Effects of aqueous leaves extract of *Holoptelea integrifolia* (Roxb) Planch on liver and kidney histopathology of albino rats

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Abstract: Histopathological studies are an essential element to ascertain comprehensive safety profile of a drug. Unfortunately limited data are available about the toxicity of herbal remedies. Since a popular medicinal plant *Holoptelea integrifolia* (Roxb) Planch. contains various bioactive molecules, the present study is aimed to assess the histopathological alterations induced by aqueous extract of *Holoptelea integrifolia* on liver and kidney of wistar albino rat. In this study 60 rats divided in two groups; control and treated with aqueous extract of *Holoptelea integrifolia* (250mg/kg body weight) for 5 days. Histopathlogical studies by hematoxylin and eosin (H&E) staining were done on the liver and kidney tissues at the end of dosing by using standard procedure. Microscopic examination was then carried out to observe any pathological changes in the animals. The result showed that there is no significant variation in the basic architecture of liver and kidney as compared to control male wistar albino rats. In conclusion, aqueous extract of leaves of *H. integrifolia* may be safe and nontoxic. Further work on pharmacological aspects is required to evaluate the clinical potential of this plant for different ailments.

Keywords: Histopathology, *Holoptelea integrifolia* (Roxb) Planch., aqueous extract, albino rat.

INTRODUCTION

The plant *Holoptelea integrifolia* (Roxb) Planch. (Ulmaceae) known as Indian Elm, a large deciduous tree. Indian Elm has been indigenous to tropical regions of Asia including Pakistan, India, Nepal, Sri Lanka, Indo-China, Cambadia, Laos, Myanmar, Vietnam, Burma and China. The plant exists in India, the lower Himalayas to Tranvancore, and in Pakistan in different places especially in Karachi and some other parts of Sindh. *Holoptelea integrifolia* is the only species of this genera found in this region (Prajapati and Patel, 2010; Sharma *et al*., 2001; Vaidyasala. 1995; Vaidya, 2000). This plant posessess anti-inflammatory, anti-diarrheal, antibacterial antitumor, adaptogenic, antidiabetic and antioxidant activity, anthelmintic and wound healing potential, etc. (Lakshmi *et al*., 2010; Maheshwari and Singh, 1990; Mudgal and Pal, 1980; Pulliah, 2006; Saxena, 2012; Singh and Ali, 1994).

The bark and leaves of *Holoptelea integrifolia* have shown anti-inflammatory, digestive, carminative, laxative, bitter, astringent, anthelmintic, acrid and repulsive activity against various diseases. Seeds possess antiulcer activity and have been employed as deodorant for bed smell of body. It has been reported that seeds and stem bark were applied externally to cure ringworms (Durga and Paarakh 2011; Sharma *et al*., 2001; Sharma and Singh, 2012; Prajapati *et al*., 2007, Prajapati and Kumar, 2003). Paste of bark and leaves has been effectively applied externally to treat leucoderma (Benjamin and Christopher, 2009; Mahumud *et al*., 2010; Sharma *et al*., 2010). Friedelin and friedelin related constituents were isolated from leaves have anti cancer activity to treat bladder carcinoma, epilepsy, inflammation, ulcers, arthritis, fever and dysentery (Saraswathy *et al*., 2008).

Preliminary phytochemical screening revealed that this plant contains variety of medicinally valuable constituents such as carbohydrates, proteins, flavonoids, amino acids, steroids, glycosides, alkaloids, tannins, myristic, lauric, palmitic, stearic, behenic, hexadecenoic, arachidic, and oleic acids. Many other compounds have been isolated from various parts of this plant species including friedlin, epifredlin, holoptelin-A, holoptelin-B, 1,4-napthale nedione, β-amyrin, stigmasterol, β-sitosterol, betulin, betulinic acid, hexacosanol and octacosanol. β-sitosterol, 2α, 3α-dihydroxyolean-12-en-28 oic acid and hederagenin has been found in heartwood (Benjamin and Christopher, 2009; Mahumud *et al*., 2010).

It has been reported that aqueous extract of the leaves of *H. integrifolia* have anti-inflammatory activity that significantly inhibited paw edema approximately same as percentage inhibition with indomethacin (Shirinivas *et al.*, 2009; Srinivas *et al*., 2009). Similarly, methanol and petroleum ether extracts of leaves of *H. integrifolia* have antidiabetic activity compared to the standard drug Glibenclamid (Sharma *et al*., 2010).

