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PHYTOPHARMACOLOGICAL EVALUATION OF *SOLANUM SURATTENSE BURN*: USED IN FOLK MEDICINES OF CHOLISTAN DESERT PAKISTAN.

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ABSTRACT

Ethanolic extracts of aerial parts of *Solanum surattense* burn. (SS), were subjected to *in vivo* study, by means of serum biochemical parameters and histopathological observations, in order to verify its traditional use in Hepatobiliary disorders. All groups of rabbits received a single dose of CCl_4 subcutaneously on 7th day except group I which was served as normal control . Group II served as CCl_4 control. While groups III, IV and V were served as silymarin control , test group 1 and test group 2 respectively. Results showed that CCl_4 control group had raised levels of SGOT, SGPT and ALP significantly but TB level was not raised as compared to a normal control group. Pre-treatment with SS extract showed hepatoprotection as obvious by significant reinstatement of levels of SGOT, SGPT and ALP, while TB level was not changed significantly when compared with CCl_4 control group. SS extract was more effective as compared to both silymarin and SS extracts because SS extract only significantly reduced SGOT level but unable to restore SGPT, ALP and TB levels. Histopathological examination of the liver tissue was further corroborated these results. Thus the outcome of the present study supports the conventional believes on hepatoprotective effects of *Solanum surattense* .

KEY WORDS*: Solanum surattense*, Hepatoprotection, Carbon tetrachloride, Serum biochemical parameters, Histopathology of liver.

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INTRODUCTION

Cholistan desert is present on the Eastern side of the Punjab province, Pakistan (Baig et al., 1980). It surrounds thirty kilometers from Bahawalpur, Punjab, Pakistan and cover an area of 16,000 km^2 (Chouhan et al., 2002). The majority of plants grows in the desert has therapeutic properties and native people utilize these plants to treat various diseases (Shafi et al., 2001). *Solanum surattense* burm. (Family: Solanaceae) is one of those plants and is commonly known as “kanderi/kanderi wal”. It is a herbecious plant. The whole plant is used for medicinal purposes. In folk medicine, Decoction of its root is used to treat phlegmatic cough and fever. Whole plant decoction is very effective in skin diseases. Decoction of its fruit is given in bronchial asthma. Leaves juice with few seed of black pepper is a very useful remedy in joint pain. Whole plant decoction is given in case of jaundice (Qureshi, 2004). In some places, whole plant and fruit are used for food purposes (Nohara et al., 2007). Native people of Cholistan desert use this plant in hepatobiliary disorders. Experimental studies shows that SS possess antifungal activity against *A. fumigates*, *A. flavus, A. niger* and *C. albicans*. It also shows significant anti-microbial activity especially against *S. typhi, E. coli, P. aeruginosa, S. aureus* (Dabur et al., 2007). SS shows significant anti-inflammatory and anti asthmatic activities because of its steroidal constituents (Maiti et al., 1979). It is also known to possess Strong anti-proliferative activity against many cancer cells, anti-herpes and hypoglycemic activities (Nohara et al., 2007). However**,** to the best of our knowledge, no previous work has been published on hepatoprotective effects of this plant. Therefore, the present study was aimed to evaluate the hepatoprotective activity of aerial parts of SS against CCl_4-induced hepatotoxicity.

