Effect of Chicory (*Cichorium intybus* L.) Root Extract on Some Blood Parameters of Broiler Chicks Under Carbon Tetrachloride-Induced Toxicity

Aref Jahani Bahnemiri¹, Mohammad Hassan Bozorgmehri-Fard^{1*}, Seyyed Mohammad Mahdi Kiaei¹, Saeed Hesarakhi², Nariman Sheikhi¹

¹Department of Poultry Diseases, Science and Research Branch, Islamic Azad University, Tehran, Iran.

²Department of Pathobiology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

THIS work was aimed to study effect of *C. intybus* root extract on blood parameters of chicks under CCl₄-induced toxicity. Totally, 240 one-day-old broiler chicks were divided into diverse groups of control, CCl₄ (1 mL/kg body weight IM injection), *C. intybus* root extract (500 mg/kg body weight/daily orally from days 20 to 29) and finally both *C. intybus* and CCl₄. Blood samples are collected daily from age of 20 to 31 days. Levels of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase and glutathione peroxidase and superoxide dismutase were assessed by commercial kits. Decrease in the levels of AST and ALT and increase in the levels of SOD and GPx were observed. The lowest levels of AST, ALP and ALT in day 31 of blood sampling were achieved in chicks treated with *C. intybus* (500 mg/kg BW/daily from days 20 to 29) (130.91±31.18 U/L), CCl₄ (1 mL/kg BW IM on days 24 and 25) (346.59±83.75 U/L) and *C. intybus* (from days 20 up to 29) (4.51±2.03 U/L) groups. The highest amounts of SOD and GPx in the stage 2 of blood sampling were achieved in chicks treated with *C. intybus* (500 from days 20 up to 29) and CCl₄ (1 mL/kg BW on days 24 and 25) (590.33±73.22 U/L) and those treated with *C. intybus* (from days 20 up to 23) and CCl₄ (on days 24 and 25) (261.91±32.18 U/L) groups, respectively. Oral prescription of chicory extract for 10 days can be used as a hepatoprotective agent on poultry.

Keywords: *Cichorium intybus*, Hepatoprotective effects, Broilers, Carbon tetrachloride, Blood parameters.

Introduction

Poultry production is one of the most economical and profitable livestock industry in the world. Convenient food conversion ratio, ease of production and no need for advanced facilities have caused the industry to expand greatly [1]. In keeping with this, modern commercial broiler chicks are prone to diverse issues. Oxidative Stress (OS) is one of the most critical threats in growth of commercial broiler chicks [2]. OS is mainly happened when reactive oxygen species (ROS) synthesis exceeds from their control [3]. Subsequently, the most susceptible tissue in

oxidative circumstances is liver which can face with fibrosis, degeneration, necrosis and failure[4].

Cichorium intybus L. (*C. intybus*) (chicory), has been introduced as an antioxidant natural plant in Iranian folk medicine. It is considered as an imperative food additive with anti-diabetic, antimicrobial, anti-hyperuricemia, anti-hepatotoxic, immunoenhancement and anti-hypertriglyceridemic agent[5, 6]. *C. intybus* has a boost hepatoprotective effects, predominantly in stress-induced toxicities, particularly OS[7]. Thus, it can use as a protective agents against hepatic toxicity in poultry industry.

Carbon tetrachloride (CCl₄) is a toxicant model with broad administration in both *in vitro* and *in vivo* toxicological researches. Hepatic intoxication induced by CCl₄ is mainly used to occur the OS and then assess the protective effects of therapeutic components [8]. CCl₄ exposure in poultry industry is accidental and causes cirrhosis and necrosis of liver which can lead to leakage of the liver enzymes into the blood stream [8]. Thus, measurement of enzymatic blood parameters such as hepatic alkaline phosphatase (ALP), aspartate aminotransaminase (AST) and alanine aminotransferase (ALT) can clearly determine the common condition of liver [8].

