Effects of Cinnamon extract on biochemical enzymes, TNF-α and NF-κB gene expression levels in liver of broiler chickens inoculated with *Escherichia coli*[1]

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ABSTRACT.- Tabatabaei S.M., Badalzadeh R., Mohammadnezhad G.R. & Balaei R. 2015. **Effects of Cinnamon extract on biochemical enzymes, TNF-α and NF-κB gene expression levels in liver of broiler chickens inoculated with *Escherichia coli.*** *Pesquisa Veterinária Brasileira 35(9):781-787.* Department of Physiology, Faculty of Medical Sciences, Tabriz Branch, Islamic Azad University, Tabriz, Iran. E-mail: smt@iaut.ac.ir

Infection with *Escherichia coli* (*E. coli*) is a common disease in poultry industry. The use of antibiotics to treat diseases is facing serious criticism and concerns. The medicinal plants may be effective alternatives because of their multiplex activities. The aim of this study was to investigate the effects of cinnamon extract on the levels of liver enzymes, tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa B (NF-κB) gene expressions in liver of broiler chickens infected with *E. coli*. Ninety Ross-308 broilers were divided into healthy or *E. coli*-infected groups, receiving normal or cinnamon extract (in concentrations of 100 or 200mg/kg of food) supplemented diets. *E. coli* suspension (10^8cfu) was injected subcutaneously after 12 days cinnamon administration. Seventy-two hours after *E. coli* injection, the blood samples were taken for biochemical analysis of liver enzymes in serum (spectrophotometrically), and liver tissue samples were obtained for detection of gene expression of inflammatory markers TNF-α and NF-κB, using real-time PCR. Infection with *E. coli* significantly increased the levels of TNF-α and NF-κB gene expressions as well as some liver enzymes including creatine-kinase (CK), lactate-dehydrogenase (LDH), alanine-transferase (ALT) and aspartate-transferase (AST) as compared with control group ($P<0.05$). Pre-administration of cinnamon extract in broilers diet (in both concentrations) significantly reduced the tissue levels of TNF-α and NF-κB gene expressions and enzymes CK and ALT in serum of broiler chickens inoculated with *E. coli* in comparison with *E. coli* group ($P<0.05$ and $P<0.01$). The levels of LDH and AST were significantly decreased only by 200mg/kg cinnamon extract in infected broilers. The level of alkaline-phosphatase (ALP) was not affected in any groups. Pre-administration of cinnamon extract in diets of broiler chickens inoculated with *E. coli* could significantly reduce the gene expression levels of pro-inflammatory mediators and liver enzymes activities, thereby protecting the liver against this pathologic condition.

INDEX TERMS: Cinnamon, *Cinnamomum zeylanicum*, tumor necrosis factor-alpha, nuclear factor-kappa B, broiler chickens, *Escherichia coli.*

RESUMO.- [**Efeitos de extrato de canela sobre os níveis de enzimas bioquímicos, e expressão de genes de TNF-α e NF-κB em fígado de frangos de corte inoculadas com *Escherichia coli*.**] Infecção ocasionada por *Escherichia coli* (*E. coli*) é uma enfermidade comum na indústria avícola. O uso de antibióticos para tratar doenças bacterianas vem enfrentando sérias críticas e preocupações. As plantas medicinais podem ser alternativas eficazes por causa de suas atividades sinérgicas. O objetivo deste estudo foi investigar os efeitos do extrato de canela sobre os níveis de as enzimas hepáticas bem como sobre a expressão dos genes relativos, fator de necrose tumoral-alfa (TNF-α) e fator nuclear -kappa B (NF-κB) em fígado de frangos de corte infectados com *E. coli*. Noventa frangos Ross-308 fo-

