Vol 3 | Issue 2| 2012 | 68-71.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

ANTI-ULCEROGENIC ACTIVITIES OF *TREWIA NUDIFLORA* IN DIFFERENT EXPERIMENTAL MODELS

***V. Rajalakshimi, S. Chaithanya Kumar, P. Rajeswary, A. Madhuri, U. Chandrasekhar**

*Department of Pharmacology, Jagan's College of Pharmacy, Nellore, Andhra Pradesh, India.

ABSTRACT

The purpose of the present study is to investigate the acute oral toxicity and anti-ulcerogenic activities of the ethanol Extract of *Trewia nudiflora (*EETN) leaf extract in albino rats. Study on acute toxicity of extract found to be safe at the doses 2000mg/kg body weight orally as per OECD guidelines No.423. EETN at the doses of 200 and 400 mg/kg body weight orally was administered to evaluate anti-ulcer activity by using indomethacin and cold-restraint stress induced gastric ulcer models in Albino rats. Ethanol Extract of *Trewia nudiflora* dose dependent inhibition in indomethacin induced gastric lesions, and it also dose dependent inhibition in Cold-restraint stress induced gastric lesions. All the results are found to be statistically significant ($p \leq 0.05$). Hence we suggest that Ethanol Extract of the leaves of *Trewia nudiflora* possess anti-ulcerogenic properties that may be due to cytoprotective mechanism.

Key words: *Trewia nudiflora*, Anti-ulcer activity, Indomethacin, Cold-restraint stress.

INTRODUCTION

Peptic ulcer disease (encompassing gastric ulcer and duodenal ulcer) affect a large portion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies, and ingestion of non-steroidal anti-inflammatory drugs [1]. The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection [2,3]. There has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Modern approach to this includes proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog [4]**.** Development of tolerance and incidence of relapses and side effects on clinical evaluation make their efficacy arguable. This has been the basis for the development of new antiulcer drugs, which includes herbal drugs.

Trewia nudiflora (Family: Euphorbiaceae), which is tall arbor and distributes in the tropical districts of India, Malaysia and China. Some maytansinoids isolated from the *Trewia nudiflora* seeds are tumor inhibitors and may be responsible for the resistance of the seeds to fungal degradation. However, there were no reports about the isolation of antibacterial components from the seeds of *Trewia nudiflora* from the seed crusts [5]. This present study carried out to assess the validity of the folkloric uses of this plant in antiulcerogenic property and establish the possible mechanisms of pharmacological action. The present investigation was carried out to investigate the constituents and anti-ulcer profile of the ethanol extract of *Trewia nudiflora* (EETN) is being reported here.

METERIALS AND METHODS

Plant collection

The leaves of *Trewia nudiflora* has been collected from Sri Venketeswara University near Tirupati, Andhra Pradesh during the month of June 2011 and dried under shade. The plant was authentified by Mr. K. Madhava chetty, Assistant Professor, Department of Botany of S. V. University, Tirupati. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of extracts

Leaves of *Trewia nudiflora* were shade dried, and the dried leaves were powdered to get coarse granules. The coarse powder was subjected to continuous hot extraction in Soxhlet apparatus using ethanol. The solvent was removed by distillation under reduced pressure, which produced a greenish sticky residue (yield 13.5%w/w with respect to dried plant material). The concentrated crude extract were stored and used for the further study.

Corresponding Author: **V. Rajalakshimi** Email id: rajii.vasudevan86@gmail.com

Animal Used

Albino Wistar rats, weighing 220–250 g were used. The selected animals were housed in acrylic cages in standard environmental conditions (20–25° C), fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the Institutional Ethical Committee.

Acute Toxicity Study

The acute toxicity of Ethanol extract of leaves of *Trewia nudiflora* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at the 2000 mg/kg doses. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose was selected for further study [6].

Indomethacin induced gastric ulcer

Animals were divided into four groups each of six rats. Group I treated with 4% v/v tween 80 (10 ml/kg p.o), Group II & III treated with Ethanol extract of *Trewia nudiflora* (200and 400mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14^{th} day, Gastric ulcer were induced with indomethacin (40 mg/kg p.o) administered to all groups after fasting for 24 h. The animals were sacrificed 4 h after treatment with the ulcerogenic agent to assess the antiulcer activity and ulcer index were examined on the dissected stomachs as described below.

