

SPASMOLYTIC ACTIVITY OF *THUJA OCCIDENTALIS* (CUPRESSACEAE)

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ABSTRACT: The crude extract of aerial parts (including leaves and branches) and its fractions (ethyl acetate, chloroform, *n*-butanol and aqueous fractions) of *Thuja occidentalis* were studied on isolated rabbit intestine. Dose dependent spasmolytic effects of crude extract and ethyl acetate fractions were observed at 1mg to 25mg doses, while aqueous and *n*-butanol fractions showed less spasmolytic activity. Prominent spasmolytic effect was observed, in chloroform fraction. The results of crude extract and its fractions were compared with acetylcholine and adrenaline. The contraction produced by acetylcholine was gradually decreased by chloroform fraction showing its effective use in irritable bowel syndrome (IBS) and other GIT complaints.

Key Words Index: *Thuja occidentalis*, Spasmolytic & Spasmogenic activity, Cupressaceae, Smooth muscles.

INTRODUCTION

Thuja occidentalis (TO) is an evergreen tree and shrubs, growing to 60 feet in height. It introduced in India from North America. Thujas are normally pyramidal trees and some times low and bushy in habit. Seeds and cuttings propagate it. Branches are horizontal and needles are bright green in colour, cones are 1/2 inch long and brownish yellow seeds are winged, it grows in moist and damp areas and also on steam banks. Northern white-cedar begins producing cones as young as 6 years of age and begins producing large quantities by age 30. The best production occurs after age 75. Good crops occur at 2- to 5-year intervals with intervening years having fair to medium crops. Seeds have lateral wings and are disseminated by wind. Seeds are dispersed adistance of 150 to 200 feet (45-60 m) from the source tree [Curtis, 1946; Curtis, 1959; Johnston, 1990]. Four species of *Thuja* are cultivated in India. White cedar is an attractive landscape tree. It is grown as Christmas tree in plains of India. Oil of *Thuja* is colourless to yellow or yellowish green liquid. The seed oil of thuja contains oleic, linoleic, α -linoleic and L15 acids (Gunstone, 1996). Heartwood of thuja contains Monooxygenated sesquiterpene compounds, occidentalol and three occidol isomers, occidenol and α -p-y-eudesmols (Andersen, 1995). The essential oil of foliage show the presence of α -thujone (39-56%), fenchone (6-15%), *p*-thujone (7.2-11%), sabinene (2.1-8.9), beyerene (1.3-5.95%), bornyl acetate (1.2-4.38%) and camphor (1.5-3.3%) (Kamdem, 1993). Oil is used as mild counter

irritant and heart stimulant. It has also abortifacient effect. Oil is used as a perfume ingredient, in room sprays, disinfectants, paints, insecticides, and cleansers as soft soap. Its essential oil has antifungal activity (Delespaul, 2000), antioxidant activity (Mallet, 1994), antimutagenic activity (Alwan, 1989), it is also used to treat damage in immune system (Reischle, 1994). Powdered leaves are used to kill the flies. Leaf decoction is used as diuretic, emmenagogue and uterine stimulant. It is also found effective in cough, fever and gout. It is found that it increases the perspiration. Ointment of fresh leaves treats the rheumatism. Heartwood of TO produces an antibiotic substance, which is non-toxic and is used as food preservative. Tagiev (1967) reported the treatment of infected wounds by plant antibiotics including phytocides of garlic, essential oil of thuja, anise and other plants.

MATERIALS AND METHODS

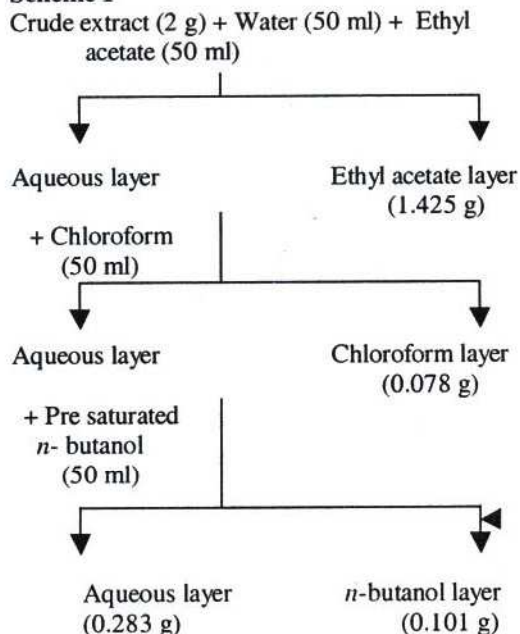
The leaves and stem of TO were collected from the Karachi university, in the beginning of March 2003. The voucher specimens were identified by Dr Mansoor Ahmad head of the department of Pharmacognosy and a sample voucher specimen has been deposited in the herbarium of the department.

