Original Article

The Effect of Abietic Acid (Cedrus Deodara Compound) on the Histopathology of Rat Stomach in Comparison with Ulcer Healing Drugs

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ABSTRACT

Purpose of study: The aim of this study is to observe the anti-ulcer effects of Abietic acid (a compound of Cedrus deodara) on the histopathology of rat's stomach in comparison with standard anti-ulcer drugs such as Femotidine (H_2 receptor blocker) and Protonix (a proton pump inhibitor).

Design of study: Experimental study.

Place and Duration of Study: This study was conducted in the department of Pharmacology, Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi.

Materials and Methods: This study was carried out on 50 albino rats of Wistar Strain for experimental purpose. The animals were divided into five groups and each group comprised of 10 rats (i.e. 5 male and 5 female). The experimental procedure was repeated for three times. The compound of abietic acid was provided by the chemistry department of Karachi University. The ulcer was induced in the animals by giving 1ml of 100% ethanol after 48 hrs of fasting. The induction of ulcer in animal was then treated with abietic acid (25 mg/kg for 2 weeks) orally through feeding tube. The ulcer healing effects of this compound were then compared with the effect of known anti-ulcer drugs.

Results: Abietic acid used in this study showed the healing effects on the mucosal epithelium of stomach, decreased inflammatory cells and formation of granulation tissues on the sub mucosal layer during histopathological examination.

Conclusion: It is thus concluded that abietic acid has an anti-ulcerative effects when given in the required dose and may be adapted in the management of gastrointestinal disorders particularly in peptic ulcer.

Key Words: Histopathology, Abietic acid, Stomach, Anti-ulcer drugs, Albino rats.

INTRODUCTION

Abietic acid which is one of the terpenoids found in many dietary and herbal related plants[1], producing many functional and biological activities such as anti-tumor proliferation, antihypercholestrolemia and antidiabeties[2-3]. Abietic acid an abietane diterpenoids has also shown some other biological roles in various ailments like anti-allergic[4], anti-inflammatory[5], phytoalexin effects[6] and anti-convulsant activities[7]. A study indicated that abietic acid exerts *in vitro anti-inflammatory* activity after oral or topical administration and also having partial ability to prevent the production of some inflammatory mediators[8]. It has also been reported that abietic acid which is the main component of rosin fraction of oleoresin synthesized by confier species also have anti-inflammatory effect. It is also indicated that one of the compound 12- sulfo dehydroabietic acid mono sodium salt exhibit potent anti-pepsin and anti-ulcer activity with low toxicities[10]. Another compound of abietic acid, Ecabet sodium which is an anti-ulcer agent may cause epigastric fullness in patients with dysmotility - like function dyspepsia. Catechols derived from abietic acid were also evaluated for several biological activities mainly antifungal, antitumoral, antimutagenic, antiviral, antiproliferative and inhibition of nitric oxide[12]. Studies have shown that much work have been done on different lines of investigation in relation to the effects of abietic acid but no such studies have been conducted so far in Pakistan and other countries in relation to its histopathological effects of abietic acid on animal tissues. Therefore, present study has been carried out to observe the anti-ulcer effects of abietic acid histopathologically on stomach tissues of albino rats in comparison with known anti-ulcer drugs Femotidine (H_2- receptor blockers) and Protonix (a proton-pimp inhibitor).

MATERIALS AND METHODS

The compound of Abietic acid was collected from Chemistry department of Karachi University and was

used to investigate its pharmacological and therapeutical characteristics. The animals used for this experimental study were adult albino rats (Wistar strain) weighing 200-250 gms. They were housed in cages (i.e., 2 rats per cage) in the animal house of Baqai Medical University, Karachi, Pakistan. They were maintained on a well balanced laboratory diet in the form of biscuits prepared in Hussain Ebrahim Jamal (HEJ) Research Institute of Chemistry, University of Karachi. They were kept in a 12 hrs light / 12 hrs dark cycles and water was given freely.

Experimental Design

A total of 50 albino rats weighing 200~250grms were taken in this study and divided into five groups. Each group comprised of 10 rats (i.e., 5 male and 5 female rats). The animals 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 sacrificed the animals.

