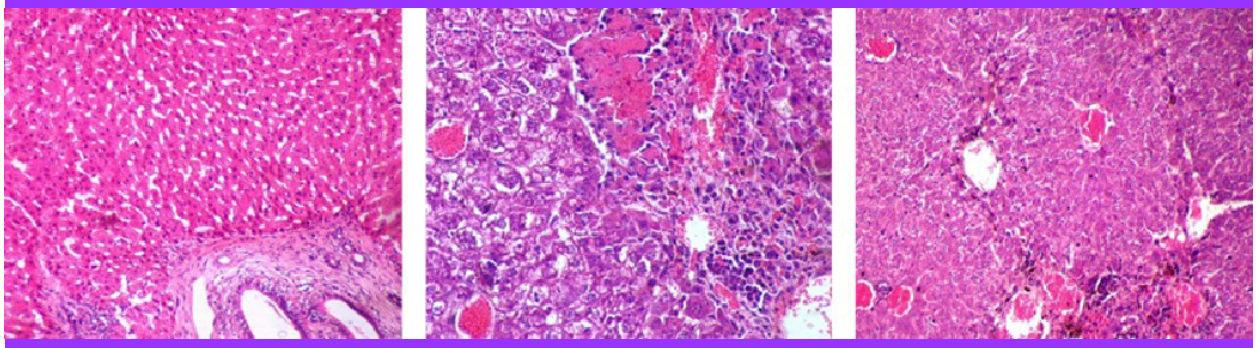

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Hepatoprotective effects of *Sapium sebiferum* in paracetamol-induced liver injury

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Abstract

Sapium sebiferum leaves were used to determine its hepatoprotective effects against paracetamol-induced hepatotoxicity in mice. A dose dependent study was conducted using two different doses (200 mg/kg and 400 mg/kg) of the extract of *S. sebiferum* against toxic effects of paracetamol (500 mg/kg) in experimental animal model. Silymarin (50 mg/kg) was used as standard drug to compare therapeutic effects of *S. sebiferum* with control and paracetamol-treated groups. Paracetamol significantly increased the serum levels of liver enzyme markers like alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, and direct bilirubin. The extract showed protective effects by normalizing the liver enzymes markers in a dose dependent manner. Histopathological results confirmed the hepatoprotective effects of leaves of *S. sebiferum*. We conclude that leaves of *S. sebiferum* have strong hepatoprotective effects against paracetamol-induced liver injury and can be used in liver injuries caused by drug-induced toxicity.

Introduction

Drug-induced hepatotoxicity is considered as a global health concern and may occur as a result of drug metabolism. Because of the lack of effective treatment options, liver diseases have extremely poor prognosis and high mortality. Though various advancements have been achieved in the field of modern medicine, drug-liver diseases still remain a major health issue. One of the most typical examples of dose-related toxicity is that of paracetamol (James et al., 2003). Therapeutic doses of paracetamol have a safety profile with no known adverse effects. Chronic hepatitis, cirrhosis and fatty liver are the liver diseases for which treatment is complicated (Rehman et al., 2014). Plant-based therapeutic strategies for the treatment of different diseases are gaining more attention owing to the presence of risk-free phytochemical constituents (Akash et al., 2011; Akash et al., 2014a,b; Akash et al., 2015; Parveen et al., 2014; Ali et al., 2014; Ibrahim et al., 2013; Akash et al., 2015; Rehman et al., 2012). Several studies have confirmed the hepatoprotective effectiveness of several plant extracts (Sadeque et al., 2010; Hegde et al., 2010; Hussain et al., 2014a,b) to cure liver diseases and unveiled their mechanisms of hepatoprotective activity.

Sapium sebiferum belongs to the family *Euphorbiaceae*. Traditionally, the leaves of *S. sebiferum* are used for a number of ailments like ascites, eczema, shingles and snakebite (Fu et al., 2013). It exhibits anti-inflammatory and antioxidant activity (Fu et al., 2013), antibacterial and anti-candidal activity (Kumar et al., 2011), anti-hypertensive activity (Hsu et al., 1994) and diuretic activity (Kee et al., 1999).

