Effect of leaf extracts of *Taraxacum officinale* on CCl_4 induced Hepatotoxicity in rats, *in vivo* study

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Abstract:*Taraxacum officinale* L is a medicinal plant, which has enormous medicinal values against various types of liver disorders and it has traditionally been used for the treatment of liver problems by people from the South East Asia. Previously we have screened the crude methanolic extract of *T. officinale* against cytotoxicity induced by CCl_4.Present study was designed to compare the protective effect of ethanolic and n-hexane extract of leaves in carbon tetrachloride (CCl_4) induced liver toxicity in rats. The extract (200 mg/kg and 400mg/kg body weight) along with silymarin (100 mg/kg) a standard drug was administered to experimental animals. It was observed that ethanolic plant extract has significantly reduced the negative effect of CCl_4 as compared to n-hexane extract and effect of extract was increased with increasing dose level. Although both leaf extracts decreased the concentration of TBARS, H_2O_2 and nitrite contents which enhance due to CCl_4 toxicity but effect was higher in ethanolic extract. The results clearly indicated that *Taraxacum officinale* ethanolic leaves extract has better protective effect against CCl_4 induced liver tissues toxicity. This claim was also supported by histopathological results obtained during this study and this might be due to presence of various polar phytochemicals that might be more prevent in this extract.

Keywords: *Taraxacum officinale,* ethanolic extract, n-hexane extract, histopathology.

INTRODUCTION

The healthy state of the body depends on proper functioning of liver for excretion of wastes, xenobiotic metabolism and its dysfunction by toxic chemicals results in serious health problem (Ahmed *et al*., 1987, Sing and Rao, 2008). Synthetic drugs available in market for liver treatment cause many complications (Sanjiv, 2002). The liver damages can be indicated by assessing the level of liver enzymes and proteins as well as assessment histopathological changes in liver tissues. Liver enzymes that are used in detection of liver malfunction are; alanine amino transferase (ALT) and Aspartate aminotransferase (AST). The liver tissue damage can also be accessed through histological studies as increased permeability of liver cells is an important indicator of liver damage (Sing and Rao, 2008; Edet *et al*., 2011). The liver therapy can be achieved by traditional medicines from medicinalas they are safer, easily reachable, economical and have less toxicities and side effects compared to synthetic medicines (Nair and Chanda, 2007). Therefore, there is growing interest in herbal medicines (Hussain *et al*., 2009).

In Pakistan, medicinal plants are used extensively in folk medicines including medicines to cure liver diseases. The medicinal activities of plants are attributed to their bioactive compounds that include Phenolics, flavonoids, terpenoids, glycosides and alkaloids asthey are proved to be efficient precursors for drugs formation. These Phytochemicals act additively, individually or in synergic way for the progress of Human health (Schutz *et al*., 2006) due to their anti-allergic and anti-tumor activities (Zhang *et al*., 2005).

Taraxacum Officinalis of family Asteraceae, (local name: Dandelion) is used in rural rareas of Pakistan to cure liver and kidney disorder and various other ailments including cough, bronchitis, asthma, GIT infection, and inflammation (Ahmed *et al*. 2013, Dirlesi *et al*., 2012; You *et al*., 2010). The present work was conducted to prove scientifically hepatoprotective activity of *T. officinale* in animal model.

MATERIAL AND METHODS

Materials

Fresh leaves of *T. officinalis* L. were collected in April 2009 from PMAS-AAUR Rawalpindi, Pakistan. After identification, the samples were air-dried and grinded finely. 200g of the powder samples were soaked in 1 L ethanol and n-hexane respectively for 48 hours and filtered through Watman No.1. The filtrate were dried at 30ºC and collected as ethanolic and n-hexane extracts. For each treatment, extracts were re-suspended in distilled water and then administered to the animals at 200 mg/kg and 400 mg/kg body weight.

Chemicals and Reagents

Reagents used for assays were commercial kits/products of Randox, USA. The chemical used were of analytical

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grade and purchased from Merck and BDH. CCl_4 was purchased from Sigma-Aldrich Chemical Co.

Phytochemical analysis

Phytochemical of ethanolic and n-hexane extracts of the leaves were carried out by following established protocols (Ahmed *et al*. 2013; Harbone, 1973; Trease and Evans, 1983)**.**

Animals

Sixty male (30 for each extract) albino rats of Wistar strain, having weight of 120-160 grams and maintained under standard animal house conditions were used in this study. All animals were allowed to rat chow (Feed Mills, Islamabad) and water *ad libitum.* Total 5 experimental group each having 6 animals were treated under standard conditions. The protocol was approved by animal ethics committee of PMAS-Arid Agriculture University Rawalpindi, Pakistan.

