INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

NEUROPHARMACOLOGICAL EXPLORATION OF *THUJA OCCIDENTALIS* LINN.

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Article Received on: 12/01/2011 Revised on: 26/02/2011 Approved for publication: 11/03/2011

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ABSTRACT
In present study, oral administration of 100, 200 and 400 mg/kg doses of the aqueous extract of *Thuja occidentalis* Linn in rats and mice were evaluated for its anxiolytic, nootropic, anticonvulsant and motor coordination activity by using different animal models. The number of entries as well as the duration of stay in the open arms significantly increased (***p<0.001) in Elevated plus-maze test and in Open field test showed significant (*p<0.5, ***p<0.001) increase of open field Ambulation, Rearing, Self grooming activity at centre in dose dependent manner when comparison to vehicle treated rats, moreover, the open field faecal dropping was also decreased that indicate anxiolytic activity of extract. Significant retention and recovery as compare to control group has been recorded in scopolamine induced amnesia that support nootropic activity of extract. In Pentylenetetrazole (PTZ) - induced seizures, extract dose dependently inhibited the incidence of convulsions and significantly (*p<0.5, **p<0.01, ***p<0.001) delayed the onset of spasm and clones in PTZ treated mice compare to vehicle treated mice. Significant (***p<0.001) motor in-coordination and increased in immobility time was recorded in case of Rota rod test and Tail suspension test respectively that indicate CNS depression activity of the extract. On the basis of these investigations, we may partially conclude that aqueous extract of *Thuja occidentalis* Linn aerial part can not recommend to patients in day time or before bed due to its CNS activity, but it could be a potent anxiolytic, nootropic, anticonvulsant and CNS depressant for next generation.
KEYWORDS - *Thuja occidentalis,* anxiolytic, nootropic, anticonvulsant, motor-coordination

INTRODUCTION

World health report (WHO 2001) says approximately 450million people suffer from a Mental or behavioural disorder, yet only a small minority of them receive even the most basic treatment this amount to 12.3% of the global burden of disease and will rise to 15% by 2020.[1]

Drug acting in the central nervous system were among the first to be discovered by the primitive human and are still the most widely used group of pharmacological agents the CNS acting drugs are invaluable therapeutically , because they can produce specific physiological and psychological effects form the vast array of materia medica of the indigenous system so many plants have been reported to have activity against CNS disorders and thus act as a very useful remedies for the alleviation of human suffering.[2]

In the search of new therapeutic product for the treatment of neurological disorder medicinal plant research worldwide has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models.[1]

Thuja occidentalis (L) family capressaceae is indigenous to eastern North America. The plant was first identified as a remedy by native Indians in Canada during a 16th century expedition and was found to prove effective in treatment of weakness from scurvy.[3] In folk medicine, *Thuja occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism.[4 5 6] In India it is grown as an ornamental plant.

A literature survey reveals that *Thuja occidentalis* possess activity against HIV-1 virus & common cold[7,8], increase in the proliferation of spleen cell as well as TNF-α, IL-6 and IL-1 activity in serum[9], anti-diarrheal[10], antioxidant and antiulcer[11] effects. Thuja has also been reported to have protective effect against radiation induced toxicity.[12]

The genus Thuja was considered to consist of the species. *Thuja karoiensis* Nakai, *Thuja occidentalis*, *Thuja Orientals* L. and *Thuja plicata* D. Don.[13, 14]

Those are commonly cultivated in central Europe. Thuja contains 1.4-4% of essential oil, 60% of which is Thujone, which corresponds to 2.4% Thujone in the whole drug.[15]

In our present study, *Thuja occidentalis* evaluated for its effect on Central Nervous System (CNS) to established neuropharmacological prospective of such therapeutically important plant.

MATERIALS AND METHODS

Collection of plant

The aerial part of *Thuja occidentalis* collected from the medicinal plant garden, BR Nahata College of Pharmacy, Mandsaur (M.P.)

