

PROTECTIVE EFFECT OF *CEDRUS DEODARA* AND *PINUS ROXBURGHII* ON EXPERIMENTALLY INDUCED GASTRIC ULCERS IN RAT

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ABSTRACT

Objective: To evaluate the antiulcer activity of chloroform extract of *Cedrus deodara* and *Pinus roxburghii* wood (Cd C and Pr C, respectively).

Methods: Cd C and Pr C at doses of 50 and 100 mg/kg were given orally to fasted rats and ulcers were induced by pyloric ligation and ethanol. Number of ulcer, ulcer score, ulcer index, percentage ulcer inhibition and tissue histology were measured in both the models. The effect on volume of gastric secretion, pH, total acidity and free acidity were evaluated in the pyloric ligation model.

Results: Cd C (50, 100 mg/kg) and Pr C (50, 100 mg/kg) extracts were able to protect gastric mucosa against pyloric ligation and ethanol. In case of pyloric ligation model respective inhibition produced were 24.18, 60.44, 10.98 and 25.27%, and in case of ethanol model inhibition were 30.00, 56.36, 20.91 and 29.09%. Like famotidine, Cd C 100 significantly decreased the gastric content (2.40 ± 0.313 to 2.09 ± 0.095 ml), total acidity (473.85 ± 25.774 to 246.04 ± 32.602 meq./l) and free acidity (357.21 ± 30.496 to 182.25 ± 25.774 meq./l) and increase the pH of gastric content (2.99 ± 0.097 to 3.68 ± 0.221) in pyloric ligation model. The protective effect of this extract was associated with marked reduction in gastric hemorrhage and maintenance of tissue integrity.

Conclusion: The present investigation provides the rationale for the use of *Cedrus deodara* in the management of peptic ulcer.

Keywords: Antiulcer, Antisecretory, Percentage ulcer inhibition, Pylorus ligation, Ethanol, Famotidine.

INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice with increasing incidence and prevalence attributed to an imbalance between the protective (mucus, bicarbonate, and prostaglandins) and the aggressive (Reactive oxygen species and acidity) factors [1]. The common causes are stress, continuous use of tobacco, alcohol abuse, non-steroidal anti-inflammatory drugs and infection by *Helicobacter pylori*. The current medical treatment for peptic ulcer is based upon the inhibition of gastric acid secretion by controlling H⁺, K⁺-ATPase, a proton pump for acid secretion in the parietal cells of gastric mucosa. Histamine H₂ receptor blockers are being used to control acid secretion. Considering the several side effects (arrhythmias, impotence, gynaecomastia, and haematopoietic changes) of modern medicine indigenous drugs possessing fewer side effects should be looked for as a better alternative to the treatment of peptic ulcer [2, 3]. This has been the rationale behind the development of new antiulcer drugs and search for novel molecule. Drugs of plant's origin are gaining popularity and investigating for the various disorders including peptic ulcer. Since decades, many indigenous drugs have been known to possess antiulcer activity [4]. The plants *Cedrus deodara* Loud. and *Pinus roxburghii* Sarg. (Pinaceae) have long been known for its medicinal value, including antiulcer activity. The plant *Cedrus deodara* has a long history of numerous traditional and ethnobotanical applications in diverse cultures [5, 6, 7]. Many tribes considered it as a cure for all ailments. Recently, various *in-vivo* and *in-vitro* studies of *Cedrus deodara* have demonstrated that this plant exhibits anti-inflammatory and analgesic [8], antioxidants [9], anxiolytic and anticonvulsant [10], antidiabetic [11], antispasmodic, antibacterial and insecticidal [12], immunomodulatory [13], anticancer [14] and molluscicidal [15] activities.

The plant *Pinus roxburghii* is pungent, heating, oleaginous, intestinal antiseptic and several parts have been used in the traditional system of medicine for disease of eye, ear, throat, skin, bronchitis,

tuberculosis, diaphoresis, diuretic, rubefacient, stimulant, skin diseases, vermifuge, giddiness, ulcer, inflammation and itching [5, 16]. Recently, various *in-vivo* and *in-vitro* studies of *Pinus roxburghii* have demonstrated that this plant exhibits anti-dyslipidemic and antioxidant [17], anti-inflammatory and analgesic [18, 19], hepatoprotective [20] and antimicrobial [21] activities.