Cold-restraint stress-induced ulcers

Animals were divided into four groups each of six rats. Group I treated with 4% v/v tween 80 (10 ml/kg p.o), Group II & III treated with Ethanol extract of *Trewia nudiflora* (200and 400mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole (20 mg/kg p.o). On the 14^{th} day, One hour after drug treatment, the experimental rats were immobilized by strapping the hind limbs on a wooden plank and kept for 1 h 30min, at temperature of 3–5 ◦C [7-10]. One hour later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs as described below.

Measurement of ulcer index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%*I*) was calculated [11] using the following formula:

$$\%I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control and USt = ulcer surface area in treated animals.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

Acute toxicity study in which the animals treated with the EETN at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of* EETN *on gastric ulcer induced by Indomethacin

The EETN showed significant anti-ulcer effect against ulcers induced by *Indomethacin* in a dose dependent manner. In *Indomethacin* induced ulcer model, EETN at a dose of 200 and 400 mg/kg body weight showed protective effect of 60.95 and 71.65%, respectively, whereas Omeprazole showed protection index of 79.41 % at a dose of 20 mg/kg body weight (Table 1).

Effect of* EETN *on gastric ulcer induced by Cold-restraint stress

The EETN showed significant anti-ulcer effect against ulcers induced by *Cold-restraint stress* in a dose dependent manner. In the gastric ulcer induced by *Cold-restraint stress*, EETN at a dose of 200 and 400 mg/kg body weight showed again significant activity. EETN at a dose 200 and 400 mg/kg body weight showed dose-dependent protective effect of 49.57 and 73.43% respectively, whereas Omeprazole showed protection effect of 83.05% at a dose of 20 mg/kg body weight, in both the above models (Table 2).

DISCUSSION & CONCLUSION

The results of this study show that the Ethanol extracts from the leaves of *Trewia nudiflora* exert protective effects against indomethacin and cold restraint stress-induced gastric mucosal damage. Their anti-ulcerogenic potency was tested against indomethacin-induced ulcer. Indomethacin is a cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogeneous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow in animals**.** It is also well known that prostaglandins synthetized in large quantities by the gastrointestinal mucosa can prevent experimentally induced ulcers by ulcerogens. Thus, when the ulcers lesions are induced by indomethacin, the cytoprotective effect of the anti-ulcer

agent can be mediated through endogeneous prostaglandins**.** The results obtained show that the mean ulcer index was significantly reduced in the ethanol extracts from the leaves of *Trewia nudiflora* treated groups, compared to their respective controls [12]. *Trewia nudiflora* extracts may be stimulate the secretion of prostaglandins or possess prostaglandins like-substances. To further confirm its anti-ulcerogenic effect we have evaluated the efficacy of EETN against Cold-restraint stress -induced ulcer model. Gastric ulceration induced by stress is probably mediated by the presence of acid, increase in gastric motility, mast cell degranulation, decreased gastric mucosal blood flow, decreased prostaglandin synthesis and augmented excretion of glycoproteins in the mucus [13]. Moreover, stress-induced ulcer can be prevented partially or entirely by vagotomy; vagal over activity has been suggested to be the principal factor in stress-induced ulceration [14]. Any of these factors could play a role in genesis of stress-induced ulcers. Oral administration of the ethanol extracts of *Trewia nudiflora* showed dose dependent inhibition of gastric ulceration induced by Cold-restraint stress.

The ethanol extracts of *Trewia nudiflora* at a dose of 400mg/kg showed similar activity to that of omeprazole (a proton pump inhibitor, which is used to heal stomach and duodenal ulcers). The gastro protective effect of omeprazole is mediated through block of acid secretion by inactivation of H+/K+-ATPase [15,16]. This study reveals that the ethanol and methanol extracts from the leaves of *Trewia nudiflora* are potent inhibitors of gastric mucosal lesions caused by indomethacin, and cold-restraint stress in rats [17].