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

Group 'B' : In this group 1 ml of 100 % ethanol was given after 48 hrs of fasting for the induction of ulcer. The animals were anaesthetized by the chloroform. The rats were sacrificed and stomach was removed out for histopathological study 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 given for 02 weeks to observe its effect on the stomach in comparison with test groups (i.e., D & E groups)

Group 'D': After induction of ulcer Abietic acid was given (25-100mg/kg) accordingly[7]. Dose of abietic acid i.e. 25 mg / kg was dissolved in 1 ml of distilled water and given for two weeks to see its anti-ulcer effects with comparison of known anti-ulcer drugs i.e. Femotidine (20 mg) and protonix tablet (40 mg).

Group 'E': After induction of ulcer by 1 ml of 100 % ethanol, known anti ulcer drugs i.e. Femotidine (20 mg) and protonix tablet (40 mg) was given orally and the dose was also calculated according to the weight of rat.

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

Routine 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 given on 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 the period of 1 hrs, twice in absolute alcohol (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 ^{0}C. This was done for two times (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 ^{0}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[8]. The stain was prepared by dissolving hematoxylin and absolute alcohol. 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 Abietic acid to observe the anti-ulcer effects on rat stomach (Fig 4). Animals of group 'E' were given known anti-ulcer drugs i.e. Femotidine 20 mg (H_2 – receptor blocker) and protonix 40 mg (Proton Inhibitor) to observe their anti-ulcer effects on rat stomach (Fig 5) Therefore, during the present study, anti-ulcer effects of drugs and samples were observed as compared to the protective effect used by some other authors[7,10].

RESULTS

The general characteristic features of the stomach tissue section of the animals examined microscopically are presented in Table-1.

Normal stomach tissues of control group 'A' (i.e. not treated with drugs) were observed as normal mucosa,

sub-mucosa, muscularis and adventitia (Fig 1). 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 for 48 hrs fasting. Ulceration was confirmed in group 'B' both macroscopically and microscopically during histopathological studies(Fig 2). Animals of group 'C' was given peanuts oil as check to note the changes in the histopathology of the stomach tissue. (Fig 3). Animals of group 'D' after the induction of ulcer by giving 1 ml of 100% ethanol were treated with Abietic acid i.e., 25 mg dissolved in 1 ml of distilled water to observe the anti-ulcer effects on rat stomach (Fig 4), where as animals of group 'E' were given known anti-ulcer drugs i.e. Femotidine 20mg (H_2 – receptor blocker) and Protonix 40 mg (Proton-pump Inhibitor) to observe their anti-ulcer effects on rat stomach (Fig 5).

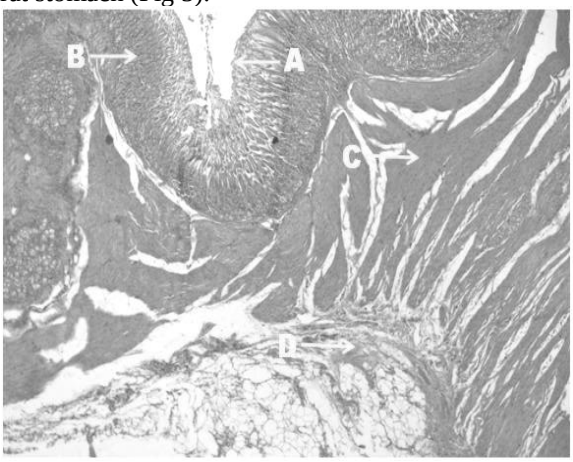

Figure No. 1: Photomicrograph of a 5 micron thick H & E stained paraffin section from the stomach of normal untreated rat (group A) showing normal mucosa (A), sub mucosa (B), muscularis (C) and adventitia (D). X 100 magnification.