MATERIALS AND METHODS

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. *Solanum surattense* (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 to grinding. 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. The 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 a temperature below 60 °C. At the end a dark brownish green solid residue was obtained and approximate yield was 310.2gms. For convenient administration, the dry extract powder was encapsulated after weighing.Healthy rabbits of either sex (local breed), weighing from 1.5-2 kg was purchased from local markets. 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 standard pellet diet and water ad libitum. All procedures were performed according to the institutional animal Ethics Committee`s approval.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 minutes after drug administration, on the 7^{th} day of the 8 days study period to all the groups except group I which was served as normal control and received only normal saline. Group II-V received the following treatments from 1^{st} to 7^{th} 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 *Solanum surattense* extract (500 mg/kg/day)
Group V *Solanum surattense* 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. 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). 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 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 the central vein more damage than level +1 more fat vacuoles in hepatocytes than level +1.
- Grade +3 (Severe degree): 3-4 hepatocyte rows around the 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. The results were presented as Mean ± Standard error of the 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, s.c.) 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 SS aerial parts extracts on CCl_4 induced hepatotoxicity are summarized in Table 1. Pretreatment with SS extract (500 mg/kg), caused a significant reduction in the levels of SGOT, SGPT and ALP while TB level remained unchanged, as compared to CCl_4 control group. Pretreatment with SS extract (750 mg/kg), produced less significant results than SS extract (500 mg/kg) and decrease only SGOT level with no restorative effect on SGPT and ALP levels as compared to CCl_4 control. SS extract (500 mg/kg) was also significantly more effective than Silymarin.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 the SS extract (500 mg/kg) significantly preserved the almost normal hepatocellular architecture from the damaging effects of CCl_4 as compared to both Silymarin (100 mg/kg) and SS extract (750 mg/kg). The scoring of histological damage is

presented in Table 1. duced acute hepatocellular damage is frequently used indicator to date for the assessment

$CC1_4$-induced acute hepatocellular damage is frequently used indicator to date for the assessment of the hepatoprotective potential of drugs or medicinal flora and their extracts, both via *in vivo* and *in vitro* techniques (Weber et al., 2003). The hepatic damage in 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 are 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 of 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 has highly raised levels of serum enzyme markers (SGOT, SGPT and ALP) along with damaged liver architecture. The SS extract (500 mg/kg) was found to be produced more significant hepatoprotection, both structurally and functionally as compared to both Silymarin (100 mg/kg) and SS extract (750 mg/kg). 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 during damage with CCl_4. According to phytochemical analysis, SS Berries contain solanocarpine, solanocarpidine (Qureshi, 2004). Berries and leaves contain solasodine (Maiti et al., 1979). Plant also contains glycosides such as spirosolane, spirostane, furostane, diosgenin, pregnane (Nohara et al., 2007), and β-solanargine (Jawahar et al., 2004). In addition, steroidal glycoside indioside D, triterpenoid glycosides, alkaloids, iridoids, flavonoids, lignans and quinines are also reported (Haribal et al., 2006). Vitamin C, α- and β-carotenes are present in *Solanum nigrum* (Sultana et al., 1995). So there is also a possibility that Vitamin C, α- and β-carotenes may also be present in other species of *Solanum*. The observed protective effects of SS ethanolic extract against CCl_4 induced liver damage might be due to the presence of these polyphenolic compounds (flavonoids, quercitin etc…), carotenoids, lignans, quinines, Vitamin C and steroid glycosides among other plant constituents. Phenolic compounds amongst many other constituents have been shown to possess hepatoprotective and calcium antagonist activities (Khalid et al., 2002) and the presence of such constituents in the extract, may be responsible for some of the pharmacological activities observed in this study. Another possibility is that SS may prevent lipid peroxidation by metal chelation and increased electron trapping capability probably due to the concurrent occurrence of both quinine skeleton and phenolic groups (Shiang et al., 1995). 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 SS extract may be due to the presence of some of the above mentioned compounds which cause down regulation of *CYP2E1* gene expression but it must be confirmed after a detail phytochemical analysis of the plant.To be brief, the possible hepatoprotective mechanism ot SS 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.

In conclusion, our present study offers scientific basis for the traditional use of *Solanum surattense* in hepatobiliary diseases in Eastern system of medicine, particularly at a dose of 500 mg/kg. However, higher concentrations are less efective. It is suggested that further studies should be carried out to determine the therapeutic index and exact mechanism of hepatoprotection offered by the plant.

