The Effects of *Taraxacum officinale* L. and *Berberis vulgaris* L. Root Extracts on Carbon Tetrachloride Induced Liver Toxicity in Rats

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Abstract

Background: ***Taraxacum officinale*** **and** ***Berberis vulgaris*** **have long been used as herbal remedies for treatment of a variety of complaints including liver dysfunction and gallbladder disease. However scientifically reliable data are needed to verify their minimum effective doses.**

Objective: In present study, the effects of ***Taraxacum officinale*** **L. and** ***Berberis vulgaris*** **L. root extracts at the different doses 10, 20 and 30 times higher than average dose (THD) used in traditional systems of medicines were tested against carbon tetrachloride (CCl_4) induced liver toxicity in rats.**

Methods: The root extracts of ***T. officinale*** **at doses of 250, 500 and 750 mg/kg/day and** ***B. vulgaris*** **at doses of 300, 600 and 900 mg/kg/day, relative to 10, 20 and 30 THD average doses used in traditional systems of medicines were prepared by dissolving dry extracts in 5% dimethyl sulfoxide in distilled water. Eighty male Wistar rats, 5 months old, were divided in 8 groups of 10 rats each. Liver intoxication was induced in 7 groups by intraperitoneal injection of 1 ml/kg of 1:1 CCl_4 in olive oil for two successive days. One group was kept as control and six different doses of medicinal plants extracts were administered to six groups simultaneously with CCl_4 administration. After three days the serum levels of ALT, AST and ALP, liver tissue glutathione level and catalase activities as well as liver tissue microvesicular steatosis and pericentral coagulation necrosis were determined.**

Results: In control group the blood levels of ALT, AST, ALP and liver tissue injury were increased whereas the serum GSH level and catalase activity decreased significantly after 3 days of beginning of carbon tetrachloride liver toxicity as compared to normal group.
In ***T. officinale*** **treated group at the dose of 750 mg/kg/day, the serum ALT and ALP levels and in** ***B. vulgaris*** **at the dose of 900 mg/kg/day, the serum ALP levels reduced significantly as compared to control group. The liver micro vesicular steatosis was inhibited significantly in both groups at the doses of 30 THD as compared to control group.**

Conclusion: In the present study administration of ***T. officinale*** **and** ***B. vulgaris*** **root extracts at with 30 THD ameliorated CCl_4 induced liver damage.**

Keywords: ***Taraxacum officinale, Berberis vulgaris,*** **Medicinal plants, Liver toxicity, Carbon tetrachloride**

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Introduction

A number of medicinal plants including *T. officinale* and *B. vulgaris* root extracts have been used by traditional medical practitioners for treatment of liver disorders for centuries [1, 2]. *T. officinale* and *B. vulgaris* roots are the main components of several herbal hepatotprotective preparations [3, 4]. In the folk medicine of many countries *T. officinale* is combined with other herbs to treat hepatitis and to enhance body immune response [1]. The European Scientific Cooperative on Phytotherapy recommends *T. officinale* roots for restoration of hepatic and biliary function, dyspepsia, and loss of appetite [5]. Some modern naturopathic physicians assert that *T. officinale* can detoxify the liver and gallbladder, reduce side effects of medications metabolized by the liver, and relieve symptoms associated with liver disease [6]. All parts of the Berberis vulgaris plant have long been used as a herbal remedy for the treatment of a variety of complaints including liver dysfunction, gallbladder disease, diarrhea, indigestion and urinary tract diseases [1, 7, 8]. The hepatoprotective effects of *T. officinale* and berberine extracted from *B. vulgaris* have been reported in experimental studies with different and some in very high doses [8 -11]. In the present study, to investigate the hepatoprotective effects of *T. officinale* and *B. vulgari* root extracts at lowest effective doses, three different doses of each extract were tested against CCl_4 induced liver toxicity in rats.

Materials and Methods

Chemicals

Dimethyl sulfoxide (DMSO), ERBA test kits, sulphosalicylic acid, 5,5'-dithio-bis (2-nitrobenzoicacid), ethylenediaminetetraacetic acid (EDTA), bovine serum albumin, CCl_4, GSH, 2-thiobarbituric acid, and other reagents of the highest grade were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals were used without further purification.

