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Full Length Research Paper

Experimental evaluation of *Echinops echinatus* as an effective hepatoprotective

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Aerial parts of *Echinops echinatus* (Family: Asteraceae) were subjected to *in vivo* hepatoprotective study in order to validate its traditional use in hepatobiliary disorders, by native people of Cholistan Desert, Pakistan. Hepatoprotective effects of pre-treatment with aerial parts (ethanolic extract) of *E. echinatus* (EE), (500 and 750 mg/kg/day, p.o., for 7 days) against CCl_4 (0.75 ml/kg., s/c.) intoxicated rabbits were evaluated 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 raised levels of SGOT, SGPT and ALP significantly but TB level was not raised as compared to normal control group. EE Extract (500 mg/kg) produced more significant results as compared to extract (750 mg/kg). However, (both doses of 500 and 750 mg/kg) showed hepatoprotection as obvious by significant reinstatement of levels of SGOT, SGPT, ALP and even TB as compared to CCl_4 and Silymarin control groups. Histopathological examination of the liver tissue further corroborated these results. Therefore, the outcome of the present study supports the traditional beliefs on hepatoprotective effects of *E. echinatus* (aerial parts).

Key words: *Echinops echinatus*, hepatoprotection, Carbon tetrachloride, serum biochemical parameters, histopathology of liver.

INTRODUCTION

Cholistan Desert is present on the Eastern side of the Punjab province, Pakistan (Baig et al., 1980). The majority of plants grown in desert have therapeutic properties and native people utilize these plants to treat various diseases (Shafi et al., 2001).

Echinops echinatus (Family: Asteraceae) commonly known as “Kanderi Bhattar, ont katara” is an herbaceous plant, widely distributed in desert regions of Pakistan. Its root, leaves, fruit and bark are most commonly used parts (Qureshi, 2004). In folk medicine, its root powder with honey is given as general tonic. Whole plant decoction is very useful in scrofula, dyspepsia and as nervine tonic. Root decoction is given in polyurea. Root powder with milk is used to treat spermatorrhoea (Qureshi, 2004). Herb is used as liver stimulant (Panhwar and Abro, 2007). Native people of Cholistan desert use this plant in hepatobiliary disorders. Many experimental studies on this plant reveal the presence of antibacterial, hypoglycemic, diuretic, antispasmodic and antifungal actions (Somashekar and Mishra, 2007). The herb is also reported to possess vermicidal, anthelmintic, strong molluscicidal (Hymete et al., 2005), anti-inflammatory (Sing et al., 2006), and anti-fertility activities (Padashetty and Mishra, 2007). However, to the best of our knowledge, no previous work has been published on hepatoprotective effectiveness of this plant. Therefore, the present study was aimed to evaluate the hepatoprotective activity of aerial parts of EE against

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CCl_4-induced hepatotoxicity.

MATERIALS AND METHODS

Pharmacological materials

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

Plant material and extraction procedure

E. echinatus (aerial parts) was collected from Cholistan Desert and authenticated by a Taxonomist. Plant material was dried under shade, cut into small pieces and then subjected for grinding. The coarse powder (3000 g) of plant material was macerated in 9 L of ethanol for approximately 15 to 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. These semisolid residues collected from extraction were combined and evaporated to dryness by vacuum at temperature below 60°C. At the end, a dark greenish black solid residue was obtained and approximate yield was 273.1 g. For convenient administration, the dry extract powder was encapsulated after weighing.

Animals

Healthy rabbits of either sex (local breed), weighing from 1.5 to 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, Pakistan. The animals were maintained at standard housing conditions and fed standard pellet diet and water ad libitum. All procedures were performed according to the institutional animal Ethics Committee`s approval.

CCl_4-induced hepatotoxicity and extract treatment

Hepatotoxicity was induced subcutaneously by CCl_4 at a dose of 0.75 ml/kg body weight, suspended in olive oil (1:1). The animals were randomly divided into five groups, containing ten rabbits in each. CCl_4 was injected 30 min 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, III, IV and 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: *Echinops echinatus* extract (500 mg/kg/day)
Group V: *Echinops echinatus* extract (750 mg/kg/day

Twenty-four hours after administration of CCl_4, blood samples (3 ml) 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 (7) rabbits per each group were selected randomly for histopathological examination. Histopathological assessment was done according to the standard method (Humason, 1979). The pathological changes of fatty liver and degeneration of liver cells were graded (Figures 1 to 5) as given below ;

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Grade 0 (Normal): Normal liver morphology; hepatocytes with round nucleus centrally with homogenous cytoplasm, flat endothelial cells around central vein and sinusoid (Figure 1).

