



Pharmacological Evaluation of Sedative activity of methanolic extract of *Thuja occidentalis* in mice.

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ABSTRACT

The methanolic leaf extract of *Thuja occidentalis* was evaluated for possible sedative activities in mice. Sedative activity was evaluated by using hole cross, open field, thiopental sodium-induced sleeping time and elevated-plus maze (EPM) tests at 200mg/kg and 400 mg/kg. The extract decreased the locomotor activity of mice in hole cross, open field and EPM test and showed the remarkable results as compared to the standard at both mentioned doses. Moreover, the extract significantly minimized onset of sleep and maximized the duration of sleeping time when administered with thiopental sodium and statistically it was significant ($p < 0.05$).

Key words: *Thuja occidentalis*, Sedative, Thiopental sodium.

INTRODUCTION

The World Health Organization (WHO) encourages the inclusion of herbal medicine in health care because of the great potentials they possess (Amos *et al*, 2001). Also, the long historical use of medicinal plants has demonstrated the safety and efficacy of traditional medicine. However, scientific research is needed to provide evidences of their safety and efficacy (WHO, 2000). A number of medicinal plants are traditionally endowed with anxiolytic or sedative properties (Wheatley, 2005). *Thuja occidentalis*, commonly known as Arbor vitae or white cedar, is indigenous to North America and is grown in Europe as an ornamental tree. In folk medicine, *Thuja occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhoea and rheumatism (Chang *et al*, 2000). Extract of this plant has shown anti viral, anti diarrhoeal activity (Nam SH & Kang MY, 2005), (Deb *et al*, 2007). It has been reported to increase the proliferation of spleen cells as well as increase in TNF α , IL-6 and IL-1 activity in serum and also have protective effect against radiation-induced toxicity (Belal *et al*, 2005).

Today it is mainly used in homeopathy as mother tincture or dilution. The aim of the present investigation was to evaluate the possible sedative activity of *Thuja occidentalis* aerial part.

MATERIALS AND METHODS

Collection of plant material and Preparation of extracts

The plant material was collected from surroundings of Multan and was identified by a senior taxonomist at Al-Manara college of Pharmacy, Multan, Pakistan with a Voucher No. 12-E.C-13. The plant material was made free from foreign adulterants and vegetative debris by hand picking and leaves were detached from the plant. Washed and shade dried. Within 8 days leaves became crispy. Special electrical herbal Grinder was used to form coarse powder. Uniform dark green powder was obtained with characteristic smell. The powdered plant material (1 kg) was subjected to maceration in 70% methanol in amber coloured glass bottle at room temperature for 8 days with occasional shaking (Aziz *et al.*, 2013). The soaked material was passed through muslin cloth to remove the Vegetative material and the fluid obtained was filtered through Whatman-1 Filter paper. The filtrate was evaporated on a rotary evaporator (Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland) at 37°C under reduced pressure to thick paste like consistency (approximate yield was 11%) and the extract obtained was stored at -4°C in air tight jars in lab refrigerator.



Figure:1. *Thuja occidentalis*

Animals

Male mice (20–25 g) were purchased from the experimental animal unit of Islamia university Bahawalpur. The animals were fed *ad libitum* with standard food and water except when fasting was required in the course of the study. The animals were housed under standard laboratory conditions (relative humidity 55–65%, room temperature 23.0±2.0°C and 12 h light: dark cycle) and acclimatized for 7 days. The animals were fed with standard diet and water. The set of rules followed for animal experiment were approved by the institutional animal ethical committee.

Sedative Activity

Hole Cross Test

We used the method described earlier by Aziz and Imran (2013). The animals were divided into four groups containing three rats in each group.

- Group 1. Negative control, (dist. Water)
- Group 2. Positive control (Lorazepam) and
- Group 3 Methanolic extract To.cr 200 mg/ kg body weight
- Group 4. Methanolic extract To.cr 400mg/ kg body weight

A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after the oral treatment with test drugs. Lorazepam was used in the positive control group as reference standard at the dose of 1 mg/kg (i.p). Number of crossings from the hole was calculated along with time of sedation induction.

Open Field Test

The experiment was carried out according to the methods described earlier by Aziz and Imran (2013). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the mice was counted for 3 min, on 0, 30, 60 and 120 min during the experiment.

