Protective action of *Taraxacum officinale* on CCl₄ induced hepatotoxicity in rats

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Plants are important source of medicines, especially in developing countries, where people use plant based traditional medicines for their health care. Leaves of *Taraxacum officinale* L. are being used against various human disorders in folk medicine since past. Current study was conducted to explore the hepatoprotective activity of methanolic leaves extract of *T. officinale* L. against carbon tetrachloride (CCl₄) induced toxicity in rats. Various concentrations of plant extracts like 150 and 300 mg/kg of body weight as well as silymarin (100 mg/kg), a standard drug, was given to experimental animals. The results of this study indicates that the methanolic leave extracts has significantly reduced (P < 0.05) the level of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) which was increased due to induction of CCl₄. It was also observed that due to CCl₄ induction, the level of bilirubin, lipid profile and antioxidants enzymes was also increased, that was significantly lowered after the treatment with the leave extracts. These results revealed that the leave extracts of *T. officinale* L. has protective effects against CCl₄ induced liver toxicity and damage. Furthermore, histopahtological results obtained during this study also supported this claim and was probably due to presence of valuable phytochemicals in the leave extracts.

**Key words:** Medicinal plants, *Taraxacum officinale*, leaves extract, animals, liver enzymes.

**INTRODUCTION**

Liver is a vital organ which plays a major role in different metabolisms and excretion of xenobiotics from the body. Liver dysfunction is a major health problem which is a challenge not only for health care professionals but also for the pharmaceutical industry and drug regulatory agencies. Liver cell injuries are caused by various toxic chemicals such as antibiotics, chemotherapeutic agents, carbon tetrachloride etc (Allies, 1990; Sing and Rao, 2008). Liver cells are also damaged due to excessive alcohol consumption and microbial action (Ahmed et al., 1987). The available synthetic drugs to treat liver disorders are also causing further damage to the liver (Singh and Rao, 2008). Furthermore, in the recent past, it was observed that the dosage of drugs might be adversely affecting the liver tissues. Many plasma proteins are synthesized in the liver and their plasma...
levels therefore depend on the balance between synthesis and catabolism and/or loss from the body (Edet et al., 2011; Ismail et al., 2010).

The liver enzymes and proteins are important biomarkers in the body utilized in the diagnosis and assessment of normal function of liver. Major or minor changes in the integrity of cellular membranes in tissues or organs have culminated changes in enzyme activities. For instance alanine amino transferase (ALT) and aspartate aminotransferase (AST) are useful in detecting alterations in liver disease while ALT and some other parameters are implicated in extra hepatic or intra-hepatic obstruction (Chatterjea and Shinde, 2002; Sing and Rao, 2008; Edet et al., 2011). In recent years, due to inadequacy of liver protective agents, researchers and traditional medicine practitioners concentrated on herbal based remedies for various liver disorders (Chaterrjee, 2000). Many folk remedies from plant sources are available for the protection of hepatic damages starting from ancient period. Medicinal plants possess valuable bioactive compounds that protects human from various complications. World Health Organization estimated that about 80% population in Africa and majority of population in Asia and Latin America still use traditional medicines for their primary health care. Phytotherapy play vital role in detoxification of oxidative stress and other degenerative disorder with minimum or no side effects comparatively to other types of drugs (Reilly and Bulkley, 1990).

*T. officinalis* (Asteraceae) locally known as Dandelion has been commonly used by rural people against many human disorders including liver and kidney diseases (Dirlesi et al., 2012; You et al., 2010; Agarwal, 2001). The present work was conducted to prove scientifically hepatoprotective activity of *T. officinalis* in rats.

**MATERIALS AND METHODS**

**Plant and chemicals**

Carbon tetrachloride (CCl₄) was purchased from Aldrich Chemical Co. All other chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for all the assays were commercial kits and products of Randox, USA. Fresh leaves of *T. officinalis* L. were collected in April, 2010 from different locations of Rawalpindi areas. The samples were identified and authenticated by a taxonomist and registered as a specimen (voucher specimen numbers 217).