Grade +1 (Mild degree): 1-2 hepatocyte rows around central vein was shown; 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 showed 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 was demonstrated; hepatocytic degeneration and necrosis, degeneration of 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 AND DISCUSSION

Administration of CCl_4 (0.75 ml/kg, p.o.) produced a significant increase in serum enzyme levels, namely SGOT, SGPT and ALP. However, TB level was remained unchanged when compared with normal control. The protective action of EE aerial parts extracts on CCl_4 induced hepatotoxicity are summarized in Table 1. Pretreatment with EE extract (500 mg/kg), caused a significant reduction in the levels of SGOT, SGPT, ALP and even TB as compared to CCl_4 control group. EE extract at 500 mg/kg dose produced more significant results as compared to its dose at 750 mg/kg. Moreover, EE extract at both doses (500 mg/kg and 750 mg/kg) was more efficient and protective as compared to Silymarin.

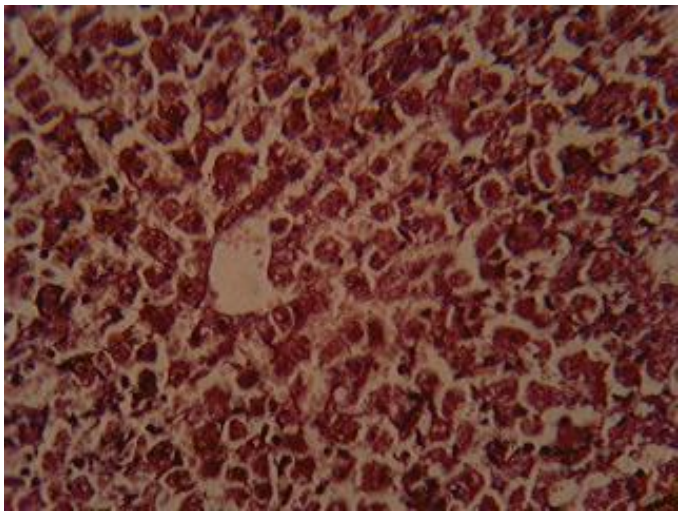

Figure 1. A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from Normal control group (Normal saline). Liver section shows normal liver morphology; Hepatocytes have round nucleus with centrally plus homogenous cytoplasm, flat endothelial cells around central vein and sinusoid.

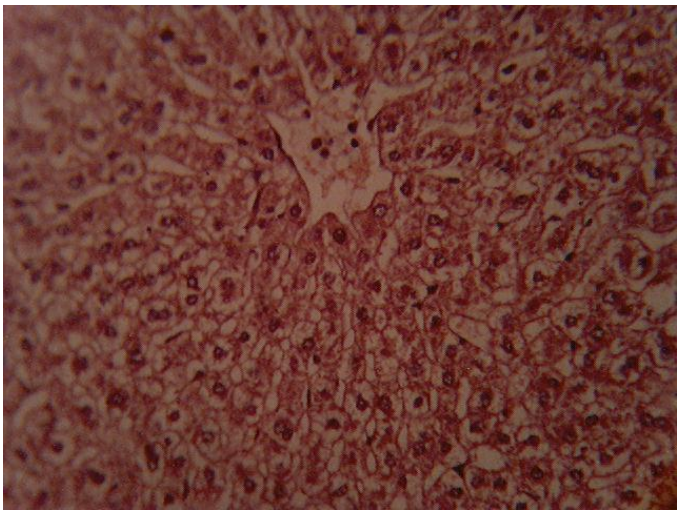

Figure 3. A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from Silymarin control group (100 mg/kg + CCl_4). In liver section, 1-2 hepatocytes rows around central vein showed; hepatic cell degeneration along with necrosis (loss of nucleus), less injury of endothelial cells around central vein and less fat vacuoles in hepatocytes.