Materials and Methods

Plant materials

Healthy and fresh roots of *C. intybus* were collected from the Mazandaran, North of Iran. At the outset, roots of *C. intybus* were identified by the Pars Imen Daru company (Iran). Identification of collected plants was performed by an expert professor of the field of medicinal plants. At that moment, dried roots (prepared in shed at room temperature) were ground into a fine powder. Afterward, 100 mg powdered were soaked in ethanol (95%, Merck, Germany) for about 72h. Succeeding solution was filtered (paper filter, Whatman No. 1.). An achieved extract was concentrated by rotary evaporator (50 °C). Subsequently, an attained extract was dissolved by Dimethyl Sulfoxide (DMSO, Merck, Germany) and weighed and stored at 4 °C.

Broiler chicks and maintenance circumstances

A total of 240 healthy one-day-old Ross 308 broiler chicks (50 g body weight) were used in this research. 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 slowly reduced to 22°C at day 28. Totally, 23 h light and 1 h dark was applied for chicks. Basal diet was formulated according to NRC (1994) [9]. No antibiotic therapies were given to chicks during the experiment. Chicks were checked twice daily for mortality. All healthy one-day-old Ross 308 broiler chicks without any types of skeletal, muscular and infectious diseases and disorders were included in the study. Mortality was the only exclusion criteria.

Study design and treatments

The duration of the experiment was 31 days. Broilers were divided into 8 varied groups, with three replicates per treatment and 10 chicks per replicate. Table 1 represents the classification of broiler chicks. All chicks were kept thirsty for about 4 h and then *C. intybus* root extract was presented orally to chicks of treatment groups. To normalize the stress condition, the chicks of the control group received 0.9% sodium chloride solution (1 mL/kg BW) via IM injection and also distilled water (1 mL/Kg BW) via oral gavage. Weight, feed intake and mortality factor of chicks were controlled.

TABLE 1. Broiler chicks classification.

No	Groups	Introduction
1	Control	Without any supplementation
2	Carbon tetrachloride control (CATC)	CCl ₄ (1 mL/kg BW IM) on days 24 and 25
3	<i>C. intybus</i> protective control (CIPC)	<i>C. intybus</i> (500 mg/kg BW/daily orally) from days 20 up to 23
4	<i>C. intybus</i> therapeutic control (CITC)	<i>C. intybus</i> (500 mg/kg BW/daily orally) from days 26 up to 29
5	<i>C. intybus</i> protective-therapeutic control (CIPTC)	<i>C. intybus</i> (500 mg/kg BW/daily orally) from days 20 up to 29
6	<i>C. intybus</i> protective treatment (CIPT)	<i>C. intybus</i> (500 mg/kg BW/daily orally from days 20 up to 23 (for 4 days before the toxicity)) and CCl ₄ (1 mL/kg BW IM on days 24 and 25)
7	<i>C. intybus</i> therapeutic treatment (CITT)	CCl ₄ (1 mL/kg BW IM on days 24 and 25) and <i>C. intybus</i> (500 mg/kg BW/daily orally from days 26 up to 29 (for 4 days after the toxicity)
8	<i>C. intybus</i> protective-therapeutic treatment (CIPTT)	<i>C. intybus</i> (500 mg/kg BW/daily orally from days 20 up to 29) and CCl ₄ (1 mL/kg BW IM on days 24 and 25)

Evaluation of enzymatic blood parameters

At the age of 20 days (before the start of the test, stage 1) and at the age of 31 days (the final day of the test, stage 2), 4 chickens from each replicate group (12 in total) were selected and subjected to blood sampling (5 mL) through cardiac puncture. Samples were poured into a 10-mL anticoagulant-free vacutainer tube and then centrifuged ($3000 \times g$ for 15 min at 4 °C) to serum achievement. Sera were stored at -20 °C away from light for further analysis. Hepatic alkaline phosphatase (ALP), aspartate aminotransaminase (AST), and alanine aminotransferase (ALT) were analyzed and measured by an auto analyzer (Autolab, PM 4000, Italy), rendering the directions of the producer.

Evaluation of antioxidant indices

Whole heparinized blood (2 mL) was assayed for glutathione peroxidase (GPx) and Superoxide dismutase (SOD) factors. Blood samples were washed and centrifuged ($748 \times g$ for 10 min) triplicate with NaCl (0.9%) [10]. Two commercially kits of RANSEL (at 340 nm absorbance) and RANDOX (at 505 nm absorbance) (Ransel, RANDOX/RS-504, Randox Lab, UK) were used to assess the amounts of GPx and SOD, respectively.

