



The Effect of *Cichorium intybus L.* Ethanol Extraction on the Pathological and Biomedical Indexes of the Liver and Kidney of Broilers Reared Under Heat Stress

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ABSTRACT

The use of compounds with antioxidant properties as a source of phenol compounds is highly recommendable in the poultry industry. Therefore, the effect of *Cichorium intybus L.* herb on pathobiochemical indexes of chicken under heat stress was studied. After exposure to heat stress (from day 21 to day 42 of growth), hydroalcoholic extraction was provided to 270 broiler chicks randomly divided into six groups and placed in two distinct poultry houses (heat stress and normal conditions). The three groups were recipient group of *Cichorium intybus L.* (1); recipient group of vitamin C (2) and control group (3). The birds in one of the houses were exposed to heat stress conditions (35 °C for 8 hours) for a time period between 22 to 42 days and the birds in the other house were reared under normal conditions (20-22°C) for the same time period. Blood samples collected from the birds showed that *Cichorium intybus L.* herb caused significant decrease in uric acid, Triglyceride, Alanine aminotransferase (ALT), total body clearance factors (CL- factors) and right ventricular failure index (RVF) and significant increase in K⁺ under heat stress condition ($p < 0.05$). Vitamin C caused significant decrease in uric acid, ALT, CL- factors and RVF index and significant increase in K⁺ and Na⁺ under heat stress condition ($p < 0.05$). A significant decrease in cholesterol and triglyceride in recipient group of *Cichorium intybus L.* was observed compared to the recipient group of vitamin C under heat stress condition ($p < 0.05$). In a pathologic examination normal observations were in recipient group of *Cichorium intybus L.* and recipient group of vitamin C compared to the control group. According to this study, use of *Cichorium intybus L.* extract and vitamin C in chicken under heat stress induced improvement in liver, kidney activity and fat metabolism.

INTRODUCTION

Heat stress is defined as a combination of high environmental temperature and humidity disturbing proper thermoregulatory processes (Nolan *et al.*, 1999). It greatly affects poultry production by reducing carcass quality, egg production, feed intake, immune response, weight gain, mineral balance, and it increases panting, mortality and fertility by affecting semen quality (Ayo *et al.*, 1996; Vathana *et al.*, 2002). Heat stress is the main cause of hepatic dysfunction in broiler production (Lara *et al.*, 2013). The supplementation with Vitamin C can ameliorate the negative effects of heat stress in poultry (Vathana *et al.*, 2002). Vitamin C is responsible for the biosynthesis of corticosterone, an important glucocorticoid hormone that participates in gluconeogenesis to increase energy supply during stress (Frandsen, 1986; Bain, 1996). Nevertheless, under significantly high ambient temperatures, the release of Vitamin C in broilers is insufficient for optimal performance (Daghir, 1995).



Therefore, it is necessary to investigate the use of alternative natural herbs, such as *Cichorium intybus* (Kasni), to provide the support for efficient liver function (Hanaa *et al.*, 2010). The *Cichorium intybus* plant has been used as forage for domestic livestock in recent years in many parts of the world. The forage is fed to supply high quality dry matter, and animal productivity obtained with *C. intybus* is comparable to that with legumes and superior to solely grass-based pastures (Shad *et al.*, 2013). Moreover, *C. intybus* plant has been recently used as a natural feed additive in poultry diets (Abaza *et al.*, 2008).

C. intybus is known to have antibacterial, digestive, bitter tonic, anti-inflammatory, diuretic, anti-hypercholesteremic and laxative properties and does not cause any side effects (Saeed *et al.*, 2015). Several authors have commended the medicinal importance of *C. intybus* due to the presence of a number of compounds such as inulin, sesquiterpene lactones, alkaloids, coumarins, chlorophyll pigments, flavonoids unsaturated sterols, vitamins, saponins and tannins (Muthusamy *et al.*, 2008; Atta *et al.*, 2010; Shad *et al.*, 2013). This study was aimed at determining the effect of *Cichorium intybus* L. ethanol extract in comparison with vitamin C on the pathobiochemical indices of the liver and kidney of broilers reared under heat stress.

