



Study The protective Effects of *Cichorium intybus* L. Root Extract Against Carbon Tetrachloride-Induced Hepatotoxicity in Broiler Chickens



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AN existing survey was performed to assess the hepatoprotective effects of *C. intybus* root extract on carbon tetrachloride-induced hepatotoxicity in broiler chickens. A total of 240 one-day-old broiler chickens were divided into diverse groups of control, carbon tetrachloride (1 mL/kg body weight IM injection), *C. intybus* root extract (500 mg/kg body weight/daily orally) and finally both *C. intybus* root extract and carbon tetrachloride. Liver samples were dissected and examined histopathologically at the day 31 of maintenance. Numerical grading of histopathological findings was performed according to the types of lesions at different criteria. Liver samples of carbon tetrachloride-treated group harbored moderate fatty degeneration and necrosis. Those treated with *C. intybus* extract showed normal cellular and tissue structure. Chicks faced with carbon tetrachloride and treated with *C. intybus* extract exhibited mild fatty degeneration and normal tissue structure. Chicks treated with *C. intybus* extract displayed the lowermost scores of necrosis (1.08±0.28), degeneration (1.33±0.49), inflammation (1.16±0.38) and total pathologic indexes (3.58±0.79). Broilers faced with carbon tetrachloride displayed the uppermost scores of necrosis (2.83±0.57), degeneration (3.66±0.49), inflammation (2.16±0.38) and total pathologic indexes (8.66±0.98). *C. intybus* extract application to broilers faced with carbon tetrachloride caused statistically significant decrease in the scores give to necrosis, degeneration, inflammation and total pathologic indexes ($P < 0.05$). Findings suggested that 10 days administration of *C. intybus* extract caused significant decrease in the formation of centrilobular hepatic necrosis, fatty changes, inflammation and degeneration of the hepatic cells of broilers faced with CCl₄-induced hepatotoxicity.

Keywords, *Cichorium intybus*, Root extract, Protective effects, Hepatotoxicity, Broiler chicken.

Introduction

Commercial broiler chickens are disposed to oxidative stress throughout their growth stages [1]. Oxidative stress happens when synthesis of reactive oxygen species (ROS) surpasses from its elimination by antioxidants [1, 2]. Consequently, liver is the most susceptible tissue which will face with degeneration, fibrosis, necrosis and even hepatic failure [3]. Thus, it is crucial to balance the broiler antioxidant diet to prevent from the occurrence of oxidative stress.

Medicinal plants have adequate potential to act as complete antioxidant agents. Antioxidants of these natural sources have no adverse carcinogenic, mutagenic and teratogenic effects of synthetic antioxidants and are readily available at low cost [4]. In recent years, there has been a great expansion in the application of these natural products as an antioxidants in the broiler industry [5].

Cichorium intybus L. (*C. intybus*), also recognized as chicory, has been employed in Iranian

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traditional medicine as an important food additive with high beneficial and therapeutic effects such as antimicrobial, anti-diabetic, anti-hepatotoxic, anti-hyperuricemia, anti-hypertriglyceridemia and immunoenhancement activities [5, 6]. It is mainly full from therapeutic chemical components with high antioxidant effects [5,6]. *C. intybus* extract is mostly used to purify liver and blood disorders [5,6]. Documented researches revealed that *C. intybus* extract has a great hepatoprotective activity, particularly in some kinds of stress-induced toxicities [7,8]. Nevertheless, some aspects of the administration of *C. intybus* extract as an antioxidant and protective agent on stress-induced toxicities, particularly hepatotoxicity are still unidentified.

Hepatic intoxication induced by carbon tetrachloride (CCl₄) is mainly used as an experimental model to evaluate the oxidative stress in both *in vivo* and *in vitro* circumstances [9]. It has been described that CCl₄ endures metabolic activation over hepatic microsomal cytochrome P450 enzymes to procedure toxic trichloromethyl radical with high oxidative stress potential which mainly caused destruction of hepatocytes and eventually hepatic failure [10].