EXTRACTION & FRACTIONATION

Fresh leaves (1 kg) chopped were successfully soaked at 25°C in 5 lit ethanol (85 %) as solvent for 15 days at

room temperature. The whole extract was collected in a conical flask, filtered and solvent was evaporated to dryness under reduced pressure in an Eyela rotary evaporator (Japan) at 40-45°C. About 76.8gm crude extract was obtained from 1kg of fresh plant parts. The preliminary phytochemical test of the whole extract was also performed following the standard methods. Then fractions of crude extract of TO were made as shown in the scheme 1.

Scheme 1



The fractions of *Thuja occidentalis* were separated in separating funnel. Dried and weighed and stored in a desiccators. The phytochemical tests of the whole extract were performed by qualitative analysis and confirmed by thin layer chromatography.

Drugs Used

Acetylcholine, adrenaline and histamine (Merck industries) and atropine sulphate (Abbott laboratories) were used in experiments.

Animals Used

Male albino rabbits (1.5-2kg) were housed in RIPS in groups of two per kg (standard metal cages). Prior to pharmacological studies with free access to standard diet and water and libitum for at least two weeks on a 12/12hr light/dark cycle (from 8:00 to 20:00 hr). Rabbits were fasted for 24 hours before test, tap water and libitum was supplied.

PHARMACOLOGICAL STUDIES

The spasmolytic activities of plant materials were tested on isolated rabbit duodenum. The rabbit was killed by cervical dislocation and the tissues of interest were removed immediately and rinsed in warm tyrode's solution. 2cm long segments of rabbit duodenum were suspended in a 50ml organ bath containing tyrode solution (mM) of composition NaCl 24g, KCl 0.6g, NaHPO₄ 0.195g, MgSO₄ 7H₂O 0.78g, CaCl₂ 1.05g, glucose 3.3g, NaHCO₃ 3g in three litre distilled water maintained at 37°C and bubbled with 95% oxygen and 5% carbon dioxide. Each tissue was equilibrated for one hour before adding the drug in the organ bath [Churchill, 1970]. Isometrically intestinal response was recorded on a polygraph (Physiograph, MK-IV-P, Narco-Biosystems) using isometric transducers (560myograph, Narco-Biosystems). To determine the spasmolytic effect on spontaneous movements of intestine, different doses of crude extract (1ml) were added to the organ bath after the equilibration period.

Statistical Analysis

The data were expressed as the mean ± SEM. Significance was evaluated by student's t-test in all the experiments for muscle relaxant activity. A value less than 0.05 were considered significant.

RESULTS AND DISCUSSIONS

Thuja occidentalis itself causes relaxation of isolated rabbit duodenum. It interacts with the activity of acetylcholine and changes the pattern of actions of acetylcholine on the rabbit duodenum. TO exhibits less effect at low doses. At high doses it completely relaxes the duodenum as shown in graph 1. It has dose depended effect. The ethyl acetate (EA) and chloroform (CHCl₃) fractions of TO produce significant dose dependant relaxation on the rabbit duodenum as shown in graph 2. In this study the ethanol extract of the TO was shown to abolish the spontaneous movements of rabbit intestine and acetylcholine induced contractions. This inhibition was concentration dependant. When a pretreated tissue with histamine was treated with ethyl acetate (5 mg) it inhibited the effect of histamine slowly, when the same tissue was treated with acetylcholine 1x10⁻⁴M, it was unable to produce the effect of actual potency as it produce on normal tissue as the muscarinic receptors were blocked. When a pretreated tissue with

Table 1
Effects of the crude extract of *Thuja occidentalis* on isolated rabbit's jejunum

S.No.	Dose mg/ml	Observation Before Dose C	Observation After Dose T	% ReSponse	t - value
1.	1	1.06 :t 0.090	0.83 :t 0.090	22	1.808*
2.	5	0.90 :t 0,057	0.23 :t 0.066	74	7.683 **
3.	10	0.96 :t 0.034	0.43 :t 0.088	55	5.509**
4.	15	0.90 :t 0.057	0.23 :t 0.066	74	7.683**
5.	20	0.96 :t 0.034	-0.3 :t 0.057	131	18.984**
6.	25	0.86 :t 0.034	-0.4 :t 0.057	147	18.984**

