

# Hepatoprotective evaluation of aqueous-ethanolic extract of *Capparis decidua* (Stems) in paracetamol induced hepatotoxicity in experimental rabbits

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**Abstract:** Liver diseases are the most common ailment all over the world mostly caused by viruses, toxic chemicals, excessive intake of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents and peroxides oil, etc. There is a great need to assess the scientific basis for the medicinal plants that are claimed to have hepatoprotective activity. We investigate the hepatoprotective effects of aqueous-ethanolic extract of *Capparis decidua* (Stems) against paracetamol induced liver injury in experimental animals. To observe the level of improvement, biochemical parameters such as SGPT, SGOT, ALP and Total bilirubin levels as well as histopathological changes in liver tissues were studied. Silymarin (50mg/kg, p.o.) was used as reference drug. The levels of the biochemical parameters were increased in rabbits which were intoxicated by paracetamol. *Capparis decidua* extract (750mg/kg, b.w.) treated rabbits showed maximum reduction of biochemical parameters in a significant ( $p < 0.001$ ) manner. Histopathological examination of the liver tissues of control and treated groups also confirmed the hepatoprotective activity. The phytochemical screening of the extracts revealed the presence of tannins, alkaloids, saponins and flavonoids. The results of the present study therefore suggest that the different doses of *Capparis decidua* possess significant hepatoprotective effect and this effect might be due to the presence of flavonoids and tannins.

**Keywords:** *Capparis decidua*, hepatoprotective activity, paracetamol, silymarin, histopathology.

## INTRODUCTION

Liver diseases are the most serious complaint of the world, mostly caused by viruses, excessive intake of alcohol, high doses of paracetamol, carbon tetrachloride and chemotherapeutic agents etc. In spite of the remarkable advances made in modern medicines, no effective hepatoprotective medicine is available until now (Adewusi *et al.*, 2010). Unfortunately, the available synthetic drugs used in liver diseases are not enough and sometimes can exert serious adverse effects (Kanauija *et al.*, 2011). In view of undesirable side effects of synthetic drugs, attempt has been made to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. Several medicinal plants have been reported previously possessing hepatoprotective effects such as *Suaeda fruticosa* roots (Rehman *et al.*, 2013), *Zingiber officinale* roots (Jamil *et al.*, 2013) *Juniperus phoenicea* aerial parts (Ibrahim *et al.*, 2013) and leaves of *Cleome viscosa* (Gupta and Dixit 2009).

In the present study, we evaluated the hepatoprotective effects of *Capparis decidua* (Stem) against paracetamol induced hepatotoxicity in rabbits. From the traditional knowledge it was evident that the stems of *Capparis*

*decidua* possess hepatoprotective potential. But still no scientific investigations have been reported in the literature regarding its hepatoprotective activity.

*Capparis decidua* (Family: Capparidaceae) is a branching shrub of the Thar Desert. It is also found in the subtropical, tropical and arid regions in southern Asia. In the traditional system of medicine, its bark is used in coughs, asthma and inflammation. Root has antipyretic activity. Its leaves are appetizer and used in cardiac trouble, vomiting, piles, ulcers, cough, asthma, swelling, boils, urinary disorders, antidote to poison, inflammation of joints and cardiac diseases (Arshad *et al.*, 2003). The plant is found to contain the number of alkaloids, terpenoids, glycosides and some fatty acids.  $\beta$ -Sitosterol is isolated from the root. The stem contains two alkaloids n-triacontanol 2-carboxy-1, 1 demethylpyrrolidine (stachydrine). Six oxygenated heterocyclic constituents like Capparis-esterpenolide (3-carboxy-6, 17-dihydroxy-7, 11, 15, 19-tetramethyleicos-13-ene-d-lactone) and deciduaterpenolides (d-lactone derivatives of 1, 3, 3-trimethyl, 1, 4-cyclohexadien-6-one) A, B, C, D and E from root bark. The root bark also contains alkaloids 14-N-acetyl isocodonocarpine, 15-N-acetyl capparisine, Cadabicine, Stachydrine, Rutin, capparisine and codonocarpine (Ratheel *et al.*, 2010).