Rendering the high importance of oxidative stress in the broiler chicken industry and lack of experimental examination about the protective roles of natural medicinal plants on hepatotoxicity in this model, an existing research was performed to assess the protective effects of *C. intybus* root extract on CCl₄-induced hepatotoxicity in broiler chickens.

Materials and Methods

Plant materials

Healthy and fresh *C. intybus* roots were prepared from the Mazandaran province, North of Iran. First of all, extraction was done on Pars Imen Daru company (Iran). *C. intybus* roots were confirmed by an expert professor in the field of medicinal plants. At that time, roots were dried in shed at room temperature for about 15 days. Dried roots were ground into a fine powder. After that, 100 mg powdered roots were soaked in 95% ethanol (Merck, Germany) for about three days. Subsequent solution was filtered using a filter paper (Whatman No. 1.). The attained extract was concentrated using rotary evaporator at 50 °C. After that, achieved extract was dissolved using Dimethyl Sulfoxide (DMSO, Merck, Germany), weighed and stored at 4 °C.

Broiler chickens

A total of 240 healthy one-day-old broiler chickens (Ross 308) with the same body weight (50 g) were used in this experiment. Broilers were divided into 8 diverse groups, with three replicates per treatment and 10 chicks per replicate. Broilers were raised in floor cages with free access to feed and water and controlled ventilation. Temperature was kept at 32°C for the first 4 days and then gradually decreased according to the normal management practices in a way that the temperature reach to 22°C at day 28. The light program was 23 h of light and 1 h of dark. Basal diet was formulated according to NRC (1994) [11] for broilers from 0 to 31 days of age. Through the examination, no antibiotics were given to chicks. Chicks were checked twice daily for mortality if any. Chicks were kept under the same hygienic, managerial, and environmental circumstances. The research had 31 days duration.

Study design and treatments

Table 1 characterizes the groups of broiler chickens used in the current experiment. The chicks were kept thirsty for about 4 hours and then *C. intybus* root extract was presented orally to chicks. Mortality factor, feed intake and weight of the groups were measured.

Histopathological examination

All broiler chickens were slaughtered humanely at the end of the experiment (day 31). The liver of broiler chicks were dissected out for supplementary examination. Liver after being removed from broiler chicks were rinsed in saline solution for 2-3 times to remove any blood debris attached on the external surface. At that moment, the liver tissue was cut into small pieces of approximately 4-5 mm and fixed in neutral buffered formalin (10%, pH 7, 20–24°C) for 24 hours, dehydrated in ethyl alcohol and xylene, inserted in paraffin, cut into 5 µm sections, and stained with Haematoxylin and Eosin (H & E) for microscopic investigation [12]. In order to assess and record the histopathologic changes of liver, observed damages were divided into four different scales according to the Table 2 [13].

Statistical analysis

All tests were performed in triplicate. Statistical analysis was done using the SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA). Normality of achieved data was assessed using the Kolmogorov-Smirnov test. One-way Analysis of Variance tests (One-way ANOVA) was used to compare the mean of achieved data.

TABLE 1. Groups of broiler chickens used in the current experiment.