**Preparation of plant samples**

The leaves were air-dried for 2 weeks and then ground into fine powder using an electric dry mill. A total of 200 g of the ground powder was soaked in 1 L of distilled water for 48 h at room temperature. The mixture was filtered into 500 ml conical flask with Whatman filter paper (No.1). The filtrate was dried at a temperature of 30°C for 10 h to produce a extract, which weighed 20.0 g. Appropriate concentration of the extract was then subsequently made by dilution with methanol into 150 and 300 mg/kg/body weight and administered to the animals.

**Phytochemical analysis**

Preliminary phytochemical tests were carried out on the methanol extract of the leaves using standard methods (Harbone, 1973; Trease and Evans, 1983).

**Animals**

Twenty five (25) male albino rats of Wistar strain were used in this study. The rats having weight of 100 to 150 g were maintained under standard animal house conditions and were fed with commercial rat chow (Feed Mills, Islamabad) and allowed water ad libitum. Animals were divided into 5 groups having 5 animals in each group and maintained in standard lab conditions with 12-hours cycle of light and dark. Room temperature was kept at 22 ± 2°C and humidity was maintained at 50 ± 5%. The protocol was approved by animal ethics committee of the University.

**Acute toxicity tests**

The acute toxicity tests were carried out by following a method reported by Lorke (1983). The extract was found to be relatively safe. Doses of 150 and 300 mg/kg of extracts were then chosen and administered to the rats.

**Hepatoprotective activity**

A total of 25 animals were divided into 5 groups of 5 animals in each group (n = 5). Group I (control) received olive oil orally for 14 days. Group II (hepatotoxic control) received a single dose of 5 ml/kg of CCl₄ diluted in olive oil, 1:1 ratio for 14 days alternatively. Group III (Test group 1) were administered with single dose of 5 ml/kg of CCl₄ along with vehicle alternatively for 14 days and it was followed by the treatment with 150 mg/kg of *T. officinalis* leaves extract orally for 21 days. Group IV (Test group 2) animals were administered with single dose of 5 ml/kg of CCl₄ for 14 days, followed by treatment with 300 mg/kg of *T. officinalis* leaves extract for 21 days. Group IV (hepatoprotective agent control) animals were administered with CCl₄ for 14 days and followed by the treatment with 100 mg/kg of known hepatoprotective agent (silymarin). On 22nd day, the animals were anaesthetized using chloroform and blood was collected by cardiac puncture.

**Preparation of serum**

Blood was obtained from the rats by heart puncture technique into centrifuge tubes. Serum was prepared by centrifugation for 10 min at 3000 rev/h in a bench centrifuge. The clear supernatant was used for the biochemical tests.

**Biochemical analysis**

Liver enzymes such as AST, ALT, ALP and lipid profile - total cholesterol, very low density lipoprotein (VLDL), high density lipoprotein (HDL), triglycerides were estimated by using commercially available kits based on the methods reported by Reitman and Frankel (1957), King and Kind (1954). Whereas, bilirubin was determined by using method reported by Jendrassic and Grof (1938). The activities of anti oxidants enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase...
21 days showed hepatoprotective activity in liver enzymes like hepatic damage in rats. Results show increases in the administration at a dose of 5 ml/kg body weight showed control group of rats (Table 2). Treatment of CCl4 induced intoxicated animals when compared with that of the control group of rats (p < 0.05). However, the treatment of CCl4 induced group of rats with extracts of T. officinalis at a dose of 150 and 300 mg/kg and also, a known hepatoprotective agent silymarin (100 mg/kg), showed significant reduction in level of liver cholesterol, triglyceride, VLDL and LDL, whereas HDL level was increased as compared to CCl4 treated group (Table 3).

The effects of methanolic leaves extracts of T. officinalis on the antioxidants enzymes like catalase, GPx and SOD in the serum of control and CCl4 treated group showed significant reduction (p < 0.05). The methanolic leaves extracts of T. officinalis and silymarin increased the revised activities of these antioxidants in the liver of CCl4 induced group on dose dependent manner as compared to control and the change was significant (p < 0.05). These results suggested that the free radicals released in the liver were effectively scavenged in the animals treated with T. officinalis. Malondialdehyde (MDA) content in liver of CCl4 treated group was significantly higher than that of the control group. However, MDA levels were significantly lowered in CCl4 treated group followed by treatment with methanolic leaves extracts of T. officinalis and silymarin (p < 0.05) (Table 4).

