Liver, the second largest organ of body responsible for a number of important functions including production of bile, urea and plasma proteins, excretion of bilirubin, cholesterol, hormone and drugs and also metabolism of protein, vitamin, minerals, carbohydrates and fats (1), is most susceptible to toxicity (2). Hepatotoxicity is caused by chemicals that damage hepatic vasculature, hepatocytes and biliary epithelial cells either directly or indirectly by producing their reactive metabolic species, hence eliciting an immune reaction and resulting in hepatic injury (3). Most of the toxins have minimum liver damaging potential which can be reversed by terminating the use of offending agent. In overdose, causative mediator results in hepatic necrosis and if not treated timely and effectively, may lead to death (3). Moreover, it is estimated that over 900 drugs have potential to cause hepatic damage that is why most marketed drugs with hepatotoxic potential have been withdrawn (4). Drug induced liver injury can be either intrinsic or idiosyncratic (4) and results from inhalation, ingestion and parenteral administration of drugs. Certain drugs cause hepatic injury even at therapeutic doses (5), for instance paracetamol, diclofenac, isoniazid, halothane, erythromycin, penicillamine and phenytoin. Moreover, reactive metabolites of various drugs interact with essential macromolecules such as proteins, lipids or nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage and oxidative stress and finally resulting in hepatic damage (6). Around the world, every year about 20,000 deaths occur followed by hepatic diseases (5).

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Current therapy of liver disorders include interferons, lamivudine, adefovir, ribavirin and corticosteroids. But these medications owe many limitations such as low efficacy, high risk of adverse effects and high price. Therefore, use of herbal remedies in treating liver maladies has been increasing nowadays. Various herbs are known for pos-
sessing hepatoprotective properties, derived from different cultural sources (7). *Euphorbia prostrata* Ait is an annual herb, mainly found in tropic and subtropic areas naturally present in Asia, Africa and many other parts of world. It is used traditionally as anti-oxidant (8) and to inhibit HIV-1 and hepatitis C virus proteases (9, 10). It has been validated for pharmacological effects like anti-inflammatory activity (11), hemorrhoid (12), diarrhea (13), antibacterial, anti-fungal activity (14) and diabetes (15).

The purpose of present study was to pharmacologically investigate the hepatoprotective effect of *Euphorbia prostrata* using animal models of liver injury thus supporting its customary use in liver ailments.

**MATERIALS AND METHODS**

**Chemicals**

The chemicals used were methanol, chloroform, ether, normal saline, paracetamol, carbon tetrachloride, silymarin (all from Sigma Aldrich). All the other chemicals used were of analytical grade.

**Plant material**

The aerial parts of *Euphorbia prostrata* Ait (2 kg) were collected from Dhillam Ballaggan, Sialkot, Punjab, Pakistan from April to May 2015. Plant was identified and authenticated by Professor Dr. Ashiq, Department of Botany, University of Agriculture, Faisalabad. The washed and shade dried plant material was ground into powder with a Chinese herbal grinder.

**Preparation of plant extract**

Aqueous methanolic (30 : 70) extract of aerial parts of *Euphorbia prostrata* was prepared using cold maceration technique. Coarse powder of plant was soaked in 3 L of 70% methanol and kept at room temperature for 3 days (72 h) with occasional stirring daily, followed by filtration after 3 days. This process was repeated thrice. Afterwards, all the filtrates were combined and again filtered through muslin cloth and Whatman filter paper I. The filtrate was dried and concentrated under reduced pressure in rotary evaporator at 50°C. The solid extract thus formed was stored in a capped container in refrigerator. The color of crude extract was dark brown (16).

**Animals used**

Young and healthy albino mice (20-40 g) of both sexes (4-5 months) were used. The mice were housed under standard conditions of temperature (23 to 25°C), relative humidity (55%) with 12 h light and 12 h dark cycle at animal house of University of Sargodha, Sargodha. They were fed with standard pellet diet and tap water ad libitum. All the experiments performed complied with the rules of National Research Council (17).