Identification

The plant was identified by Dr. H.S. Chatree (Ex. Prof. Botany) Dept. of Botany, Govt. P.G. College Mandsaur (M.P.). The voucher specimen herbarium BRNCP/A/002/2005 was deposited in the Dept. of Pharmacognosy, BR Nahata College of Pharmacy. Mandsaur (M.P.)

Extraction

The aerial parts were cleaned properly and washed with distilled water to remove any kind of dust particle, aerial parts were air shade dried and then processed for extraction with distilled water by decoction method.

Experimental Animals

Wistar rats (150-200gms) and albino mice (20-30gm) of either sex provided by the Institutional Animal House of B.R. Nahata College of Pharmacy, Mandsaur were used. Animals were maintained under standard environmental condition: (Room temperature - 27 ± 3°C, Relative humidity - 65 ± 10 %, 12 hours light / dark cycle).

All the animals were fed with synthetic diet (gold Mohr, Lipton India Ltd., Bangalore) and water was allowed ad-libitum under strict hygienic conditions. Experiments were performed in accordance with the current guidelines of CPCSEA India. All the animal experiments were conducted according to the protocol approved by Institutional Animal Ethics Committee. (Approval No: BRNCOP/IAEC/B.Ph/25/2006/date – 04 -05 - 2006).

Pharmacological screening of extract

Determination of acute toxicity

The acute toxicity for aqueous extract of *Thuja occidentalis (TOA)* was determined in wistar mice, maintained under standard condition. The animals were fasted overnight prior to the experiment, fixed dose method of CPCSEA was adopted for toxicity studies.

Evaluation of Neuropharmacological Activity

Elevated Plus-maze Test

Anxiolytic activity was evaluated using the elevated plus maze. [16] The elevated plus maze consisted of two open arms (50x10 cm) crossed with two closed arms (50x10x40 cm). The arms were connected together with a central square (10x10 cm). The apparatus was elevated to the height of 70 cm in a dimly illuminated room. The rats were divided into 5 groups each containing five animals. Group III, IV and V were administered orally with 100, 200, 400 mg/kg body weight respectively of *Thuja occidentalis* (suspended in water). While animals in group II administered in proportionally with 1 mg/kg diazepam as standard and group I were served as control. One hour post administration, each rat was placed individually at center of the elevated maze. The number of entries in the open and closed arm of the elevated maze during a period of 5 min and the duration of the stay in open arm, close arm and number of entries in both arms were noted.

Open field test (OFT)

The open field apparatus of squares (61x61 cm), the entire apparatus was pointed black except for 6 mm thick white lines, which divided the floor into 16 squares. Open filed was lighted by a 40 w bulb focusing onto the filed from a height of about 100 cm. The entire room except the open field was kept dark during the experiment; each animal was centrally placed in the test apparatus for 5 min and the following behavioral aspects were noted.

- **Ambulation**: This was measured in terms of the number of squares crossed by the animal.
- **Rearing**: Number of times the animal stood on its hind limbs.
- **Self grooming**: Number of times the animal scratched various parts of its body.
- **Activity in centre**: Number of central squares crossed by the animal; and
- **Faecal 2droppings**: Number of faecal droppings excreted during the period. [17]

Scopolamine induced amnesia

The nootropic activity of *Thuja occidentalis* was evaluated by using the conditioned avoidance response (CAR) in rats as described by cook and widely.[18]

Rats were divided into 4 groups each containing five animals. Group II, III and IV were administered orally with 100, 200 and 400 mg/kg body weight respectively of *Thuja occidentalis* while animals in group I were served as control. After 45 minutes; all the animals were subjected to a training schedule individually by placing inside the Perspex chamber of the apparatus. After an accustomed period of five minutes to the chamber, a

buzzer was given followed by a shock through the grid floor.

