

THE EFFECT OF *CEDRUS DEODARA* ROOT OIL ON THE HISTOPATHOLOGY OF RAT STOMACH

Ijaz Hussain Zaidi^{1*}, Syed Naeemul Hasan Naqvi², Qamar Aziz³, R.M. Tariq⁵, Aneela Qureshi⁴,
Mazhar Hussain Hijazi³, Muhammed Ahmed Azmi⁴, Shabih Zehra⁴ and Rehana Parveen⁶

¹Department of Casualty, P.N.S Shifa Hospital, DHA, Phase 2, Karachi

^{2,3,6}Department of Pharmacology, Anatomy & Physiology, Baqai Medical University, Toll Plaza, Gadap Town, Karachi

⁴Departments of Physiology, Pathology & Radiology, Al-Tibri Medical College, Old Thana Gadap, Malir Town, Karachi

⁵MAH Qadri Biological Research Centre, University of Karachi

Abstract

The aim of this study was to observe the anti-ulcer effects of *Cedrus deodara* root oil on the rat's stomach and compare it with standard anti-ulcer drugs, famotidine and protonix. The study was conducted on 50 albino Wistar rats in three different doses i.e. 30, 40, and 50 mg/kg. The animals were divided into five groups, each group comprised of 10 rats (5 male and 5 female). The oil was extracted from the plant root by dry destructive distillation method and the dose was calculated by dissolving 1.25 gms of *Cedrus deodara* in 25ml of 10% ethanol. The drugs were administered to the treated animals orally through feeding tube for two weeks. Animals received the dose of 50 mg cedar oil only, showed the healing effects on the mucosal epithelium of stomach, decreased inflammatory cells and granulation tissues on the submucosal layer upon histopathological examination. Therefore it may be concluded that *Cedrus deodara* root oil has anti-ulcerative effects and may be used in the management of gastrointestinal disorders particularly in peptic ulcer.

Key words: Histopathology, *Cedrus deodara* root oil, Stomach, Anti-ulcer drugs, Albino rats.

INTRODUCTION

Cedrus deodara (Roxb) Loud. (Fig. 1) belongs to Deodar – Pinaceae mostly found in the hilly areas of Khyber Pukhtoonkhwa of Pakistan and also in the Western Himalaya as well as in the Mediterranean region. It is a tall coniferous tree and has a conic crown with spreading horizontal boneless drooping branch-lets (Saxena *et al.*, 1964; Bhattacharyya *et al.*, 1988). In the Indus Unic System (Greek System), various parts of the plant, plant's extracts, oils etc. have been used for a long time in the treatment of patients for various ailments. Its inner wood is aromatic and is distilled into essential oil that is used as insects repellent on the feet of horses, cattle and camels (Gamble, 1902). The wood extract is carminative, diaphoretic, antipyretic and has also been used to treat flatulence, pulmonary and urinary disorders, rheumatism, piles and kidney stones. In addition, the bark extract is astringent and is useful for treating fever, diarrhea and dysentery (Baquar, 1989). The root extract or oil also contains terpenoids such as himachalol, atlantone and trans-atlantone similar to those found in trunk oil (Khan and Naheed, 1988, Onoda *et al.* 1989, Parveen, 2006). Some other plants are also in use as medicinal plants e.g Tariq *et al.* (2010) reported the essential oil of root stock the modified stem (rhizome) of *Acorus calamus* as a medicine for stomach complaints, snake bite, insect repellent and for remittent fever.

