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Histopathological effects on stomach, liver and kidney associated with *Cedrus deodara* root oil in ulcer induced rats (Wistar strain)

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Abstract: *Cedrus deodara* have been used traditionally in ayurvedic system against peptic ulcer. Present work is concerned with the determination of histopathological effects in ethanol induced ulcer on rats (Wistar Strain) treated with *Cedrus deodara* root oil at a dose of 200mg/kg and comparison of its antiulcer activity against control, positive control and standard anti-ulcer drug (Omeprazole). The aim was to find out the antiulcer effect of *Cedrus deodara* root oil and to observe histopathology of liver, kidney as well. 120 Albino rats were taken and divided into four groups i.e. A, B, C and D designated as control, positive control, standard and treated groups respectively. Normal and intact general architecture of mucosa and submucosa layers of stomach observed. No significant changes observed in thickness of epithelium, no inflammatory cells were present on the mucosa and submucosal layer and gastric glands were normal. Liver of albino rats, showed no dilation and congestion in central as well as portal vein. Kidney of albino rats exhibited no shrinkage in glomeruli, no congested and dilated renal corpuscles, neither hemolysis nor congested and dilated renal tubules were seen. It is concluded that *C. deodara* root oil has anti-ulcer properties without effecting kidney and liver tissues

Keywords: Anti-ulcer, *Cedrus deodara*, histopathological, omeprazole, peptic ulcer.

INTRODUCTION

Advancement in the research on medicinal plants and their various parts. The efficacy of the extracts and against numerous diseases is of great interest to botanists as well as pharmacologists. *Cedrus deodara* is cedar species and have its place in the family Pinaceae with a botanical name *Cedrus deodara (Roxb. ex D.Don) G.Don*. It`s English name is deodar or Himalaya cedar with a local name deodar cedar and is recognized as Burada deodar in Urdu language (Gupta *et al*., 2011). The plant is a native to Karakoram areas of North Western Pakistan, Hindu Kush at East Afghanistan and North India at Kashmir and Himalaya (Shah, 2006). *Cedrus deodara (C. deodara)* is among those herbs that has been used traditionally in eastern medicine as it`s being found very effective in treating gastrointestinal, pulmonary and urinary illnesses, kidney stones and piles. It has also been used in dysentery, diarrhea and fever (Baquar, 2000). Traditionally, the plant *Cedrus deodara* have been known for its curative action against ulcer and have been famous in olden times for its number of traditional as well as ethnopharmacological applications in various cultures (Shah, 2006; Hussain *et al.,* 2006; Kunwar *et al.,* 2009).

The anti-inflammatory, analgesic (Shinde *et al.,* 1999a) and immunomodulatory activities (Shinde *et al*., 1999b), an anxiolytic as well as anticonvulsant activities (Viswanathan *et al*., 2009, Dhayabaran *et al*., 2010) of the plant wood oil have also been testified. It possess antifungal activity against Aspergillus fumigatus at concentration of 150µg/disc, however at the same dose it is not effective against *Candida albicans* (Rehana *et al*., 2010). Moreover, the ulcer healing effect of *Cedrus deodara* have also been conveyed by Avadhesh and collogues (Avadhesh *et al*., 2011). It have anti-spasmodic activity which is because it's constituent himachalol (Kar *et al*., 1975).

The anti-ulcer activity of the *Cedrus deodara* root oil have been observed at a dose of 50mg/kg of rats which showed curing effect on stomach mucosal epithelium on histopathological examination (Zaidi *et al.,* 2011). However, the adverse effects of *Cedrus deodara* root oil *on the stomach linings have also been reported at a dose of 0.5ml/kg and 2.5ml/kg but therapeutically, these effects were not lethal (Rehana *et al*., 2013). In vitro studies have shown that the root oil of *Cedrus deodara* known to have histopathological changes specifically at a dose of 0.5 ml/kg and 2.5ml/kg orally in wistar rats and may effect liver and kidney tissues (Rehana *et al*., 2010).

Aim of current study is to evaluate the anti-ulcer activity of *Cedrus deodara* root oil at a dose of 200mg/kg and compared it against control, positive control, and standard anti-ulcer drug i.e. Omeprazole. The study was performed

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on Albino rats and histopathological effects on different organs of the rats including stomach, kidney and liver were observed.

