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ANTICONVULSANT ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF LEAVES OF *CALOTROPIS GIGANTEA* LINN

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ABSTRACT

Calotropis gigantea linn is a perennial shrub, commonly known as milkweed or wasteland weed. It belongs to family Asclepiadaceae and was evaluated for its anticonvulsant activity. The leaves were extracted with 70% ethanol and concentrated to obtain residue. The hydro-alcoholic extract (HLE) at a dose of 400 mg/kg b.w, 300 mg/kg b.w, 200 mg/kg b.w and 100 mg/kg b.w by i.p route were tested for their anticonvulsant property against Maximum Electric Shock (MES) induced convulsion model in *Swiss albino* mice of either sex weighing between 18 g -22 g. A significant ($p < 0.01$) anticonvulsant activity had been observed in the HLE (400 mg/kg b.w and 300 mg/kg b.w) followed by HLE (200 mg/kg b.w and 100 mg/kg b.w) when compared to control group. Phytochemical screening reveals the presence of alkaloids, cardiac glycosides, flavonoids, steroids, tannins, triterpenoids, carbohydrates and saponins in the hydro-alcoholic leaf extract of *Calotropis gigantea* linn.

Key words: Anticonvulsant, *Calotropis gigantea* linn, Hydro-alcoholic extract, Maximum electric shock.

INTRODUCTION

Calotropis gigantean linn. commonly known as milkweed or a wasteland weed belongs to family Asclepiadaceae. Being native to India, it grows wild up to 900 m throughout the country on a variety of soils and in different climates[1]. It is a slow growing variety of *Calotropis*. Leaves are simple, opposite and sub-sessile ovate and cordate at base. *Calotropis gigantea* contains latex in almost all parts of the plant. Flowers are beautiful, white in color, in umbellate lateral cymes. Fruits are fleshy follicles, green; seeds attached with abundant white coma[2]. Various chemical constituents have been reported from different

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parts of the plant[3]; Cardenolide glycosides such as Calotropin, Frugoside and 4`-0-beta-D glucopyranosyl frugoside were isolated from the roots of *Calotropis gigantea*[4]. The stem bark of *C. gigantea* yields resin and wax. The wax contains β-amyrin and its isovalerate, calotropeols-a and b, mixture of tetracyclic triterpene, traces of sterols, C31 and C33 hydrocarbons, fatty acids and giganteol[5]. The leaf contains ascorbic acid, ortho-pyrocatechic acid and also contains β-amyrin, taxasterol, tarasterol and β-sitosterol[1]. Two new cardenolides, 19-Nor and 18,20-epoxy-cardenolides were isolated from the leaves of *C. gigantea*[4]. Two 15β-hydroxycardenolides and a 16α-hydroxycalactinic acid methyl ester along with eleven known compounds were isolated from the polar fraction of the CH_2Cl_2 extract and *n*-BuOH extract of the *C. gigantea* leaves[6]. Ayurveda system of medicine recommends the use of *Calotropis gigantea* in the treatment of cutaneous diseases, intestinal worms, cough, ascites, asthma, bronchitis, dyspepsia, paralysis, swellings, intermittent fevers, anorexia, inflammations and tumours. In large doses, it acts as a purgative and emetic[7]. The leaves are useful in conditions like paralysis, swellings, wounds, ulcers, eczema, skin eruptions, cold sweats, asthma and intermittent fevers. The flowers are used to treat asthma, catarrh, anorexia, inflammations and fever[3,7]. Siddha system of medicine recommends the use of root, bark, leaf, flower, latex of *C. gigantea* in diseases of vattam and kapham, snake bite, rat bite poisonings, leprosy, convulsions, swelling in joints, worm infestations and skin diseases[8].

EXPERIMENTAL

Materials and methods

The leaves of *Calotropis gigantea* linn. were collected, washed thoroughly with water, dried under shade at room temperature and powdered using hand mill to make a coarse powder and stored in well-closed light resistant container until further used.

Preparation of the extracts

Powdered plant material is subjected for cold maceration with ethanol (70%), the solvent was then separated by filtration and the marc is air-dried. The air dried marc was subjected for extraction with alcohol using Soxhlet apparatus at 50^{o}C. Materials were extracted until liquid in the side arm of the Soxhlet apparatus became colorless. Mecilla were collected and combined with the macerates and subjected for solvent recovery using rotary evaporator. The extract is then dried in reduced pressure using vacuum. The dried extract is then stored at

low temperature (4°C) for further use. The extract is screened for phytochemical investigation as per protocol[9,10].