MATERIALS AND METHODS

This study was conducted at the Poultry Research Unit of the department of Veterinary Medicine, Islamic Azad University, Shahrekord branch, Shahrekord, Iran. All experiments were carried out under the ethical guidelines of the Islamic Azad University of Shahrekord Branch (93/061, in 2014).

Collection and drying of the plant

Leaves and stems of the *Cichorium intybus* L. plant of the family Asteraceae (Kasni in Persian) were used in the study. *Cichorium intybus* (Kasni) was purchased from Pharmaceutical Plants Bazaar in Iran. Plant identification was confirmed according to the book collection of Iran Herbs (Ghahreman, 1973). The stem and leaves of *Cichorium intybus* were dried under shade at low humidity at 25-32°C. After drying the plant was ground to the particle size of 0.5- 1.5 inches using a ceramic mortar.

Extraction

The concentrate of *Cichorium intybus* L. was extracted by soaking the ground particles in 96% alcohol. Thereafter, the soaked material was milled

through a 0.5-inch electrical mill sieve. The *Cichorium intybus* alcohol mixture was left for 48 hours in a glass jar before removing the filtrate. *Cichorium intybus* filtrate was transferred to a flask and solvent was extracted by a rotary and vacuum pump at 48-50 °C. The high-density liquid extract was dried at 37°C for 24 hours. The residual powder was used to formulate the *Cichorium intybus* L. hydroalcoholic extract doses (Saggu *et al.*, 2014).

Experimental procedure

A total of 270 one-day-old male Ross broiler chicks were obtained from a commercial hatchery and housed in a concrete-floor, cross-ventilated windowless broiler house. Chicks were randomly separated into six groups with three replicates of 15 chicks each, and placed in 2×1 m cages. From day 1 to day 21, birds were reared under normal brooding conditions (temperatures gradually being reduced from 36 to 22°C) in the two houses. A three-phase feeding program, made up of starter, grower and finisher feeds containing 21 %, 19 % and 18 % crude protein, respectively, was applied. After 22 days, the environment in one of the houses (days 22-42) was changed to heat stress by exposing the birds to temperatures of 35 °C for eight hours (09:00-17:00) until day 42. Therefore, the groups under heat stress and the control groups (under thermoneutral temperature) were reared in two separate houses. Body weight and feed intake were recorded weekly on pen basis and feed conversion ratio (feed intake to body weight gain) was then calculated.

Treatments

Both heat-stressed and control birds were divided into the following treatments: A = fed *Cichorium intybus* solvent 100 mg.1000 mL⁻¹, B = fed vitamin C 100 mg.1000 mL⁻¹, C=negative control with no additional material in the feed. The other chickens were under thermal stress D=on *Cichorium intybus* 100 mg.1000 mL⁻¹, E=on vitamin C 100 mg.1000 mL⁻¹, F=negative control chickens with no additional material in their feed from day 22 to day 42.

Data collection

The effects of supplemental *Cichorium intybus* L. ethanol extract and vitamin C on broiler chickens' growth performance and liver and kidney pathobiochemical indices were determined. Chickens were weekly weighed using a digital scale and the feed conversion ratio was calculated by dividing body weight by feed intake.



On day 42, all chickens were sacrificed after weighing and collection of the blood samples (with no anticoagulant to allow serum collection). The liver and kidneys were collected and fixed in 10% formalin for pathological studies.

Blood samples

Blood samples (1-2 mL) were collected from the jugular vein from individual birds, and the tube was tilted to coagulate the blood. After collecting the serum, tubes were centrifuged for 5 minutes at 1500 r.min⁻¹. The obtained serum was placed in identified microtubes and frozen until further analyses.

Right ventricular failure index (RVF)

After sacrifice, the whole heart was removed, weighed, and the auricles were removed. Total weight of the two abdomens was obtained before removing the right abdomen and weighing it separately to obtain the proportion of right abdomen to total weight of two abdomens.

