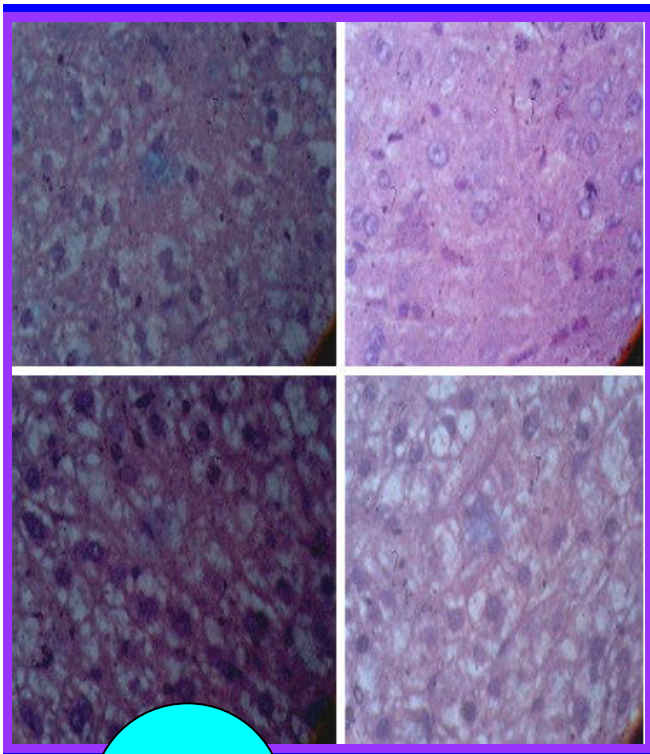

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Hepatoprotective activity of aqueous-methanolic extract of *Suaeda fruticosa* in paracetamol-induced hepatotoxicity in rabbits

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Abstract

In the present study, we evaluated the protective effects of the aqueous-methanolic extract of *Suaeda fruticosa* (500 and 750 mg/kg b.w., p.o) against paracetamol-induced hepatotoxicity in rabbits. Silymarin 100 mg/kg, p.o. was served as standard. The degree of protection was determined by measuring the levels of biochemical marker such as SGOT, SGPT, ALP and bilirubin (total). The results showed that extract of *S. fruticosa* significantly ($p<0.05$) decreased the paracetamol-induced increased levels of liver enzymes and total bilirubin in a dose-dependent manner. Histopathological studies also revealed the hepatoprotective effects of *S. fruticosa*. It is concluded that the aqueous-methanolic extract of *S. fruticosa* possesses hepatoprotective activity.

Introduction

Liver is a vital organ present in vertebrates and other animals which performs detoxification of the exogenous xenobiotic, drugs, viral infection and chronic alcoholism. The liver is involved in wide range of functions including all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Ward et al., 1999). Problems due to liver diseases are worldwide and are associated with significant morbidity and mortality.

Herbal drugs play a vital role in the management of different liver disorders most of which fasten the natural healing processes of the liver. Several medicinal plants and their formulations are used for liver disorders in traditional system of medicine from a prolonged period of time (Subramoniam et al., 1998). Some plants have hepatoprotective activity like *Trianthema decandra* (Balamurugan and Muthusamy, 2008), *Cocculus hirsutus* (Thakare et al., 2009), *Carica papaya* (Sadeque and Begum, 2010), *Carissa spinarum* (Hegde and Joshi, 2010), *Convolvulus arvensis* (Ali et al., 2013), *Dodonaea viscosa* (Khan et al., 2013), *Trichodesma sedgwickianum* (Saboo et al., 2013), *Ofpomoea staphylina* (Bag and Mumtaz, 2013) and Khamira Gaozaban Ambri Jadwar Ood Saleeb Wala (Akhtar et al., 2013).