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MATERIALS AND METHODS

Collection and identification

The fresh leaves of *H. integrifolia* (Roxb) Planch. were collected from the premises of University of Karachi, Pakistan. After the identification by Prof. Dr. Ghazala H. Rizwani, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, a voucher specimen No.01 of *Holoptelea integrifolia* was deposited at herbarium, Department of Pharmacognosy, Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi.

Extraction and fractionation

Plant material (500g) was dried, chopped into small pieces and percolated with methanol (5L) for 15 days. After that the solvents from extract were evaporated at reduced pressure and controlled temperature, to obtained methanolic extract (70g). Methanol extract powder of plant leaves (52g) was then partitioned with an equal quantity (500ml) of distilled water and ethyl acetate (EtOAC). Two layers, ethylacetate and water were obtained, solvents were removed on rotary evaporator at controlled temperature i.e. 40°C and under reduced pressure. The aqueous extract obtained was then lyophilized to powder form (35g). The aqueous extract *H. integrifolia* (Roxb) Planch. was used for histopathological screening (Perveen *et al*., 2010; Shareef *et al*., 2014).

Animals

Adult albino rats (200±10g) of male sex were used for experimental work provided by the animal house facility of Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi. Animal were housed at temperature of 25-27°C in the animal house along with standard environment condition for 12 hours light and 12 hours dark cycle. They were fed with standard balance laboratory diet and water. The study was approved by Ethical Committee of Baqai Medical University.

Histopathological study

Aqueous extract show significant effects on inflammation (Khalid *et al*., 2013, Shirinivas *et al*., 2009; Sharma *et al*., 2009) and organ histopathology in wistar albino rats (200g±10) was determined. A total of 60 albino rats were divided in two groups i.e. group I was marked as control and no treatment was given, in group II. Aqueous extract of *H. integrifolia* (Roxb) Planch. (250mg/kg body weight) was administered for five days. After long term exposure minimal five days dosing rats were dissected, kidney and liver tissues were isolated and preserved proceeding to section and staining (Bancroft and Stevens, 1990).

Routine tissue process

The fixation of tissue was done by keeping the organ in normal saline for 24-48 hours. Tissues processing were carried out by using different concentrations of ethyl alcohol from 70%, 80% and 95%, for a duration of 60 minutes for these strengths.; twice in absolute alcohol (for 1hour each); and twice in xylene (for 1hour) in order to retain transparency of component of tissues. Molten paraplast was used to infilterate the tissues at 58°C; twice (for 1hour on each occasion). After clearing all the components from the tissues, a solid mass of paraplast was used to embed the transparent tissues. Labeled were placed, cool and removed metal blocks. By using rotary microtome, about four microns thick longitudinal sections were cut. Cleaned gelatinized slides were used for mounted sections and the slides were kept on hot plates at 37°C for 24h for proper fixation. The H&E stain were employed for staining according to the prescribed staining method (Bancroft and Stevens, 1990). For the preparation of stain, hematoxylin was dissolved in absolute alcohol and boiled immediately and mercuric oxide was added. The stain was placed in cold water bath to allow to cool; then glacial acetic acid was added and stain was prepared for use. Several slides were prepared accordingly. The stained slides, after drying and labelling, were preserved and stored for histopathological studies which entailed microscopical examination for comparative morphological and pathological changes in the tissues of the animals.

RESULTS

Histopathological analysis on Holoptelea integrifolia

The overall changes the appeared in organs such as the kidney and liver were noted with a magnifying glass and microscope in a row to make changes to both morphological and histological in comparison with the treatment of animal that are as mentioned in Table 1.

Liver: normal control

The liver is located in the abdomen cavity just in the right upper region and appears like a bi-lobed dark brown organ. Stained liver section is visualized as normal hexagonal lobular pattern consisting of portal and sparse collagenous tissue (fig. 1a). Portal system comprises of terminal portal venule, terminal branch of hepatic artery and bile ductules embedded in fibrous stroma. Blood in the central vein comes from portal system. At the lobule margin and between the plates of hepatocytes sinusoids were originated as three dimensional structure like a sponge. These sinusoids were lined by a discontinuous, fenestrated endothelium. Hepatocytes consist of large polyhedral cells with round nuclei with peripherally scattered chromatin and prominent nucleoli which are varied in size, demonstrate cellular component of the cell. Some binucleated cells were also seen. The cytoplasm of hepathocytes was extensive, granular and strongly eosinophilic.