In addition, the root oil also has antiseptic and antifungal

properties and has some potential for the control of fungal deterioration (Parveen, 2006). In the Khyber Pukhtoonkha, the root oil is used as an anti-ulcer drug by Hakims (unic-medical practioners). As very little pharmacokinetic and pharmacodynamic studies have been conducted on the root oil on its toxicity, curative effect, side effects and adverse effects particularly in relation to its therapeutic use as an anti-ulcer drug, further investigations are needed in this respect. However, in the developed countries much work is being done on the different lines of investigations on *Cedrus deodara* extracts or oil. One of the major component in the wood of *Cedrus deodara*, himachalol, has antispasmodic and spasmolytic activities similar to those of papaverine that also induces spasm in different tissues such as ileum, jejunum and bronchial muscles when given in different doses (Kar *et al.*, 1975) Similarly, mast cell-stabilizing and lipoxygenase inhibitory activities as well as the immunological properties of *Cedrus deodara* oil in allergy cause by pollen antigens, have also been evaluated (Shinde *et al.*, 1999b; Rawat *et al.*, 2000). In vitro cytotoxic activities against human cell lines, in vivo induction of intracellular caspases, DNA fragmentation and DNA cell cycle analysis have also been reported (Dirami and Sharma, 2004; Shashi *et al.*, 2006; Singh *et al.*, 2007). Recently, a study is conducted on the lipid contents, specific surface area and stomata density as well as the levels of accumulation of polycyclic aromatic hydro carbons (PAHs) in the kinds of pine i.e. *Cedrus deodara* and *Pinus thunbergi* needles (Yang *et al.*, 2008).

*Corresponding author: E-mail: col_zaidi@yahoo.com

In Pakistan, considerable work has been done on the chemistry of stem bark for the analysis of stem bark extract or oil (Khan and Naheed, 1988; 1990; Adinarayana and Seshadri, 1965; Agarwal and Rastogi, 1981). Antifungal activity of *C. deodara* root oil and its pure compounds against *Candida albicans* and *Aspergillus fumigatus* has been reported by Parveen *et al.* (2010), but to the best of our knowledge, no scientific studies have been reported so far in the literature in relation to its histopathological effects of *Cedrus deodara* root oil on the animal tissues.

Therefore, the present work aims to discuss the anti-ulcer effects of *Cedrus deodara* root oil on stomach tissues of albino rats in comparison with the known anti-ulcer drugs mainly famotidine and protonix.

MATERIALS AND METHODS

Materials

a) Drugs/Chemicals:

- *Cedrus deodara* root oil
- Anti-ulcer drugs i.e famotidine (20mg) and protonix (40 mg)



Fig. 1. Showing the different parts of *Cedrus deodara* plant i.e branches, stem, male and female cone.

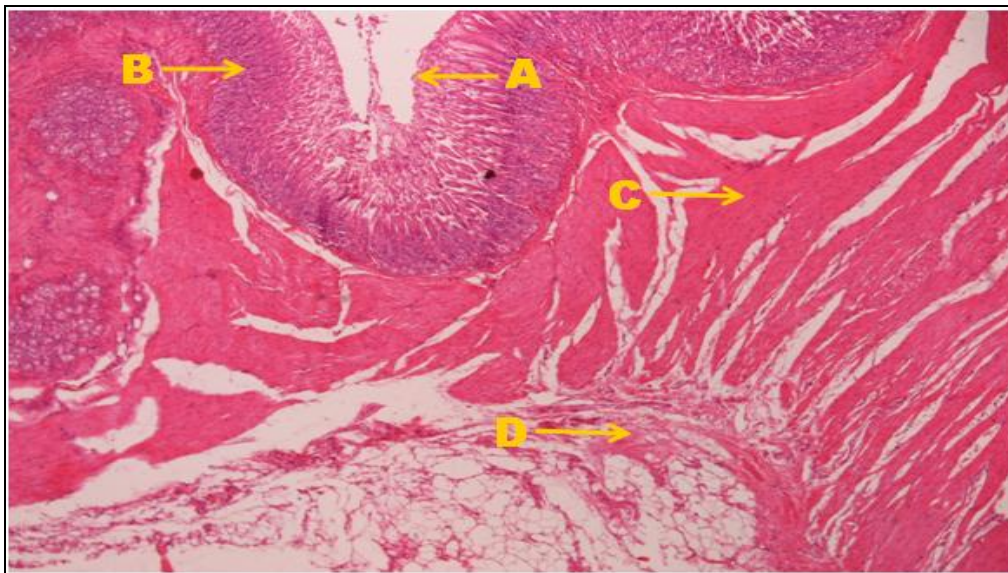


Fig. 2: Photomicrograph of a 5 micron thick H & E stained paraffin section from the stomach of normal untreated rat (group A) showing normal mucosa (A), sub mucosa (B), muscularis (C) and adventitia (D). X 100 magnification