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

Results

Amounts of enzymatic blood parameters

Table 2 reveals the mean of enzymatic blood parameters in chicks of the control and treatment groups under toxicity with CCl₄. Findings revealed that there were no significant statistical analysis in control and CITT groups between all examined enzymatic blood parameters (*P*>0.05). The amount of ALP had a statistically significant difference between the first and second stages of blood sampling in CATC group (*P*=0.05). The amount of ALT had a statistically significant difference between the first and second stages of blood sampling in CIPC, CITC and CIPTC groups (*P*=0.00). The amount of AST had a statistically significant difference between the

first and second stages of blood sampling in CIPT group (*P*=0.005). The amount of ALP and ALT had statistically significant differences between the first and second stages of blood sampling in CIPTT group (*P*=0.05 and *P*=0.01, respectively). The highest amounts of AST, ALP and ALT in the stage 1 of blood sampling were obtained in CIPT (223.91±65.21 U/L), CIPT (383.38±104.71 U/L) and CIPC (15.87±3.96 U/L) groups. The highest amounts of AST, ALP and ALT in the stage 2 of blood sampling were obtained in CATC (241.75±151.48 U/L), CIPT (432.00±64.05 U/L) and CIPTT (90.80±3.90 U/L) groups. The lowest amounts of AST, ALP and ALT in the stage 1 of blood sampling were obtained in CITT (138.91±65.94 U/L), CATC (304.72±134.06 U/L) and control (11.50±6.29 U/L) groups. The lowest amounts of AST, ALP and ALT in the stage 2 of blood sampling were obtained in CIPTC (130.91±31.18 U/L), CATC (346.59±83.75 U/L) and CIPTC (4.51±2.03 U/L) groups.

Amounts of antioxidant indices

Table 3 reveals the mean of antioxidant indices in chicks of the control and treatment groups under toxicity with CCl₄. Findings revealed that there were no significant statistical analysis in control and CIPC groups between all examined antioxidant indices (*P*>0.05). The amount of GPx had a statistically significant difference between the first and second stages of blood sampling in CATC group (*P*=0.02). The amount of SOD had a statistically significant difference between the first and second stages of blood sampling in CITC, CIPTC, and CITT groups (*P*=0.00). The amount of SOD and GPx had a statistically significant difference between the first and second stages of blood sampling in CIPTC group (*P*=0.001 and *P*=0.03, respectively). The amount of GPx had statistically significant differences between the first and second stages of blood sampling in CIPT group (*P*=0.04). The highest amounts of SOD and GPx in the stage 1 of blood sampling were obtained in CITT (496.33±96.06 U/L) and CIPTT (252.00±31.77 U/L) groups, respectively. The highest amounts of SOD and GPx in the stage 2 of blood sampling were obtained in CIPTT (590.33±73.22 U/L) and CIPT (261.91±32.18 U/L) groups, respectively. The lowest amounts of SOD and GPx in the stage 1 of blood sampling were obtained in CIPTT (365.00±55.25 U/L) and CITC (189.41±17.15 U/L) groups, respectively. The lowest amounts of SOD and GPx in the stage 2 of blood sampling were obtained in CIPTC (130.91±31.18 U/L), CATC (374.07±42.58 U/L) and CATC (189.91±15.99 U/L) groups, respectively.

TABLE 2. Mean of enzymatic blood parameters in chicks of the control and treatment groups under toxicity with CCl₄.