Internal organs and microscopic sections

Liver, kidney and muscle samples (1×1 cm) were collected and fixed in 10% formalin buffer for 48 hours. The samples were then observed under an optical microscope for histopathological examinations as done by Biró *et al.* (2002).

Statistical analyses

Qualitative data (pathological observations) was analysed using the Chi-square test whilst quantitative data that is growth index, rate of liver enzymes and hematology indexes was analysed by ANOVA and means were compared using the Tukey post hoc test using SigmaStat software.

RESULTS

Collected blood samples showed that *Cichorium intybus L.* herb caused a significant decrease in uric acid, Triglycerid, ALT, CL- factors and RVF index and a significant increase in K+ under heat stress condition ($p<0.05$) in comparison to under normal condition (Table 1 and 2). Vitamin C caused a significant decrease in uric acid, ALT, CL- factors and RVF index and a significant increase in K+ and Na+ under heat stress condition ($p<0.05$) compared to under normal condition (Tables 1 and 2). Uric acid was significantly ($p<0.05$) lower in birds under heat stress receiving *Cichorium intybus L.* extract and vitamin C compared to birds receiving same treatments under normal conditions (Table 1). Triglyceride, ALT, AST and Cl were significantly higher in the control group under heat stress compared to birds receiving vitamin C and *Cichorium intybus L.* under the same conditions. Na and K were significantly ($p<0.05$) higher in birds under heat stress receiving vitamin C and *Cichorium intybus L.* compared to birds in the control group. RVF was significantly ($p<0.05$) lower in birds receiving vitamin C and *Cichorium intybus L.* under heat stress compared to birds in the control group (Table 2). Liver weight was significantly higher in birds in the control group under heat stress compared to birds on *Cichorium intybus L.* and vitamin C. A significant decrease in cholesterol and triglyceride in the recipient group of *Cichorium intybus L.* compared to the recipient group of vitamin C under heat stress condition was observed ($p<0.05$). In the pathologic examination normal observations were in recipient group of *Cichorium intybus L.* and recipient group of vitamin C compared to the control group (Table 3). Use of *Cichorium intybus L.* extract and vitamin C in chicken under heat stress showed improvement in liver, kidney activity and fat metabolism.

Table 1 – Effect of heat stress on serum biochemical parameters in broilers on *Cichorium intybus L.* and vitamin C.

Group Elements	Normal conditions			Under heat stress		
	Control	Receiving <i>Cichorium intybus L.</i>	Receiving vitamin C	Control	Receiving <i>Cichorium intybus L.</i>	Receiving vitamin C
Uric acid	5.50±1.40 ^{ab}	5.28±1.43 ^{ab}	4.90±1.25 ^{ab}	6.31±1.73 ^b	4.33±0.61 ^a	4.15±0.88 ^a
Cholesterol	123.33±18.98 ^{ab}	111 ±13.5 ^a	125.33±25.27 ^{ab}	125.66±9.62 ^{ab}	114±10.712 ^a	131±19.32 ^b
Triglyceride	120±22.24 ^c	74.5±22.8 ^b	83±8.29 ^{ab}	140.33±30.86 ^a	98.16±20.01 ^{bc}	110±26.19 ^{cd}
Total protein	3.43±0.68	3.36±0.65	3.68±0.6	3.38±0.62	3.51±0.43	3.18±0.27
ALT	8.50±1.94 ^a	7.00±1.18 ^a	6.38±2.14 ^a	11.66±4.62 ^b	6.11±2.11 ^a	7.66±1.58 ^a
AST	305.16±63.49 ^a	272.33±36.83 ^a	281.16±29.8 ^{ab}	229.83±67.68 ^b	277.66±49.76 ^{ab}	292.50±36.09 ^{ab}
Na	160.33±18.03 ^a	153.5±5.65 ^a	151.22±3.11 ^a	138.11±18.20 ^b	153.33±5.75 ^a	152.00±5.94 ^a
K	8.43±0.48 ^a	8.17±0.58 ^a	8.05±0.98 ^a	7.11±0.77 ^b	8.10±0.61 ^a	8.43±0.48 ^a
Cl	122.83±18.93 ^a	124.66±10.37 ^a	127±7.94 ^a	136.16±11.23 ^b	122.83±3.27 ^a	124.66±5.09 ^a