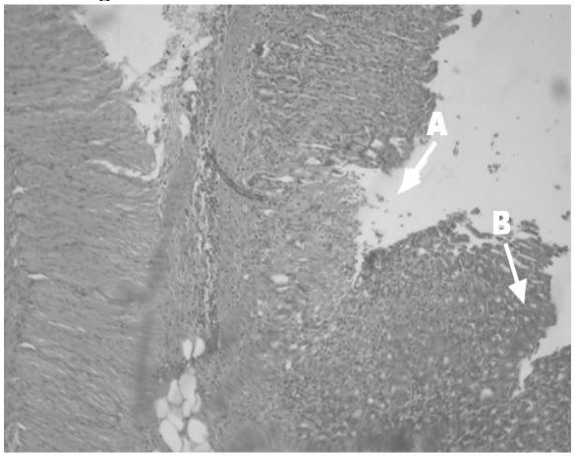

Figure No. 2: Photomicrograph of a 5 micron thick (H & E) stained paraffin section from the stomach of treated rats with 100% ethanol (dose 1 ml) showing superficial ulceration and erosion on mucosa (A) and inflammatory cells (B). X 100 magnification.

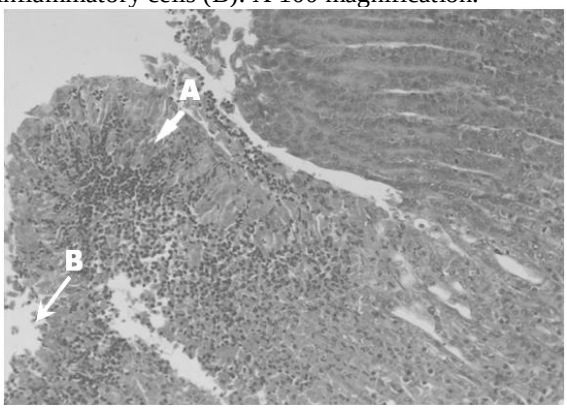

Figure No. 3: Photomicrograph of a 5 micron thick (H & E) stained paraffin section from the stomach of treated rats with 100% ethanol and 1 ml pea-nut oil showing inflammation of mucosal layer with inflammatory cells (A). Disruption of luminal epithelium (B). X 100 magnification.

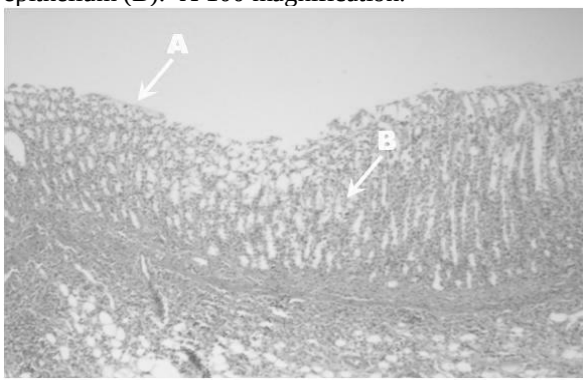

Figure No. 4: Photomicrograph of a 5 micron thick (H & E) stained paraffin section from the stomach of treated rats with 1 ml of 100% ethanol and abietic acid. (Group D). Showing healing of mucosal ulcer i.e. re-epithelization (A) and decreased inflammmatory cells (B). X 100 magnificati

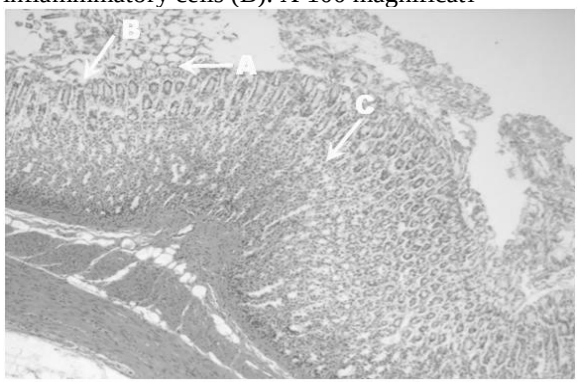

Figure No. 5: Photomicrograph of a 5 micron thick (H & E) stained paraffin section from the stomach of treated rats with 100% ethanol and Femotidine (H_2 receptor blocker) (Group E). Showing healing of gastric mucosa with re-epithilization (A). Formation

of gastric pits (B). Decreased inflammatory cells (C).
X 100 magnification.