Suaeda fruticosa Forssk. (Amaranthacea) is one of such plant abundantly grow along coastal salt marshes and salt deserts of Pakistan and used as important medicinal plant. By literature survey it is found that the plant contains high contents of phenols gallic acid, flavonoids and ascorbic acid. *S. fruticosa* has been proven for anti-cancer, anti-oxidant and antimicrobial activities. It anti-cancer activity against human lung carcinoma was reported (Ullah et al., 2012). This plant contains flavonoids hence we planned to determine its hepatoprotective activity. Keeping this in view it was thought that the plant *S. fruticosa* which is abundantly grown and used as medicinal plant may have a protective role in hepatic toxicities induced by paracetamol.

Materials and Methods

Plant collection and identification: The plant was collected from Cholistan Desert (Bahawalpur) and was identified by Dr. Shazia Anjum, Director, Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur. A specimen has been preserved in the Herbarium of the Cholistan Institute of Desert Studies, The Islamia University Bahawalpur (Specimen no. 3469/CIDS/IUB).

Extraction preparation: In the present work, the authenticated shade dried plant *S. fruticosa*, approximately (500 g), was powdered to coarse particle size No. 40. The plant materials were soaked in 70%aqueous methanol for 72 hours with occasionally shaking and stirring in 5 L beaker. The extracts were separated from the residues by filtering 1st through several layers of muslin cloth for coarse filtration and then through Whatman No. 1 filter paper. The residues were extracted twice with the same fresh solvent and extracts combined. The filtered extracts were concentrated and solvents were evaporated under reduced pressure at 40°C, using a rotary evaporator (EYELA, CA-1111, Rikakikai Company Limited, Japan). The dried crude concentrated extracts were weighed to calculate the percentage yield and stored in a refrigerator (-8°C), until used for analyses.

Chemicals: Ethanol, methanol, distilled water, paracetamol, diagnostic kits (TB, SGPT, SGOT and ALP) by Human (Germany), xylene, paraffin wax, sodium chloride, sodium hydroxide, potassium hydroxide, aluminum chloride, benzene, ether, sulfuric acid, hydrochloric acid, potassium iodide, potassium bromide, mercuric chloride, eosin, hematoxylin and Canada balsam. These chemicals were purchased from the Merck, Germany. Silymarin and pentothal sodium were obtained from the Abbott laboratories, Pakistan. All of the above mentioned chemicals were of analytical grade.

Animals: Healthy adult rabbits of (1,000 — 1,200 g) were housed in cages with free access to standard rodents chow diet and water *ad libitum* and acclimatized to the surroundings for one week prior to the experiment. Animals were harbored on a light/dark cycle (12/12 hours) at a constant temperature (25 ± 3°C) and relative humidity (50 ± 20%).

Hepatoprotective activity: In the dose response experiment, rabbits were randomly assigned into 5 groups of 6 individuals each. Group I: Animals (+ve control) were administered 1 mL distilled water p.o., for 7 days. Group II: Animals (-ve control) were administered 1 mL distilled water p.o., for 7 days. Group III: Animals were administered with silymarin 50 mg/kg p.o., for 7 days. Group IV: Animals were administered with 70% methanolic extract 500 mg/kg p.o., for 7 days. Group V: Animals were administered with 70%methanolic extract 750 mg/kg p.o., for 7 days. PCM 2 g was administered orally 30 min after the administration of last dose of each group except Group 1 on 7th day in divided doses of 400 mg/kg at 0, 6, 12, 18 and 24 hours. Animals were observed for 24 hours after the PCM induction. Animals were sacrificed under mild ether anesthesia. Blood samples were collected by heart puncture for evaluating the serum bio-chemical parameters like SGOT, SGPT, ALP, total bilirubin (Huang et al., 2010). The liver samples were separated out, washed with normal saline and preserved it in 10% formalin and proceeded for histopathological studies to evaluate microscopically the details of hepatic architecture in each group (Luna, 1986).

Histopathology: Small pieces of liver tissues were collected and immediately fixed in 10%formalin. These tissues were went through automatic processor for dehydration in ascending grades of alcohol (ethanol) 70, 80, 95% and absolute alcohol for 2 changes each. The tissues were cleared in xylene and embedded in paraffin wax. Serial section of 5-6 microns in thickness were obtained using rotary microtome and stained with hematoxylin and eosin. The stained sections were examined under microscope for the analyzing of any change in the architecture of the liver tissue due to paracetamol challenge and improved liver architecture due to pretreatment with test extracts and standard drug (Luna, 1986).