These plants are such medicinal plants whose therapeutic application no doubt has a folkloric background and enjoy widespread reputation as a remedy for ulcer. Hence, a scientific verification of its use would be important in establishing a pharmacological basis for some of the claimed ethno medicinal uses of the plants. It is therefore, not surprising that we set out to investigate the scientific rationale for the efficacy of these plants for the treatment of ulcer using animal models. The need for safer and effective antiulcer drug and the lack of enough scientific data to support the claims made in ancient literature prompted the present study. In the present study, the chloroform extract of *Cedrus deodara* and *Pinus roxburghii* wood were evaluated for antiulcer activity in the pylorus-ligated and ethanol induced gastric lesion's models in rats.

MATERIALS AND METHODS

Collection and authentication of plant material

The stem wood of *Cedrus deodara* and *Pinus roxburghii* were collected from naturally growing regions of Pauri Garhwal, Uttarakhand, India. The stem wood of *Cedrus deodara* was authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen has been deposited at the RHMD (NISCAIR/RHMD/Consult/-2011-12/1711/11 dated April 11, 2011). The stem wood of *Pinus roxburghii* was authenticated by Dr. E. Roshni Nayar, Principal Scientist, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. A voucher specimen has been

deposited at the NBPGR Herbarium (NHCP/NBPGR/2011-14/7288 dated April 07, 2011).

Drying and comminution of plant materials

The wood of *Cedrus deodara* and *Pinus roxburghii* were thoroughly washed, and then shade dried under $25 \pm 2^\circ\text{C}$ for 10 days. The dried plant samples were grounded well into a fine powder in a mixer grinder and sieved to give particle size 50-150 mm.

Preparation of plant extracts

The powder plants material (stem wood of *Cedrus deodara* and *Pinus roxburghii*) were extracted successively with petroleum ether and chloroform using Soxhlet extraction apparatus. The solvents were completely removed at reduced pressure until the semi solid mass was obtained. The extracts were stored in amber-colored bottle and kept in refrigerator until further use.

Preliminary phytochemical testing and TLC profiling of extracts

The extracts were subjected to preliminary phytochemical screening for the presence of alkaloids, glycosides, flavonoids, terpenoids, steroids, carbohydrates, proteins, fats, fixed oils and phenolic compounds [22]. The presence of phytochemicals were further confirmed by thin layer chromatography (TLC). The extracts were applied to silica gel G plates for TLC profiling. For TLC profiling plates were developed using various ratio of solvent system in n-Hexane: ethyl acetate as mobile phase. The spots were visualized using anisaldehyde sulphuric acid at 120°C [23].

Animals

Wistar rats (180-220 g) were obtained from Central animal house, S.V. Subharti University, Meerut, U.P, India. The animals were housed in the polypropylene cage under standard conditions ($25 \pm 2^\circ\text{C}$, 12 h light and dark cycle) and animals were fed on standard chow diet and water *ad libitum*. All the experimental procedure and protocol involving animals were reviewed and approved by the Institutional Animal Ethical Committee and were in accordance with the guidelines of CPCSEA.

Treatment schedules

To test the effect of chloroform extract of plants *Cedrus deodara* and *Pinus roxburghii* on ulcer, two paradigms such as pylorus induced and ethanol induced gastric ulceration test in rats were employed. The rats were divided in two sets of six groups each. In both the set, the animals were treated as follows: Group 1 (Control) received vehicle {mixture of acacia and tragacanth in distilled water}, 10 ml/kg of body weight. Group 2 (Standard) received famotidine, 20 mg/kg of body weight. Group 3 (Cd C 50) and 4 (Cd C 100) received chloroform extract of *Cedrus deodara* in doses of 50 and 100 mg/kg. Group 5 (Pr C 50) and 6 (Pr C 100) received chloroform extract of *Pinus roxburghii* in doses of 50 and 100 mg/kg.