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ram divididos em grupos saudáveis ou infectados com *E. coli*, que receberam dietas controle ou com suplementação contendo extrato de canela (em concentrações de 100 ou 200 mg/kg de alimento). Suspensão de *E. coli* (10^8UFC) foi injetado por via subcutânea, após 12 dias de administração do extrato de canela. Setenta e duas horas após a injeção de *E. coli*, amostras de sangue foram colhidas para análise bioquímica das enzimas do fígado no soro (espectrofotometria), e amostras de tecido de fígado foram obtidas para a determinação da expressão de genes de marcadores inflamatórios TNF-α e NF-κB, através PCR em tempo real. A infecção com *E. coli* aumentou significativamente os níveis de expressão dos genes TNF-α e NF-κB, assim como algumas enzimas hepáticas, incluindo creatina-quinase (CK), lactato-desidrogenase (LDH), alanina-transferase (ALT) e aspartato-transferase (AST), em comparação com o grupo de controle (P<0.05). A pré-administração do extrato de canela na dieta dos frangos (em ambas as concentrações) reduziu significativamente os níveis de expressão tecidual de TNF-α e NF-kB, da mesma forma que das enzimas CK e ALT no soro de frangos infectados com *E. coli*, em comparação com o grupos somente infectado com *E. coli* (P<0.05 e P<0.01). Os níveis de LDH e AST foram significativamente menores apenas para o grupo suplementado com extrato de canela na concentração de 200mg/kg. O nível de fosfatase alcalina (ALP) não foi afetado em nenhum grupo. A pré-administração do extrato de canela em rações para frangos infectados com *E. coli* pode reduzir significativamente os níveis de mediadores pró-inflamatórios e as atividades das enzimas hepáticas, desse modo protegendo o fígado contra esta condição patológica.

TERMOS DE INDEXAÇÃO: Canela, fator de necrose tumoral-alfa, fator nuclear kappa B-, frangos de corte, *Escherichia coli*.

INTRODUCTION

The prevalence of bacterial infections in poultry industry and encountering with drug resistance challenge in the use of antibiotics have led researchers to find out an alternative treatment other than antibiotics, such as probiotics, organic acids and herbal extracts (Griggs & Jacob 2005). Many herbs, spices and plant extracts are used for thousands of years to make savory taste in foods and treat several diseases in animals as well as humans (Chang-Liang et al. 2011). Recently, the growing interest has been gained in the use of natural materials such as feed additives to prevent or treat many animal diseases and to improve the production of poultry, because they have multipotent activities including antimicrobial, anti-inflammatory and antioxidant properties which make them useful as natural additives in animal feeds, instead of drug-resistant antibiotics.

One of the widely used food additives is cinnamon, which its medicinal properties have not been deeply investigated. It has been reported that the cinnamon have antioxidant and anti-inflammatory effects and has potential benefit in poultry production (Faix et al. 2009, Sang-Oh et al. 2013). The cinnamon extracts prevented *Helicobacter pylori* infection in birds in a concentration similar to those of antibiotics; the antimicrobial properties of the extract *in vitro* are mostly attributed to its components cinamaldehyde, eugenol and carvacrol (Taback et al. 1999). Cinnamon oil has been reported to have antibacterial activity against other bacteria such as *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, and *Enterococcus faecalis* (Chang at al. 2001, Griggs & Jacob 2005*)* and anti-fungal properties against *Aspergillus flavus* (Montes-Belmont & Carvajal 1998).

E. coli is one of the major bacteria causing burden to poultry industry. Pathogenic *E. coli* can cause colibacillosis, a local or systemic disease which in very acute and serious form may lead to the septicemia and mortality (Rodriguez-Siek et al. 2005, Ewers et al. 2007, Barnes et al. 2008). Colibacillosis is a common disease in all stages of life birds, resulting in a substantial economic losses worldwide (Bisaillon et al. 1988, Lutful-Kabir 2010). If antibiotics use is restricted in poultry production, it would be anticipated that colibacillosis would become an even greater burden. If antibiotic use is restricted in poultry production, it would be expected that colibacillosis may become an even greater burden (Huff et al. 2002). Exposure to *E. coli* and its lipopolysaccharide endotoxin results in edema and inflammatory in tissues, elevated levels of acute phase proteins, and increased production of particularly active pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and transcription factor, necrotic factor-kappa B (NF-κB) (Turkozkan et al. 2005, Helwig et al. 2006, Teo & Tan 2006, Demir et al. 2007). *E. coli* can also lead to increased lipid peroxidation of cell membranes and impaired antioxidant defense of the body, increased vascular permeability and accumulation of proteins and fluid outside cells, and tissue damages (Celik et al. 2007, Shen et al. 2010). These changes (oxidative stress and inflammatory processes) would be responsible for a variety of diseases in animals and humans. Considering the fact that the *E. coli* infections arise the uninvited economic costs in poultry industry, and due to the high resistance of bacteria to the antibiotics and other chemical agents, it is rationale to survey the alternative approaches including natural and safe materials to limit these adverse complications.