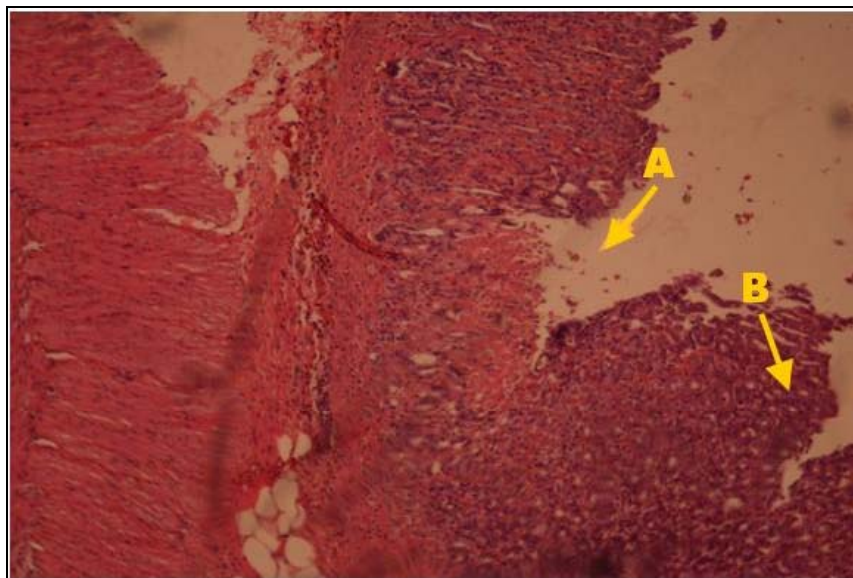


Fig. 3. Photomicrograph of a 5 micron thick (H & E) stained paraffin section from the stomach of treated rats with 100% ethanol (dose 1 ml) showing superficial ulceration and erosion on mucosa (A) and inflammatory cells (B). X 100 magnification.

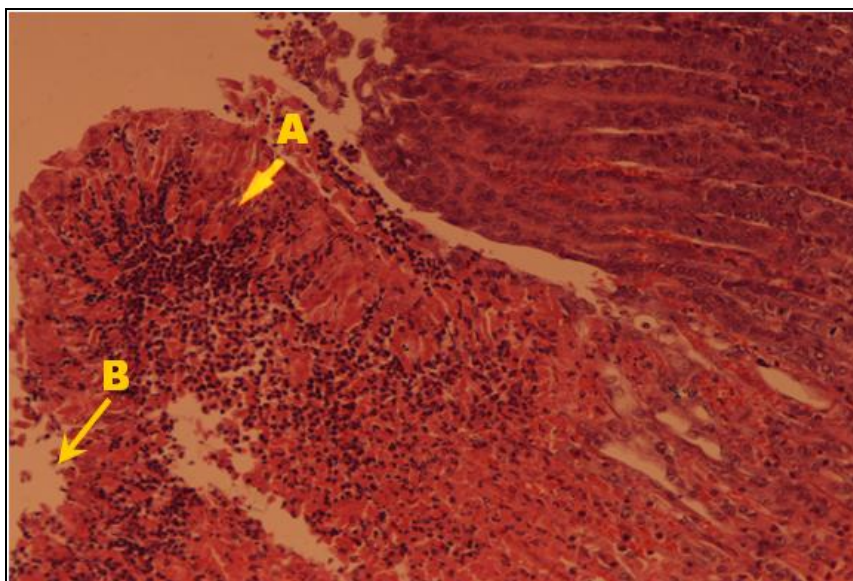


Fig. 4. Photomicrograph of a 5 micron thick (H & E) stained paraffin section from the stomach of treated rats with 100% ethanol (dose 1 ml) and 1 ml peanut oil showing inflammation of mucosal layer with inflammatory cells (A). Disruption of luminal epithelium (B). X 100 magnification.

b) Animals:

The animals used for this experimental study were adult albino rats (Wistar strain) weighing 200-250 gm. They were housed in cages (2 rats per cage) in the animal house of Baqai Medical University, Karachi, Pakistan. They were on a well balanced laboratory diet prepared by Hussain Ebrahim Jamal (HEJ) Research Institute of Chemistry, University of Karachi. They were kept in a 12 hrs light / 12 hrs dark cycle and water was given freely, at a temperature of 25-30°C.