Statistical analysis: Results were expressed as Mean ± SEM (n = 6). Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by tukey's multiple comparison test by using GraphPad Prism Software. p value less than 0.05 was considered to be statistically significant.

Results

A significant increase ($p<0.05$) in serum SGPT, SGOT, ALP, total bilirubin, levels was observed in animals treated with paracetamol (2 g/kg s.c.) as compared to normal. Pretreatment with *S. fruticosa* (500 and 750 mg/kg p.o.) for 7 days decreases the above parameters significantly ($p<0.05$) as compared to paracetamol-treated group. Silymarin pretreatment produced significant decrease ($p<0.05$) in the above parameter when compared to paracetamol ($p<0.05$) treated group (Table I).

Histopathological examination of liver section of normal rabbits showed normal hepatic cells with cytoplasm and nucleus whereas paracetamol treated group showed various degree of fatty degeneration like ballooning of hepatocytes, infiltration of lymphocytes and the loss of cellular boundaries. Administration of a *S. fruticosa* at higher doses (750 mg/kg, p.o.) significantly normalized these defects in the histological architecture of the liver (Figure 1).

Discussions

In paracetamol-induced hepatotoxicity, reduction of hepatic glutathione is (Gupta et al., 2006; Moron et al., 1979) common; SGPT and SGOT enzymes are present in high concentration in liver. Due to necrosis of hepatic

Table I

Effect of aqueous methanolic extract of *S. fruticosa* on biochemical markers

Groups	Treatment	SGPT IU/L	SGOT IU/L	ALP IU/L	Total bilirubin mg/dL
I	Normal	68.3 ± 1.2	57.6 ± 1.8	195.5 ± 3.5	0.6 ± 0.03
II	Paracetamol	291.0 ± 47.6	263.3 ± 44.3	300.6 ± 6.9	1.2 ± 0.2
III	Sylimarine	181.0 ± 24.0[b]	165.0 ± 22.1[b]	188.5 ± 15.2[b]	0.9 ± 0.1[b]
IV	AMESF 500 mg/kg	213.6 ± 9.2[a]	192.5 ± 6.7[a]	243.1 ± 8.6[b]	0.9 ± 0.1[b]
V	AMESF 750 mg/kg	189.5 ± 8.0[b]	163.0 ± 7.1[b]	188.5 ± 2.9[b]	1.0 ± 0.1[b]

Each value expressed as mean ± SEM (n = 6). Values in the parentheses indicate p values. $p<0.05$; [a]$p<0.01$; [b]$p<0.0001$; compared to paracetamol group. One-way ANOVA followed by Tukey's multiple comparison tests; AMSEF (aqueous methanolic extract of *S. fruticosa*)

