Vol. 7/2 (2007) 207 - 213

JOURNAL OF NATURAL REMEDIES

Evaluation of anti-ulcer properties of the leaf extract of *Juniperus communis* L. in animals

Kartick Chandra Pramanik[1], Ria Biswas[1], Durba Bandyopadhyay[1], Moumita Mishra[1], Chintamoni Ghosh [2], T. K. Chatterjee[1*]

1. Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata - 700 032, India.

2. State Pharmacopoeial Laboratory and Pharmacy for Indian Medicine, Kalyani, Nadia - 741 235, India.

Abstract

Objective: The purpose of this present study is to elicit the anti-ulcer property of *Juniperus communis* (Linn.). Materials and Methods: In the present communication, models of acetyl salicylic acid, serotonin, indomethacin, alcohol and stress-induced gastric ulcerations in rats and histamine-induced duodenal lesions in guinea pigs were adopted to study the anti-ulcer activity of the extract. Biochemical parameters like pH, total acidity and peptic acidity of the gastric juice was also studied. Results: The crude leaf extract at doses of 50 mg and 100 mg/kg (i.p) significantly inhibited aspirin, serotonin, indomethacin, alcohol and stress-induced gastric ulcerations in rats and histamine-induced duodenal lesions in guinea pigs. The healing rate of acetic acid induced ulcer in rats was also enhanced significantly by the leaf extract. The results were at par with the standard drug ranitidine. Biochemical analysis of gastric juice revealed that the extract significantly decreased its volume and total acidity, but did not alter its pH and peptic activity. Conclusion: The present study revealed that the leaf extract of *J. communis* is a potent ulcer pain reliever and also promotes healing, which are the two ultimate goals of therapy for ulcers.

Key words: *Juniperus communis* (L.); anti-ulcer; leaf extract; gastric lesion.

1. Introduction

The lifetime prevalence of peptic (gastric and duodenal) ulcer disease is approximately 10% and it is estimated that 50% of healthy individuals experience heart-burn on daily basis. It is also known that anti-inflammatory drugs are often ulcerogenic, which restricts their use in long-term therapy in pain management. Moreover, drugs that reduce gastric acid secretion (H_2 histamine receptor antagonists and covalent inhibitors of the H^+, K^+ - ATPase of the parietal cell) effectively promote healing of ulcers, often leaving behind other detrimental effects on the body. All these factors have prompted us to study in detail the anti-ulcer property of *Juniperus communis* (L.) and evaluate its preventive and healing action. *J. communis* Linn. (Family:

* Corresponding author
Email: tkchatterjee81@yahoo.co.in

Cupressaceae) is a plant widely available in the high altitudes of North India. Different parts of the plant have been claimed to possess medicinal properties, in the traditional medical system. Some reports concerning the different activities of *J. communis* are available [1, 2]. Preliminary studies in our laboratory revealed that the plant possessed anti-inflammatory, antipyretic and anti-microbial properties [3, 4]. Hence, the present work was undertaken to evaluate the anti-ulcer properties of the leaf extract of *J. communis*.

2. Materials & Methods

2.1. Plant material

Fresh leaves were collected from Chhitkul (Baspa Valley) in Himachal Pradesh, India, at an altitude of 3425m. The specimen was identified and authenticated by Dr. R. B. Ghosh, Regional Botanist, Botanical Survey of India, Shibpur, where a voucher specimen was submitted at the Central National Herbarium, bearing the number BSI. NC. No. 47375.

2.2. Preparation of extract

Freshly collected leaves were washed with distilled water and dried at 60°C in a forced air oven (SICO Make). The dried powder (200gms) was extracted in a soxhlet extractor with 80% methanol. A sticky blackish-green material yield (11%w/w) was obtained after vacuum drying. The dried crude methanolic extract (20gms) was chromatographed through a silica gel (G-60, 30gms) column by eluting with 1:1 methanol: chloroform and yielded a yellow-green coloured solid substance (13.5gms).