Because of anti-inflammatory and anti-oxidative activities of cinnamon on body systems, pretreatment with this material can prevent the adverse and detrimental effects of pathogenic conditions such as infection with *E. coli.* There are some reports regarding the effects of cinnamon on blood biochemistry of broiler chickens (Faix et al. 2009, Sang-Oh et al. 2013); however, its influence on the *E. coli* challenge as an important infectious disease in birds has not been investigated yet. Therefore, the purpose of this study was to investigate the effects of cinnamon on alterations of liver enzymes in serum and the expression of key mediators involved in inflammation, ie NF-κB and TNF-α in liver of broiler chickens infected with *E. coli.*

MATERIALS AND METHODS

Animals. In this study, 90 Ross-308 one-day-old broiler chickens were used. The experimental protocol was approved and strictly followed by the local Committee for animal ethics (approval number: 17487-5-11-13). Animal diets were adjusted based

Table 1. Composition of the basal diets fed to broiler chickens during the experiment

Components (%)	Growing periods		
	Starter diet	Growing diet	Finisher diet
Corn	62.37	68.92	70.96
Soybean	29.06	22.59	19.91
fat powder	3.00	3.00	4.00
shell powder	1.18	1.44	1.38
Salt	0.35	0.35	0.35
vitamin supplementation	0.25	0.25	0.25
mineral supplementation	0.25	0.25	0.25
di-calcium phosphate	2.08	1.54	1.34
DL-methionine	0.24	0.27	0.27
lysine monohydrochloride	0.21	0.23	0.29
natrium salinomycinate	0.50	0.50	0.50
arabinoxylanase	0.50	0.50	0.50
Sand	0.01	0.16	0.00
Calculated analysis			
Metabolic energy (kcal/kg)	2834	2894	2978
Protein (%)	19.95	17.86	16.92
Calcium (%)	0.95	0.90	0.83
Phosphorus (%)	0.47	0.45	0.41
Sodium (%)	0.17	0.17	0.17
Potassium (%)	0.84	0.74	0.69
Chloride (%)	0.24	0.24	0.24
Methionine (%)	0.27	0.24	0.22
Methionine+Cysteine (%)	0.83	0.80	0.77
Lysine (%)	1.14	1.00	0.98
Tryptophan (%)	0.28	0.24	0.23

on NRC International Organization guidelines. Diets were set for three courses, including a starter diet (1-10 days), a growing diet (11-21 days) and a finisher diet (day 22 up to slaughter time). The basal diet of chickens was obtained from Dansazan (Tabriz, Iran). The composition of the diet has been shown in Table 1. The dimensions of cages allocated for chickens were 0.9×1.5×1 meters with a floor area of 1.5 m2. Chickens had free access to food and water. The initial room temperature 32-33°C was reduced weekly by 1°C to a final temperature of 25°C which kept until the end of the experimental period. The environmental humidity of the room was adjusted at 65-70% at the start of the experiment.

Animal grouping and experimental protocol. All broilers were firstly weighted to get an overall mean weight of all animals. Then, the birds were allocated into different groups so that the mean weights of each group is close to the overall mean of total. The broiler chickens were divided into 6 groups with 3 replicates, 5 birds per treatment as following:

Group 1: Normal diet (Control);
Group 2: Normal diet with cinnamon 100 mg/kg of food (NC100);
Group 3: Normal diet with cinnamon 200mg/kg of food (NC200);
Group 4: Normal diet plus *E. coli* injection (*E.coli*);
Group 5: Normal diet with cinnamon 100 mg/kg of food, plus *E. coli* injection (EC100);
Group 6: Normal diet with cinnamon 200 mg/kg of food, plus *E. coli* injection (EC200).

All broilers were fed with normal diet until day 20. After day 21, the powder extract of cinnamon at concentrations of 100 or 200mg/kg of food were added to the diet of the broilers in corresponding groups. Then on day 32, the amount of 0.2ml suspension of *E. coli* bacteria at a concentration of 10^8 cfu were injected subcutaneously to animals in related groups (The groups untreated with *E. coli* were injected with equal volumes of distilled water) and 72 hours after the end of the experimental period, the blood samples were obtained from their wing veins and transferred to the test tube containing EDTA. In addition, all animals were sacrificed and liver samples were taken and immediately stored at -80°C until biochemical analysis.

Cinnamon extract preparation. The cinnamon barks were purchased from the local market form Tabriz in Azarbaijan province, Iran. After scientific identification and confirmation of the *Cinnamomum zeylanicum* species by an expert of herbarium of the university, the plant samples were cut into small pieces, dried at room temperature in the shade, and then finely grinded into powder. The extract was made by soaking the powder (350 g) with methanol (90%) at 25°C for 48 h. The extraction was repeated three-times and the solvent was completely evaporated in vacuum, and dried extract was stored at -20°C until use.