Acute toxicity tests

The method of Lorke (1983) was followed for acute toxicity tests. Doses of 200mg/kg and 400mg/kg were then chosen as concentrations of the extracts to be administered to the rats (Ahmed *et al*., 2013).

Experimental design for hepatoprotective activity

A total of 60 animals (30 for each extract) were taken and were divided into 5 groups of 6 animals each (n=6/group). Group I (control) received olive oil orally for 20 days. Group II (hepatotoxin control) received a single dose of 5ml/kg of CCl_4 diluted in olive oil, 1:1 ratio for 20 days alternatively. Group III (Test group 1) were administered with single dose of 5 ml/kg of CCl_4 along with vehicle alternatively for 20 days and it was followed by the treatment with 200 mg/kg of *T. officinale* ethanolic and n-hexane leaves extract orally for 28 days. Group IV (Test group 2) animals were administrated with single dose of 5 ml/kg of CCl_4 for 20 days, followed by treatment with 400 mg/kg of *T. officinale* ethanolic and n-hexane leaves extract for 28 days. Group V (Hepatoprotective agent control) animals were administered with CCl_4 for 20 days and followed by the treatment with 150 mg/kg of known hepatoprotective agent (silymarin). On 29 days, the animals were anaesthetized using chloroform and blood was collected by cardiac puncture (Ahmed *et al.*, 2013).

Preparation of serum

Heart puncture technique was exploited to collect blood samples into centrifuge tubes. Serum was prepared by centrifugation for 15 min at 3500 rev/hr and biochemical analyses were performed from serum (Ahmed *et al.* 2013; Harbone, 1973; Trease and Evans, 1983).

Biochemical analysis

Liver pathophysiological enzymes such as "aspartatetransaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lipid profile - total cholesterol, VLDL, HDL, triglycerides" were estimated by commercially available kit methods of Reitman and Frankel (1957), King and Kind (1954). Bile pigments were determined by using Jendrassic and Grof (1938) methods. The activities of anti oxidant enzymes such as" SOD, catalase and 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 hepatic tissues of experimental animals (Ahmed *et al*., 2013; Harbone, 1973; Trease and Evans, 1983).

Histopathological Studies

Normal histological procedures were performed on liver tissues and were stained with Hematoxylin-Eosin followed by microscopic examination for any morphological changes (Ahmed *et al*. 2013; Kleiner *et al.*, 2005).

STATISTICAL ANALYSIS

Data obtained was analyzed by one way analysis of variance (ANOVA) followed by Bonferoni test for comparison using SPSS software version 17.0. The $P<0.05$ was considered as statistically significant.

RESULTS

Phytochemicals

Polyphenols, alkaloids, flavonoids, glycosides, reducing sugars, saponins and tannins were found during preliminary screening of Methanolic leaf extract of *T. officinale* (Ahmed *et al*., 2013). Here, results of phytochemical analysis of the ethanolic and n-hexane fractions of *T. officinale* are shown in table 1. The result indicates that proportion of phenolics and flavonoids was higher in ethanol extract and this might be because of their more hyrophilicty (table 1). These high phenolic contents in ethanolic extract suggest their hepatoprotective effects.

Table 1: Phytochemical analysis of n-hexane and ethanolic leaves extract of *T. officinale*

Phytochemicals	Availability	
	n-hexane extract	Ethanolic extract
Polyphenols	+	+ +
Flavonoids	+	+ +
Alkaloids	+	+
Glycosides	+	+
Reducing sugar	-	++
Saponins	+ +	+ +
Tannins	+	+
+ = present, - = absent		

Table 2: Effect of ethanolic and n-hexane extracts of *T. officinalis* leaves on liver enzyme and Bilirubin

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Control	73.6±0.21	35.1±0.26	115.23±0.48	0.13±0.08	1.81±0.05
CCl_4	215.43±0.25[a]	112.57±0.95[a]	221.96±0.51[a]	0.38±0.01[a]	2.19±0.04[a]
CCl_4+200mg/kg ethanolic extract	154±0.25	86±0.71	131.38±0.19	0.29±0.04	1.91±0.05
CCl_4+400mg/kg ethanolic extract	71.3±0.21[b]	34.45±0.28[b]	102.71±1.82[b]	0.21±0.07[b]	1.68±0.05[b]
CCl_4+200mg/kg n-hexane extract	162±2[*++]	107±1[**++]	182±6[**++]	0.31±0.91[*++]	1.84±0.24[++]
CCl_4+400mg/kg n-hexane extract	94.7±2[++]	74.7±4[++]	123±3[++]	0.26±0.73[++]	1.78±0.09[++]
CCl_4+ Silymarin (100 mg/kg)	148.71±z.50[b]	87±0.48[b]	139.82±0.61[b]	0.24±0.07[b]	1.81±0.06[b]

