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EVALUATION OF ANTIEPILEPTIC ACTIVITY OF ETHANOLIC EXTRACT OF POPULUS DELTOIDES LEAF IN MICE

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ABSTRACT

Populus deltoides leaf commonly known as eastern cottonwood belongs to the Salicaceae family. In the present study, Ethanolic extract of *Populus deltoides* leaf (EEPL) was tested for anticonvulsant activity in albino mice, using different convulsive models. The Ethanolic extract of *Populus deltoides* leaf produced significant anticonvulsant activity in all models studied. The present study also revealed that EEPL increase the concentration of nor-adrenaline and dopamine in brain of mice which attributed to the significant protection against MES induced seizures. Significant results were observed in the estimated parameters thereby justifying the use of this medicinal plant in the treatment of epilepsy.

KEYWORDS: Epilepsy, *Populus deltoides*, maximal electroshock, pentylenetetrazole, strychnine, seizures, Nor-adrenaline, Dopamine.

INTRODUCTION

Epilepsy is defined as recurrent and unprovoked epileptic seizures.[1] The loss of consciousness, violent spasmodic contractions of skeletal muscles (convulsions) and autonomic hyper activity is usually associated with epilepsy.[2] Epilepsy is one of the central nervous system disorder that affect wide range of peoples.[3] The exact cause of epilepsy is not known however, it is believed that it occurs due to uncontrolled high frequency discharge of impulses by a group of neurons in the brain.[4]

The various mechanisms which involves the development of seizures either normal or pathologic brain are, 1) Diminition of inhibitory mechanism (especially synaptic inhibition due to GABA) 2) Enhancement of the excitatory synaptic mechanism (especially those

mediated by NMDA). 3) Enhancement of endogenous neuronal burst firing (usually by enhancing voltage dependent calcium currents).[5]

Many mechanisms involved in overcoming to the seizure like enhancement of GABA-mediated inhibition, suppression of rapid repetitive firing, reduction of current through T-type Ca^{++} channels, reduction of excitatory glutaminergic neurotransmission.[6]

In this modern era of medicine there are 20 medications suggested by the Food and Drug Administration for the use of treatment of epileptic seizures. But, these drugs posseses various limitations in the form of serious side effects.[7] Thus due to prevalent side effects and tolerance development of the presently available treatment for epilepsy, it is a necessary to develop a new drug without or less side effect.[8, 9]

MATERIALS AND METHOD

Plant material

The leaf of *Populus deltoides* were collected in spring (Jun to September) from Puranpur, Pilibhit district, Bareilly, Uttar Pradesh, India. The plant was identified and authenticated by Dr. N. M. Ganesh Babu, senior botanist at FRLHT (Foundation for Revitalisation of Local Health Traditions) Jarakabande Kaval; post Attur, Yehalanka, and Bangaluru (560106). A herbarium specimen was preserved in the college museum for future reference.

Extraction procedure[10-12]

The leaf of *Populus deltoides* was dried under shade and then powdered with a mechanical grinder. The powder was stored in an air tight container for further use. The coarse powder was extracted directly with 65% ethanol (hydro-ethanolic extract) after defating with petroleum ether by Soxhelet extraction for 72 hours. The extracts were concentrated under reduced pressure and stored in a dissector until further use.

Animals

Swiss albino mice (20-25g) were selected and procured from Bioneeds, Daverhosaholli, Sonapura hobli, Nelamangala (Tq), Tumkur (Dist.), Karnataka, India. They were acclimatized for one week under standard laboratory conditions (20-25°C, and elimination cycle set to 12 hour light and 12 hour in dark) and fed with standard Amrut brand pellet diet and UV purified and filtered water was provided *ad libitum* in polypropylene bottles with stainless steel sipper tubes. Each group contains six animals and was used for the mentioned

research activities. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Office of institutional animal ethical committee (IAEC) of Mallige College of pharmacy, Bangalore (IAEC 1432/PO/a/11/CPCSEA.).

Drug and Chemicals

The ethanolic extract of Populus deltoides leaf was used as experimental extract. Phenytoin (Karnataka fine chem- Bangalore, India) used as standard antiepileptics agent. Pentylenetetrazole, (PTZ) (Karnataka fine chem- Bangalore), Strychinine (S D fine Chemicals – Mumbai) were all dissolved in normal saline. Dopamine, Nor-adrenaline (Neon Laboratory LTD – Mumbai), Adrenaline (Medlife Health Science LTD – Mumbai), Methanol (Nice Chemicals Private Limited, Kerala, India).