Preparation of *E. coli* suspension and *E. coli* challenge. The preparation of *E. coli* suspension (O157: H7) for inoculating chickens was performed as the method described previously. Briefly, one loopfull of the stock culture was plated on Luria–Bertani (LB) agar and incubated at 37°C for 18 h. A single colony of this fresh culture was inoculated in 5ml of LB broth and incubated at 37°C overnight with agitation.

Then, the cultures were diluted 1:100 in fresh LB broth and grown at 37°C with agitation and shaking. After incubation, cultures were harvested by centrifugation at 5000g for 10 min and the cell pellet was re-suspended in phosphate-buffered saline (PBS) to a concentration of about 10^8 colony forming unit (cfu)/ml. For verifying the suspension count and number of bacteria, the turbidity of suspension was adjusted with the McFarland 0.5 turbidity standard to attain 10^8 cfu/ml of bacteria. At day 32, the challenged chickens were inoculated subcutaneously with PBS containing approximately 10^8 cfu of a pathogen strain of *E. coli* to induce the pathologic changes in poultry.

Measurement of biochemical enzymes in serum and liver function indications. Blood samples were obtained at the end of the experiment and before surgery by wing vein blood collection. The blood sample was immediately centrifuged, and serum was stored at -20°C until measurement of liver enzymes concentrations. The concentrations of enzymes creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP) in serum were determined spectrophotometrically by using the enzymes-specific detection kits (Roche Diagnostica, Basel, Switzerland) according to the manufacturer's specifications. The absorbance of CK and LDH were determined by a spectrometer (pharmacia biotech, Sweden) at 340nm wavelength, and the absorbance of AST, ALT and ALP were determined at 590nm wavelength. Concentrations were determined in duplicates of each sample.

Supernatant preparation from liver tissue. Certain weight of liver tissue were homogenized on ice by a homogenizer in 1ml of lysis buffer containing (10mM Nacl, 1.5mM MgCl2, 20mM HEPES, 20% glycerol, 0.1% Triton X-100, and pH 7.4). Homogeneous solution was then centrifuged (rpm1000, for 10 minutes at 4 °C), Supernatant was collected and a protease inhibitor cocktail (104 mM AEBSF, 0.08% mM aprotinin, 2 mmol leupepin, 4mmol bestatinbestatin, 1.5mM pepstatin A, and 1.4mM E-64) (P840, Sigma-Aldrich, St Lois, MO) was added on it and kept at -80°C until use (Rothermel et al. 2000). Protein concentration in the supernatant was determined using a total protein kit, according to the manufacturer's instructions (Randox Lab. Crumlhn, UK).

Detection of liver pro-inflammatory genes expression: Real-time PCR. The quantitative real-time PCR was used to validate the expression levels for selected genes (Wyss & Kaddurah-Daouk 2000). RNA samples were prepared from approximately 100 mg of tissue using TRIzol® Reagent. cDNA was synthesized from 2 µg of RNA using AccuPower® RT PreMix (Bioneer, Daejeon, Korea) primed with random hexamer-primers under conditions of 70°C for 15 min, 42°C for 60 min, and 95°C for 5 min. The Rotor-Gene

TM 6000 system (Corbett Life Science, Australia) was utilized for performing all amplification reactions. cDNAs were diluted 1:5 in nuclease-free distilled water and 5µL of diluted cDNA was added to 20µL of PCR mixture containing SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) and 0.2µmol/L of each primer. Primer sequences were for for TNF-α forward: GAGCTGTGGGGAGAACAAAAGGA and reverse: TTGGCCCTTGAAGAGGACCTG; for NF-κB forward: CAAGGCAGCAAATAGACGAG and reverse: GTTGAGAGTTAGCAGTGAGGCA; for β-actin forward: CCTGGAGGAGAG CTACGAG and reverse: TTCATGATGGAGTTGAAGGT. The PCR conditions for pro--inflammatory genes and β–actin were 95°C for 10 min followed by 35 cycles at 94°C for 25 sec and 60°C for 50 sec. Relative gene expressions of TNF-α and NF-κB were calculated with the $2^{(-\Delta Ct)}$, using β-actin mRNA expression levels as the endogenous housekeeping gene.

