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Neuropharmacological screening of fronds of *Adiantum Capillus Veneris Linn*

Sachin Kumar Jain[1]*, Talever Singh[1], Milind Pande[1], Nitin Nema[2]

[1]*Department of Pharmacy, Institute of Biomedical Education & Research, Mangalayatan University, Aligarh*
[2]*Department of pharmacology, Sagar Institute of Pharmaceutical Sciences, Sagar*

ABSTRACT

The present study was aimed to evaluate neuropharmacological profile of ethanolic extract of Adiantumcapillusveneris (L) by using wide pharmacological activities, i.e. anticonvulsant activity, antidepressant activity, skeletal muscle relaxant activity and analgesic activity. Neuropharmacological profile of ethanolic extract of Adiantumcapillusveneris (L) was evaluated by using different screening models, i.e. anticonvulsant activity by Maximal electro shock induced seizures and Pentylenetetrazole induced convulsions model, antidepressant activity by forced swim test, skeletal muscle relaxant activity by Rota rod apparatus and analgesic activity by Eddy's hot plate method and tail immersion method in wistar albino mice. The ethanolic extract of Adiantumcapillusveneris (L) showed anticonvulsant activity by prolonging the onset of seizures and reducing the duration of seizures in a significant manner when compared with control group in PTZ induced convulsion model, also by reducing the time for various phases of seizure in MES induced seizures test. Ethanolic extract showed significant depressant activity by increasing the immobility time of mice in forced swim test. The extract was not showed significant skeletal muscle relaxation in mice. Significant increase in onset of paw licking to heat stimuli in Eddy's hot plate method and increase in reaction time to tail withdrawal in tail immersion test confirms the analgesic activity of extract. The dose dependent response was recorded in all models. Results obtained from present study revealed that the ethanolic extract of Adiantumcapillusveneris (L) possess significant neuropharmacological properties. The extract at dose 400 mg/kg body weight was found to be more effective.

Key words: Neuropharmacology, Adiantum species, Epilepsy, Central analgesic.

INTRODUCTION

Drug acting in central nervous system were among first to be discovered by primitive human and are still most widely used group of pharmacological agents. The CNS acting drugs are invaluable therapeutically, because they can produce specific physiological and psychological effects from vast array of material medica of the indigenous system so many plants have been reported to have activity against CNS disorders and thus act as very useful remedies for alleviation of human suffering[1]. The strategy used for discovery of new drugs based on the screening of plants having medicinal uses relevant to the treatment of a particular disease, is referred to as ethanopharmacological screening [2].

The genus Adiantum belongs to family *Adiantaceae*, which consists of 150 to 200 species worldwide distributed in North America, United States, South Dakota, British Columbia, Canada and India[3]. Ethno medicinally, the genus is

important and popularly known as "Hansraj" in Ayurvedic System of Medicine. About nine species of Adiantum are found in India[4]. *Adiantumcapillusveneris (L)*is one of most common species with potential importance for medicinal and nutritive purpose [5].

The fresh or dried leafy fronds are antitussive, astringent, demulcent, depurative, emetic, emollient, laxative, pectoral, refrigerant, stimulant, sudorific, tonic skin diseases and hard swellings[6, 7]. Syrup is made from the plant - it makes a refreshing summer drink[8]**.** The genus has been commonly used for the treatment of inflammatory diseases such as gastritis, bronchitis, nephritis, dermatitis and cystitis[9].

MATERIALS AND METHODS

Animals: All the experimental studies were carried out under standard conditions in animal house of Mangalayatan University according to Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA), Gov. of India(Reg. No. 1341/a/10/CPCSEA) and Institutional Animal Ethical Committee (Reference No. MU-IBMER-PH/IAEC-Clear/2012-2013). All the animals were procured from Indian Veterinary Research Institute (IVRI) Bareilly.

All the animals were housed in standard polypropylene cages and maintained under environmentally controlled room provided with a 12:12 hr. light and dark cycle for each 24 hr. period at a temperature of approximately 25°C ± 2^{o}C. They were given free access to water and standard rat feed but 12 hr. prior to an experiment, the rats were deprived of food but not water.