Gastric ulceration in pylorus-ligated rats

Gastric secretion content, pH, total acidity, free acidity, number of ulcer, ulcer score and ulcer index were measured according to the method of Shay *et al.*, 1945 [24]. Animals were fasted for 36 h before the study, but had free access to water. One hour after oral administration of chloroform extracts of *Cedrus deodara* and *Pinus roxburghii* (50 and 100 mg/kg) or famotidine (20 mg/kg) or vehicle, the animals were subjected to pylorus ligation under thiopental sodium anaesthesia. The animals were sacrificed with over dose of thiopental sodium after 4 h of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out, and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1ml of each) were taken for the determination of pH, total and free acidity. Total acidity and free acidity were determined using titrimetry [25]. The inner surface of free stomach was examined for gastric lesions. The number of ulcers was counted. Ulcer scoring was done according to the method by Vogel & Vogel, 1997 [26] as given below.

The scores were: 0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcer, 3 = perforation.

Ulcer index was measured by using following formula:

$$UI = U_N + U_S + U_P \times 10^{-1}$$

UI = Ulcer Index

U_N = Average number of ulcers per animal

U_S = Average number of severity score

U_P = percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ inhibition of ulceration} = \frac{(\text{Ulcer index Control} - \text{Ulcer index Test}) \times 100}{\text{Ulcer index Control}}$$

Gastric lesions induced by ethanol

Lesions were induced according to the method of Vogel & Vogel, 1997 [26]. Rats, fasted for 18 h but had free access to water were used. One hour after the treatments (*Cedrus deodara* and *Pinus roxburghii* at 50 and 100 mg/kg or Famotidine at 20 mg/kg or vehicle at 10 ml/kg), 1 ml absolute ethanol were administered orally. After 1 h of ethanol treatment, the animals were sacrificed under high dose of anesthesia. The stomach of each animal was excised and opened along the greater curvature. The numbers of ulcer, ulcer score, ulcer index and percentage inhibition of ulcer were determined.

Histopathological evaluation

The stomach samples from the pylorus ligated and ethanol treated groups were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Using a rotary microtome, sections of thickness of about $5 \mu\text{m}$ was cut and stained with haematoxylin and eosin. These were examined under the microscope for histopathological changes such as degeneration, hemorrhage, edematous appearance, erosion and necrosis.

Statistical analysis

All values were expressed as mean \pm SD. The data obtained from the various groups were statistically analyzed using One-way ANOVA followed by Dunnett's multiple comparisons test. * $p < 0.05$, ** $p < 0.01$, ^{ns} $p > 0.05$ vs control.

RESULTS

Yield of wood extracts

The 260 g wood powder of *Cedrus deodara* produced 17.00 g dry chloroform extract resulting a yield of 6.8% w/w. Whereas, the 400 g wood powder of *Pinus roxburghii* produced 8.00 g of dry chloroform extract resulting a yield of 2.0% w/w.

Phyto-constituents and TLC Profiling of extracts

Phytochemical testing showed that the chloroform extract of *Cedrus deodara* contains alkaloids, glycoside, flavonoids, terpenoids, steroid, phenolic compound and carbohydrates. Whereas, the chloroform extract of *Pinus roxburghii* contains alkaloids, glycosides, flavonoids, terpenoids, steroids and carbohydrate. It was evident from TLC profiling that comparatively a large number of compounds, including terpenoids and flavonoids were present in the chloroform extract of *Cedrus deodara* than chloroform extract of *Pinus roxburghii*.

Gastric secretion in pylorus-ligated rats

Gastric secretion measurements of pylorus-ligated rats showed that chloroform extracts of *Cedrus deodara* (50 and 100 mg/kg) and *Pinus roxburghii* (100 mg/kg) significantly decreased the gastric content ($p < 0.05$), total and free acidity ($p < 0.01$), while only *Cedrus deodara* (100 mg/kg) significantly increase the pH ($p < 0.01$). Famotidine (20 mg/kg), the reference compound used also showed significant ($p < 0.01$) change of all these secretory parameters. Results showed that among the tested doses of *Cedrus deodara* and *Pinus roxburghii* for antisecretory activity, the higher dose of *Cedrus deodara* (100 mg/kg) showed similar levels of action as that of reference compound (Table 1).

