

Histopathologic changes in liver and kidney of male sprague dawley rats treated with extract of *Cardiospermum halicacabum* L.

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Abstract: In traditional medicine *Cardiospermum halicacabum* L. (*Sapindeaceae*) is used against various ailments such as rheumatism, nervous diseases, stiffness of the limbs and snakebite. Leaves are crushed and made into a tea, which aids itchy skin. Salted leaves are used as a poultice on swellings. Young leaves can be cooked and used as vegetables. The leaf juice has been used as a treatment for earache as well. In this study we evaluate acute toxicity (10, 50, 100 and 500 mg/kg) and pathologic changes in esophagus, stomach, liver and kidney tissues with a magnifying glass and microscope in a row to mark changes to both morphological and histological in comparison to control with the treatment of ethyl acetate extract (dose of 40mg/kg) in male Sprague Dawley rats. The rats were divided into 4 groups consisting of 3 rats per group for acute toxicity and histopathological change. In conclusion, no lethality was observed in acute toxicity study for 7 days. The treatment of ethyl acetate extracts at 40 mg/kg did not show lethal toxicological changes as observed by histopathological examination in the kidney and liver tissues.

Keywords: Histopathology, ethylacetate extract, rats, toxicity.

INTRODUCTION

Nature always situated like a golden mark to demonstrate the marvelous occurrence of symbiosis. Natural yields from plants, animals and minerals have been provided the basis for the treatment of human diseases (Verma and Singh, 2008). Now a days the global market share or trade of traditional medicine was about 2500 species internationally and estimated at US\$ 83 billion annually in 2008, with an increase rate of sale per year and estimates suggest that it will reach up to the US\$ 5trillion by 2050 (Kamboj, 2000). Therefore, this recommendation of wide spread sales of medicinal plants, toxicological assessment of herbal medicine become more necessary to check the potencies that can be reflect on secure formulation and raw material for clinically efficient remedies (WHO, 2011).

Cardiospermum halicacabum L. (CH) belongs to the family *Sapendaceae*, commonly known as love in puff. It is originated all through the plains of Africa, America, Bangladesh, India, Malacca and Pakistan. The whole plant is diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific in folk. It is also used in the treatment of rheumatism, chronic bronchitis and stiffness of the limbs and snakebite (Huma *et al.* 2012).

The aim of this study to identify the dose related histopathological changes for the development of safety protocol of CH extract for the utilization of herbs safely.

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MATERIAL AND METHOD

Collection and identification

Cardiospermum halicacabum L. was collected in June 2010 from the garden of University. After the identification of plant material by Prof. Dr. Ghazala H Rizwani, Faculty of Pharmacy, University of Karachi. A voucher specimen no. 036 of the CH was deposited at herbarium, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi.

Extraction and fractionation

Shade dried whole plant of CH (2kg) was crushed coarsely and percolated in 70% ethanol (Merck, Germany) at room temperature for 20 days. After that, percolate was filter through Whatmann filter paper and repeated this process for three times and then combined all three filtrate. Ethanolic solvent was evaporated under reduced pressure and controlled temperature (40°C) and a dark brown thick semi-solid residue (25.2gm) was obtained.

The gummy ethanol extract was first partitioned with ethyl acetate and distilled water (H₂O) by 1:1 ratio, ethyl acetate and water layers were obtained. All three filtrates of ethyl acetate evaporated further on rotary evaporator at 40°C separately to obtain ethyl acetate (11.4 gm) fraction.

Animals

Wistar and Sprague Dawley rats (220-380gm) and mice (20-25gm) either sex were purchased from the animal house facility of International Center for Chemical and Biological Sciences, University of Karachi throughout the

study. Animals were housed in a standard environmental conditions of Baqi Institute of Pharmaceutical sciences with temperature of 25-27°C and 12:12 light/dark photo period in ventilation plastic cages (length=42.5cm, breadth =27cm and height =19.2cm) serviced with plastic bottles having stainless steel cannulas and food hoppers. They were fed with a standard diet and water was supplied *ad libitum* to the animals.

Chemicals and their solubility

Ethanol extract and its ethyl acetate fraction were solubilized in 10% dimethyl sulfoxide (DMSO), 0.9% normal saline

Toxicity studies

Acute toxicity

Mice of either sex were used. Each group (n=5, mice) were treated with physiological solution served as control, ethanol extract of whole plant administered in the doses of 10, 50, 100, 500mg/kg by intraperitoneal route. Changes in various autonomic and behavioral responses were noted up to 4 hrs. with the gap of 15 minutes (Zaoui *et al.*, 2002) The animals were kept under observation for 7 days, gross effect and mortality, if any occurred was observed during this period.

