antiepileptic activity results were statistically analyzed by one-way ANOVA analysis of variance followed by Tukey's multiple range comparison tests using GraphPad Prism software version 5.0. $P < 0.05$ was considered as statistically significant.

RESULTS

Acute Toxicity Study Analysis

The acute toxicity of the different extracts of *L. camara* was determined as per the OECD guidelines no. 423 (acute toxicity class method). This study showed the non-toxic nature of all the extracts even at the highest dose of 2000 mg/kg body weight of the animal (OECD, 2001). Further, no signs of toxicity were observed during short-term (2 days) and long-term (14 days) observation period. Hence, 100 mg/kg and 200 mg/kg of this dose were selected for the further study.

Table 1: Yield of different extracts of *Lantana camara* flowers

Extracts	Yield (% w/w)
Petroleum ether	0.93
Chloroform	1.69
Ethanol	2.81
Water	3.91

Yield of Extracts

Yield of different extracts, namely, petroleum ether, chloroform, ethanol, and water of *L. camara* flowers was calculated as 0.93, 1.69, 2.81, and 3.91%, w/w, respectively. Results are reported in Table 1.

Phytochemical Screening

Phytochemical analysis of *L. camara* flowers showed those bioactive ethanol extract possesses flavonoids and tannins. Results of phytochemical screening of various extracts are presented in Table 2.

Pharmacological Evaluation (Antiepileptic Activity)

MES method

Among, the different extracts prepared, namely, petroleum ether, chloroform, ethanol, and aqueous, ethanol extract exhibited maximum antiepileptic activity at a dose of 200 mg/kg in MES model of epilepsy (76% protection). Whereas ethanol extract (100 mg/kg), chloroform extract at a dose of 100 and 200 mg/kg showed 61%, 34, and 48% protection, respectively. Effects of different extracts of

Table 2: Phytochemical screening of different extracts of L*antana camara* flowers

Phytochemical screening	Pet. ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids				
Dragendorff's reagent	−	+	−	−
Hager's reagent	−	+	−	−
Wagner's reagent	−	+	−	−
Mayer's reagent	−	+	−	−
Carbohydrates				
Barfoed's reagent	−	−	+	+
Molisch reagent	−	−	+	+
Fehling's Sol^{n} A	−	−	+	+
Fehling Sol^{n} B	−	−	+	+
Tannins				
$FeCl_3$ test	−	−	+	−
Lead acetate test	−	−	+	−
with dilute HNO_3	−	−	+	−
With acetic acid	−	−	+	−
With Br_2 water	−	−	+	−
with dilute I_2 Sol^{n}	−	−	+	−
Proteins				
Warming test	−	−	−	−
Biuret test	−	−	−	−
Heller's test	−	−	−	−
Xanthoproteic test	−	−	−	−

(Contd...)

Table 2: (*Continued)*

Phytochemical screening	Pet. ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Glycosides				
Legal's test	−	−	−	−
Liebermann's test	−	−	−	−
Keller-killani test	−	−	−	−
Brontrager's test	−	−	−	−
Modified Brontrager's test	−	−	−	−
Foam test	−	−	−	−
Salkowski test	+	−	−	−
Lieberman–Burchard test	+	−	−	−
Lieberman test	+	−	−	−
Amino acids				
Ninhydrin test	−	−	−	−
Million's test	−	−	−	−
Fats	+	−	−	−
Terpenoids	+	−	−	−
Flavonoids	−	−	++	−

Table 3: Effect of different extracts of *Lantana camara* on maximum electric shock-induced convulsions

Groups	Drug treatment	Type of convulsions			% Protection
		Flexion (sec)	Stupor (sec)	Extensor (sec)	
I	Control (vehicle)	7.59±0.26	128.52±1.41	17.56±0.13	0
II	Phenytoin (25 mg/kg) (standard)	2.11±0.16	48.09±1.15a	0±0[a]	100
III	Ethanol extract of *Lantana camara* (100 mg/kg)	4.2±0.59	84.99±1.24[a]	6.81±0.34[a]	61
IV	Ethanol extract of *Lantana camara* (200 mg/kg)	3.72±0.17	60.28±1.19[a,b]	4.14±0.14[a,b]	76
V	Chloroform extract of *Lantana camara* (100 mg/kg)	5.59±0.28	110.61±1.28	11.57±0.54[a]	34
VI	Chloroform extract of *Lantana camara* (200 mg/kg)	4.90±0.19	98.88±1.35	9.13±0.45[a]	48