Treatment with aqueous extract of Holoptelea integrifolia

Following observation has been made while interpretation of liver architecture under microscope (table 1).

Table 1: Architectural layout of untreated and treated liver and kidney of albino rats

Tissue type	Normal control	Treated with Aqueous extract of *Holoptelea intergrifolia*
Liver	Dark brown bilobed organ hexagonal lobular pattern: Normal central vein [C], sinusoids [S] and hepatic cells [H]: Normal (fig. 1a.) Sinusoids lined by fenestrated endothelium. Hepatocytes: large polyhedral cells with round nuclei dispersed chromatin and prominent nucleoli. Cytoplasmic hepatocytes: granular and deep eosinophilic.	No significant changes were found General architect were found normal Few inflammatory cell were showed Minimal periportal inflammation No fatty deposition No fibrosis No necrosis (fig. 1b,c,d)
Kidney	Cortex and medulla: Normal Cortex: normal glomeruli appeared as dense rounded structure (fig. 2a) Vascular basement and glomerular basement membranes have uniform thickness and uninterrupted. Afferent and efferent arterioles were seen at vascular pole of renal corpuscles. Proximal convoluted tubule (PCT): simple cuboidal epithelium with brush border and narrow lumen. Cytoplasm of PCT stained dark pink. Distal convoluted tubule (DCT) contains few cuboidal cells with wide lumen. Cytoplasm of DCT stained light pink. Medulla consists of loop of Henle, collecting tubules vasa recta and collecting duets.	Intact cortex and medulla No significant changes was observed General architect were found normal Minimal inflammatory cell were observed No tubular atrophy No necrosis (fig. 2b,c,d)

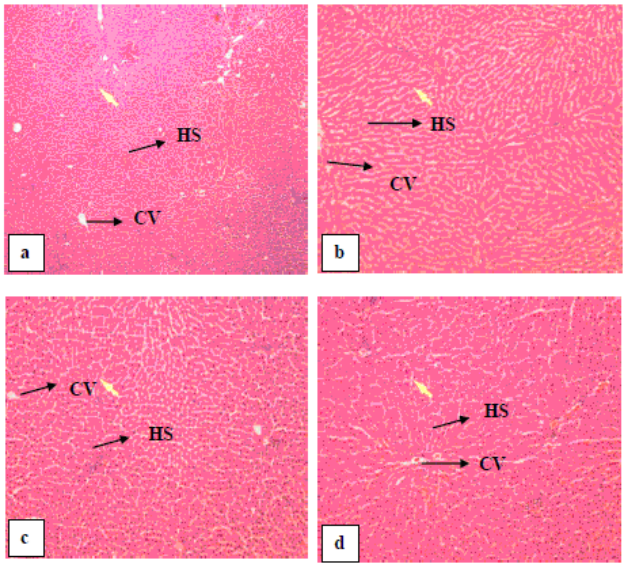

Fig. 1: Photomicrograph of liver sections of albino rats stained with Hematoxylin and Eosin (H and E). (a) section of the liver shows normal histology for the control group: (b, c and d) section of the liver of the treated albino rats with 250mg/kg of aqueous showing normal histology. CV- central vein HS- Hepatic sinusoid

Kidney: Normal control

Normal H& E stained sections of kidney appeared with normal cortex and medulla containing normal glomeruli with dense rounded structures (fig. 2a). Glomeruli were situated beside the surface of cortex at the junction of medulla and in between the surface of cortex and medulla. Inside the glomerular tufts, the nuclei of endothelium, mesengial cells and visceral layer of Bowman's capsules were viewed as associated with each other. Glomerular basement membrane and the vascular basement membrane came into view with uniform thickness and uninterrupted.