Groups	Mean $\pm$ SD of enzymatic blood parameters (U/L)							
	AST			ALP			ALT	
	Stage 1	Stage 2	P*	Stage 1	Stage 2	P	Stage 1	Stage 2
Control	179.25 $\pm$ 77.85	145.16 $\pm$ 60.45	0.237	307.90 $\pm$ 156.24	399.24 $\pm$ 92.41	0.095	11.50 $\pm$ 6.29	9.30 $\pm$ 6.31
CATC	162.41 $\pm$ 66.24	241.75 $\pm$ 151.48	0.1	304.72 $\pm$ 134.06	346.59 $\pm$ 83.75	0.05	14.15 $\pm$ 3.34	12.62 $\pm$ 7.20
CIPC	195.50 $\pm$ 63.19	143.91 $\pm$ 43.90	0.49	321.50 $\pm$ 157.28	394.58 $\pm$ 98.13	0.18	15.87 $\pm$ 3.96	8.52 $\pm$ 4.22
CITC	166.40 $\pm$ 47.49	135.91 $\pm$ 42.75	0.11	319.52 $\pm$ 109.59	392.95 $\pm$ 105.79	0.09	15.49 $\pm$ 5.46	7.25 $\pm$ 3.72
CIPTC	174.16 $\pm$ 73.23	130.91 $\pm$ 31.18	0.16	344.83 $\pm$ 104.54	392.08 $\pm$ 119.11	0.31	14.50 $\pm$ 5.26	4.51 $\pm$ 2.03
CIPT	223.91 $\pm$ 65.21	154.41 $\pm$ 41.69	0.005	383.38 $\pm$ 104.71	432.00 $\pm$ 64.05	0.18	12.34 $\pm$ 3.78	11.04 $\pm$ 7.37
CITT	138.91 $\pm$ 65.94	149.00 $\pm$ 56.09	0.69	349.31 $\pm$ 86.24	414.96 $\pm$ 95.12	0.09	14.39 $\pm$ 5.85	10.41 $\pm$ 4.43
CIPTT	152.50 $\pm$ 46.00	145.91 $\pm$ 51.53	0.74	310.35 $\pm$ 105.74	408.65 $\pm$ 125.73	0.05	14.08 $\pm$ 4.34	90.80 $\pm$ 3.90
<i>*P value</i>								

*P value

TABLE 3. Mean of antioxidant indices in chicks of the control and treatment groups under toxicity with CCl₄.

Groups	Mean $\pm$ SD of antioxidant indices (U/L)						
	SOD		GPx				P
	Stage 1	Stage 2	P*	Stage 1	Stage 2	Stage 2	
Control	465.66 $\pm$ 70.60	476.75 $\pm$ 112.18	0.95	235.83 $\pm$ 24.55	253.75 $\pm$ 28.34		0.1
CATC	402.25 $\pm$ 64.89	374.07 $\pm$ 42.58	0.22	215.58 $\pm$ 34.58	189.91 $\pm$ 15.99		0.02
CIPC	453.75 $\pm$ 97.71	486.66 $\pm$ 78.19	0.37	193.75 $\pm$ 14.30	201.83 $\pm$ 14.86		0.18
CITC	402.15 $\pm$ 50.53	543.33 $\pm$ 82.31	0.00	189.41 $\pm$ 17.15	202.08 $\pm$ 19.91		0.10
CIPTC	420.05 $\pm$ 83.55	533.50 $\pm$ 63.47	0.001	209.91 $\pm$ 15.28	228.66 $\pm$ 25.32		0.03
CIPT	369.25 $\pm$ 56.74	423.33 $\pm$ 86.43	0.08	222.83 $\pm$ 20.63	246.50 $\pm$ 31.60		0.04
CITT	496.33 $\pm$ 96.06	556.83 $\pm$ 58.63	0.00	223.08 $\pm$ 29.35	240.66 $\pm$ 34.84		0.57
CIPTT	365.00 $\pm$ 55.25	590.33 $\pm$ 73.22	0.00	252.00 $\pm$ 31.77	261.91 $\pm$ 32.18		0.45

*P value

Discussion

OS induced by ROS is one of the major factors that caused functions of internal organs, particularly liver into the failure. CCl₄-induced OS is involved in the cytochrome P450-Nicotinamide-adenine dinucleotide phosphate (NADPH) system during the reactive radicals metabolism. These radicals attack alkalizing proteins, fatty acids, and other macromolecules which cause cell membrane's lipid peroxidation, failure of enzymatic function, and cell damage through necrosis [11]. Thus, it is important to control the hepatic toxicity caused by the CCl₄ molecules in sensitive animals like poultries.