Table 1: Effect of chloroform extract of *Cedrus deodara* and *Pinus roxburghii* on gastric content, pH, total acidity and free acidity in pylorus ligation induced ulceration in rats.

Treatment	Dose (mg/kg)	Gastric content (ml)	pH of gastric content	Total acidity (meq./l)	Free acidity (meq./l)
Control	10 ml/kg	2.40 ± 0.313	2.99 ± 0.097	473.85 ± 25.774	357.21 ± 30.496
Standard	20	2.07 ± 0.156**	3.83 ± 0.220**	233.28 ± 41.559**	174.96 ± 30.496**
Cd C 50	50	2.12 ± 0.082*	3.18 ± 0.080 ^{ns}	320.76 ± 30.496**	240.57 ± 32.602**
Cd C 100	100	2.09 ± 0.095*	3.68 ± 0.221**	246.04 ± 32.602**	182.25 ± 25.774**
Pr C 50	50	2.28 ± 0.120 ^{ns}	3.07 ± 0.075 ^{ns}	408.24 ± 30.497 ^{ns}	328.05 ± 36.450 ^{ns}
Pr C 100	100	2.13 ± 0.071*	3.13 ± 0.096 ^{ns}	342.63 ± 19.965**	247.86 ± 30.496**

Values are represented as mean ± SD. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparisons test.

* $p < 0.05$ as compared to control., ** $p < 0.01$ as compared to control., ^{ns} $p > 0.05$ as compared to control.

Gastric lesions in pylorus-ligated rats

Gastric lesion measurements in pylorus-ligated rats showed that *Cedrus deodara* (50 and 100 mg/kg) and *Pinus roxburghii* (100 mg/kg) significantly ($p < 0.01$) decreased the number of ulcer and ulcer score, while only *Cedrus deodara* (100 mg/kg) significantly decrease the ulcer index ($p < 0.01$). Famotidine (20 mg/kg), the reference compound used also showed significant ($p < 0.01$) reduction of all these gastric lesion parameters. The ulcer inhibition with *Cedrus deodara* and *Pinus roxburghii* were found to be 60.44% and 25.27% at a dose of 100 mg/kg, while standard drug showed 73.62% ulcer inhibition in comparison to control group. The results showed that among the different tested doses of *Cedrus deodara* and *Pinus roxburghii* for antagastric lesion activity, the higher dose of *Cedrus deodara* (100 mg/kg) showed the similar levels of action as that of reference compound (Figure 1).