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## MATERIALS AND METHODS

### **Plant collection and authentication**

The plants used in the study were collected from Cholistan Desert of Bahawalpur and authenticated by Dr. Shazia Anjum, Director, Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur. A specimen has been saved in the Herbarium of the Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur (Specimen no. 3469/CIDS/IUB).

### **Preparation of extracts**

The authenticated shade dried plant, *Capparis decidua* approximately (500g) was coarsely powdered. The plant materials were soaked in 70% aqueous ethanol for 72h with occasionally shaking and stirring in 5 liter glass beaker. The extracts were filtered 1<sup>st</sup> 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 Tokyo, Japan). The dried crude concentrated extracts were weighed to calculate the percentage yield and stored in a refrigerator (-8°C), until used for analysis.

### **Chemicals**

Ethanol, Distilled water, Paracetamol, Diagnostic kits (TB, SGPT, SGOT and ALP) by Human (Germany), Potassium iodide, Potassium bromide, Mercuric chloride, Eosin, Hematoxylin, Xylene, Paraffin wax, Sodium chloride, Sodium hydroxide, Potassium hydroxide, Aluminum chloride, Benzene, Ether, Sulphuric acid, Hydrochloric acid, and Canada balsam. These chemicals were purchased from the Merck, Germany. Silymarin and Pentothal sodium was obtained from the Abbott laboratories, Pakistan. All of the above mentioned chemicals were of analytical grade.

### **Animals**

Healthy adult rabbits of (1000gm-1200gm) were obtained from laboratory animal center, The Islamia University of Bahawalpur and were maintained in standard housing conditions. The animals were on standard rodents chow diet and water *ad libitum*. Animals were harbored on a light/dark cycle (12/12hr) at a constant temperature (25°C  $\pm$  3°C) and relative humidity (50 $\pm$ 20%) and were acclimatized for one week prior to the experiment.

### **Preliminary phytochemical screening**

The preliminary phytochemical screening was done by standard qualitative chemical methods (Evans *et al.*, 2009). The extract of *Capparis decidua* was screened for the presence of alkaloids, anthraquinones saponins, tannins and flavonoids.

### **Test for alkaloids**

Plant extract (500 mg) was dissolved in 8ml of 1% HCl, heated on water bath and then filtered. Dragendroff's reagent and Mayer's reagent were used separately to detect the presence of alkaloids in 2ml of the filtrate. Presence of any turbidity or precipitate formation shows the incidence of alkaloids

### **Test for tannins**

Plant extract (500 mg) was dissolved in 20 ml of water in a test tube, and then it was warmed and filtered. A few drops of 0.1% ferric chloride were added in it and check the appearance of blue-black or brownish green coloration.

### **Test for saponins**

Plant extract (500mg) was dissolved in a test tube containing boiling water. Then it was allow to cold and test tube was shaken vigorously which produces the froth on shaking (Tona *et al.*, 1998). Appearance of frothing indicates the presence of Saponins.

### **Test for flavonoids**

5 ml of dilute ammonia solution was added to a portion of each plant extract then concentrated H<sub>2</sub>SO<sub>4</sub> was added into it along the walls of the test tube. Presence of yellow coloration was observed in each extract which indicates the presence of flavonoids. This yellow coloration disappears on standing.

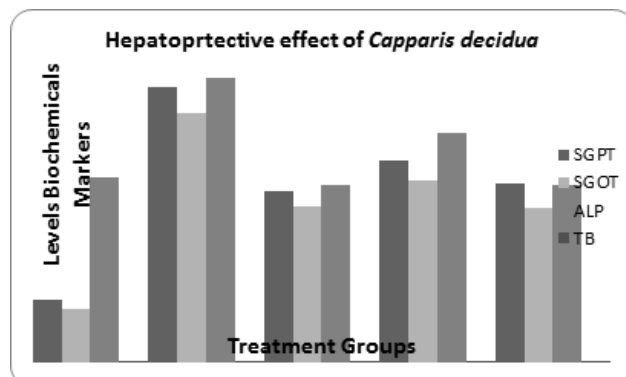
### **Test for anthraquinones**

Plant extract (1.0gm) was boiled in 6 ml of 1% HCl and then filtered. Then 5ml of benzene was added in the filtrate and it was shaken. After shaking remove the benzene layer, and then added 10% ammonia solution into it. Appearance of red color or pink/violet color indicates that anthraquinones are present.