The rat had to jump on the pole to avoid foot shock. Jumping on the pole functionally terminates the shock and this was classified as an escape while such jumping prior to the onset of the shock was considered as avoidance. The session was terminated after completion of 30 trials with an interval of 20-30 seconds given for each trial. This procedure was repeated at 24 h intervals until all groups reach 95 to 99% avoidance. After attaining complete training of a particular group, the animals were treated with a single dose of scopolamine butyl bromide (1 mg/kg body weight; i.p.) thirty minutes before the next day dosing. The training schedule was continued further with the vehicle or extract until they returned to normal level from scopolamine induced amnesia.

Pentylenetetrazole (PTZ) induced seizures

An 80 mg/kg dose of PTZ was i.p. administered 1 hrs at the injection of the aqueous extract of *Thuja occidentalis* (100, 200, 400 mg/kg p.o.). Latencies to the onset of mioclonic, generalized clonic – tonic and tonic seizures were evaluated during 30 min after PTZ injection (80 mg/kg). Control animals were administered with vehicle and diazepam as reference drug (4 mg/kg).[19, 20]

Tests for motor coordination

Rota rod test

Untreated fresh mice were placed on a horizontal metal rod (75 mm diameter) rotating at a speed of 30 rpm. The animals remaining on the rod for 3 min or more in two successive trials were selected for the test and were divided into 4 groups of 5 animals each. The first were injected with vehicle (10 mg/kg p.o.) as control while the group 2^{nd}, 3^{rd} and 4^{th} were administered with *Thuja occidentalis* extract (100, 200, 400 mg/kg p.o.) respectively. Then the animals were placed on the rod. The time taken for the mice to fall from the rotating rod was noted.[2]

Tail suspension test

According to Steru et al 1988 with some modification rats weigh 150-200gm are used preferentially .They are housed in plastic cage for at least 10 days prior to testing in a 12 hours light cycle with food and water freely available. Animals are transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 hrs before testing group of five animals are treated with the aqueous extract of *Thuja occidentalis* in different doses (100,200,400 mg/kg p.o.) or the vehicle (5 ml/kg p.o.) 45 minutes prior to testing. For the test the rats are suspended on the edge of a shelf 60 cm above platform by thread placed approximately 1 cm from the tip of the tail. The duration of immobility is recorded for a period of 5 minutes Rats are considered immobile when they hang passively and completely motionless for at least 1 min.[21]

RESULTS

Elevated plus-maze test

The extract of the dose levels (100, 200, 400 mg/kg p.o.) produce – significant increase in the number of entries and the duration of stay in the open arm. When compared with the vehicle control group, the reference drug diazepam (1 mg/kg i.p.) significantly increased ($p < .001$) the number of entries as well as the duration of stay in the open arms indicating anxiolytic activity. The results are given in table-1.

Open field test (OFT)

Rats treated with different doses of aqueous extract (*Thuja occidentalis*) showed dose dependent significant increase in open field Ambulation, Rearing, Self grooming and Activity in centre in comparison to vehicle treated rats evincing significant anxiolytic activity of extract where the pen field faecal dropping was also decreased. (Table – 2)

Scopolamine induced amnesia

On challenging with the scopolamine butyl bromide (1 mg/kg body weight),the amnesia was less in group treated with the aqueous extract doses of 100,200,400 mg/kg (p.o.)and showed significant retention and recovery as compare to control group.(Table-3)

Pentylenetetrazole (PTZ) - induced seizures

The aqueous extract of *Thuja occidentalis* dose dependently inhibited the incidence of convulsions and significantly delayed the onset of spasm and clones in PTZ treated mice (Table -4).

Rota rod test

From the results of the Rota Rod test, it was noted that, the extract dose dependently produced significant motor in coordination in mice. (Table – 5)

Tail suspension test

The aqueous extract in doses of 100, 200, 400 mg/kg (p.o.) significantly increased in immobility time which indicate CNS depression .This effect was dose dependent. (Table-6)

Statistical Analysis

Result were expressed as mean $\pm$ SEM Statistical Analysis were performed with one way analysis of variance (ANOVA) followed by student's 't' test P value less than <0.05 was considered to be statistically significant *P<0.05, **<0.01 and ***<0.001, When compared with control and toxicant group as applicable

DISCUSSION

Despite the widely popular use of *Thuja occidenalis* for treating central disorders there is an absence of scientific reports about the evaluation of its neuropharmacological

effects .In this project, it was demonstrated that the administration of different doses of the aqueous extract of *Thuja occidentalis* in rats and mice were able to induce anxiolytic effects, nootropic and anticonvulsant effect without modifying significantly the spontaneous motor activity.

