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Evaluation of Phytochemical Screening Anti-Convulsant Activity of Ethanolic Extract of Plumbago Zeylanica

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ABSTRACT
The purpose of this study is to explore the anticonvulsant effect of the leaf gall extract of *Plumbago zeylanica* using pentylene tetrazole-induced convulsion (PTZ) in rats *Plumbago zeylanica* is an indigenous plant belonging to the family Fabaceae (Papilionaceae) commonly known as Karanj. Freshly powdered flowers were extracted with 70% ethanol. The convulsion is induced by the administration of pentylene tetrazole (80 mg/kg, i.p.) to wistar albino rats, and those showing response was divided into three groups of six animals each. The group I treated with 1% normal saline (1ml/100gm, orally), Groups II treated with phenytoin sodium (25\mg/kg, i.p.) and Groups III treated with ethanolic extract at a dose of (250 mg/kg, i.p.). The ethanolic extract showed significant anticonvulsant activity by lowering the duration of the extension phase (3.72 ± 0.65) when compared to the control group (8.94 ± 0.42). From the experiment, we can say *Plumbago zeylanica* had significant anticonvulsant activity.

KEYWORDS: *Plumbago zeylanica* Phenytoin sodium, Pentylene tetrazole induced convulsion, anticonvulsant activity

INTRODUCTION

Plumbago zeylanica is an herbaceous plant with glabrous stems that climb prostrate, or erect. The leaves are petiolate or sessile and have ovate, lance-elliptic, or spatulate to oblanceolate blades that measure 5-9 × 2.5–4 cm in length. Bases are attenuated while apexes are acute, acuminate, or obtuse. Inflorescences are 3–15 cm long, and have glandular, viscid rachises. Bracts are lanceolate and 3-7 × 1–2 mm long. The heterostylous flowers have white corollas 17–33 mm in diameter and tubes 12.5–28 mm in length. Capsules are 7.5–8 mm long and contain reddish-brown to dark-brown seeds.[1] Plumbago zeylanica L. (Synonym: P. viscosa Blanco)

(chromosome number 2n=24) is a multipurpose medicinal herb of the family Plumbaginaceae. A native of South Asia, the species is distributed throughout most of the tropics and subtropics; growing in deciduous woodland, savannas' and scrublands from sea-level up to 2000 m altitude[2,-5]

It has been recognized in different systems of traditional medicines for the treatment of different diseases and ailments of humans[6]. It contains several phytoconstituents belonging to category flavonoids and fixed oils. These candidates show antimicrobial and anthelmintic activity[7,8].

Leaf galls are a disturbing sight but are not usually as serious as they appear. These bumps and deformities are generally the result of feeding insects or some other foreign organism such as bacteria, fungi, mites, nematodes, and even viruses. The bumps are generally not caused by a disease. They are usually insect damage. For galling aphids and their hosts, tannins are crucial for plant-insect interactions and for protecting the host plant from herbivory. Due to their peculiar chemical characteristics, tannins from plant galls have been used for medical and chemical purposes for more than 2000 years. In this study, hydrolyzable tannin concentrations in galls increased from gall initiation (38.34% on June 21) to maturation (74.79% on August 8), then decreased gradually thereafter (58.83% on October 12). A total of 81 genes (named as GTS1-81) with putative roles in Gallo tannin biosynthesis and 22 genes (TS1-22) in condensed tannin biosynthesis. Multiple genes encoding 1-Beta-D- glucosyl transferases may play a vital role in Gallo tannin accumulation in plant galls [9]. Crown galls form as a result of wound inoculation on many species of plants by the soil-dwelling bacterium Agrobacterium tumefaciens.

Tannins are water-soluble polyphenols that are present in many plants and these are more abundant in vulnerable parts of plants, such as new leaves and flowers [10] In all vascular plants studied, tannins are manufactured by a chloroplast–derived organelle, the tannosome. Tannins are mainly physically located in the vacuoles or surface wax of plants. The two most important components of tannins are tannic acid and Gallic acid. Tannic acid is widely used in therapeutics, and it is derived from galls on the leaves, which are the result of parasitic insect activity. They can be effective in the treatment of various diseases by the multitude of their beneficial activities such as anti- inflammatory11, anti-oxidative [12], anti-convulsant [13], and antitumor behaviors[14]. The pivotal neuroprotective effects of tannins are related to their capacity to act as free radical scavengers and to activate the antioxidant system in the body, thereby including protection against neurotoxins and oxidative-stress-induced neuronal damage, neuronal inflammation, and some other factors.