Results were expressed as Mean± S.E.M (n= 6); 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 ethanolic and n-hexane extracts of *T. officinale* leaves on liver lipid profiles

Groups	Cholesterol (mg/dl) dl)	Triglyceride (mg)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	87.86±0.31	76.32±0.24	28.37±0.31	51.5±0.17	17.48±0.34
CCl_4	128.51±0.18[a]	131.46±2.16[a]	21.72±0.31[a]	97.36±0.24[a]	28.48±0.18[a]
CCl_4+200mg/kg ethanolic extract	111.38±0.11	91.51±0.21	22.58±0.21	68.56 0.13	16.8±0.41
CCl_4+400mg/kg ethanolic extract	82.53±0.23[b]	73.8±0.24[b]	21.51±0.18[b]	49.51±0.32[b]	14.38±0.19[b]
CCl_4+200mg/kg n-hexane extract	115.5±1.79*[++]	113.6±0.28[*++]	19.7±0.98[++]	73.8±0.47[**++]	21.34±0.49
CCl_4+400mg/kg n-hexane extract	102.94±1.14[++]	93.3±1.47[**++]	21.94±0.57[++]	62.85±0.39[*++]	19.32±0.43
CCl_4+ Silymarin	98.45±0.19[b]	80.56±0.24[b]	23.6±0.18[b]	63.32±0.25[b]	15.1±0.15[b]

Results were expressed as Mean± S.E.M (n= 6),
[a] P<0.05 compared with control group of rats, [b] P<0.05 compared with CCl_4 induced group of rats

Table 4: Effect of ethanolic and n-hexane 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	15.35±0.05	2.87 ± 0.06	47.43±0.05	2.96±0.71
CCl_4	9.82±0.02[a]	1.75±0.05[a]	28.5±0.08[a]	8.71±.83[a]
CCl_4+200mg/kg ethanolic extract	13.65±0.05	1.96±0.05	41.18±.02	3.89±0.72
CCl_4+400mg/kg ethanolic extract	14.98±0.08[b]	2.68 ±0.05[b]	44.38±.05[b]	2.92±0.71[b]
CCl_4+200mg/kg n-hexane extract	11.21±0.09	1.79±0.09	33.12±.07	5.13±.21
CCl_4+400mg/kg n-hexane extract	12.21±0.15	2.02±0.08	37.32±.09	3.32±.23
CCl_4+ Silymarin	11.31±0.06[b]	1.78±0.02[b]	46.80±.09[b]	3.61±0.62[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± S.E.M (n= 6),
[a]P<0.05 compared with control group of rats, [b]P<0.05 compared with CCl_4 induced group of rats

Assessment of hepatoprotective activity

Both leaves extracts of *T. officinale* (200 and 400 mg/kg of bow) given orally for 28 days showed hepatoprotective activity in CCl_4 induced hepatic damage in rats. Results showed increases in the liver enzymes like ALT, AST, ALP and bile pigments in CCl_4 intoxicated animals compared to control group (table 2).

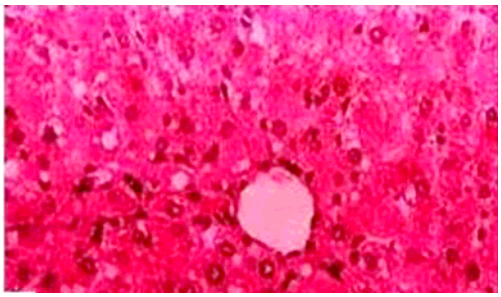

Fig. 1: Liver tissues of normal rat.

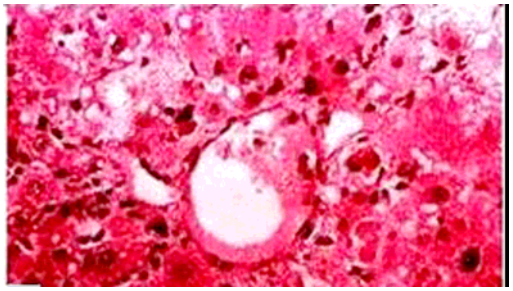

Fig. 2: Liver tissue of rat treated with CCl_4.