Histopathological study

We were studied the organ pathology of ethylacetate extract of CH in albino rats at the dose of 40 mg/kg (Huma *et al.*, 2014). For this instance we were separated rats into two groups A and B (3 in each). Group 'A' marked as a control while group 'B' treatment and ethyl acetate extract was orally administered 40mg/Kg. The dose was repetitive thrice. The chloroform was used to anesthetised the rats and after 24 hours rats were dissected and organs i.e. oesophagus, stomach, liver and kidney were isolated and preserved in 10% neutral buffered formalin prior to sectioning and staining (Bancroft and Stevens, 1990). The routine tissue preparation and staining with hematoxyline and eosin dies were according to (Rehana *et al.* 2010).

RESULTS

The results of ethyl acetate extract of CH exposed no gross morphological variations were observed in the liver and Kidney organ.

DISCUSSION

To attain the desired advantage from herbal preparations, an individual must take the requisite dose up to a certain span of time. Although it is usually believed that most herbal preparations are safe for consumption but research shows that some herbs are containing biologically active substances that could be toxic and produced undesirable side effects (Bisset, 1994). In this connection, we were

evaluated the acute toxicity and pathological effect of ethylacetate fraction of CH at the dose of 40 mg/Kg on rat organs including oesophagus, stomach, liver and kidney. No research has been reported so far to outline the pathological effect of ethylacetate CH extract on liver and kidney in relation to the dose.

Intraperitoneal administration of 10, 50,100 and 500mg/kg of ethanol extract of CH did not show any changes in gross behavior of the animals as compared to the control group. As far as the mortality was concern no toxic effect was detected up to 500mg/kg for 7 days while pathological effect on the organs showed no serious damage at the dose of 40mg/kg.

Histological section of the oesophagus obtained from rats treated with the dose of 40mg/Kg, showed a normal appearance of oesophagus and no evidence of intraepithelial dysplasia. Keratinization was also not seen which indicated that there was no deficiency of vitamin A (Sommer, 1983). Ethyl acetate extract of CH showed normal epithelium without apoptotic bodies and vasculated cells. No local irritation to the oesophagus produced by the extract, which means no sensitize the local epithelium and it can be used by inhaled or targeted site drug delivery. The cells were spongy and columnar attributes were preserve, which is clearly shown in the fig. 1b. The therapeutic attribute in particular that ethyl acetate extract of CH can locally protect the oesophageal cell in risk subjects e.g. smoker, people prone to environmental toxin.

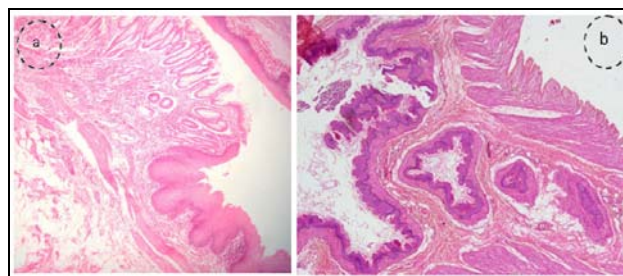


Fig. 1: Photomicrograph of a 5 micron thick H & E Stained paraffin section of esophagus (10x magnification) a) Normal histology b) Normal squamous mucosa of esophagus.

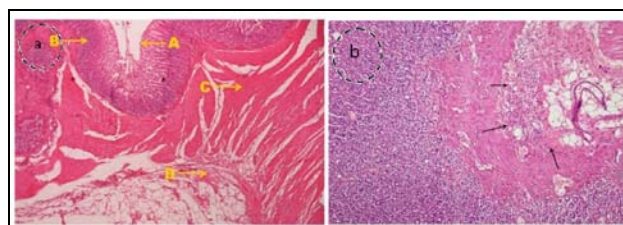


Fig. 2: Photomicrograph of a 5 micron thick H & E stained paraffin section of stomach (40x magnification) a) Showing normal mucosa (A), sub mucosa (B), muscularis (C) and adventitia (D) b) Focal acute inflammation (neutrophils) in the submucosa.

From this exploration we also give an evidence for, the oral route is appropriate selected route because of no irritating or damaging property of ethylacetate extract of CH on the endothelial lining of the passage organs oesophagus and stomach or the processing organ liver (epithelial cells). Thus proving the oral dosage form is preferable route for CH extract.

The pathological slide of stomach tissues showed (fig. 2) that the majority of mucous cells have dense basophilic, which are healthier than the cell of control group. Epical convex portion is clear and dilated oxyntic glands showed cleared and prominent basophilic glandular epithelium. Amorphous soluble mucous is thick and contain few cell debris. The proliferative cells are constituted of fibroblast with well-differentiated myoblast. Tunica serosa was devoid of mesothelial layers and no penetration of lesion in muscularis mucosa and sub mucosa muscularis was similar to normal cells.

