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Research Article

EVALUATION OF ANTI EPILEPTIC ACTIVITY OF *GMELINA ARBOREA ROXB.* FRUIT EXTRACTS BY STRYCHNINE INDUCED TONIC CONVULSION IN MICE

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ABSTRACT

Gmelina arborea roxb. is found in the tribal areas of Koraput district, Odisha, India. It is extensively used traditionally by the tribal people as anthelmintic, antimicrobial, antidiabetic, hepatoprotective and antiepileptic. The present study is an attempt to explore the antiepileptic activity of different fruit extracts of plant *G. arborea* using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. The extracts were screened for nontoxic properties examined by acute toxicity study and evaluated for their antiepileptic activity. The antiepileptic activity of above extracts was evaluated by using strychnine induced tonic convulsion in Swiss albino mice. The extracts were found as nontoxic. All extracts were able to show antiepileptic activity at a single dose of 200 mg/kg body weight (b.w.). The activities are comparable with the standard drug such as Diazepam. The dose of ethyl acetate, n-butanol and petroleum ether extracts of *G. arborea* showed lesser antiepileptic activity than the standard drug diazepam except ethanol extract. Among all the solvent extracts, the ethanol extract showed better antiepileptic activity even in comparison with the standard drug. The data were verified as statistically significant by using one way ANOVA at 1 % level of significance ($p < 0.01$) followed by z-test.

Keywords: *Gmelina arborea*, antiepileptic, diazepam, strychnine, Swiss albino mice.

INTRODUCTION

Epilepsy is a brain disorder in which a person has repeated seizures (convulsions) over time. Seizures are episodes of disturbed brain activity that cause changes in attention or behavior, with or without characteristics body movement and loss of consciousness[1,2]. Epilepsy is a common neurological abnormality affecting about 1 % of the world population[3]. Strychnine acts by blocking the post synaptic inhibition produced by the inhibitory transmitter glycine, which leads to loss of synaptic inhibition resulting in generalized excitation and tonic convulsion (Seizure)[4,5]. The antiepileptic drugs acts against seizure either by inhibition of use dependent Na^+ channel, enhancement of GABAergic action (Gamma Amino Butyric Acid) or by blockage of NMDA receptors (N-methyl-D-aspartate)[6]. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects. Approximately 30 % of the patients continue to have seizures with current antiepileptic drugs therapy[7-9]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects[10]. The fruits of the plant *Gmelina arborea* roxb. are oval in shape, ¾ inches in length and are yellow in color. The fruits are sweet in taste and some times astringent[11,12]. The plant, *G. arborea* was reported to have several medicinal properties such as aphrodisiac, astringent, analgesic, antipyretic, antidiabetic, diuretic, anti-inflammatory and tonic characteristics[12]. The literature survey reveals that fruits of *G. arborea* contain cardiac glycosides and steroids. The ethanol extract contains alkaloids, carbohydrates, anthraquinone glycosides, gums, mucilages, tannins, phenolic compounds and flavonoids. The ethyl acetate extract contains gums, mucilages, proteins and amino acids. The n-butanol extract contains alkaloids, anthraquinone glycosides, gums, mucilages, tannins, phenolic compounds, triterpenoids, saponins and flavonoids. The petroleum ether extract contains alkaloids, carbohydrates, anthraquinone glycosides, proteins, amino acids, triterpenoids and saponinns[13]. The literature survey reveals that there were no reports scientifically on the antiepileptic activity of the fruit extracts of *G. arborea*. This prompted us to investigate the antiepileptic activity of *G. arborea* fruit extracts.

MATERIALS AND METHODS

Drugs and Chemicals

Diazepam was procured as gift sample from Sun Pharma, Gujurat, India. Strychnine (Ref. no. RM9828-25G and CAS No. 57-24-9) was purchased from Himedia Laboratory Pvt. Ltd., Mumbai, India. The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. n-butanol GR 80°C and petroleum ether AR 40-60°C were procured from Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals and reagents were procured from authorized dealer.

Collection of plant materials, identification and size reduction

The fruits of *G. arborea* were collected from local area of Koraput district (India) in the month of April and May 2008. The plant was identified 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/DBT (08)/1067, dated 05.09.2008). The fruits were shade dried under normal environmental conditions. The dried fruits were pulverized to form coarse powder by using electrical grinder and stored in a closed air tight container for further use.