2.3. Animals

Adult male Wistar albino rats (250-300 gms) and random strain guinea pigs weighing (400-450 gms) from local suppliers were used. They were housed in steel cages and maintained under standardized conditions (12 hr light / 12 hr dark cycle, 25±3°C temperature, 35-60% humidity). Animal feed with standard diet and tap water was provided *ad libitum*. The protocols applied in this study have been approved by the Departmental Animal Ethics Committee.

2.4. Evaluation of anti-ulcer properties

The selected rats were divided into four groups, each group consisting of 6 animals for each pharmacological screening.

Group I : Injected with control vehicle intraperitoneally (i.p.)

Group II : Treated with plant extract [50mg/kg, i.p.] and ulcerogenic materials.

Group III : Treated with plant extract [100mg/kg, i.p] and ulcerogenic materials.

Group IV : Treated with Ranitidine [Glaxo Limited, Mumbai] [40mg/kg, i.p] and ulcerogenic materials.

Three types of tests were performed with above-mentioned groups of animals as per the methods listed below.

2.4.1. Preventive tests

The tests were performed with the following experimental models.

i) Acetyl salicylic acid (ASA)-induced gastric ulcer in rats [5].

Rats were fasted for 24 hours with water *ad libitum*, prior to the experiment. ASA (250 mg/kg) were administered orally to all the animals (n=6) of the four groups (Gr.I, Gr.II, Gr.III and Gr.IV). Six hours later the animals were sacrificed and ulcer index was measured. [6].

ii) Serotonin-induced gastric ulcer in rats [7].

Serotonin creatinin sulfate (20 mg/kg) was administered subcutaneously to all the animals

of three groups (n=6; fasted for 24 hours). Drugs or control vehicle was administered 10 minutes prior to serotonin injection. Eighteen hours later the animals were sacrificed and ulcer index was noted [6].

iii) Indomethacin-induced gastric ulcers in rats

The test was performed following the standard method [8]. Rats (fasted for 24 hours) were given 20 mg/kg of indomethacin (p.o). Groups of animals were treated with leaf extract or control vehicle intraperitoneally 30 minutes prior to the indomethacin treatment. The animals were sacrificed after 7 hours of drug treatment and ulcer index was noted [6].

iv) Alcohol-induced ulcers in rats

Rats were fasted for 24 hours but had water *ad libitum*. Test substances were administered before 30 minutes to the alcohol treatment. Ethanol 50% (5ml/kg) was administered orally to all the animals of the three groups. The animals were sacrificed after 1 hour of administration of ethanol [9] and ulcer index was noted [6].

v) Stress-induced ulcer

Rats were fasted for 48 hours prior to each treatment. Each animal of the three groups was restrained in a small cylindrical wire cage, and placed in cold chamber (2-4°C) for a period of 2 hours after which the animals were sacrificed, the stomachs were removed and opened along the greater curvature for the measurement of ulcer index [6].

vi) Acetyl salicylic acid (ASA)-induced gastric lesions in pylorus ligated rats [7].

Rats were deprived of food but allowed free access to water for 24 hours prior to the experiment. Under proper anaesthesia, the abdomen was incised, pylorus ligation was done and the abdomen was sutured and closed. The water was withdrawn 1 hour before pylorus ligation. ASA (100mg/kg) suspended in 1%w/v carboxymethyl-cellulose (CMC) solution was given orally to the rats of each group, 15 minutes after pylorus ligation. The animals were sacrificed after 7 hours and the stomach were removed. Following 1% formalin treatment, each stomach was examined for probable lesions in the glandular portion, and lesion index was prepared.

vii) Histamine-induced duodenal ulcers in Guinea pigs [10].