The results of histopathological study of the liver tissues of the control and CCl4 treated rats are given in Figures 1 to 4, respectively. The liver section of the animal in control group showed normal hepatic cells, well defined cytoplasm prominent nucleus, nucleolus and a central vein with prominent small-sized (Figure 1). While liver section of CCl4 induced animal showed total loss of hepatic architecture with centrilobur hepatic necrosis, fatty changes vacuolization and congestion of sinusoids (Figure 2). However, treatment of animals with 300 mg/kg of methanolic leaves extracts of T. officinalis (Figure 3) and 100 mg/g of silymarin (Figure 4) represent normal condition of liver tissues and it is assumed that treatment returned that injury towards normal side. Therefore, results of histopathological study also provided support for biochemical analysis carried out during this work.

### DISCUSSION

Carbon tetrachloride (CCl4) is assumed to initiate the biochemical processes leading to oxidative stress, which is the direct cause of many pathological changes in liver, kidney, testes, lungs, nervous system and blood tissues by producing free radicals (Abraham et al., 1999; ATSDR, 2003). These free radicals frequently damage different cell organelles through lipid peroxidation (Ahmed et al., 1987). For example, the acute exposure of CCl4 to the liver tissues can cause damage of liver and lead to elevated levels of its enzymes (Sing and Rao, 2008; Okonkwo et al., 2004). These liver enzymes can then be
Table 2. Effect of methanolic extracts of *T. officinalis* leaves on liver enzyme and bilirubin.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Direct bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>72.4±0.24</td>
<td>38.1±0.21</td>
<td>114.21±0.42</td>
<td>0.15±0.07</td>
<td>1.73±0.07</td>
</tr>
<tr>
<td>CCl4</td>
<td>217.43±0.23a</td>
<td>115.17±0.93a</td>
<td>224.93±0.54a</td>
<td>0.39±0.01a</td>
<td>2.17±0.05b</td>
</tr>
<tr>
<td>CCl4+150 mg/kg extract</td>
<td>155±0.27</td>
<td>88±0.61</td>
<td>173.38±0.19a</td>
<td>0.31±0.03a</td>
<td>1.94±0.04</td>
</tr>
<tr>
<td>CCl4+300 mg/kg extract</td>
<td>72.6±0.24b</td>
<td>35.15±0.27b</td>
<td>123.73±1.71b</td>
<td>0.24±0.06b</td>
<td>1.79±0.04b</td>
</tr>
<tr>
<td>CCl4+ Silymarin (100 mg/kg)</td>
<td>149.81±1.53b</td>
<td>88±0.47</td>
<td>138.83±0.61b</td>
<td>0.25±0.07b</td>
<td>1.83±0.05b</td>
</tr>
</tbody>
</table>

Results were expressed as Mean± SEM (n= 5); a P<0.05 compared with control group of rats; b P<0.05 compared with CCl4 induced group of rats.

Table 3. Effect of methanolic extracts of *T. officinalis* leaves on liver lipid profiles.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.76±0.32</td>
<td>77.35±0.23</td>
<td>29.38±0.35</td>
<td>54.7±0.19</td>
<td>19.41±0.33</td>
</tr>
<tr>
<td>CCl4</td>
<td>132.54±0.19a</td>
<td>135.48±1.18a</td>
<td>23.83±0.34a</td>
<td>98.37±0.23a</td>
<td>27.46±0.16a</td>
</tr>
<tr>
<td>CCl4+150 mg/kg extract</td>
<td>112.29±0.14</td>
<td>97.11±0.24</td>
<td>20.78±0.27</td>
<td>58.57±0.14</td>
<td>16.9±0.43</td>
</tr>
<tr>
<td>CCl4+300 mg/kg extract</td>
<td>84.59±0.26b</td>
<td>75.9±0.25b</td>
<td>21.11±0.17b</td>
<td>51.38±0.31b</td>
<td>13.39±0.18b</td>
</tr>
<tr>
<td>CCl4+ Silymarin</td>
<td>99.41±0.18b</td>
<td>81.57±0.23b</td>
<td>22.51±0.17b</td>
<td>62.31±0.24b</td>
<td>14.13±0.16b</td>
</tr>
</tbody>
</table>