Expression of target genes/β-actin = $[1+E]^{-Ct}$ target gene/ $[1+E]^{-Ct}$ β-actin. The specificity of the real-time PCR reactions for each primer set was verified by generation of a melting curve analysis and checked by gel electrophoresis. The amplification efficiency of PCR reactions was determined by running standard curves, which was derived from the 10-fold serial dilution of a positive PCR product by a customary RT-PCR. Logarithms of concentrations were plotted against target gene cycling threshold (Ct) of serial dilution. TNF-α, NF-κB, and β-actin efficiencies were 99%, 98% and 99% respectively.

Statistical analysis. Data are reported as mean ± SE. The data between groups' differences were analyzed using *one-way ANOVA* followed by *Tukey's* test as a post hoc. The statistical level of P<0.05 was considered significant.

RESULTS

Effect of Cinnamon extract on the enzyme creatine kinase (CK) level in different experimental groups

The liver enzymes activities in serum as indicators of liver function were measured in healthy and *E. coli*-inoculated broiler chickens previously treated or untreated with cinnamon extract. Injection of the bacterium could increase the serum levels of enzymes in comparison to the control group. The CK levels in *E. coli*-inoculated group was significantly increased as compared to controls (P<0.05) (Fig.3). Adding the powdered cinnamon extract at doses of 100 and 200 mg/kg of diet in groups NC100 and NC200 did not significantly change the levels of this enzyme as compared to those of control group. However, both doses of cinnamon extract in broilers inoculated with *E. coli* significantly reduced the level of CK as compared with *E. coli* group and brought the level of that enzyme to control values (P<0.05). Additionally, the decreasing effect of 200 mg/kg cinnamon (P<0.01) was greater than those of 100mg/kg extract (P<0.05) (Fig.1).

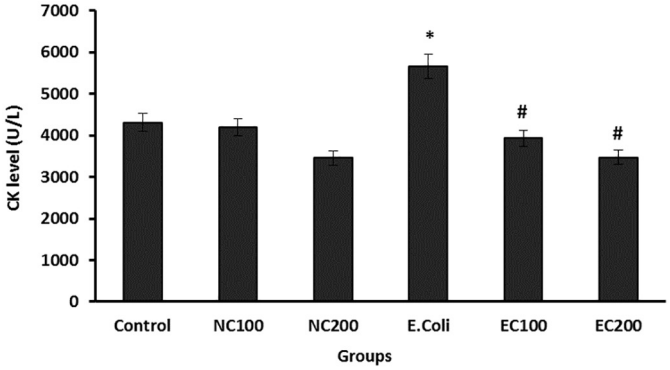

Fig.1. Changes in the levels of creatine kinase (CK) among experimental groups. *P<0.05 as compared with control group, #P<0.05 as compared with *Escherichia coli* group. In all figure: NC100 = cinnamon 100mg/kg of diet; NC200 = cinnamon 200mg/kg of diet; EC100 = *E. coli* plus cinnamon 100mg/kg of diet; and EC200 = *E. coli* plus cinnamon 200mg/kg of diet.

Effect of Cinnamon extract on the enzyme lactate dehydrogenase (LDH) level in different experimental groups

The effect of cinnamon on the activity of enzyme lactate dehydrogenase (LDH) in various groups is showed in Figure 2. The level of LDH in *E. coli* group was not significantly varied as compared to the control group. In addition, the results showed that the level of this enzyme in groups NC100 and NC200 was near to control values. In animals inoculated with *E. coli*, the findings indicated that only the concentration of 200 mg/kg extract led to decrease in the level of LDH as compared to *E. coli* group (P<0.05).

Effect of cinnamon extract on the enzyme aspartate transferase (AST) level in different experimental groups

The levels of aspartate transferase (AST) in *E. coli* group as well as in healthy chickens receiving 100 and 200 mg/kg cinnamon in diet were not significantly changed from the control value, while only the dose of 200 mg/kg cinnamon could significantly reduce the level of this enzyme

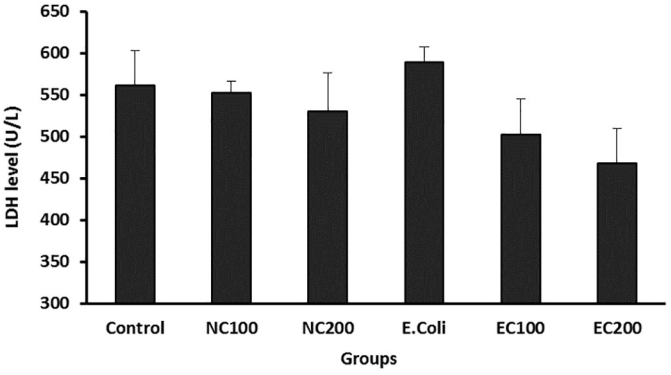

Fig.2. Changes in the levels of lactate dehydrogenase (LDH) among experimental groups. #P<0.05 as compared with *E. coli* group.