The plant extract induced anxiolytic effect in dose dependent manner. An increase of the most important variable of the Elevated plus-maze (EPM) test was found, as follows: the time that rats spend in the open arms as well as the number of entries in to these arms. The EPM model is useful for modeling anxiety , and it has been assumed that the time rats spend in the illuminated side of the box is the most useful and consistent parameter of anxiety. [22] The anxiolytic effect was also evidenced through the open field test. As animals are taken from there home cage, and placed in a novel environment, they express their anxiety and fear by decrease in ambulation, rearing, and other exploratory behaviors.[20] All these behaviors are increased by anxiogenic agents and attenuated by anxiolytics under experimental conditions.

The process of nootropic activity involves acquisition, retention and retrieval and is measured using condition avoidance response; the acquisition was quicker in extract treated rats (100,200,400 mg/kg body weight p.o.) in comparison to control. When challenged with scopolamine butyl bromide (1 mg/kg body weight) the amnesia was less in treated group showing better retention and recovery then control group. This scientifically evaluated a traditional mindset of people of M.P. about Thuja occidentalis. As field survey about tradition uses of *Thuja occidentalis* in central India noted that the peoples especially students are use to keep aerial parts of *Thuja occidentalis* (Vidhya mean goodness of knowledge) keep in books till it dry could easily memories the matter in books. Present study supports that concept of memory enhancement.

The immobility display by rodents, when subjected to an unavoidable & inescapable stress has been hypothesized to reflect behavioural despair which in turn may reflect depressive disorders in human.[21]

In present study aqueous extract (100,200,400 mg/kg p.o.) significantly increased in immobility time dose dependently compared to vehicle control. Indicate CNS depression effect of extract.

Nearly all of the neuroleptic agents used in psychiatry diminish spontaneous motor activity in animals and increase muscle tone.[2]

In the present study the plant extract treated mice showed a significant lack in motor co-ordination and exhibited significant muscle relaxant activity.

The aqueous extract of *Thuja occidentalis* prolonged the latency and reduced severity of seizure induced by PTZ.

The PTZ test was used because this is one of the first assays developed to conventionally accepted anticonvulsant screening procedure. It is used to identify chemical substances that alter seizure threshold.[23, 24]

The mice treated with aqueous extract of *Thuja occidentalis* were protected from convulsions induced by PTZ, which can be indicative of a more specific effect due to action on GABA system.[25]

CONCLUSION

In the present investigation aqueous extract of *Thuja occidentalis* has shown promise as a memory enhancing agent on rats in scopolamine induced amnesia model.

The neuropharmacological effects determine in present study also suggest that aqueous extract of *Thuja occidentalis* produces a significant CNS depressant activity, anticonvulsant and muscle relaxant activity. So the aqueous extract of *Thuja occidentalis* cannot recommend to patients for day time or before bed due to its CNS activity, though it's a useful therapeutic agent.

A further studies can be done on isolated the active constituents responsible for these activities.