### **Hepatoprotective activity**

The animals were divided into 5 groups of 6 individuals each. Group-I: Animals (+ve control) were administered 1ml distill water p.o., for 7 days. Group-II: Animals (-ve control) were administered Paracetamol 2gm/kg/body weight on day 7. Group-III: Animals were administered with silymarin 50mg/ kg p.o., for 7 days. Group-IV: Animals were administered with aqueous-ethanolic extract 500mg/kg p.o., for 7 days. Group-V: Animals were administered with aqueous-ethanolic extract 750 mg/kg p.o., for 7 days. Paracetamol 2gram was administered orally 30 mints after the administration of last dose of each group except group 1 on 7<sup>th</sup> day in divided doses of 400mg /kg at 0hr, 6hr, 12hr, 18hr and 24hrs. Animals were observed for 24 hours after the Paracetamol induction. Animals were sacrificed under mild ether anesthesia. Blood samples were collected by heart puncture for evaluating the serum biochemical parameters like SGOT, SGPT, ALP, and Total Bilirubin. Parameters

were evaluated by using the diagnostic kits of Human, Germany. Results were obtained by operating the individual samples, standard and reagent blanks on Merck Microlab 300 (Merck Germany). For the evaluation of these parameters i.e. TB, ALP, SGPT and SGOT wide procedures enclosed in kits were adopted (Huang *et al.*, 2010). The liver were separated out, washed with normal saline and preserved in 10% formalin and preceded for histopathological studies (Luna *et al.*, 1986).



**Fig. 1:** Effect of aqueous-ethanolic extract of *Capparis decidua* on serum enzyme levels

### Histopathology

Small pieces of liver tissues were cut and fixed in 10% formalin. These liver 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 made using rotary microtome and stained with hematoxylin and eosin. The stained sections were observed under microscope to see any change in the architecture of the hepatic tissue due to paracetamol toxicity and improved liver architecture due to pretreatment with *C. decidua* extracts and standard drug silymarin (Luna, 1986).

### STATISTICAL ANALYSIS

All results are expressed as Mean $\pm$ SEM, (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by turkey's multiple comparison test by using Graph Pad Prism Software.  $P < 0.05$  was considered to be statistically significant.

### RESULTS

#### Phytochemical screening

The phytochemical analysis of the extract showed the presence of flavonoids, alkaloids, tannins and saponins (table 1).

#### Hepatoprotective activity

The level of serum SGPT, SGOT, ALP, Total bilirubin were significantly increase in animal treated with

paracetamol (2gm/kg s.c) compared to normal. Pretreatment with *C. decidua* (750mg/kg p.o.) for 7 days decreases the biochemical parameters significantly ( $p < 0.05$ ) as compared to paracetamol treated animals. The standard drug silymarin give significant decrease in the above parameter when compared to paracetamol ( $p < 0.05$ ) treated group (table 2).

### Histopathological studies

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

### DISCUSSION

*C. decidua* is the traditional medicinal plant of Bahawalpur Cholistan desert which has been reported to have hepatoprotective effect. Stems of *C. decidua* showed hepatoprotective effect in rats against  $\text{CCl}_4$ -induced hepatotoxicity when it's aqueous and methanolic extracts were given orally (Ali *et al.*, 2009). We designed our research study to evaluate hepatoprotective potential by using the aqueous-ethanolic extracts of *C. decidua* stems in paracetamol intoxicated rabbits.