Preparation of the extract

The crude plant (frond) of *Adiantumcapillusveneris*(L.) (Hansraj) was collected from Ayurveda and Medicinal plant supplier in market of Aligarh Uttar Pradesh. The plant was identified and confirmed by Dr. (Mrs.) SunitaGarg, at Raw Material Herbarium and Museum, Delhi (RHMD), Council of Scientific and Industrial Research (CSIR) - National Institute of Science Communication and Information Resources (NISCAIR). Authentication No. of *Adiantumcapillusveneris*(L.) is NISCAIR/RHMD/Consult/2013/2238/19, dated 02/05/2013.

The Plant material was subjected to size reduction to get coarse powder of desired particle size. The powdered material (100 gm.) was subjected to successive extraction in a Soxhletapparatus using solvent ethanol. After completion extract was concentrated to ¾th of its original volume by using rotary evaporator apparatus. The concentrated extract was then evaporated to form a thick paste. Percentage yield obtained was approx.12-15% w/w.

Acute Toxicity Study

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD).Acute toxicity studies were performed on Albino Mice of either sex weighing between 20-30 gm. Mice were fasted overnight prior to the experimentation.

1. Pharmacological Screening

A. Anticonvulsant Activity

1. Pentylenetetrazole (PTZ) Induced Conulsions

PTZ at the dose of 80 mg/kg was injected s.c. to induce clonic-tonic convulsions in mice. The test animals (n=5) received 200mg/kg, 400 mg/kg of ethanolic extract orally as a suspension prepared in 1% CMC solution and standard group received phenytoin (10 mg/kg) injected i.p., PTZ was injected s.c. 60 min after the administration of drug [10]. Each animal was placed in individual plastic cage for observation lasting forty five minutes [11]. The parameters noted were mean onset time of convulsions, duration of clonus and recovery/death (% recovery or % survival) due to PTZ challenge[12]**.**

2. Maximum Electro Shock Induced Seizure Method

The electrical shock applied (120 mA for 0.2 s) through corneal electrodes to wistar albino mice preliminary to produced convulsion and those showing response were divided into four groups of 5 animals each. The animals of group I was treated with 1% CMC solution served as control, group II with Phenytoin as standard, while group III and IV treated with ethanolic extract of test drug at 200 and 400 mg/kg body weight respectively. Drug pretreatment was given 60 min prior to the electric shock and animal were observed for hind limb tonic extension (HLTE) in seconds [10].

3. Antidepressant Activity

Forced Swim Test (FST)

Forced swim test, the most frequently used behavioral model for screening antidepressant-like activity in rodents, was first proposed by Porsolt et al.Mice were individually forced to swim in open glass chamber (25 × 15 × 25cm) containing fresh water to a height of 15 cm and maintained at 26°±1°C. At this height, animals were not able to support themselves by touching the bottom or the side walls of chamber with their hind-paws or tail. Water in the chamber was changed after subjecting each animal to Forced swim test because "used water" has been shown to alter behavior. Each animal showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Following swimming session, mice were towel dried and returned to their housing conditions[13]**.** The test was conducted in a dim lighted room and each mouse was used only once in the test[14]**.**

4. Skeletal Muscle Relaxant Activity

Rotarod Test:

In 1956, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod[15]**.** Untreated fresh mice were placed on a horizontal metal rod rotating at a speed of 30 rpm. The animals remaining on the rod for 3 min or more in two successive trials were selected for the test and were divided into 4 groups of 5 animals each. Group 1st was treated with 1% CMC (5ml/kg p.o.) solution, Group 2nd with Standard drug Diazepam (4mg/kg p.o.), while group 3rd and 4th received the extract (200, 400 mg/kg p.o.) respectively. The time taken for the mice to fall from the rotating rod was noted[16, 17]**.**

5. Analgesic Activity

1. Eddy's Hot Plate Method

The mice of either sex were weighed and divided into four groups (n = 5 in each groups). Group I served as control. Group II (pentazocine 10 mg / kg body weight) served as standards and group III and IV were treated with extracts at a dose of 200 and 400 mg/kg body weight, respectively. Reaction time of animals was noted down in hot plate at0, 30, 60, 90, 120 and 180 minutes after the treatment. The basal reaction time taken by observing hind paw licking or jump response (whichever appear first) in animals while placed on hot plate, which was maintained at constant temperature $55^0C \pm 1^0C$. A cut off period of 10 seconds was observed to avoid damage to the paws[18]**.**

2. Tail Immersion Method

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice.The animals were treated as discussed above; 1 to 2 cm of tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by mice to deflect their tails. A latency period of 12s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The reaction time of the tail-flick response at 0, 30, 60, 120, 180 and 240 min after the administration of drugs was recorded[19]**.**

Statistical Analysis

The results were expressed as the mean ± S.E.M. The results obtained from present study were analyzed using One Way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis by using Graph pad Prism (version 5) Software. P values <0.05 were considered statistically significant.

