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PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTICONVULSANT ACTIVITY OF ETHANOLIC EXTRACT OF GMELINA ARBOREA ROXB ROOT

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ABSTRACT

Medicinal plants are widely used by the traditional medicinal practitioners to cure various diseases due to their world-wide availability and less side effect. The present investigation was aimed at investing phytochemical constituents and anticonvulsant activity of ethanolic root extracts of Gmelina arborea Roxb. The phytochemical investigation is done by using various chemical analysis and standard methods. The anticonvulsant effect of ethanolic extract was studied by using maximum electroshock (MES) or pentylenetetrazole (PTZ) in male mice. The root extract of G. arborea (orally) was administered in mice at the doses of 100 and 200 mg/kg. The extract suppressed hind limb tonic extensions (HLTE) induced by MES and also exhibited protector effect in PTZ-induced seizures, at 200 mg/kg dose. Data from this study show that the extract significantly increases the onset time and decreases the duration of seizures by electroconvulsive shock. The study also revealed that the onset of tonic convulsion produced by PTZ was significantly delayed and also duration of seizures was prolonged. it is concluded that the ehanolic root extract of G. arborea exert their anticonvulsant effect.

KEYWORDS: Gmelina arborea, Anticonvulsant effects, Maximal electroshock, Pentylenetetrazole.

INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons.[1] Globally, there are nearly 50 million people suffering from epilepsy, 80%

of which are in the developing countries and 90% of these do not receive appropriate treatment. India alone has approximately 8-10 million epileptics. Epilepsy affects not only the individual, but also has consequences for the family and the rest of society.[2] In developing countries like India, a majority of people who live in the rural areas almost exclusively use traditional medicines in treating all sorts of diseases. Gmelina arborea Roxb. belonging to family (Verbenaceae) locally named as Gambhari (Oriya), were frequently distributed and extensively used traditionally by the tribal people for curing their aliments. Gmelina arborea Roxb. (Verbenaceae) belongs to a genus of trees and shrubs distributed chiefly in South East Asia, tropical Australia and tropical Costa Rica.[3,4] Gmelina arborea Roxb. belonging to family Verbenaceae locally named as Gambhari (Oriya), Gambhar (Hindi), Gambhar (Bengali), Sriparni (Sanskrit) and Gummadi (Telgu).[5] Flowering takes place during February to April when the tree is more or less leafless whereas fruiting starts from May onwards up to June. Flowers occur in narrow branching clusters at the end of branches. The yellow flower, tinged with brown, is trumpet shaped, 3-4 cm long. The trumpets flare open into a gaping mouth with 5 distinct lobes.[6] The root of this plant has been used in traditional Indian systems of medicines as a demulscent, stomachic, bitter tonic, refrigerant, laxative, and galactagogue. The tender leaves are used as demulscent, in headache, fevers, gonorrhea, cough etc. The whole plant is used in snake bite and scorpion sting throughout India.[7] As per the folkore medicine the root decoction is used in folk remedies for, demulcent, stomachic, and tonic, diarrhea, dropsy, dyspepsia, epilepsy, fever, gout, headache, hemorrhage, rheumatism, smallpox, snakebite, sores, sore throat, stomachic and urticaria. Ayurvedics. prescribe them for alopecia, anemia, consumption, leprosy, thirst, and vaginal discharges; the flowers for blood disorders and leprosy; the root, deemed anthelmintic, laxative and stomachic, for abdominal pains, burning sensations, fever, hallucinations, piles and urinary discharges.[8,9] According to scientific studies, the root decoction is used as a folk remedy for abdominal tumors. The roots are useful in hallucination, piles, abdominal pains, fevers, ‘tridosha’ and urinary discharge.[10,11] Traditional people are using to get relieve from Post delivery weakness. They are using half glass of boiled root extract. The extract is prepared by boiling roots with one glass of water till it gets reduced to half aglass. The plant has also been reported to have anti-inflammatory activity hypoglycaemic and anti-viral activities against Ranikhet disease virus.[12]

MATERIAL AND METHODS

Collection and authentification of plant

The G. Arborea roots were collected from the campus of Jeypore college of pharmacy, Jeypore, Koraput district. (India) in the month of sept. 2016. The plant was identified, confirmed and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-517/16, dated (10.11.2016).

Drugs and Chemicals

Diazepam was procured from Ranbaxy, India, and Pentylenetetrazole from Sigma, USA as a gift sample. The ethanol AR procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. All other chemicals and reagents used in present work were procured from authorized dealer.

Animals

Albino mice of either sex weighing between 20-30g were used from the experiment from the animal houhse of Jeypore college of pharmacy, Jeypore, Odisha. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet. Water was allowed ad libitum under hygienic conditions at room temperature with 12h light and dark cycles. All the studies conducted were approved by the Institutional Animal Ethical Committee (1906/PO/Re/S/16/CPCSEA), Jeypore college of pharmacy, Jeypore, Odisha according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. and Technology University.