Paracetamol is an antipyretic and analgesic agent, and it is safe in therapeutic doses, but can produce lethal hepatic necrosis in man and experimental animals like mice, rabbits and rats with toxic doses. It is used as hepatotoxic agent in studies conducting on animals (Gujrati *et al.*, 2007). The hepatic cytochrome P450 enzyme system metabolizes paracetamol, forming a metabolite known as NAPQI. NAPQI is irreversibly conjugated with the sulfhydryl groups of glutathione (Mayuren *et al.*, 2010). NAPQI initiates covalent binding to cellular proteins. This disrupts the calcium homeostasis, mitochondrial dysfunction, and oxidative stress and may eventually lead to cellular damage and death.

So in the present study, paracetamol was used as toxic agent and the protective effect of *C. decidua* stem against the paracetamol induced hepatotoxicity was studied. The extent of toxicity was determined by histopathological studies and biochemical parameters like SGOT, SGPT, ALP and TB levels. These are present in hepatic cells. When there is injury to hepatic cells, these enzymes leak into the blood stream in conformity with the extent of liver damage (Nkosi *et al.*, 2005). The elevated level of these biochemical parameters was observed in the group II, Paracetamol treated rabbits in this study corresponded to the extensive liver damage induced by toxin.

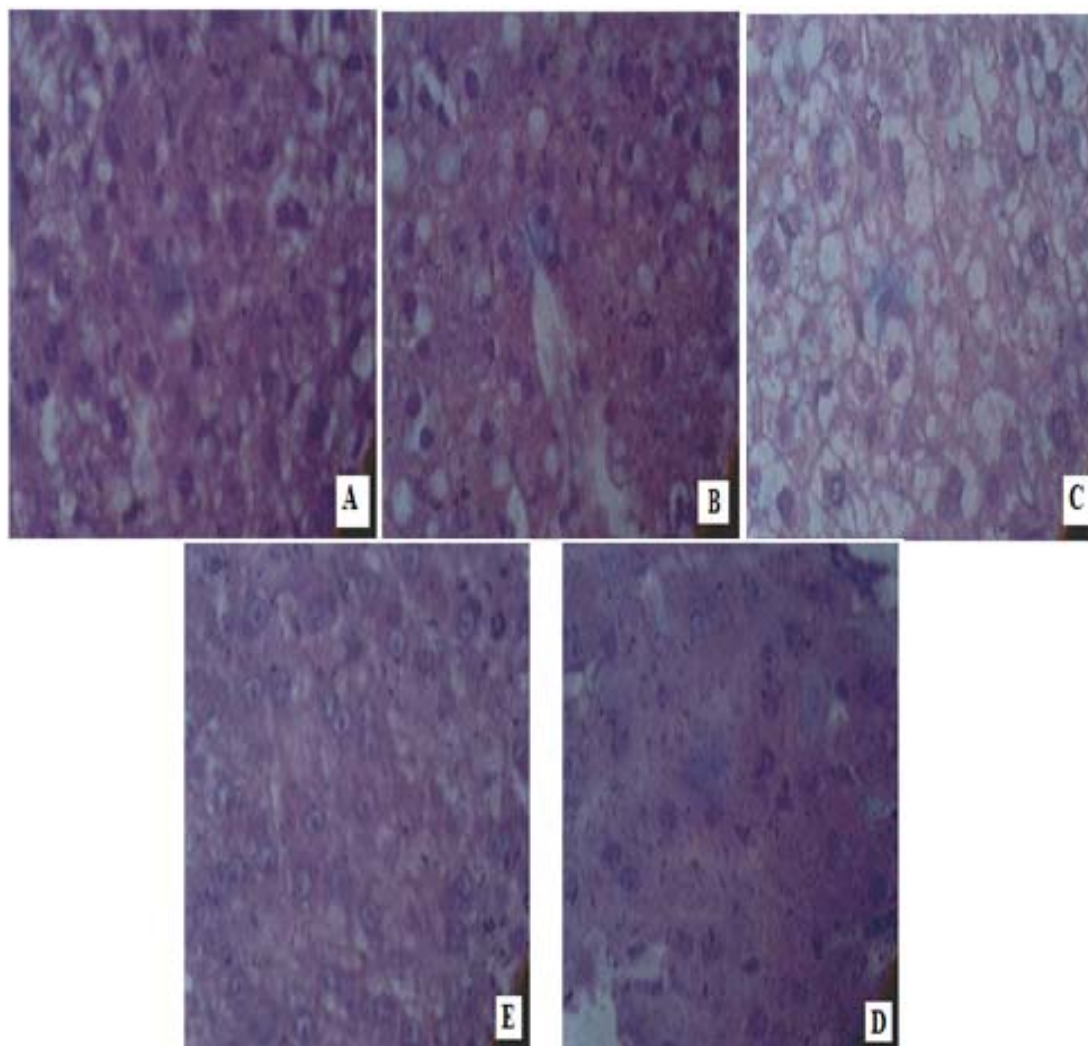
**Table 1:** Phytochemical constituents of *Capparis deciduas*