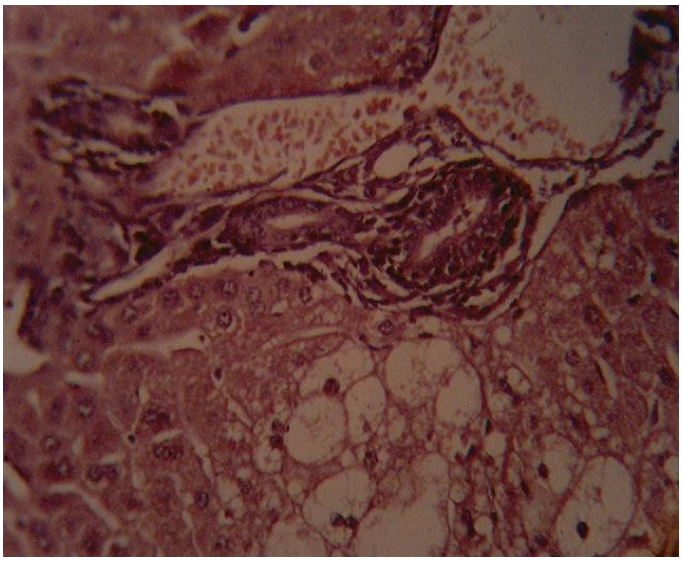

Figure 2. A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from CCl_4 control group (Normal saline + CCl_4). In liver section, 3-4 hepatocytes rows around central vein was demonstrated; hepatocytes degeneration 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.

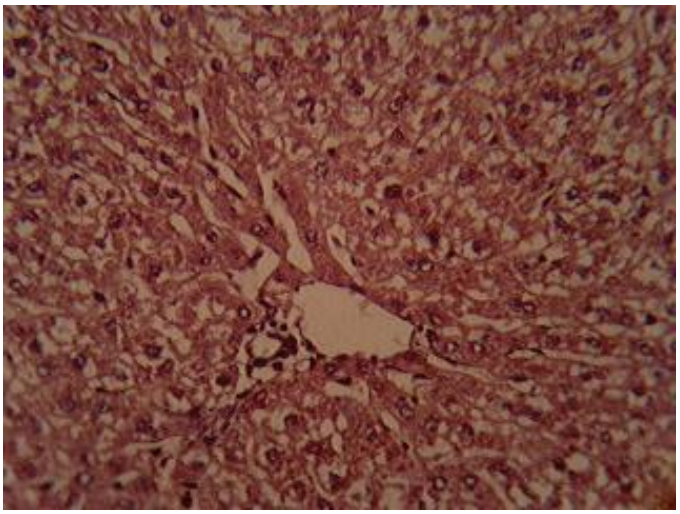

Figure 4. A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from Test group 1 (*Echinops* 500 mg/kg + CCl_4). In liver section, 1-2 hepatocytes rows around central vein showed; hepatic cell degeneration along with necrosis (loss of nucleus), less injury of endothelial cells around central vein and less fat vacuoles in hepatocytes.

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. Administration of both doses of EE extract (500 and 750 mg/kg) significantly preserved the almost normal hepatocellular architecture from damaging effects of CCl_4 as compared to Silymarin (100 mg/kg). The scoring of histological damage is presented in Table 1. CCl_4-induced acute hepatocellular damage is frequently used indicator to date for assessment of hepatoprotective potential of drugs or medicinal flora and their extracts,

Table 1. Effects of ethanolic extract of EE (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 *Echinops echinatus* extract (500 mg/kg + CCl_4)	52.65 ± 25.45°	103.4 ± 22.17°	187.6 ± 42.10°	0.09 ± 0.06*°	+1
Test group 2 *Echinops echinatus* extract (750 mg/kg + CCl_4)	127.8 ± 59.92°	136.8 ± 45.46°	196.7 ± 40.28°	0.12 ± 0.04*°	+1

Values are represented as Mean ± S.E.M. (n=10). 0 = Normal. +1 = Mild. +2 = Moderate. +3 = Severe.* $P < 0.05$ compared with normal control group; ° $P < 0.05$ compared with CCl_4 control group.

