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Protective action of *Taraxacum officinale* on CCl_4 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 carbontetrachloride (CCl_4) 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 CCl_4. It was also observed that due to CCl_4 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 CCl_4 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

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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. Phytotheraphy 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

Carbontetrachloride (CCl_4) 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 (hepatotoxin control) received a single dose of 5 ml/kg of CCl_4 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 CCl_4 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 administrated with single dose of 5 ml/kg of CCl_4 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 CCl_4 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

Table 1. Phytochemical analysis of leave extracts of *T. officinalis*.

Constituent	Relative abundance
Polyphenols	++
Flavonoids	++
Alkaloids	++
Glycosides	+
Reducing sugar	++
Saponins	+
Tannins	+

++ = Highly present, + = Present

(GPx) were assayed in the hepatic tissue of control and experimental group of animals by using methods reported by Kakkar et al. (1984), Sinha (1972) and Rotruck et al. (1973), respectively. Whereas, the level of lipid peroxide-malondialdehyde (MDA) was also determined by using serum, as well as liver tissue of control and experimental groups of animals (Berton et al., 1998).

Histopathological studies

The liver tissues were subjected to normal routine histological procedures, stained with hematoxylin-eosin and examined using the light microscope for any morphological changes (Kleiner et al., 2005).

Statistical analysis

Data obtained was analyzed by one way analysis of variance (ANOVA), followed by Bonferoni test for comparison by using SPSS software version 16.0, and the $P < 0.05$ was considered as statistically significant.

RESULTS

The preliminary screening of phytochemicals from leaf samples of *T. officinalis* revealed the presence of polyphenols, alkaloids, flavonoids, glycosides, reducing sugar, saponins and tannins. Results indicate that proportion of polyphenols, flavonoids and alkaloids found in the extract was relatively higher than the other phytochemicals (Table 1). Methanolic leave extracts of *T. officinalis* (150 and 300 mg/kg bw) when given orally for 21 days showed hepatoprotective activity in CCl_4 induced hepatic damage in rats. Results show increases in the liver enzymes like ALT, AST, ALP and bilirubin in CCl_4 intoxicated animals when compared with that of the control group of rats (Table 2). Treatment of CCl_4 induced animals with different concentrations of plant extracts (150 and 300 mg/kg) significantly reduced ($P < 0.05$) CCl_4 induced elevations in enzymes on dose dependent manner as compared to control. The recovery of hepatic injury was observed in animals treated with plant extracts as well as with silymarin. Hepatic injury caused by CCl_4 administration at a dose of 5 ml/kg body weight showed significant increase in the lipid profile (total cholesterol, triglycerides, LDL and VLDL) levels in liver tissues. Whereas HDL level was decreased as compared to that of control group of rats ($p < 0.05$). However, the treatment of CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 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.

DISSCUSION

Carbon tetrachloride (CCl_4) 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 CCl_4 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.

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Control	72.4±0.24	38.1±0.21	114.21±0.42	0.15±0.07	1.73±0.07
CCl_4	217.43±0.23[a]	115.17±0.93[a]	224.93±0.54[a]	0.39±0.01[a]	2.17±0.05[a]
CCl_4+150 mg/kg extract	155±0.27	88±0.61	173.38±0.19	0.31±0.03	1.94±0.04
CCl_4+300 mg/kg extract	72.6±0.24[b]	35.15±0.27[b]	123.73±1.71[b]	0.24±0.06[b]	1.79±0.04[b]
CCl_4+ Silymarin (100 mg/kg)	149.81±1.53[b]	88±0.47[b]	138.83±0.61[b]	0.25±0.07[b]	1.83±0.05[b]

Results were expressed as Mean± SEM (n= 5); a P<0.05 compared with control group of rats; b P<0.05 compared with CCl_4 induced group of rats.

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

Groups	Cholesterol (mg/dl)/dl)	Triglyceride (mg)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	88.76±0.32	77.35±0.23	29.38±0.35	54.7±0.19	19.41±0.33
CCl4	132.54±0.19[a]	135.48±1.18[a]	23.83±0.34[a]	98.37±0.23[a]	27.46±0.16[a]
CCl_4+150 mg/kg extract	112.29±0.14	97.11±0.24	20.78±0.27	58.57±0.14	16.9±0.43
CCl_4+300 mg/kg extract	84.59±0.26[b]	75.9±0.25[b]	21.11±0.17[b]	51.38±0.31[b]	13.39±0.18[b]
CCl_4+ Silymarin	99.41±0.18[b]	81.57±0.23[b]	22.51±0.17[b]	62.31±0.24[b]	14.13±0.16[b]

Results were expressed as Mean± S.E.M (n= 5). [a]P<0.05 compared with control group of rats. [b]P<0.05 compared with CCl4 induced group of rats.