Thiopental sodium induced sleeping time test

The experiment was conducted following the method described by Aziz and Imran (2013). The animals were randomly divided into three groups consisting of five mice each. The test groups received methanol extract from the leaves of *Thuja occidentalis* at dose 200 and 400 mg/kg (p.o) body weight while the standard group was treated with lorazepam (1 mg/kg, p.o) and control group with distill water. Twenty minutes later, thiopental sodium (40 mg/kg, i.p) were administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep *i.e.* time between the loss and recovery of righting reflex.

Elevated plus-maze (EPM) test

The method initially suggested by Handley and Mithani was employed with minor modifications (Lister RG, 1987). The apparatus consist of two open arms (5 × 10 cm) and two closed arms (5 × 10 × 15 cm) radiating from a platform (5 × 5 cm) to give the apparatus a plus sign appearance. The apparatus was situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The maze floor and walls were constructed from dark opaque wood. Sixty minutes after administration of the test drugs, each animal was placed at the center of the maze facing one of the enclosed arms. During the 5-min test period, the number of open arms entries was recorded. Entry into an arm was defined as the point when the animal places all four paws onto the arm. The procedure was conducted in a sound attenuated room; observations made from an adjacent corner.

Statistical analysis

Data are expressed as mean \pm STD and were analyzed statistically by one-way ANOVA procedures, followed by using Dunnett's test. A difference was considered significant at $p < 0.05$.

Results**Sedative Activity****Hole cross test**

The number of hole crossed from one chamber to another by mice of the control group was similar from 0 to 120 min (Table 1). In the hole cross test, the extracts showed a decrease in locomotion in the test animals from the second observation period as evident by the reduction in number of hole crossed by the treated mice compared to the control group. The result was comparable to the reference drug lorazepam. At 200mg/kg To.cr show less significant result but at dose 400mg/kg the result was statistically significant ($p < 0.05$) as shown in table 1.

Open field test

In the open field test, the number of squares traveled by the mice was suppressed significantly in the test group at dose of 400mg/kg. The CNS depressant activity obtained for extract was more than that of standard drug and the result was statistically significant as shown in Table 2.

Thiopental sodium induced sleeping time test

In the thiopental sodium induced sleeping time test, the test group treated with the extract at 400 mg/kg showed significant ($p < 0.05$) decrease in onset of action and increased the duration of sleep. The extract showed better sedative activity at dose 400mg/kg than the standard drug lorazepam regarding both onset of sleep and duration of sleep (Table 3).

Elevated plus-maze (EPM) test

Result of EPM test is presented in Table 4. The methanol extract of *T. occidentalis*. at the dose of 400 mg/kg body weight, significantly decreased the percentage of entries of mice into the open arms and the percentage of time spent in the open arms of the EPM as compared to the dose of 200mg/kg as shown in table 4.

Table 1. CNS depressant activity of methanolic extract of leaves of *T. occidentalis*. on hole cross test in mice

Group	Treatment	Dose	Number of movements				
			0min	30 min	60 min	90 min	120 min
Control	Dist.water	10ml/kg	17.10± 1.42	16.5±0.5	15.64±1.81	15.27±2.14	16.43±1.95
Standard	Lorazepam	1mg/kg	17.68±2.14	6.36±0.29	4.18±2.41	3.94±1.2	3.67±0.43
Methanolic extract	To.cr	200mg/kg	17.81±1.2	12.67±2.9	11.64±2.8	10.74±2.0	9.6±1.8
	To.cr	400mg/kg	17.24±1.2	7.61±1.5	6.51±1.87	5.41±1.84	4.31±2.01

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

Table 2. CNS depressant activity of methanolic extract of leaves of *T. occidentalis*. on open field test in mice

Group	Treatment	Dose	Number of movements				
			0min	30 min	60 min	90 min	120 min
Control	Dist.water	10ml/kg	54.64±1.5	56.35±2.1	58.35±1.12	60.35±0.24	58.64±1.91
Standard	Lorazepam	1mg/kg	55.64±1.2	36.34±1.34	24.36±1.57	19.61±2.34	16.37±0.24
Methanolic extract	To.cr	200mg/kg	57.35±1.24	55.61±0.64	42.61±1.6	38.64±1.64	29.64±1.3
	To.cr	400mg/kg	56.54±0.6	54.36±1.81	33.67±0.64	22.91±0.67	14.61±2.03

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

Table 3. CNS depressant activity of methanolic extract of leaves of *T. occidentalis*. on thiopental sodium induced sleeping time test in mice

Group	Treatment	Dose	Onset of sleep (min.)	Duration of sleep (min.)
Control	Dist.water	10ml/kg	39.6±0.65	44.61±1.6
Standard	Lorazepam	1mg/kg	26.6±2.04	236.14±1.81
Le.cr	-	200mg/kg	22.6±0.9	91.6±0.41
Le.cr	-	400mg/kg	19.41±1.43	271±0.68

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

Table 4. CNS depressant activity of methanolic extract of leaves of *T. occidentalis*. on elevated plus maze test in mice.

