Screening of Anti-Convulsant Activity of Methonalic Extract of Aerial Parts of *Canna indica*

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ABSTRACT

Aim: The aim of the present study is to explore the "Screening of anticonvulsant activity of methanolic extract of aerial parts of *Canna indica*" in albino mice.

Materials and method: Collection and authentication of aerial parts of *Canna indica* L. Preparation of methanolic extract of aerial parts of *Canna indica* L. Assesment of toxicity studies of *Canna indica* L. Screening of anticonvulsant activity by:

- Maximal electroshock method
- Isoniazid induced seizures
- Strychnine induced seizures

Results: Methanolic extract of aerial parts of *Canna indica* L. decreased the duration of tonic hind leg extension in maximal electroshock-induced seizures probably by acting on voltage gated sodium ion channels. The latency of convulsion and decreased the seizure threshold by acting on the GABAergic system, glutaminergic mechanism and Na^+, Ca^+ channels. Methanolic extract of aerial parts of *Canna indica* L. did not Showed any protection against strychnine induced convulsions even at highest dose, 400 mg/kg probably acting on glycinergic transmission. Methanolic extract of aerial parts of *Canna indica*. L did not Showed any protection against Isoniazid induced convulsions even at highest dose, 400 mg/kg probably acting on glycinergic transmission.

Discussion: In the present study we have evaluated the effect of methanolic extract of aerial parts of *Canna indica* L. against seizures induced by maximal electroshock (MES), isoniazid (INH) and strychnine in mice.

Conclusion: The findings of the present study lend pharmacological credence to the suggested folkloric, ethno medical uses of *Canna indica* L. as a natural supplementary remedy for the reveals that plants of *Canna indica* shows MES induced seizures which could be by interfering with GABA, glutaminergic mechanism and Na^+, Ca^+ channels. However, the exact mechanism and the active principle by which these extracts exert their action remain unclear. Further studies are required to study the individual mechanism of actions.

Keywords: Toxicity studies, *Canna indica*, Isoniazid, Anticonvulsant activity

INTRODUCTION

Epilepsy is the second most common serious neurological disorder after stroke, which affects a wide range of people throughout the world. Medicinal plants are the important source for the new chemical substance with potential therapeutic effects. Several plants used for the treatment of epilepsy in the system of traditional medicine and many such plants are yet to be scientifically investigated. Traditional medicines occupy an important place in the health care systems of developing countries. The people in developing countries depend on traditional medicine, because it is cheaper and more accessible than orthodox medicine. Herbal drugs have preparation are still popular in developing

countries in spite of great advance observed in medicines in recent decades [1-21]. These drugs do not have any side effects and do not show any drug interactions.

The drugs which are extensively used in the treatment of epilepsy in ayurvedic and unani system of medicine are *Bacopa monnieri* [9], *Ficus platyphylla* [22], *Viscumcapense* [23], *Clerodendrum infortunatu* [24], *Carissa carand* [25], *Spondias mombin* [26], *Boerhaavia diffusa* [27] and *Opuntia vulgar*. The saponins which are present in these drugs are mainly thought to be responsible for their anti-epileptic activity. In the present study, *Canna indica* L. contains the saponins and flavonoids as their main chemical constituents so the scope of these plants to treat epilepsy will be evaluated because of the presence of saponins and flavonoids as their main chemical constituent. However the plants containing saponins or flavonoids exhibits anticonvulsant activity [28]. Apart from this the *Canna indica* L. aerial parts will be prepared and evaluated for their anti-epileptic activity.

The attempt to discover the antiepileptic potential of these plants will lead to development of new herbal preparations and open new vistas in treatment of epilepsy.

A review of literature how ever did not reveal any scientific information of *Canna indica* L. plant on anticonvulsant activity.

Therefore, the present study is an attempt to assess the efficacy of anticonvulsant activity of aerial parts of *Canna indica* L. in mice.