RESULTS

Pentylene Tetrazole Induced Convulsion Test

Results of anticonvulsant activity by PTZ induced convulsion test is shown in Table No. 4.1.

Both the dose of ethanolic extract of *Adiantumcapillusveneris* (L) was found to be statistically significant at value $P < 0.001$ and $P < 0.01$. The anticonvulsant activity of dose 400 mg/kg of extract was found to be better than the extract at dose 200 mg/kg with reference to prolongation of onset of seizures and reduction of duration of seizures.

Maximal Electro Shock Induced Seizures Model

Results of anticonvulsant activity by MES induced seizures test is shown in Table No. 4.2.

Both the dose of ethanolic extract was found to be statistically significant at value P < 0.001 and P < 0.01. The anticonvulsant activity of dose 400 mg/kg of extract was found to be better than the extract at dose 200 mg/kg with reference to decrease in time for various phases of convulsions.

Antidepressant activity

Forced swim test

Time taken by the mice in seconds to become immobile at different time interval was recorded after drug administration.Results of forced swim test to evaluate antidepressant activity are shown in Table No. 4.3.

It was found that extracts increases the immobility time and produce CNS depressant effect in mice in forced swim test.

Skeletal muscle relaxant activity

Rota rod test

Time taken by mice in seconds to fall from the rotating rods at different time interval was recorded after drug administration.Results of Rota rod test to evaluate the skeletal muscle relaxant activity are shown in Table No. 4.4.

The ethanolic extract of *Adiantumcapillusveneris* (L) with 200 mg/kg and 400 mg/kg dose was not showed significant skeletal muscle relaxation, that of diazepam when compared to control group. From the result it was found that extract failed to show any significant skeletal muscle relaxant activity in Rota rod test.

Analgesic activity

Eddy's hot plate method

The reaction time of mice is shown in Table No. 4.5 at different time interval.

It was found that the ethanolic extract of *Adiantumcapillusveneris*(L) with both doses i.e., 200 mg/kg and 400 mg/kg possesses the analgesic activity in mice, where extract with dose 400 mg/kg was found to be better in increasing the response time.

Tail Immersion Test

The tail withdrawal reflex time of mice is shown in Table No. 4.6 at different time interval.

It was found that the ethanolic extract at both dose i.e., 200 mg/kg and 400 mg/kg possesses the analgesic activity in mice, where extract with dose 400 mg/kg was found to be more potent than 200 mg/kg dose in increasing the tail withdrawal reflex time.

DISCUSSION

PTZ induces convulsion by antagonizing the γ-aminobutyric acid ($GABA_A$) receptor chloride (Cl^-) channel complex to attenuate GABA-dependent inhibition[10]. PTZ is a convulsant known to act on the reticular activating system of brain stem and also on motor cortex. PTZ convulsions are used to evaluate anti-epileptic drugs likely to be used in petitmal epilepsy[20].It is found that the anticonvulsant action occur either by reducing T-type calcium currents or by enhancing $GABA_A$ receptors mediated synaptic inhibition[21]. Drugs protecting against tonic-clonic seizures induced by PTZ are considered useful in controlling myoclonic and absence seizures in humans[10]**.** The ethanolic extract of *Adiantumcapillusveneris* (L.) delayed the latency of seizures induced by pentylenetetrazole suggesting that the extract is useful in suppressing absence seizures.

Inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures[10]**.** MES test correlates well with the ability to prevent generalized tonic clonicseizures and it is said that this model evaluates the capacity to prevent seizure spread. Drugs that are active against MES test often possess an effect on voltage dependent sodium channels (prolongation of Na^+ channel inactivation) as that of phenytoin[21]**.** Ethanolic extract of *Adiantumcapillusveneris* (L) also effective in MES test like phenytoin and may prolong the Na^+ channel inactivation. It was found from the above observations that ethanolic extract of *Adiantumcapillusveneris*(L) has shown anticonvulsant activity against seizures induced by MES in a dose dependent manner.

In the conventional version of the animal model, an antidepressant effect is evaluated by a decrease in immobility during exposure to the un-escapable water tank[22]**.** The decrease in the immobility time is accompanied with the increase in swimming time[23]. The results obtained from experiment clarify that the ethanolic extract of *Adiantumcapillusveneris* (L) with both doses showed a significant increase in immobility time, which indicates CNS depression, and the response is opposite to that of imipramine standard drug used to assess antidepressant activity.

