HEPATOPROTECTIVE STUDIES ON *HALOXYLON SALICORNICUM:* A PLANT FROM CHOLISTAN DESERT

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ABSTRACT

The objective was to study the *in-vivo* hepatoprotective effect of aerial parts of *Haloxylon salicornicum* (Moq.) Bunge (Family: Chenopodiaceae) in order to validate its traditional use in hepatobiliary disorders, by native people of Cholistan desert, Pakistan. Aerial parts (ethanolic extract) of *Haloxylon salicornicum* (HS), (500 and 750 mg/kg/day, p.o. for 7 days) were evaluated on CCl_4 intoxicated rabbits (0.75 ml/kg., s/c.) by serum biochemical parameters and liver histopathological observations. Silymarin (100 mg/kg/day, p.o. for 7 days) was used as a standard hepatoprotective drug. CCl_4 intoxicated group had elevated levels of SGOT, SGPT and ALP significantly but TB level was normal as compared to control group. HS extract (both doses of 500 and 750 mg/kg) showed hepatoprotective effect by significant restoration of SGOT, SGPT, ALP and TB levels as compared to CCl_4 control. 500 mg/kg doses of HS extract produced more significant results as compared to 750 mg/kg doses and Silymarin. Histopathological examination of liver tissues further substantiated these findings. Therefore, outcome of the present study validate the traditional claims on hepatoprotective effects of *Haloxylon salicornicum* (aerial parts).

Keywords: *Haloxylon salicornicum*, Hepatoprotection, Carbon tetrachloride, Serum biochemical parameters, Histopathology of liver.

INTRODUCTION

Haloxylon salicornicum (Moq.) Bunge (Chenopodiaceae), commonly known as "Khar" (Shafi *et al.*, 2001) is a common shrub in desert areas of Pakistan. In folk medicine, its decoction is recognized for its antiseptic and anti-inflammatory effects. Traditional healers are using it to treat intestinal ulcers (Shafi *et al*., 2001). Some native people also claim about this whole plant as poisonous plant on the basis of local non-scientific knowledge (Ahmad *et al.*, 2004). Cholistan desert is present on the Eastern side of the Punjab province, Pakistan (Baig *et al.*, 1980). The majority of plants grow in desert have therapeutic properties and native people utilize these medicinal plants to treat various diseases like rheumatism, GIT and respiratory disorders (Shafi *et al*., 2001).

Native people of Cholistan desert use this plant in hepatobiliary disorders. Previous studies revealed that the ethanolic extract of HS have anti-diabetic and anti-coagulant activities (Qasheesh, 2004). The reported experimental studies on its aqueous extract have confirmed its larvicidal (Sathiyamoorthy *et al*., 1997), anti-cancer and anti-plasmodial potentials (Sathiyamoorthy *et al*., 1999). Its volatile oil is known to possess anti-microbial action especially against *Staphylococcus aureus* and *Bacillus subtilis* (Qasheesh, 2004). Its few alkaloids are recognized as strong agonists for nicotinic receptors (El-Shazly *et al*., 2005). However, to the best of our knowledge, no work has been published previously to investigate its medicinal importance as hepatoprotective agent. Therefore, the present study was designed to evaluate its hepatoprotective activity of aerial parts of HS against CCl_4-induced hepatotoxicity.

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MATERIALS AND METHODS

Chemicals

Ethanol, CCl_4, Formalin, Diagnostic kits (SGPT, SGOT, ALP, and TB), Xylene, Paraffin wax, Eosin, Hematoxylin and Canada balsam were purchased from Merck, Darmstadt, Germany. Silymarin and Pentothal sodium were acquired from Abbott Laboratories, Pakistan. Olive oil was purchased from the local market prepared by P. Sasso, Italy. All the chemicals were of analytical grade.

