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Evaluation of Antiepileptic Potential of Ethanolic Extract of *Thuja Occidentalis* Leaves in Mice

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ABSTRACT

The present investigation was carried out with an objective to evaluate and establish the anti epileptic action of ethanolic extract of *Thuja occidentalis* leaf. The ethanolic extraction was carried out using soxhlet apparatus and the yield of extract was obtained to be 34.1 %. The findings of the phytochemical analysis suggest the presence of alkaloids, phenolics, terpenoids, sterols, and flavonoids in the ethanolic extract leaves. The total flavonoid content of the extract was determined as quercetin equivalent and was found to be 0.497 mg/100 mg quercetin equivalent. The acute toxicity study revealed a dose of 750 mg/kg body weight as maximum safe dose. The anticonvulsant action was determined using maximal electroshock induced seizure model and pilocarpine induced convulsions model. Two different dose levels (75 mg/kg and 150 mg/kg body weight) of *Thuja occidentalis* leaf extract were selected for evaluation of the antiepileptic action. The results indicated that the extract of *Thuja occidentalis* leaf was able to decrease the onset of hind limb extension significantly and also it abolished the occurrence of clonus in the test animals.

Key words: *Thuja occidentalis*, extract, epilepsy, pilocarpine, latency

1. INTRODUCTION

Epilepsies are a group of CNS disorders characterized by paroxysmal cerebral dysrhythmia, manifesting as brief episodes (seizures) of loss or disturbance of consciousness, with or without characteristic body movements(convulsions), sensory or psychiatric phenomena¹. A seizure is a sudden surge of electrical activity in the brain. A convulsion is a condition in which body muscles contract and relax rapidly and repeatedly, results in an uncontrolled shaking of the body². A number of plants have been used by the folkloric practitioners world-wide and the potential of many such plants is yet to be scientifically explored. *Thuja* is a genus of coniferous trees in the Cupressaceae. It is widely cultivated and grown as an ornamental tree worldwide. It has been reported to contain terpene thujone along with other constituents like isopircodeoxy podophyllotoxin, deoxypodophyllotoxin 7-oxo-13epi-pimara-8,15-dien-18-oic acid³. The plant has been reported to possess the hepatoprotective, antioxidant, antidiabetic, antimicrobial and anticancer activities^{4,5}. The oil of the different parts of the plant as well as the extracts obtained using different solvents has been investigated. In folk medicine, *Thuja occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism.

The objective of the present investigation herein is to investigate the antiepileptic potential of the ethanolic extract of *Thuja occidentalis* leaves.

2. MATERIALS AND METHODS

The leaves of *T. occidentalis* were collected from the garden of Technocrats Institute of Technology-Pharmacy, Madhya Pradesh a voucher specimen of same has been deposited in the herbarium of the institute.

2.1 Preparation of the plant material

The authenticated plant leaves were washed with distilled water and were dried under shade. The dried leaves were powdered using a hand blender at low speed. The powdered leaves were stored in air tight container until taken for use.

2.1.1 Extraction of leaves⁶

As previous studies have indicated the presence of flavonoids in the alcoholic extract, ethanolic extraction was carried out using soxhlet apparatus. The powdered leaves were used for the extraction process. 350 g of powder was evenly packed in the extractor of the soxhlet apparatus and extracted using ethanol by hot continuous extraction process for about 6 h. The extract was filtered while hot using Whatman filter paper to make it free from impurities. The volume of solvent was reduced using a rotary vacuum evaporator. The remaining solvent was evaporated on water bath to obtain the resinous extract which was dried in desiccator to obtain dry extract.

2.1.2 Preliminary phytochemical screening⁷

The extract was evaluated by phytochemical qualitative reactions for identifying the presence or absence of usual plant secondary metabolites. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests.

2.1.3 Total flavonoid Content⁸

The determination of total flavonoids content was carried out by aluminium chloride method.

2.2 Preparation of standard

10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. The absorbance of these solutions at 420 nm was determined after mixing each with 1 mL of 2% AlCl₃ solution and standing for 1 hour. Calibration curve was plotted for concentration against absorbance and the regression equation was used for calculation of the flavonoid content of the extract equivalent to quercetin.

2.3 Preparation of extract

10 mg extract dissolved in 10 ml methanol and filter. 3 ml of this extract solution was for the estimation of flavonoid. 1 ml of 2% AlCl₃ methanolic solution was added to 3 ml of extract and allowed to stand for 60 min at room temperature. The absorbance was measured at 420 nm.