MATERIALS AND METHODS

Brook`s Pharma (Pvt.) Ltd. provided the root oil of *Cedrus deodara* as gift sample which was obtained by the method of dry destructive distillation.

Animals

Wistar strain albino rats were obtained from Hussain Ebrahim Jamal Research Institute of Chemistry. These rats were housed 12/12 light and dark cycle and room temperature was set $25^0C \pm 2^0C$. Hundred and twenty (120) rats of weight 200 grams $\pm$ 5were separated for conducting the experiment. The research work was performed in accordance with internationally accepted principles for laboratory animal use and care as found in European Community guidelines. Ethical approval from the Ethics Committee of Baqai Medical University was obtained prior to conducting experiments.

Experimental design

120 rats were divided equally into four groups i.e. A, B, C and D and each experiment was repeated three times for the purpose of accuracy. Ulcer was induced by the help of 1ml of 1% ethanol given orally to the rats of group B, C and D. Group A, that was control (n=10) was given only 1ml normal saline orally for 2weeks. Group B served as positive control received ethanol only. Group C received the standard anti-ulcer drug i.e. Omeprazole at dose of 20mg/kg of body weight (Al- radhey *et al*., 2012) orally for 2 weeks after ulcer induction. Group D, served as treated group to which extract of *Cedrus deodara* root oil was given at dose of 200 mg/kg (Shinde *et al*., 1999c) for 2 weeks. Then animals were sacrificed and the samples were preserved in 10% formalin solution for histopathological analysis.

Routine tissue processing

Each group of rats was fasted overnight before scarification. The animals were anaesthetized from chloroform. A dissection was made to remove the required organs like stomach, liver and kidney after opening abdomen. Tissues of the stomach, liver and kidney were fixed in 10% formalin for 24-48 hours to avoid autolysis. Then the tissues were processed in different strengths of ethyl alcohol i.e. 70, 80, and 95% to remove water. The slides were prepared according to the suggested staining method with the help of Hematoxylin and Eosin (H&E) Stain (Bancroft and Stevens., 1990).

RESULTS

In the present research work, histopathological analysis of all the four groups of animals was intensely studied. The histopathological investigation of the stomach, liver and kidney tissues of albino rats were examined microscopically, which are in brief summarize in table 1. Animals were divided into four groups i.e. Group A for control, B for positive control, C for omeprazole treated group and D for treated with *Cedrus deodara* root oil.

Tissues of stomach in control group, omeprazole group and *C. deodara* treated group showed intact lining of gastric mucosa, submucosa, muscularis externa and serosa whereas in positive control group, moderate degree shedding and edema were found. Similarly, positive control group also reported dilated and congested blood vessels as well as mild necrosis. Whilst the group treated with *C. deodara* represents normal characteristic of stomach tissues as the control group (table 1).

Tissue of liver showed normal and intact general architecture in all four groups, however, congestion and dilation of central vein and portal tract along with occurrence of inflammatory cells was only observed in positive control group. Liver tissues of group treated with *C. deodara* doesn't show any variation as compared to control group hence reported to be normal (table 1).

Tissues of kidney also represent normal and intact general architecture in all four groups whereas dilation and congestion of renal tubules and corpuscles with shrink glomeruli was observed in positive control group. The *C. deodara* treated group represents normal characteristic of kidney tissues as compared to the control group (table 1).

Group A, served as control, showed normal characteristics of gastric pits, gastric mucosa, submucosa, and surface epithelium as well as the normal gastric glands of stomach tissues as shown in fig. 1. Liver tissues were observed normal as no dilation or congestion observed in central vein, sinusoids and hepatocytes were also normal as presented in fig. 2. In kidney tissues, renal tubule and corpuscles were observed to be normal as represented in fig. 3.

Group B served as positive control, rats of which received 1ml of 100% ethanol for induction of ulcer. On examination of stomach tissues microscopically, inflammatory cells in the mucosa were observed as well as congested blood vessels. Necrosis and erosion of epithelium can also be observed from fig. 4. In liver tissues dilation in the central vein and portal tract was detected. Inflammatory cells were also observed (fig. 5). Kidney tissues showed congestion and dilation in the renal tubules and shrinkage in glomeruli (fig. 6).