All values are expressed as mean±standard error of the mean (n=6) observation; mean of various groups were statistically compared by one-way ANOVA followed by Tukey's multiple comparison test using GraphPad version 5.0. [a]P<0.05 when compared with control, [b]P<0.05 when compared with 100 mg/kg dose of ethanol extract of L*antana camara*

Table 4: Effect of different extracts of *Lantana camara* on pentylenetetrazole induced-induced convulsions

Groups	Drug treatment	Onset of clonic convulsion	Duration of clonic convulsion	% Protection
I	Control (vehicle)	38.83±0.27	47.05±0.29	0
II	Diazepam (4 mg/kg) (standard)	316.51±1.04	0±0[a]	100
III	Ethanol extract of *Lantana camara* (100 mg/kg)	170.03±1.28	20.28±0.55[a]	57
IV	Ethanol extract of *Lantana camara* (200 mg/kg)	195.76±1.15	12.09±0.27[a,b]	74
V	Chloroform extract of *Lantana camara*(100 mg/kg)	95.23±1.28	32.09±0.61[a]	32
VI	Chloroform extract of *Lantana camara* (200 mg/kg)	113.83±1.31	25.82±0.50[a]	45

All values are expressed as mean±standard error of the mean (n=6) observation; mean of various groups were statistically compared by one-way ANOVA followed by Tukey's multiple comparison test using GraphPad version 5.0. [a]P<0.05 when compared with control, [b]P<0.05 when compared with 100 mg/kg dose of ethanol extract of *Lantana camara*

L. camara on MES-induced convulsions on mice are shown in Table 3 and Figure 1.

*PTZ-induced method (PTZ method***)**

Similarly, among, the different extracts prepared namely, petroleum ether, chloroform, ethanol, and aqueous, again ethanol extract at a dose of 200 mg/kg showed significant antiepileptic activity (74% protection). While ethanol extract (100 mg/kg), chloroform extract at a dose of 100 and 200 mg/kg showed 57, 32, and 45% protection, respectively. Results of different extracts of *L. camara* on PTZ-induced convulsions on mice are presented in Table 4 and Figure 2.

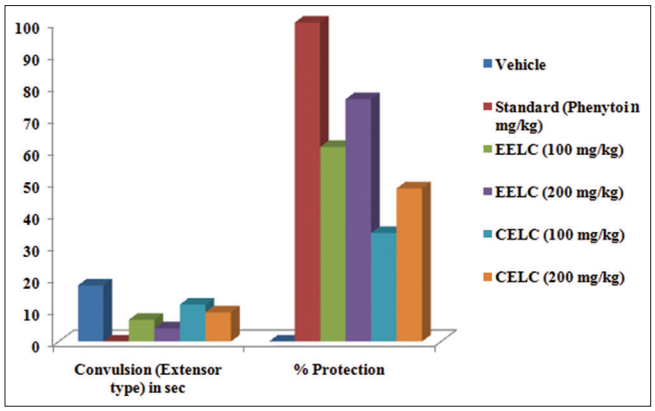

Figure 1: Effect of different extracts on maximum electric shock-induced convulsions. All values are expressed as mean ± standard error of the mean (n = 6) observation; mean of various groups were statistically compared by one-way ANOVA followed by Tukey's multiple comparison test using GraphPad version 5.0