**Paracetamol (PCM) induced hepatotoxicity**

In the dose response experiment, albino mice were randomly divided into 5 groups (n = 4). Group I (Normal control group) animals received normal saline 1 mL/kg, p.o. for 7 days. Group II (Diseased control) mice were given normal saline, 1 mL/kg p.o. for 7 days. Group III (Standard group) mice were administered silymarin, 100 mg/kg p.o. for 7 days. Group IV and V (Experimental groups) animals were given *Euphorbia prostrata* extract, 250 mg/kg/d and 500 mg/kg/d p.o. for 7 days, respectively.

On 7th day, 1 h after administering normal saline (disease control group), silymarin, 250 mg and 500 mg of *Euphorbia prostrata* extract to Group II, III, IV and V, respectively, paracetamol (250 mg/kg) was given orally. After 24 h of administration, mice were sacrificed under mild ether anesthesia and hepatoprotective activity was assessed (18).

**Carbon tetrachloride (CCL4) induced hepatotoxicity**

Albino mice were randomly divided into 5 groups (n = 4). Group I (Normal control group) was given distilled water 1 mL/kg, p.o. for 7 days followed by administration of olive oil (1 mL/kg, s.c.) on 7th day, 1 h after feeding distilled water. Group II (Diseased control) mice were administered distilled water (1 mL/kg, p.o.) for 7 days. Group III (Standard group) animals received silymarin, 100 mg/kg p.o. for 7 days. Group IV and V (Experimental groups) mice were given *Euphorbia prostrata* extract, 250 mg/kg/d and 500 mg/kg/d p.o. for 7 days, respectively.

On 7th day, 1 h after administration of distilled water, sylimarin, 250 mg and 500 mg of *Euphorbia prostrata* extract to Group II, III, IV and V, respectively, carbon tetrachloride (20% v/v in olive oil) 1 mL/kg was given subcutaneously. After 24 h of administration, mice were sacrificed under mild ether anesthesia and hepatoprotective activities were evaluated (19).

**Assessment of hepatoprotective activity**

The hepatoprotective activity was appraised biochemically as well as histopathologically. After
24 h of drug treatment, animals were anesthetized using ether. Blood was withdrawn from each mouse through carotid artery and collected in centrifugation tubes and was allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 3000 rpm for 15 min and biochemical parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, bilirubin and total protein were evaluated. The hepatic tissues were also excised quickly, washed with saline and stored in 10% formalin. Four representative sections were taken and submitted in one block. The histopathological examination of these sections revealed architecture of liver (5).

Statistical analysis
The results were expressed as the means ± standard error of mean (S.E.M). One-way ANOVA followed by Dunnet test was applied using Graphpad prism software. Significance was set at 95% (p < 0.05).

RESULTS
The present study revealed that in paracetamol induced intoxication, Euphorbia prostrata extract exhibited highly significant reduction in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (p < 0.01) and alkaline phosphatase (ALP) (p < 0.001) at 250 mg/kg dose. Moreover, Euphorbia prostrata extract caused highly significant (p < 0.001) reduction in activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) at 500 mg/kg as compared to disease control group. However, Euphorbia prostrata extract produced non-significant changes in levels of alkaline phosphatase (ALP) at 500 mg/kg. Also, non-