The results are expressed in Mean!: SEM at P ~ 0.05 * = Significant ** = Highly significant

Table 2
Effects of the fractions of *Thuja occidentalis* on isolated rabbit's jejunum

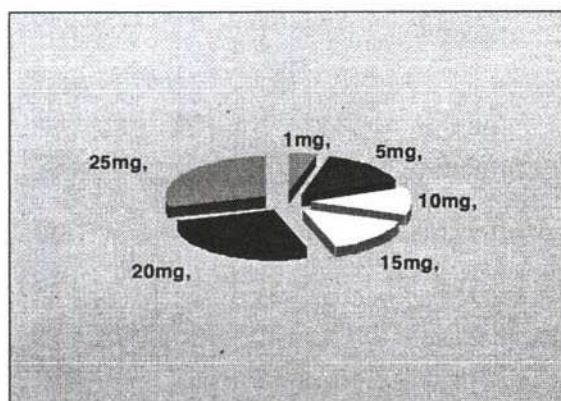
S.No.	Fraction	Dose mg/ml	Observation before dose C'	Observation after dose T	Response in percentage	t - value
1.	Ethyl acetate	1	1.06 :t 0.067	0.50:t 0.120	53	4.076*
2.	CHC13	5	0.66 :t 0.034	'0.16:t 0.033	76	10.570**
3.		1	0.63 :t 0.033	0.37 :t 0.088	43	2.818*
4.	n-Butanol	5	0.86 :t 0.034	0.13 :t 0.033	85	15.433**
5.		1	0.83 :t 0.090	0.70 :t 0.060	16	1.202*
6.	Aqueous	5	0.96 :t 0.034	0.43 :t 0.088	55	5.509**
7.		1	'0.66 :t 0.034	0.47 :t 0.088	30	2.079*
8:		5	0.70:t 0.060	0.47 :t 0.088	33	2.163*

acetylcholine $1 \times 10^{-4}M$ was treated with ethyl acetate (10mg) it produced less effect it is due to the higher susceptibility of ethyl acetate to muscarinic receptors. When the same tissue was treated with histarrune (0.1mg), the histamine did not show the effect, showing that histaminic receptors were also blocked. When the same tissue was treated with acetylcholine

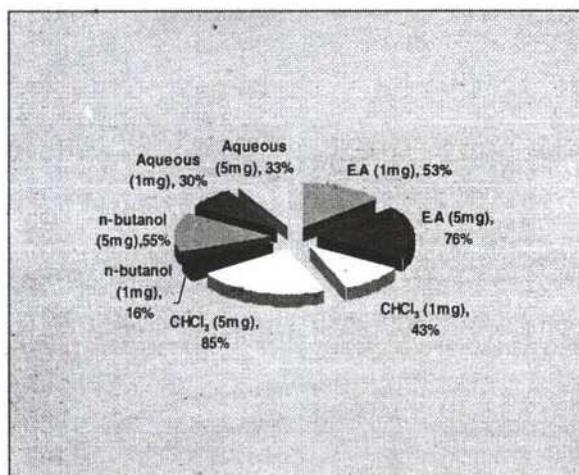
$1 \times 10^{-2}M$, a sudden rise and transient fall occurred as shown in graph 3, this may be by some other mechanism as the muscarinic and histaminic both receptors were already blocked.

The effect of chloroform fraction of *Thuja* in comparison with the standards is shown in graph 4.

When a pretreated tissue with acetylcholine $1 \times 10^{-4}M$, was treated with chloroform (10 mg), the tissue was unable to produce the effect as a result of blockage of muscarinic receptors. The contraction produced by acetylcholine was gradually decreased by chloroform fraction showing its effective use in irritable bowel syndrome and other GIT complaints.