After induction of ulcer, Group C and D received the standard anti-ulcer drug (omeprazole) and tested compound (*Cedrus deodara* root oil) respectively to observe their ulcer healing properties. Their histopathological examination of stomach, rats of group C showed partial therapeutic effect on the ulcerated stomach

Table 1: Gross features of stomach, liver and kidney tissues of rats

Features	Control (A)	Positive control (B)	Omeprazole (C)	*Cedrus deodara (D)*
Tissue of Stomach				
General Architecture Mucosa, submucosa, muscularis externa and serosa	Normal and intact	Normal and intact	Normal and intact	Normal and intact
	Epithelium Thickness			
	Normal	Normal	Normal	Normal
	Changes in Epithelium			
	No shedding	Moderate degree shedding	No shedding	No shedding
Mucosal and submucosal layer	Edema			
	No edema	Edema seen on submucosal layer	No edema	No edema
	Inflammatory cells			
	Not found	Inflammatory cell were present	Mild leucocytes found	Not found
Blood vessels	Dilation and Congestion			
	No congestion and no dilation	Dilated and congested	Mild congested found in submucosa	No congestion and no dilation
Necrosis	Not found	Mild	Mild	Not found
Tissue of Liver				
General Architecture	Normal and intact	Normal and intact	Normal and intact	Normal and intact
Central Vein	No congestion and dilation was present	Congestion and dilation was observed	Congestion and dilation was not observed	Congestion and dilation was not observed
Inflammatory cells	Not present	Present	Not observed	Not observed
Necrosis	Not present	Not observed	Not observed	Not observed
Fibrotic changes	Not present	Not found	Not found	Not found
Fatty changes	Not present	Not found	Not found	Not found
Portal tract	No congestion and dilation	Congestion and dilation observed	No Congestion and dilation observed	No Congestion and dilation observed
Tissues of Kidney				
General Architecture	Normal and intact	Normal and intact	Normal and intact	Normal and intact
Renal tubules	Normal	Dilated and congested	Normal	Normal
Renal corpuscles	Normal	Dilated and congested	Normal	Normal
Glomeruli	Normal	Shrinkage present	Normal	Normal
Blood vessels	Normal in size and appearance	No change observed	Normal	Normal
Necrosis	Not found	Not found	Not found	Not found
Inflammatory cells	Not present	Not observed	Not observed	Not observed
Cellularity	Normal	Normal	Normal	Normal
Basement membrane	Normal	No change observed	Normal	Normal

Effect of normal saline on different tissues of albino rats (Group A)

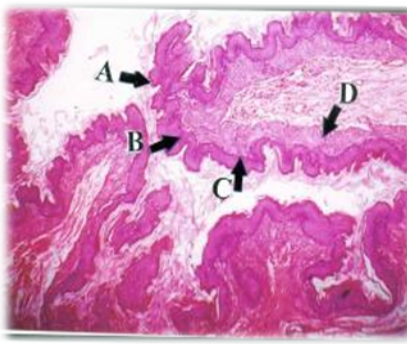

Fig. 1: Stomach Tissues normal gastric pits (A), mucosa (B), muscularis mucosa (C) and submucosa (D)

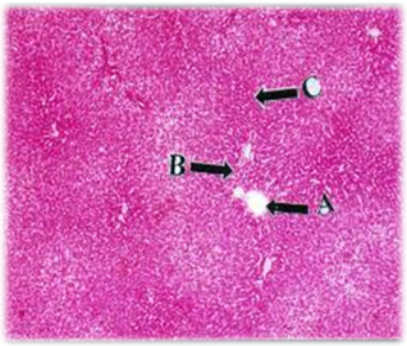

Fig. 2: Liver Tissues showing normal central vein (A), sinusoids (B) and hepatocytes (C)

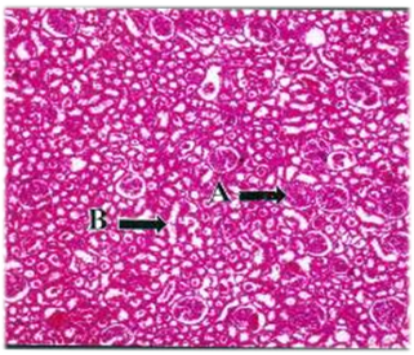

Fig. 3: Kidney Tissues showing normal renal corpuscles (A) and renal tubules (B)

Effect of ethanol on different tissues of albino rats (Group B)