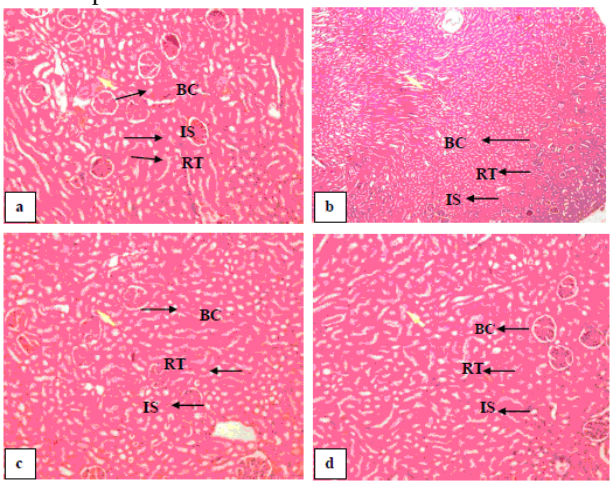

Fig. 2: Photomicrograph of the Kidney sections of albino rats stained with H and E (a) a section of the control group showing normal histology with numerous glomeruli, renal tubules and blood vessels. The interstitial space appears unremarkable. (b, c and d) A section of the kidney of the treated albino rats with 250mg/kg of aqueous showing normal histology. BC= Bowman's capsule; RT= Renal tubules; IS= interstitium.

The glomerular extra cellular mesengial matrix showed normal appearance. At the vascular pole of renal corpuscles, the afferent and efferent arterioles were observed lined with endothelium and their walls with normal thickness. Proximal convoluted tubules (PCTs) were lined with smooth large number of cuboidal epithelium with prominent a narrow lumen and brush border. The distal convoluted tubules (DCT) were sheathed with some uncomplicated cuboidal epithelial cells along with a wide lumen. The cytoplasm of PCT cells was filled and stained dark pink with H&E stain. The cytoplasm of DCT cells was smaller in amount and stained light pink with H&E stain. The medulla of kidneys is contained with segments of loop of henle, collecting tubules, vasa recta and collecting ducts. They were lined with their specific epithelium.

Kidney: Treatment with aqueous extract of Holoptelea integrifolia

Microscopical interpretation of the kidney architecture is presented in table 1.

DISCUSSION

Histopathological studies are considered as an important part of research to evaluate the toxicity profile of the drug which produced alteration or damage to some organs either acute or prolonged used (Saxena *et al*., 1987). It was noticed that some of the drugs lead to produce changes in stomach, liver and kidney (Perveen *et al*., 2010; Shareef *et al*., 2014). To develop the safe and effective use of the drug it is required to evaluate the undesirable effects of that drug. Generally liver functioned as the detoxifying organ for all type of chemical substances and kidney is responsible for the excretion of drugs (Abdulrahman *et al*., 2007).

In this regard pathological effects of aqueous extract of *H. integrifolia* at the dose of 250mg/kg body weight have been observed which produce anti-inflammatory effect on albino rats. In the present study the histopathological effects on aqueous extract of plant were reported first time. No research has been reported for the histopathological changes on liver and kidney so far.

The pathological slides were prepared and group II was compared with normal control. Liver and kidney of albino rats were observed to evaluate the overall changes in organs after chronic exposure of aqueous extract at dose of 250mg/ kg. The histological section of liver has shown that general architecture with portal vein is found to be intact (fig. 1a). No necrosis and fibrosis were observed with minimal periportal inflammation. The results were compared with group I (figs. 1b, c, d). No significant changes and no focal abnormalities were found in liver.

In addition to this, no abnormalities in shape and variation in size with no shrinkage in cells were visualized. It may be due to no degenerative changes and no fatty deposition within cells. It means that the aqueous extract of plant can be safely administered in patient on risk with nonalcoholic fatty liver diseases. The histopathological screening of liver cells was done, that showed no noticeable alteration and damages in liver cells.

Similarly histopathological examination of kidney of same albino rats i.e. group aqueous extract of *Holoptelea integrifolia* have been compared with normal or untreated albino rats i.e. group normal control (Fig 2a). Minimal inflammatory cells were seen with intact cortex and medulla. Kidney showed no tubular atrophy and no necrosis. In overall view no significant changes were noticed. Abnormalities in shape and size of kidney cells were not found. It can be suggested that the aqueous extract of plant may be used safely without any alteration renal function (figs. 2b, c, d).

CONCLUSION

Histopathological screening supports the present research regarding the safety profile of aqueous extract of *Holoptelea integrifolia* which is reported first time. Further work on pharmacological aspects is required to evaluate the clinical potential of this plant to used again for different ailments. In future, critical evaluation of the aqueous extract of *Holoptelea integrifolia* is required regarding its safety and efficacy profile.

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