The Protective Effect of Medicinal Herbs Extracts Including *Cynara scolymus* L., *Cichorium intybus* L. *Taraxacum officinal* L. and *Berberis vulgaris* L. in Single and in Combination Form in CCl_4 Induced Rat Liver Toxicity

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Received: 14 Nov. 2011 **Accepted:** 10 Mar. 2012

Abstract

Background: Several herbal remedies are used in traditional medicine for treatment of liver disorders, but their efficacy, safety, and dosage have not been investigated so far.

Objective: The present study was aimed to evaluate the liver protective effects of medicinal herbs extracts such as *Cynara scolymus* leave (artichok), *Cichorium intybus roots* (chichory), *Taraxacum officinale* root (dandelium), *Berberis vulgaris* (barberry) root and stems extract in single and in combination form against carbon tetrachloride (CCl_4) induced liver toxicity.

Methods: Total 70 wistar male rat, aged 5 months were divided in 7 groups of 10 rats each. One group kept as normal and out of six CCl_4 intoxicated groups one served as control, one received mixture of four extract and four groups received artichok, chichory, dandelium, and barberry in the dosage of 150, 300, 300 and 250 mg/kg/day respectively. The plant extracts were injected intra peritoneal simultaneously with intoxication for three days. Three days after intoxication and extract treatments the serum liver enzymes levels such as ALT, AST and ALP as well as serum gluthathion and catalase were determined.

Results: In group receiving mixture of 4 herbal extract the blood level of ALT, AST and ALP were reduced significantly as compared to control group. In all the groups receiving herbal extracts the serum glutathione and catalase levels did not differ as compared to control groups.

Conclusion: In the present study administration of chichory, artichoke, dandelium and barberry in combination form prevent liver intoxication withought influence on serum antioxidant properties.

Keywords: Artichoke, Chichory, Dandelium, Barberry, Herbal medicine, Liver toxicity

[DOR: 20.1001.1.2717204.2012.11.41.9.5]

Introduction

Numbers of plant remedies have been used by traditional practitioners for the treatment of liver disorders for centuries [1, 2]. Artichoke, chichory, dandelium and barberry are the plants used widely in hepatotprotective preparations [3, 4]. Artichoke leave extract is one of the phytopharmaceuticals who's experimential and clinical studies show to be effective for improving digestion and liver function as well as lowering blood cholesterol level [5 - 8]. Chichory root is an herb that has been known for its curative benefits since the first century A.D. This herbal medicine continues to be a popular herbal remedy due to its healing effects on several ailments including liver diseases, loss of appetite, jaundice, gallstones, gout and rheumatism [3, 4, 9]. Dandelium is combined with other herbs to treat hepatitis, to enhance immune response to upper respiratory tract infections, and others [10]. The Commission E Monographs recommends dandelium root for restoration of hepatic and biliary function, dyspepsia, and loss of appetite [11]. Some modern naturopathic physicians assert that dandelium can detoxify the liver and gallbladder, reduce side effects of medications metabolized by the liver, and relieve symptoms associated with liver disease [12]. All parts of the barberry plant have long been used as an herbal remedy for the treatment of a variety of complaints including liver dysfunction, gallbladder disease, diarrhea, indigestion and urinary tract diseases [1-3]. The hepatoprotective effects of these plants have been reported in several experimental studies [13-17]. In present study the liver protective effect of hydroalcholic extract of artichoke leaf, chichory roots, dandelium roots and barberry roots and stems were determined in liver intoxicated rats in single form and in combination.

Materials and Methods

Animals

Total 70 Male 5 month old wistar rats (150 – 200 g), were purchased from central animal house of Shaheed Beheshti University (Tehran, Iran). The animals were housed under standard conditions of light and dark cycle with free access to food (Behparvar products) and water. The experimental protocols were approved by the Institutional Ethical Committee of Jahad Daneshgahee, Tehran Iran.