2.4 Pharmacological Study

2.4.1 Animals

Healthy adult Wistar albino rats of either sex weighing 180-250g were selected for the study. The animals were housed in large, spacious, hygienic cages during the course of experimental period. The animal house was maintained properly and the animals had 12 ± 1 hour day and night schedule with a temperature [64-79°F] maintained at standard experimental condition. The animals were been fed with standard rodent pellet feed and water ad libitum. The animals were fasted 12 hours prior to the experiment with free access to only water.

3. EVALUATION OF ANTI EPILEPTIC ACTION

3.1 Maximal electroshock seizure [MES] model⁹

3.1.1 Experimental design

Wistar albino rats weighed around 150-250g were used for the study. Rats were divided into four groups of 5 animals each.

Group I -	Vehicle control [Equivalent normal saline, oral]
Group II -	Standard [Diazepam 5 mg/Kg, oral]
Group III -	<i>Thuja occidentalis</i> ethanolic extract (TOEE), 75 mg/kg orally
Group III -	<i>Thuja occidentalis</i> ethanolic extract (TOEE), 150 mg/kg orally

Animals in the control group [Group 1] were been administered equivalent volume of normal saline by oral route. Animals in Group 2 were been administered standard drug Daizepam. In Groups 3 and 4 *Thuja occidentalis* ethanolic extract (75 and 150 mg/kg) was administered by oral route in 1% Sodium lauryl sulphate solution respectively.

After 30 minutes of administration of above drugs, all the rats were been given electroshock with electro convulsimeter through ear electrodes [after moistening the ear of animals with drop of normal saline] at intensity of 150 mA, 60Hz for 0.2 seconds. Thereafter the animalwere observed for number of convulsions. The percent protection and duration of tonic hind limb

extension (i.e., the hind limbs of animals outstretched at 180° to the plane of the body axis) was observed. Protection was defined as complete absence of tonic hind limb extension.

3.2 Pilocarpine Induced Convulsion model⁹

3.2.1 Experimental design

Wistar albino rats weighed around 150-250 g were used for the study. Rats were divided into four groups of 5 animals each.

Group I -	Vehicle control [Equivalent normal saline oral]
Group II -	Standard [Diazepam 5 mg/kg, oral]
Group III-	<i>Thuja occidentalis</i> ethanolic extract (TOEE), 75 mg/kg orally
Group III-	<i>Thuja occidentalis</i> ethanolic extract (TOEE), 150 mg/kg orally

The control group received vehicle and the standard group was treated with diazepam 5 mg/kg, per oral. The tests groups were treated with 75 & 100 mg/kg *Thuja occidentalis* ethanolic extract, orally.

Thirty minutes after administration of the test drugs, the mice were treated with pilocarpine, 8 mg/kg, subcutaneously. Immediately after injection of the convulsant, mice were individually placed in plastic boxes and observed for duration of 30 min. Latency to the first convulsion, percentage of animals exhibiting convulsions, latency to death, and percentage of deaths were the parameters measured. Mice that did not show clonic or tonic convulsions within 30 minutes of pilocarpine administration were considered protected.

4. RESULTS AND DISCUSSION

The branchlets of *Thuja occidentalis* grow flattened with the upper surface dark green in color and the lower surface appears to be light green. The leaves on the upper part of the branchlets are slightly convex while that on the underside are slightly concave.

The yield of the dried ethanolic extract was found to be 34.1% (119 g) with reference to the weight of the dried leaf powder used for extraction. The extract was dark brown in color and completely dry in powder form.

4.1 Phytochemical Screening

The findings of the phytochemical analysis suggest the presence of alkaloids, phenolics, terpenoids, sterols, and flavonoids

in the ethanolic extract leaves (Table 1). The presence of tannic acid, β -thujone, limonene, coumaric acid and umbelliferone along with other chemical constituents in the leaves of the *T. occidentalis* has also been reported by Naser et al¹⁰ in their review on *T. occidentalis*.