Animals

Eighty 230-250 g male Wistar rats aged 5 months were purchased from Shahid Beheshti University animal house, Tehran, Iran. The animals were maintained under a daily controlled 12 / 12 hr light dark cycle at 23°C and 50% humidity with free access to rat chow and water. All animals received humane care in compliance with the guidelines of the Institutional Ethical Committee of ACECR, Tehran Iran.

Medicinal plants extracts

Preparation of dry hydro-alcoholic (80%) extracts of *T. officinale*, *B. vulgaris* roots were performed by Institute of Medicinal Plant Tehran Iran. Briefly the plants materials were powdered and immersed in hydro-alcoholic (80%) solvent for 24 hours and filtered. Repeat the procedure twice more and mixed the filtrate. Concentrate the filtrate in rotary instrument and then dry it in frieze dryer. The herbal extracts dry powders were dissolved in 5% DMSO in distilled water. Three concentrations of each extract were prepared according to 10, 20, 30 THD average doses used in traditional medicine i.e. 250, 500 and 750 mg/kg/day for *T. officinale* root extracts respectively and 300, 600 and 900 mg/kg/day for *B. vulgaris* root extracts respectively.

Experimental protocol

Administration of CCl_4 and plant extracts

The total 80 rats were divided into eight groups of 10 animals each. One group was kept as normal and liver damage was induced in 7 groups by intra-peritoneal injection of 1

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ml/kg body weight of 1:1 carbon tetrachloride in olive oil for two successive days according to modified Zimmerman method [12]. Rats without CCl_4 treatment (normal group) received an equal volume of olive oil in the same manner. One of the 7 intoxicated groups was kept as control and herbal extracts were administered to other 6 groups. The herbal extracts were injected intraperitonealy for three days following carbon tetrachloride administration.

Experimental design

Normal group: Rats in this group received intraperitoneal injections of vehicles i.e. olive oil and DMSO in distilled water.

Control group: The CCl_4 treated rats received DMSO in distilled water as vehicle for three days.

***T. officinale* groups:** *T. officinale* root extract at the doses of 250, 500 and 750 mg/kg/day was administered to three groups for three days.

***B. vulgaris* groups:** *B. vulgaris* root extract at the doses of 300, 600 and 900 mg/kg/day was administered to three groups for three days.

Serum biochemical study

Three days after induction of liver damage with CCl_4 and medicinal plants extracts treatment, the blood samples were collected from the animal's hearts under chloroform anesthesia. The serum liver enzymes including ALT, AST, and ALP levels were estimated in all groups by International Federation of Clinical Chemistry (ERBA test kits) method [13, 14] and expressed as international units per liter (IU/L).

Liver anti-oxidative study

Sample preparation

Immediately after the blood samples were collected under chloroform anesthesia, the animals were sacrificed and the residual blood in liver as much as possible was removed by ice cold 0.9% NaCl perfusion and then liver was removed from the body. The isolated livers were weighed and kept at −80°C as soon as possible until use. For the assays of liver tissue GSH level and catalase activity, a part of liver was homogenized in 9 volumes of ice-cold 0.15 M KCl containing 1.0 mM EDTA using a glass homogenizer with a Teflon pestle. The homogenate was sonicated on ice twice for 30 s and centrifuged at 12,000 g for 15 min at 4°C. The supernatant was dialyzed against 100 volumes of the same buffered solution at 4°C for 60 min.

Determination of GSH level and catalase activity in liver tissue

The liver tissue reduced GSH levels were determined by the method of Jollow et al [15]. Briefly, the supernatant samples were kept at 4°C for at least 1 hour and then centrifuged at 1200 g for 5 minutes at 4°C. The assay mixture contained 0.1 ml filtered aliquot, 2.7 ml phosphate/EDTA buffer (0.1 M, pH 7.4) and 0.2ml of 5,5'-dithio-bis (2-nitrobenzoic acid) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm on a spectrophotometer. At each determination, a standard curve of GSH was prepared. The catalase activity was measured by Bergmeyer method [16]. The enzymatic method of H_2O_2 decomposition was used for determination of catalase activity. The protein in the liver tissue samples was measured by using bovine serum albumin as a standard according to method of Lowry et al [17].

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Determination of liver tissue histopathological injury

Small pieces of liver were removed and fixed in 10% buffered formalin for 24 hour, then dehydrated in ascending grades of alcohol cleared in xylene and embedded in paraffin wax (58 - 60%) all in automatic tissue processors. Sections were cut at 3µm, double stained with hematoxylin - eosin and examined under the light microscope. The microvesicular steatosis and pericentral coagulation necrosis were determined as indication of CCl_4 induced liver injury. The microvesicular steatosis and pericentral coagulation necrosis were graded as 0 for no injury, grade 1 for low or below 30% injury, grade 2 for moderate or 30- 60% injury and grade 3 for severe or above 60% injury [18].