Group	Treatment	Dose	%age entry into open arm
Control	Dist.water	10ml/kg	58.34±0.41
Standard	Lorazepam	1mg/kg	21.64±0.6
Le.cr	-	200mg/kg	34.21±1.68
Le.cr	-	400mg/kg	23.64±0.98

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, significant compared to control.

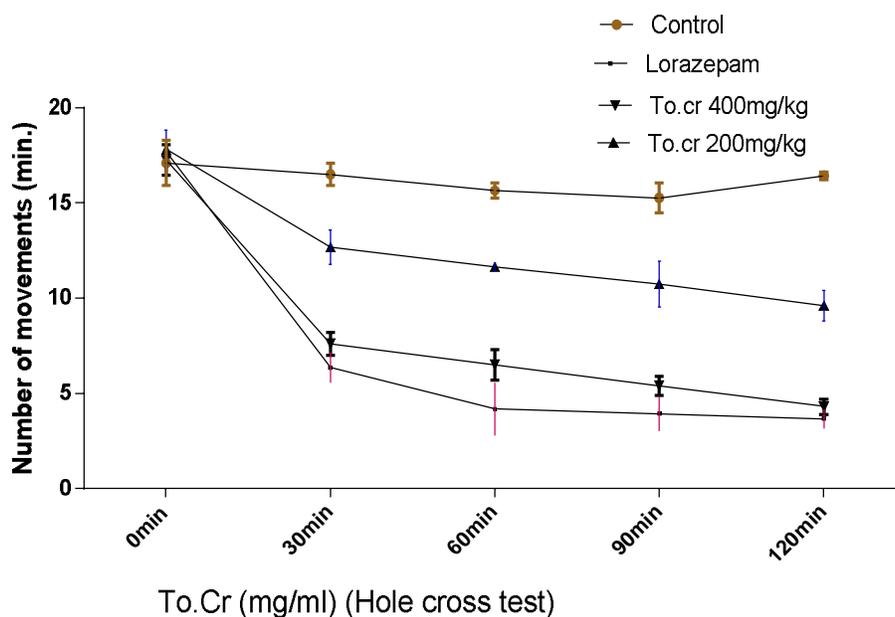


Figure 2. Graph showing CNS depressant activity of methanolic extract of leaves of *T. occidentalis*. on hole cross test in mice

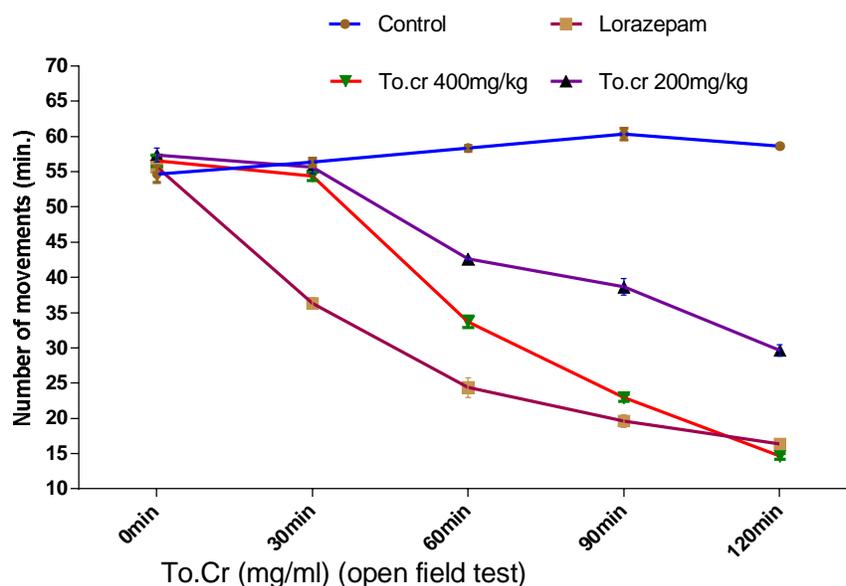


Figure 3. Graph showing CNS depressant activity of methanolic extract of leaves of *T. occidentalis*. on open field test in mice