MATERIALS AND METHODS

Collection of Drugs

The aerial parts of *Canna indica* L. were collected from the local area and authenticated by Dr. Prallubha, Department of Botany, Osmania University.

Chemicals Used (Table 1)

Table 1. Chemicals used for extraction and pharmacological screening.

S. No.	Name of the chemical	Source
1	Methanol	S.D Fine Chemicals, India
2	Phenytoin	Cadilla Healthcare Ltd., India
3	Diazepam	Ranbaxy Research Laboratories, India
4	Strychnine nitrate	Shakun Enterprises Private Limited, India
5	Isoniazid	Shakun Enterprises Private Limited, India

Plant Extract

Methanolic extract of aerial parts of *Canna indica* L.

Preparation of Extract

The aerial parts of *Canna indica* L. were collected and shade dried and powdered using mechanical grinder and passed through sieve #no 40 to get the powder of desired coarseness. The powdered material was preserved in a desiccator for further use.

Extraction Procedure

A weighed quantity (500 g) of the powder was subjected to continuous hot extraction using methanol as a solvent in Soxhlet apparatus. The extract was concentrated under reduced pressure and stored in desiccator. Percentage yield of methanol (99%) extract of aerial parts of *Canna indica* was found to be 9.6%.

Animals Used

Albino mice of either sex, weighing about 25-35 g were used in experiments. They are obtained from the animal house facility of Shadan Institute of Medical Sciences with an approval from institution ethical committee. Animals were housed in polypropylene cages maintained under standard condition (12 h light/dark cycle; 25 ± 3°C) and had free access to

standard rat/mice feed (Hindustan Lever Ltd., India) and water ad libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment.

Acute Toxicity Studies [29]

Acute toxicity study will be conducted to determine median lethal dose (LD50) of the methanol extract. Acute toxicity study of extract. Acute toxicity study will be carried out in albino Mice by "Up and Down method" (OECD guidelines 425).

Different dose levels (Up to 2000 mg/kg body weight) of the extract will be administered orally to overnight fasted Mice to different groups consisting of three animals in each group. Following the administration of the extract, animals will be observed continuously for 2-3 h.

For general behavioral, neurological, autonomic profiles and to find out percentage of mortality observations were tabulated according to Irwin's table. For this the following check list was employed:

Stimulation

Hyperactivity, piloerection, twitching, rigidity, irritability, jumping, clonic convulsions, tonic convulsions.

Depression

Ptosis, sedation, loss of righting reflex (sleep), loss of traction, loss of pinnal reflex, catatonia, ataxia, loss of muscle rigidity, analgesia.

Autonomic reflexes

Straub's tail, laboured respiration, cyanosis, reddening, abnormal secretions, balancing.

METHODS EMPLOYED IN SCREENING OF ANTICONVULSANT ACTIVITY [30]

Maximal Electro Shock Induced Seizures [31]

Instrument used

Electro convulsometer.

Standard drug used

Phenytoin.

Procedure

In the electrically-induced seizure experiment, the maximal electroshock (MES) method will be employed. In brief, tonic convulsions of the hind extremities of the mice was induced by passing alternating electrical current of 50 Hz and 150 mA for 0.2 s through corneal electrodes. The animals were divided into five groups containing six animals each group.

Group 1-was treated as control and administered with Normal saline (p, o).

Group 2-was treated with methanolic extract of aerial parts of *Canna indica* (100 mg/kg, p, o).

Group 3-was treated with methanolic extract of aerial parts of *Canna indica* (200 mg/kg, p, o).

Group 4-was treated with methanolic extract of aerial parts of *Canna indica* (400 mg/kg, p, o).

Group 5-was treated with Phenytoin (50 mg/kg, i.p).

For 7 days prior to the induction of convulsion. The number of animals protected from hind limb tonic extension seizure and the time spent in this position were determined for each dose group.

Strychnine Induced Seizures [32]

Standard drug employed

Diazepam (1 mg/kg body weight) intraperitoneally

Procedure

The animals were randomly divided into five groups containing six animals each.