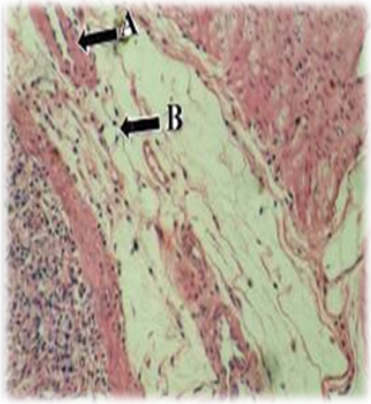

Fig. 4: Stomach Tissues showing congested blood vessels (A) and inflammatory cells (B)

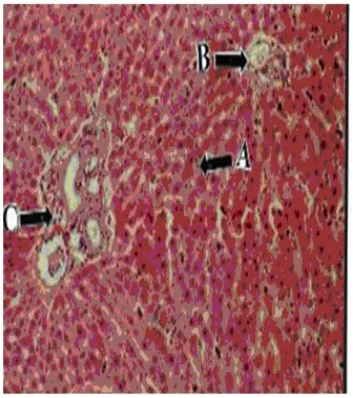

Fig. 5: Liver Tissues showing inflammatory cells (A), congested and dilated central vein (B) and portal tract (C)

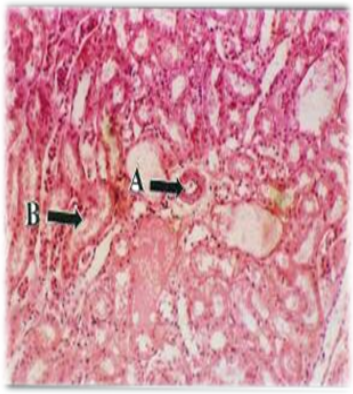

Fig. 6: Kidney Tissues showing shrinkage of glomeruli (A) and congested and dilated renal tubules (B)

Effect of omeprazole on different tissues of albino rats (Grouup C)

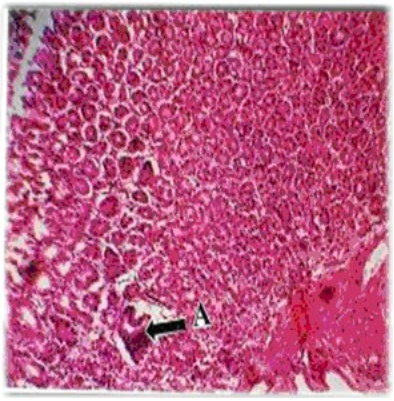

Fig. 7: Stomach Tissues showing inflammatory cells (A)

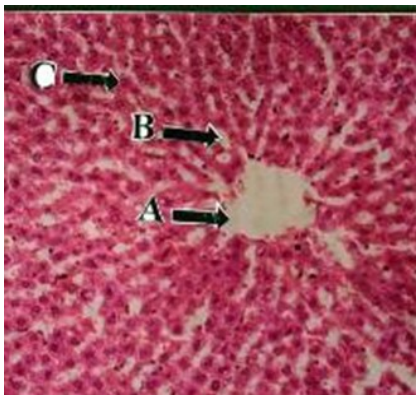

Fig. 8: Liver Tissues showing normal central vein (A), sinusoids (B) and hepatocytes (C)

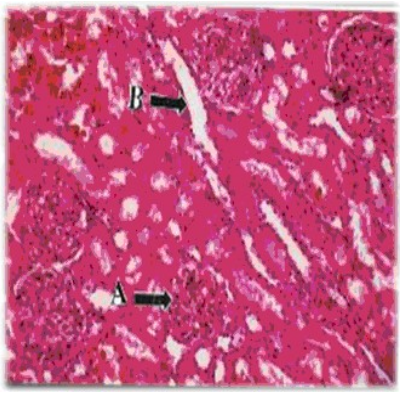

Fig. 9: Kidney Tissues showing normal renal corpuscles (A) and renal tubules (B)

Effect of Cedrus deodara root oil on different tissues of albino rats (Group D)

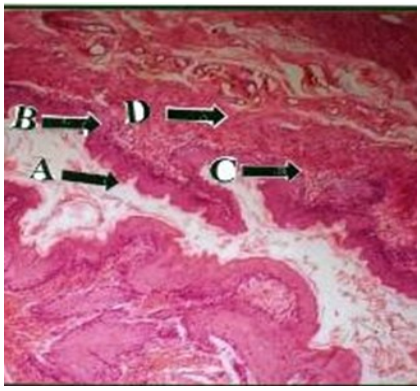

Fig. 10: Stomach Tissues showing normal intact epithelium layer (A), mucosa (B), muscularis mucosa (C) and submucosa (D)