Preliminary phytochemical studies[10-13]

Ethanolic extract of *Populus deltoides* was subjected to qualitative chemical tests for the identification of their active constituents. Test for the presence of carbohydrates, glycoside, resin, tannin, alkaloid, flavanoid, terpenoid, protein, saponin, anthraquinone, Coumaric, narcission, ascorbic acid, fibers, vitamins, iron, minerals, protein and amino acid were conducted as per the standard procedure. And the results obtained are presented in table 5.

Screening of Antiepileptic activity

Electroshock Induce Convulsion

Electro-convulsion shock, induce Hind Leg Tonic Extension (HLTE) in 99% of the animal. The MES test, developed by the Toman and Collaborators more than 60 years ago and probably the best validated test that predict drugs effective against generalized seizure of the tonic-clonic type. MES induce tonic extension has been suggested to identify drug that are effective in preventing seizure spread through neural tissue.[14]

Mice weighting 20-30g received an electrical stimulus of 50 mA for 0.2 sec through auricular electrode to induce maximal seizure of their hind limbs, with tonic extension as the endpoint of the test.

The MES induced convulsion in animal represent grandmal type of epilepsy. This test can easily be conducted and requires a minimal investment in equipment and technical expertise, and is well standardized.[15]

Experimental design

Animal were divided in 4 groups of 6 animals in each. Group I served as the control (0.9% saline, *p.o.*), Group II served as standard treated with phenytoin (25mg/kg, *i.p.*) and group III and IV served as the low dose of EEPL (250mg/kg, *p.o.*) and high dose of EEPL (500mg/kg, *p.o.*) respectively. Treatment of EEPL to Group III and Group IV was given for 21 days. On 21st day after 30 minutes of *i.p.* injection of phenytoin and 60 minutes of oral administration of extract electric shock was given. Immediately after giving the electric shock, hind leg extension was observed. Decreased in the duration of hind leg extension was considering as a protective action and determine for each group.

Group	Treatment
Group I	Vehicle control (0.9%saline + electric stimulus(50mA;2 sec-duration)
Group II	Phenytion (25mg/kg, *i.p.*) + electric stimulus(50mA;2 sec-duration)
Group III	Test drug (250mg/kg, *p.o.*) + electric stimulus(50mA;2 sec-duration)
Group IV	Test drug (500mg/kg, *p.o.*) + electric stimulus(50mA;2 sec-duration)

Pentylenetetrazole Induce Convulsion

Pentylenetetrazole (PTZ) has been used widely to induce the convulsion since this chemical is highly sensitive for comparing different chemical under standard condition.[16] It is consider as the model for the petit-mal epilepsy. PTZ may elicit seizure by inhibiting gabaminergic mechanism.[17-19] The dose of PTZ (80 mg/kg, s.c.) used in this study was determined and found to be approximate minimal dose(CD_{100}) that induce convulsion in 100% mice (i.e. control group). Mice that received PTZ were observed for 30 minutes.[15]

Experimental design

Swiss albino mice of either of sex were divided in the 4 group, each containing 6. Group I was consider as the vehicle control (0.9% saline, *p.o.*), group II was consider as the standard treated with Phenytion (25mg/kg, *i.p.*) and group III and group IV was consider as the low dose of EEPL (250mg/kg, *p.o.*) and high dose of EEPL (500mg/kg, *p.o.*) respectively. On the 21st day of the experiment, after the administration of the phenytoin and Ethanolic extract of *Populus deltoides*(EEPL), 30 minutes and 60 minutes respectively, PTZ injection 80mg/kg, *i.p.* was given. The animals were observed individually in the cage for an hour. The time interval between PTZ-injection and occurrence of seizures were measured. The delay of onset was calculated in comparison with the control group.