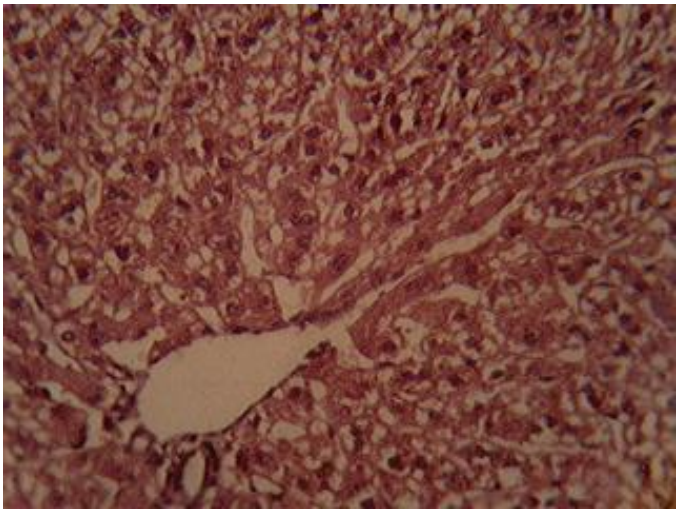

Figure 5. A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from Test group 2 (*Echinops* 750 mg/kg + CCl_4). In liver section, 1-2 hepatocytes rows around central vein showed; hepatic cell degeneration along with necrosis (loss of nucleus), less injury of endothelial cells around central vein and less fat vacuoles in hepatocytes.

both via *in vivo* and *in vitro* techniques (Weber et al., 2003).

The hepatic damage is evident by increase in the level of released cytoplasmic transaminases (SGOT and SGPT), alkaline phosphatases (ALP), in circulation which is an 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 levels of serum total bilirubin (TB) is the most sensitive tool that reflects the severity of jaundice (Sturgill and Lambert, 1997). So, the degree and type of hepatocellular damage is evaluated 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 towards respective normal values, which were raised by a hepatotoxin, is an unambiguous sign of its hepatoprotective effects.

In our study, CCl_4 treated group have highly raised levels of serum enzyme markers (SGOT, SGPT and ALP) along with damaged liver architecture. The EE extract (500 mg/kg) was found to produce more significant hepatoprotection as compared to EE extract (750 mg/kg). However, both doses of EE extract (500 and 750 mg/kg) were more effective, both structurally and functionally than Silymarin (100 mg/kg). This reduction in serum enzymes level by EE 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, EE contains alkaloids, echinopsine, echinopsidine, and echinozolinone etc; many flavonoids like apigenin, echinacin and triterpenoids like taraxasterol, lupeol, tannins, sugars, amino acids, phenols and steroids (Somashekar and Mishra, 2007). The flavonoids are well-reputed for their anti-oxidant, free radical scavengers and anti-lipo-peroxidant actions (Zhou and Zheng, 1991). Saponins inhibit lipid peroxidation by scavenging reactive oxygen species (Tran et al., 2001). Tannins and lignans (Faure et al., 1990) are also well renowned for their hepatoprotective effects. Moreover, alkaloids (Vijayan et al., 2003) and triterpenoids (Xiong et al., 2003) also have hepatoprotective activity. So, it is reasonable to think that the observed protective effects of EE extract might be due to the presence of these polyphenolic compounds (flavonoids, quercitin etc., alkaloids, tannins, saponins and steroid among other plant constituents. Moreover, phenolic compounds amongst many other constituents have been shown to possess hepatoprotective and calcium antagonist activities and the presence of such constituents in extract, may be responsible for some of the pharmacological activities observed in this study.

It is reported that the mice knocked out of *CYP2E1* gene show resistance against CCl_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 EE extract may be due to the presence of some of the above mentioned compounds which cause down regulation of *CYP2E1* gene expression.

To be brief, the possible hepatoprotective mechanism of EE aerial parts ethanolic extract (500 mg/kg) on CCl_4-induced liver injuries may be through one of the following actions:

1. Prevention of process of lipid per oxidation.
2. Free radical scavengers.
3. Down regulation of *CYP2E1* gene expression.

Conclusion

The present study provides scientific root for the conventional use of *E. echinatus* in hepatobiliary diseases in Eastern system of medicine, more significantly at a dose of 500 mg/kg. An extremely imperative observation is that both doses of EE 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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