No	Groups	Definition
1	Control	Chicks were fed under standard conditions and diets without any supplementation
2	Carbon tetrachloride control (CATC)	Chicks were received carbon tetrachloride (1 mL/kg body weight IM) on days 24 and 25
3	<i>C. intybus</i> protective control (CIPC)	Chicks were received <i>C. intybus</i> root extract (500 mg/kg body weight/ daily orally) from days 20 up to 23 (for 4 days)
4	<i>C. intybus</i> therapeutic control (CIIC)	Chicks were received <i>C. intybus</i> root extract (500 mg/kg body weight/ daily orally) from days 26 up to 29 (for 4 days)
5	<i>C. intybus</i> protective-therapeutic control (CIPTC)	Chicks were received <i>C. intybus</i> root extract (500 mg/kg body weight/ daily orally) from days 20 up to 29 (for 10 days)
6	<i>C. intybus</i> protective treatment (CIPT)	Chicks were received <i>C. intybus</i> root extract (500 mg/kg body weight/ daily orally from days 20 up to 23 (for 4 days before the occurrence of toxication)) and also carbon tetrachloride (1 mL/kg body weight IM on days 24 and 25)
7	<i>C. intybus</i> therapeutic treatment (CITT)	Chicks were received carbon tetrachloride (1 mL/kg body weight IM on days 24 and 25) and also <i>C. intybus</i> root extract (500 mg/kg body weight/ daily orally from days 26 up to 29 (for 4 days after the occurrence of toxication))
8	<i>C. intybus</i> protective-therapeutic treatment (CIPTT)	Chicks were received <i>C. intybus</i> root extract (500 mg/kg body weight/ daily orally from days 20 up to 29 (for 10 days)) and also carbon tetrachloride (1 mL/kg body weight IM on days 24 and 25)

TABLE 2. Types of lesions evaluated and the criteria used for their grading.

Criteria	Evaluation criteria	Points
Vacuolar degeneration*	<5%	1
	Between 5% to 33%	2
	Between 33% to 66%	3
	66%<	4
Necrosis	Lack of necrosis	1
	1-3 necrotic cells	2
	3-6 necrotic cells	3
	Higher than 6 necrotic cells	4
Kupffer cell enhancement and inflammation	Lack of inflammation	1
	Mild	2
	Average	3
	Severe	4

*The criterion of obtaining of table numbers is based on the mean of the factors evaluated in 3 ranges with a magnification of $\times 400$ in each tissue section.

Duncan test was also used to assess the significant level within treatments. P value <0.05 was considered as statistical significant level.

Results

Figure 1 discloses the histopathological properties of hepatic cells of broiler chickens of the control group No 1). Normal cellular and tissue structure were presented in the hepatic cells. Figure 2 discloses the histopathological properties of hepatic cells of broiler chickens of the CATC group (group No 2). Moderate fatty degeneration (arrows) and also necrosis (arrowheads) were presented in the liver tissues of broiler chicks of this group. Figure 3 discloses the histopathological properties of hepatic cells of broiler chickens of the 3, 4 and 5 groups (as

a candidate of CIPC, CIIC and CIPTC groups). Normal cellular and tissue structure were presented in the hepatic cells. Figure 4 discloses the histopathological properties of hepatic cells of broiler chickens of the 6, 7 and 8 groups (as a candidate of CIPT, CITT and CIPTT groups). Mild fatty degeneration (arrows) and normal tissue structure were presented in the liver tissues of broiler chicks of this group.

Table 3 signifies the histopathologic findings of the anti-hepatotoxic effects of *C. intybus* root extract on liver samples of broiler chickens under toxicity with carbon tetrachloride. Group 2 exhibited the highest complications compared to other groups ($P <0.01$). Broilers of group 2 had the higher scores of necrosis, degeneration,

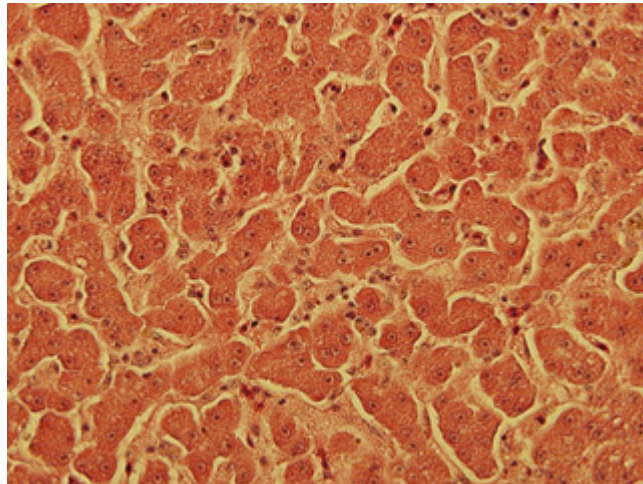


Fig. 1. Histopathological properties of hepatic cells of broiler chickens of the control group No 1) (H&E $\times 400$).