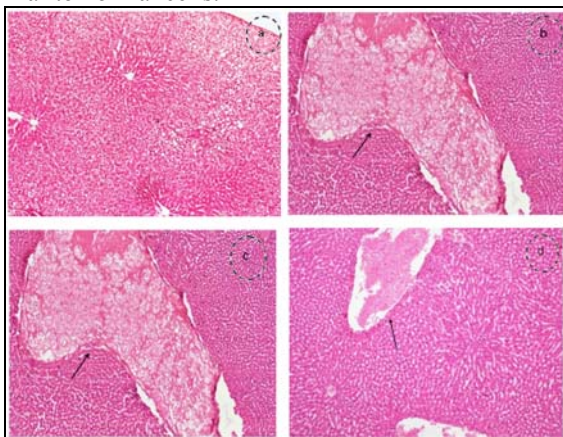


Fig. 3: Photomicrograph of a 5-micron thick H & E stained paraffin sections of liver. a) Normal histology with normal hepatocyte (40x) b-d) massively dilated and congested terminal hepatic venules (10x, 20x and 40x respectively)

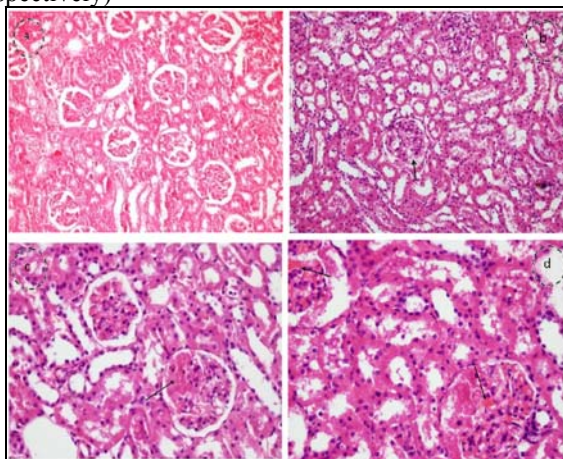


Fig. 4: Photomicrograph of a 5-micron thick H & E stained paraffin sections of Kidney. a) normal histology (40x) b-d) renal glomeruli show focal hemorrhage in the Bowman's capsule (10x, 20x and 40x respectively)

Results of many sub chronic toxicity tests of various plant extracts give an idea about the major organs (liver and kidneys), which were usually affected. Hepatotoxic and nephrotoxic effects are mostly to be expected, as the liver acts as the main detoxifying organ for chemical substances, while the kidney is a principal route of excretion for many drug substances in their active and/or inactive forms (Abdulrahman, 2007). Therefore, significant changes to these organs were observed particularly.

The general architect with portal vein in liver was intact. Steatosis, necrosis and fibrosis were not seen (fig. 3). No focal abnormalities reported which means cells were non-inflammatory. Furthermore shrinkage of cell was not seen and cell did not exhibit features of pleomorphism, abnormalities in shape and size variation in cytoplasm to nucleus ratio was also not observed. Because, no fatty deposition and degenerative sequence was noticed in the cell this extract is safe and to be administered in risk patients of non-alcoholic fatty liver disease like steatohepatitis, cirrhosis etc. or drug induced fatty liver changes. The selected route of administration of ethylacetate extract of CH is oral because of its first pass hepatic metabolism. Therefore it was important to assess any degree of histological damage imparted to hepatic tissues. The histopathological assessment of liver cell, therefore conducted, show that no substantial or remarkable hepatic damage exhibited in the hepatocytes. This finding also supports the (Arjuman *et al.* 2009) that the drug-induced hepatotoxicity may protect the liver by ethylacetate extract of stem of *C. halicacabum*.

The dose of 40mg/kg ethylacetate fraction of CH showed few signs of suspected treatment induced renal toxicity on the cortical region of kidney. Although no significant changes in the basement membrane were visible and few inflammatory cells were present which indicated that the inflammation was negligible and no focal necrosis was seen. Abnormalities in the shapes and the size of the cells were not seen despite marginal inflammation. The glomeruli are apparently shrunken which is suggestive of treatment induced atrophy which can lead to apoptosis due to the metabolic changes from the inhibition of the enzyme when treated with CH and can induce kidney cell death (underlying pathogenesis of renal failure) (Bohle *et al.*, 1989) but that was only a possible complication at higher doses of extract.

However, critical evaluation of the therapeutic efficacy of ethyl acetate CH extract is more important therefore toxic attributes must be reassessed for the appropriate indication for ethyl acetate extract of CH in elucidation of the risk and benefit ratio and minimize the renal toxicity of CH extract required additional investigations with modification in the dose (reduction in doses).

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