Group 1-was treated as control and administered with normal saline (p, o).

Group 2-was treated with methanolic extract of aerial parts of *Canna indica* (100 mg/kg, p, o).

Group 3-was treated with methanolic extract of aerial parts of *Canna indica* (200 mg/kg, p, o).

Group 4-was treated with methanolic extract of aerial parts of *Canna indica* (400 mg/kg, p, o).

Group 5-was treated with diazepam (1 mg/kg, i.p).

Seizures were induced in mice with standard convulsing agents, strychnine (2 mg/kg, i.p) 30 min after drug treatment and the animals were observed for 1 h for tonic convulsion episode. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period was noted. The ability of the plant extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity.

Isoniazid-Induced Seizures [33]

Standard drug employed

Diazepam (1 mg/kg body weight) intraperitoneally.

Procedure

The animals were randomly divided into five groups containing six animals each.

Group 1-was treated as control and administered with Normal saline (p, o)

Group 2-was treated with methanolic extract of aerial parts of *Canna indica* (100 mg/kg, p, o).

Group 3-was treated with methanolic extract of aerial parts of *Canna indica* (200 mg/kg, p, o).

Group 4-was treated with methanolic extract of aerial parts of *Canna indica* (400 mg/kg, p, o).

Group 5-was treated with Diazepam (1 mg/kg, i.p).

For 7days prior to the induction of convulsion. The number of animals protected from onset of clonic convulsion and death, and the time spent in this position were determined for each dose group up to 2 h.

Statistical Analysis [34]

The results for electrically induced seizures, isoniazid induced seizures and strychnine induced seizures were expressed as Mean ± Standard Error of Mean. Paired Student's t-test was used to analyze the level of significance. A p-value of <0.05 was considered as statistically significant. The results for biogenic amines were expressed as Mean ± Standard Error of Mean. The Significance of differences among the group was assessed using one way analysis of variance (ANOVA). The test followed by Dunnet's test p-values less than 0.05 were considered as statistically significant. It was done using Graph pad 5.0 software versions.

RESULTS

Percentage Yield of the Extracts

The methanolic extract of aerial parts of *Canna indica* L. was prepared by the soxhlation method. The percentage yield of the extracts was 9.6%.

Preliminary Qualitative Phytochemical Screening of Extracts

The preliminary qualitative phytochemical analysis of methanolic extract of aerial parts of *Canna indica* L. was carried out. The results were tabulated in Table 2. The methanolic extract of aerial parts of *Canna indica* L. showed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids, steroids, fats and oils and saponins, phenols, starch, anthraquinones glycosides.

Table 2. Preliminary qualitative phytochemical screening of the extracts of aerial parts of *Canna indica*.

Alkaloids		
1	Dragendroff's test	+
2	Mayer's test	+
3	Hager's test	-
4	Wagner's test	+
Carbohydrates		
1	Molish's test	-
2	Fehling's test	-
3	Benedict's test	+
Flavonoids		
1	Shinoda test	+
2	Alkaline reagent test	+
Proteins		
1	Biuret test	-
2	Xanthoproteic test	-
3	Trichloroacetic acid test	-
Amino Acids		
1	Million's test	-
2	Ninhydrin test	-
Tannins		
1	Ferric chloride test	+
2	Bromine water test	+
Steroids		
1	Liebermann-Burchard test	+
2	Salkowski test	+
3	Sulfur powder test	+
Triterpenoid		
1	Liebermann-Burchard test	-
2	Salkowski test	-
Fats and Oils		
1	Spot test	-
2	Saponification test	+
Saponins		
1	Forth formation tests	+
2	Hemolytic test	+

Glycosides		
1	Borntrager's test	-
2	Baljet's test	-
3	Keller-Killani test	-
4	Picric acid test	-

Acute Toxicity Studies

Acute toxicity was performed in mice by staircase method. Mice were divided in to five groups with six animals per dose. A safe oral dose of aerial parts of *Canna indica* L. was determined by the procedure as described by the organization of economic co-operation and development (OECD) as per 423 guidelines. The aerial parts of *Canna indica* L., at different doses starting from 100-2000 mg/kg, was prepared by dissolving the extract in distilled water and the concentration was adjusted in such a way that it does not exceed 1 ml/100 g body weight of experimental animals. The extract was then administered and animals were observed individually for behavioral changes, mortality and toxicity up to 48 h with special supervision given during first 4 h and thereafter periodically.