Table 1: Phytochemical screening of *Thuja occidentalis* leaf extract

Chemical Tests	Observation	Result	Inference
Alkaloids			
Mayer's reagent	cream colour precipitate	+	Alkaloid Present
Hager's reagent	yellow colour precipitate	+	
Wagner's reagent	reddish brown precipitate	+	
Dragendorff's reagent	reddish brown precipitate	+	
Glycosides			
Froth test	Frothing is seen	-	Glycoside Absent
Kedde's Test	No color	-	
Bontrager's Test	Rose pink or red color in the ammonical layer not found	-	
Keller-Kiliani	No color in acetic acid layer	-	
Phenols/Tannins			
Ferric chloride	Blue green color	+	Phenolics and Tannins Present
Gelatin Solution	White precipitate	+	
Alkaline reagent test	Yellow to red precipitate	+	
Vanillin HCl test	Purplish red color	+	
Flavonoids			
Shinoda test	red color	+	Flavonoids Present

<i>Alkaline reagent test</i>	Yellow color that turns red on acidification	+	
<i>Zinc HCl reductino test</i>	red color	+	
Proteins			
<i>Millon's Test</i>	white precipitate, turns red on heating	-	Protein Absent
<i>Ninhydrin Test</i>	Voilet color	-	
Sterols/triterpenoids			
<i>Salkowski Test</i>	Yellow color in lower layer	+	Sterol Present

+ indicates a positive observation; - indicates a negative observation

4.2 Total flavonoid Content

The content of total flavanoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract. The total flavonoid content of the extract of *Thuja occidentalis* leaf was calculated to be 0.497 mg/100 mg quercetin equivalent.

4.3 Antiepileptic Activity

Table 2: Effect of *Thuja occidentalis* extract on onset of hind limb extension an clonus in MES model

Group	Onset Time (sec)		Recovery/ Mortality
	Extension	Clonus	
I (Control)	3.37 ± 0.139	30.13 ± 2.135	Recovery
II (Standard)	16.84 ± 0.539	0	Recovery
III (Test low dose)	9.96 ± 0.444	0	Recovery
IV Test (High Dose)	13.28 ± 0.108	0	Recovery

Values represented as average ± standard deviation of 5 readings; 0 represents no occurrence of clonus

4.3.1 MES Method

The maximal electroshock induced convulsion in animals represents grand mal type of epilepsy. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure¹¹. The result of the present study shows that the chloroform extract of *Thuja occidentalis* at doses 75 and 150 mg/kg significantly belated the onset of hind limb extension and decreased the duration of extension (Table 2).

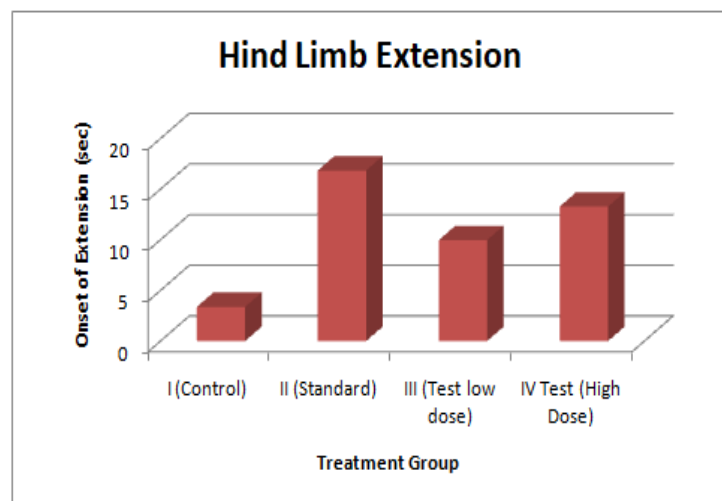


Figure 1: Effect of *Thuja occidentalis* on hind limb extension onset in MES model

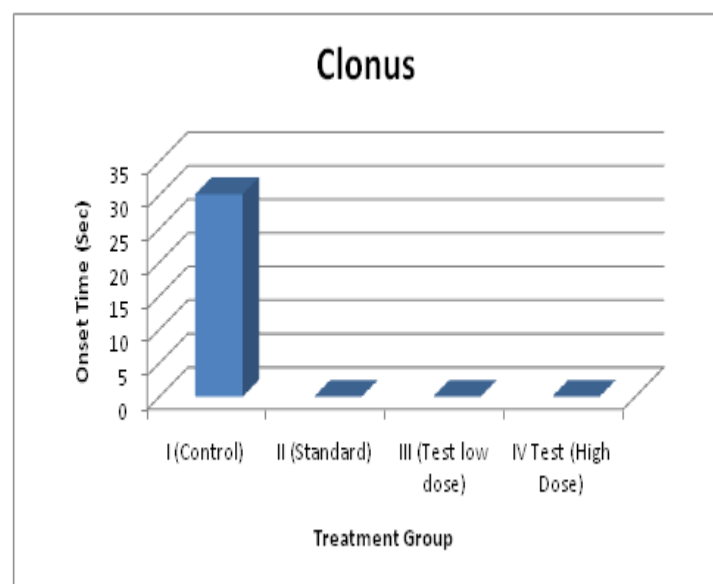


Figure 2 : Effect of *Thuja occidentalis* on clonus onset in MES model

As seen from the figure 2 both doses of *Thuja occidentalis* leaf extract (ethanolic) were able to completely abolish the phase

of convulsion in MES induced convulsion (clonus, involuntary muscle movement) models.