Table No.1: Gross features of un-treated and treated stomach tissues of albino rat

TISSUE TYPE	ASSESS	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 + 25 mg / kg of Abietic acid	1 ml of 100% Ethanol + Anti-ulcer drugs
		♦ Dilated part of gastro-intestinal tract. ♦ consists of cardia, fundus, body, pylorus	♦ Multiple hemorrhagic red patches seen on greater curvature	♦ Multiple hemorrhagic spots also seen on the mucosal surface.	No hemorrhagic spots were noticed	♦ No hemorrhagic spots were seen
	Microscopic	♦ Showed normal architecture i.e. Normal gastric mucosa (A), Sub-mucosa(B), Muscularis mucosae(C), Adventitia(D) … Fig 1.	♦ Superficial ulceration and erosion seen on mucosal surface ♦ Inflammatory cells also seen Fig 2	♦ Inflammation seen on mucosal and sub-mucosal layer ♦ Few inflammatory cells (A) ♦ Disruption of luminal epithelium Fig 3.	♦ Re-epithelization of mucosal layer (A) ♦ Decreased inflammatory cells (B). ♦ Formation of granulation tissue Fig. 4	♦ Re-epithelization of mucosal surface (A) ♦ Formation of gastric pits (B) ♦ Decreased inflammatory (C) cells Fig 5.

DISCUSSION

In the present study anti-ulcer effects were seen on the rats' stomach, when 25 mg / kg Abietic acid was given orally in comparison with anti-ulcer drugs i.e. Femotidine and Protonix. The findings were observed histopathologically and was reconfirmed by using scanning electron microscopic method (submitted somewhere else).

The effects of Abietic acid were noted on stomach tissues with anti-ulcer drugs e.g, Femotidine 20 mg (H_2 – receptor blocker) and Protonix 40 mg (a proton inhibitor). The dose 25-100 mg / kg of Abietic acid was calculated[7] and was dissolved in 1 ml of distilled water. Abietic acid was then given to the rats after producing ulcer with 1 ml of 100% ethanol by keeping them on fasting to 48 hrs. Histopathological changes were observed in the rat stomach tissues i.e. healing effects on the mucosal epithelium of stomach, decreased inflammatory cells and the formation of granulation tissue seen in the sub-mucosal layer (Fig 4) In relation to the anti-ulcerative effect of the Abietic acid, only few references have been found in the scientific literature, however, some researchers have worked on the activity of Abietic acid but on different lines of investigations[10]. Abietic acid when given orally at the doses of 25-100 mg / kg body weight reported significant reduction of pepsin as well as prostaglandin mediated process[7,10]. The present work is also found more closely to the clinical approach in which anti-ulcer activity of Abietic acid was studied when given at the doses of 25 mg dissolved in 1 ml of distilled water orally to albino rats and significant healing effects on the mucosal surface of rats' stomach were noted. As very little work or investigation has been done on abietic acid to observe

its protective effects, so more research is needed in relation to the scientific and medicinal use of this compound.

CONCLUSION

Gastric ulcer is a serious gastro – intestinal disorder that requires a well targeted therapeutic strategy. As number of drugs like H_2 – 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, drugs of plant origin are gaining popularity. On this basis Abietic acid is used in this study in order to assess the anti-ulcerative and healing effects when intorduced with the dose of 25 ml / kg orally to the albino rats. Therefore through this study it is suggested and recommended to medical personals that obietic acid can be used as a drug with appropriate doses for the management of peplic ulcer therapeutically.

Acknowledgment

The author gratefully acknowledge the assistance of Dr. Firdous, Assistant Professor of Chemistry, department of Karachi University in providing Abietic acid compound for this study. We also grateful to the Professor Dr. Syed Azhar Ahmed, Vice Chancellor Baqai Medical University and Professor Dr. Fazal Hussain , of Baqai Institute of Pharmaceutical Sciences for their cooperation and partial financial support for provision solvents from HEC project no. 20/1127/2009. We are also gratefully acknowledge services of Mr. Arif jawaid of Baqai Institute of Hematology, Baqai Medical University and Noman Ahmed Issani of Al-Tibri Medical College & Mr. Junaid PNS Shifa also thankfully acknowledge for assistance in writing this manuscript.

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