Group	Treatment
Group I	Vehicle control (0.9%saline + PTZ (80mg/kg, *i.p.*)
Group II	Phenytion (25mg/kg, *i.p.*) + PTZ (80mg/kg, *i.p.*)
Group III	Test drug (250mg/kg, *p.o.*) + PTZ (80mg/kg, *i.p.*)
Group IV	Test drug (500mg/kg, *p.o.*) + PTZ (80mg/kg, *i.p.*)

Strychnine induce convulsion

Experimental design

Swiss albino mice of either of sex were divided in the 4 group, each containing 6. Group I was consider as the vehicle control (0.9% saline), group II was consider as the standard treated with Phenytion (25mg/kg, *i.p.*) and group III and group IV was consider as the low dose of EEPL (250mg/kg, *p.o.*) and high dose of EEPL (500mg/kg, *p.o.*) respectively. On the 21st day of the experiment, after the administration of the phenytoin and Ethanolic extract of *Populus deltoides*(EEPL), 30 minutes and 60 minutes respectively, strychnine nitrate (2mg/kg, *i.p.*) was given. The time until occurrence of tonic extensor convulsion and death were noted during one hour period.

Group	Treatment
Group I	Vehicle control (0.9%saline + strychnine nitrate (2mg/kg, *i.p.*)
Group II	Phenytion (25mg/kg, *i.p.*) + strychnine nitrate (2mg/kg, *i.p.*)
Group III	Test drug (250mg/kg, *p.o.*) + strychnine nitrate (2mg/kg, *i.p.*)
Group IV	Test drug (500mg/kg, *p.o.*) + strychnine nitrate (2mg/kg, *i.p.*)

Statistical Analysis

All the values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey's posthoc tests. P values < 0.01 were considered as stastically significant.

RESULTS

Table 1: Effect of Ethanolic extract of *Populus deltoidesleaf* (EEPL) on Maximal electroconvulsive shock (MES) induced seizures in mice.

Group	Treatment	Flexion (sec)	Extensor (sec)	Clonus (sec)	Stupor (sec)	% protection against extensor	% protection against mortality
I	Vehicle control (0.9% saline, *p.o.*)	2.217±0.1078	18.00 ±1.592	11.60±0.7638	16.65±3.435	0	40
II	EEPL (250mg/kg, *p.o.*)	1.683±0.1138**	12.33±1.022**	9.217±0.5974*	11.13±1.415*	28.64	65.66
III	EEPL (500mg/kg, *p.o.*)	1.163±0.1054***	8.600±0.6708***	5.333±0.1978***	6.550±0.2527**	54.26	65.66
IV	Phenytoin (25mg/kg, *i.p.*)	0.7000±0.09661***	1.923±0.7177***	3.283±0.1046***	4.533±0.1783***	89.35	100

Values are expressed as mean ± SEM (n=6)

Statistical significant test for comparison was done by ANOVA, followed by Tukey's posthoc Test

*p<0.05, **p<0.01 and ***p<0.001 as compared to control group.

Table 2: Effect of ethanolic extract of *Populus deltoidesleaf* (EEPL) on PTZ induced epilepsy in mice.

Group	Treatment	Onset of convulsion (sec)	% protection against convulsion	% mortality
I	Vehicle control (0.9% saline, *p.o.*)	154.8±8.101	0	100
II	EEPL (250mg/kg, *p.o.*)	206.3±7.531**	33.05	66.66
III	EEPL (500mg/kg, *p.o.*)	226.3±11.14***	51.66	50
IV	Phenytoin (25mg/kg, *i.p.*)	282.5±15.15***	87.74	33.33

Values are expressed as mean ± SEM (n=6)
Statistical significant test for comparison was done by ANOVA, followed by Tukey's posthoc Test
*p<0.05, **p<0.01 and ***p<0.001 as compared to control group.

Table 3: Effect of Ethanolic extract of *Populus deltoidesleaf* (EEPL) on Strychnine induced epilepsy in mice.

Group	Treatment	Onset of convulsion (sec)	% protection against convulsion	% mortality
I	Vehicle control (0.9% saline, *p.o*)	153.2±7.236	0	100
II	EEPL (250mg/kg, *p.o.*)	194.0±8.383**	26.45	50
III	EEPL (500mg/kg, *p.o.*)	229.8±5.231***	49.67	33.33
IV	Phenytoin (25mg/kg, *i.p.*)	289.0±12.38***	88.06	16.66

Values are expressed as mean ± SEM (n=6)
Statistical significant test for comparison was done by ANOVA, followed by Tukey's posthoc Test
***p<0.001 as compared to control group.