| Sr. No. | Phytochemical constituents | Results |
|---------|----------------------------|---------|
| 1       | Alkaloids                  | Present |
| 2       | Tannins                    | Present |
| 3       | Saponins                   | Present |
| 4       | Flavonoids                 | Present |
| 5       | Anthraquinones             | Absent  |

**Table 2:** Effect of aqueous-ethanolic extract of *Capparis decidua* on serum enzyme levels

| Groups    | Treatments        | SGPT (IU/L)  | SGOT (IU/L) | ALP (IU/L)    | Total Bilirubin (mg/dl) |
|-----------|-------------------|--------------|-------------|---------------|-------------------------|
| Group I   | Normal            | 68.3±1.22    | 57.6±1.82   | 195.5±3.54    | 0.63±0.03               |
| Group II  | Paracetamol       | 291±47.56    | 263.3±44.3  | 300.6±6.87    | 1.17±0.15               |
| Group III | Silymarine        | 181.0±24.0** | 165±22.05** | 188.5±15.22** | 0.89±0.13**             |
| Group IV  | CDE dose 500mg/kg | 213.6±9.17*  | 192.5±6.72* | 243.1±8.61**  | 0.93±0.07**             |
| Group V   | CDE dose 750mg/kg | 189.5±7.98** | 163±7.11**  | 188.5±2.91**  | 0.95±0.07**             |

All values are expressed as Mean ± SEM, (n=6). \* $P < 0.01$ ; \*\* $P < 0.001$ . Where CDE denotes *Capparis decidua* extract



**Fig. 2:** Photograph of architecture of liver in Paracetamol induced hepatotoxicity in rabbits, Fig. A (Liver architecture of Normal), Fig. B (Liver architecture of Paracetamol treatment + 50mg/kg Silymarin treatment), Fig. C (Liver architecture of Paracetamol treatment), Fig. D (Liver architecture of Paracetamol treatment + 500 mg/kg of *C. decidua*), Fig. E (Liver architecture of paracetamol treatment + 750 mg/kg of *C. decidua*).

SGOT, SGPT, ALP, Total bilirubin was found to be lower in *C. desidia* (extract dose 750mg/kg) treated group, indicating that extract protects the paracetamol induced hepatic damage. Silymarin is a flavonoids isolated from *Silybum marianum* is a well-known hepatoprotective compound. It has protective effect on plasma membrane of hepatic cells and shown to possess number of inhibitory effects against different hepatotoxins. Silymarin has antioxidant effects and cell regenerating functions. Antioxidant effect of *C. desidia* is believed to be due to the presence of flavonoids and tannins that regenerate the hepatic cells and stabilize the plasma membrane of these cells. The histopathological study of tissues reveals that the plant extract showed significant protection to liver architecture almost comparable to the Silymarin treated groups, showing its potential hepatoprotective activity in experimental animal. Therefore, the study shows that *C. desidia* at a dose of (750 mg/kg p.o.) possess hepatoprotective activity and the effect was almost comparable to standard drug Silymarin. This effect was might be due to presence of flavonoids and tannin. Further research is required to explore phytochemical constituent responsible for the hepatoprotective response and its mechanism of action. From the above preliminary study, we conclude that aqueous-ethanolic extract of *C. desidia* is proved to be one of the herbal remedies for liver diseases.

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