Plant material and extraction procedure

Haloxylon salicornicum (aerial parts) was collected from Cholistan Desert and authenticated by a Taxonomist (Dr. Mohammad Arshad, Director, CIDS. Herbarium number is 903/CIDS/IUB). Plant material was dried under shade, cut into small pieces and then grinded. The coarse powder (3000 g) of plant material was macerated in 9 L of ethanol for approximately 15-20 days with frequent shaking. The extract was filtered and marc left behind. Extract was concentrated under reduced pressure on Rotary evaporator until a semisolid residue is obtained. Marc was further extracted under the same conditions twice. The semisolid residues, collected after extraction, were combined and evaporated to dryness by vacuum at temperature below 60 °C. At the end, a dark greenish brown solid residue was obtained and approximate yield was 261.3 g. For

convenient administration, the dry extract powder was encapsulated after weighing. Silymarin (a standardized extract from *Silybum marianum*) was used as a reference hepatoprotective agent, which has been administered previously from 50-200 mg/kg in various animal studies (Sonia *et al*., 2005; Eminzade *et al*., 2008; Arshad *et al*., 2010)

Animals

Healthy rabbits of either sex (local breed), weighing from 1.5-2 kg were purchased from local market. They were kept in the animal house of Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur. The animals were maintained at standard housing conditions and fed a standard pellet diet and water *ad libitum*. All procedures were performed as per approval of the Institutional Animal Ethics Committee.

CCl_4-induced hepatotoxicity and extract treatment

Hepatotoxicity was induced by subcutaneous administration of CCl_4 (suspended in olive oil at 1:1) at a dose of 0.75ml/kg body weight. The animals were randomly divided into five groups, containing ten rabbits in each. CCl_4 was injected (30 minutes after drug administration) on the 7th day of the 8 days study period to all the groups except group-I which served as normal control and received only normal saline. Group II-V received the following treatments from 1st to 7th day of the study.

Group II	CCl_4 control (normal saline at 5 ml/kg/day)
Group III	Silymarin control (100 mg/kg/day)
Group IV	*Haloxylon salicornicum* extract (500 mg/kg/ day)
Group V	*Haloxylon salicornicum* extract (750 mg/kg/ day

Twenty-four hours after administration of CCl_4, blood samples (3ml) from all the five groups were drawn from Jugular vein by sterile disposable syringe. Blood samples were allowed to coagulate at room temperature for 45 min into sterile dry centrifuge tubes. Serum was separated by centrifugation at 2500 rpm for 15 min and subjected to biochemical analysis.

Assessment of liver functions

Biochemical estimations

Merck diagnostic kits and UV-VIS Spectrophotometer (U2020 IRMECO, Germany) were used to measure serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP) and total Bilirubin (TB).

Histopathological studies

Seven rabbits from each group were selected randomly for histopathological examination. The selected rabbits were euthanized by intravenous administration of pentothal sodium (0.5 ml/kg). Histopathological assessment was carried out according to the reported standard method (Humason, 1979). The pathological changes of fatty liver and degeneration of liver cells were graded as given below:

- Grade 0 (Normal): Normal liver morphology; hepatocytes with round nucleus centrally with homogenous cytoplasm, flat endothelial cells around central vein and sinusoid.
- Grade +1(Mild degree): 1-2 hepatocyte rows around central vein showed; hepatic cell degeneration along with necrosis (loss of nucleus), less injury of endothelial cells around central vein, less fat vacuoles in hepatocytes.
- Grade +2 (Moderate degree): Some hepatocyte rows around central vein showed; swelling, intracytoplasmic vacuolar degeneration in centrilobular, midzonal and periportal areas endothelial cells around central vein more damage than level +1 and more fat vacuoles in hepatocytes than level +1.
- Grade +3 (Severe degree): 3-4 hepatocyte rows around central vein demonstrated; hepatocytic degeneration and necrosis, degeneration cells including centrilobular, midzonal and periportal areas (diffuse intra-cytoplasmic vacuolar degeneration), endothelial lining of central vein showed more cell damage, increased fat vacuoles in hepatocytes than level +2, marked focal necrosis.

STATISTICAL ANALYSIS

The results were presented as Mean ± Standard error of means (S.E.M). Multiple comparisons were performed by student's *t*-test. Differences were considered statistically significant when $P < 0.05$.

RESULTS

$CC1_4$-induced acute hepatotoxicity model is commonly used to assess the hepatoprotective potential of drugs or medicinal flora and their extracts, both via *in vivo* and *in vitro* techniques (Weber *et al*., 2003).