After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 h and 24 h to detect changes in the autonomic and behavioral response and also for tremors, convulsion, salivation and diarrhea, lethargy, Sleep and coma and then monitored for any mortality for the following 7 days. According to the results of the acute toxicity test, the doses were chosen for experiments, i.e., 100 mg/kg, 200 mg/kg, 400 mg/kg [34-40].

Effect of Extracts of Aerial Parts of *Canna Indica* L. on Maximal Electroshock Induced Seizures in Mice

Table 3. Effect of extracts of aerial parts of *Canna indica* L. on maximal electroshock induced seizures in mice; *P<0.05. The values are expressed in mean ± SEM (n=6); (compared with control using student's t-test), ***P<0.0001(compared with control using student's t-test).

S. No.	Treatments	Dose, p, o	Duration (s)		Quantal protection	% protection
			Tonic Flexion	Tonic extensor		
1	Control	Normal saline	8.83 ± 1.95	12.16 2.95	2/6	33.67
2	Test Group-1	100 mg/kg	3.16 ± 0.40*	20.83 0.47	3/6	50.33
3	Test Group-2	200 mg/kg	3.33 ± 0.49**	16.83 1.37	4/6	66.67
4	Test Group-3	400 mg/kg	2.4 ± 0.22*	12.33 0.42	4/6	66.67
5	Phenytoin	50 mg/kg	2.5 ± 0.51*	2 0.44	6/6	100

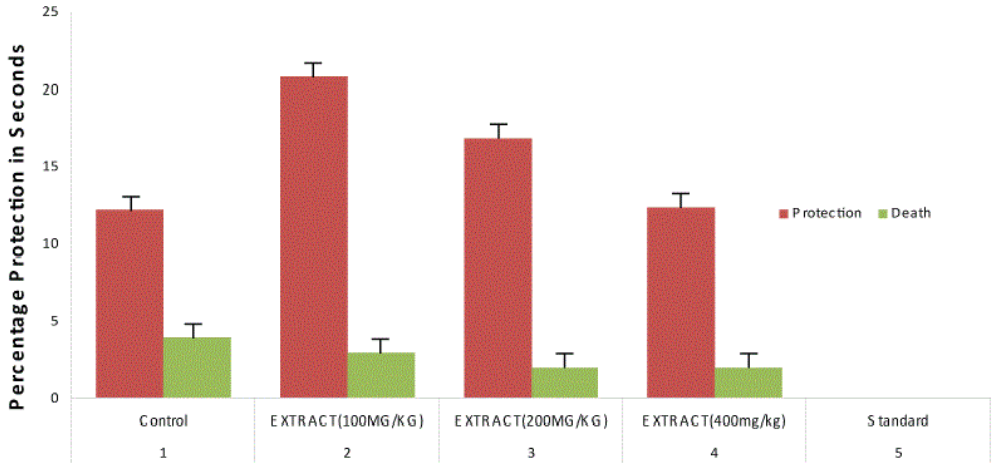

Figure 1. Effect of extracts of aerial parts of *Canna indica* L. on maximal electroshock induced seizures in mice.