Table 1. Effect of methanolic extract of Euphorbia prostrata on biochemical parameters in paracetamol induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total bilirubin (mg/dL)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Albumin (g/dL)</th>
<th>Total protein (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCM (250 mg/kg)</td>
<td>0.83 ± 0.01</td>
<td>82.66 ± 15.45</td>
<td>85.33 ± 18.98</td>
<td>209.33 ± 5.20</td>
<td>4.30 ± 0.88</td>
<td>7.53 ± 0.62</td>
</tr>
<tr>
<td>Normal control (N.S 1 mL/kg)</td>
<td>0.53 ± 0.01*</td>
<td>79.33 ± 4.05*</td>
<td>72.33 ± 2.33*</td>
<td>135.66 ± 5.36*</td>
<td>3.00 ± 0.05*</td>
<td>5.86 ± 0.46*</td>
</tr>
<tr>
<td>Silymarin + PCM (100 mg/kg/10mL)</td>
<td>0.53 ± 0.03**</td>
<td>19.00 ± 1.52**</td>
<td>14.66 ± 1.85**</td>
<td>193.33 ± 4.41**</td>
<td>3.90 ± 0.57**</td>
<td>6.53 ± 0.31**</td>
</tr>
<tr>
<td>EP + PCM (250 mg/kg)</td>
<td>0.46 ± 0.03***</td>
<td>21.00 ± 0.57***</td>
<td>32.00 ± 1.73***</td>
<td>206.00 ± 12.74***</td>
<td>2.83 ± 0.06***</td>
<td>6.13 ± 0.13***</td>
</tr>
<tr>
<td>EP + PCM (500 mg/kg)</td>
<td>0.60 ± 0.00***</td>
<td>21.00 ± 0.57***</td>
<td>32.00 ± 1.73***</td>
<td>206.00 ± 12.74***</td>
<td>2.83 ± 0.06***</td>
<td>6.13 ± 0.13***</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SEM (n = 4), where, ns = (p > 0.05), * = (p < 0.05), ** = (p < 0.01), *** = (p < 0.001) vs. PCM (250 mg/kg). PCM=Paracetamol, EP=Euphorbia prostrata

Table 2. Effect of methanolic extract of Euphorbia prostrata on biochemical parameters in carbon tetrachloride induced hepatotoxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total bilirubin (mg/dL)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Albumin (g/dL)</th>
<th>Total protein (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCl4 (1 mL/kg)</td>
<td>1.60 ± 0.15</td>
<td>175.00 ± 15.37</td>
<td>72.00 ± 16.16</td>
<td>441.00 ± 28.93</td>
<td>6.70 ± 1.35</td>
<td>9.33 ± 0.17</td>
</tr>
<tr>
<td>Normal control (N.S 1mL/kg)</td>
<td>0.53 ± 0.06ns</td>
<td>79.33 ± 4.05ns</td>
<td>72.33 ± 2.33ns</td>
<td>135.66 ± 5.36ns</td>
<td>3.00 ± 0.05ns</td>
<td>5.86 ± 0.46ns</td>
</tr>
<tr>
<td>Silymarin + CCl4 (100 mg/kg/10mL)</td>
<td>0.63 ± 0.03**</td>
<td>47.66 ± 4.63**</td>
<td>42.33 ± 9.06**</td>
<td>162.00 ± 7.57**</td>
<td>3.10 ± 0.10**</td>
<td>5.83 ± 0.44**</td>
</tr>
<tr>
<td>EP + CCl4 (250 mg/kg)</td>
<td>0.50 ± 0.05**</td>
<td>51.66 ± 5.36**</td>
<td>42.66 ± 1.76**</td>
<td>186.66 ± 4.41**</td>
<td>3.46 ± 0.27**</td>
<td>6.46 ± 0.12**</td>
</tr>
<tr>
<td>EP + CCl4 (500 mg/kg)</td>
<td>0.63 ± 0.03**</td>
<td>34.66 ± 2.40**</td>
<td>29.00 ± 1.73**</td>
<td>158.66 ± 4.66**</td>
<td>3.20 ± 0.15**</td>
<td>6.30 ± 0.25**</td>
</tr>
</tbody>
</table>

Results are expressed as the means ± SEM (n = 4), where, ns = (p > 0.05), * = (p < 0.05), ** = (p < 0.01), *** = (p < 0.001) vs. CCl4 (1mL/kg). CCl4=carbon tetrachloride, EP=Euphorbia prostrata
significant changes in levels of total bilirubin, albumin and total protein at both concentrations were observed as illustrated in Table 1.

Likewise, in carbon tetrachloride induced hepatotoxicity model, methanolic extract significantly (p < 0.001) decreased the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) at both doses but there were non-significant changes in the levels of bilirubin, albumin and total protein at both doses as presented in Table 2.