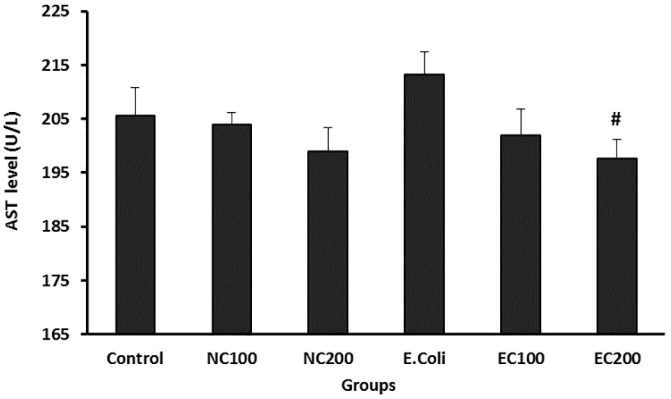

Fig.3. Changes in the levels of aspartate transferase (AST) among experimental groups. #P<0.05 as compared with *E. coli* group.

in *E. coli*-inoculated chickens compared with un-inoculated chickens (P<0.05) (Fig.3).

Effect of cinnamon extract on the enzyme alanine transferase (ALT) level in different experimental groups

The effects of cinnamon and *E. coli* on the activity of enzyme alanine transferase (ALT) in different groups are showed in Figure 4. *E. coli* injection increased the level of ALT in comparison with untreated controls (P<0.05). Although the administration of cinnamon at the concentrations of 100 or 200 mg/kg in food reduced this enzyme from control value, this effect was not statistically significant. However, administration of cinnamon extract in both concentrations significantly lowered the levels of ALT as compared with those of *E. coli* group (P<0.05) (Fig.4).

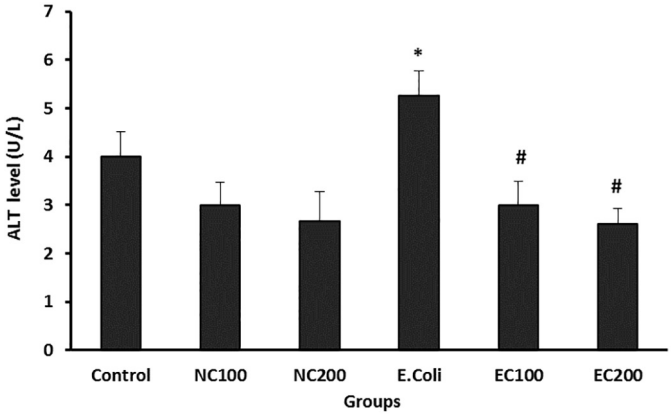

Fig.4. Changes in the levels of alanine transferase (ALT) among experimental groups. *P<0.05 as compared with control group, #P<0.05 as compared with *E. coli* group.

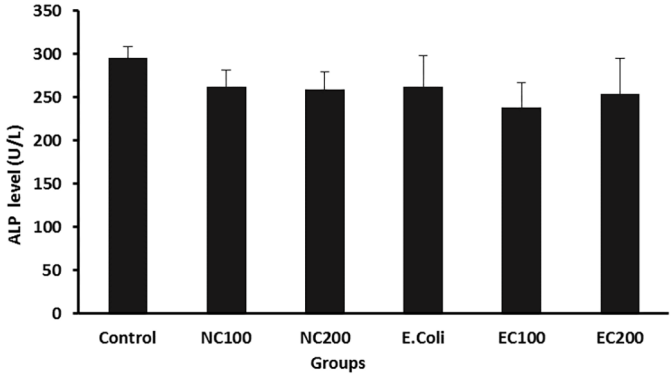

Fig.5. Changes in the levels of alkaline phosphatase (ALP) among experimental groups.

Effect of cinnamon extract on the enzyme alkaline phosphatase (ALP) level in different experimental groups

Figure 5 shows the alterations of alkaline phosphatase (ALP) in serum of broiler chickens treated or untreated with *E. coli* and/or cinnamon. The findings indicated that the administration of cinnamon extract could not influence on this enzyme and there were no statistically significant changes in the level of ALP either in control or in *E. coli* injected chickens.