Administration of CCl_4 (0.75 ml/kg, p.o.) produced a significant rise in serum enzyme levels, namely SGOT, SGPT and ALP. However, TB level was remained unchanged when compared with normal control. The protective action of HS extracts on CCl_4 induced hepatotoxicity has been summarized in table 1. The groups (pretreated with HS extracts) showed the reduced levels of SGOT, SGPT, ALP and even TB as compared to CCl_4 control group. HS extract at 500 mg/kg dose produced more significant results as compared to its dose at 750 mg/kg, when given prophylactically. Moreover, HS extract at 500 mg/kg and 750 mg/kg doses showed more hepatoprotection as compared to Silymarin (100mg/kg), this effect might be due to difference in doses.

Histopathological changes after 24 h of CCl_4-induced liver injury included hepatocytes necrosis, inflammatory cell infiltration, fatty degeneration, hydropic degeneration, vacuole generation and micro-vascular steatosis. Histopathological changes have been shown in figures A-D. Administration of both doses of HS extract (500 and 750 mg/kg) and Silymarin (100 mg/kg) significantly preserved the almost normal hepatocellular architecture from damaging effects of CCl_4. The scoring of histological damage is presented in table 1.

DISCUSSION

A significant rise in cytoplasmic transaminases (SGOT and SGPT), alkaline phosphatases (ALP) in circulation was a clear indication of cellular leakage, loss of functional integrity of the cell membrane and necrosis in the liver (He and Aoyama, 2003) and the rise in the level of serum total bilirubin (TB) is most sensitive tool that reflects the severity of jaundice (Sturgill and Lambert, 1997). The degree and type of hepatocellular damage can be estimated by level of numerous above mentioned biochemical parameters in circulation, along with histological assessment of liver sections. Thus the alleviation in serum enzyme levels by a drug toward their respective normal values was an obvious sign of its hepatoprotective effects.

In the present study, CCl_4 treated group had highly elevated levels of serum enzyme markers (SGOT, SGPT and ALP) along with damaged liver architecture. The HS extract was found to be more effective hepatoprotective at 500 mg/kg dosage level as compared to HS extract (750 mg/kg) from both aspects of liver function and structure. This reduction in serum enzymes level by HS is attributed to a decrease in the lipid peroxidation induced by the metabolites ($CCl_3^{\bullet}$) and ($CCl_3\ OO^{\bullet}$). Decreased levels of SGOT and SGPT seem to protect the structural integrity of the hepatocellular membrane or accelerated regeneration/repairing of damaged hepatocytes produced by CCl_4, while decreased ALP and TB levels proposed the constancy of the biliary functions in the duration of damage with CCl_4.

According to phytochemical analysis, HS contains many alkaloids like Piperidine, haloxynine, hordenine, aldotripiperideine, smipine, haloxine, halosaline, anabasine (El-Shazly *et al*., 2005). Other alkaloids are Nicotine, Tryptamine, dipterine, N-methylisosalsoline, carnegine, isosalsoline, salsolidine, dehydrosalsolidine isosalsolidine, *N*-methyltyramine, Oxedrine, tyramine and many more. In addition to these alkaloids, it also contains coumarins like scopoletin, scopolin, umbelliferone, xanthotoxol, isooxyimperatorin and esculetin. Plant also contains saponins, cardiac glycosides, tannins, anthraquinones, sterols and volatile oils (Ghazanfar, 1994). Coumarins are also well documented for their antioxidant and hepatoprotective actions (Oh *et al*., 2002). Tannins and lignans (Faure *et al*., 1990) are also well renowned for their hepatoprotective effects. Saponins inhibit lipid peroxidation by scavenging reactive oxygen species (Tran *et al*., 2001). Moreover, alkaloids (Vijayan *et al*., 2003) also have hepatoprotective activity. However, the role of steroids cannot be ruled out. So, it is reasonable to think that the observed protective effects of *Haloxylon salicornicum* ethanolic extract against CCl_4 induced liver damage might be due to the presence of these coumarins, alkaloids, tannins, saponins and steroid among other plant constituents.