Statistical analysis

All values obtained are expressed as mean ± SD. All data were analyzed by SPSS ver.11.5. The group's data means were compared by one-way analysis of variance and Tukey's post hoc test. The level of significance was set at <0.05.

Results

The serum liver enzymes levels, liver tissue GSH levels and catalase activities are given in table 1 and the data of liver histopathological injury are given in table 2.

Table 1 – The serum liver enzymes levels and liver tissue catalase activities and GSH levels in normal and CCl_4 treated rat groups

Groups of 10 rats	Aspartate transaminase (U/L)	Alanine transaminase (U/L)	Alkaline phosphatase (U/L)	Glutathione (nM/mg protein)	Catalase (U/mg protein)
Normal	112 ± 23	63 ± 12	340 ± 57	22.87 ± 4.3	19.31 ± 4.1
Control	1561 ± 196	1225 ± 254	1200 ± 223	10.72 ± 1.0	13.29 ± 3.6
T. officinale 250 mg/kg/day	1530 ± 132	1100 ± 192	1215 ± 199	11.33 ± 2.2	13.23 ± 1.8
T. officinale 500 mg/kg/day	1344 ± 173	1052 ± 201	1012 ± 174	13.78 ± 1.9	13.95 ± 0.8
T. officinale 750 mg/kg/day	946 ± 71*	763± 91	565 ± 47**	13.71 ± 1.0	13.33 ± 1.9
B. vulgaris 600 mg/kg/day	1403 ± 148	1361 ± 255	1216 ± 232	13.21 ± 1.4	14.05 ± 2.4
B. vulgaris 300 mg/kg/day	1312 ± 115	1249 ± 161	1312 ± 218	11.55 ± 1.9	13.05 ± 2.9
B. vulgaris 900 mg/kg/day	1510 ± 105	1231 ± 178	604 ± 51*	14.31 ± 2.1	13.55 ± 2.1

Values are given as Mean ± SD. $p<0.01^*$, $p<0.0001^{**}$ as compared to control group.
The medicinal plants treated groups were compared to control group.

Table 2- The liver tissue histipathological injury parameters in normal and CCl_4 treated rat groups.

Groups of 10 rats	Microvesicular Steatosis (%)	Pericentral Coagulation necrosis (%)
Normal	0.222 ± 0.01	0.111 ± 0.01
Control	2.83 ± 0.40	2.50 ± 0.54
T. officinale 750 mg/kg/day	1.82 ± 0.16**	2.33 ± 0.81
B. vulgaris 900 mg/kg/day	1.83 ± 0.31 *	2.52 ± 0.74

Values are given as Mean ± SD. $p<0.01^*$, $p<0.001^{**}$ as compared to control group.
The medicinal plants treated groups were compared to control group.

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Serum liver enzymes

In control group, a significant increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphates (ALP) concentration (1561 ± 196 U/l, 1225 ± 254 U/l and 1200 ± 223 U/l, respectively) were recorded three days after CCl_4 administration as compared to the normal group (112 ± 23 U/l, 63 ± 12 U/l and 340 ± 57 U/l respectively). The statistical comparisons of these two groups were not showed in Table 1.

In *T. officinale* and *B. vulgaris* root extracts treated groups at the doses of 10 and 20 THD the serum liver enzymes levels did not differ as compared to the control group.

In *T. officinale* root extract treated group at the dose of 750 mg/kg/day (30 THD) the serum AST and ALP levels (946 ± 71 and 565 ± 47 respectively) were significantly reduced ($p<0.01$ and $p<0.0001$ respectively) as compared to the control group.

In *B. vulgaris* root extract treated group at the dose of 900 mg/kg/day (30 THD) the serum ALP level (604 ± 51) were significantly reduced ($p<0.01$) as compared to control group.

Liver tissue GSH level and catalase activity

In control group, a significant decrease in liver tissue GSH level and catalase activity occurred three days after CCl_4 administration (10.72 ± 1.0 nmol/mg protein and 13.29 ± 3.6 U/mg protein respectively), that were significantly lower as compared to the normal CCl_4-untreated group (22.87 ± 4.3 nmol/mg protein and 19.31 ± 4.1 U/mg protein respectively).

