

**Research Article**

**Evaluation of analgesic and anti-inflammatory activity of *Thuja occidentalis* leaves in chemically induced animal models**

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**Abstract**

**Objective:** *Thuja occidentalis* (family: Cupressacae) is commonly known as American Arbor vitae or white cedar, is grown in Europe and indigenous to North America. The plant was first identified as a remedy effective in the treatment of weakness from scurvy. In folk medicine, *T. occidentalis* has been used to treat bronchial catarrh, psoriasis, uterine carcinomas and rheumatism. The objective of present study was to evaluate analgesic and anti-inflammatory efficacy of *T. occidentalis* leaves extract in acetic acid-induced writhing response, formalin test and carrageenan-induced rat paw edema. **Material and methods:** Ethanol extract of *T. occidentalis* leaves was screened for chemical constituents and evaluated for analgesic activity using acetic acid-induced writhing response, formalin test. Anti-inflammatory activity was tested by using carrageenan-induced rat paw edema. Both extracts were tested in 200 and 400 mg/kg dose on both activities. **Results:** The phytochemical screening of ethanol extract was confirmed the presence of terpenes, tannins, carbohydrates and flavonoids. The paw edema was reduced significantly by treatment with 400 mg/kg ethanolic extract of *T. occidentalis*. The results of study was confirmed that ethanolic extract of *T. occidentalis* leaves showed significant (*P*<0.05) analgesic and anti-inflammatory activity in dose dependent manner. **Conclusion:** The extract showed potent activity with 400 mg/kg dose and activity may be responsible due to presence of flavonoids. In future, it required to identify and evaluate individual chemical constituent for that activity with possible mechanism.

**Keywords:** Analgesic, carrageenan, acetic acid, anti-inflammatory, *Thuja occidentalis*

**Introduction**

Inflammation is a complex pathophysiological process mediated by a variety of molecules produced by leukocytes, macrophages and mast cells and mediator release resulted edema formation by extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site (White, 1999). Many inflammatory diseases are associated with the synthesis of prostaglandins, which are responsible for a sensation of pain. The primary enzyme responsible for prostaglandins synthesis is the membrane-associated cyclooxygenase, which occurs in two isoforms, COX-1 and COX-2 (Vane and Botting, 1996). COX-1 is constitutively expressed while COX-2 is induced in the inflamed tissue. Modulation of the activity of the enzyme implies that the inflammation process can be modified.

*Thuja occidentalis* (family: Cupressacae) is commonly known as American Arbor vitae or white cedar, is indigenous to eastern North America and is grown in Europe as an ornamental tree (Chang et al., 2000). The plant was first identified as a remedy by native Indians to prove effective in the treatment of weakness from scurvy (Milspaugh, 1974). In folk medicine, *Thuja occidentalis* it has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhoea and rheumatism (British Herbal Pharmacopoeia, 1983; Shimada, 1956). In combination with other immunomodulating plants, such as *Echinacea purpurea*, *Echinacea pallida* and *Baptisia tinctoria*, it is also used as phyotherapy for acute and chronic infections of respiratory tract (Baran, 1991) and as an adjuvant to antibiotics in severe bacterial infections such as bronchitis, angina and pharyngitis. Dubey and Batra (2008 and 2009), reported hepatoprotective activities and antioxidant activity of *Thuja occidentalis*. Anti-
proliferative and apoptosis-inducing properties of *Thuja occidentalis* has been evaluated by Biswas et al., (2011). The fresh *T. occidentalis* contains 0.6% essential oil, 2.07% reducing sugar, 4.9% water-soluble polysaccharides, 2.11% minerals, 1.67% free acid and 1.31% tannic substances (Madaus, 1938). The essential oil contains 65% thujone, 8% iso(thujone, 8% fenchone, 5% sabines and 2% α-pinene as monoterpenic constituents (Harnischfeger et al., 1983). Other monoterpenic constituents are carvotanacetone, origanol, origanones, myrcen and camphen, reported (Berlin et al., 1984). Hansel et al. (1994) reported that dried leaves contain 1.4–4% essential oil, out of which 60% thujone was present. The present study was aimed to investigate analgesic and anti-inflammatory activity of *T. occidentalis* leaves on different animal models.

Materials and methods

Plant material and phytochemical study

The plant material was identified and authenticated by botanist, SCSCOP, Harapanahalli, Karnataka. Leaves of *T. occidentalis*, approximately 1 kg were collected during January-February duration for extraction and phytochemical analysis. Leaves were well shade dried at room temperature and made fine powder for extraction by soxhlet apparatus. Powdered materials were directly extracted with ethanol by continuous hot extraction method for 12 hours. The extract was filtered and concentrated by rotary vacuum evaporator at 50-60°C. The yield of ethanol extract was calculated. Phytochemical analysis was carried out by using different chemical test to detect qualitatively for alkaloids, glycosides, terpenoids, flavonoids, volatile oils, carbohydrates and tannins (Shukla et al., 2016; Kokate, 1999).