Table 5: Phytoconstituents present in 65% Ethanolic extract of *Populus deltoides*leaf.

Serial no.	Phytoconstituents	Result
2	Alkaloids	Present
3	Saponins	Present
4	Flavonoids	Present
5	Phenolics	Present
6	Total Flavonoids	Present
7	Glycosides	Present
8	Proteins	Present
9	Amino acids	Present
10	Phytosterol	Present
11	Tannins	Present
12	Terpenoids	Present
13	Polypherol	Present

DISCUSSION

Epilepsy is a chronic neurological condition, characterized by recurrent seizures that are caused by abnormal cerebral nerve cell activity. The treatment of epilepsy is always a challenge for researchers and clinical practitioners. However, several new drugs have been introduced for the treatment of epilepsy. Despite this progress, about 30% of patients with epilepsy are resistant to current pharmacotherapies and many of the available antiepileptic drugs. Based on this reason, there has been a continuous attempt to find new antiepileptic drugs which possessing multiple mechanisms of action with lesser adverse effect.[20]

MES induced seizures in the animal is most frequently used model for the identification of anticonvulsant activity of the drugs against generalized tonic-clonic seizure "grand mal" and coritical focal seizures. PTZ is a Chemoconvulsants, which induces seizures by the inhibition of GABA-A receptors and it is widely accepted experimental model for "generalized absence seizure"[21-23] and also a valid model for human generalized myoclonic seizures and generalized seizures of the petitmal type.

MES induced tonic extension can be prevented by AEDs that inhibit either voltage gated channels or by blocking glutamergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor.[24] AEDs have the ability to open K^+ channels in neuronal cells; can prevent seizure induced by PTZ and MES. Currently used anticonvulsant drugs (e.g. phenytoin, carbamazepines) effective in therapy of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action in MES test.[25-28]

The treatment with EEPL on mice significantly reduced tonic hind leg extension in MES induced epilepsy. Since, EEPL significantly inhibited generalized tonic-clonic seizures in MES test; which may be either inhibiting voltage gated channel or blocking glutamergic transmission.

PTZ is non-competitive antagonist which blocks GABA mediated Cl^- influx and leads to enhanced release of excitatory neurons to release NTs such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and N-methyl-D-aspartic acid (NMDA). This results in neuronal excitotoxicity leading to seizures and convulsions characterized by clonic and tonic phases.[29] so the NMDA receptor antagonist ketamine, diazocilpine have reported the effect against PTZ induced seizure.[30]

The antagonism of PTZ-induced seizures suggests the interaction of the Ethanolic extract *Populus deltoidesleaf* either with the potentiating of GABAergic neurotransmission system and/ or inhibition of NMDA receptors.

Strychnine may cause seizures by activation of excitatory amino acid NMDA receptor. Since Strychnine has been shown to interact with the NMDA neurotransmission thus Strychnine-induced seizures can be prevented by drugs that antagonize NMDA-receptor-mediated excitatory neurotransmission.[31]

There are five established biogenic amine neurotransmitters, of which there are three catecholamines Dopamine, Norepinephrine (Nor-adrenaline) and Epinephrine (Adrenaline), and other biogenic amines are histamine and serotonin.[32]

The brain amine estimation was done by the HPLC method in which increase of amine level in the forebrain region of treated mice shows the significant antiepileptic activity. Increase of brain monoamine level by inhibiting the monoamine oxidase (MAO), an enzyme responsible for the seizure by destruction of amines in brain. Inhibition of prostaglandin synthesis increases the levels of dopamine and nor-adrenaline, which also cause an inhibition of seizure activity.[33, 34]

In the present study the administration of EEPL significantly increased the brain levels of dopamine and nor-adrenaline attributed to the significant protection against MES induced seizures (Table 5).

CONCLUSION

The present study suggests that the Ethanolic extract of *Populus deltoidesleaf* has significant antiepileptic activity against various models of epilepsy in experimental mice. This activity of *Populus deltoides leaf* might be due to presence of flavonoids (rutin & quercetin active principles of extract). Further studies and tests are required to evaluate the exact mechanisms, specific active principles, and the safety profile of the plant as a medicinal remedy for convulsion.

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