Drugs

Chemicals employed in these studies were reagent grade. Artichoke leave, chichory and dandelium roots and barberry root and stems were collected from Institute of Medicinal Plant Farm and their identity was authenticated by a botanist (Y. Ajani). One voucher specimen of the each plant (numbers 711, 329, 1654, 1655 respectively) has been deposited in the Central Herbarium of the Research Institute of Medicinal Plants affiliated with the ACECR (Iranian Academic Center for Education, Culture and Research).

Preparation of plants extract: The extracts preparation was performed by Institute of Medicinal Plant Tehran Iran. Briefly the dry plants materials were powdered and extracted with 70% aqueous ethanol using percolation method at room temperature. The extracts were filtered through Whatman no. 1 filter paper and evaporated to dryness under reduced pressure at a maximum of 40ºC using a rotary evaporator instrument.

[DOR: 20.1001.1.2717204.2012.11.41.9.5]

Experimental protocol

The rats were divided in 7 groups of 10 rats each. One group kept as normal and liver intoxication were induced in 6 groups by intra peritoneal injection of 1 ml/kg of 1:1 carbon tetrachloride in olive oil for two successive days. The plant extracts were dissolves in distilled water and injected intraperitonealy simultaneously with intoxication.

Experimental groups

Normal: Rats in this group received no treatment.

Control: One group of CCl_4 intoxicated rats received IP injection of distilled water.

Artichoke: One group of CCl_4 intoxicated rats received IP injection of artichoke 150 mg/kg/day.

Chichory: One group of CCl_4 intoxicated rats received IP injection of chichory extract 300 mg/kg/day.

Dandelium: One group of CCl_4 intoxicated rats received IP injection of dandelium 300 mg/kg/day.

Barberry: One group of CCl_4 intoxicated rats received IP injection of barberry 250 mg/kg/day.

Mixture of 4 herbal extract: One group of CCl_4 intoxicated rats received IP injection of artichoke 150, chichory 300, dandelium 300 and barberry 250 mg/kg/day in combination.

Serum biochemical study

Three days after liver intoxication and extract treatments to rats, the blood samples were collected from rat heart under chloroform anesthesia. The serums were used for assessment of liver function and serum antioxidative activity.

Assessment of liver function: The serum liver enzymes such as serum ALT, AST and ALP were determined in all groups.

Assessment of serum anti oxidative activity: The reduced serum glutathione and catalse enzyme levels were measured as indication of serum antioxidative activity.

Serum glutathione: Serum reduced glutathione (GSH) in the serum was assayed by the method of Jollow et al [18]. Briefly, 1.0 ml of PMS (10%) was precipitated with 1.0 ml of sulphosalicylic acid (4%). The samples were kept at 4°C for at least 1 hour and then subjected to centrifugation at 1200 g for 15 minutes at 4°C. The assay mixture contained 0.1 ml filtered aliquot and 2.7 ml phosphate buffer (0.1 M, pH 7.4) in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm on a spectrophotometer.

Serum catalase activity was assayed by the method of Claiborne et al [19, 20]. Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml PMS (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of k $minutes^{-1}$.

Statistical analysis

All values obtained are expressed as the mean ± SD. Each mean value is compared by one-way analysis of variance and Fisher's protected significant difference for multi comparison as the post hoc test. The level of significance was set at $p<0.05$.

Results

The average finding of blood parameters in all experimental groups at the end of the study are summarized in table 1. Results showed that

[DOR: 20.1001.1.2717204.2012.11.41.9.5]

Table 1- Serum liver enzymes and antioxidative parameters levels in CCl_4 intoxicated rat treated with herbal extracts