Methodology

a) Preparation of extract

The root oil of *Cedrus deodara* was provided by Abdul Haseeb Khan, Chairman, Brookes' Pharma (Pvt.) Ltd., Karachi-Pakistan and has been used to investigate its pharmacological and therapeutic characteristics.

b) Grouping of animals& administration of drugs:

A total of 50 albino rats weighing 200~250grms were used in this study and divided into five groups. Each

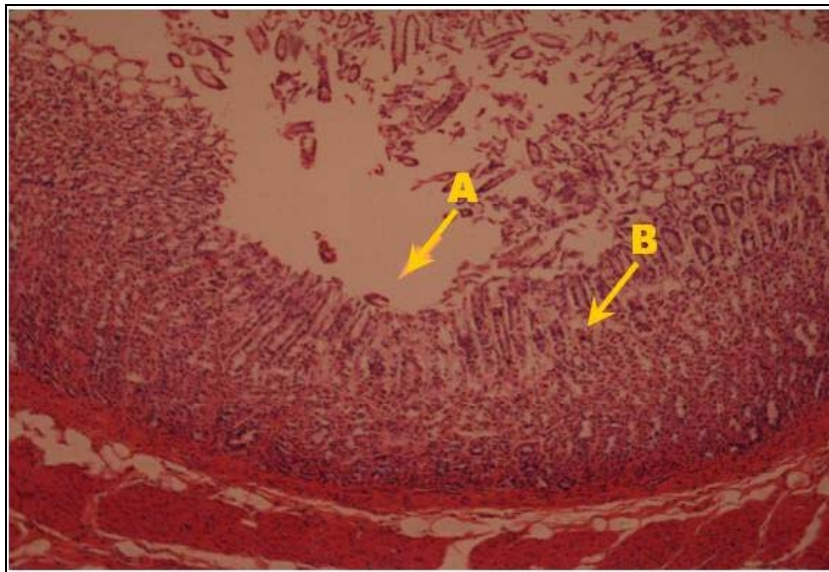


Fig. 5. photomicrograph of a 5 micron thick (H & E) stained paraffin section from the stomach of treated rats with 1 ml 100% ethanol and 50 mg of cedar wood oil showing healing of mucosal layer (a), decreased number of inflammatory cells (b). X 100 magnification.

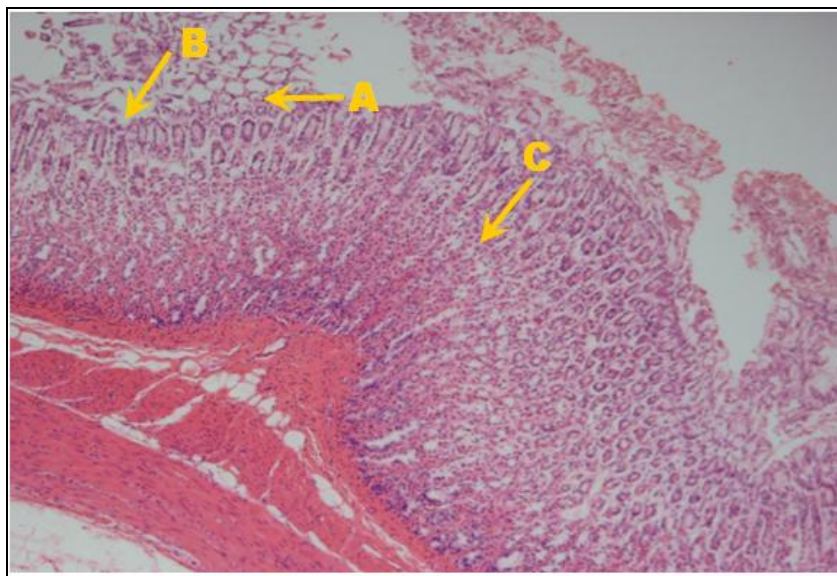


Fig. 6. Photomicrograph of a 5 micron thick (H & E) stained paraffin section from the stomach of treated rats with 100% ethanol and Femotidine (H_2 receptor blocker) (Group E). Showing healing of gastric mucosa with re-epithilization (A). Formation of gastric pits (B). Decreased inflammatory cells (C). X 100 magnification.

group comprised of 10 rats (5 male and 5 female). Rats were fasted except group 'A' for 48 hrs before oral administration of 1 ml of 100 % ethanol with metallic feeding tube for induction of gastric ulcer. All the animals were fed with special diet and water was given freely before the animals were scarified. The experiments were repeated three times.