The present research was carried out to determine the hepatoprotective effects of *C. intybus* root extract on the enzymatic blood parameters and antioxidant indices of chicks under toxicity with CCl₄. Findings revealed that The levels of AST have been decreased in stage 2 experiment than stage 1 in CIPC, CITC, CIPTC, CIPT and CIPTT groups. Additionally, The levels of ALT have been decreased in stage 2 of experiment than stage 1 in all studied groups. However, decrease in the levels of AST was significant only in CIPT group ($P<0.05$). Furthermore, decrease in the levels of ALT was significant only in CIPC, CITC, CIPTC, and CIPTT groups ($P<0.05$). Thus, it can conclude that Treatment of chicks with *C. intybus* (500 mg/kg BW/daily orally from days 20 up to 23 (for 4 days before the toxicity)) can significantly decrease the levels of AST. Additionally, Treatment of chicks with *C. intybus* (500 mg/kg BW/daily orally from days 20 up to 29 (4 days before and 4 days after the CCl₄ prescription)) can significantly decrease the levels of ALT.

Similar to our survey, Jamshidzadeh et al. [12], Khodadadi et al. [13] and Li et al. [14] reported antioxidant and hepatoprotective effects of *C. intybus* in toxicity, particularly CCl₄-induced toxicities. Since the CCl₄ toxicity is as a result of free radicals formation and OS occurrence, accordingly *C. intybus* protective effects is possibly occurred by an inhibition of the P450 cytochrome activity. Additionally, presence of antioxidant chemical components in the *C. intybus* extract is another possible reason for its hepatoprotective effects. Polyphenols,

anthocyanins, Vitamin C, and flavonoids are major phytochemical components of the *C. intybus* extract which all have antioxidant effects [15]. Furthermore, tannins, inulin, sesquiterpene lactones, coumarins, and monomeric are another chemical components of the *C. intybus* which all have scavenging effects toward OS [16-18]. Baradaran et al. [19] reported that treatment of broiler chicks with silymarin caused significant decrease in ALT, AST and ALP parameters compared to the ccl₄ groups after 42 days of the experiment. Additionally, Khodadust et al. [20] reported that a significantly higher amounts of liver enzymes in chicks treated by CCl₄ than those by *Mentha piperita* ($P<0.05$). Furthermore, treatment of chicks with *Mentha piperita* (2 mL) CCl₄ (1 mL) caused significant decrease in the liver enzymes than CCl₄ groups ($P<0.05$) [20]. Decrease the promotion of liver failure's specific markers, antioxidant enzymes expansion and reduction of uric acid production may be the possible mechanism of protective actions of *C. intybus* toward CCl₄-induced hepatotoxicity in broilers [21].

Findings also revealed that the amounts of SOD and GPx antioxidant enzymes have been increased in the chicks of CIPC, CITC, CIPTC, CIPT, CITT, and CIPTT groups after treatment with *C. intybus*. This finding may show the boost antioxidant effects of *C. intybus* in broilers with CCl₄-induced hepatotoxicity. Moradi et al. [22] reported that treatment of Japanese quail suffered from CCl₄-induced hepatotoxicity with silymarin caused significant increase in the levels of SOD and GPx antioxidant enzymes. Zafar and Mujahid [23] and Sadeghi et al. [24] reported the antioxidant effects of *C. intybus* extract in CCl₄-induced hepatotoxicity in rats which introduced with decrease in the ALT, AST and ALP enzymes and increase in the SOD and GPx antioxidant enzymes.

Limitations of the present survey are lack of analysis of the effect of chicory on other blood parameters, absence of group of broilers faced with hepatotoxicity caused by therapeutic options routinely used in aviculture and finally lack of evaluation of the effect of chicory in other age groups. The strengths of the present survey are adequate numbers of samples, adequate numbers

of treatment groups and analysis of the groups from days 20 to 31.

Conclusion

In conclusion, the present research revealed that application of *C. intybus* root extract for 10 days (500 mg/kg body weight/daily orally) caused decrease in the ALT, AST and ALP liver toxicity enzymes and increase in the SOD and GPx antioxidant enzymes of broiler chicks compared to the control and CATC group. 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 chicks. Finally, chicory can be used as a therapeutic feed in broiler industry.

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Conflict of interest

Authors declared that they have no conflict of interest.

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