The purpose of present study was to evaluate the hepatoprotective effects of leaves of *S. sebiferum* against paracetamol-induced liver toxicity. We used methanolic extract of *S. sebiferum* leaves to determine the protective effects on liver enzyme markers and confirmed the hepatoprotective effects by histopathological examination of liver tissues of treated Swiss albino mice.

Materials and Methods

Collection and authentication of plant

Leaves of S. sebiferum were collected from the botanical garden of Government College University Lahore, Pakistan in July 2012. The plant was verified and given the voucher number (GC Herb. Bot. 2289) at Government College University Lahore, Pakistan and plant was kept in the college herbarium for future reference. The leaves were washed, dried and grinded by mechanical pulverizer to form coarse powder.

Preparation of plant extract

The powdered leaves (1 kg) was soaked in aqueous methanol (30:70), kept at room temperature for seven days with constant stirring. After soaking for seven days, the extracts were separated by filtration and concentrated under reduced pressure using rotary evaporator at 70°C. The crude extract was stored in air tight container. The extract was suspended in normal saline to prepare two different doses (200 mg/Kg and 400 mg/kg) of plant.

Pharmacological studies

Experimental animals

Swiss albino mice of either sex, weighing between 20-30 g were used for this study, procured from animal house of University of Agriculture Faisalabad, Pakistan. These animals were kept in the animal housed of Government College University Faisalabad, Faisalabad, Pakistan for research purpose. After randomization into various groups, the animals were house for the period of 7 days under standard husbandry conditions at room temperature. The animals were fed under strict hygienic conditions with rodent pellet diet and water *ad libitum*. The experimental studies were performed in accordance with the guidelines of Ethical committee of Government College University Faisalabad and were approved by Advanced Studies and Research Board (ASRB).

Hepatoprotective activity

The experimental protocol was designed for seven days study period. A total of 25 animals were equally divided into 5 groups of five rats in each group. The test substances were administered by oral gavage using a stomach tube. Prior to dosing, animals were kept for 12 hours of fasting. After giving the dose, food was withheld for further 3-4 hours. Group I served as normal control received normal saline (1 mL/kg, p.o) daily for 7 days. Group II served as paracetamol control, receiving paracetamol (250 mg/kg, p.o) at the same time for seven consecutive days. Group III served as reference control and received silymarin (50 mg/kg, p.o) daily. Group IV and V animals were treated with two different doses of aqueous methanolic extract of *S. sebiferum* (200 mg/kg and 400 mg/kg) respectively. Groups III, IV and V were further intoxicated with paracetamol (250 mg/kg) 3 hours after the administration of silymarin and extracts for seven consecutive days. After 24 hours of adminis-tration of last dose, all the animals were anesthetized with chloroform and sacrificed on 8th day. The blood was collected in eppendorf tubes. Blood was allowed to clot and serum was separated with the help of centrifuge at 4,000 rpm for 20 min. Serum was used for the estimations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin (total, direct and indirect).

Histopathological evaluation

All the mice were killed mercifully and their liver tissues were dissected out. A small cross section of the liver was separated out, washed with ice-cold normal saline and embedded in paraffin after fixing with 10% buffered formalin. These tissues were then dehydrated with graded ethanol and cleaned by xylene following paraffin infiltration. Finally, tissue sections were cut in size of 4-5 µm, deparaffinized with xylene and then rehydrated with graded isopropyl alcohol and a drop of water. Water was removed and slides were oven dried. After tissue fixation, staining was done with hematoxylin and eosin. The stained sections of slides were examined under high-resolution microscope by blind observer and photographs were taken.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test using Graph Pad Prism 5 (Graph Pad, Software Inc., USA). The value of significant difference was considered at $p<0.05$.

Results

Initially, paracetamol significantly increased the serum levels of ALT, AST and ALP compared to the serum levels of these liver enzyme markers in control group animals (Figure 1). Similarly, we also measured the toxic effects of paracetamol on liver by estimating the serum levels of different forms (total, direct and indirect) of bilirubin in animals treated toxic doses of paracetamol and/or standard drug silymarin and extracts of *S. sebiferum* (Figure 2). Extracts of *S. sebiferum* significantly exhibited protective effects on liver by preventing the deleterious effects of paracetamol on liver as evidenced by the decreased serum levels of different forms of bilirubin in animals treated with *S. sebiferum* compared to those treated with paracetamol (Figure 2).