Here, we can say that the ethanolic extract possesses CNS depressant activity and it supports the anticonvulsant activity of the extract against the stimulus.

In the present study Rota rod test was used for assessment of skeletal muscle relaxation. From the results it is clear that the ethanolic extract of *Adiantumcapillusveneris* (L) with both doses 200 mg/kg and 400 mg/kg body weight did not showed a significant relaxation of skeletal muscle as like that of diazepam which was used as a standard reference drug.

The hot plate test is considered to be selective for opioid-like compounds, which are centrally acting analgesics in several animal species. The increase in the reaction time of the mice on the hot plate following administration of the extracts suggests that the extracts possess central analgesic activity. Prostaglandins and bradykinins were suggested to play an important role in analgesia. Flavonoids and sterols are reported to inhibit prostaglandin synthesis. A number of flavonoids have been reported to produce analgesic activity[24]**.** Presence of flavonoids sssin extract might suppress the formation of prostaglandins and bradykinins and exert its activity.

In hot plate test and tail immersion test, the extract increases mean basal latency which indicates that it may act via centrally mediated analgesic mechanism as because the hot plate method and tail immersion test are considered to be selective to examine compounds acting through opioid receptors[25].

It is also reported that the inhibition of pain could arise not only from the presence of opioid or opiodiomimetics but could also arise from the presence of phenolic constituents and also steroidal constituents. Hence similar type of constituents may present in the extract. There are reports on the role of flavonoid in analgesic activity primarily by targeting prostaglandins[25].

4.1 Effect of ethanolic extract of *Adiantumcapillusveneris*(L) frond on pentylenetetrazole induced convulsion in albino mice.

S. No.	Treatment	Onset of seizures (Sec)	Duration of seizures (Sec)
1.	Control (1 % CMC Suspension)	629 ± 17.5	37.8 ± 1.85
2.	Phenytoin (10 mg/kg)	859 ± 10.2***	9.20 ± 0.735***
3.	Ethanolic extract 200 mg/kg	696 ± 7.24**	29.4 ± 1.89**
4.	Ethanolic extract 400 mg/kg	724 ± 12.7***	24.2 ± 1.39***
	P Value	<0.0001	<0.0001

4.2 Effect of ethanolic extract of *Adiantumcapillusveneris*(L) frond on Maximal electro shock induced convulsion in albino mice.

S. No.	Treatment	Flexion Sec	Extensor Sec	Clonus Sec	Stupor Sec	Recovery Sec
1.	Control (1 % CMC Suspension)	5.46 ± 0.353	15.0 ± 0.495	19.2 ± 0.989	54.9 ± 2.07	125 ± 2.69
2.	Phenytoin (10 mg/kg)	2.39 ± 0.337***	0.0 ± 0.0***	13.2 ± 0.496***	30.1 ± 0.743***	81.3 ± 2.97***
3.	Ethanolic extract 200 mg/kg	4.20 ± 0.338*	12.4 ± 0.441*	16.4 ± 0.762*	49.5 ± 1.32*	111 ± 4.07*
4.	Ethanolic extract 400 mg/kg	2.63 ± 0.312***	10.6 ± 0.857***	14.7 ± 0.641***	33.4 ± 1.21***	95.6 ± 2.10***
P Value		<0.0001	<0.0001	0.0003	<0.0001	<0.0001

4.3 Effect of ethanolic extract of *Adiantumcapillusveneris*(L) frond on immobility period in albino mice (Forced Swim Test).

S. No.	Treatment	Immobility Time in sec (Mean ± SEM)
1.	1 % CMC Suspension	181 ± 4.68
2.	Imipramine (10mg/kg)	124 ± 3.83***
3.	Ethanolic extract 200mg/kg	203 ± 4.69 **
4.	Ethanolic extract 400mg/kg	225 ± 4.25***
P Value		<0.0001

4.4 Effect of ethanolic extract of *Adiantumcapillusveneris*(L) frond on skeletal muscle relaxation in albino mice (Rotarod Test).