Pharmacological studies

Swiss albino mice of either sex weighing between 18-22 g were used for the experimental work. Animals were maintained under standard conditions husbandry, room temperature 24 ± 2°C, relative humidity of 45-55%, 12 hours dark-light cycle, in an animal house. The animals had free access to standard diet and water and housed in the poly-propylene cages. All the animals were kept for fasting 12 hours prior to the experiment but allowed to free access to water. The acute toxicity study was performed according to the OPPTS (Health Effect Test Guideline 2004, Office of prevention, pesticide and toxic substance) by Up and Down procedure using *Swiss albino* mice of either sex[11]. The hydro-alcoholic extracts were suspended in Tween 80 (1%w/v) and administered intraperitoneally. The safe dose was found to be up to 4500 mg/kg b.w.

Evaluation of anticonvulsant activity

In maximum electric shock induced convulsions (*in vivo*) model, the animals were divided into six groups with six animals in each group. The animals of I group served as solvent control, received distilled water (1 mL/100 gm b.w); II group received phenytoin (25 mg/kg b.w), treated as positive control; III, IV, V and VI groups treated with hydro-alcoholic leaf extract (HLE) at the dose of 100 mg/kg b.w, 200 mg/kg b.w, 300 mg/kg b.w and 400 mg/kg b.w, respectively. All the treated groups of animals were administered intraperitoneally 30 min prior to the electroshock. The electroshock was induced in the entire animals by passing a current of 45 mA for 0.2 sec duration through electro convulsiometer (Techno India) using ear electrodes. The duration of flexion, extensor, clonus and stupor phases were noted[12-14].

RESULTS AND DISCUSSION

The present study attempts the evaluation of leaf of *C. gigantea* for preliminary phytochemical studies and pharmacological screening. In this study, an attempt has been made to evaluate the anticonvulsant activity of hydro-alcoholic leaf extract of *C. gigantea* by using maximum electric shock induced convulsions (*In vivo*) in mice. Preliminary phytochemical screening reveals the presence of alkaloids, cardiac glycosides, flavonoids, steroids, tannins, triterpenoids, carbohydrates and saponins in the hydro-alcoholic leaf extract of *C. gigantea* (Table 1).

Table 1: Preliminary phytochemical constituents present in the hydro-alcoholic leaf extract of *Calotropis gigantea* Linn.

S. No.	Chemical constituent	Test	HLE
1.	Alkaloids	Mayer's test	+
		Wagner's test	+
		Dragendorff's test	+
		Hager's test	+
2.	Glycosides	Chrysorbin test	+
		Legal test	+
3.	Carbohydrates	Molisch test	+
		Fehling test	+
		Benedict's test	+
		Barfoed's test	+
4.	Proteins	Biuret's test	_
		Xanthoproteic test	_
		Libermann buchard test	+
5.	Steroids	Salkowski test	+
		Sulphur test	+
		Acetic anhydride plus H_2SO_4 test	_
6.	Tannins	Ferric chloride test	+
		Salkowski test	+
7.	Triterpenes	Libermann starch morawski test	+
		Hirschorn test	+
		Tschujawes test	+
8.	Flavanoids	Ferric chloride test	+
		Shinoda test	+
		10% NaOH	+
		10% Lead acetate	+
		Mineral acid test	+
		Zinc dust test	+
9.	Saponins	Liberman buchard sterol reaction	+
		Salkowski reaction	+

Evaluation of anticonvulsant activity

Convulsion is one of the CNS disorder affecting brain, which is characterized by paroxysmal cerebral dysarrythmia, brief episodes of loss or disturbance of consciousness with or without characteristic body movements with excessive EEG discharge. Convulsion occurs due to imbalance between excitatory and inhibitory influences in brain. Inhibitory influence involves GABA as the neurotransmitter and increases the extra-cellular K^+ and excitatory neurotransmitters involves opening of voltage dependant Na^+ channels.

Maximum electric shock induced convulsions (*in vivo*)

Swiss albino mice were used for the screening of anticonvulsant activity in MES induced convulsions. The experiment had been performed, where convulsions were induced after 30 mins to each group of animals, following the i.p administration of hydro-alcoholic leaf extract as well as the standard drug phenytoin. Hydro-alcoholic leaf extract exhibited a significant reduction in various phases of epileptic seizures on comparison with the standard drug phenytoin (25 mg/kg b.w). There was also a significant reduction in the time required for righting reflex (recovery) in treated groups (Table 2).

Table 2: Effect of hydro-alcoholic leaf extract of *Calotropis gigantea* Linn. on maximal electric shock induced convulsion in mice after 30 mins.