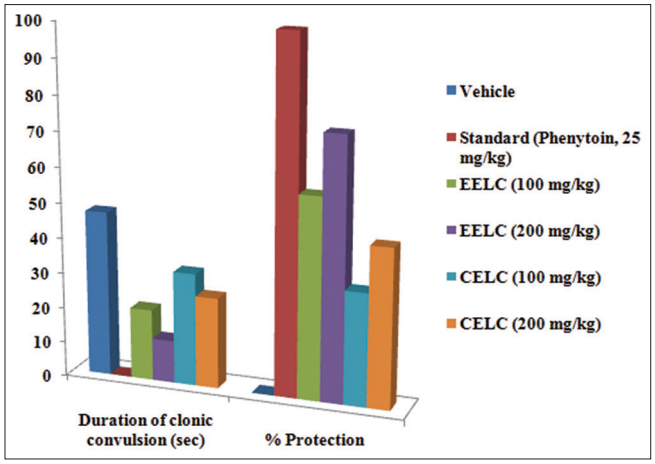

Figure 2: Effect of different extracts on pentylenetetrazole-induced convulsions. All values are expressed as mean ± standard error of the mean (n = 6) observation; mean of various groups were statistically compared by one-way ANOVA followed by Tukey's multiple comparison test using GraphPad version 5.0

DISCUSSION

Epilepsy is one of the chronic and most common neurological disorders, affecting approximately 50% people worldwide. Currently, marketed anticonvulsant drugs are able to effectively reduce epileptic seizure in about 50% of the patients, later 25% may show improvement whereas the remains 25% left ineffective by the drugs.[18] These marketed drugs have more side effects sometimes which are difficult to treat so the need to develop new anticonvulsant exists. Hence, as the part of that one of the base is studying naturally available compounds for better treatment and effective treatment of epilepsy.[19]

The present study was at aim to evaluate antiepileptic potential of *L. camara*. Among, the various extracts prepared, namely, petroleum ether, chloroform, ethanol, and aqueous, only ethanol and chloroform extract showed significant antiepileptic activity in MES and PTZ model of epilepsy. In the MES model, electric shock is delivered to potentiate sodium influx through the opening of sodium channels and also increases glutamate level which induces the symptoms that exactly mimic the petit mal epilepsy. Phenytoin treated animals have shown 100% protection against MES-induced seizures whereas EELC 100 mg/kg, EELC 200 mg/kg, CELC 100 mg/kg, and CELC 200 mg/kg have shown 61%, 76%, 34%, and 48% protection, respectively, against MES-induced seizures. EELC is found more effective than CELC. EELC 200 mg/kg dose was found more effective than EELC 100 mg/kg [Figure 1, Table 3]. In this MES model, anticonvulsant activity may be due to voltage-gated sodium channel blockade or NMDA antagonistic activity.

In the PTZ model, PTZ is delivered to decrease the GABA levels and density of GABA-a receptors in the various parts of the brain which leads to continuous stimulation of cortical neurons and results are similar to absence seizures. Diazepam treated animals have shown 100% protection against PTZ-induced seizures whereas EELC 100 mg/kg, EELC 200 mg/kg, CELC 100 mg/kg, and CELC 200 mg/kg have shown 57%, 74%, 32%, and 45% protection, respectively, against PTZ-induced seizures. Again EELC is found more effective than CELC. EELC 200 mg/kg dose was found more effective than EELC 100 mg/kg [Figure 2 and Table 4].

In PTZ-induced convulsion anticonvulsant activity of *L. camara* would be due to increase in the GABA level by inhibiting GABA transaminase or increases the frequency of opening of GABA-chloride channel.

On the other hand, petroleum ether and aqueous extract did not show any antiepileptic effect may be due to absence of antiepileptic phytoconstituents.

Identification of the different classes of phytochemical constituents of the plant is an important parameter which gives an indication of the pharmacological active metabolites present in that plant. The phytochemical screening of different extracts of *L. camara* flower was performed and different phytoconstituents are reported in Table 2.

In the present study, phytochemical screening of *L. camara* flowers showed that ethanol extract possess flavonoids and tannins. Several studies have reported that plants containing flavonoids have significant anticonvulsant activity. Many flavonoids have been found to be ligands for the $GABA_A$ receptors and hence can act like benzodiazepine-like molecules.[20] Therefore, these phytoconstituents may be responsible for the anticonvulsant activity. However, further study is needed to find the exact mechanism of anticonvulsant effect.

CONCLUSION

The findings of the current investigations, suggest that EELC flowers have anticonvulsant activity may be due to inhibited and/or attenuated seizures by interfering with GABAergic neurotransmission. Thus, the present study validates the traditional claim of *L. camara flower* to have antiepileptic potential.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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