# Assessment of *Cedrus deodara* root oil on the histopathological changes in the gastrointestinal tissues in rats

**Rehana Perveen[1], Mohammad Ahmed Azmi[2*], Ijaz Hussain Zaidi[2], Syed Naeemul Hassan Naqvi[1], Syed Mohammad Mahmood[3], Kiran Ajmal[1] and Mohammad Usman[1]**

[1]Institute of Pharmaceutical Sciences and Hematology, Baqai Medical University, Karachi, Pakistan
[2]Department of Physiology, Pharmacology, Al-Tibri Medical College, Isra University, Karachi Campus, Malir, Karachi, Pakistan
[3]Department of Medicine, Agha Khan University Hospital, Karachi, Pakistan

**Abstract**: The present study was designed to investigate the effect of *Cedrus deodara* root oil on the histopathology of different gastrointestinal organs of Wistar rats. This oil was used traditionally as an anti-ulcer agent in the Indus Unic System and extracted from the plant root by destructive distillation method. A total of 90 rats were taken and divided into groups A, B and C, each comprising of 30 animals. The animals of group B and C were given 0.5 ml/kg and 2.5 ml/kg of *C. deodara* oil respectively while group A served as control and administered vehicle only. The treatment was given to the animals ones only for 24 hours. All animals were sacrificed and the organs like esophagus, stomach and ileum were taken out. Tissue processing and staining procedure was then carried out for any pathological changes in the animal tissues during microscopic examination. The results indicated that *Cedurs deodara* root oil at both doses 0.5ml/ kg and 2.5 ml/kg exhibited some adverse effects such as erosion of epithelium, edema on sub-mucosal and mucosal layers, congestion of blood vessels as well as presence of inflammatory cells on esophagus, stomach and ileum were seen. Moreover shortening of villi was also seen at both doses. A study conducted on mammalian toxicity previously on rats revealed that the *C. deodara* root oil used is not very toxic and comes under least toxic group as standardized by toxicologists. Based on the results obtained it was concluded that *C. deodara* root oil produced some adverse changes in the tissues of GIT when given at 0.5 ml/kg and 2.5 ml/kg doses but the effects were not lethal therapeutically at this dose $LC_{50}$ 16.5 ml/kg. The plant oil showed some toxicity and needs further detailed studies to assess its potential toxicity and therapeutic status before using this material as drug.

**Keywords**: Histopathology, *Cedrus deodara* root oil, gastrointestinal tissues, Albino rats.

## INTRODUCTION

*Cedrus deodara* is a tall ever green tree belonging to the family Pinaceae. It is commonly known as Deodar mostly found in the Frontier Province of Pakistan where the root extract or oil is used as anti-ulcer drug by the old Unic-Medical Personals. This roots extract is diaphoretic composed mainly of three main active compounds as himachalol, atlantone and trans-atlantone same to that of trunk oil (Rehana, 2006). One of the important constituent identified as himachalol acts as antispasmodic and spasmolytic similar to paperverine that also has the same characteristics when given in different doses (Kar *et al*., 1975). *Cedrus deeodara* 15% in castor oil when applied on the skin of rabbit to treat skin infection produce better therapeutic effect (Tandan *et al*., 1989*).* Mast cell stabilizing, immunological and lipoxygenase inhibitory activities of *Cedrus deodara* oil have been documented (Shinde *et al.,* 1999; Rawat *et al., 2000).* The *in vitro* cytotoxic and *in vivo* induction of intracellular caspases, DNA fragmentation as well as DNA cell cycle analytical activities of *Cedrus deodara* oil have also been reported that may be attributed to the presence of the active compounds of the plant materials mainly himachalol (Dimri *et al*., 2004; Shashi *et al.,* 2006; Singh *et al.,* 2007). Regarding the chemistry of stem bark for the analysis of stem bark extract or oil, much analytical work has been done in India and Pakistan (Khan and Naheed 1988; Khan and Naheed 1990; Adinarayana *et al., 1965;* Agarwal *et al*., 1981).