The flowers and sprouts of *Plumbago zeylanica* were used in folk remedies for tumors[15] *Plumbago zeylanica*, commonly known as Ceylon leadwort, doctor bush or wild leadwort is a species of Plumbago with a pantropical distribution. Seed extract of this plant has hypotensive effects and produces uterine contractions. Powdered seed is used in bronchitis, chronic fever, whooping cough, and chronic skin diseases and painful rheumatic joints[16]. Leaves are active against Micrococcus; their juice is used for cold, cough, diarrhea, dyspepsia, flatulence, gonorrhea, and leprosy[17]. Roots are used for cleaning gums, teeth and ulcer. ***Plumbago zeylanica*** plant is used for anti-inflammatory[18], anti-plasmodial[19], anti-nonciceptive[20], anti-hyperglycemic, anti-lipid oxidative, antidiarrheal, anti-ulcer, anti-hyperammonic, CNS depressant activity[21] and antioxidant activity.

2. MATERIALS AND METHODS

2.1 Collection of Plant material

Collection and authentication of plant material The leaf galls of ***Plumbago zeylanica*** was collected from Maruthi Nagar, Guntur, Andhra Pradesh, India and material was identified and authenticated by Dr. B.R.C Murthy, reader in Botany, Hindu college, Guntur, India.

Preparation of test extracts: Crushed powders of species were successively soxhlet extracted. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were cooled individually. Each filtrate was concentrated to dryness in vitro and re-dissolved in respective solvents, were stored at 4°C in a refrigerator, until screened for phytochemical activity. Phytochemical Screening: Phytochemical screening was performed using standard procedure: Test for Reducing sugar (Fehling's Test): The aqueous extract (0.5gm in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a color reaction[22].

Test for Flavonoids: 4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was

warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid were added and red color was observed for flavonoids and orange color for flavones[23].

Test for Alkaloids: Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent. The alcoholic extract was heated in a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The sample was then observed for turbidity or yellow precipitation[23].

Test for Tannins: About 0.5 g of extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration[24].

Test for Terpenoids (Salkowski Test): To 0.5 gm each of the extracts was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish-brown coloration of the interface indicates the presence of terpenoids[25].

Test for carbohydrates: Phytochemical screening Plumbago zeylanicum shows that the plant has abundant amount of Secondary metabolites in it. The following table shows the results of tests performed. Preliminary phytochemical screening of the plant Plumbago zeylanica leaves (chitrak) [(+: present) (-: absent)[26].

Preparation of *Plumbago zeylanica* extract

After collection, the leaf galls and flowers were shade-dried, pulverized using an electrical grinder and passed through the sieve (40 mesh size). The extract was prepared cold maceration method by taking 100g of powder in ethanol and soaked about 7 days. The extract was filtered using muslin cloth, followed by Whatman filter paper (No.1) and concentrated at reduced temperature on rotary evaporator. The extract was evaporated to dryness to achieve the yield of 8% (w/w) of the extract and kept in a desiccator[27]. We made experiments to collect the fraction of extract using column chromatography with different solvents of varying polarity and finally the ethanolic fraction was selected for the study as it has given number of colored bands (Table 1). The 39th fraction (C-39) of ethanolic extract was collected and used for the evaluation of anti-convulsant activity.

Table no :1 Phytochemical screening of leaf gall and flower extract of Plumbago zeylanica.