Additionally, histopathologic studies demonstrated that, in normal control group, normal architecture of hepatocytes was observed with intact cell nuclei and regular portal vein. While, in paracetamol intoxicated group, severe histopathological changes were observed such as infiltration of inflammatory cells, fatty changes, necrosis and ballooning degeneration of hepatocytes, granuloma and malignancy. Silymarin (standard) treated group unveiled that hepatocytes were arranged in single plate with central veins and porta hepatis and no inflammatory or necrotic changes were noticed in standard group. Moreover, no granuloma or malignancy was evident. While, among extract treated groups, 250 mg/kg exhibited partial protection of hepatocytes and prevented histopathological changes associated with hepatotoxicity induced by acetaminophen, but hepatocytes were mildly infiltrated by inflammatory cells. However, no necrosis, granuloma or malignancy was seen. While, at 500 mg/kg, hepatocytes were arranged in single plate, central vein and porta hepatis were also clearly discernible. There were no signs of inflammatory and necrotic changes, granuloma or malignancy (Fig. 1).

The liver sections of carbon tetrachloride treated mice divulged characteristic centrilobular pattern of degeneration, liver fibrosis, hyperemia around central vein, wide vacuolar degeneration of hepatocytes, lymphocyte infiltration, derangement of hepatocyte cord and necrosis at periphery of central vein. Whereas, in normal control group, central vein, portal space and hepatocytes were normal. However, silymarin distinctly protected liver from carbon tetrachloride induced damage as revealed by histopathological analysis. Similarly, *Euphorbia prostrata* extract provided hepatic protection comparable to standard group particularly at 500 mg/kg dose (Fig. 2).

**DISCUSSION**

Liver is the central organ having astounding role in metabolism, excretion and detoxification of
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chemotherapeutic agents, xenobiotics and environmental pollutants. It is involved in maintenance, regulation of body homeostasis and various biochemical pathways to growth, nutrient provision and fight against disease, energy generation and reproduction. Various liver maladies like hepatitis, cirrhosis and fatty liver disease originate because of exposure to environmental toxins, generation of free radicals, deprived food habits, alcohol abuse and numerous prescribed and over-the-counter drugs (20). The available synthetic drugs are less effective in treating hepatic diseases owing to their numerous adverse effects and low therapeutic potential. Therefore, use of herbal medicines has been increasing gradually in both developed as well as developing countries as they are economical with substantial biological effects and free from toxic profile. Furthermore, plant based preparations play a noteworthy role in regeneration of liver cells and acceleration of healing process and hence management of numerous liver ailments (21). Currently, most effective therapy for hepatic ailments are constituents derived from herbal drugs such as glycyrrhizin (*Glycyrrhiza glabra*) and silymarin (*Silybum marianum*) in Japan, catechin (*Anacardium occidentalis*) in Europe, and chizandrins (*Schizandra chinensis*) in China (22). Similarly, in present study, hepatoprotective activity of aqueous methanolic extract of *Euphorbia prostrata* was evaluated using paracetamol (PCM) and carbon tetrachloride (CCl4) as hepatotoxins to scientifically support its folkloric use.

These hepatotoxic agents cause liver damage resulting in elevation of marker enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Carbon tetrachloride is a hepatotoxic agent that undergoes biotransformation in endoplasmic reticulum by CYP 450 and produces trichloromethyl free radical i.e. CCl3 that interact with cellular proteins and lipids. The trichloromethylperoxyl radical more potentially attacks lipids of endoplasmic reticulum causing lipid peroxidation and alter calcium homeostasis resulting in cell death and release of enzymes in circulation. In current study, methanolic extract of *Euphorbia prostrata* markedly decreased levels of liver marker such as enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), total bilirubin and albumin comparable to that of standard drug silymarin. However, in carbon tetrachloride intoxication, *Euphorbia prostrata* extract reduced the levels of total bilirubin in dose independent manner, which might be due to either enzyme/receptor saturation or genetic variations. Likewise, paracetamol is a well-known and most commonly used analgesic and anti-pyretic drug. Hepatotoxic doses of paracetamol deplete normal stores of hepatic glutathione. CYP 450 enzymes such as CYP2E1 and CYP1A2 metabolize paracetamol and forms NAPQI (N-