Antifungal activity (Rehana *et al.,* 2010), mammalian toxicity (Perveen *et al*., 2008) and anti-ulcerative effects (Zaidi *et al*., 2011) of *Cedrus deodara* root oil on rat's stomach was also reported. As very little study has been conducted in relation to its pathological effect on animal tissues therefore, present study has been carried out to investigate the effects of *Cedus deodara* root oil particularly on the histopathological changes in the intestinal tissues of albino rats.

## MATERIALS AND METHODS

### *Animal and plant material*

Male albino rats of Wistar Strain (200~250 gms) were procured from the animal house of Baqai Medical University, Karachi. They were maintained on well balanced laboratory diet. The animals were kept in 12/12

**Corresponding author:* e-mail: azmiahmed@hotmail.com

hrs light-dark cycle and water was given freely throughout the experimental period. Ninety albino rats were divided into three groups A, B and C (i.e., 30 rats per group). The approval for conducting experiment on albino rats was taken by Institutional Ethics Committee of Baqai Medical University, Karachi. The tested oil was obtained from the roots of *Cedrus deodara* by destructive distillation method. This plant material was collected from Brooke's Pharma (Pvt) Ltd, Karachi for the experimental work.

***Experimental design***

***Treatment***

The animals of group A did not receive any treatment and kept as control or untreated, while the dose of 0.5 ml / kg and 2.5 ml / kg of *Cedrus deodara* root oil were given orally to the animals of B and C groups. All the animals were treated once with a single dose for 24 hours. They were then kept fasted for overnight before sacrifice.

***Sacrifice of animals***

Animals in each group were anaesthetized by chloroform inhalation. A dissection was made and the organs like oesophagus, stomach and ileum were removed out after opening abdomen. The organs were dried by blotting paper and then the tissues were kept in normal saline immediately for histopathological examination.

***Tissue processing*** (Bancroft and Stevens, 1990)

Samples from the oesophagus, stomach and ileum were fixed in normal saline for 24-48 hours. All the tissues were then processed by keeping in ethyl alcohol solution of different concentrations 70%, 80% and 95% for one hour in each strength respectively. The tissues were also processed in absolute alcohol and xylene for one hour in each for rendering the tissue elements transparent. The transparent tissues were infiltrated with molten paraplast at 58$^{o}$C and then enclosed in a solid mass of paraplast. The blocks containing tissue samples were properly labeled and cooled. The excess of paraplast were removed out but leaving some free margins around the embedded tissues.

Thick longitudinal section of three microns were made on rotary microtome. The sections were mounted on well cleaned and gelatinized slides and maintained at 37$^{o}$C for 24 hours for proper fixation of sectioned tissues. The slides were then stained by Hematoxylin and Eosin (H & E) stain using Bancroft and Stevens, 1990 method for histopathological changes in the animal tissues during microscopic examination (Bancroft and Stevens, 1990)

## RESULTS

In the present work, histopathological studies were done to find any pathological effects of *Cedrus deodara* root oil on different organs of gastro intestinal tract such as esophagus, stomach and ileum when treated with two different doses of 0.5 ml/kg and 2.5 ml/kg orally for animals of group-'B' and group 'C' respectively. Gross examination of different tissues in treated and untreated animals were studied both morphologically and histologically. The findings observed were noted and summarized in table 1 and figs. 1-3.

Initial work has been done on the chemistry of *Cedrus deodara* root oil which contained compounds e.g., himachalol, allohimachalol and trans-atlantone similar to that of trunk oil. After isolation, the purity and structure of these compounds were reconfirmed using thin layer chromatography (TLC), gas chromatography and mass spectroscopy techniques. Histopathological effects of *Cedrus deodara* root oil were carried out during the present study on different tissues of GIT (i.e., esophagus, stomach and ileum) of treated albino (Wistar Strains) rats with animals of untreated group. Mild degree of edema was also noted on the sub-mucosal layer in oesophagus, stomach and on the villi of ileum when a dose 0.5 ml/kg of *Cedrus deodara* oil was applied. Moreover, prominent edematous changes were also seen on the sub-mucosal layer of oesophagus when given at the dose of 2.5 ml / kg. In addition some changes such as erosion of epithelium on mucosal and sub-mucosal layers of oesophagus, congestion of blood vessels on sub-mucosal layer, shortening of villi in case of ileum as well as atropic changes with slight increase in cellularity in stomach were observed. This indicates that *Cedrus deodara* root oil is not very safe because of causing damage to GIT organs when *Cedrus* oil at 0.5 ml/kg and 2.5 ml/kg was given orally (figs. 2a to 3d).

