

Short Communication

EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC POTENTIAL OF AQUEOUS METHANOLIC EXTRACT OF *THUJA ORIENTALIS* IN ALBINO RATS

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ABSTRACT

This study was conducted for evaluating a natural source to treat inflammation and pain, to avoid the severe side effects of currently used agents for these ailments. *Thuja Orientalis* (More Pankh) is commonly used for the treatment of pain and inflammatory disorders in traditional medicine. Carrageenan induced inflammatory model, acetic acid induced writhing test and hot plate methods were used to evaluate anti-inflammatory, peripheral and central analgesic properties of aqueous methanolic extract of *Thuja Orientalis* fruit (*To-Cr*) in albino rats. Completely randomized design (CRD) was constructed for the study and one way ANOVA was applied to compare means. The results showed that TO-Cr has significant anti-inflammatory and analgesic properties.

Key words: Analgesic, inflammation, oedema, anti-inflammatory, carrageenan, *Thuja Orientalis*

INTRODUCTION

Plants have been used by human being as remedies to many ailments since ancient times. The medicines of plant origin are being used in many traditional systems of medicine. Now a day the science of ethnobotany and ethnopharmacognosy are the main guidelines for the chemist for the search of new drugs (Gurib-Fakim, 2006). Recently, multidimensional approaches have been used combining phytochemical, biological, botanical and molecular technologies, for the discovery of new drugs from medicinal plants (Balunas and Kinghorn, 2005). The popularity of herbal traditional medicine in the treatment of different chronic diseases has increased in the recent years in world as compared with the western medicine. Many researchers have carried out investigational studies to assess the efficacies of various herbal medicines against chronic inflammation and pain (Ahmed *et al.*, 2005). Inflammation is the protective response of the living tissue to any foreign injurious stimulus. And inflammation is always associated with the redness, pain, oedema, heat formation and dysfunctioning of the tissue temporarily. Sometimes the inflammation process is prolonged without any apparent benefit. The external stimulus may be of physical (trauma), chemical (acids, alkalies etc.) or microbial (pathogens) origin (Brunton *et al.*, 2008). *Thuja Orientalis*, commonly known as "More Pankh" in local language, is commonly used as anti-inflammatory agent in traditional medicine. *Thuja Orientalis* (= *Platycladus orientalis* or *Biota orientalis*) is evergreen tree which is coniferous. It belongs to family Cupressaceae. It is grown

as an ornamental plant in Asia. Traditionally, it is used for variety of problems by folk healers but majority of medicinal uses have not been scientifically proved. The present study was conducted to evaluate the analgesic and anti-inflammatory properties of fruit of *Thuja Orientalis*. (Usmanghani *et al.*, 1997).

MATERIALS AND METHODS

Plant Material: The fruit of *Thuja Orientalis* was collected in the month of November. The fruit was cleaned physically by rubbing in muslin cloth. The fruit was dried under shade.

Preparation of plant extract: The dried fruit material was ground with mechanical grinder to reduce particle size and increase surface area for maximum extraction. The ground material in the form of powder was soaked in 70 % methanol for three days and filtered through muslin cloth. The filtrate obtained after three successive macerations was filtered through filter paper. The filtrate was dried to semisolid mass in rotary evaporator under reduced pressure. The semisolid mass obtained was weighed and percentage yield was calculated (Jabeen *et al.*, 2009). The extract was stored at temperature below 8°C for later use.

Experimental Animals: The wistar albino rats of either sex (220- 250 grams) were used for the study. The animals were maintained in identical laboratory conditions i.e. 25-30 °C and relative humidity of 55-65 %. The animals had free access to feed and water.

Carrageenan induced paw oedema: To study the anti-inflammatory properties, carrageenan induced rat paw oedema model was used. The animals were divided into five groups (n=6). Carrageenan solution in concentration of 1% w/v in normal saline, was used to produce acute inflammation. 100 microliter of carrageenan solution was injected into plantar region of right hind paw of animal. The paw volume was measured at 0 and 3 hours after the injection of carrageenan. All the groups were pre-treated 1 hour before injection as follows: group I was given normal saline 10 ml / kg p.o. (Chao et al., 2009), group II was given indomethacin 10 mg / kg p.o. Groups III, VI and V were given TO-Cr orally at doses of 50 mg, 100 mg and 300 mg / kg respectively.

Determination of paw volume: The volume of the paw was determined by a novel method called liquid immersion method (Fereidoni et al., 2000). In this method liquid filled in a beaker is placed on a weighing balance. When the paw is immersed in the liquid, a force F is applied to the balance due to the displacement of liquid in beaker. This force F results in an increase in reading on the balance, and is equal to the increase in weight on balance.