Figure 2. Histopathological examination of liver after administration of *Euphorbia prostrata* in CCl4 treated mice: A) The Normal control, B) CCl4 intoxicated group, C) silymarin 100 mg/kg, D) EP (250 mg/kg), E) EP (500 mg/kg)
acetyl-p-benzo-quinoneimine) that is an alkylating metabolite. The P450 gene is highly polymorphic in nature as CYP2D6, third isoenzyme, is associated with individual variation towards paracetamol toxicity. Paracetamol is metabolized into NAPQI to a lesser extent by CYP2D6 but in ultra-rapid metabolizers, this enzyme causes hepatotoxicity. Glutathione is a natural anti-oxidant, but in paracetamol toxicity NAPQI becomes irreversibly conjugated with sulfhydryl group of glutathione, thus causing its depletion. NAPQI causes hepatic damage by releasing inflammatory mediators such as TNF-α, responsible for tissue necrosis (23). In paracetamol intoxicated mice, plant extract showed a dose dependent decrease in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), which might be due to stabilization of plasma membrane, as well as repair of hepatic tissue damage caused by carbon tetrachloride and paracetamol (24). Moreover, plant extract significantly decreased albumin level, but surprisingly it exhibited concentration independent behavior in case of total bilirubin which might be due to hereditary disparity or receptor saturation producing less than expected response at higher dose as in carbon tetrachloride model. However, in previous studies it has been revealed increase in serum total bilirubin level to be owing to defective bile excretion by liver, associated with loss of integrity of liver and eventually tissue necrosis. Hence, this results in increase in binding, conjugating and excretory capacity of liver cells, relative to erythrocyte degeneration rate. Moreover, depletion in serum bilirubin level by plant extract might be due to its ability to alleviate biliary dysfunction during paracetamol induced hepatotoxicity in prophylactic studies (24). Besides, histopathological examination also supported hepatoprotective ability of Euphorbia prostrata.

Since, it has been found that flavonoids through their free radical scavenging activity are accountable for hepatoprotection. Thus, liver damage induced by carbon tetrachloride and paracetamol is oxidative in nature, usually reversed by antioxidants by stabilizing cell membrane and repairing liver tissue damage. Moreover, various studies revealed hepatoprotective action of alkaloids due to their anti-oxidant property (25). Also, hepatoprotective agents act by inhibiting aromatase activity of CYP 450 which favors liver regeneration (26). Hence, current study has revealed hepatoprotective aptitude of Euphorbia prostrata against carbon tetrachloride (CCL₄) and paracetamol (PCM) induced liver damage, which could be due to its anti-oxidant activity, as it contains glycosides, flavonoids, phenols, polysaccharides, anthraquinones, phlobatannins, saponins and various other alkaloids (27, 28). Furthermore, in another study it has been ascertained that aqueous methanolic extract of E. prostrata possesses noticeable scavenging properties and scavengers DPPH, which might be owing to the presence of phenolic and polyphenolic compounds that significantly inhibit oxidative stress caused by free radicals (29). Moreover, it has been avowed that Euphorbia halmifolia possesses hepatoprotective potential due to marked anti-oxidant and inhibitory lipid peroxidation (LPO) activity (30, 31). So, Euphorbia prostrata being the member of same family and genus might have exerted hepatoprotective effect due to its strong anti-oxidant and lipid peroxidation inhibition activity and owing to the presence of flavonoids and phenolics. Further studies and investigations are required to prove hepatoprotective potential of active constituents of plant extract.

CONCLUSION

Euphorbia prostrata is a medicinally valuable plant and its hepatoprotective activity might be due to flavonoid and phenolic constituents which exhibit anti-oxidant and anti-lipid peroxidation properties. However, actual mechanism is not known and it needs to be investigated.

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