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Table 1: **Effect of *Thuja occidentalis* aqueous extract on Elevated Plus-maze test**

SL NO	Treatment	Time spent (in sec)		Number of Entries	
		Open arm	Closed arm	Open arm	Closed arm
1	Vehicle	24.33±18.30	274.33±16.73	1.00±0.44	2.00±0.44
2	diazepam	131.77±31.7***	168.83±31.7***	9.00±2.6***	5.00±0.9***
3	*TOA* 100	37.6±1.6***	262.4±1.6***	2.8±0.37***	2.4±0.24***
4	*TOA* 200	52.6±1.66***	247.4±1.66***	4.2±0.37***	3±0.31***
5	*TOA* 400	86.8±6.14***	213.2±5.78***	7.4±0.50***	3.6±0.50***

All values are mean ± SEM (n=5), *p<0.5, ***p<0.001 compare to vehicle

Table 2: Effect of *Thuja occidentalis* aqueous extract on Open Field Test

SL NO	Treatment	Ambulation	Rearing	Self grooming	Activity in center	Faccal drop in no.
1	Vehicle	31.50±5.30	8.66±1.20	6.66±0.66	3.00±0.36	3.66±0.42
2	*TOA* 100	41.71±6.31 *	1.00±0.57*	1.66±0.33***	9.35±0.92	2.5±0.56
3	*TOA* 200	47.32±3.29*	1.33±1.33*	3.5±0.61*	6.72±0.66	1.83±0.30*
4	*TOA* 400	58.42±7.42*	2.33±1.85*	4±0.81*	5.21±0.42	1.50±0.42*

All values are mean ± SEM (n=5), *p<0.5, ***p<0.001 compare to vehicle

Table 3: Effect of *Thuja occidentalis* aqueous extract on Scopolamine Induced Amnesia

SL NO	Treatment	% Performance		% Reduction in performance after scopolamine
		Before scopolamine	After scopolamine	
1	Control	98.54 ± 4.32	03.8 0± 3.12	97.81
2	*TOA* 100	96.55 ± 5.23	86.61 ± 4.54 ***	13.01
3	*TOA* 200	96.90 ± 1.21	88.06 ± 3.29 ***	11.07
4	*TOA* 400	97.81 ± 4.33	93.04 ± 1.67 ***	8.02

All values are mean ± SEM (n=5), ***p<0.001 compare to Control

Table 4: Effect of *Thuja occidentalis* aqueous extract on Pentylenetetrazole (PTZ) Induced Seizure.

SL NO	Treatment	Dose mg/kg	Convulsion			Death/ recovery
			Onset (sec)	Nature of severity	Clonic time in sec.	
1	Vehicle + PTZ	0.2 ml + 80 mg/kg	46.6±1.50	Jerky movement and straub`s tail	64±1.92	5/5
2	Diazepam + PTZ	100+80	135.8±4.32 ***	Jerky movement and straub`s tail	576±20.71 ***	2/5
3	*TOA* + PTZ	100+80	83.4±4.90 *	straub`s tail	256±9.160 ***	2/5
4	*TOA* + PTZ	200+80	88.60±5.00 **	straub`s tail	372.2±11.56 ***	1/5
5	*TOA* + PTZ	400+80	106.4±4.03 ***	straub`s tail	472.2±11.60 ***	0/5

All values are mean ± SEM (n=5), *p<0.5, **p<0.01, ***p<0.001 compare to vehicle

Table 5: Effect of *Thuja occidentalis* aqueous extract on Rota Rod Performance

SL NO	Treatment	% reduction in performance	
		After 45 min.	After 1 hr.
1	Vehicle	4.23±0.64	6.06±0.44
2	Diazepam	71.66±3.44***	76.97±2.54***
3	*TOA* 100	42.90±5.70***	51.95±4.57***
4	*TOA* 200	67.11±1.60***	81.06±1.08***
5	*TOA* 400	82.01±2.22***	87.53±1.58***

All values are mean ± SEM (n=5), ***p<0.001 compare to vehicle

Table 6: Effect of *Thuja occidentalis* aqueous extract on Tail Suspension Test

SL NO	Treatment	Immobility time (in sec.)
1	Vehicle	37.33±9.06
2	*TOA* 100	70.33±13.37
3	*TOA* 200	128.00±16.28*
4	*TOA* 400	131.33±31.75*

All values are mean ± SEM (n=5), *p<0.5 compare to vehicle

Source of support: Nil, Conflict of interest: None Declared