



Comparison of Hepatoprotective Activity of *Cichorium Intybus* and *Cynara Scolymus* Extracts Against Paracetamol Induced Hepatotoxicity in Broiler Chicken



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THIS study focused on the effects of *Cichorium intybus* (Chicory), and *Cynara scolymus* (Artichoke) in reducing the hepatotoxicity effects of paracetamol in broiler chickens. Eighty four 21-d-old male commercial broilers were randomly allotted to 4 groups (n=21) and treated as follows for 2 weeks: Control group: basal diet alone, paracetamol group: similar to control group, in addition to intraperitoneal (IP) administration of paracetamol as 0.5 mg/kg of BW on 4th day. *Cichorium intybus* group: similar to paracetamol group and also daily oral administration of *Cichorium intybus* as 0.1 % ethanolic extract in drinking water, for 2 weeks. *Cynara scolymus* group: similar to paracetamol group and oral administration of *Cynara scolymus* as 0.1 % ethanolic extract in drinking water, for 2 weeks. The SGOT, SGPT, CPK, LDH, Uric acid, Creatinine and GGT serum levels were analyzed on 25th, 27th and 34th days of study. The mortality rates and body weight changes were measured in the end of study. Ethanolic extracts of *Cichorium intybus* and *Cynara scolymu* were found to decrease both the mortality rate and body weight loss. Also ethanolic extract of *Cichorium intybus* exhibited protective lowering effects of the serum enzyme levels, SGOT, GGT and CPK to a significant extent (P<0.05). In conclusion, the results showed the protective activity of *Cichorium intybus* and *Cynara scolymus* on paracetamol induced toxicity in broiler chickens. Based on the results in the present study, the ethanolic extract of *Cichorium intybus* showed significant better hepatoprotective effect as compare to *Cynara scolymus*.

Keywords: *Cichorium intybus*, *Cynara scolymus*, Hepatotoxicity, Broiler chickens.

Introduction

Some drugs especially in high doses can cause hepatitis. Moreover, drug-induced hepatotoxicity is one of the most important causes of mortality and morbidity both in human and some animals [1, 2, 3]. Drug-induced hepatitis can be classified into intrinsic and idiosyncratic types. The intrinsic type such as acetaminophen (paracetamol) toxicity is dose dependent, whereas the idiosyncratic type does not depend directly on dose and is unpredictable [4]. Some studies in

United States showed that more than 50% of cases of acute liver failure are caused by drug-induced hepatitis, and more than 75% of idiosyncratic drug reactions undergo liver transplantation or die[5]. In the present study, experimental hepatotoxicity by paracetamol was performed on the broiler chickens. In addition to the importance of hepatotoxicity as a major complication in poultry industry [6, 7], this model can be noticed as an animal model for more research studies. Heat stress as the main cause of hepatic dysfunction in

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broiler production[8], is an inducer of oxidative stress, and causes mitochondrial dysfunction[9]. On the other hand, with attention to oxidative stress involvement in paracetamol-induced hepatotoxicity[10], paracetamol can be used as a similar pattern for heat stress-hepatotoxicity. Paracetamol-induced hepatotoxicity is the most frequent cause of acute liver failure in human in many countries. Further studies have shown the Paracetamol-induced hepatotoxicity can magnify in some conditions such as in patients with alcohol abuse [11]. *Cichorium Intybus*, commonly known as chicory historically was grown by the ancient Egyptians as a medical plant. Some research studies have shown the effect of aqueous and/or alcoholic extracts on several disorders. Antimicrobial, anthelmintic, antimalarial, gastroprotective, anti-inflammatory, antioxidant, antidiabetic, tumor-inhibitory and hepatoprotective activities are the most recognized pharmacological activities of *Cichorium intybus*[12]. One of the important and well-documented use of *Cichorium intybus* as a hepatoprotectant agent in traditional medicine. It was found to decrease both the hepatotoxicity and mortality in acetaminophen and carbon tetrachloride-induced toxicity in mice[13]. Similar studies have established the hepatoprotective effect of *Cichorium intybus* in albino rats without significant fat accumulation or necrosis in hepatocytes after the treatment[14, 15]. *Cynara scolymus* is another medicinal plant with hepatoprotective activity used in the present study. *Cynara scolymus* leaf extracts with choleric, diuretic and hypocholesterolemic activities have long been used in traditional folk medicine[16]. The extract of *Cynara scolymus* significantly can reduce hepatotoxicity and liver damage via its antioxidant and anti-apoptotic properties[17]. In this study, we examined the possible protective effect of *Cichorium intybus* and *Cynara scolymus* extracts on hepatotoxicity induced by paracetamol in broiler chickens.

Materials and Methods

Preparation of Cynara scolymus and Cichorium intybus extracts.