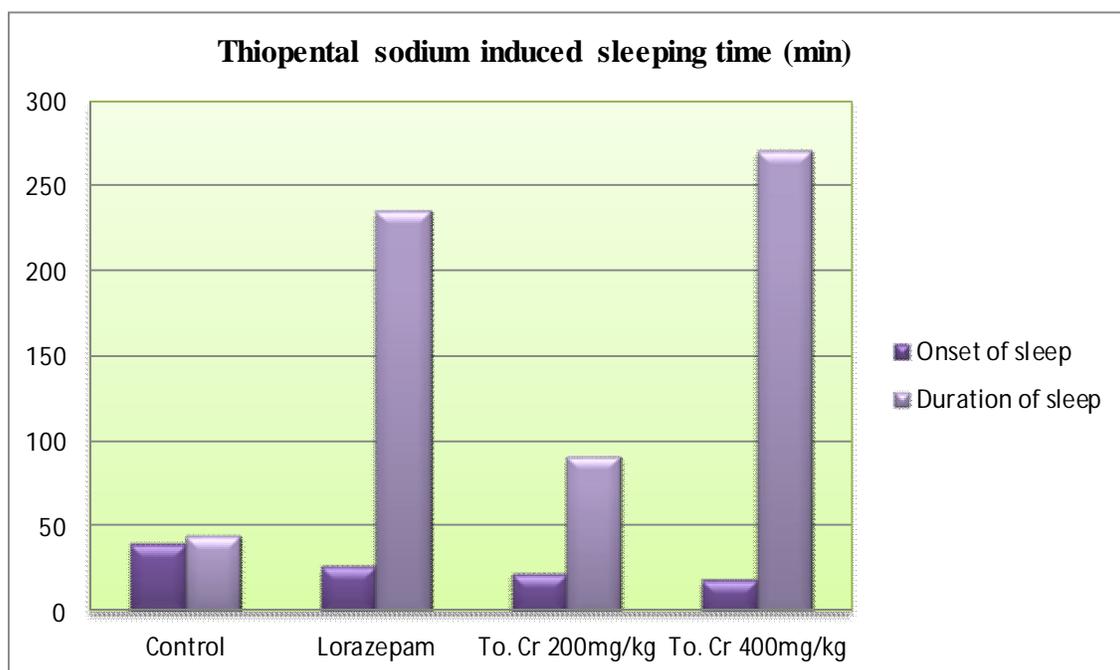


Figure 4. Graph showing CNS depressant activity of methanolic extract of leaves of *T. occidentalis*. on thiopental sodium induced sleeping time test in mice

DISCUSSION

The study has examined some neuropharmacological activities of methanolic extract of *T. occidentalis*. The plant extract possessed central nervous system depressant activity as indicated by the decrease in locomotor activity in mice in hole cross, open field and EPM test as shown in figure 2 and 3. The marked sedative effect of the extract was also found by the reduction in sleeping latency and increase of thiopental sodium induced sleeping time. The data presented in this study showed that the extract produced a dose-dependent reduction in the onset of sleep and prolongation of the total sleep duration induced by lorazepam. The activity of the methanolic extract of *T. occidentalis* was dose-dependent as shown in figure 4. This may be due to the fact that they contain different constituents. GABA (γ -amino butyric acid) systems are known to play an important role in sleep and positive allosteric modulators of GABAA receptors (e.g. Benzodiazepines) are widely used to promote restful sleep. Lorazepam binds to GABAA receptor and potentiates its activation. This enhancement of neuronal inhibition by GABA produces sedation (reduction of motor activity) which is mediated via $\alpha 1$ GABAA receptors (Tobler *et al.*, 2001). Many herbal preparations like chamomile tea and valerian have been shown to enhance the positive allosteric modulating effects of benzodiazepines on GABAA receptors (Johnston, 2005). The ability of the To.cr to potentiate the sedative property of lorazepam suggests that they may possibly act by interacting with GABA-mediated synaptic transmission. Phytochemical constituents like flavonoids and terpenoids which are active ingredients of herbal hypnotics are known to moderate GABAA receptor function (Johnston, 2005). Therefore, the sedative properties shown by To.cr might be due to the presence of flavonoids, saponins and other phytochemical constituents in them.

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