Results were expressed as Mean± S.E.M (n= 5). aP<0.05 compared with control group of rats. bP<0.05 compared with CCl4 induced group of rats.

Table 4. Effect of Methanolic extracts of *T. Officinalis* leaves on antioxidants enzyme.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase U/mg of protein</th>
<th>GPX U/ mg of protein</th>
<th>SOD U/mg of protein</th>
<th>MDA nm/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.32±0.06</td>
<td>2.65±0.05</td>
<td>46.45±0.04</td>
<td>2.87±0.75</td>
</tr>
<tr>
<td>CCl4</td>
<td>9.74±0.04a</td>
<td>1.64±0.04a</td>
<td>29.5±0.07a</td>
<td>8.83±0.13a</td>
</tr>
<tr>
<td>CCl4+150mg/kg extract</td>
<td>14.15±0.04</td>
<td>1.98±0.03</td>
<td>42.19±0.03</td>
<td>3.88±0.73</td>
</tr>
<tr>
<td>CCl4+300mg/kg extract</td>
<td>15.67±0.09b</td>
<td>2.78±0.06b</td>
<td>45.34±0.08b</td>
<td>2.81±0.48b</td>
</tr>
<tr>
<td>CCl4+ Silymarin</td>
<td>12.43±0.08b</td>
<td>1.65±0.08b</td>
<td>47.83±0.08b</td>
<td>4.51±0.81b</td>
</tr>
</tbody>
</table>

Catalase (U/mg of protein), glutathione peroxidase (U/mg of protein) superoxide dismutase (U/mg of protein), MDA- nm/mg of protein. Results were expressed as Mean ± SEM (n=5). aP<0.05 compared with control group of rats and bP<0.05 compared with CCl4 induced group of rats.

Figure 1. Liver tissues of normal rat.
bilirubin, total cholesterol LDL, VLDL and triglycerides were also restored towards their normal levels by the treatment of _T. officinalis_ leaves extract. The reason was that the bioactive compounds (phytochemicals) in the extract minimized the adverse affects of CCl₄ by chelating with by-products produced from CCl₄ metabolites. This demonstrates the hepatoprotective role of plant extract which not only involved in considerably decrease in the effect of CCl₄ induced damage but also resulted in the recovery of damaged liver at a significant level (Chungma et al., 2007; You et al., 2010; Dirleise et al., 2012; Sing and Rao, 2008).

Moreover, the previous studies have reported that the oxidative damage to tissues and their cellular components can be prevented by certain antioxidant metabolites present in the plants (Khan and Ahmed, 2009). Therefore, the results obtained from this study clearly indicate that the antioxidant effect of _T. officinalis_ extract resulted in the protection of liver against CCl₄ induced injury (Allis et al., 1990). It was found that the antioxidant activities of enzymes like SOD, GSH-Px and catalase were considerably decreased in the liver in response to CCl₄ administration compared with control group of animals, which indicates the CCl₄ induced oxidative damage of liver (Guven et al., 2003). The level of antioxidant enzymes was significantly improved by administration of 300 mg/kg of leaves extract to CCl₄ intoxicated rats. This further proves that in addition to hepatoprotective affect, _T. officinalis_ has the ability to restore the antioxidant enzyme activities in CCl₄ damaged liver (Kakkar et al., 1984; Sinha, 1972; Rotruck et al., 1973).

From the whole discussion, it is concluded that the current study provides an important platform for the cure of liver damage caused by CCl₄ intoxication in rats. However, more studies are required to prove the availability of lead compounds from _T. officinalis_ leaves extract having hepatoprotective nature.

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