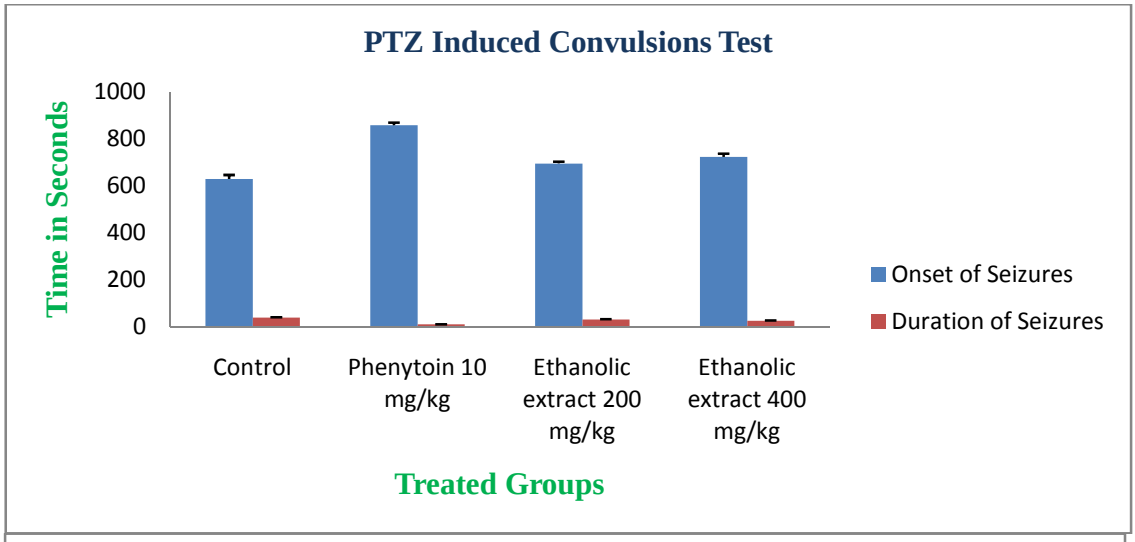
S. No.	Treatment	Time in seconds (to fall from rotating rod) Mean ± SEM			
		0 min	30 min	60 min	120 min
1.	Control (1 % CMC Suspension)	260 ± 8.85	259 ± 10.4	272 ± 2.95	270 ± 6.15
2.	Diazepam (4 mg/kg)	254 ± 8.86	42.5 ± 4.02***	25.7 ± 1.65***	108 ± 9.07***
3.	Ethanolic extract 200mg/kg	263 ± 15.2	267 ± 6.87	265 ± 7.64	284 ± 9.10
4.	Ethanolic extract 400mg/kg	255 ± 9.22	251 ± 7.30	265 ± 9.76	261 ± 13.0
P Value		0.9135	<0.0001	<0.0001	<0.0001

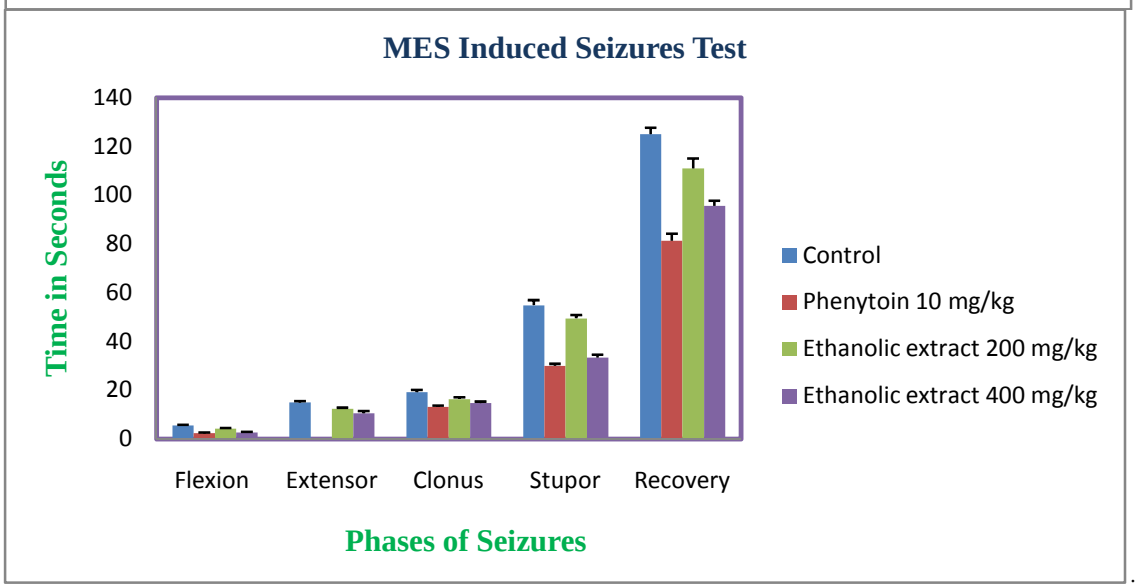
4.5 Effect of ethanolic extract of *Adiantumcapillusveneris*(L) frond in albino mice by Eddy's hot plate test.