In Maximal electro shock-induced convulsions model, the methanolic extract of aerial parts of *Canna indica* at three doses of 100 mg/kg, 200 mg/kg and 400 mg/kg MES produced hind limb tonic extension and hind limb tonic flexion seizures in all the animals used. The control mice showed tonic limb extension for the duration of 12.16 ± 2.95 s, tonic limb flexion 8.83 ± 1.95 s. The test group at the dose of 100 mg/kg protected 3 of mice and alter the incidence of seizures elicited by MES to a significant extent. The test group at the dose of 200 mg/kg protected 4 of mice and considerably decreased the duration of hind limb tonic extension and hind limb tonic flexion produced by MES **(Figure 1)**. A dose of 400 mg/kg protected 4 of the animals and significantly reduced the duration of the seizure. The standard antiepileptic drug, phenytoin (50 mg/kg) also protected all the animals and significantly reduced the duration of hind limb tonic extension and hind limb tonic flexion **(Table 3)**.

Isoniazid produced onset of tonic seizures in all the animals used. A dose of 100 mg/kg and 200 mg/kg of methanolic extract of aerial parts of *Canna indica* L. protected 2 animals against isoniazid induced seizures and did not affect the onset of seizures to any significant extent. Methanolic extract of aerial parts of *Canna indica* L. at the dose of 400 mg/kg protected 83.33% of mice and significantly delayed the latency of seizures **(Figure 2)**. The standard antiepileptic drug, Diazepam (5 mg/kg) profoundly antagonized the seizures produced by isoniazid. The above results are tabulated in **Table 4**.

Table 4. Effect of aerial parts of *Canna indica* L. extraction on isoniazid induced seizures in mice; values are mean ± SEM (n=6); ns-p value not significantly different (compared with control using student's t-test), **P<0.01(compared with control using student's t-test), ***P<0.0001(compared with control using student's t-test).

S. No.	Treatments	Dose, p.o	Onset of seizures (s)	Quantal protection	% protection
1	Control	Normal saline	141.61 ± 8.29	0/6	16.67
2	Test Group-1	100 mg/kg	130.17 ± 6.69 ns	0/6	33.33
3	Test Group-2	200 mg/kg	152.83 ± 3.47 ns	1/6	83.33
4	Test Group-3	400 mg/kg	290 ± 3.084 **	3/6	100
5	Diazepam	1 mg/kg	360.3 ± 27.38 ***	5/6	100

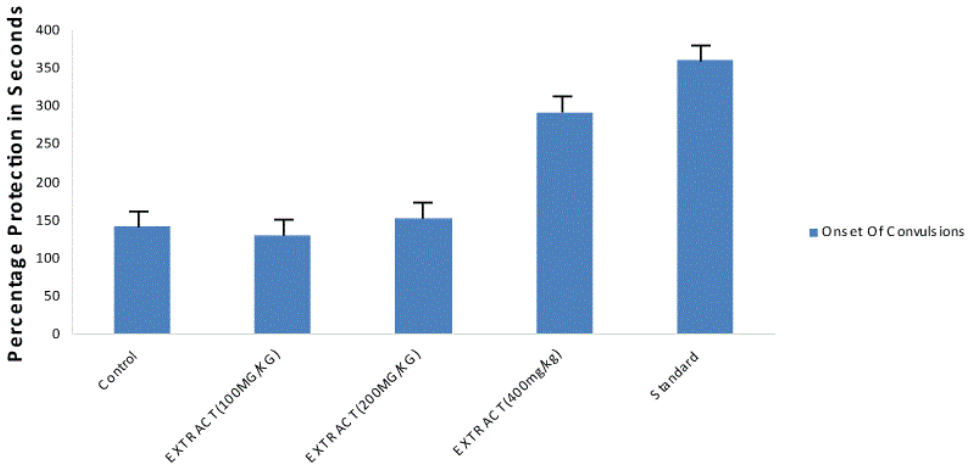

Figure 2. Effect of aerial parts of *Canna indica* L. extraction on isoniazid induced seizures in mice.

Effect of Extracts of Aerial Parts of *Canna Indica* L. on Strychnine Induced Seizures In Mice

Table 5. Effect of extracts of aerial parts of *Canna indica* L. on strychnine induced seizures in mice; *P<0.05 The values are mean ± SEM (n=6);(compared with control using student's t-test), ***P<0.001 (compared with control using student's t-test).