Groups (n=10)	Serum parameters				
	Alanine transamina (U/L)	Aspartate transaminase (U/L)	Alkaline phosphatase (U/L)	Glutathione (µM)	Catalase (µM)
Normal	112 ± 23	63 ± 12	340 ± 57	24.16 ± 4.3	18.31 ± 4.1
Control	1561 ± 196	1225 ± 254	1200 ± 223	2.35 ± 1.0	3.29 ± 0.6
Artichok 150 mg/kg	1470 ± 109	1129 ± 203	1194 ± 230	4.52 ± 1.6	4.02 ± 0.9
Chichory 300 mg/kg	1431 ± 121	1221 ± 180	1116 ± 193	4.62 ± 1.8	4.78 ± 2.1
Dandelium 300 mg/kg	1530 ± 132	1100 ± 192	1215 ± 199	4.33 ± 2.2	5.23 ± 1.8
Barberry 250 mg/kg	1312 ± 115	1249 ± 161	1312 ± 218	3.95 ± 1.9	4.45 ± 2.0
Herbal extracts combination	505 ± 52**	291 ± 53**	655 ± 41*	6.52 ± 1.2	6.74 ± 1.6

Values are given as Mean ± SD.
$p<0.01^{*}$, $p<0.001^{**}$ The data of herbal extract treated groups were compared to control group at the end of the study.

carbon tetrachloride (CCl_4) administration to rats caused a marked rise in serum levels of ALT, AST and ALP and marked reduction in GSH and CAT enzyme levels.

In group treated with mixture of 4 herbal extract the blood level of ALT, AST, and ALP were reduced significantly (p=0.0009, p=0.0008 and p=0.003 respectively) as compared to control group. In four groups treated with artichok, chichory, dandelium and barberry extract the blood level of ALT, AST, and ALP did not differ as compared to control group. Determination of GSH and CAT indicate that in all the groups receiving herbal extracts as well as in combination therapy GSH and CAT levels did not differ as compared to control group.

Discussion

Carbon tetrachloride (CCl_4) is a well-known hepatotoxin and exposure to this chemical is known to influence the antioxidant enzymes such as peroxidase superoxide dismutase, catalase and glutathione peroxidase in the body and causes liver injury by the formation of free radicals [21, 22]. Liver injury by CCl_4 induced inflammation of the hepatic cells results in serum elevation in the ALT, AST levels and inflammation of the biliary tract cells results predominantly in an elevation of the serum ALP levels.

In present study the herbal medicine artichoke leaf, chichory root, dandelium root, and barberry root and stems extract did not influence the CCl_4 induce liver toxicity in rats. Interesting finding is that four herbal combination treatments reduced ALT, AST and ALP liver enzyme. All herbal medicine extract at above dosage and combination did not influence antioxidative profile in serum.

The mechanism underlying the protective effect these herbal medicine remains to be elucidated. It is established that plant phenolic such as flavonoids and other antioxidative compound have an important role in the process of many chronic diseases including liver disorders [23-26].

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Artichoke contains cynarin and other flavonoids, polyphenols compound with antioxidant and hepatoprotective effect [27, 28]. Several research works reported that hepato protective effect of *artichok* is due to antioxidant property of its chlorogenic acid and cynarin content [29].

Chicory contains esculetin a phenolic compound found in chicory was investigated for its anti oxidative and anti-hepatotoxic activity [30, 31].

Dandelium 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 bitter constituents like taraxecerin and taraxcin[32, 33]. Its anti-inflammatory liver protective and antioxidant activity were reported in research studies [34-36].

Barberry contains isoquinoline alkaloids such as berberine, as well as carbohydrates, organic acids, some vitamin, poliphenolic compounds, pectin tannin, mineral elements [37]. Antioxidant, cytoprotective and hepatoprotective properties of berberine is reported in experimental studies [38- 40].

Our results support the hepato protective effect of artichoke, chicory, dandelium, and barberry reported by other researcher [27, 30, 35, 38]. But in those studies the authors proposed that, the antioxidative properties of these plants on liver tissue may be an important factor for their protective effects.

Conclusion

The artichoke, dandelium, barberry and chichory extrct at low dosage are not hepatoprotective as single therapy but on combination at low dosage induced hepatoprotective effect. Our suggestion is that, hepatoprotective effects of above plants also investigate at different combination in future studies.

Acknowledgements

This study was supported by a research Grant 445/23 from the ACECER Tehran, Iran. We thank the administration of the Institute of Medicinal Plants for their support in providing the necessary facilities for conducting this study.

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