Group 'A' (Control): Animals in this group received only 1 ml of normal saline for two weeks orally.

Group 'B': Ulcer was induced by administration of 1 ml 100 % ethanol after 48 hrs of fasting. Then rats were anaesthetized by chloroform and sacrificed. Stomach was then removed for histopathological examination to observe for gastric ulceration.

Group 'C' (Check): After induction of ulcer with 1 ml of 100% ethanol, 1 ml of Pea-nuts oil was administered for two weeks to observe its effect on the stomach in comparison with test groups (D & E groups)

Group ‘D’: After induction of ulcer with 1 ml of 100% ethanol, *Cedrus deodara* oil was given (50, 100, 200 ml per kg body weight) according to Shinde *et al.*, 1999c. The dose for *Cedrus deodara* oil was calculated according to the weight of the animal (1.25 gm oil was dissolved in 25 ml of 10% ethanol), from which 30, 40 and 50 mg was given to the rats to observe its anti-ulcer effects in comparison with two anti-ulcer drugs, famotidine 20 mg and protonix 40 mg.

Group ‘E’: After induction of ulcer with 1 ml of 100 % ethanol, anti ulcer drugs, famotidine (20 mg) and protonix tablet (40 mg) was given orally as reference drugs.

The approval for conducting the experimental procedures on the animals was taken by the Board of Advance Studies and Research (BASR) and Ethical Committee of Baqai Medical University, Karachi-Pakistan.

Table 1. Gross features of un-treated and treated stomach tissues of albino rat

TISSUE TYPE	ASSESS-MENT	UN-TREATED CONTROL GROUP	TREATED GROUPS			
		A	B	C	D	E
Stomach	Macroscopic	1 ml Normal Saline	1 ml of 100% Ethanol	1 ml of 100% Ethanol + 1 ml peanut oil	1 ml of 100% Ethanol + 50 mg of <i>C. deodara</i> oil	1 ml of 100% Ethanol + Anti-ulcer drugs
		<ul style="list-style-type: none"> ◆ Dilated part of gastro-intestinal tract. ◆ consists of cardia, fundus, body, pylorus 	<ul style="list-style-type: none"> ◆ Multiple hemorrhagic red patches seen on greater curvature 	<ul style="list-style-type: none"> ◆ Multiple hemorrhagic spots also seen on the mucosal surface. 	<ul style="list-style-type: none"> ◆ No hemorrhagic spots were noticed 	<ul style="list-style-type: none"> ◆ No hemorrhagic spots were seen

Table 2. Gross features of un-treated and treated stomach tissues of albino rat.

TISSUE TYPE	ASSESS-MENT	UN-TREATED CONTROL GROUP	TREATED GROUPS (B to E)			
		A	B	C	D	E
Stomach	Microscopic	1 ml Normal Saline	1 ml of 100% Ethanol	1 ml of 100% Ethanol + 1 ml peanut oil	1 ml of 100% Ethanol + 50 mg of <i>C. deodara</i> oil	1 ml of 100% Ethanol + Anti-ulcer drugs
		<ul style="list-style-type: none"> ◆ Showed normal architecture i.e. Normal gastric mucosa (A), Sub-mucosa(B), Muscularis mucosae (C), Adventitia (D) as shown in Fig 2. ◆ Showed normal gastro duodenal junction (A) and Normal gastric glands (B). 	<ul style="list-style-type: none"> ◆ Superficial ulceration and erosion seen on mucosal surface ◆ Inflammatory cells also seen as shown in Fig 3 ◆ Mucosal ulceration extended up to muscularis with necrotic slough. 	<ul style="list-style-type: none"> ◆ Inflammation seen on mucosal and sub-mucosal layer ◆ Few inflammatory cells (A) ◆ Disruption of luminal epithelium as shown in Fig4 ◆ Diffuse chronic inflammatory infiltrate seen on mucosa, sub-mucosa and muscularis. 	<ul style="list-style-type: none"> ◆ Re-epithelization of mucosal layer (A) ◆ Decreased inflammatory cells (B). ◆ Formation of granulation tissue as shown in Fig 5 ◆ Development of granulation tissue comprising of numerous capillaries, inflammatory cells (A). 	<ul style="list-style-type: none"> ◆ Re-epithelization of mucosal surface (A) ◆ Formation of gastric pits (B) ◆ Decreased inflammatory cells as shown in Fig 6