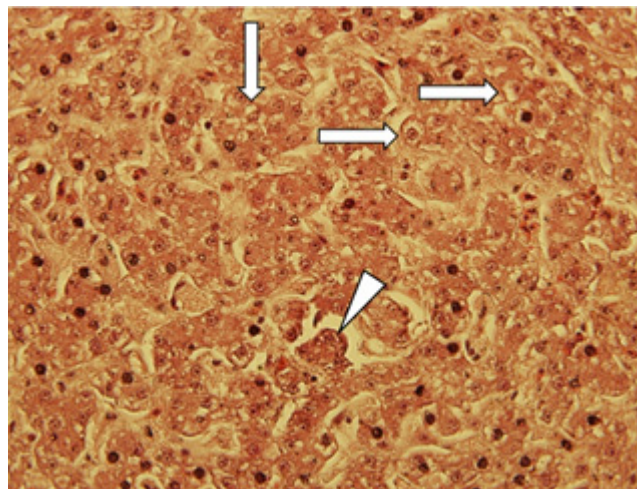


Fig. 2. Histopathological properties of hepatic cells of broiler chickens of the CATC group (group No 2) (H&E $\times 400$). Arrows show degeneration and arrowheads show necrosis.

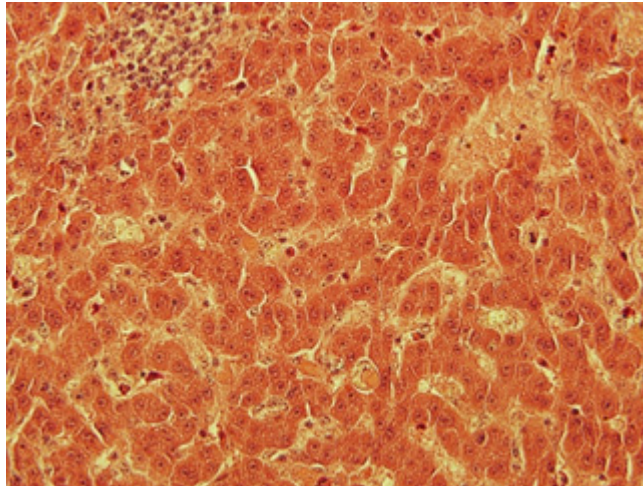


Fig. 3. Histopathological properties of hepatic cells of broiler chickens of the 3, 4 and 5 groups (as a candidate of CIPC, CHC and CIPTC groups). (H&E $\times 400$).

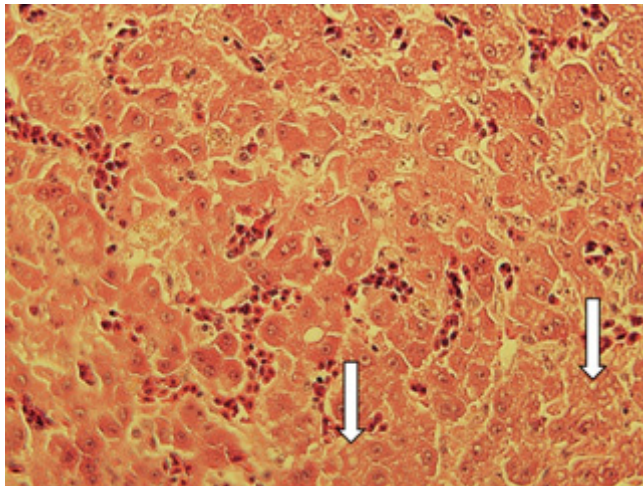


Fig. 4. Histopathological properties of hepatic cells of broiler chickens of the 6, 7 and 8 groups (as a candidate of CIPT, CITT and CIPTT groups) (H&E $\times 400$). Arrows show mild fatty degeneration.