The volume of the paw (or any other object immersed) can be calculated from the following formula;

$$\text{Volume} = \text{weight} / \text{specific gravity of liquid}$$

In this case the distilled water was taken as the liquid on balance, so the value of specific gravity was taken as 1. And increase in weight was taken as the increase in volume according to the following factor.

$$1 \text{ gram} = 1 \text{ ml or cubic centimeter}$$

Determination of paw oedema: The oedema was calculated by subtracting the paw volume measured immediately after injection (V_0) from the volume measured at specific time (V_t) thereafter.

$$\text{Oedema at time } t = \text{volume at } t \text{ hour} - \text{volume at 0 hour} (V_t - V_0)$$

Assessment of Peripheral analgesic effects: The peripheral analgesic activity of *Thuja orientalis* was evaluated using the acetic acid induced writhing test. Each group consisted of 6 animals. All groups were pretreated as follows:

Group I (negative control): was given normal saline 10ml/Kg p.o.

Group II (positive control): was given Aspirin 300 mg/Kg p.o.

Group III (TO-Cr): was given TO-Cr 100 mg/Kg p.o.

The writhing movements were observed and counted for 20 min after the administration of acetic acid.

Assessment of Central analgesic effects: The central analgesic action of *Thuja orientalis* was assessed by hot plate at 55±2 °C. 3 groups of animals (n=6) were used for hot-plate method (Hajare et al. 2000) with following pre-treatments.

Group I (negative control): was given normal saline 10ml/Kg p.o.

Group II (positive control): was given Tramadol 20 mg/Kg p.o.

Group III (TO-Cr): was given TO-Cr 100 mg/Kg p.o.

The response time (latency time) for lifting hind paw or licking was noted before administration and after 20, 60 and 90 min following oral administration of the standard drug and methanolic extract of *Thuja Orientalis* (Vogel, 2007).

Statistical analysis: All the data was expressed as mean ± S.D. Experimental design was completely randomized design (CRD). The result values were analyzed for statistical significance by One-way ANOVA and treatment means were compared then by Post Hoc test.

RESULTS AND DISCUSSION

Carrageenan induced paw oedema: The anti-inflammatory effect of aqueous methanolic extract of *Thuja Orientalis* (TO-Cr) is shown in Fig. 1. The aqueous methanolic extract of *Thuja Orientalis* showed dose dependant effects as compared with standard drug. The standard drug Indomethacin showed 79.70 % inhibition, whereas, TO-Cr showed 13.04 %, 34.00 % and 59.57 % inhibition at doses of 50, 100, 300 mg/kg of extract after 3 hours of carrageenan injection (Fig. 1).

Peripheral analgesic effect: In acetic acid-induced writhing test TO-Cr reduced the number of writhings significantly. Number of writhings was reduced by 40 % with the extract dose (100 mg / kg) while it was reduced by 65 % with the standard drug i.e. Aspirin (300 mg / kg) as compared with the negative control (Fig 2).

Central analgesic activity: In hot plate test, latency times were recorded at 0, 20, 60 and 90 min after treatment. The observations showed that latency time with extract of *Thuja Orientalis* was significantly increased till 60 min but after 60 min, there is no significant difference till 90 min. Whereas the latency time was maximum (22.48±1.84) with Tramadol at 90 min. (Table 3).

The anti-inflammatory activity was studied by using carrageenan-induced paw oedema model, as this is the most widely used model for this study among other models (Das et al., 2010). The standard drug used for anti-inflammatory study was indomethacin. The volume of the paw was measured using a novel method, liquid immersion method (Fereidoni et al., 2000). There was no major difference among groups in the paw volume at 0 hour (Fig 1). But it is evaluated from the study of paw volumes after 3 hours that a significant decrease in oedema is observed in group treated with standard drug i.e. indomethacin (79.70 % decrease) as compared with the negative control (Fig. 1). And the response of the

extract under study was dose related. There was 13 % decrease in paw oedema as compared with negative control with 50 mg / kg dose of TO-Cr (Fig 1). Similarly

there was 34 % and 59.57 % decrease in paw oedema as compared with negative control with 100 mg / kg and 300 mg / kg doses of TO-Cr (Fig. 1).