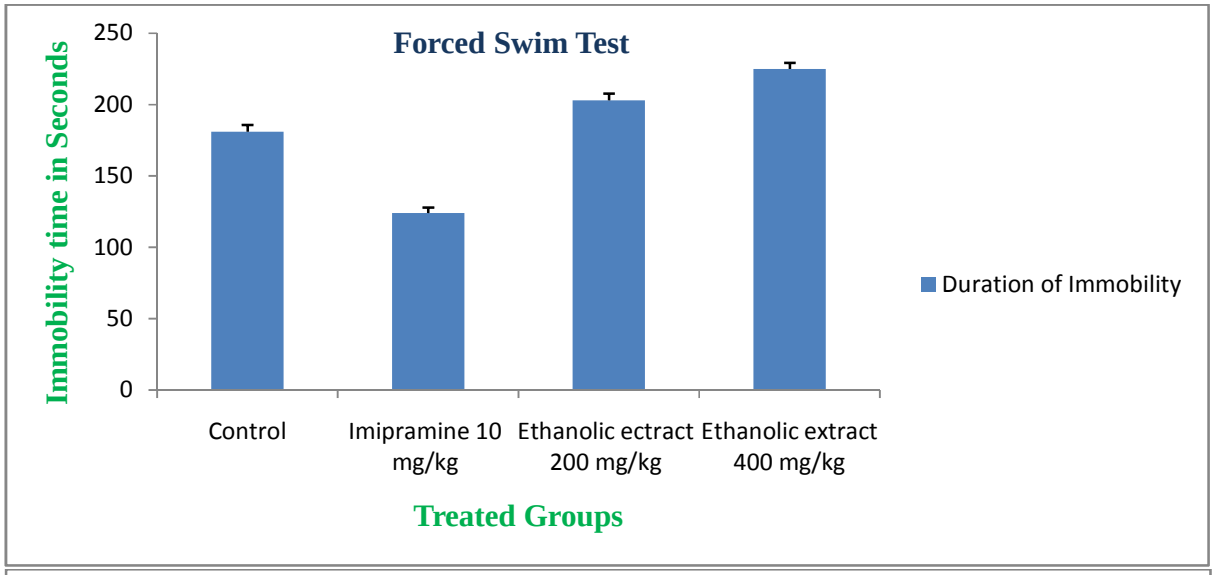
S. No.	Treatment	Reaction time in seconds (Mean ± SEM)					
		0 min	30 min	60 min	90 min	120 min	180 min
1.	Control (1 % CMC Suspension)	4.13 ± 0.189	4.11 ± 0.125	4.22 ± 0.173	4.13 ± 0.0882	4.28 ± 0.129	4.25 ± 0181
2.	Pentazocin (4 mg/kg)	4.04 ± 0.0896	4.74 ± 0.148*	7.40 ± 0.208***	8.92 ± 0.250***	9.86 ± 0.134***	7.01 ± 0.0954***
3.	Ethanolic extract 200 mg/kg	4.21 ± 0.199	4.37 ± 0.159	5.27 ± 0.150**	5.55 ± 0.162***	5.85 ± 0.188***	5.16 ± 0.206**
4.	Ethanolic extract 400 mg/kg	3.92 ± 0.106	4.09 ± 0.0934	5.74 ± 0.173***	6.78 ± 0.207***	7.27 ± 0.268***	5.53 ± 0.195***
P Value		0.5684	0.0114	<0.0001	<0.0001	<0.0001	<0.0001