S. No.	Treatments	Dose, p.o	Onset of seizures (min)	Quantal protection	% protection
1	Control	Normal saline	139.83 ± 2.71	0/6	16.67
2	Test Group-1	100 mg/kg	142.33 ± 4.22	0/6	16.67

3	Test Group-2	200 mg/kg	121.67 ± 1.68 ns	0/6	83.33
4	TesstGroup-3	400 mg/kg	132.22 ± 2.22 ns	0/6	100
5	Diazepam	5 mg/kg	310.33 ± 4.50***	4/6	100

Strychnine (2 mg/kg) elicited tonic seizures in all the animals used. The methanolic extract of aerial parts of *Canna indica* L. 100 mg/kg, 200 mg/kg and 400 mg/kg significantly delayed the latency, but did not alter the incidence of seizures produced by strychnine to any significant extent. The standard anti-epileptic drug diazepam significantly delayed the latency of seizures **(Figure 3)**. These results are given in **Table 5**.

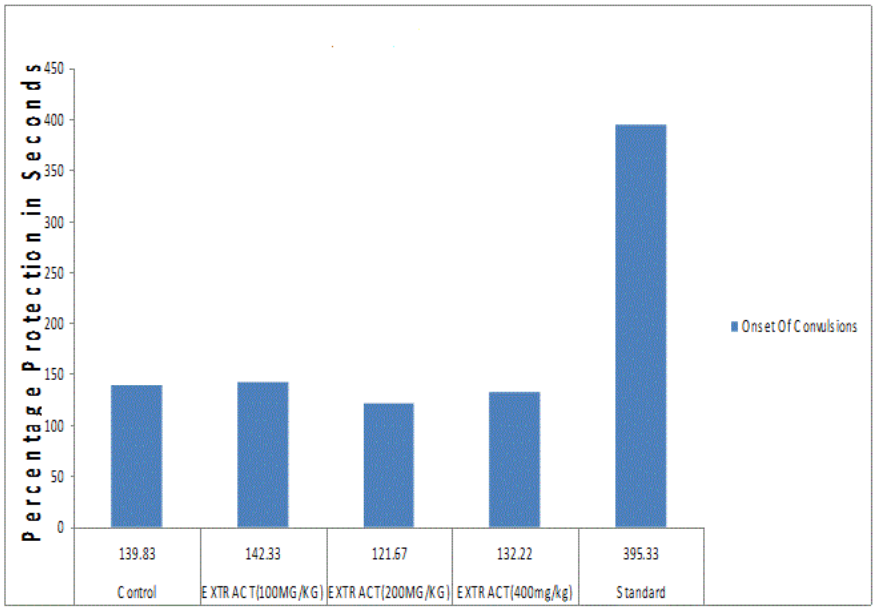

Figure 3. Effect of extracts of aerial parts of *Canna indica* L. on strychnine induced seizures in mice.

DISCUSSION

Epilepsy is the second most common neurological disorder which affects an estimated 7 million people in India and 50 million people worldwide (approximately 1-2% of the world population).Although several anti-epileptic drugs are available to treat epilepsy, the treatment is still far from adequate .Unfortunately most of the synthetic drugs not only fail to control seizures in some patients, but they frequently cause side effects. Due to these problems research focus has shifted towards natural products for new and better sources of drugs. In this process, medicinal plants serve as an alternative source for the development of new anti-convulsant drugs. Various plants are being studied based on the traditional knowledge of their pharmacological properties and confirmed to be useful in treating and managing various diseases. Medicinal plants are believed to be an important source of new chemical substance with potential therapeutic effects [40-50]. Several plants used for the treatement of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassay for the detection of anti-convulsant activity and many such plant are yet to be scientifically investigated.

In the present study we have evaluated the effect of methanolic extract of aerial parts of *Canna indica* L. against seizures induced by maximal electroshock (MES), isoniazid (INH) and strychnine in mice.