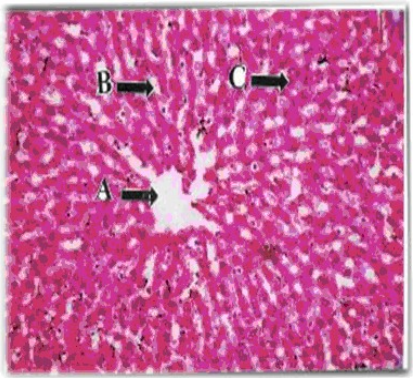

Fig. 11: Liver Tissues showing normal central vein (A), sinusoids (B) and hepatocytes (C)

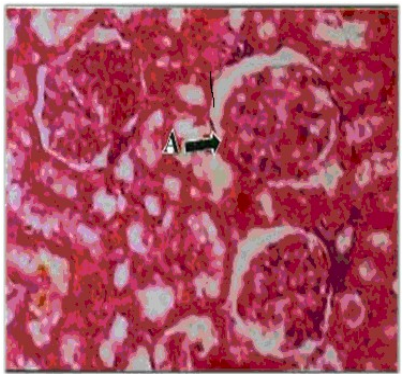

Fig. 12: Kidney Tissues showing normal renal corpuscles (A)

of rats as inflammatory cells can be seen in fig. 7. Liver tissues showed normal central vein as no dilation observed. Sinusoids and hepatocytes were normal (fig. 8). In kidney tissues, renal corpuscles and tubules were observed normal (fig. 9).

Group D which received *Cedrus deodara* root oil showed progressive effects in stomach tissues complete healing effect against ulcerated stomach, the epithelium layer was normal and intact. The other layers of stomach i.e. mucosa, muscular is mucosa and sub mucosa is also normal fig. 10. In liver tissues central vein, sinusoids as well as hepatocytes were observed normal (fig. 11). In kidney tissues renal corpuscles and renal tubules were detected normal (fig. 12).

DISCUSSION

Scientific significance of this study is worthy as it indicate anti-ulcer activity of *Cedrus deodara* root oil and its safety on stomach, liver and kidney tissues. A study has been done which report the anti-secretory antiulcer activities of *Cedrus deodara* wood and supports its traditional use in peptic ulcer (Avadhesh *et al*., 2011). Outcomes of the current study are noteworthy as the herb oil showed more promising results in contrast to omeprazole, as it reduced the gastric ulcer by reducing inflammatory cells in mucosa, congestion and dilation blood vessels in submucosa without necrosis and shedding of epithelium.

Ethanol is extensively used to produce gastric lesions by interfering with the normal function of mucosa and activating mast cells that releases mediators which initiates a series of reactions that ultimately lead to produce ulcer (Robert *et al*., 1979; Enerbäck, 1976). In present study ethanol is used to produce ulcer

Histopathological adaptation including erosion of epithelium, mucosal and sub-mucosal edema, presence of inflammatory cells and congested blood vessels have been reported in the stomach tissues of rat at doses 0.5 and 2.5ml/kg (Rehana *et al*., 2013). In current study we have also assessed the histopathological changes in stomach tissues of albino rats at a dose of 200mg/kg.

In the present study, *Cedrus deodara* root oil at a dose of 200mg/kg has shown more prominent ulcer healing effects in comparison to omeprazole. This study also reveals that this antiulcer dose is safe to other organs as well, namely liver and kidney as the compound was administered orally and it does not show any major toxicity.

CONCLUSION

A number of drug therapies have been used for the treatment of gastric ulcer including proton pump inhibitors, h_2 receptor blocker but all these have side effects and interactions. Herbal therapies are nowadays attaining great acceptance as showing promising results with no or very less side effects.

Histopathological effects of *Cedrus deodara* root oil on liver, kidney and stomach were evaluated at a dose of 200mg/kg. It is concluded in present study that *Cedrus deodara* root oil has anti-ulcer properties without producing side effect in stomach, kidney and liver tissues. It is thus suggested that the compound should further be analyzed on human beings to make safe use of the root oil used in treatment of ulcer.

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