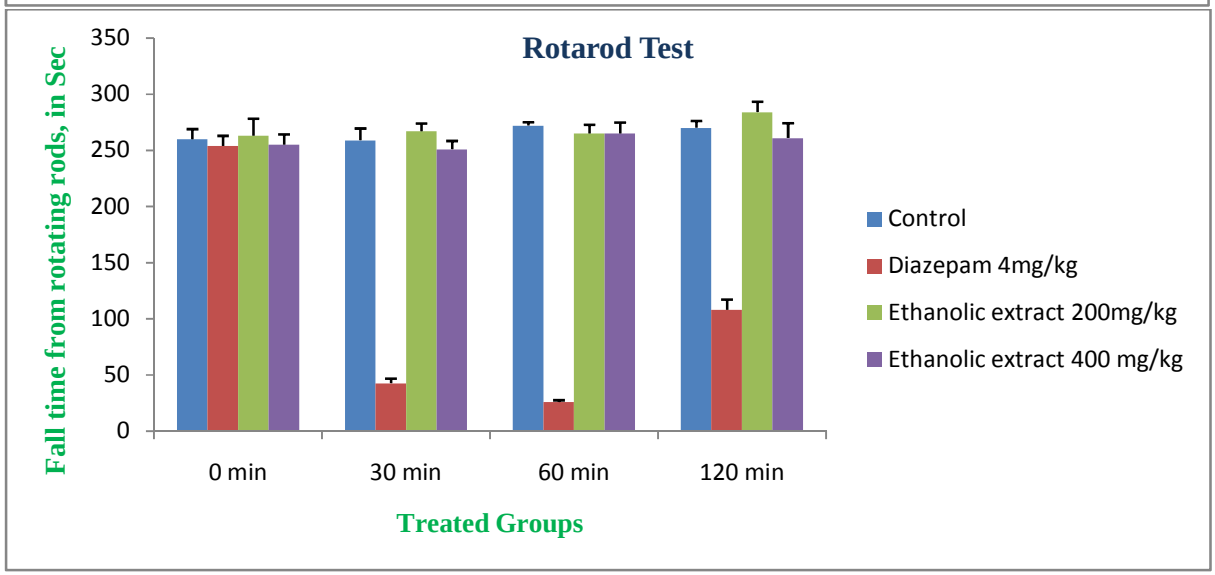
4.6 Effect of ethanolic extract of *Adiantumcapillusveneris*(L) frond in albino mice by Tail Immersion test.