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

Groups	Catalase U/mg of protein	GPX U/ mg of protein	SOD U/mg of protein	MDA nm/mg of protein
Control	16.32±0.06	2.65±0.05	46.45±0.04	2.87±0.75
CCl_4	9.74±0.04[a]	1.64±0.04[a]	29.5±0.07[a]	8.83±0.13[a]
CCl_4+150mg/kg extract	14.15±0.04	1.98±0.03	42.19±0.03	3.88±0.73
CCl_4+300mg/kg extract	15.67±0.09[b]	2.78±0.06[b]	45.34±0.08[b]	2.81±0.48[b]
CCl_4+ Silymarin	12.43±0.08[b]	1.65±0.08[b]	47.83±0.08[b]	4.51±0.81[b]

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). [a]P<0.05 compared with control group of rats and [b]P<0.05 compared with CCl4 induced group of rats.

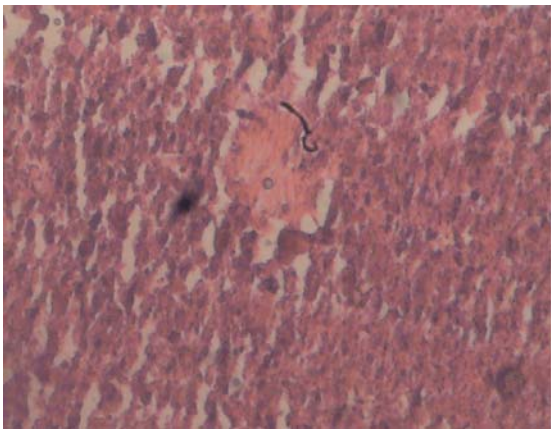

Figure 1. Liver tissues of normal rat.

released into blood stream and cause cellular necrosis, which is used as a diagnostic measure of liver damage (Alexander and Griffiths, 1993).

This study clearly indicates that a significant reduction in CCl_4 elevated liver enzymes was occurred after treatment with *T. officinalis* leaves extract in a dose dependent manner (Friday et al., 2010), which represents a protective effect of the extract on the damaged liver tissues (Chioma et al., 2008; Sumitha and Thirunalasundari, 2011). It was further investigated that the elevated level of serum marker enzymes (AST, ALT and ALP) produced by CCl_4 treatment was returned towards the normal level in the sample group of animals treated with plant extract compared with control (Friday et al., 2010). Furthermore, the biochemical parameters like

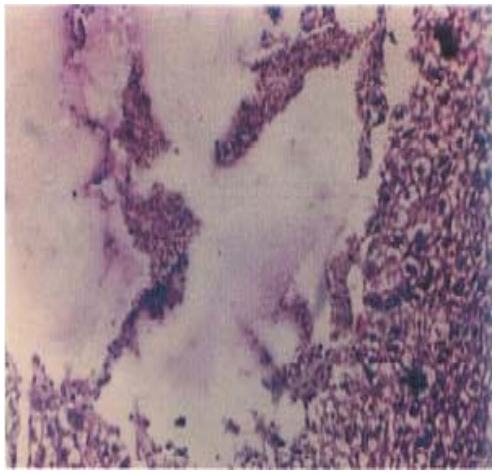

Figure 2. Liver tissue of rat treated with CCl4.

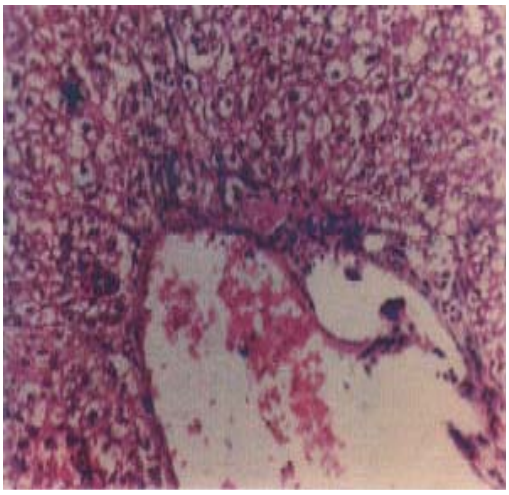

Figure 4. Liver tissue of rat treatments with 100 mg/kg of silymarin.

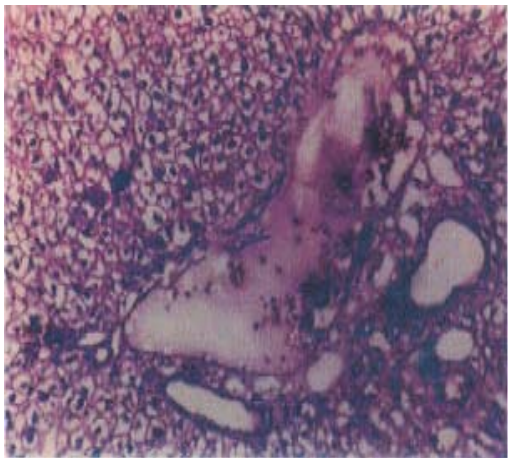

Figure 3. Liver tissue of rat treated with 300 mg/kg of methanolic leaves extract of *T. officinalis.*

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 CCl_4 by chelating with by-products produced from CCl_4 metabolites. This demonstrates the hepatoprotective role of plant extract which not only involved in considerably decrease in the effect of CCl_4 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 CCl_4 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 CCl_4 administration compared with control group of animals, which indicates the CCl_4 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 CCl_4 intoxicated rats. This further proves that in addition to hepatoprotective affect, *T. officinalis* has the ability to restore the antioxidant enzyme activities in CCl_4 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 CCl_4 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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