Table 1: Effects of ethanolic extract of HS (aerial parts) on rabbits serum biochemical parameters after CCl_4 administration

Group	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	TB (mg/dl)	Liver damage (Histological scores)
Normal control (5 ml/kg Normal saline)	40.69 ± 19.94	41.66 ± 23.35	264.5 ± 49.72	0.83 ± 0.22	0
CCl_4 control (5 ml/kg Normal saline + 0.75 ml/kg)	455.2 ± 37.12*	434.2 ± 34.30*	394.3 ± 29.56*	1.32 ± 0.20	+3
Silymarin control (100 mg/kg + CCl_4)	176.5 ± 56.77*°	205.9 ± 36.59*°	257.0 ± 41.03°	1.01 ± 0.42	+1
Test group 1 *Haloxylon salicornicum* extract (500 mg/kg + CCl_4)	69.05 ± 50.95°	189.8 ± 51.37*°	234.1 ± 49.32°	0.24 ± 0.12*°	+1
Test group 2 *Haloxylon salicornicum* extract (750 mg/kg + CCl_4)	169.3 ± 51.05*°	324.1 ± 49.25*	181.3 ± 34.11°	0.22 ± 0.07*°	+1

Values are represented as Mean ± S.E.M. (n=10). 0 = Normal. +1 = Mild. +2 = Moderate. +3 = Severe.

* $P < 0.05$ (significant change when compared with normal control group).

° $P < 0.05$ (significant change when compared with CCl_4 control group).

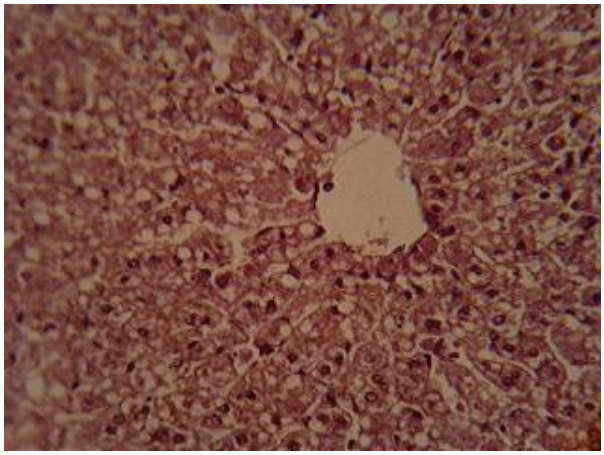

Fig. A: A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from Normal control group (Normal saline). Liver section shows normal liver morphology; Hepatocytes have round nucleus with centrally plus homogenous cytoplasm, flat endothelial cells around central vein and sinusoid

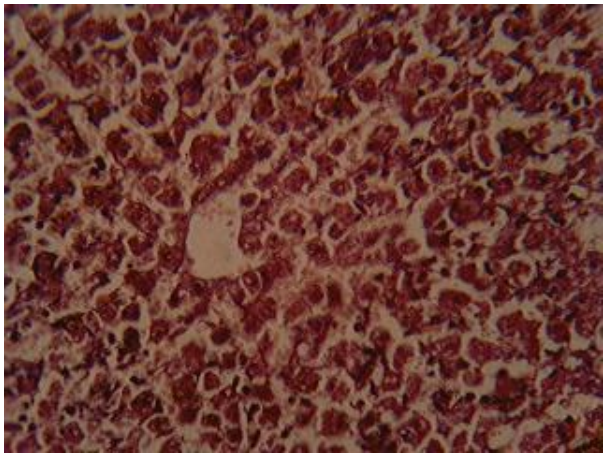

Fig. B: A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H & E-stained liver section of rabbit from CCl_4 control group (Normal saline + CCl_4). In liver section, 3-4 hepatocytes rows around central vein demonstrated; hepatocytes degeneration and necrosis, degeneration cells, endothelial lining of central vein showed more cell damage increased fat vacuoles in hepatocytes than level +2, focal necrosis and Bile duct proliferation.

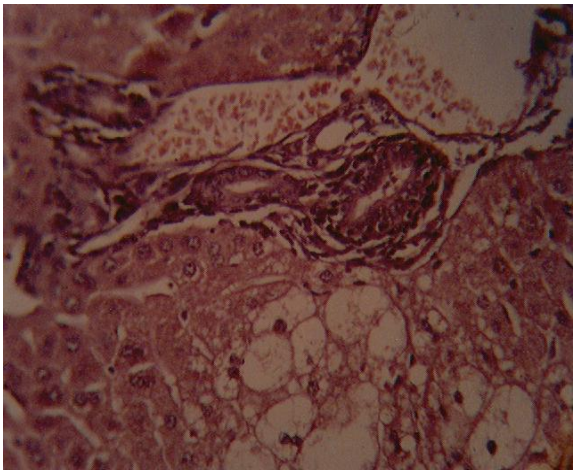

Fig. C: A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from Silymarin control group (100mg/kg + CCl_4). In liver section, 1-2 hepatocytes rows around central vein showed; hepatic cell degeneration along with necrosis (loss of nucleus), less injury of endothelial cells around central vein and less fat vacuoles in hepatocytes