^{a,b} Numbers with different superscripts in the same column differ significantly $p<0.05$

Aspartate Aminotransferase (AST) Alanine aminotransferase ALT



Table 2 – Effect of heat stress on liver and heart weight and RVF index in broilers on *Cichorium intybus* L. and vitamin C

Group Elements	Normal conditions			Under heat stress		
	Control	Receiving <i>Cichorium intybus</i> L.	Receiving vitamin C	Control	Receiving <i>Cichorium intybus</i> L.	Receiving vitamin C
RVF	0.18±0.02 ^a	0.18±0.02 ^a	0.16±0.01 ^a	0.21±0.04 ^b	0.18±0.03 ^a	0.18±0.03 ^a
Heart weight	9.46±1.03	9.15±1.26	8.67±1.15	9.91±1.93	9.29±0.52	8.97±1.86
Liver weight	53.00±16.22 ^a	59.83±6.55 ^{ab}	58.91±11.58 ^{ab}	64.50±9.93 ^b	57.83±12.33 ^{ab}	53.58±5.08 ^a

^{a,b} Numbers with different superscripts in the same column differ significantly $p < 0.05$

DISCUSSION

Diagnosis of the effects of *Cichorium intybus* and vitamin C was based on liver enzymes because they are regarded as the most sensitive biochemical markers employed in the diagnosis of hepatic dysfunction in broiler production (Johnkennedy *et al.*, 2010). Analysis of data revealed significant effects of *Cichorium intybus* on uric acid, triglyceride, ALT, CL- factors and RVF index and K+. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are transaminase enzymes used to detect liver injury or to diagnose liver disease. The significant decrease in ALT was also reported by Noreen (2009). However, Marzouk *et al.*, (2011) observed no significant effect of *Cichorium intybus* leaf extract on ALT.

In this study both *Cichorium intybus* and vitamin C showed significant effects on ALT. In the presence of *Cichorium intybus* and vitamin C, a decrease in ALT was observed. Sultan *et al.*, (2009) also observed reduction in ALT concentration in rats whereas Mishra and Kamal, (2009); Hassan and Yousef (2010) and Abd El-Mageed (2011) showed reduction in the concentration of AST in rats when fed (10 %) *Cichorium intybus* root powder.

Cichorium intybus extracts are known to enhance the endogenous antioxidant defense status, ultimately resulting in reduced ALT concentration in birds. ALT is responsible for decreasing enzyme activity which is the most vital index related to abnormal liver histopathology.

In this study, *Cichorium intybus* additionally showed an effect on total cholesterol level whilst,

total protein, heart weight and liver weight were not significantly affected. Several authors observed a significant decrease in the total cholesterol level in birds given *Cichorium intybus* ethanol extract. This is due to the stimulation of lactic acid producing bacteria that secrete bile deconjugating enzymes responsible for converting bile salts into deconjugated bile acids, eventually resulting in reduced serum cholesterol levels (Hinton *et al.*, 2000; Safamehr *et al.*, 2013). Moreover, the reduction in cholesterol levels caused by *Cichorium intybus* is attributed to the isoflavones in this herb which may reduce intestinal absorption of cholesterol by competing for absorption sites and consequently leading to a higher excretion of cholesterol consequential higher concentrations of un-absorbable cholesterol excreted (Chen *et al.*, 2005; Lin *et al.*, 2014).

There was no significant effect of *Cichorium intybus* on total protein and these findings are in agreement with Rezaei *et al.*, (2010), Behboud *et al.*, (2011), Velasco *et al.*, (2012) and Sarwar (2013) where supplementation of *Cichorium intybus* extracts showed no significant effect on protein level. Hanaa *et al.* (2010) also observed no significant change in total protein and albumin when *Cichorium intybus* was supplemented in rats under stress. Lin *et al.* (2014) mentioned that *Cichorium intybus* stimulates a positive effect on protein metabolism and lipid profiles. The evaluation of the levels of total protein and its fractions supply the information required to interpret the occurrence of dehydration under heat stress and inflammatory responses (Bounous, 2000).