inflammation and also total pathologic indexes compared to other examined groups ($P < 0.01$). The lowest scores of inflammation and also total pathologic indexes were found in broilers of the group 1 ($P < 0.01$). Statistical significant difference was found for the necrosis index between group 2 (CATC) and other examined groups ($P < 0.01$) which showed higher necrosis damages of broilers of group 2 compared with others. Statistical significant difference was found for the degeneration index between group 2 (CATC) and other examined groups ($P < 0.01$) which showed that treatments of *C. intybus* root extract significantly decreased the degeneration damages of liver cells of the

broiler chicks. Statistical significant difference was found for the inflammation index between group 2 (CATC) and other examined groups ($P < 0.01$) which showed higher inflammation of broilers of group 2 compared with others. There were no significant statistical difference for the total pathologic indexes between groups No 1, 3, 4 and 5 and also between groups No 6, 7 and 8. Group No 2 had the highest total pathologic indexes compared to others ($P < 0.01$). Additionally, statistically significant difference was found for the total pathologic indexes between control and treatment groups ($P < 0.01$).

TABLE 3. Histopathologic findings of the anti-hepatotoxic effects of *C. intybus* root extract on liver samples of broiler chickens under toxicity with carbon tetrachloride.

Groups	Necrosis	P Value	Degeneration	P Value	Inflammation	P Value	Total pathologic indexes	P Value
1	1.16±0.38 ^{a*}		1.41±0.66 ^a		1.0±0.00 ^a		3.58±0.66 ^a	
2	2.83±0.57 ^b		3.66±0.49 ^c		2.16±0.38 ^b		8.66±0.98 ^a	
3	1.08±0.28 ^a		1.33±0.49 ^a		1.25±0.45 ^a		3.66±0.88 ^a	
4	1.08±0.28 ^a	0	1.25±0.45 ^a	0	1.25±0.45 ^a	0	3.58±0.99 ^a	0
5	1.08±0.28 ^a		1.33±0.49 ^a		1.16±0.38 ^a		3.58±0.79 ^a	
6	1.41±0.51 ^a		2.25±0.62 ^b		1.41±0.51 ^a		5.08±1.24 ^a	
7	1.33±0.49 ^a		2.25±0.45 ^b		1.25±0.45 ^a		4.83±0.71 ^a	
8	1.25±0.45 ^a		2.33±0.49 ^b		1.33±0.49 ^a		4.91±0.99 ^a	

*Dissimilar letters in each column signifies the statistical difference about $P < 0.05$.

Discussion

Liver is an imperative tissue in the body. It normalizes metabolic functions including cleansing and detoxification and plays a crucial portion in biological adaptations such as excretion, and producing a diversity of coagulation factors. Hepatotoxicity is a severe toxicity of the liver tissue mostly caused by exposure with over dosages of toxic chemicals and drugs [14]. Oxidative stress is the loss of redox homeostasis which caused the commencement and development of hepatic damages mainly familiar with inflammation, necrosis and degeneration [15]. In the broiler chicken industry, it is indispensable to monitor and manage of the oxidative stress because this event can directly affect the rate of production and increase the mortality rate of chicks [15]. Several investigations announced the administration of natural medicinal plants to control and decrease the adverse effects of oxidative stress on hepatic functions [16, 17].