4.4 Pilocarpine Induced Convulsions Method

Injection of pilocarpine induces a status epilepticus that is characterized by tonic-clonic generalized seizures. After several hours of status epilepticus, pilocarpine-treated animals remit spontaneously and go into a seizure-free period, known as latent period, before displaying the SRSs that characterize the chronic epileptic condition. The latency to occurrence of convulsion was observed (Table 3).

Table 3 Effect of *Thuja occidentalis* on pilocarpine induced convulsion

Group	Onset Duration (sec)		Recovery/ Mortality
Group	Extension	Clonus	
I (Control)	6.34 ± 0.126	19.03 ± 2.035	Recovery
II (Standard)	19.23 ± 0.148	0	Recovery
III (Test low dose)	10.36 ± 0.147	0	Recovery
IV Test (High Dose)	14.74 ± 0.362	0	Recovery

Values represented as average ± standard deviation of 5 readings; 0 represents no occurrence of clonus

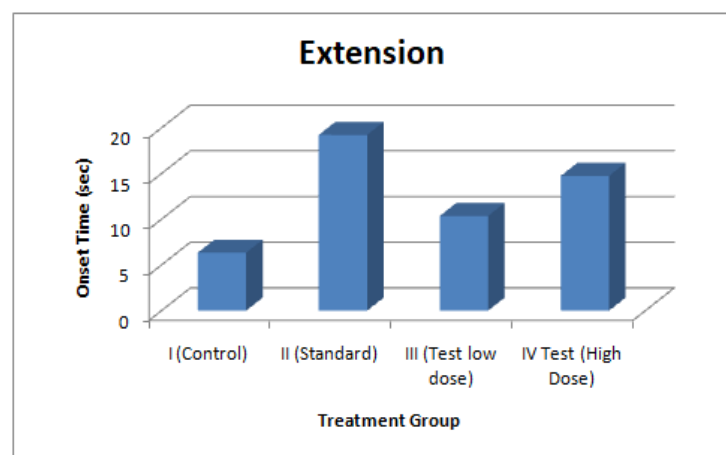


Figure 3: Latency to hind limb extension in treatment groups

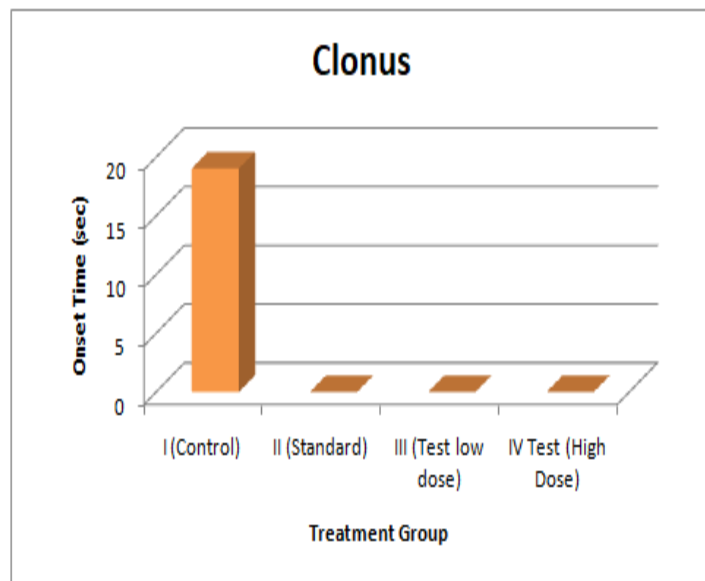


Figure 4: Effect of *Thuja occidentalis* extract on clonus in pilocarpine induced epilepsy model.

As evident from the figure 4 both doses of *Thuja occidentalis* leaf extract (ethanolic) were able to completely abolish the phase of convulsion in MES induced convulsion (clonus, involuntary muscle movement) models.

5. CONCLUSION

The ethanolic extract of *Thuja occidentalis* delayed the onset and reduced the duration of convulsion in MES and pilocarpine induced convulsion models and can be used as an adjuvant therapy in convulsions. Further studies are needed to explore the mechanism as well as the active principle responsible for the anticonvulsant activity of *Thuja occidentalis*

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