Table 3 – Pathological lesions

Lesions Group	Diffuse cytoplasmic vacuolation	Hematopoietic foci Increased	Hepatic venous hyperemia	Renal capillary congestion	Exudation of Lymphocytes around the tubules	Increased mesenchymal cells of glomerular capsule
Control / Normal	-	1	1	1	1	-
Vitamin C / Normal	-	3	2	-	4	-
<i>Cichorium intybus</i> / Normal	-	3	1	1	1	-
Control / Stress	4	5	3	2	1	1
Vitamin C / Stress	5	2	2	2	4	1
<i>Cichorium intybus</i> / Stress	3	2	1	4	-	-

The numbers suggest that the severity of injury in six samples from each group



Zhu *et al.*, (2015) assessed uric acid-lowering activities using high-performance liquid chromatography fingerprints and reported that *Cichorium intybus* has uric acid-lowering activities. Furthermore, Lin *et al.*, (2014) reported that *Cichorium intybus* could reduce serum uric acid levels and inhibit liver xanthine dehydrogenase and xanthine oxidase.

However, Coles (1986); Kaneko *et al.* (1989) and Carlson *et al.* (2002) showed that the use of *Cichorium intybus* leaves as feed additive in horses under normal conditions had no effect on serum levels or factors related to hepatic function. In a study conducted by Kim & Shin, (1998) and Najafzadeh *et al* (2011) in horses, the concentration of factors (ALT, AST, ALP, and LDH, conjugated and total bilirubin, total protein and albumin) was observed in the normal range whereas the level of uric acid was outside the normal range. Additionally, research by Soder *et al.*, (2006) showed that the blood urea nitrogen and nonesterified fatty acids in dairy cows were not affected by *Cichorium intybus* consumption for 12 weeks.

In a study where different fractions of alcoholic extracts of *Cichorium intybus* were used, the histopathological study of the liver showed almost complete normalization of the tissues as neither fatty acid accumulation nor necrosis was observed (Ahmed *et al.*, 2003). Furthermore, in the pathologic examination no histopathological changes were observed in the recipient group of *Cichorium intybus* L and the recipient group of vitamin C when compared to the control group. The findings are in agreement with results obtained by Fallah *et al.* (2011), who indicated that the administration of *C. intybus* L. root extract in doses of 900 mg kg⁻¹ day⁻¹ to CCl₄ intoxicated rats prevents liver toxicity and liver histopathological changes. These results may be due to the action of antioxidant compounds such as anthocyanins, vitamin C, polyphenols and flavonoids that might contribute to protection against carcinogenic effects of nitrosamines and free radical generation (Kocsis *et al.*, 2003; Mulabagal *et al.*, 2009). Nayeemunnisa, (2009) also reported that feeding with *Cichorium intybus* has a significant role in up-regulating the endogenous antioxidant defense system by reducing the oxidative stress and inducing gene expression, thereby causing overexpression of the activity of the violent antioxidant enzyme CAT and restoring GSH levels (Li *et al.*, 2014).

The most common cause of ascites is increased vascular hydraulic pressure in the venous system, which most commonly is caused by right ventricular failure (RVF), also associated with hepatic fibrosis.

Elevated K⁺ and Na⁺ levels were observed in this study in chickens receiving vitamin C under stress conditions while *Cichorium intybus* only showed an increase in K⁺. High levels of Na⁺ may cause oedema, ascites and pulmonary hypertension-induced ascites in broilers (Mirsalimi and Julian, 1993).

In conclusion, *Cichorium intybus* has shown hepatoprotective effects against heat stress-induced hepatotoxicity in broiler chickens. The observed improvement of antioxidant enzymes and reduction in uric acid are the main mechanisms of action of *Cichorium intybus* in the prevention of stress induced liver fibrosis. However, additional work is required to establish the efficacy of *Cichorium intybus* as a potent anti-hepatic fibrosis herb under heat stress conditions in broiler chickens.

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