An existing survey disclosed that the chicks treated with *C. intybus* root extract for 10 days exhibited the lowest scores of necrosis (1.08±0.28), degeneration (1.33±0.49), inflammation (1.16±0.38) and total pathologic indexes (3.58±0.79) of the hepatic tissues compared to other tested groups. Reversely, those treated with carbon tetrachloride on days 24 and 25 exhibited the highest scores of necrosis (2.83±0.57), degeneration (3.66±0.49), inflammation (2.16±0.38) and total pathologic

indexes (8.66±0.98) of the hepatic tissues compared to other tested groups. Statistically significant differences were obtained for scores of necrosis, degeneration, inflammation and total pathologic indexes between chicks of the group 5 (treated with *C. intybus* root extract for 10 days) with other groups. Additionally, mild fatty degeneration and normal tissue structure were presented in the liver sections of chicks treated with both carbon tetrachloride and *C. intybus* root extract. Thus, oral administration of *C. intybus* root extract can reduce the hepatotoxic effects of CCl₄ in broiler chickens. In a similar survey, Jamshidzadeh et al. (2006) [18] stated that the *C. intybus* leave extract with concentrations of 60 to 600 µg/ml protected the rat hepatocyte's cells toward CCl₄-induced cytotoxicity. In a similar survey, Khodadadi et al. (2016) [19] conveyed that *C. intybus* has hepatoprotective activities besides improvement in kidney activity and fat metabolism toward heat stress-induced hepatotoxicity in broilers. Additionally, Li et al. (2014) [8] described that the *C. intybus* extract at oral doses of 6, 18, and 54 g/kg/daily presented a significant hepatoprotective effects with significant reduction in the levels of aspartate aminotransferase, hexadecenoic acid, laminin and hydroxyproline enzymes of the on rat model.

Because the toxicity of CCl₄ is supposed to be owing to formation of some free radicals and occurrence of oxidative stress, consequently protective effects of the *C. intybus* extract

is probably occurred by prevention of the activities of P450 cytochrome (accountable for CCl₄ metabolism to free radicals) and also presence of certain chemical components in the *C. intybus* extract with high antioxidant effects and scavenging activities against free radicals. Vitamin C, anthocyanins, flavonoids and polyphenols are some of the main chemical components of the *C. intybus* extract which all have considerable antioxidant effects [20]. Presence of these chemical compounds can diminish and even stop the formation of oxidative stress-induced by CCl₄. Similar findings were also described by Fallah et al. (2011) [21], Abd El-Mageed (2011) [22] and Saeed et al. (2015) [23]. Inulin, tannins, coumarins, sesquiterpene lactones and monomeric flavonoids are some of the major chemical components of the *C. intybus*.

In a current research, hepatoprotective effects of *C. intybus* extract on liver function associate with the changes in the histopathological properties of broiler chicks. Broiler chicks treated with CCl₄ harbored centrilobular hepatic necrosis, some levels of fatty changes, and inflammation and degeneration. Treatment with *C. intybus* root extract prohibited the above-mentioned histopathological changes. Thus, reducing the promotion of specific markers of liver failure may be the probable mechanism of protective action of *C. intybus* root extract toward CCl₄-induced hepatotoxicity in broilers. Additionally, development of antioxidant enzymes and decrease in production of uric acid are considered as probable mechanisms of action of *C. intybus* root extract in inhibition of oxidative stress induced by CCl₄ [19].

Conclusion

In conclusion, the current research revealed that application of *C. intybus* root extract for 10 days (500 mg/kg body weight/daily orally) caused decrease in the scores of necrosis, degeneration, inflammation and total pathologic indexes of the hepatic tissues compared to other tested groups. Application of *C. intybus* root extract decreased the formation of centrilobular hepatic necrosis, some levels of fatty changes, and inflammation and degeneration in broilers faced with CCl₄-induced hepatotoxicity. However, further researches are required to assess other mechanisms of function of *C. intybus* root extract as a hepatoprotective agent toward CCl₄-induced hepatotoxicity in broiler chickens.

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Conflict of interest

Authors declared that they have no conflict of interest.

Funding statement

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Ethical consideration

The study was approved by the ethical council of the Faculty of Veterinary Medicine. All effort were applied to minimized the pains of the examined broiler chicks.

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