Cichorium intybus root and *Cynara scolymus* leave ethanolic extracts (containing 8.5% inulin and 0.38 % chlorogenic acid, respectively) were supplied by Barij Essence Pharmaceutical Co. (Kashan, Iran). Briefly the dry plants materials were powdered and extracted with 50% 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 40°C using a rotary evaporator instrument.

The extracts were diluted into 0.1% suspensions with drink water (1 mL extract in 1000 mL drink water). This suspension was used as drinking water for the chickens during the trial.

Birds and Experimental design

Healthy, vaccinated, 21 day-old Ross male broiler chickens (n=84) weighing 700±30g, were randomized divided into four groups of 21 birds in each, and maintained under standard laboratory conditions. Group I (fed with basal diet from the first day of trial to end of period), served as the control group and received 50% ETOH (2.5 ml/kg, IP) at 24 day of age, while group II(fed with basal diet from the first day of trial to end of period +IP injection of paracetamol as 0.5 g/kg of paracetamol dissolved in 2.5 cc 500 ethanol, at 24 day of age), group III(fed with basal diet from the first day of trial to end of period +IP injection of paracetamol as 0.5 g/kg of paracetamol dissolved in 2.5 cc 500 ethanol , at 24 day of age + and received 0.1% alcoholic extract of *Cynara scolymus* through their water from 21nd to 34nd day of age) and group IV(fed with basal diet from the first day of trial to end of period +IP injection of paracetamol as 0.5 g/kg of paracetamol dissolved in 2.5 cc 500 ethanol , at 24 day of age + and received 0.1% alcoholic extract of *Cichorium intybus* through their water from 21nd to 34nd day of age), were considered as the experimental groups. Regular diet (61% corn, 31.64% soybean meal, 2% fish meal, 1% soybean oil, 1.16% calcium carbonate, 1.96% dicalcium phosphate, 0.2% sodium bicarbonate, 0.2% salt, 0.6% vitamin-mineral, 0.3% methionine and 0.12% lysine) for broiler chickens was purchased from Sarshardaneh CO., Ltd. (Tehran, Iran). The chickens were in a conventional open-sided house with cyclic temperatures (minimum, 25°C, maximum, 31°C). The area of each pen was 2 m². Feed and water were provided ad libitum and lighting was continuous. The study was approved by the Institutional Review Animal Care Committee.

Measurement of enzymes and weight

To determine the biochemical parameters in broiler chickens, on days 25, 27 and 34 of age 7 animals were randomly selected from each group and then blood samples without anticoagulant were collected from jugular vein. Blood samples for serum collection were allowed

to stand at room temperature for 30 min to form clot and consequently serum was separated by centrifugation at 1800 g for 10 min and stored at -20°C until analysis. Serum Creatinine, Creatinine phosphokinase (CPK), Alanine Aminotransferase (SGPT or ALT), Aspartate Aminotransferase (SGOT or AST), Lactate Dehydrogenase (LDH), Gamma-Glutamyl Transferase (GGT) and Uric acid were measured by specific commercial kits (Pars Azmoon Co. Iran) using a standard auto analyzer with veterinary software (Eppendorf, EPOS 5060), and also body weight of the chickens were recorded on day 34.

Statistical analysis

Results are expressed as mean values \pm standard deviation (SD). Comparison of means of three measurements, using a significance level of $P < 0.05$, was performed by one-way analysis of variance (ANOVA). SPSS, version 19, was used, and the means were compared using the Duncan test.

Results

The effects of paracetamol, *Cichorium intybus*, and *Cynara scolymus* extract on body weight and some biochemical parameters in broiler chicken are presented in Table 1. In clinical survey, ethanolic extracts of *Cichorium intybus* and *Cynara scolymus* found to decrease the mortality rate. The mortality rates were 0%, 20%, 5% and 0% in I, II, III and IV group, respectively. Therefore, the most mortality rate was in paracetamol group. A significant gain in body weight was observed in *Cichorium intybus* and *Cynara scolymus* groups when compared to the paracetamol group. There was a significant difference for AST on 25th and 27th day, GGT on 25th day, CPK on 25th, 27th and 34th day, LDH on 25th day and weight on 34th day between treatments groups. There was no significant difference between the treatments for ALT, Creatinine and Uric acid throughout the trial.

Discussion

The effects of many medical plants as natural products in different diseases of broiler chickens are still unclear. Therefore, the aim of this study was to verify the hepatoprotective effects of *Cynara scolymus* and *Cichorium intybus* on paracetamol-induced hepatic damage in broiler-chicks, by analyzing their serum biochemical profile and recording the gain and mortality rate.

In the present study the mortality rates were

TABLE 1. Biochemical values (Mean \pm SD) on 25th, 27th and 34th days and weight values (Mean \pm SD) on 34th day of age in broiler chickens.