Effect of Cinnamon extract on TNF-α gene expression in different experimental groups

The gene expressions of TNF-α in different experimental groups were measured using the technique of real-time PCR (Fig.6). The results of real-time PCR showed that the injection of *E. coli* significantly increased the expression of this gene as compared with those of control healthy broilers (P<0.05). In NC100 and NC200 groups in which two different doses of cinnamon extract (that is; 100 and 200 mg per kg of food, respectively) were added to the diets of control broilers, gene expression of TNF-α was not significantly differ from control group and the level of this gene was similar to those of controls.

To investigate the protective effect of cinnamon extract on the expression of this gene in broiler chickens inoculated with *E. coli* bacteria, it was found that both doses of 100 and 200 mg extract of cinnamon caused a significant reduction in TNF-α expression levels as compared to the group injected only with *E. coli* bacteria (P<0.05). It was also observed that the decreasing effect of dose of 200 mg/kg extract was greater than those of 100 mg/kg extract (Fig.6).

Effect of Cinnamon extract on the expression of NF-κB in different experimental groups

The results of real-time PCR showed that the expression level of NF-κB gene in group inoculated with *E. coli* was significantly increased as compared with those of control group (P<0.05) (Fig.7). Administration of cinnamon extrac-

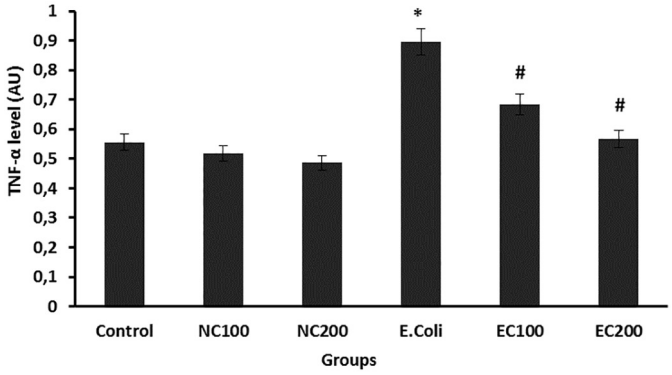

Fig.6. Changes in the gene expression of TNF-α among experimental groups. *P<0.05 as compared with control group, #P<0.05 as compared with *E. coli* group.

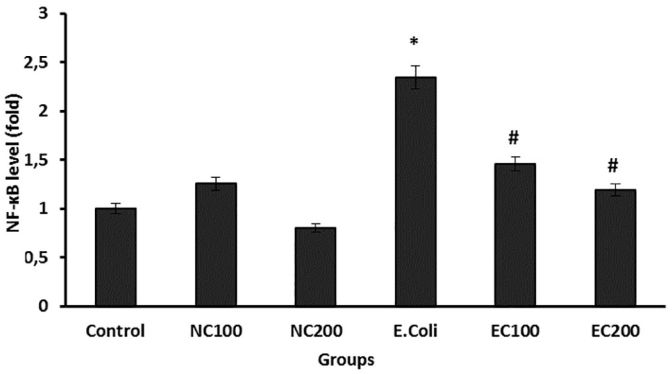

Fig.7. Changes in gene expression of NF-κB in experimental groups. *P<0.05 as compared with control group, #P<0.05 as compared with *E. coli* group.

tion at the concentration of 100 and 200 mg/kg to the food of healthy control chickens could not significantly change the expression level of NF-κB gene in comparison with untreated control group. However, in *E. coli* injected broiler chickens, both doses of extract significantly prevented the *E. coli*-induced increase of NF-κB expression ($P<0.05$) and reduced the level of expression toward the levels of control group (Fig.7).

DISCUSSION

Natural products, especially medicinal plants are increasingly used to cure various diseases such as infectious diseases, especially because of their multiplex properties and minimal adverse effects on the body. Alterations in the nutrient intake of living organisms could improve their metabolisms, leading to the protection against serious diseases. Bacteria with multiple antibiotic resistance are a growing concern in human and animal medicine. In this study, the effects of cinnamon extract on broiler chickens inoculated with *E. coli* were studied. Reduced liver enzymes activity as well as reduced gene expression levels of TNF-α and NF-kB by pre-treatment with cinnamon in broiler chickens inoculated with *E. coli* indicates the tissue protective effects and anti-inflammatory properties of this medicinal plant in pathological circumstances. Similarly, previous studies have revealed that cinnamon has anti-oxidative, anti-inflammatory and anti-microbial properties (Faix et al. 2009, Stefan et al. 2009, Sang-Oh et al. 2013). Chang et al. (2001) have also showed that cinnamon extract has anti-bacterial effects against *E. coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella* and *Vibrio parahaemolyticus* (Chang et al. 2001, Griggs & Jacob 2005). In addition, it has been suggested that cinnamon enhances phagocytic activity of macrophages, playing a role in modulating the immune defensive system. Cinnamon essential oils have showed certain anti-oxidative action in overweight chickens and its extract may have protective influences in oxidative stress-related diseases in humans (Stefan et al. 2009). However, the majority of previous research has been performed on the healthy subjects not on pathologic conditions. In addition, infection with *E. coli* is a common disease among poultry industry which its pathophysiology may be related to the oxidative stress and inflammatory responses. In this study, we used *E. coli*-inoculated chickens as a common pathologic condition to delineate the effects of the cinnamon on this condition as well as in healthy chickens.