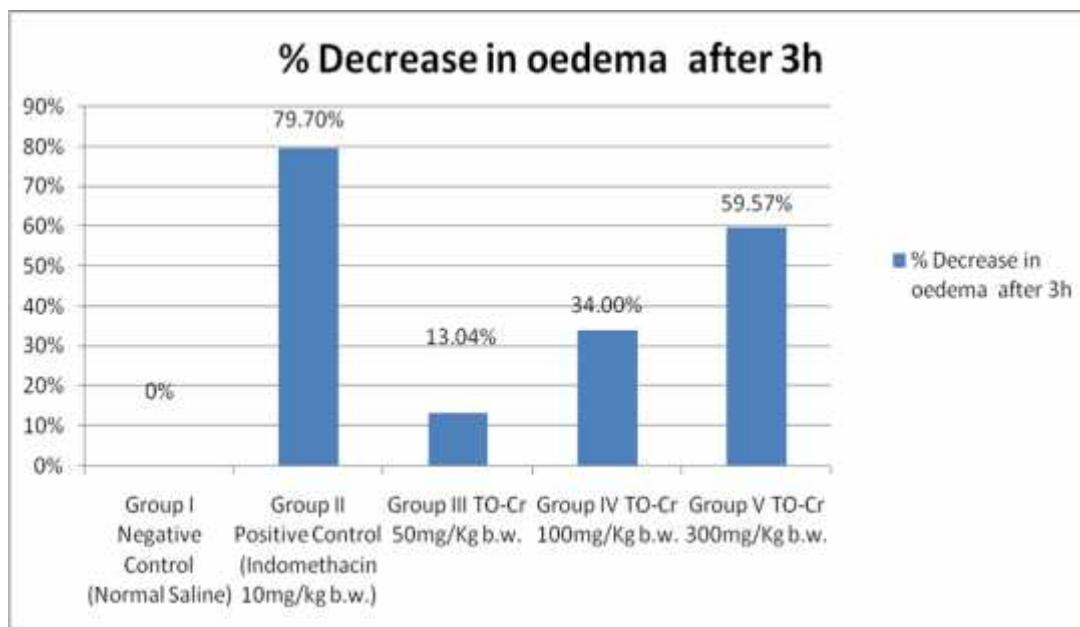


Fig. 1: Graph of Average oedema (ml), and % decrease in oedema in +ve Control and Treated groups at 3 hour after the treatment as compared with -ve control

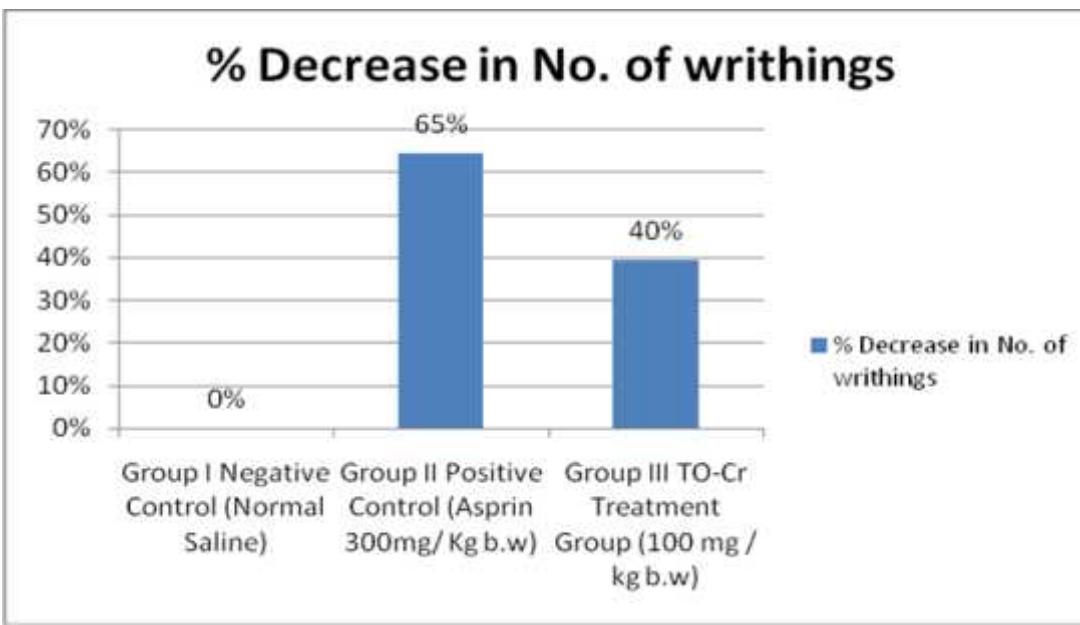


Fig. 2: Percent Decrease in writhings of Positive Control and Treated group as compared with Negative control

The central analgesic study was done by using hot plate method. The latency time in negative control was approximately the same at 0, 20, 60, and 90 min, but there was significant increase in latency time in positive control group at 60 min interval and then it remained almost same after 90 min (Table 1). The latency times

noted at 0, 20, 60 and 90 min after treatment showed that aqueous methanolic extract of *Thuja orientalis* TO-Cr has significantly increased the latency time till 60 min, but there is no significant difference in latency times at 60 and 90 min after treatment (Table 1).

Table 1. Effect of TO-Cr extract and Tramadol compared with negative control as central analgesic.

Treatment	Dose (mg / Kg)	Latency time in sec. at time (min.)			
		0	20	60	90
Negative Control		7.32±0.71	7.52±1.26	6.87±1.18	6.97±0.38
Tramadol	20	7.40±0.61	11.40±1.12	15.93±1.13	22.48±1.84
TO-Cr	100	7.07±0.90	9.47±0.59	13.58±1.43	13.72±1.41

The peripheral analgesic activity was determined by acetic acid induced writhing test. The writhings were counted for 20 min after treatment. The writhings were compared with that of negative control. There was 65 % decrease in positive control with standard drug i.e. aspirin and 40 % decrease in writhings as compared with negative control (Fig. 2). Since prostaglandins are considered to play an important role in perception of pain so it may be suggested that extract of plant TO-Cr may contain constituents like flavonoids which inhibit the synthesis of prostaglandins. That's why the analgesic effects of the plant extract are observed.

It is finally concluded from the results of the present study that the oral administration of aqueous methanolic extract of *Thuja Orientalis* has significant anti-inflammatory and analgesic properties. Thus study confirms its use as folk medicine.

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