c) Tissue Processing

All the groups of animals were fasted over-night prior to being sacrificed. The animals were anesthetized with chloroform and placed on a dissection board. A midline incision was made in the abdomen to expose out the abdominal organs. The stomach tissue was taken out and preserved in 10% formalin before microscopic examination. The tissues were then fixed in normal saline for 24-48 hrs and processed through a series of ethyl alcohol of ascending strength (70, 80 and 95%) for period of 1 hrs, twice in absolute ethanol (for 1 hr each) and twice in xylene (for 1 hr) in order to render the tissue elements transparent. The tissues were then infiltrated with molten paraplast at 58°C twice (1 hr on each occasion). The transparent tissues after clearing all elements from it were embedded in a solid mass of paraplast. The blocks were labeled, allowed to cool and the metal blocks were removed. The solid mass was then trimmed free of excess paraplast, leaving some free margins around the embedded tissues.

Five microns thick longitudinal sections were cut with a rotary microtome. The sections were mounted on thoroughly cleaned gelatinized slides and were placed on hot plates at 37°C for 24 hrs for proper fixation. The slides were then stained by Hematoxylin and Eosin (H & E) stain according to the prescribed staining method (Bancroft & Stevens., 1990). The stain was prepared by dissolving hematoxylin in absolute ethanol. The mixture was boiled rapidly and mercuric oxide was then added. The stain was cooled rapidly in cold water bath; glacial acetic acid was then added and the stain was ready for immediate use. Several slides were prepared accordingly. The stained slides, after drying and labeling were preserved and stored for histopathological studies before microscopic examination for comparative morphological and pathological changes in the gastric tissues of the animal *Cedrus deodara* root oil to observe the anti-ulcer effects on rat stomach (Fig 5). Animals of group 'E' were given known anti-ulcer drugs i.e. famotidine 20 mg (H₂ – receptor blocker) and protonix 40 mg (proton-pump inhibitor) to observe their anti-ulcer effects on rat stomach (Fig 6).

RESULTS

The general characteristic features of the section of stomach tissue of the animals examined macroscopically and microscopically are presented in Tables 1 & 2. Normal stomach tissues of control group 'A' (i.e. not treated with drugs) were observed as normal mucosa, sub-mucosa, muscularis and adventitia (Fig 2). The tissues also showed normal gastro – duodenal junction and normal gastric glands. The animals of remaining groups were induced for ulcer by giving 1 ml of 100% ethanol after keeping them on fasting for 48 hrs. Ulceration was confirmed in group 'B' both macro and microscopically

during histopathological studies (Fig 3). Animals of group 'C' was given peanuts oil as check to note the changes in the histopathology of the stomach tissue (Fig 4). Animals of group 'D' after the induction of ulcer by giving 1 ml of 100% ethanol were treated with 50 mg of *Cedrus deodara* oil to observe the anti-ulcer effects on rat stomach (Fig 5), where as animals of group 'E' were given known anti-ulcer drugs i.e. famotidine 20mg and protonix 40 mg to observe their anti-ulcer effects on rat stomach (Fig 6).

DISCUSSION

In the present study anti-ulcer effects were seen on the rats' stomach, when 50 mg of *Cedrus deodara* oil was given orally in comparison with known anti-ulcer drugs i.e. famotidine / protonix. The observations were noted histo-pathologically and were reconfirmed by using scanning electron microscopic method (submitted elsewhere).