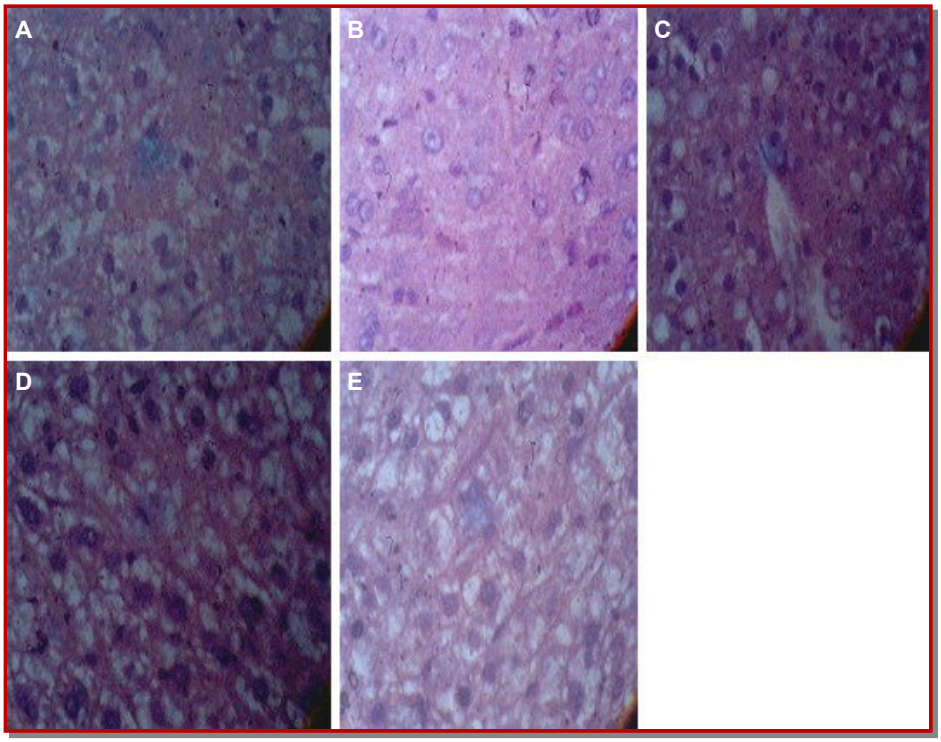

Figure 1: Photograph of architecture of liver in paracetamol-induced hepatotoxicity in rabbits. (A) Liver architecture of normal; (B) Liver architecture of paracetamol-treatment showing inflammation, ballooning degeneration, apoptotic cells, and fibrosis; (C) Liver architecture paracetamol-treatment plus 100 mg/kg silymarin treatment showing limited ballooning degeneration and inflammation; (D) Liver architecture paracetamol treatment plus extract 500 mg/kg showing reduced ballooning degeneration and inflammation; (E) Liver architecture paracetamol treatment plus 750 mg/kg showing limited area of ballooning degeneration and inflammation

cells or abnormal membrane permeability, these enzymes are released from the cells and their levels increases in the blood. ALT is a susceptible indicator of acute liver damage and elevation of this enzyme in the blood in non hepatic diseases is uncommon. Liver function can be assessing by estimating the activities of serum ALT, AST, ALP and bilirubin. These are the enzymes present in higher concentration in cytoplasm. When there is any damage to liver cells, these enzymes leak into the blood stream and give indications the extent of liver damage (Nkosi et al., 2005).

The elevated level of these entire marker enzymes observed in the Group II, paracetamol-treated rabbits, in the present study corresponded to the extensive liver damage induced by toxin. In the present study, pretreatment with aqueous-methanolic extract of *S. fruticosa* (500 and 750 mg/kg) attenuated the increases in the activities of SGOT, SGPT, ALP, Total bilirubin, was found to be lower than the paracetamol treated group indicated that (500 mg/kg and *S. fruticosa* 750 mg/kg) protects the paracetamol-induced hepatic damage.

Silymarin is a well-known hepatoprotective compound isolated from *Silybum marianum* is shown to have a protective effect on plasma membrane of hepatocytes and possess multiple inhibitory effects against different hepatotoxic agents (Bagban et al., 2012). The antioxidant effects and cell regenerating functions as a

consequence of increased protein synthesis were considered as most important actions of silymarin. Anti -oxidant potential of aqueous-methanolic extract of *S. fruticosa* is believed to be due to the presence of flavonoids and tannins that regenerate the hepatocytes and stabilize the plasma membrane of hepatocytes by scavenging free radicals. The study shows that aqueous -methanolic extract of *S. fruticosa* at higher dose (750 mg/kg p.o.) is comparable with silymarin.

The histopathological study of tissues reveals that the phytoconstituents like flavonoids and tannins which are present in the plant extract showed excellent protection to liver architecture almost comparable to the silymarin-treated groups, showing its potential hepatoprotective activity in animal model. Thus, the extract also exhibited the significant hepatoprotective activity in a dose-dependent manner by the reducing the elevated level of biochemical enzyme when they are treated with paracetamol.

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