Preparation of extract

The coarse powder form of dried fruits was extracted by Soxhlation method by using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. In this extraction process, a total amount of 1500 g coarse powdered fruits was extracted with 1200 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude

extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study.

Acute toxicity studies

To study the toxic effect (if any) of *G. arborea* fruit extracts, Albino mice of either sex weighing 20-25 g were used. The animals were kept in the standard polypropylene cages at 25 ± 2°C/ 60 % relative humidity in normal day and night photo cycle (12: 12 h). The animals were acclimatized for a period of 14 days prior to performing the experiments. Prior to the study, the experimental protocols were approved by the Institutional Animal Ethics Committee of Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha, India (Ethical Committee No 1339/ac/10/CPCSEA). Acute oral toxicity study was performed as per OECD–423 guidelines[14-16]. The animals were kept fasting overnight but allowed free access to water *ad libitum*. The fasted mice were divided into different groups of six animals each. Each solvent extract solution was administered orally at a dose of 10 mg/Kg b. w., using normal saline water as vehicle and mortality in each group was observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the same procedure was repeated in each group for each extract for further higher doses such are 100, 300, 600, 1000, 2000 and 3000 mg/Kg b.w. Antiepileptic activity

Animals

Healthy Swiss albino mice of either sex were used in the present study. They were housed in standard conditions of temperature (25 ± 2°C), relative humidity of 45-55 % in animal house of Gayatri College of Pharmacy. They were fed with a standard pellet diet and water *ad libitum*. Animals were caged in polypropylene cages and all operations on animals were done under aseptic condition.

Drugs

The extract of *G. arborea* was tested in a single dose in each group of experimental model (200 mg/kg b.w.). Diazepam was used as the standard drug in strychnine induced tonic convulsion of Swiss albino mice model in a dose of 5 mg/kg b.w. of mice[17].

Experimental protocol

Animals were selected, weighed (20-25 g) and divided in to six groups (n = 3), namely control, standard drug and four groups belonging to four different extract of *G. arborea*. Approval for the research work was obtained from the Institutional Ethical Committee of Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha, India.

Experimental Method

The convulsion inducing agent strychnine along with normal saline water was used to evaluate the acetylcholine level lowering capacity of plant extract in Swiss albino mice[17,18]. Swiss albino mice were divided into six groups (3 each). The group (I) served as normal control (Vehicle) which received normal saline water (2 ml/kg b.w.) only. The group (II) served as standard control which received Diazepam (5 mg/kg b.w.). Groups (III) to (VI) received a single dose of extracts (Test drugs) of ethanol, ethyl acetate, n-butanol and petroleum ether (200 mg/kg b.w.). The standard and test drugs were administered orally one hour before the intraperitoneal administration of strychnine in the dose of 5 mg/kg of b.w. of mice. The time required for the onset of tonic extensor convulsions, number of convulsions and time of death of the animals were noted during one hour period after strychnine injection.

Statistical analysis

Each value is expressed as mean ± standard deviation (n = 3). For determining the statistical significance, standard error mean and one way analysis of variance (ANOVA) at 1 % level significance was employed followed by z-test. P values < 0.01 were considered significant[19,20].

RESULTS AND DISCUSSION

Extraction (Soxhlation)

The Soxhlation method was found to be efficient for extraction of phytochemicals from fruit coarse powder by using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. The percentage yield of all the solvent crude extracts were found in the order of ethanol > n-butanol > ethyl acetate > petroleum ether.

Acute toxicity study

Acute toxicity study revealed that no mortality was found in any solvent extract at any dose in Swiss albino mice, which confirmed that *G. arborea* fruit extract would be non toxic in living body.