Preparation of extracts

After authentification, the collected plant parts barks were separated from undesirable materials & washed thoroughly with water several times. They were sun-dried for one week and then dried in an oven to make it suitable for grinding. The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol as solvent. A total amount of 650 g coarse powder was extracted with 1000 ml of solvent.10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain the crude extract. The crude extracts were kept in closed air tight containers under cool and dark place for further study.[13,14]

Priliminary phytochemical investigation

The crude ethanol, extracts of the root of G. arborea were subjected to preliminary phytochemical analysis showed the presence of Alkaloids, Carbohydrates, Flavonoids, glycoside, Proteins and amino acids, Steroids, tannins, Saponins, Triterpinoid etc by chemical analysis.[15,16]

Table 1: Phytochemical screening of Gmelina arborea root extract.

Extract	Alkaloids	Flavonoids	Steroids	Glycoside	Protin & aminoacid	Tannins	Saponins	Terpenoids
Ethanol extract	+	+++	+	++	---	++	++	--

+++, Strong; ++, moderately; +, poor presence; --, absence

Determination of LD 50

The acute toxicity of root extracts of G. arborea was determined by using albino mice of either sex weight between (20-25 g), maintained under standard conditions. The animals were fasted for 3 hr prior to the experiments. Animals were administered with single dose of ethanol root extract of G. arborea and observed for its mortality up to 48 hr study period (short term toxicity). Based on the short-term toxicity profile, the next dose was decided as per OECD guidelines No 425. Since no mortality was observed upto dose 2000mg/kg From the LD50 dose, 100 mg/kg and 300 mg/kg doses were selected and considered as low and high doses respectively.[17]

Experimental Methods

In maximum electroshock induced seizure model, Electroconvulsive shock (50 mA for 0.2 sec) was delivered through ear electrodes to induce hind limb tonic extensions (HLTE) in mice. The extract was administered orally at the doses of 100 and 200mg/kg into test groups. Gum acacia in water and Diazepam (4mg/kg) were administered orally into two groups of animals as control and positive control groups, respectively. Electroconvulsive shock was delivered 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted closely for 2 min. The animals that did not exhibit HLTE were considered protected Percentage of inhibition of seizures relative to controls was calculated and in PTZ Induced seizures method, PTZ at the dose of 80 mg/kg (minimal dose needed toinduce convulsions) was injected i. p. to induce clonictonic convulsions in mice. Doses of 100 and 200mg/kg of the extract were administered orally into test groups. Gum acacia in water and Diazepam (4 mg/kg) were administered orally into two groups of animals as

control and positive control groups, respectively. PTZ was injected i. p. 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected. Percentage of inhibition of seizures relative to controls was calculated.[18,19]

Table 2: Effect of Ethanolic Root Extract of Gmelina arborea on tonic seizures induced by maximal electroshock in mice.

Treatment Group	Dose mg/kg (p. o)	Onset time (Sec)	Duration of HLTE (Sec)	Percentage inhibition of convulsions
Control (Group-I)	1ml/kg	2.18±0.42	107.5±2.71	-----
Diazepam (Group-II)	4	0	0	100
Ethanolic extract (Group-III)	100	5.47±0.69*	61.17±1.7*	43.09
Ethanolic extract (Group-IV)	200	12.06±0.76*	33.56±4.08*	68.78

Values are given as mean + SEM for six rats in each group. Results are statistically significant at *P<0.001 as compared with control.

Table 3: Effect of Ethanolic Root Extract of Gmelina arborea on pentylenetetrazole induced Seizures in mice.

Treatment Group	Dose mg/kg (p. o)	Onset time (Sec)	Duration of HLTE (Sec)	Percentage inhibition of convulsions
Control (Group-I)	1ml/kg	58.63±2.27	47.18±3.12	-----
Diazepam (Group-II)	4	0	0	100
Ethanolic extract (Group-III)	100	86.23±2.12*	32.21±1.63*	31.72
Ethanolic extract (Group-IV)	200	97±1.44*	18.06±1.53*	61.72

Values are given as mean + SEM for six rats in each group. Results are statistically significant at *P<0.001 as compared with control.

Statistical analysis

The data are represented as mean ± SEM, and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey posttest where $P < 0.001$) was considered statistically significant.[20]

RESULTS AND DISCUSSION

The preliminary phytochemical screening of G.arborea root extracts showed that alkaloids, flavonoids, saponins, steroids. glycoside, tannin and saponin were present but terpinoid, Protin & aminoacid were abscent, Which shown in (Table no.1)**.** In maximum electroshock induced seizure model, Albino mice pretreated with the ethanolic extract have been significantly protected from convulsions induced by electroshock one hour post-dosing. The percentage inhibition achieved at the doses 100 and 200mg/kg were 43% ($p<0.001$) and 69% ($p<0.001$) respectively. Extract at both the doses, prolonged the onset of convulsions in the extract treated group compared to vehicle treated control group (Table no 2).all so in PTZ methods, Animals treated with ethanolic extract at a dose of 200mg/kg showed alteration in the occurrence of HLTE and duration of seizures significantly as related to controls in the model of convulsion induced by pentylenetetrazole in mice but did not alter at 100mg/kg. Percentage of inhibition of seizures for 200 mg/kg relative to controls was 44.21% (Table no 3).

CONCLUSION

Based on the above investigations, it may be concluded that the ethanolic extract of root of G. arborea exhibited significant anticonvulsant activity. These findings justify the traditional use of root of this plant in the control and/or treatment of convulsions and epilepsy. The presence of flavanoids may partially contribute the significant activity. Further detailed phytochemical investigations are required to identify the phytoconstituents responsible for the anticonvulsant activity.

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