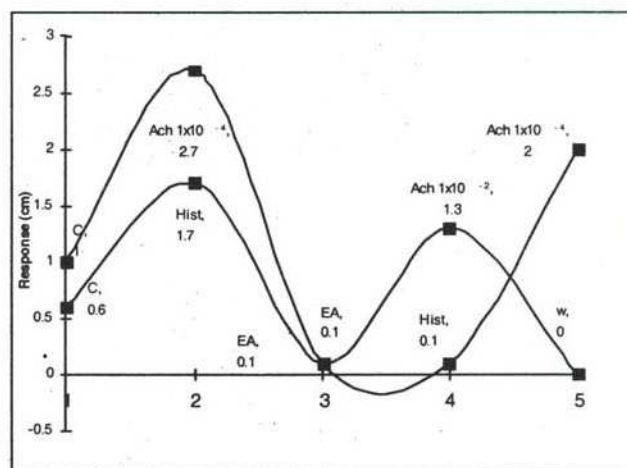
Graph 1: Pie graph showing the inhibitory percentage response of the crude extract of *Thuja occidentalis* on isolated rabbit's jejunum.



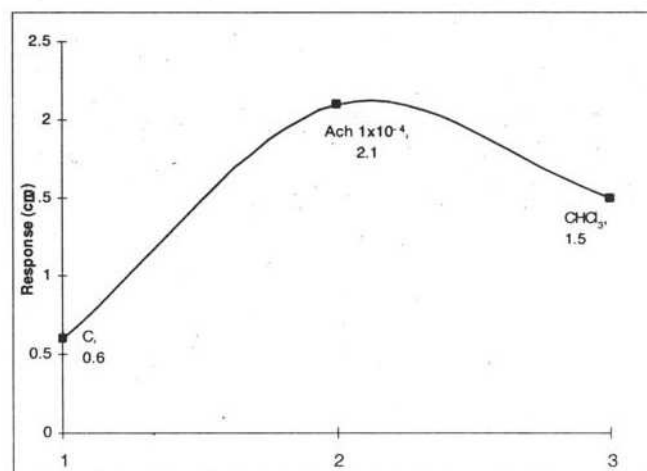
Graph 2: Pie graph showing the percentage response of the fractions of *Thuja occidentalis* on isolated rabbit's jejunum.

The effect of *n*-butanol fraction of thuja with the standard acetylcholine $1 \times 10^{-2}M$ is shown in graph 5. When acetylcholine was administered to the pretreated tissue with *n*-butanol (15mg), it was unable to produce the effect as the muscarinic receptors were blocked.

Graph 5 also shows the effect of *n*-butanol fraction with atropine. When *n*-butanol (5mg) was administered to the pretreated tissue with atropine 1×10^{-2} , it produced no effect as the muscarinic receptors were blocked, confirming that the inhibitory effect of *n*-butanol was due to the effect on muscarinic receptors.

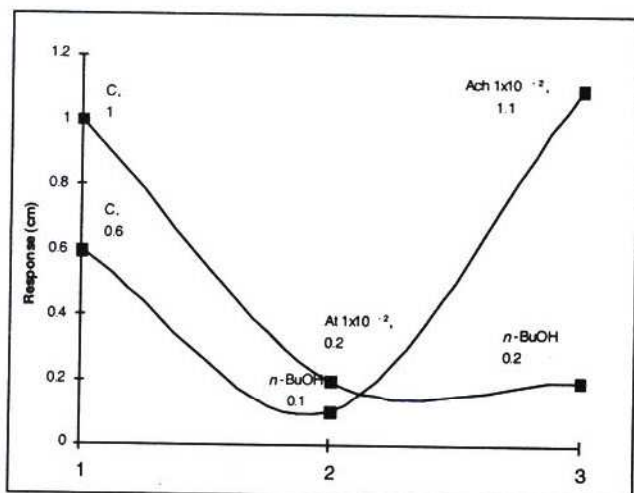


Graph 3: Effects of ethyl acetate fraction of *Thuja occidentalis* on rabbit's jejunum pretreated with histamine (0.1mg) and post treated with acetylcholine $1 \times 10^{-2}M$ and the series 2 also shows the effect of ethyl acetate fraction pretreated with acetylcholine $1 \times 10^{-4}M$ and post treated with histamine 0.1 mg and acetylcholine $1 \times 10^{-4}M$.