Table 1: Antiepileptic activity of *G. arborea* fruit extracts in strychnine induced seizure in mice

Group	Drugs	Dose (mg/kg)	O.O.A. (min) (X ± SD)	C.N. (X ± SD)	Quantal death	Survival (%)	T.O.D. (min)
I	Control (NSW)	2 ml/kg	6.2 ± 0.62	10.5 ± 0.16	All	0	8.4 ± 0.94
II	Diazepam	5	29.4 ± 0.31	2.7 ± 0.22	0	100***	-
III	Ethanol extract	200	31.2 ± 0.44	4.6 ± 0.20	0	100***	-
IV	E.A. extract	200	26.3 ± 0.70	6.1 ± 0.13	0	100***	-
V	n-butanol extract	200	24.6 ± 0.51	8.2 ± 0.11	1	80**	42.2 ± 1.02
VI	Pet. ether extract	200	23.5 ± 0.38	10.4 ± 0.1	2	60*	53.6 ± 0.83

ANOVA						
Source of variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	811.8075	1	811.8075	17.93251	0.00173	4.964602701
Within Groups	452.7017	10	45.270166			
Total	1264.509	11				

NSW- Normal saline water, O.O.A. – Onset of action, C.N. – Convulsion no, T.O.D. – Time of death, E.A. – Ethyl acetate; each values is represented as mean ± standard deviation (n = 3), Standard error of mean < 0.589. *P < 0.05, **P < 0.01 and ***P < 0.001 (test of significance between two proportions by z-test) in comparison to control. Data are found to be significant (F value < F crit) by testing through one way ANOVA at 1 % level of significance (p < 0.01 that is p = 0.0017309)

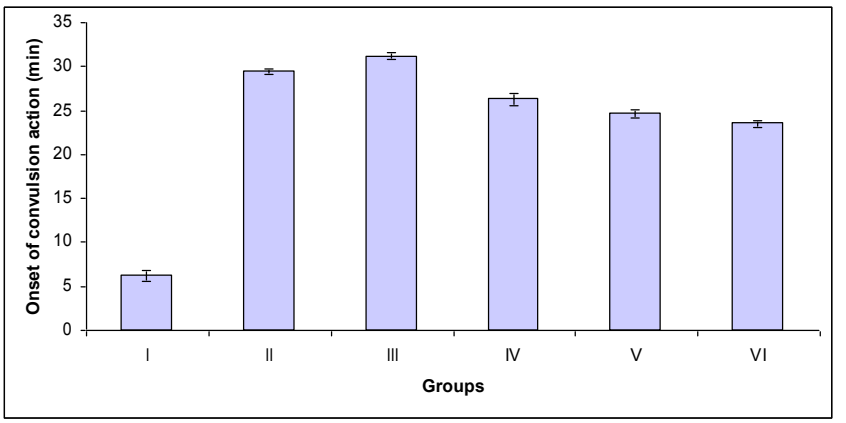

Figure 1: Onset of convulsion action in strychnine induced mice by administration of different drugs

Each bar is represented as mean ± standard deviation (n = 3). Group I – Control (Normal saline water), group II – Standard (Diazepam – 5 mg/kg), group III – Ethanol extract, group IV – Ethyl acetate extract, group V - n-butanol extract and group VI – Petroleum ether extract respectively at 200 mg/kg of b.w.

Antiepileptic activity

The extracts of *G. arborea* produced a significant anticonvulsant activity after 23 min in the dose of 200 mg/kg b.w., as shown in Table 1. The anticonvulsant activity of all the extracts were found in the order of ethanol > ethyl acetate > n-butanol > petroleum ether. The anticonvulsant effect of the extract obtained from different solvents was well comparable with the standard drug and control used in this study. It will be worth mentioning that although different constituents were extracted in different solvents as per polarity but ethanol fraction is more effective as compared to other solvent extracts, as the onset of convulsant action of strychnine was delayed in case of ethanol fraction with minimum convulsion number, having no quantal death showing 100 % survival. The activity shown by ethanol extract is of considerable importance and has justified its use in controlling the convulsion as suggested in the folklore medicine. By employing one-way ANOVA, all data were found to be statistically significant (F value < F crit) at 1 % level of significant ($p < 0.01$ that is $p = 0.0017309$) followed by z-test. The extent of activity shown by the ethanol crude extract is more than that of the standard drug Diazepam and many fold more than the control, which justifies its activity (As shown in Figure 1) by testing through one way ANOVA at 1 % level of significance.

CONCLUSION

It could be concluded that the plant *Gmelina arborea* is having anticonvulsant activity and better result is obtaining from extract of ethanol even more potent than standard drug Diazepam. Further studies are required to identify the actual chemical constituents present in the crude extracts of this herb which claims to produce anticonvulsant activity.

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