Parameters	25 th day				27 th day				34 th day			
	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
ALT(U/L)	8.28 \pm 0.6	9.57 \pm 1.8	6.86 \pm 0.8	7.1 \pm 0.7	7.86 \pm 1.3	8.85 \pm 2.2	6.62 \pm 0.49	8 \pm 0.8	6.69 \pm 0.54	6.12 \pm 0.99	5.83 \pm 0.83	5.7 \pm 0.54
AST(U/L)	304 \pm 25 ^{a*}	445 \pm 54 ^b	398 \pm 23 ^{ab}	312 \pm 9 ^a	259 \pm 17 ^a	343 \pm 20 ^b	296 \pm 14 ^{ab}	318 \pm 24 ^{ab}	301 \pm 12	324 \pm 15	291 \pm 29	308 \pm 19
CPK (U/L)	4650 \pm 913 ^{ab}	7150 \pm 934 ^b	7570 \pm 859 ^b	3395 \pm 395 ^a	4112 \pm 965 ^{ab}	5367 \pm 945 ^b	3553 \pm 425 ^{ab}	2106 \pm 133 ^a	4628 \pm 474 ^a	4467 \pm 678 ^a	1770 \pm 159 ^b	2776 \pm 210 ^{ab}
LDH(U/L)	1715 \pm 116 ^a	2012 \pm 149 ^{ab}	2485 \pm 211 ^b	2020 \pm 196 ^{ab}	2431 \pm 330	3035 \pm 301	2855 \pm 247	2485 \pm 141	1909 \pm 127	2063 \pm 245	2155 \pm 334	1646 \pm 132
GGT (U/L)	15.7 \pm 1 ^{ab}	19 \pm 0.8 ^b	18.6 \pm 1.5 ^b	13.3 \pm 1.5 ^a	20.4 \pm 1.8	28.7 \pm 4.4	22.5 \pm 2.1	21.3 \pm 2	26.8 \pm 1.6	23.5 \pm 1.9	24 \pm 2.1	22.1 \pm 1.4
Uric Acid(mg/dl)	5.89 \pm 0.4	7.71 \pm 0.6	6.3 \pm 2.2	4.9 \pm 0.5	5.79 \pm 0.65	5.59 \pm 0.55	4.31 \pm 0.4	5.2 \pm 0.8	5.66 \pm 0.3	5.88 \pm 1	4.74 \pm 0.4	4.5 \pm 0.5
Creatinine(mg/dl)	0.26 \pm 0.02	0.26 \pm 0.03	0.24 \pm 0.02	0.28 \pm 0.03	0.026 \pm 0.04	0.21 \pm 0.04	0.25 \pm 0.01	0.25 \pm 0.02	0.24 \pm 0.01	0.29 \pm 0.01	0.28 \pm 0.01	0.21 \pm 0.02
Weight (gr)									1704 \pm 42 ^a	1212 \pm 66 ^b	1467 \pm 52 ^c	1513 \pm 37 ^{ac}

* Figures bearing different superscripts in a row (in a same day and between different groups) differ significantly ($P < 0.05$).

I: Control group, II: Paracetamol group, III: Paracetamol + *artichoke* group, IV: Paracetamol + *Cichoriumintybus* group

20%, 5%, 0% and 0% in II, III, I and IV groups, respectively. Therefore, the most mortality rate was in paracetamol group, and treatment of animals with *Cynara scolymus* and *Cichorium intybus* reduced the mortality rate to 5% and 0% respectively. In a research study, paracetamol produced 100% mortality at the dose of 1 gr for each 1 kg of body weight (1g/kg) in mice while pre-treatment of animals with esculetin (a phenolic compound found in *Cichorium intybus* with hepatoprotective effects) as 6 mg kg⁻¹ reduced the death rate to 40%[18]. Acute intoxication of broilers by paracetamol induced significant increases ($P<0.05$) in serum levels of liver and muscle enzymes AST, GGT, and CPK when compared to the control group. Compared to the paracetamol group, the oral administration of *Cynara scolymus* significantly ($P<0.05$) lowered the elevated serum levels of CPK, and the *Cichorium intybus* extract significantly ($P<0.05$) lowered the elevated serum levels of CPK, AST and GGT. Disproportionate generation of reactive oxygen species (ROS) and oxidative stress are important factors in the pathogenesis of many diseases as they can cause damage to fundamental cellular components including DNA, protein, and lipid. ROS are generated both by-products of normal cellular metabolic activities and drug-induced oxidative stress. Although ROS have normal physiological roles in many cells, high formation of ROS can overwhelm internal defense system resulting in cellular damage[9, 10]. Paracetamol is a nonsteroidal anti-inflammatory drug, and is widely used in human as an analgesic and antipyretic drug[10]. Hepatotoxicity as a common cause of severe metabolic disorders and even death can be caused by excessive use or overdose of the paracetamol. Paracetamol-induced hepatotoxicity has been attributed to the formation of toxic highly reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) which causes oxidative stress and glutathione depletion[19, 20]. In the present study, oral administration of *Cichorium intybus* extracts to paracetamol - intoxicated broiler chickens exhibited hepatoprotective effect. This effect was evident from the significant decreases in the elevated serum levels of liver enzymes (AST, and GGT) in paracetamol - intoxicated broiler chicks, and also the reduction of mortality rate to 0% and a significant gain in body weight. This finding agreed with that previously reported by Li *et al.* [21] in CCL4 - intoxicated rat. Also Ahmed *et al.*, have found similar results. They have shown