At the end of treatment period, we also estimated the hepatoprotective effects of *S. sebiferum* by histopatho-

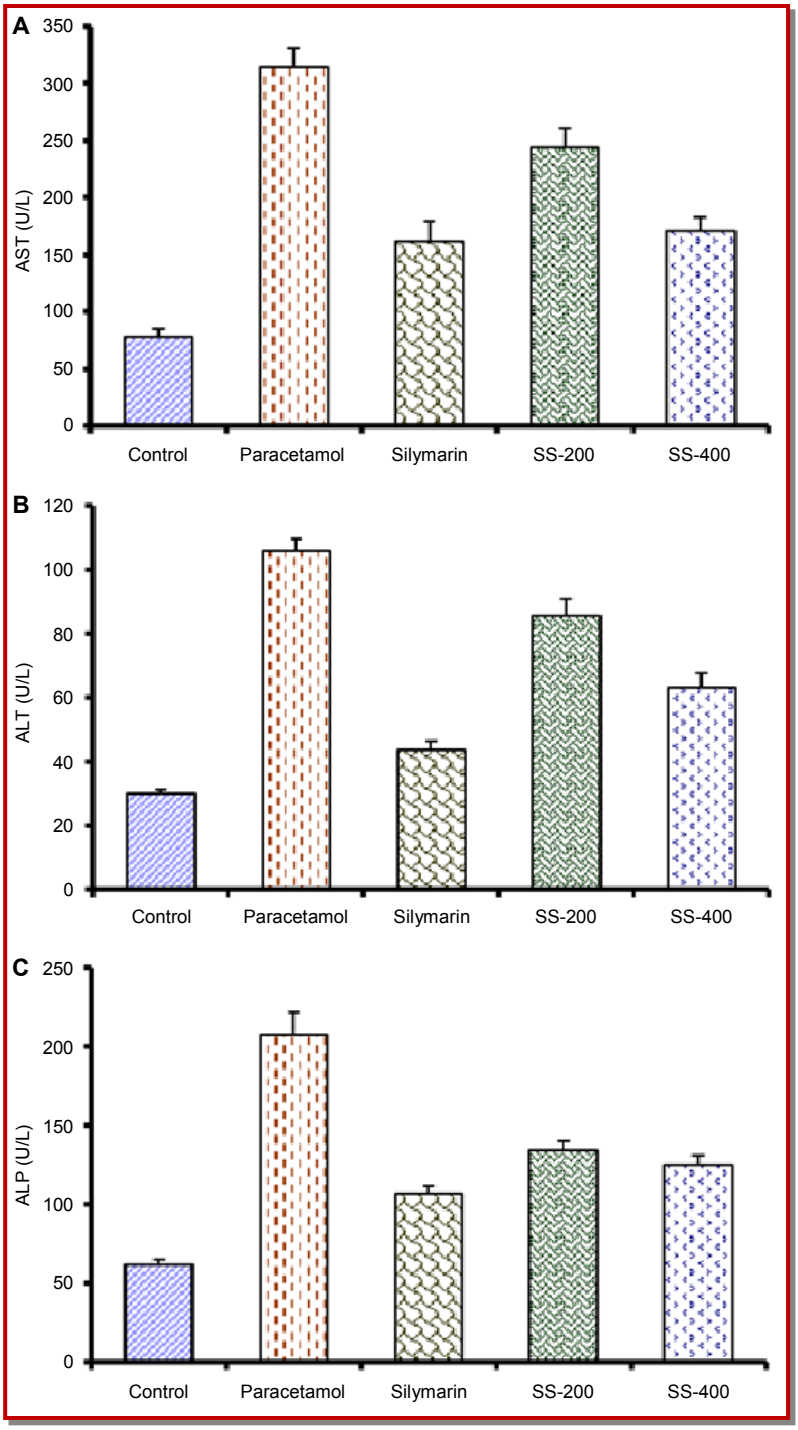

Figure 1: Effect of the methanolic extract of aerial parts of *Sapium sebiferum* on AST (A), ALT (B) and ALP (C). SS-200; 200 mg/kg of *S. sebiferum*, SS-400; 400 mg/kg of *S. sebiferum*