## DISCUSSION

Results from the present study revealed that the herbal material i.e., *Cedrus deodara* root oil at the doses of 0.5 ml / kg and 2.5 ml / kg used experimentally produced some adverse effects on the different gastrointestinal tissues of albino rats. In view of the changes observed, the current study also indicated that the *Cedrus deodara* root oil is not very safe due to its cytotoxic activities.

Our results also correlated with the findings of other researchers to assess its pathological effects on different tissues of the body. The anti-secretory and anti-ulcerative effects of aqueous extract of neem bark have also been studied in rats (Bandyopadhyay *et al*., 2002). The Cedar wood tree also has anti-ulcer effect at appropriate doses that has been noticed by the present study during microscopic examination (figs. 2a to 3d). The extract of neem for ulcer was also used in Harlingen human patients by the oral administration of 40 mg lyphoysed powder twice a day, causing marked reduction in acid secretion (Bandyopadhyay *et al*., 2004). Therefore, it is claimed that the present oil (i.e., *Cedrus deodara* root oil) at a

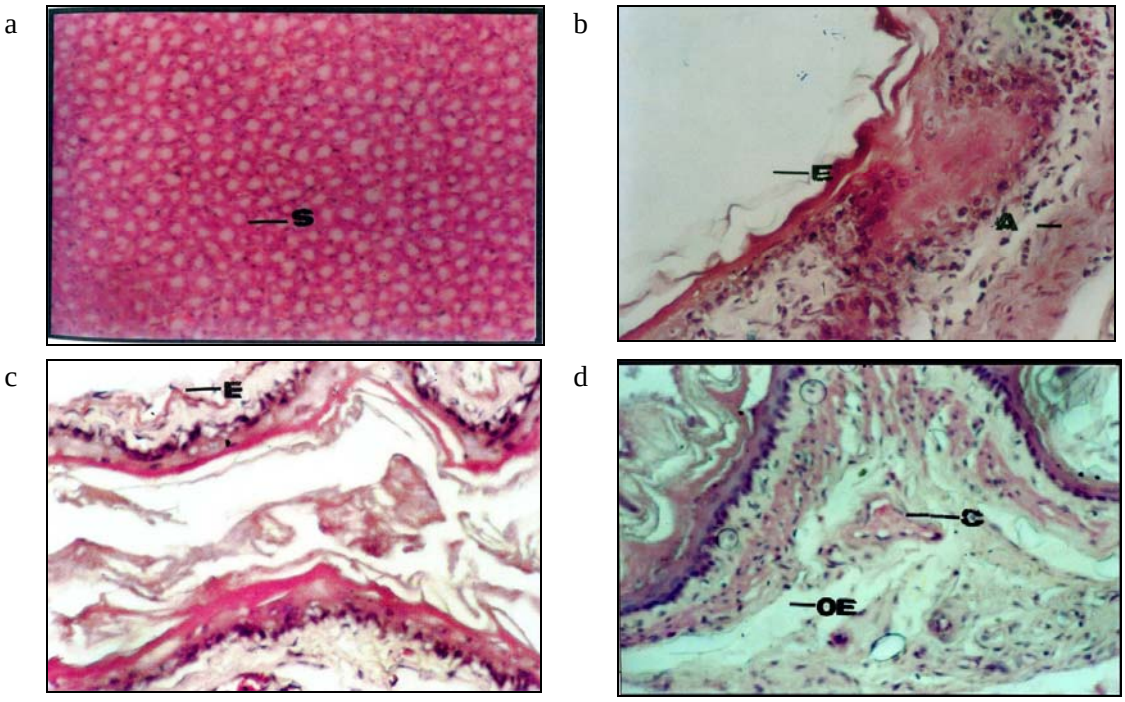



**Fig. 1**: Photomicrographs of control and treated rat esophagus.
[a] represent control esophagus showing normal stratified squamous epithelium (S).[b] represent treated esophagus (0.5 ml kg-1) showing erosion on epithelium (E) and edematous submucosal layer (A). [c and d] represents treated esophagus (2.5 ml kg-1) showing erosion on epithelium (E) edematous (OE) and congestion of blood vessels (C) on submucosal layer. (Original magnifications 200X).

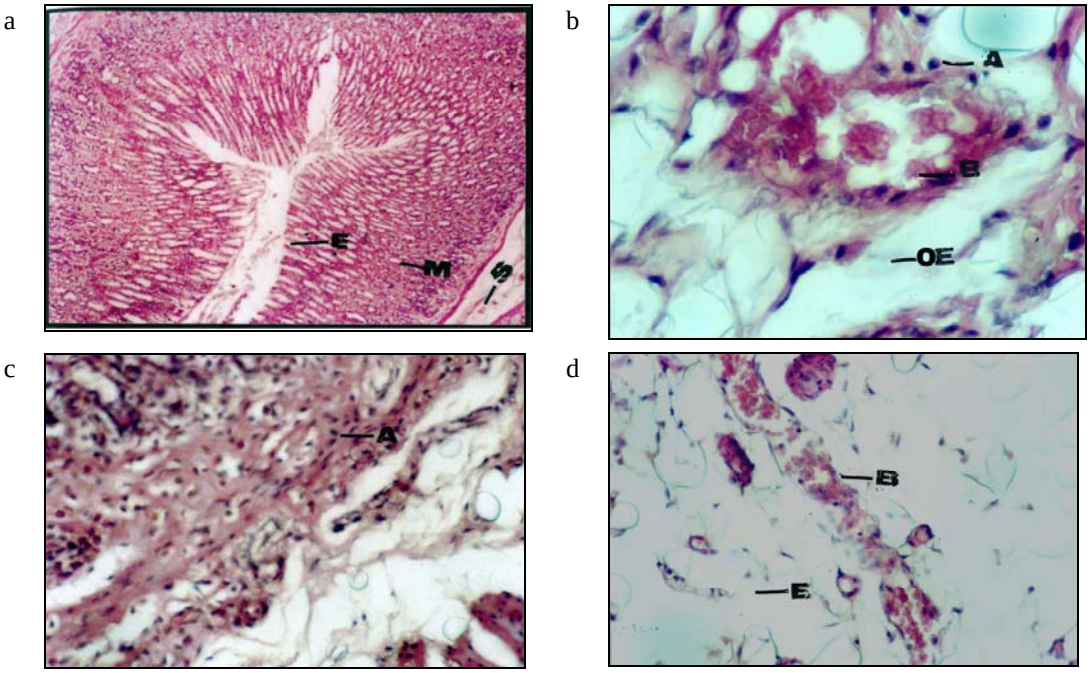
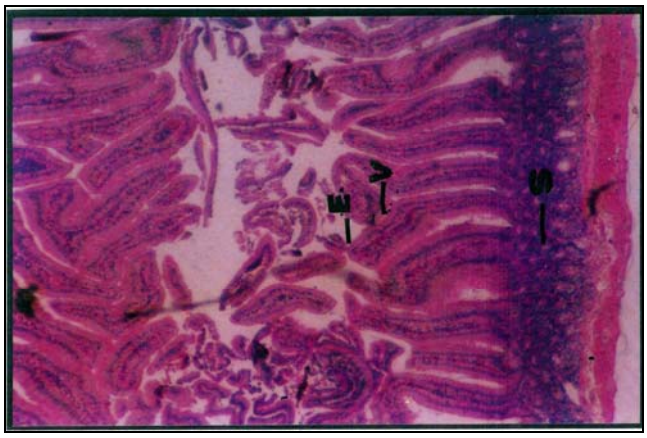
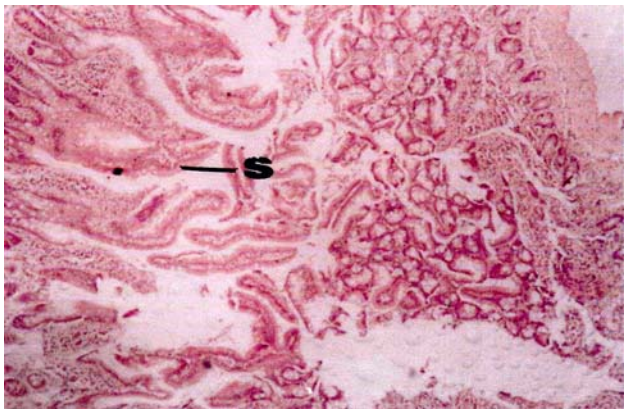