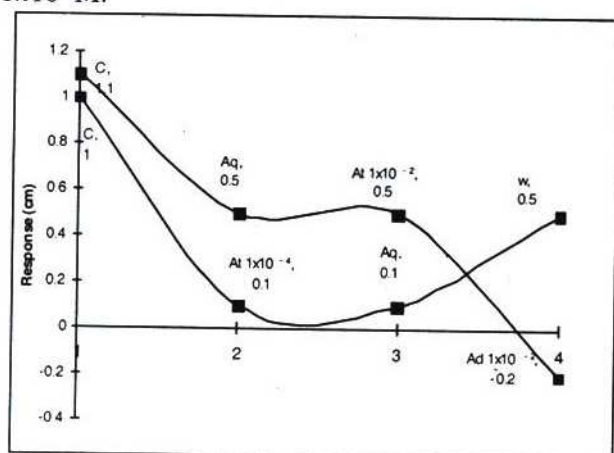


Graph 4: Effect of chloroform fraction of *Thuja occidentalis* on rabbit's jejunum pretreated with acetylcholine $1 \times 10^{-4}M$.

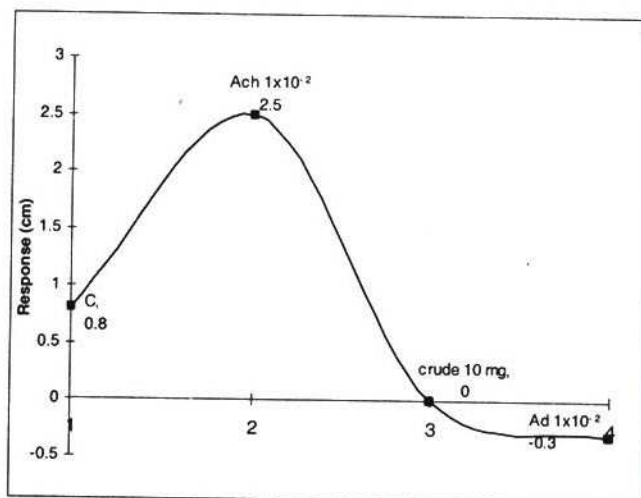
When aqueous fraction 10 mg was administered to the pretreated tissue with atropine $5 \times 10^{-2} \text{M}$, it was unable to give the effect as the muscarinic receptors were blocked. When the atropine $5 \times 10^{-2} \text{M}$ was administered to the pretreated tissue with aqueous fraction (10mg), it produced a little effect as the muscarinic receptors were blocked. When the adrenaline 1×10^{-2} was administered to the same tissue, it produced its full inhibitory effect as the adrenergic receptors were not blocked. When acetylcholine 1×10^{-2} was administered to the same tissue it was unable to any effect as shown in graph 6 because the muscarinic receptors were blocked.



Graph 5: Effects of *n*-butanol fraction of *Thuja occidentalis* on rabbit's jejunum post treated with acetylcholine $1 \times 10^{-2} \text{M}$, and pretreated with atropine $1 \times 10^{-2} \text{M}$.



Graph 6: Effects of aqueous fraction of *Thuja occidentalis* on rabbit's jejunum pretreated with atropine $1 \times 10^{-4} \text{M}$, and post treated with atropine $1 \times 10^{-2} \text{M}$ and adrenaline $1 \times 10^{-2} \text{M}$.



Graph 7: Effect of crude extract of *Thuja occidentalis* on rabbit's jejunum pretreated with acetylcholine $1 \times 10^{-2} \text{M}$ and post treated with adrenaline $1 \times 10^{-2} \text{M}$.

The effect of crude extract of *Thuja occidentalis* with the standards is shown in graph 7. When a pretreated tissue with acetylcholine $1 \times 10^{-2} \text{M}$, was treated with the crude extract (10mg), it was unable to produce the inhibitory effect, showing that the inhibitory effect of *Thuja occidentalis* was due to the effect on muscarinic receptors. When the same tissue was treated with adrenaline $1 \times 10^{-2} \text{M}$, it produced its full effect showing that crude extract has no effect on adrenergic receptors. As the receptors were fully saturated so when acetylcholine 1×10^{-2} was administered to that tissue it was unable to give any effect.

Results obtained from our studies on isolated smooth muscle preparations have shown that the extract contains substances, which relax smooth muscles. These data's suggest that TO may have some potentially useful antispasmodic activity. The *n*-butanol and aqueous fractions at 1mg and less doses give less spasmolytic and then slight spasmogenic activity while at high doses they give dominant spasmolytic activity and less spasmogenic activity. The inhibitory effect was minute at 1mg dose but maximum at 20 to 25mg doses. The inhibitory effects in a dose dependant manner reaches to maximum within one minute and were maintained as long as it was present in organ bath and persisted for 15-30 minutes after washing.

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