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Preventive effect of *Thuja occidentalis* (Linn) on gastric ulcer - a novel role of free radical scavenger

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Abstract

In this study, 95% ethanolic extract of *Thuja occidentalis* Linn (*TOE*) was evaluated for its anti-ulcer potential by using hard liquor (42.8 % v/v ethanol, 1 ml/150 g body weight) and aspirin (200 mg/kg) induced gastric ulcer models on rats, where oral administration of 200 and 400 mg/kg of *TOE* showed significant (***$p<0.001$) ulcer protective effect on both the models in a dose dependent fashion. The *TOE* was also evaluated for its *in-vitro* free-radical scavenging activity and anti-inflammatory activity. The studies showed significant (***$p<0.001$) free radical scavenging activity of *TOE*, when compare to control, whereas *TOE* failed to exhibits any significant (ns $p>0.05$) anti-inflammatory activity on Carrageenan - induced paw oedema and Cotton pellet granuloma models. Since the extract fail to show significant anti-inflammatory activity, the inhibitory role on prostaglandin synthesis and over production of leukotriene may be ruled out, so we can suggest that gastro-protective activity against aspirin induced ulcer by inhibiting the back diffusion of H^+ ions. However, further studies are needed to confirm this hypothesis. On the basis of these investigations, we may partially conclude that ulcer-protection may be due to free radical scavenging activity of *TOE* and *TOE* could be a potent antiulcer agent for next generation.

Keyword: *Thuja occidentalis*, Anti ulcer, Thujone, Anti oxidant, Anti-inflammatory.

1. Introduction

Gastro intestinal disorders are one of the severe classes of human ailments causing maximum discomfort, morbidity and mortality. Peptic ulcer is one such GIT disorder. Peptic ulcer is a being lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to acid and pepsin. There are several causes including stress, alcohol consumption, cigarette smoking, *H. pylori* infection, ingestion of drugs and chemicals. The role of free radicals in the pathogenesis of peptic ulcer due to mucosal damage is established. If it is not treated properly it may results in perforation in the wall of the gastro intestinal tract.

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Therefore, normally chronic dietary control and phramacotherapeutic management is adopted for treatment of peptic ulcer. However, several side effects like, arrhythmias, impotence, gynaecomastia, hematopoietic changes etc. associated with the various synthetic drugs like H_2 blockers, proton pump inhibitors, anticholinergic agents etc. used for the management of peptic ulcer is restricting the prolonged uses of these agents [1]. In such context now a global trend to review the traditional system of medicines and renewed interest in the natural remedies for treating human ailments. With this background we thought of finding a remedy available at a hand's stretch for the treatment and management of peptic ulcer. In this regard we concentrated on certain herbs which are used in various human ailments.

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. In folk medicine, *Thuja occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism [2]. In India it is grown as an ornamental plant.

A literature survey reveals that Thuja been reported to possess activity against HIV-1 virus [3, 4] and common cold. It has also been reported to increase in the proliferation of spleen cell as well as TNF-α, IL-6 and IL-1 activity in serum [5].

Thuja has also been reported to have protective effect against radiation induced toxicity [6]. The genus Thuja was considered to consist of the species. *Thuja karoiensis* Nakai, *Thuja occidentalis*, *Thuja orientals* L. and *Thuja plicata* D.Don [7, 8]. Which 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 [8].

2. Methodology

2.1 Plant Material

Thuja occidentalis, fresh aerial parts were collected from Garden of B. R. Nahata College of Pharmacy, Mandsaur (M.P.) India in August 2005 and identified by Dr. H. S. Chatree (Ex. Professor Botany Govt. P.G. College, Mandsaur) and a voucher specimen (T/001/2005) was deposited for reference to department of pharmacognosy BRNCP.

2.2 Preparation of Extract

Shade-dried aerial part powder was extracted with 95% ethanol by soxhletion. The extract was concentrated by rotary vacuum evaporator. The dried extract was stored in air tight container in refrigerator below 10°C. The extract was suspended in distilled water for pharmacological experiments.