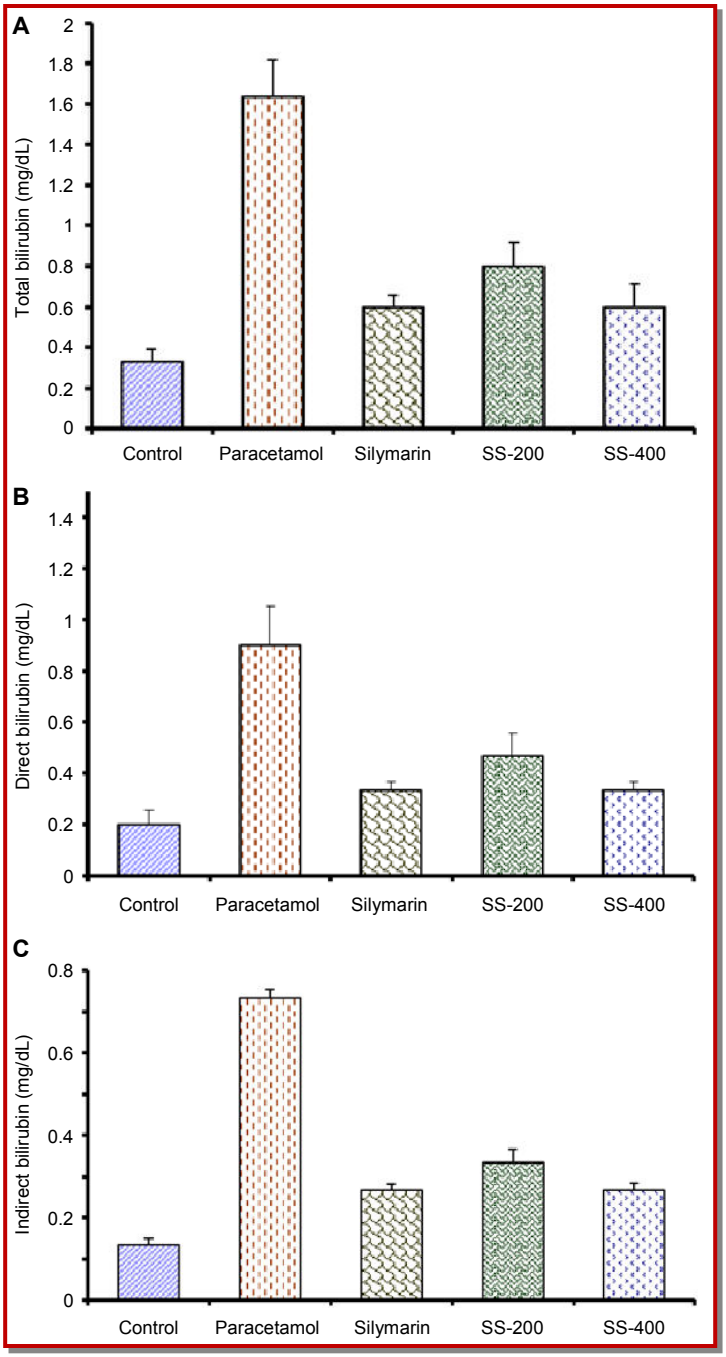

Figure 2: Effect of the methanolic extract of aerial parts of *Sapium sebiferum* on total (A), direct (B) and indirect (C) bilirubin. SS-200; 200 mg/kg of *S. sebiferum*, SS-400; 400 mg/kg of *S. sebiferum*

logical examination of liver tissues obtained from the mice of all groups (Figure 3). The liver tissue from control exhibited normal hepatocytes (Figure 3A), whereas, paracetamol intoxicated group revealed swollen, degenerative and necrotic changes in hepatocytes (Figure 3B). The mice group treated with silymarin exhibited normal hepatocytes (Figure 3C). The liver of the mice group treated with *S. sebiferum* with the dose of 200 mg/kg showed prominent hepatocytes with mild fatty changes (Figure 3D). Mostly hepatocytes were seen normal with prominent nucleus and no cytoplasmic vacuolation. Mild congestion was seen, overall showing mild sign of hepatotoxicity. In contrast, the hepatic tissue sections of mice treated with *S. sebiferum* at the dose of 400 mg/kg showed the hepatocytes of normal size and shape, arranged in cord like fashion with prominent nucleus (Figure 3E). Cytoplasmic vacuolation was not prominent. There was no sign of