Guinea pigs were deprived of food for 24 hours and histamine acid phosphate (0.25mg/kg) was injected 8 times to the animals at 30 minutes intervals in each group,(Gr.I, Gr.II, Gr.III). The test drug and control vehicle were being administered (i.p.) 30 minutes prior to the first histamine injection. After 30 minutes of the last dose of histamine injection, the animals were sacrificed and the duodenums were removed for the measurement of the ulcer index.

2.4.2. Ulcer healing test

Acetic acid-induced ulcers in rats

Solution of 0.5 ml of 30% acetic acid was injected into the gastric wall of the three groups of animals (fasted overnight). The reference drug ranitidine or control vehicle was injected intraperitoneally to the groups of animals once daily from the day after operation for 10 consecutive days. These animals were sacrificed on the 12th day after operation and examined for lesions under the dissecting microscope and the lesion index was calculated [11].

2.4.3. Biochemical tests

Biochemical analysis of the gastric secretion in pylorus ligated male rats were made. The drug treated group III and the control vehicle group I were pylorus ligated after 30 minutes of the treatment of drug (100 mg/kg) and control vehicle. Four hours later the animals were sacrificed and the abdomen were opened. The

stomach were removed and opened along the greater curvature for the measurement of ulcer index. The gastric juice was centrifuged and the supernatant liquid was measured for pH, total acidity and the peptic activity using standard procedures.

2.4.4. Statistical analysis

Results are expressed as the mean ± SEM. The results were analyzed for statistical significance by one - way analysis of variance (ANOVA) followed by Dunnett's test using computerized Graph Pad InStat version 3.05, Graph Pad software Inc., San Diego, U.S.A.

3. Results

The results have been summarized in Tables 1 - 4.

Acetyl salicylic acid-induced, serotonin-induced, indomethacin-induced, alcohol-induced and stress-induced gastric ulcers were significantly inhibited by the leaf extract (Table-1). The extract significantly inhibited the gastric lesions induced by acetyl salicylic acid in pylorus ligated rats. Histamine-induced duodenal ulcers in guinea pigs were also arrested by the leaf extract (Table-2). Acetic acid-induced ulcers were also significantly healed by the plant extract (Table-3). The total volume as well as total acidity of gastric juice was decreased by the leaf extract but it did not alter the pH and the peptic activity (Table-4).

4. Discussion

Severe gastric damage is caused to rats by 50% ethanol through oral administration. Prostaglandin helps to stop this damage if treated earlier. Prostaglandins effectively protect the mucosa against the hemorrhagic and necrotic effects of ethanol [12, 13]. On the other hand, ethanol stimulates gastric mucosal leukotriene production. It suggests a possible causal or contributing role for lipoxygenase products in the pathogenesis of such lesions [14].

The protective action of leaf extract against gastric damage in this experimental model might be assumed due to the protection against 5-lipoxygenase or leukotriene pathway. It is

Table 1. Effects of *Juniperus communis* (L.) leaf extract on salicylic acid, serotonin, indomethacin, alcohol and stress-induced gastric ulcer in rats & on acetyl salicylic acid-induced pylorus ligated rats.

Group	Treatment	Acetyl salicylic acid (250mg/kg,p.o.)	Serotonin (20mg/ kg,s.c)	Indomethacin (20mg/ kg, p.o.)	50% alcohol (5mg/ kg. P.o.)	Stress (Immobilized and cold)	Acetyl salicylic acid (100mg/kg)
I	Vehicle control	2.96±0.28	1.48±0.08	1. 56±0.15	4.33±0.21	12.19±1.47	10. 53±1.94
II	Extract (50mg/kg)	1.98±0.23a	0.82±0.11c	1.11±0.10a	2.50±0.42b	1. 37±0.28b	9.3±0.76b
III	Extract (100mg/kg)	1.16±0.06b	0.48±0.06c	0.68±0.08b	2.00±0.25b	0.49±0.11b	5.08±0.85b
IV	Ranitidine (40mg/kg)	1.01±0.004b	0.51±0.07b	0.49±0.01b	1.19±0.006b	2.05±0.003b	5.09±0.008b

Values are expressed as mean ± SEM, (n = 6), ANOVA followed by Dunnett's '*t*' test.
$p^a < 0.001$ vs Gr. I, $p^b < 0.001$vs Gr. I, $p^c < 0.001$ vs Gr. I.

known that majority of the non-steroidal anti-inflammatory agents are actively ulcerogenic in nature. Thus, on gastrointestinal ulcer models in experimental animals, the leaf extract was to examine the effect and a significant anti-ulcer action was seen. Different experiments were done for both preventive and healing action of leaf extract.