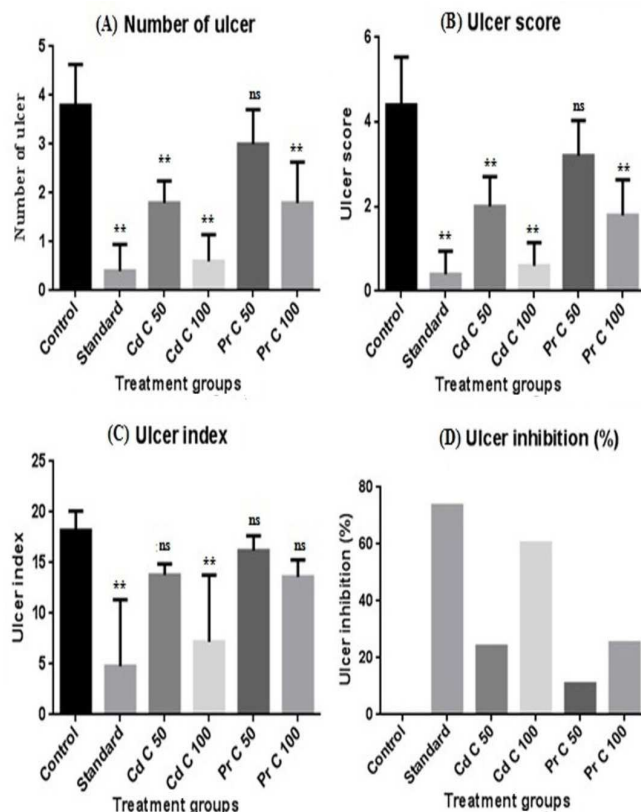


Fig. 1: Effect of chloroform extract of *Cedrus deodara* and *Pinus roxburghii* on number of ulcers (A), ulcer score (B), ulcer index (C) and percentage ulcer inhibition (D) in the ulcerated region of pylorus ligation induced ulcers in rats. Values are represented as mean ± SD. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparisons test. ** $p < 0.01$, ^{ns} $p > 0.05$ vs control

Gastric lesions induced by ethanol

In the ethanol-induced ulceration study, oral administration of ethanol produced severe ulceration while pretreatment with *Cedrus deodara* and *Pinus roxburghii* extracts (50 and 100 mg/kg) produced a significant ($p < 0.01$) decrease in number of ulcer and ulcer score, while *Cedrus deodara* (50 and 100 mg/kg) significantly ($p < 0.01$) decrease the ulcer index. Famotidine (20 mg/kg) showed significant ($p < 0.01$) reduction of all these gastric lesion parameters. The ulcer inhibition with *Cedrus deodara* and *Pinus roxburghii* were found to be 56.36% and 29.09% at a dose of 100 mg/kg, while standard drug showed 54.45% ulcer inhibition in comparison to control group. The results showed that among the different tested doses of chloroform extracts, the higher dose of *Cedrus deodara* (100 mg/kg) showed similar levels of action as that of reference compound (Figure 2).

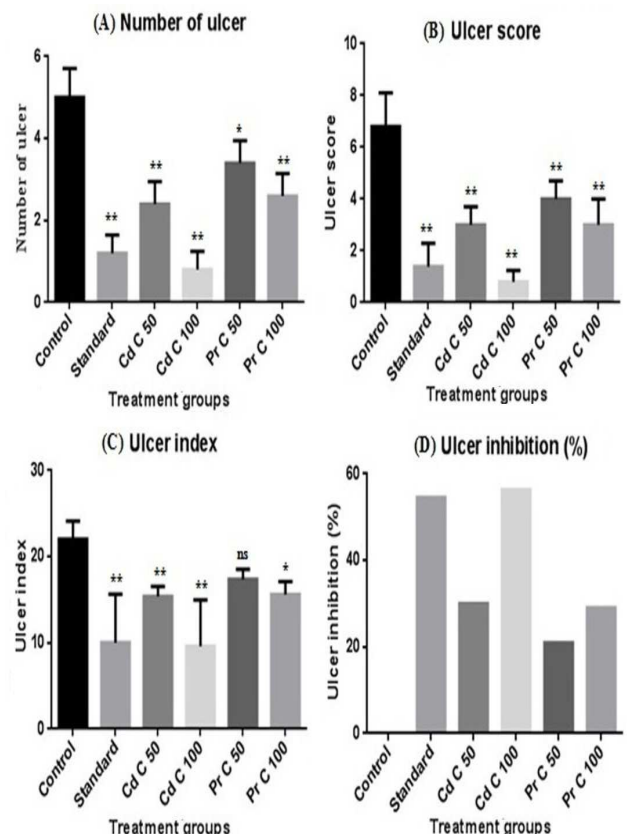


Fig. 2: Effect of chloroform extract of *Cedrus deodara* and *Pinus roxburghii* on number of ulcers (A), ulcer score (B), ulcer index (C) and percentage ulcer inhibition (D) in the ulcerated region of ethanol induced gastric ulcers in rats. Values are represented as mean ± SD. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparisons test. * $p < 0.05$, ** $p < 0.01$, ^{ns} $p > 0.05$ vs control

Histopathological evaluation

Pylorus ligation and ethanol caused histopathological lesions, including degeneration, hemorrhage, and edematous appearance of the gastric tissue. Among the different tested dose, pretreatment with *Cedrus deodara* (100 mg/kg) and famotidine (20 mg/kg) offered significant protection against all such damage to the mucosa (Figure 3 and 4).