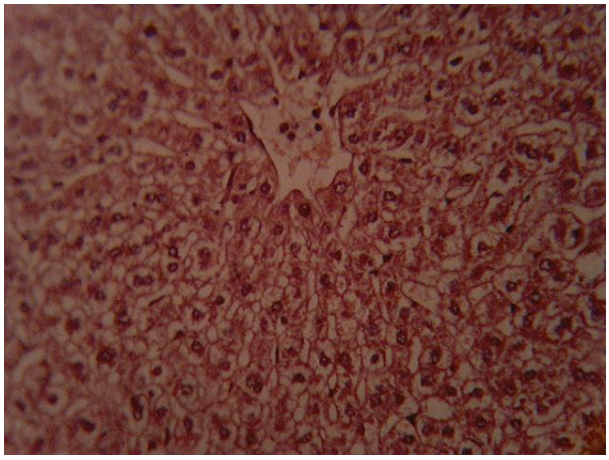

Fig. D: A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from Test group 1 (*Haloxylon* 500mg/kg + CCl_4). In liver section, 1-2 hepatocytes rows around central vein showed; hepatic cell degeneration along with necrosis (loss of nucleus), less injury of endothelial cells around central vein and less fat vacuoles in hepatocytes

It is reported that the mice knocked out of *CYP2E1* gene show resistance against $CC1_4$ induced hepatotoxicity and the level of reactive metabolites can be reduced by inhibition of *CYP2E1* gene expression, consequently tissue injury is reduced (Wong *et al*., 1998). In recent years, there has been an active search for the development of CYP_{450} inhibitors from natural products that may have therapeutic potential in prevention of liver damage.

Triterpene acids, oleanolic acid and ursolic acid inhibit CYP_{450} (Kim *et al*., 2004). So, the hepatoprotective action of HS extract may be due to the presence of some of the

above mentioned compounds which causes down regulation of *CYP2E1* gene expression.

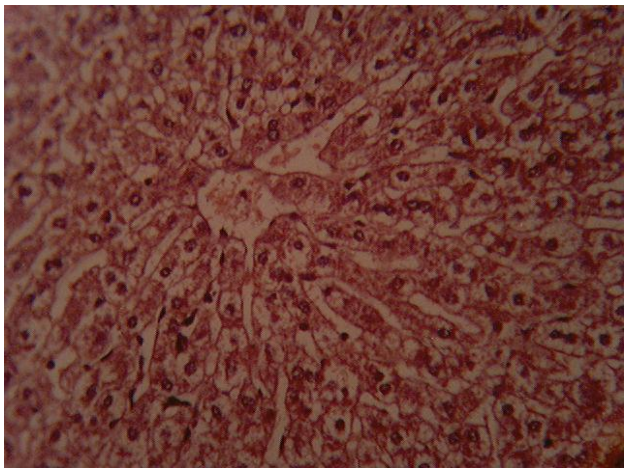

Fig. E: A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H & E-stained liver section of rabbit from Test group 2 (*Haloxylon* 750mg/kg + CCl_4). In liver section, 1-2 hepatocytes rows around central vein showed; hepatic cell degeneration along with necrosis (loss of nucleus), less injury of endothelial cells around central vein and less fat vacuoles in hepatocytes.

The possible hepatoprotective mechanism of HS aerial parts ethanolic extract (500 mg/kg) on CCl_4-induced liver injuries may be through one of the following actions; a) prevention of process of lipid per oxidation b) free radical scavengers and c) down regulation of CYP2E1 gene expression.

The conclusion of present study provides a scientific cause for the conventional use of *Haloxylon salicornicum* in hepatobiliary diseases in eastern system of medicine. An extremely imperative observation is that both doses of HS extract produced a quick decline in serum total bilirubin which suggests that the plant could be very effective in an acute form of jaundice. Further studies should be carried out to determine the therapeutic index and exact mechanism of hepatoprotection offered by the plant.

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