2.3 Experimental Animals

Wistar rats (150-200 gms) and albino mice (20-30 gm) 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 (proposal no. 31/M.Ph/06/IAEC/BRNCP) approved by Institutional Animal Ethics Committee (Reg. No. 918/ac/05/CPCSEA/BRNCP).

2.4 Determination of acute toxicity (LD_{50})

The acute toxicity for 95% ethanolic extract of *Thuja occidentalis* aerial part was determined in Wistar rats, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies [9].

2.5 Pharmacological evaluation

2.5.1 Anti ulcer activity

Gastric ulceration, according to the method described by Al-Shabanah [10] was induced in 24 hrs. fasted rats by the administration of necrotizing agent like hard liquor (42.8% ethanol at 1ml/150 g p.o. dose) and ulcerogenic drugs like Aspirin (at 200 mg/kg, p.o. dose) to group of 6 animals each were pre-treated with *Thuja occidentalis* 95% ethanolic extract (200, 400 mg/kg, p.o.) 1 hr. before the ulcerogenic procedures.

The animals were sacrificed 6 hr. after administration of ulcerogenic drugs or 2 hr. after the administration of necrotizing agent (an overdose of hard liquor). The stomachs were removed and opened along with the greater curvature of stomach; the ulcer index were evaluated according to severity and scored microscopically with the help of hand lens (10x) as follows. 0 = Normal coloured stomach, 0.5 = Red coloration, 1 = Spot ulcers, 1.5 = Haemorrhaegic streaks, 2 = Ulcer > 3 mm but < 5mm, 3 = Ulcers > 5mm Lansoprazole (8 mg/kg, p.o.) was used as an antiulcer standard [11].

2.5.2 In-vitro antioxidant activity.

The assay was based on the capacity of the sample to inhibit blue formazan formation by scavenging the super oxide radicals generated in riboflavin-light-NBT system. The reaction mixture contains 50 mM phosphate buffer, pH 7.6, 20 µg riboflavin 12 mM, reaction was started by illuminating the reaction mixture with different concentration of samples (like 5 µg, 10 µg, 25 µg, 50 µgm 100 µg) for 15 minutes. Immediately after illumination the absorbance was measured at 590 nm and EC_{50} was calculated. Ascorbic acid was used as positive control [12].

2.5.3 Evaluation of Anti-inflammatory Activity

2.5.3.1 Carrageenan - induced paw oedema.

Inflammation was induced by injecting 0.1 ml of 1% w/v carrageenan sodium salt subcutaneously in the sub-plantar region of the rat right hind paw according to X.M.liu *et.al* 2001. The *Thuja occidentalis* 95% ethanolic extract (200, 400 mg/kg) was administered orally, 1 hr. before carrageenan injection while control group received saline (10 ml/kg, p.o.). The hind paw volume was measured plethysmometrically before and after the carrageenan injection, at hourly intervals for 4hr [10].

$$\%\,\text{inhibition of edema} = \left(\frac{Vc - Vt}{Vc}\right) x100$$

Where, V_t = mean paw volume of test group. V_C = mean paw volume of control group.

2.5.3.2 Cotton pellet granuloma

A 50 mg sterilized cotton pellet was implanted subcutaneously on the back of neck in rats under ether anesthesia. Animals in test groups received the extract (200, 400 mg/kg p.o.), once daily for 14 consecutive days.

Animals in the control group received only the vehicle (10 ml/kg, i.p.) and Diclofenac sodium (4 mg/kg, i.p.) was given as reference drug. On the 14^{th} day, the animals were sacrificed and the pellets granulomas were removed, fixed from extraction tissue, dried overnight at 55 ± 0.5°C and weighed [10].

Table 1: Effect of ethanolic extract of *Thuja occidentalis* Linn (TOE) fresh aerial parts on gastric ulcers induced by ulcerogenic drugs and necrotizing agents in rats.