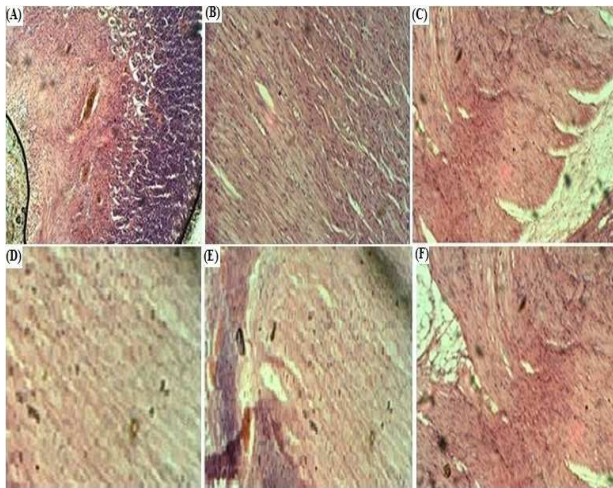


Fig. 3: Effects of chloroform extract of *Cedrus deodara* and *Pinus roxburghii* on pylorus ligation-induced gastric ulcer in rats. Stomach tissue was stained with hematoxylin and eosin (x100): (A) received vehicle, (B) received standard, (C) received chloroform extract of *Cedrus deodara* at 50 mg/kg, (D) received chloroform extract of *Cedrus deodara* at 100 mg/kg, (E) received chloroform extract of *Pinus roxburghii* at 50 mg/kg, (F) received chloroform extract of *Pinus roxburghii* at 100 mg/kg.

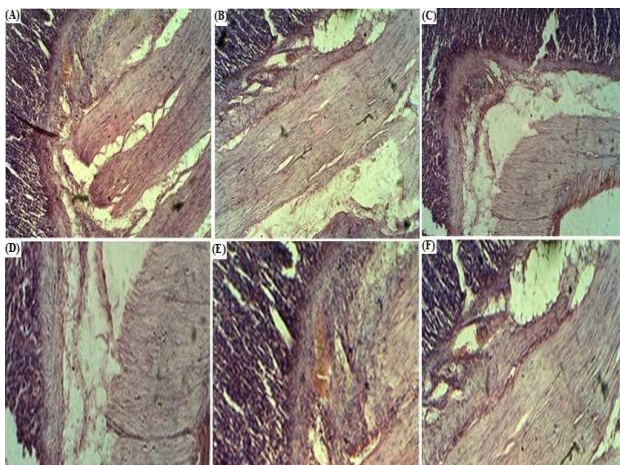


Fig. 4: Effects of chloroform extract of *Cedrus deodara* and *Pinus roxburghii* on ethanol induced gastric ulcer in rats. Stomach tissue was stained with hematoxylin and eosin (x100): (A) received vehicle, (B) received standard, (C) received chloroform extract of *Cedrus deodara* at 50 mg/kg, (D) received chloroform extract of *Cedrus deodara* at 100 mg/kg, (E) received chloroform extract of *Pinus roxburghii* at 50 mg/kg, (F) received chloroform extract of *Pinus roxburghii* at 100 mg/kg.

DISCUSSION

The present investigation was primarily undertaken to demarcate the antiulcerogenic property of two common traditional plant