Further, our results fortify the ethano pharmacological importance of EETN as an anti-ulcer agent. Etiology of ulcers produced in different ulcer models is diverse. Since EETN has been found effective in various models depicting its anti-ulcerogenic activity. EETN and its active constituents may emerge as more effective therapeutic agent to counter gastric ulcer incidence. However more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

Table 1. Effect of Ethanol Extract of *Trewia nudiflora L. (*EETN*) in* indomethacin (40mg/kg) induced gastric ulcer in rats

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (4% v/v tween 80, 10 ml/kg b.w) p.o	12.24 ± 0.22	---
II	EETN (200mg/kg b.w) p.o	4.78 ± 0.17*	60.95
III	EETN (400mg/kg b.w) p.o	3.47 ± 0.41**	71.65
IV	Omeprazole (20mg/kg b.w) p.o	2.52 ± 0.33**	79.41

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. $^{*}P < 0.01$ and $^{**}P < 0.001$ as compared to control (n = 6 in each group). EETN= Ethanol Extract of *Trewia nudiflora L.*
B.W=Body weight.

Table 2. Effect of Ethanol Extract of *Trewia nudiflora L. (*EETN*)* on Cold-restraint stress induced Gastric ulcer in Rats.

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (4% v/v tween 80, 10 ml/kg b.w) p.o	9.26 + 2.3	-
II	EETN (200mg/kg b.w) p.o	4.67 + 0.13*	49.57
III	EETN (400mg/kg b.w) p.o	2.46 + 0.52**	73.43
IV	Omeprazole (20mg/kg b.w) p.o	1.57 + 0.41**	83.05

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. $^{*}P < 0.01$ and $^{**}P < 0.001$ as compared to control (n = 6 in each group). EETN = Ethanol Extract of *Trewia nudiflora L.*
B.W=Body weight.

REFERENCES

1. Nash J, Lambert L, Deakin M. Histamine H2-receptor antagonists in peptic ulcer disease. Evidence for a prophylactic use. *Drugs*, 47, 1994, 862–871.
2. Hoogerwerf WA, Pasricha PJ. Agents used for control of gastric acidity and treatment of peptic ulcers and gastroesophageal reflux disease. In: Hardman JG, Limbird LE, Goodman Gilaman A (Eds.). The Pharmacological Basis of Therapeutics, tenth ed. Mc Graw-Hill, New York, 2001, 1005–1019.
3. Valle DL. Peptic ulcer diseases and related disorders. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. (Eds.), Harrison's Principles of Internal Medicine, vol. 16. McGraw-Hill, New York, 2005, 1746–1762.
4. Manonmani S, Viswanathan VP, Subramanian S, Govindasamy S. Biochemical studies on the antiulcerogenic activity of cauvery 100, an ayurvedic formulation in experimental ulcers. *Indian Journal of Pharmacology*, 27, 1995, 101–105.
5. Madhava chetty K. et al. "*Trewia nudiflora*" Flowering Plants of Chittoor District, Andhra Pradesh, India. 2008.
6. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris, June, 2000.

7. Hiruma-lima CA, Gracioso JS, Toma W, Almeida AB, paula. Gastroprotective effect of Aparisthman. A terpene Isolated from Aparisthmium Cordatum, on Experimental Gastric Ulcer Models in Rats and Mice. *Phytomedicine,* 8(2), 2001, 94-100.
8. Singh S, Khajuria A, Singh J, Taneja SC. The Gastric Ulcer Protective Effect of Boswellic Acids a Leukotrine Inhibitor from Boswellia Serrata in Rats. *Phytomedicine,* 15, 2008, 408-415.
9. Zayachkivska OS , Konturek SJ, Drozdowicz D, Konturek PC, Brzozowski T, Ghegotsky MR.Gastroprotective Effects of Flavonoids in Plant Extracts. *Journal of Physiology and Pharmacology,* 56, 2005, 219-231.
10. Hanifa Moursi SA, Izaldin Al Khatib MH. Effect of Melia Azedarach Fruits on Gipsing-Restraint Stress-Induced Ulcers In Rats. *Japan J Pharmacol*, 36, 1984, 527-533.
11. Alvin Berger, Peter Jones JH, Suhad Abumweis S. Plant Sterols: factors Affecting their Efficacy and Safety as Functional Food Ingredients, 2007.