The dose 100-200 mg/kg of *Cedrus deodara* oil was calculated according to Shinde *et al.*, (1999c). The different fractions of doses such as 30, 40 and 50 mg were given orally to the rats after producing ulcer with 1 ml of 100% ethanol by keeping them on fasting to 48 hrs. However those animals who received 50 mg of *Cedrus deodara* oil showed significant healing effects on the mucosal epithelium of stomach, decreased inflammatory cells and the formation of granulation tissue seen in the sub-mucosal layer (Fig 5) in Table 2. In relation to the anti-ulcerative effect of the *Cedrus deodara* root oil, only few references have been found in the scientific literature, however, some researchers have worked on the activity of *Cedrus deodara* root oil but on different aspects e.g., Shinde *et al.*, (1999a) reported the anti-inflammatory and analgesic activity of *Cedrus deodara* root oil when given orally at the doses of 50 and 100 mg/kg body weight and therefore reported the significant inhibition of carrageenan rat paw edema and inflammation, when the oil was tested at both doses. The present work is also found more closely to the clinical approach in which anti-inflammatory activity of *Cedrus deodara* oil is studied when given at the dose of 50 mg orally to albino rats for two weeks, which showed significant healing effects on the mucosal surface of rat stomach. Some researchers have evaluated the anti-secretory, anti-ulcerative, (Kumar *et al.* 2011) anti-inflammatory as well as cytotoxic and mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* root oil were reported. (Shinde *et al.*, 1999b; Bandyopadhyay *et al.* 2002,2004). The present study also in line with the agreement of the same findings of the gastro-protective effect of *Cedrus deodara* oil as well as curative and healing effects on the mucosal surface of rat's stomach has been noted. Anti-inflammatory, anti-ulcerative as well as hepato-protective studies were conducted in human and animals, when different neem based preparation was given. It is therefore

concluded that if neem-derived preparation when applied with care produce better results as insecticides (Boeke *et al.*, 2004). However, *Cedrus deodara* root oil when used at a dose of 50 mg or 1.0 ml produce significant curative effects. Therefore, *Cedrus deodara* oil by this study is considered to be much better oil as compared to other extracts or oil. Analysis of *Cedrus deodara* oil and its pharmacokinetics and pharmacodynamics has also been investigated (Perveen 2006). The present study is only concerned with the anti-ulcerative effect of *Cedrus deodara* oil when given at a dose of 50 mg orally for two weeks with comparison with commercially available known anti-ulcer drugs i.e. famotidine 20 mg / protonix 40 mg. In addition, mammalian toxicity by oral administration of *Cedrus deodara* oil was investigated against albino rats by (Parveen *et al.*, 2008). It was found that LD₅₀ was 34.4 gm / kg indicated that the oil is quite safe as compared to neem oil having LD₅₀ (i.e., 5 gm / kg). The *Cedrus deodara* root oil is still being used in Khyber Pukhtoonkha by Hakims as an anti-ulcer drug without having scientific knowledge.

CONCLUSION

Gastric ulcer is a serious gastro-intestinal disorder that requires a well targeted therapeutic strategy. As number of drugs like H₂ - receptor blocker and proton-pump inhibitor are available commercially for the treatment and healing of gastric ulcer but showing incidence of relapses, side effects and drug interactions. To overcome this problem medicinally, drugs of plant origin are gaining popularity have got much therapeutic importance. *Cedrus deodara* root oil used in this study showed anti-ulcerative and healing effects when tested with the dose of 50 mg orally to the albino rats. Therefore present investigation is however suggested that *Cedrus deodara* root oil has a potential to cure peptic ulcer.

The approach of this study is different from previous studies because research has been carried out in vivo rather than in vitro. It is therefore suggested that formulation (Liquid/Tablet) may be prepared and further work on human being may be carried out in future by researcher.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Mr. Abdul Haseeb Khan, Chairman Brooke's Pharma (Pvt.) Ltd., Karachi, Pakistan for providing the root oil of *Cedrus deodara* for this study. We are also grateful to Prof. Dr. Syed Azhar Ahmed, Vice-Chancellor, Baqai Medical University and Prof. Dr. Fazal Hussain, Baqai Institute of Pharmaceutical Sciences for their technical support and cooperation. Mr. Arif Jawaid, Baqai Institute of Hematology, Baqai Medical University and Noman Ahmed Issani of Al-Tibri

Medical College, also deserves thanks for their assistance in writing this manuscript.

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Received: 28-04-2011

Accepted: 20-05-2011