alcoholic extract of *Cichorium intybus* could exhibit a very significant hepatoprotective effect in CCL4 - intoxicated rat [21]. In other study, oral administration of *Cichorium intybus* extract and vitamin C could improve liver, kidney activity and fat metabolism in chicken under heat stress [22]. *Cichorium intybus* has anti-hepatotoxic, antioxidative and anti-inflammatory activities [13, 15, 23, 24]. Moreover, hepatoprotective agents have been revealed in the seeds of the *Cichorium intybus* [25]. Atta *et al.* have shown that cichorium root extract therapy improved the degenerative histopathological changes such as centrilobular necrosis, ballooning degeneration in hepatocytes, congestion in the central vein and sinusoids, proliferation of Kupffer cells and mononuclear leucocytes inflammatory cells infiltration in the liver induced by CCl₄ intoxication in rat [26]. In a research study, *Cichorium intybus* extract could inhibit prostaglandin E2 (PGE2) production in human colon carcinoma HT29 cells treated with the proinflammatory agent TNF- α [27]. With attention to the mechanisms involved in the paracetamol induced hepatic toxicity as previously mentioned, the antioxidant and anti-inflammatory effects of *Cichorium intybus* have the pivotal roles in hepatoprotective effects in paracetamol toxicity. Hepatoprotective action of *Cichorium intybus* extract could be due to flavonoids or polyphenolic compounds, which have potent antioxidant activity, and protect the liver against free radical injury[28]. Cichoric acid present in leaf water extract, Flavonoids present in leaf ethanolic extract and also phytoconstituents from seed alcoholic extract such as flavonoids, saponins and their glycosides may be responsible for hepatoprotective role of chicory[29]. In this study 3 days after paracetamol injection 4 chicks (20%) died in group II whereas only 1 chick (5%) died in group III received paracetamol plus *Cynara Scolymus* extract and there was no mortality in control group. *Cynara scolymus* contains large amount of lignocellulosic biomass produced during its cultural cycle, and may be used as green forage for livestock and as an energy crop[30].

Cynara scolymus extract (stem, bract and receptaculum) showed potent inhibitory activity on plasma levels of AST and ALT in hepatic lesions induced by carbon tetrachloride in rats[31]. But in the present study and at least from the point of view of the parameters analyzed in this study, *Cynara scolymus* extract did not protect the liver against paracetamol induced injury. ALT, AST and GGT animals in *Cynara scolymus* extract

group did not have significant difference with animals in paracetamol group. These results did not agree with many other studies. Using different methods of extraction and so different amount of effective agents in extract used in diverse studies can play an important role in the effectiveness of the extract. Flavones, flavanones, flavonols, coumarins, and phenolic acids in the *Cynara scolymus* extract have antioxidant properties [16]. Phenolic structures in the *Cynara scolymus* extract have a pivotal role in free radical mediated processes inhibition [16]. Speroni et al. (2003), have shown different extracts of *Cynara scolymus* with the different content in phenolic derivatives could have different effects on bile flow and liver protection. Their results showed that the extract with the highest content in phenolic derivatives exerted the major hepatoprotective effect and some extracts of *Cynara scolymus* did not protect liver injury induced by CCl₄. In the present study the use of higher concentration of the *Cynara scolymus* extract may have more hepatoprotective effects in the broiler chickens.

Conclusion

In conclusion, both extracts of *Cichorium intybus* and *Cynara scolymus* reduced the mortality rate in paracetamol - intoxicated broiler chicks and had a significant gain in body weight when compared to the paracetamol group. Moreover *Cichorium intybus* extract exhibited a hepatoprotective effect, as demonstrated by a significant decrease in AST and GGT concentrations. Thus, it is probably a promising anti-oxidative therapeutic agent, and protects against paracetamol-induced hepatic toxicity in broiler chickens. Based on the results in the present study, the ethanolic extract of *Cichorium intybus* compared with *Cynara scolymus* showed better hepatoprotective effectiveness.

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Ethical consideration

The study was conducted according to the ethical standards and institutional guides that recorded in Instructions of the Ministry of Health and Medical Education.

Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

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