Cedrus deodara and *Pinus roxburghii*. We have studied gastric antisecretory and antiulcer activities of these plants in two different models, including pyloric ligation and ethanol induced ulcer model in rat. The causes of gastric ulcer in pyloric ligation are believed due to increase in gastric hydrochloric acid secretion and/or stasis of acid, leading to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life-threatening perforation and hemorrhage. In the pylorus ligation method, chloroform extract of *Cedrus deodara* (50 and 100 mg/kg) and *Pinus roxburghii* (100 mg/kg) significantly decreased the gastric content, total acidity and free acidity induced by pylorus ligation. On the other hand, only *Cedrus deodara* (100 mg/kg) significantly increases the pH. The finding that the *Cedrus deodara* increases the gastric pH and decrease the gastric volume, total acidity and free acidity in pylorus-ligated rats suggests that antisecretory action is likely ascribed to its antigastric ulcer effect. Famotidine is standard control used here to test antisecretory mechanism. Ulcer index parameter was used for the evaluation of antiulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. The chloroform extract of *Cedrus deodara* also significantly reduced number of ulcer, ulcer score, and ulcer index at a dose of 100 mg/kg. The ulcer inhibition was found to be 60.44% at a dose of 100 mg/kg which is comparable to the 73.62% of standard drug in comparison to control group. It could be suggested that chloroform extract of *Cedrus deodara* can suppress gastric damage induced by aggressive factors. The antiulcer activity of *Cedrus deodara* was further supported by histopathological study, which showed protection of mucosal layer from ulceration and inflammation. Ethanol-induced gastric mucosal lesions are due to superficial damage to mucosal cells caused by the direct necrotizing action of ethanol through the reduction in mucus production, gastric mucosal blood flow and bicarbonate secretion and gastric acid is not involved during the formation of such lesions [27]. Endogenous glutathione and prostaglandin (PG) levels are also lowered by ethanol while the release of histamine, the influx of calcium ions, generation of free radicals and production of leukotrienes are all increased [28]. Hence a cytoprotective agent, increases mucus secretion, will be effective in this model. In our studies we have observed that chloroform extract of *Cedrus deodara* and *Pinus roxburghii* has significantly reduced the number of ulcer, ulcer score and ulcer index. Further, it is evident from our results that the chloroform extract of *Cedrus deodara* produced marked reduction in the extent of gastric mucosal damage produced by ethanol, and their protective effect was more pronounced than that of famotidine and *Pinus roxburghii* in ethanol model. This was further substantiated by histological findings where a marked reduction in gastric mucosal damage and cellular influx was observed. Such a protective effect of *Cedrus deodara* could have resulted because of the potent antioxidant property as reported earlier [9]. Reactive oxygen species are involved in the pathogenesis of pylorus ligation-induced [29] and ethanol-induced [30] gastric mucosal injury *in-vivo*. Reduced glutathione is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation [31]. Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore, it is not surprising that membrane lipids are susceptible to peroxidative attack [32]. Both *Cedrus deodara* and *Pinus roxburghii* demonstrated free radical scavenging and antioxidant properties that have been implicated in maintaining the integrity of gastric mucosa. Famotidine, on the other hand, was effective in alleviating oxidative stress in all the models. Besides antagonizing H₂ receptors, it has also been shown to exhibit oxygen radical scavenging properties [33]. The preliminary phytochemical test and TLC profile showed that comparatively a large number of compounds, including terpenoids and flavonoids were present in the chloroform extract of *Cedrus deodara* than chloroform extract of *Pinus roxburghii*. It is known that terpenoids compound have antisecretory and cytoprotective activity [34] which

partially explains the mechanism of action of *Cedrus deodara*. Flavonoids possess antioxidant property in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion [35]. The results showed that chloroform extract of *Cedrus deodara* appears more potent antiulcer extract than the chloroform extract of *Pinus roxburghii* and the mechanism(s) of action involve cytoprotective, antisecretory and acid neutralizing effects by terpenoids and flavonoids compound. However, there is a need of bioactivity guided drug discovery to isolate the lead compound responsible for antiulcer activity from the most active extract. The isolated compounds may serve as useful prototypes of antiulcer drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

CONCLUSION

The results from this study confirm the use of the *Cedrus deodara* in the traditional management of peptic ulcer disease and appear more potent antiulcer extract than the chloroform extract of *Pinus roxburghii*. The mode of action involves cytoprotective, antisecretory, and acid neutralizing effects through terpenoids and flavonoids compound, and it may pave the way for the establishment of a new gastric antisecretory and antiulcer therapy regimen that will not require the use of antacids and antisecretory agents.

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CONFLICT OF INTEREST

The authors have no conflict of financial and personal interests with other people or organization.

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