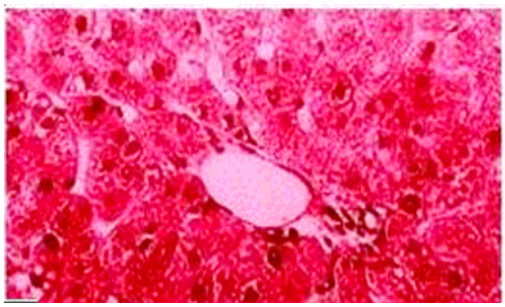

Fig. 3: Liver tissue of rat treated with n-hexane leaves extract of *T. officinalis* L

Fig. 4: Liver tissue of rat treated of ethanolic leaves extract of *T. officinalis* L

Treatment of CCl_4 induced animals with ethanolic and n-hexane extracts significantly ($P<0.05$) reduced CCl_4 induced enzyme elevations in dose dependent manner. The effect was higher in ethanolic extract as compare to n-hexane extract and this might be because of high phenolic and flavonoid contents in ethanolic extract.

The VLDL and LDL but HDL levels deviated from normal in CCl_4 induced group as compared to that of control group of rats ($p<0.05$). Whereas treatment of CCl_4 induced group of rats with ethanolic and n-hexane extracts of *T. officinale* at a dose of 200 mg/kg and 400 mg/kg restored the levels towards normal range (table 3).

The effects of ethanolic leaves extracts of *T. officinale* on the antioxidants enzymes like catalase, GPx and SOD in the serum of control and CCl_4 treated group showed significant reduction ($p<0.05$) as compared to n-hexane extrat. The ethanolic leaves extracts of *T. officinale* 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. officinale*. 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 CCl_4 treated group followed by treatment with ethanolic leaves extracts of *T. officinale* and silymarin ($p<0.05$) (table 4).

Microscopic examination

The results of histopathological study of the liver tissues of the control and CCl_4 treated rats are given in figs. 1-4 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 (fig. 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 (fig. 2) However, treatment of animals with n-hexane leaves extracts of *T. officinale* (fig. 3) and ethanolic extract (fig. 4) represent normal condition of liver tissues and it is assumed that treatment returned that injuries towards normal side.

DISSCUSION

CCl_4 is assumed to cause oxidative stress that is the cause of many pathological conditions including liver damage and death and shows elevated levels of liver enzymes in the blood following cellular necrosis/cell membrane permeability (Ahmed *et al*., 1987; Alexander and Griffiths, 1993; Sing and Rao, 2008). Results of this study show a reduction in CCl_4 elevated liver enzymes (Friday *et al*., 2010) after treatment with ethanolic and n-hexane extracts of *T. officinale* extracts (Sumitha and Thirunalasundari, 2011). During present study elevation in serum marker enzymes such as “AST, ALT and ALP” were observed, due to CCl_4 treatment. However, it was observed that elevated level was returned towards normalization in the plant treated group especially ethanolic extract (Friday *et al*., 2010). Serum enzymes level, bilirubin, total cholesterol, LDL, VLDL and, triglycerides were observed near to the normal level by the treatment of ethanolic extract of *T. officinale*. This exposed the hepatoprotective role of plant ethanolic

extract and the recovery of liver damage at a significant level (Sing and Rao, 2008). Whereas treatment of *T. officinale* extract significantly declined the effect of CCl_4 induced damage (Chungma *et al*., 2007; You *et al*., 2010). Furthermore the antioxidant action of plant extract plays an important role in protection against CCl_4 induced liver injury. Antioxidant enzymes like "SOD, GSH-Px and catalase activities" were significantly decreased in the liver in response to CCl_4 administration compared with control, which indicates that CCl_4 induced oxidative damage of liver. Whereas the level of antioxidant enzymes were significantly improved by administration of 400 mg/kg of leaves extract of *T. officinale* to CCl_4 intoxicated rats. This indicated that *T. officinale* has the potential to normalize these enzyme activities in CCl_4 damaged liver (Rotruck *et al*., 1973).

CONCLUSION

Biochemical and histological results of this study demonstrated that ethanolic leaves extract of *T. officinale* possess more hepatoprotective activity against CCl_4 induced hepatotoxicity in rats compared to n-hexane extract. However, further studies are required to prove to a lead compound with hepatoprotective nature.

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