Table 1
Effects of ethanolic extract of SS (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 *Solanum surattense* extract (500 mg/kg + CCl_4)	82.40 ± 26.85°	82.38 ± 20.10°	120.0 ± 38.06*°	1.28 ± 0.17	0
Test group 2 *Solanum surattense* extract (750 mg/kg + CCl_4)	166.8 ± 27.66*°	333.2 ± 36.24*	348.3 ± 48.06	1.54 ± 0.04*	+2

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.***

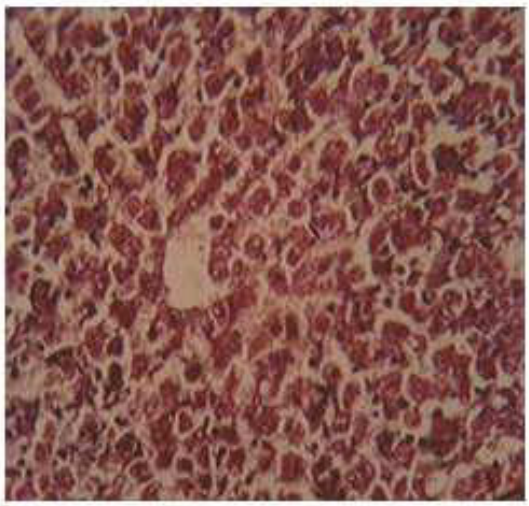

Figure A

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.

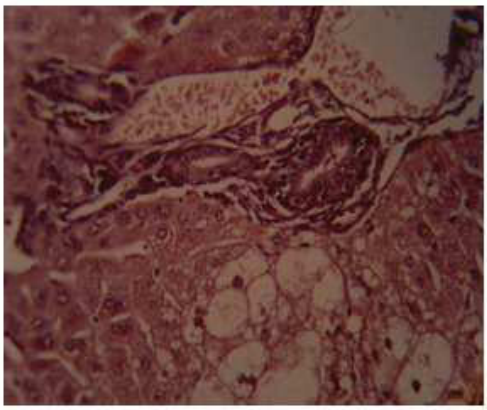

Figure B

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.

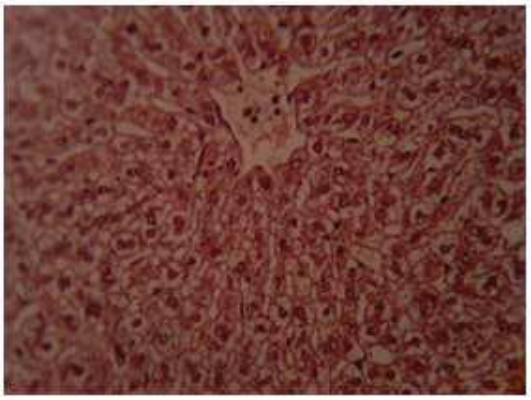

Figure C

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

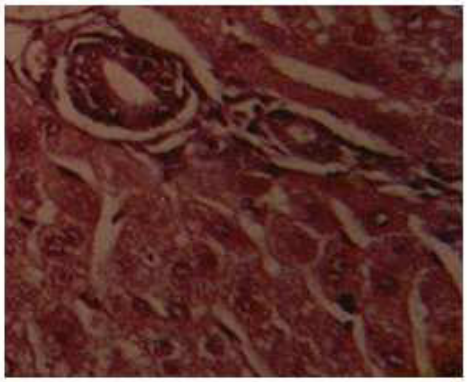

Figure D

A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from Test group 1 (Solanum 500mg/kg + CCl_4).Liver section shows normal liver morphology; Hepatocytes have round nucleus with centrally plus homogenous cytoplasm, flat endothelial cells around central vein and sinusoid.

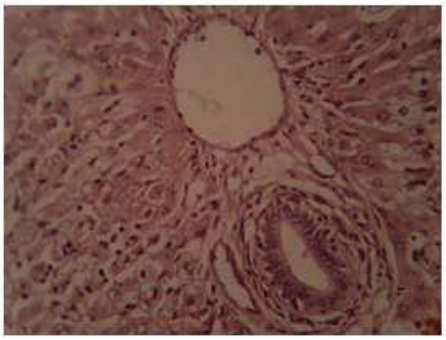

Figure E

A microphotograph of histopathological examination of randomly selected, formalin fixed, paraffin embedded, H &E-stained liver section of rabbit from Test group 2 (Solanum 750mg/kg + CCl_4). In liver section, some hepatocytes 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, more fat vacuoles in hepatocytes than level +1.

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