Animal protocol

Wistar albino rats (150-180 g) of either sex were selected for the pharmacological screening of ethanol extract of *T. occidentalis*. The rats were kept in the animal house in a controlled room temperature at 24±1°C temperature in a 12h/12h day/night cycle with free access to food and water *ad libitum*. All the experiments were carried out according to standard protocol. The experimental protocol was approved by the IAEC (Reg. No: 157/99/CPCSEA) of SCSCOP, Harapanahalli, Karanataka.

Acute toxicity study

Albino rats (150-180g) were used for acute toxicity study. The animals were acclimatized to the laboratory conditions for at least seven days prior to the experiments. Acute toxicity study was carried out according to the OECD 425 guidelines. Ethanolic extract of *T. occidentalis* leaves was administered orally in doses of 2000, 3000, 4000, 5000 mg/kg to the group of rats (n=5) and the percentage mortality was recorded for a period of 24 hours. Administration of 5000 mg/kg maximum dose did not produce mortality or general signs of toxicity for 24 hours. 1/10th of 2000 mg/kg, p.o. was taken as therapeutic dose for animals.

Analgesic and anti-inflammatory activity

Acetic acid-induced writhing response

Ethanolic extract of *T. occidentalis* was suspended with 2% Tween 80 by mixing the accurately weighed quantity of the extract. Diclofenac sodium was purchased from market and 25 mg/kg dose was given i.p. for the standard group. Vehicle, 2% v/v Tween 80 solution was administered in control group. The test groups were administered with 200 and 400 mg/kg of ethanol extract of *T. occidentalis* leaves per oral. Briefly the total number of writhes following intraperitoneal administration of 0.1 ml of 1% (v/v) acetic acid was recorded over a period of 20 min, starting 5 min after acetic acid injection. The animals were pretreated with oral dose of ethanol extract of *T. occidentalis* leaves (200 and 400 mg/kg, p.o.) or vehicle, 60 min before administration of acetic acid. Standard group of animals pretreated with diclofenac sodium with dose of 25 mg/kg, i.p. (Dev et al, 2015).

Formalin test

The initial left hind paw thickness of the rats was measured using venire caliper. Chronic inflammation was induced by administration of 0.02 ml of 2.5% freshly prepared formaldehyde solution into the sub plantar area of the rat’s hind paw (Sen and Nag-Chaudhari, 1991). All rats were given treatments as in previous acetic acid model. The left hind paw thickness of each rats was measured daily before each treatment and take observations. The total time used up on licking or biting the injured paw was measured and the activity was recorded in each five min.

Carrageenan-induced rat paw edema

Edema was induced in the hind paw of rats by the subplantar injection of 0.1 ml of a 1% carrageenan suspension in normal saline (Maswadeh et al., 2006). Test extracts or vehicle was given 1 hour prior to carrageenan injection. Paw volume was measured immediately after the carrageenan injection, at 30 min interval for 3 hour later and the difference from initial volume was taken as the edema volume, which was compared to control. The inhibition of inflammation was calculated using the following formula:

\[
\text{Percentage inhibition} = \left( \frac{V_c - V_t}{V_c} \right) \times 100
\]

Where, \(V_c\) is the paw volume of test group and \(V_t\) is the paw volume of control group.

Statistical analysis

Data are expressed as mean ±S.D. Statistical analysis was carried out by one-way analysis of variance (ANOVA).
Statistical significance is expressed as *$P < 0.05$.

**Results and discussion**

**Phytochemical study**

The phytochemical study of ethanol extract of *T. occidentalis* leaves reveals the presence of volatile oil, terpenes, flavonoids, steroids, carbohydrates and glycosides. The yield of ethanolic extract was 7.24%.

**Acute toxicity study**

Animals were observed initially after dosing at least once during the first 30 min, periodically during the first 24 hour. No specific changes like changes in skin and fur, eyes, mucous membranes and also respiratory, circulatory, autonomic, central nervous systems activity and behavioral pattern were observed. Attention was also given to observations of tremors and convulsions.