Table 2. Effects of the leaf extract of *Juniperus communis* (L.) on histamine induced duodenal ulcer in guinea pigs.

Group	Treatment	Lesion Index (Mean ± S.E.M)
I	Vehicle control	4.66 ± 0.21
II	Extract (50mg/kg)	2.33 ± 0.21[a]
III	Extract (100mg/kg)	1.83 ± 0.16[a]

Values are mean ± S.E.M, (n = 6), ANOVA followed by Dunnett's '*t*' test.
$p^a < 0.001$ vs. Gr I.

Table 3. Healing rates of gastric ulcers induced by acetic acid in rats

Group	Treatment	Lesion Index (Mean ± S.E.M)
I	Vehicle control	16. 50 ± 1. 34
II	Extract (50mg/kg)	8. 83 ± 1. 33a
III	Extract (100mg/kg)	5.41 ± 0.84 b
IV	Ranitidine (40mg/kg)	5.12 ± 0.007 b

Values are mean ± S.E.M, (n = 6), ANOVA followed by Dunnett's'*t*' test

pa < 0.001 vs. Gr. I. pb < 0.001 vs. Gr. I.

Acetyl salicylic acid and indomethacin, being non-steroidal anti-inflammatory (NSAIDs) drugs, are known to induce ulcers during the course of action i.e. prostaglandin synthesis inhibition through cyclooxygenase pathway [15]. Aspirin induces gastric ulcers caused by back diffusion of H^+ ions into the mucosal cells. From the present experiment on the aspirin and indomethacin induced ulcer, it is speculated to occur by inhibition of back diffusion of H^+ ions. Gastric mucosal microcirculation is disturbed by serotonin-induced ulcer. The development of ulcers by serotonin usually takes about 18 hours; thus the sustained action of leaf extract is evident and the active principle present in the extract might suppress gastric hyper motility and also improve local microcirculations apart from its action on gastric secretion.

Ethanol induced lesion formation is due to different factors like stasis of gastric blood flow contributing significantly to the development of hemorrhagic as well necrotic aspects of the tissue injury. The products of 5-lipoxygenase pathway may also play a key role in the development of such ulcers. Thus the possible mechanism of action of the leaf extract to cure ulcers is assumed to be occurring by inhibition of the lipoxygenase pathway. Stress increases histamine release with enhanced acid secretion, which causes ulcer and reduces mucous production. Again, stress induced ulcers can be prevented partly

Table 4. Effect of the plant extract of *Juniperus communis* (L.) [100 mg/kg, i.p.] on gastric secretion in 4 hours pylorus ligated rats.

Group	Treatment	Volume (mg/100gm)	pH	Total acidity (meq/lit)	Peptic activity (μm/ 1hr /ml)
I	Vehicle control	4. 92 ± 0.0 6	1.60 ± 0.07*	104.19 ± 3.53	101.77 ± 8.39*
III	Extract(100mg/kg)	3.11 ± 0.25[a]	1.59 ± 0.08	81.82 ± 8.14[b]	99.34 ± 8.82

Values are mean ± S.E.M, (n = 6), ANOVA followed by Dunnett's'*t*' test.
$p^a < 0.001$ vs. Gr I. $p^b < 0.001$ vs. Gr I.