In both *B. vulgaris* and *T. officinale* root extracts treated groups with different doses of plants extracts treatment, liver GSH level and catalase activity didn't differ significantly as compared to the control group (Table 1).

Liver histopathological injury

Severe microvesicular steatosis and pericentral coagulation necrosis were observed in control group (2.83 ± 0.40 and 2.50 ± 0.54 respectively), as compared to the normal group (0.222 ± 0.01 and 0.111 ± 0.01 respectively). The microvesicular steatosis and pericentral coagulation necrosis were not determined in *T. officinale* and *B. vulgaris* root extracts treated groups at the doses of 10 and 20 THD due to high serum liver enzyme levels in these groups same as control group.

The microvesicular steatosis in *T. officinale* and *B. vulgaris* root extracts treated groups at the doses of 30 THD were 1.82 ± 0.16 and 1.83 ± 0.31 respectively which were significantly lower ($p=0.001$ and $p=0.002$ respectively), as compared to the control group. The pericentral coagulation necrosis was not changed in both herbal extracts treated groups as compared to the control group (Tab. 2).

Discussion

Carbon tetrachloride is a well-known hepatotoxin and exposure to this chemical induced oxidative stress to many body tissues evidenced by deactivation of superoxide dismutase, catalase, and glutathione peroxides enzymes [19-21]. CCl_4 induced acute liver injury is characterized by liver cell necrosis and steatosis resulting in elevation of serum liver enzymes levels including alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase [12].

In the present study, CCl_4 administration to rats caused severe hepatic tissue injury evidenced as microvesicular steatosis and pericentral coagulation necrosis as well as significantly elevated levels of serum liver enzymes and decrease in liver tissue GSH level and catalase activities.

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Administration of *T. officinale*, and *B. vulgaris* root extracts at 10 and 20 times of human doses (THD) to CCl_4 treated groups, did not influence the liver toxicity, where as at higher dose i.e. 30 THD to two groups of rats attenuated the liver toxicity as indicated by lowering liver enzyme levels and amelioration of histopathological changes in the liver tissue. Both medicinal plants treatments at doses of 10, 20 and 30 THD did not influence the liver tissue GSH level and catalase activities in the present study. The underlying mechanism for hepatoprotective effects of *T. officinale*, and *B. vulgaris* root extracts remains to be elucidated.

T. officinale root extract is a rich source of vitamins A, B complex, C, and D, as well as minerals such as iron, potassium, and zinc as well as phytosterols and bitter constituents like taraxecerin and taraxcin [22]. *B. vulgaris* root extract also contain isoquinoline alkaloids such as berberine, as well as carbohydrates, organic acids, some vitamins, polyphenolic compounds, pectin, tannin, mineral elements [23]. However, the presence of these chemicals in *T. officinale* and *B. vulgaris* may produce metabolic changes in favor of liver protection. The anti-inflammatory and antioxidant activities of *T. officinale* and antioxidant, cytoprotective and hepatoprotective properties of *B. vulgaris* have also been reported in experimental studies [24-27].

In the present study, the liver protective effects of *T. officinale* and *B. vulgaris* root extracts were observed without significant effects on the liver tissue GSH level and catalase activity as markers of body anti-oxidative defense system. These indicate that protective effects on CCl_4 induced liver injury observed in this study may not be only due to anti-oxidative activities of the two extracts as demonstrated in other studies [9, 27].

It is established that several oxidative metabolic disturbances as well as inflammation and regeneration are involved in the tissue damage following CCl_4 induced acute liver injury [19]. However, the observed effects of *T. officinale* and *B. vulgaris* root extracts against CCl_4 intoxication in present study may be due to the direct or indirect favorable effects of the plants chemical constituents such as flavonoids and phytosterols as well as vitamins and minerals on liver cellular metabolism, inflammation and regeneration [28, 29].

In conclusion, *T. officinale* and *B. vulgaris* root extracts at 30 THD prevented CCl_4 induced hepatotoxicity in rats. In addition, the present findings indicate that administration of *T. officinale* and *B. vulgaris* root extracts at the doses of 30 THD to CCl_4 intoxicated rats, prevent hepatotoxicity without significant influence on hepatic antioxidant properties. Further studies are required to evaluate the efficacy of combined administration of both medicinal plant extracts at different doses in experimental studies.

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