Groups	Drug used	Flexion (in sec.)	Extension (in sec.)	Clonus (in sec.)	Stupor (in sec.)	Recovery /Death
I	Control	30.92 ± 0.02	46.0;7 ± 0.03	59.02 ± 0.02	86.02 ± 0.01	Recovered
II	Phenytoin (25 mg/kg)	Nil	Nil	Nil	Nil	Recovered
III	HLE (100 mg/kg)	1.62 ± 0.02*	9.03 ± 0.19*	21.34 ± 0.43*	35.01 ± 0.01*	Recovered
IV	HLE (200 mg/kg)	1.40 ± 0.01*	8.70 ± 0.02*	18.71 ± 0.20*	33.20 ± 0.12*	Recovered
V	HLE (300 mg/kg)	0.93 ± 0.01**	5.42 ± 0.02**	16.42 ± 0.02**	31.36 ± 0.01**	Recovered
VI	HLE (400 mg/kg)	0.20 ± 0.01**	3.10 ± 0.01**	10.37 ± 0.01**	16.42.37 ± 0.01**	Recovered

Values represent the mean ± SD of six animals HLE – Hydro-alcoholic leaf extract of *Calotropis gigantea* L, *= $p < 0.05$, ** = $p < 0.01$, (the mean difference was considered significant at 0.01 level)

Prominent anticonvulsant effect had been observed in HLE (400 mg/kg b.w and 300 mg/kg b.w) when compared to the control group. Whereas, standard drug phenytoin (25 mg/kg b.w) completely abolished the convulsion and its phases (Fig. 1). The result indicates that the extract increased the threshold of seizure in the MES test. It has been found that the drugs which raise the threshold for production of electrically-induced seizures are generally effective against absence seizures, where those that reduce the duration and spreads of electrically-induced convulsions are effective in generalized tonic-clonic seizures[15]. So, the anticonvulsant activity of HLE (400 mg/kg b.w and 300 mg/kg b.w) in maximal electroshock model indicates that they might precipitate the tonic-clonic seizures.

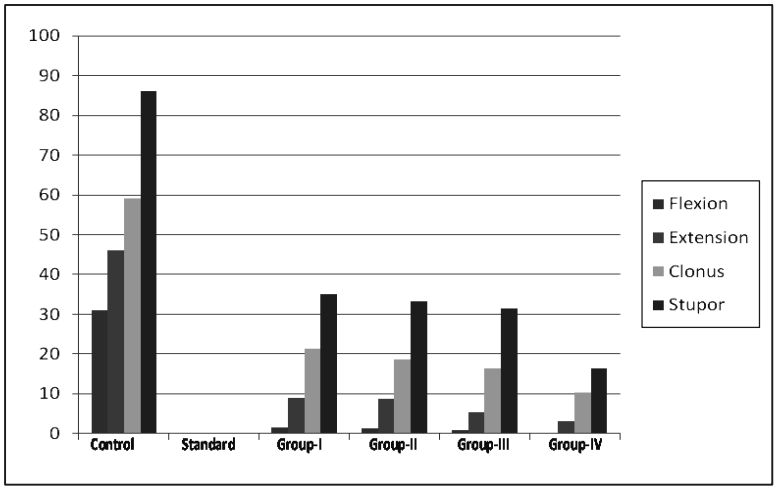

Fig. 1: Effect of hydro-alcoholic leaf extract of *Calotropis gigantea* Linn. on maximal electric shock induced convulsion in mice after 30 mins.

CONCLUSION

The preliminary phytochemical investigation of the extract revealed the presence of alkaloids, cardiac glycosides, flavonoids, steroids, tannins, triterpenoids, carbohydrates and saponins in the hydro-alcoholic leaf extract of *C. gigantea.* In acute toxicity study, the hydroalcoholic extract of *C. gigantea* were found to be safe and no mortality occurred to a dose as high as 4500 mg/kg b.w. Anticonvulsant activity was performed by using Maximal Electroshock (MES) induced model. All the treated groups showed reduction in duration of flexion, extension, clonus and stupor phases. The hydro-alcoholic extract showed significant ($p < 0.01$) activity at 400 mg/kg b.w and activity at 300 mg/kg b.w followed by 200 mg/kg b.w and 100 mg/kg b.w by abolition of all the phases of convulsion in MES model. So the

potency of anticonvulsant activity was found to be more with 400 mg/kg b.w and 300 mg/kg b.w by abolition of all the phases of convulsion in MES model. All the results indicate the broad spectrum anticonvulsant activity in absence seizures as well as tonic-clonic seizures. The actual phytoconstituents responsible for anticonvulsant activity is needed to be determined. Hence, there is a further scope for phytochemical investigation and activity guided isolation of active constituents from the leaves of *C. gigantea.*

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