Plant Extract	Reducing sugar	Flavonoids	Alkaloids	Glycosides	Tannins	Terpenoids	Carbohydrates	Saponins
Distilled Water	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Ethyl acetate	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Pet.ether	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
Benzene	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Methanol	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve
Chloroform	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve

2.3. Experimental Animals

Animals Five male white albino Wistar rats weighing 150-200g were selected and procured from the animal house of Hindu college of Pharmacy, Guntur. The animals were maintained in well-cages. They were fed with a rodent diet and water adlibitum. Animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) and guided by the Committee for Control and Supervision of Experiments on Animals (CPCSEA) with registration number 1263/PO/ac/09/CPCSEA, dated 05/05/2014. All efforts were made to minimize animal suffering and to reduce the number of animals used. The Institutional animal ethical committee has approved the protocol of the study[28].

2.4. Experimental design

The anticonvulsant activity of ***Plumbago zeylanica*** flower extract was evaluated for pentylene tetrazole-induced convulsion (PTZ) in rats[13]. The convulsion is induced by administration of pentylene tetrazole (80 mg/kg, i.p.) to Wistar albino rats. Rats showing responses were divided into three groups of six animals each. The first group of animals was administered 1% normal saline (1ml/100gm) orally which served as a negative control. Group of II animals were treated with phenytoin sodium (obtained from Merck, India) (25 mg/kg, i.p.) which served as positive control[14]. Group of III animals (Ps were treated with ethanolic extract of PPLE at a dose of (250 mg/kg i.p.). Drug pretreatment was given 1 hr the administration of Pentylenetetrazole and each animal was placed in an individual plastic cage and observed initially 30 min. and later up to 24 hrs for the duration of tonic, flexion, tonic extension, clonus, and death/recovery[29]. Statistical analysis Statistical analysis was done using a one-way analysis of variance (ANOVA) followed by a Student's t-test. Results are expressed as means ± SEM from six rats in each group[30-32].

3. RESULTS

Plumbago zeylanica flower extract was subjected for an anticonvulsant effect using PTZ induced convulsion model in rats. PPLE exhibits significant anticonvulsant activity by lowering the duration of the extension phase when compared to the control group. The duration of tonic and hind limb extension in rats with 70% ethanolic extract was **3.72±0.65** at a dose of 250 mg/kg. The activity of ethanolic extract was comparable (P>0.001) to that produced by standard drug phenytoin sodium (Table 2).

Table 2. Anticonvulsant activity *Plumbago zeylanica* flower extract on Pentylenetetrazole induced convulsion in rats.

Treatment	Dose	Time in seconds in various phases of flexion and extension				
		Flexion	Extension	Convulsion	Stupor	Recovery/ death
Control (normal saline)	100 mg /1 ml	3.83 ±0.36	3.83 ±0.368	4.65± 0.71	104.10±8.74	Recovery
Phenytoin sodium	25 mg/Kg	2.15± 0.21	9.41 ± 0.42	7.86 ± 0.35	91.55± 5.85	Recovery
Ethanolic extract	250 mg	1.90 ± 0.25	3.72 ± 0.6	8.96 ± 2.00	102 ± 8.75	Recovery

Data are expressed in mean ± SEM, n = 6 in each group *P < 0.001 vs Phenytoin sodium–treated rats by student's t-test

4. DISCUSSION

Pentylenetetrazole is a selective blocker of the chloride ionophore complex to the GABA-A receptor, and after repeated or single dose administration leads to a decrease in GABAergic function and to the stimulation and modification of density or sensitivity of different glutamate receptor subtypes in many brain regions. Pentylenetetrazole may also triggers various biochemical processes including the activation of the membrane phospholipase, proteases, and nucleases. Alteration in membrane phospholipids metabolism causes liberation of free fatty acids, diacylglycerols, eicosanoids, lipid peroxidase, and free radicals. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic-clonic seizure[11]. Phytochemicals such as kaempferol and fixed oils are the active principles responsible for the anticonvulsant activity of ***Plumbago zeylanica***; flavonoidal compounds, present in this plant may likely be involved in this action. Hence this drug may be able to modulate the function of GABA or glutamate receptors

5. CONCLUSION

The anticonvulsant potential observed in the leaf galls and flower extract of Plumbago zeylanica against chemically induced convulsions in experimental animals underscores its significance in Ayurvedic and modern drug development. The presence of compounds like quercetin, kaempferol, flavonoidal compounds, and fixed oils is implicated in the plant's phyto medicinal uses.

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