Preliminary Phytochemical Screening

The preliminary qualitative phytochemical analysis of methanolic extract of aerial parts of *Canna indica* L. showed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids, steroids, fats and oils and saponins, starch, phenols, antraquinones glycosides, tannins.

Acute Toxicity Studies

The acute toxicity studies showed that the extract of methanolic extract of aerial parts of *Canna indica* L. was found to be safe at the maximum dose of 100 mg/kg, 200 mg/kg, 400 mg/kg and 2000 mg/kg body weight, respectively by post-operative route. After 48 h mice was found to be well tolerated. There was no mortality and no signs of toxicity. The stimulatory depressive and autonomic profiles were found to be normal. No signs of mortality were observed at the doses

of 100 mg/kg, 200 mg/kg, 400 mg/kg and 2000 mg/kg body weight, respectively by post-operative route for extract of aerial parts of *Canna indica* L. respectively. The extracts were found to be safe at these doses.

Electrically Induced Seizures

The result of present study showed that the extract of aerial parts of *Canna indica* L. decreased the duration of tonic hind leg extension in maximal electroshock-induced seizures. So the methanolic extract of aerial parts of *Canna indica* seems to act on the voltage dependent sodium ion channels there by preventing the repetitive firing of action potential and thus produce their anticonvulsant effect [50-67].

Strychnine Induced Seizures

Strychnine induces convulsions by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing the spinal reflexes. The results show that methanolic extract of aerial parts of *Canna indica* L. increase the latency of convulsion more than 400 mg/kg compare to 100 mg/kg, 200 mg/kg, but all the three did not showed protection against strychnine induced convulsions which suggests that the aerial parts of *Canna indica* L. probably did not act on glycinergic transmission.

Isoniazid (Inh)-Induced Seizures

Isoniazid induce convulsion is thought to be inhibition of GABA synthesis in the CNS. So Diazepam treated group was showed 80% of protection of the animals. But the aerial parts of *Canna indica* L. not showed significant protection of the animals it was ineffective. The extract might be not having either by stimulation of L-glutamate or prevention of GABA degradation by GABA transaminase.

CONCLUSION

The present study was conducted to evaluate the anticonvulsant potential of methanolic extract of aerial parts of *Canna indica* L. in experimental mice by maximal electroshock induced seizures, isoniazid induced seizures and strychnine induced seizures.

The preliminary qualitative phytochemical analysis of methanolic extract of aerial parts of *Canna indica* showed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids, steroids, fats and oils and saponins, tannins, antraquinone glycosides, phenols, starch.

Acute toxicity as per OECD guidelines 425 was carried out and no mortality was found. No signs of mortality were observed at the doses of 100 mg/kg, 200 mg/kg, 400 mg/kg and 2000 mg/kg body weight by post-operative route for extract of aerial parts of *Canna indica* L. The extracts were found to be safe at these doses.

Methanolic extract of aerial parts of *Canna indica* L. decreased the duration of tonic hind leg extension in maximal electroshock-induced seizures probably by acting on voltage gated sodium ion channels. The latency of convulsion and decreased the seizure threshold by acting on the GABAergic system, glutaminergic mechanism and Na^+, Ca^+ channels.

Methanolic extract of aerial parts of *Canna indica* L. did not Showed any protection against strychnine induced convulsions even at highest dose, 400 mg/kg probably acting on glycinergic transmission.

Methanolic extract of aerial parts of *Canna indica* L. did not Showed any protection against Isoniazid induced convulsions even at highest dose, 400 mg/kg probably acting on glycinergic transmission.

The findings of the present study lends pharmacological credence to the suggested folkloric, ethnomedical uses of *Canna indica* L. as a natural supplementary remedy for the reveals that plants of *Canna indica* shows MES induced seizures which could be by interfering with GABA, glutaminergic mechanism and Na^+, Ca^+ channels. "However, the exact mechanism and the active principle by which these extracts exert their action remain unclear. Further studies are required to study the individual mechanism of actions.

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