Helwig et al. (2006) and Turkozkan et al. (2005) have reported that the exposure with *E. coli* lead to the inflammatory responses reactions and increase in the concentration of cytokines likes TNF-α and transcription factor NF-κB, which in turn induces the expression of other pro-inflammatory cytokines such as IL-5, IL-2, IL-3, IL-12, and IL-1β (Turkozkan et al. 2005, Helwig et al. 2006). Furthermore, Celik and colleagues (2007) and Shen and colleagues (2010) have found that infection with *E. coli* induces the oxidative stress and lipid peroxidation reactions leading to the development of tissue injuries and subsequent detrimental outcomes in the host body (Celik et al. 2007, Shen et al. 2010). The increased levels of pro-inflammatory mediators and oxidative stress markers due to the inoculation with *E. coli* may cause cell membrane damages and apoptotic reactions which play crucial roles in cell survival and cell death. In the present study, for the first time we showed that pre-administration of cinnamon to the diet of broiler chickens significantly prevented the *E. coli*-induced increase in the expression levels of inflammatory genes and liver enzymes; therefore, feeding the broiler chickens with cinnamon extract may potentially protect their body organs against detrimental influences of infection inoculation with *E. coli.*

Furthermore, the results of this study showed that the cinnamon-induced changes in gene expression of TNF-α and NF-KB were associated with the changes of liver enzymes including AST, ALT, ALP, CK and LDH in *E. coli*-inoculated broiler chickens. Elevated levels of liver enzymes in serum indicate the destruction of hepatocytes and dysfunction of the liver. The extract, especially at a concentration of 200 mg per kg of food, significantly reduced the levels of AST, ALT and CK in the serum of infected broilers. These findings indicate that the cinnamon extract has a potential to limit the tissue injuries induced by pathogenic agents. Our results are somewhat in accord with the findings of Abd-El-Rahman and colleagues (2010) which they reported that oral application of extract and powder of cinnamon in rats with type 2 diabetes mellitus decreased the levels of liver enzymes including AST (Abd-El-Rahman et al. 2010). Additionally, the results of the study of Amin and colleagues (2009) on the effect of cinnamon on fatty liver disease in rats are also along with these findings (Amin & Abd-El-Twab 2009).

Because of anti-inflammatory and anti-oxidative potentials of cinnamon and its modulating activities in immune system due to its constituents such as cinnamaldehydes and terpenoids having anti-allergic and anti-bacterial features, it can be concluded that the cinnamon would be a good substance in enhancing the immune system and reducing the toxicity of pathological conditions such as infection with *E. coli* on the body systems of humans and poultry.

CONCLUSIONS

According to findings of the present study, infection with *E. coli* might induce the expression of pro-inflammatory genes TNF-α and NF-kB in the liver and lead to the biochemical alterations in the serum of broiler chickens.

Administration of cinnamon extract, at the concentrations of 100 and 200mg/kg food, before the inoculation with *E. coli* could reduce the levels of gene expression and tissue damages indicated by liver enzymes.

Due to the extensive poultry industry and its economic costs worldwide, considering the poultry meet as a more-consumed dietary product of the families and its direct relation with humans' health, it is important to promote the growth of the poultry and reduce the related economic damages and costs.

Therefore, adding the supplements such as cinnamon to the diet of the broiler chickens may decrease the burden of pathologic conditions, reduce the medicinal costs in farms, and increase the resultant profits.

These, in turn, lead to developing a safe dietary product in which the natural compounds like cinnamon extract has been used instead of harmful antibiotics.

Due to the preventive effects of cinnamon on pathological conditions, it can be assumed that broiler chickens would be profited with pretreatment by this material, although complementary research is needed to confirm this hypothesis, especially on the standardization of the cinnamon concentration and periods of supplementation.

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