Treatment	Dose mg/kg/p.o	Mean Ulcer index ± SEM	% inhibition
Hard liquor induced ulcer			
5 ml/kg water p.o	-	0.333 ± 0.247	-
1 ml/150 g hard liquor p.o	-	18.66 ± 1.838	-
Lansoprazole + 1 ml/150 g hard liquor p.o	8	3.41*** ± 1.21	81.71
TOE + 1 ml/150 g hard liquor p.o	200	5.41*** ± 1.630	71
TOE + 1 ml/150 g hard liquor p.o	400	2.66*** ± 0.572	85.74
Aspirin induced ulcer			
5 ml/kg water p.o	-	0.166 ± 0.1054	-
Aspirin 200 mg/kg p.o	-	17.3 ± 1.229	-
Lansoprazole + Aspirin 200 mg/kg p.o	8	3.41*** ± 1.21	81.71
TOE Aspirin 200 mg/kg p.o	200	4.166*** ± 0.988	75.91
TOE Aspirin 200 mg/kg p.o	400	1.25*** ± 0.3354	92.77

Ulcer Index were expressed as mean ± Standard Error Mean (SEM), *** p<0.001 (n=6) vs. +ve control, one way ANOVA test.

Table 2 : *In vitro* antioxidant activity of ethanol extract of *Thuja occidentalis* Linn (*TOE*) fresh aerial parts in superoxide scavenging pathway.

Treatment and Dose in µg	% inhibition
Control	-
TOE 5	80.63
TOE 10	82.96
TOE 25	85.25
TOE 50	87.82
TOE 100	88.96

2.6 Statistical analysis

Result were expressed as mean ± 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<0.01, ***p<0.001), when compared with control and toxicant group as applicable.

3. Result

In acute toxicity study, 95% ethanolic extract of *Thuja occidentalis* aerial part showed no mortality at 2000 mg/kg. Therefore 2000 mg/kg dose was considered as LD_{50} cut off the dose (a safe dose), so 1/10th and 1/5th of that were selected (200, 400 mg/kg dose) for all *in vivo* experiments as sub-maximal and maximal dose. Pre treatment with 95% ethanol extract of *Thuja occidentalis* aerial part (200, 400 mg/kg, p.o.) produced significant and dose - dependent decrease in the intensity of gastric mucosal damages induced by ulcerogenic drug (Aspirin) and necrotizing agent (hard liquor) (Table 1). From the study of *in vitro* super oxide scavenging activity of the extract, it is observed that the extract has demonstrated dose dependent increase in super oxide scavenging activity and at 100 µg/ml has more reducing property (Table 2). 95% ethanol extract of *Thuja occidentalis* aerial part (200, 400 mg/kg, p.o.) neither reduced the increase in hind paw oedema induced by carrageenan (Table-3) nor the weight of the granuloma produced by cotton pellet method (Table-4) significantly.

Table 3 : Effect of ethanolic extract of *Thuja occidentalis* Linn (*TOE*) fresh aerial parts on Carrageenan induced paw oedema in rats.

Treatment and Dose (mg/kg)	Mean paw volume (ml) 0 hrs	½ hrs	1 hrs	2 hrs	3 hrs
Control	0.955 ± 0.053	1.231 ± 0.041	1.431± 0.084	1.775 ± 1.293	1.915 ± 0.105
Diclofenac 100	0.686 ± 0.055	0.81 ± 0.066	0.873 ± 0.068	0.933 ± 0.078	0.908 ± 0.078
TOE 200	1.05 ± 0.061	1.255 ± 0.056	1.35 ± 0.069	1.51 ± 0.073	1.77 ± 0.05
TOE 400	0.996 ± 0.0686	1.19 ± 0.05	1.38 ± 0.05	1.53 ± 0.05	1.60 ± 0.053

Values are mean ± SEM (n=6)

Table 4 : Effect of ethanolic extract of *Thuja occidentalis* Linn (*TOE*) fresh aerial parts on cotton pellet induced granulation in rats.