or fully by vagotomy. Vagal over activity is suggested as the principle effector in stress induced ulcers. Anti-peptic, anti-cholinergic agents and vagotomy inhibit such type of ulceration. It thus signifies that the corrosive action of secreted gastric juice is possibly neutralised by the extract. It is observed that the leaf extract significantly inhibited gastric output volume and total acidity. The pH of the gastric secretion and peptic activity are failed to be inhibited by the leaf extract, from which it seems that the leaf extract possesses marked anti-secretory property. Vascular disturbances due to histamine may be the main etiological factor of lesion in case of histamine-induced ulcer. Thus, it can be assumed that the extract possesses possible inhibitory influence on histamine induced vascular problems. The healing rate of acetic acid-induced gastric ulcer in rats is enhanced by the leaf extract. Surprisingly, the effect is more or less similar to that of the standard drug, ranitidine (40mg/kg i.p.). The ulcer healing activity of the leaf extract may be due to anti-secretory and anti-peptic activity associated with an enhancement of the local healing process. Acute and sub-acute toxicity studies revealed that the extract is safe for animal systems.

5. Conclusion

The present investigations have proved that the leaf extract of *J.communis* has a significant anti-ulcer activity. The extract has been found to prevent and heal ASA, serotonin, indomethacin, alcohol, stress and histamine-induced ulcers and lesions. The extract enhanced the healing rate of acetic acid induced ulcers. The comparison with standard drug ranitidine furthermore endorsed the leaf extract to possess potent anti-ulcer activity. The healing of histamine-induced duodenal lesions in guinea-pig also established the extract to have anti-ulcer property. The total acidity of the gastric juice was reduced significantly by the extract, whereas, its pH and peptic activity remained unchanged. The outcome of this communication has enormous scope to further explore the avenues of research. Our findings are encouraging from the clinical point of view, and so the promising anti-ulcer property of the leaf extract is worthy of further studies.

6. Acknowledgements

The authors wish to extend their gratitude to Herbicure Health-care Bioherbal Research Foundation, Kolkata, for the financial assistance rendered.

References

1. Devi G, Sisodia CS. (1969) *Indian J. Animal Sciences* 39: 345-349.

2. Srivastava SC, Sisodia CS. (1969) *Indian Veterinary J.* 46: 826-32.

3. Chatterjee T, Ghosh C, Roychoudhuri P. (1991) *Indian Drugs* 28(9): 430-432.

4. Chatterjee T, Ghosh CM, Mukherjee K, Achary PMR. (1993) *Indian J. Microbiology*. 33 (4): 273-275.

5. Aquwa C.N, Ramanujam T.R. (1984) *Jap. J. Pharmacol.* 36, 125.

6. Main IHM, Whittle BJR. (1975) *British J. of Pharmacology.* 53(2): 217-24.

7. Okabe S, takata Y, Tekuchi K, Naganuma T, Takagi K. (1976) *Digestive Diseases.* 21, 618.

8. Somogyi A, Kovacs K, Selye H. (1969) *J. of Pharmacy and Pharmacology.* 21(2): 122-123.

9. Nielsen ST, Beninati L, Chang J. (1987) *Agents and Actions*. 21(3): 320-322.

10. Eagleton GB, Watt J. (1967) *J. Pathol. Bacteriol*. 93, 694.

11. Takagi K, Okabe S, Saziki R. (1969) *Japanese J. of Pharmacology*. (19): 418-26

12. Robert A. (1979) *Gastroenterology*. 77, 761.

13. Rainsford KD. (1987) *Agents and Actions*. 21(3): 316-319.

14. Longe K, Peskar BA, Peskar BM. (1985) *Naunymschmiedebergs Archivfuer Pharmacologie Suppl*. 330, 27.

15. Brunton LL. (1996) *The Pharmacological Basis of Therapeutics*, IX Edn, 37, Agents for control of gastric acidity and treatment of peptic ulcers, In: Hardman, JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman Gilman, A, editors. The McGraw-Hill Companies, USA; 901-915.