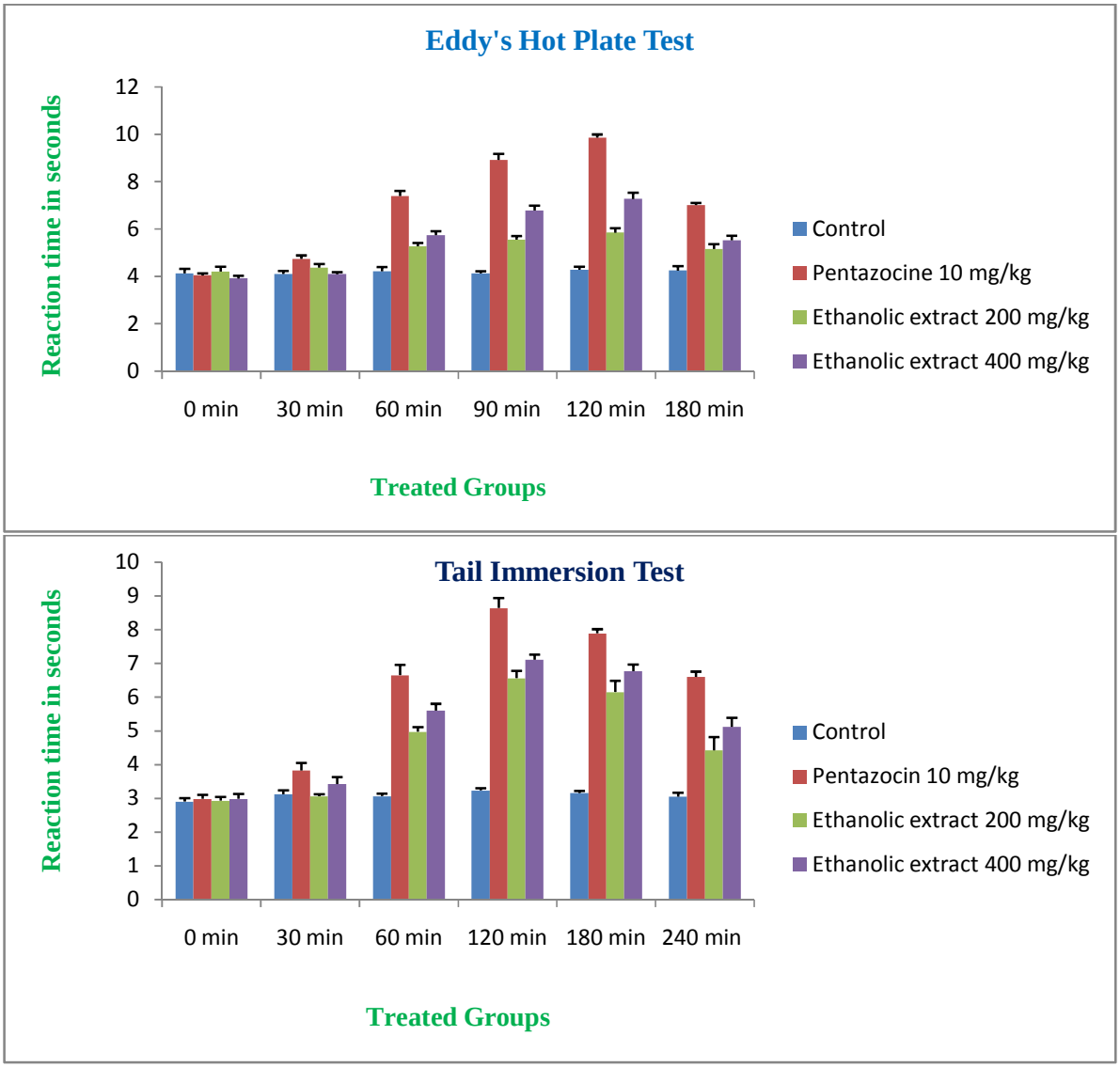
S. No.	Treatment	Tail withdrawal reflex time in seconds (Mean ± SEM)					
		0 min	30 min	60 min	120 min	180 min	240 min
1.	Control (1 % CMC Suspension)	2.90 ± 0.0998	3.12 ± 0.117	3.06 ± 0.0716	3.23 ± 0.0691	3.15 ± 0.0676	3.05 ± 0.114
2.	Pentazocin (10 mg/kg)	2.98 ± 0.118	3.82 ± 0.224*	6.65 ± 0.302***	8.64 ± 0.297***	7.89 ± 0.123***	6.60 ± 0.154***
3.	Ethanolic extract 200 mg/kg	2.93 ± 0.104	3.06 ± 0.0596	4.97 ± 0.141***	6.56 ± 0.218***	6.15 ± 0.322***	4.42 ± 0.389**
4.	Ethanolic extract 400 mg/kg	2.98 ± 0.150	3.42 ± 0.206	5.60 ± 0.199***	7.11 ± 0.147***	6.77 ± 0.197***	5.12 ± 0.262***
P Value		0.9525	0.0198	<0.0001	<0.0001	<0.0001	<0.0001






Forced Swim Test
Immobility time in Seconds
250
200
150
100
50
0
Control
Imipramine 10 mg/kg
Ethanolic ectract 200 mg/kg
Ethanolic extract 400 mg/kg
Duration of Immobility
Treated Groups


Rotarod Test
Fall time from rotating rods, in Sec
350
300
250
200
150
100
50
0
0 min
30 min
60 min
120 min
Control
Diazepam 4mg/kg
Ethanolic extract 200mg/kg
Ethanolic extract 400 mg/kg
Treated Groups



CONCLUSION

Anticonvulsant, antidepressant, skeletal muscle relaxant and analgesic effects, and also acute toxicity (LD50 for p.o route) of an ethanol extract of the fronds of *Adiantumcapillusveneris*(L) were evaluated in this study. The outcome of the present study demonstrates that *Adiantumcapillusveneris (L)* produced widespread effects on the CNS.

Phytochemical screening of the ethanolic extract showed that the *Adiantumcapillusveneris* (L) fronds contain alkaloids, phenols, flavonoids, terpenoids and some glycosides. Its anti-convulsant, depressant and analgesic activities might be due to the presence of such chemical constituents. However, flavonoids, phytosterols, phenolic compounds, tannins, fatty acid are reported in many neuro-pharmacological activities and in different experimental seizure models.

In conclusion of the experimental work, ethanolic extract of *Adiantumcapillusveneris*(L) at doses 200mg/kg and 400 mg/kg body weight of animal possess neuropharmacological properties like anticonvulsant activity, depressant and analgesic activity in mice. Finallyethanolic extract of *Adiantumcapillusveneris* (L) at dose 400 mg/kg body weight was found to more effective than extract at dose 200 mg/kg body weight.

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