Treatment and Dose in mg/kg/day	Mean weight of the granulation (mg) ± SEM
Control	100.66 ± 1.430
Diclofenac 100	64.833 ± 1.579
TOE 200	101.83 ± 1.773
TOE 400	98.16 ± 1.662

Values are mean ± SEM (n=6)

4. Discussion

In our pursuit for the search of gastro-protective plants, we came across with most popularly used plant *Thuja occidentalis* aerial part which is claimed to possess gastro-protective activity [2]. In addition there are reports that free radicals are involved in gastric ulceration in various experimentally induced gastric ulcers, keeping all these things in view the present study was carried out for screening of antioxidant and gastro protective properties of the plant and to correlate them [13].

In addition an attempt has also been made to screen *Thuja occidentalis* aerial part for it possible anti-inflammatory activities. In present study 95% ethanol extract demonstrated dose dependent superoxide radical scavenging activity. There are reports that reactive oxygen species are responsible for pathogeneses of gastric ulcer [14]. In addition, lipidperoxidation and involvement of other free radicals are demonstrated in various experimentally induced ulcers [15].

The results of alcohol induced gastric ulceration model shows that treatment with extract of *Thuja occidentalis* aerial part has reduced the ulcer index significantly. When compared with positive control decrease in mucosal resistance is considered to be most important etiological reason in alcohol induced gastric ulcers. Even, there are reports that alcohol increases the secretion of protein into the gastric juice. Similarly in ethanol induced ulcer GSH level is reduced in gastric mucosa. The gastric GPX level is also reduced. In addition, ethanol causes ulcer by producing toxic oxygen derived free radicals [16].

Since the result of present study demonstrated the gastro protective activity and superoxide anion scavenging activity, it may be suggested that gastro protection of 95% ethanol extract of *Thuja occidentalis* aerial part may be due to increase in the mucosal resistance or superoxide radical scavenging activity of the extract or both in alcohol induced gastric ulcer model.

NSAIDS are the most popular class of pain killers and are possessing side effects like gastric irritation and gastric ulceration. This is

because of inhibition COX-I and resulting in the inhibition of synthesis of prostaglandins, consequently there is enhanced lipoxygenase pathway liberating leucotrienes and these leucotrienes are reported to have role in ulcerogenesis. In addition there is some evidence that aspirin induces gastric ulcer by causing back diffusion of H^+ ions into the mucosal cells [17]. Since the extract fail to show significant anti-inflammatory activity, the inhibitory role on prostaglandin synthesis and over production of leucotrienes may be ruled out, so we can suggest that gastro-protective activity against aspirin induced ulcer by inhibiting the back diffusion of H^+ ions. However, further studies are needed to confirm this hypothesis.

Overall in both the models of ulcers studied in our lab, it was observed that there was a great imbalance between the aggressive factors of ulcers and in built protective mechanisms. Treatment with 95% ethanol extract of *Thuja occidentalis* aerial part has reduced the ulcer index in both the models. This may be due to bringing back the balance between above mentioned factors. The mechanism of this protective action may be due to the superoxide and other free radical scavenging activity also contributes to gastro protective activity of the *Thuja occidentalis*.

5. Conclusion

The present study reveals the ethanol extract of *Thuja occidentalis* shows significant protection against hard liquor and aspirin induced gastric ulcers in rats. Also the extract showed significant free radical scavenging action but did not show significant anti-inflammatory activity, which may put some light on possible mechanism of action of the active constituents present. On the basis of these investigations, we may partially conclude that ulcer-protection may be due to free radical scavenging activity of *TOE* and *TOE* could be a potent antiulcer agent for next generation. Neither the exact biological active constituent(s) responsible for the said effect nor the exact mechanism of action is reported earlier.

Further studies need the isolation of the active constituent(s) and the possible mechanism(s) of action for the said activity.

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