**Analgesic and anti-inflammatory activity**

Analgesics drugs can act on peripheral or central nervous system. Peripherally acting analgesics drugs act by inhibiting the generation of impulses at chemo-receptors site of pain, whereas centrally acting analgesics not only raise the threshold for pain and also alter the physiological response to pain and repress the patient's anxiety and apprehension. Acetic acid produces inflammatory pain by inducing capillary permeability and liberating endogenous substances that stimulate pain nerve ending. Acetic acid is also known to increase PGE1 and PGE2 peripherally (Shreedhara et al., 2009). NSAIDs can inhibit COX in peripheral tissues and as a result interfere with the mechanism of transduction of primary afferent nociceptors (Kumar et al., 2001).

The results showed that pain behavior of writhing response in the treatment of animals with ethanol extract produced a significant and dose dependent inhibition comparable to the effect produced by diclofenac sodium. The acetic acid induced writhing test is generally used to study the peripheral analgesic activity of drugs. It is a non-specific test widely used for analgesic screening and involves local cholinergic and histaminic receptors (Shibata et al., 1989). These results confirmed that the analgesic effect of ethanol extract might be mediated by its peripheral effect (Table 1). The ethanol extract of *T. occidentalis* also produced significant inhibition in the late phase of formalin induced pain (Figure 1).

**Table 1. Effects of ethanolic extract of *T. occidentalis* leaves on acetic acid induced writhing on rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of writhing</th>
<th>% inhibition of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control (2% v/v tween 80)</td>
<td>55.37±4.28</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac sodium (25 mg/kg, i.p.)</td>
<td>16.32±1.24</td>
<td>70.52</td>
</tr>
<tr>
<td>Ethanol extract of <em>T. occidentalis</em> (200 mg/kg, i.p.)</td>
<td>26.30±2.64*</td>
<td>52.50</td>
</tr>
<tr>
<td>Ethanol extract of <em>T. occidentalis</em> (400 mg/kg, p.o.)</td>
<td>19.62±1.83*</td>
<td>65.16</td>
</tr>
</tbody>
</table>

Data represented as mean ± S.D. Significant value at $P<0.05$

**Figure 1. Effects of ethanolic extract of *T. occidentalis* leaves on formalin test in rats**

The formalin test is a perfect model for analgesic and is sensitive for different analgesic drugs. This test produced

| Table 2. Effects of ethanolic extract of *T. occidentalis* leaves on carrageenan induced hind paw edema in rats |
|-------------------------------------------------------|------------------------------------------------|
| Animal groups                                        | Carrageenan induced rat paw edema Mean ± SD (%) |
|                                                      | 1h      | 2h      | 3h      | 4h      | 5h      | 24h     |
| Vehicle control (2% v/v tween 80)                    | 0.83±0.02 | 0.91±0.04 | 0.98±0.07 | 1.23±0.13 | 0.97±0.12 | 0.85±0.09 |
| Diclofenac sodium (25 mg/kg, i.p.)                  | 0.41±0.06 | 0.44±0.08 | 0.46±0.06 | 0.53±0.05 | 0.47±0.08 | 0.42±0.05 |
| Ethanol extract of *T. occidentalis* (200 mg/kg, p.o.) | 0.49±0.09 | 0.52±0.06 | 0.53±0.07 | 0.58±0.08 | 0.51±0.09 | 0.46±0.05 |
| Ethanol extract of *T. occidentalis* (400 mg/kg, p.o.) | 0.42±0.10 | 0.45±0.13 | 0.48±0.12 | 0.56±0.09 | 0.50±0.08 | 0.46±0.07 |

Data represented as mean ± S.D. Significant value at $P<0.05$
different analgesics effect may act differently in the early and late phases (Tjolsen et al., 1992). The analgesic effect of ethanol extract on the late phase of formalin test suggests that its activity can be correlated with its peripheral action and comparable with Diclofenac sodium activity. The results can be concluded that activity may be attributed to inhibition of prostaglandin release and other mediators.

The results of carrageein induced the paw edema by the administration of ethanol extract of T. occidentalis leaves (200 and 400 mg/kg) dose were shown in table 2. The ethanol extract with 400 mg/kg dose significant decreased the carrageein induced paw edema (P<0.05). The carrageenan test widely used to test nonsteroidal anti-inflammatory drugs. The maximum swelling in the carrageenan-injected paws was found after 3 hour after injection and the significant decrease in paw volume was recorded after treatment with ethanol extract by 400 mg/kg dose. The effect was approximately similar to the standard treatment group.

Conclusion

The present study indicates the ethanolic extract of T. occidentalis leaves shows that analgesic and anti-inflammatory activity in rats. The involvement of flavonoids and terpenes can be the possible chemical constituents responsible for analgesic and anti-inflammatory activity for which further research work is needed.

Conflict of interest

There are no conflicts of interest.

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References


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