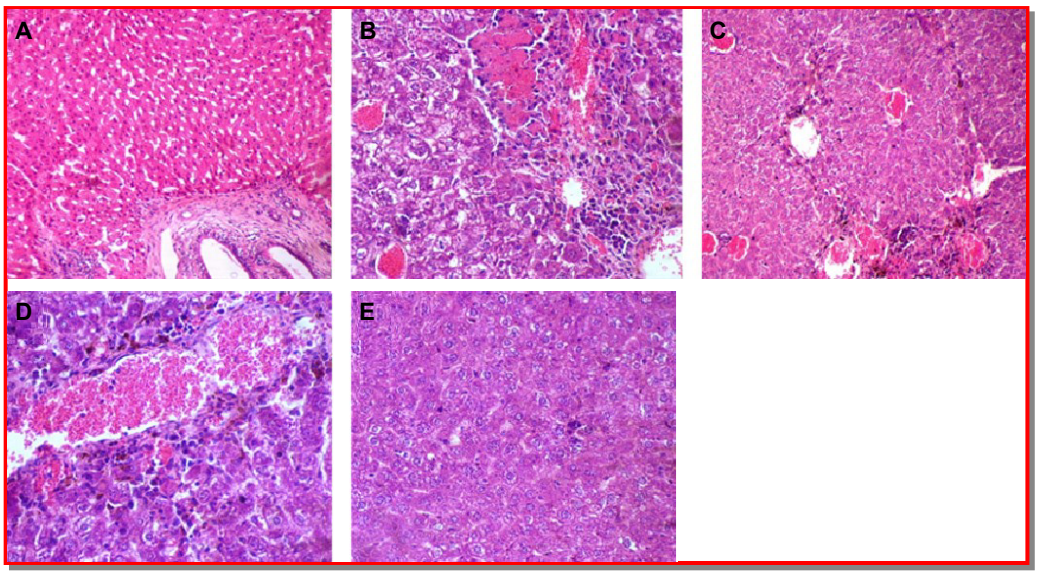

Figure 3: Photomicrograph of liver from mice administered with normal saline (A) paracetamol only, (B) paracetamol + silymarin, (C) paracetamol + SS-200, (D) and paracetamol + SS-400 (E). SS-200; 200 mg/kg of *S. sebiferum*, SS-400; 400 mg/kg of *S. sebiferum*. All figures are × 100 magnification

necrosis or fibrosis, thus proving the extract to be a good hepatoprotective agent.

Discussion

Extracts of *S. sebiferum* exhibited hepatoprotective effects on liver more or less likely to that of silymarin. We also find similar types of findings in our previous work (Hussain et al., 2014a,b). The results of our present study are highly in consistent with the findings of several studies that have reported the hepatoprotective effects of different plants including *Carica papaya* (Sadeque and Begum, 2010), *Carissa spinarum* (Hegde and Joshi, 2010), *Cocculus hirsutus* (Thakare et al., 2009), *Convolvulus arvensis* (Ali et al., 2013), *Dodonaea viscosa* (Khan et al., 2013), *Ipomoea staphylina* (Bag and Mumtaz, 2013), *Suaeda fruticosa* (Rehman et al., 2013), *Alcea rosea* and *Malva sylvestris* (Hussain et al., 2014a,b), *Trianthema decandra* (Balamurugan and Muthusamy, 2008). All of these plants have shown the more or less likely to same hepatoprotective effects against paracetamol-induced hepatotoxicity under different experimental conditions. Various studies have confirmed the hepatoprotective activity of these medicinal plants, but none of the studies reported presence of such phytochemical constituent that exactly exhibit the hepatoprotective activity. Analytical techniques and methodologies (Lei et al., 2013; Lei et al., 2014; Lei et al., 2015; Bilal et al., 2014) play a most important and significant role to detect the presence of phytochemical constituents in these plants. Therefore, the significant outcomes of plants extracts having hepatoprotective activity open a new gateway to detect the possible phytochemical constituents that are responsible the hepatoprotective effects.

Conclusion

The significant therapeutic effects of *S. sebiferum*, we bring to an end that the methanolic extract of the leaves of *S. sebiferum* has proven to have hepato-protective activity in experimental animal models.

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