**Fig. 2**: Photomicrographs of control and treated rat stomach.
[a] represents control stomach showing normal epithelial surface (E), mucosal (M) and submucosal (S). [b] represents treated stomach (0.5 ml kg-1) showing congested blood vessels (B), edema (OE) and inflammatory cells (A) on submucosa. [c and d] represents treated stomach (2.5 ml kg-1) showing inflammatory cells (A), congested blood vessels (B) and edematous cells (OE) on submucosa (Original magnifications 200X and 400X).



a

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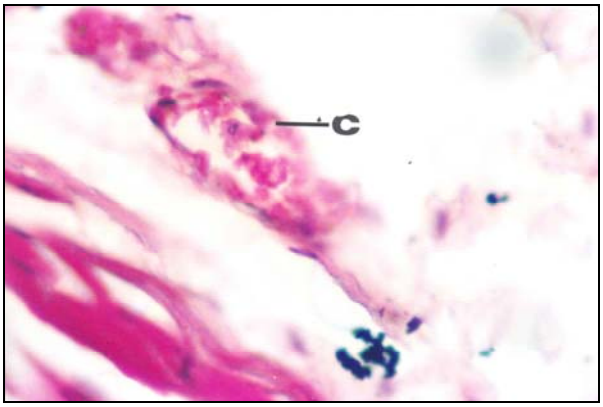


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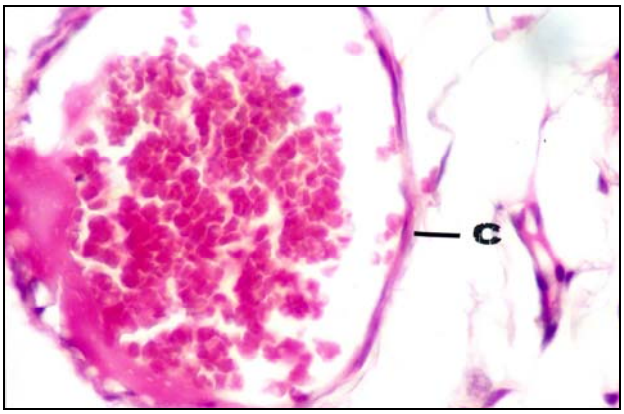


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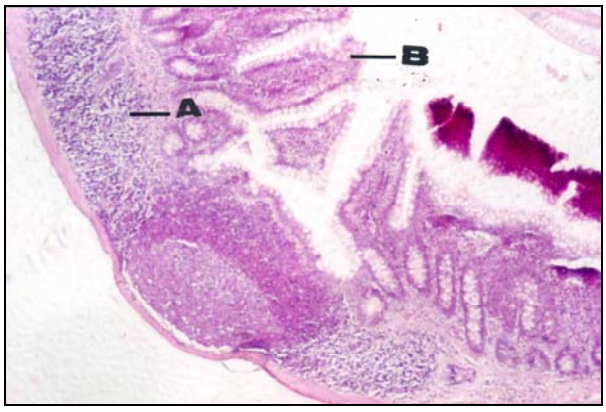


e

**Fig. 3**: Photomicrographs of control and treated rat ileum.
[a] represents control ileum showing normal villi (V), epithelial lining (E) and submucosa (S). [b and c] represents treated ileum (0.5 ml kg-1) showing shortening of villi (S) congested blood vessels (C). [d and e] represents treated ileum (2.5 ml kg-1) showing congested blood vessels (C) shortening of villi (B) and inflammatory cells (A). (Original magnifications 200X and 400X).

**Table 1**: Histological features of untreated and treated esophagus, stomach and ileum tissues of albino rats

| Tissue type | Control rats ( Group–A ) | Treated rats with *Cedrus deodara* root oil | | | | | |
|---|---|---|---|---|---|---|---|
| | | ( Group-B ) Dose: 0.5 ml / kg | | | ( Group-C ) Dose: 2.5 ml / kg | | |
| | General Architecture | General Architecture | Epithelium | Abnormal changes | General Architecture | Epithelium | Abnormal changes |
| Oesophagus | – Lined by a protective stratified squamous epithelium (Fig. 1).<br>– Consists of muscularis mucosae.<br>– Sub-mucosa is highly vascular.<br>– Muscularis propria consists of:<br>i. Outer-longitudinal fibres<br>ii. inner circular fibres | Sub-mucosal and mucosal layers were found intact. | No changes seen in the thickness. Superficial erosion was found on epithelium (fig. 1). | – Mild oedema (+) was present on the epithelium and sub-mucosa (fig. 1).<br>– Slight (+) congestion is present in sub-mucosal layer.<br>– No inflammatory cell found. | – Sub-mucosal and mucosal layers partly disrupted. | – No change in thickness<br>– Marked erosion was found (fig. 1). | – Sub-mucosal (+) edematous (fig. 1).<br>– Congestion (++) was seen on sub-mucosal.<br>– No inflammatory cell found |
| Stomach | – Consists of four regions- Cardia, Fundus, Body and Pylorus.<br>– Mucosal layer has prominent folds or rugae.<br>– Muscularis mucosae open into stomach lumen via gastric pits (fig. 2).<br>– Muscularis mucosae consists of:<br>i. Outer-longitudinal fibres<br>ii. inner circular fibres | Sub-mucosal and mucosal layers were found intact | No change in the thickness. Showed moderate degree of shedding. | – Showed edema (+) on Sub-mucosa (fig-2)<br>– Congestion (++) was found on the sub-mucosal<br>– Inflammatory cells (++) were found on the sub-mucosal. | – Sub-mucosal and mucosa layer were almost intact. | – No change in thickness.<br>– Showed atropic changes with slight increased in Cellularity. | – Few inflammatory cells (+) found on sub-mucosa (fig. 2).<br>– Marked congestion (++) was on the sub-mucosa (fig. 2).<br>– Sub-mucosa showed oedema (+) (fig. 2). |
| Ileum | – Convulated part.<br>– Mucosal layer consists of numerous folds with villi (fig. 3).<br>– Muscular mucosae has intervening crypts.<br>– Sub-mucosa is highly vascular consisting of longitudinal fibers.<br>– Mucosal layer folds give peristaltic movements. | Sub-mucosal and mucosal layers were found intact | – No change in the thickness. | – Shortening of villi (fig. 3).<br>– Mild Edema (+) found on sub-mucosa (fig. 3).<br>– Inflammatory cells (++) found on mucosa/sub-mucosal layer. | – Sub-mucosal and muscular layers found intact. | – Epithelium was<br>– stunted (fig. 3). | – Shoretening of villi (fig. 3).<br>– Sub-mucosal layer was found edematous (++).<br>– mucosal and sub-mucosal layer showed (+) congestion (fig. 3).<br>– Inflammatory cells (++) seen on mucosal layer (fig. 3). |

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