



The Protective Effect of Cinnamon against Thermally Oxidized Palm Oil in Broiler Chickens

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ABSTRACT

The present study was planned to investigate the extent of the protective effect of cinnamon against the possible damage effect of thermally oxidized palm oil on broiler chicks. A total number of 150 one d-old Cobb 500 broiler chicks were randomly divided into three treatment groups. The first group was served as control and fed the basal diet with tap water. While the 2nd group fed the basal diet supplemented with 1g cinnamon/kg diet, the 3rd group fed the basal diet supplemented with 5% thermally oxidized palm oil in combination with 2g cinnamon/kg diet. The experiment has lasted till chicks were 42 d old. Bodyweight, feed consumption, feed conversion ratio was estimated. Estimation of the effect of different treatments on the PPAR- α gene expression, Estimation of the lipid profile in serum, Estimation of the cholesterol level in the liver tissue. Correlation between the cholesterol level in both serum and liver tissue and determination of oxidative stress markers in serum. The results showed that the addition of cinnamon increases body weight and feed consumption plus improving the feed conversion ratio. Cinnamon also causes a significant increase in the PPAR- α gene expression in liver tissue, decreases the cholesterol concentrations in serum and liver, decreases triglycerides in serum and decreases the oxidative stress markers.

INTRODUCTION

Poultry production is a business which like any other business seeks to generate profit, one of the objectives of any poultry producer is to keep the balance between a cheap diet with the least cost and obtain maximum productivity (Ahiwe, *et al.*, 2018). Lipids are commonly added to the poultry rations as concentrated sources of energy to improve feed efficiency (Pettigrew and Moser, 1991). Cinnamon is one of the phytochemical feed additives which are plant-derived products used in animal feeding in order to improve livestock performance. Recently this class of feed additives has gained interest, especially in poultry production.

Cinnamon is one of the oldest medicinal plants, belonging to Lauracea family, the genus *Cinnamomum* China, India and Australia (Koochaksaraie, *et al.*, (2011). The main substance in Cinnamon is cinnamaldehyde. Cinnamon is a plant that has a variety of uses among many different cultures, from spicing up foods to deterring germs from growing. There are actually two main forms of cinnamon that are commonly found in foods. The first, *Cinnamomum verum*, also known as “true” cinnamon or Ceylon cinnamon, is commonly used in sweet pastries. On the other hand, *Cinnamomum cassia*, also known as cassia, Chinese cinnamon or “bastard” cinnamon, is used as a stronger spice in a variety of foods (Rahman, *et al.*, 2013). The cinnamon can be used to improve the health of the colon, reduce the risk of colon cancer (Wondrak, *et al.*, 2010), Coagulant prevents bleeding (Hosseini, *et al.*, 2013), increases the blood circulation in the uterus and advances tissue regeneration (Minich, & Msom, 2008), antimicrobial (Chang, *et al.*, 2001; Gende, *et al.*, 2008) antifungal (Wang, *et al.*, 2005), antioxidant (Mancini-Filho, *et al.*, 1998), antidiabetic (Kim, *et al.*, 1993; ONDEROGLU, *et al.*, 1999; Kim, 2006), anti-inflammatory (Chao, 2005), antitermitic (Tung, *et al.*, 2010), nematicidal (Park, *et al.*, 2005; Kong, *et al.*, 2007). mosquito larvicidal (Cheng, *et al.*, 2004). Insecticidal (Cheng, *et al.*, 2009), antimycotic, (Cheng, *et al.*, 2009; Bandara, *et al.*, 2012) and anticancer agent (Lu, *et al.*, 2009; Koppikar, *et al.*, 2010). tooth powder and to treat toothaches, dental problems, oral microbiota, and bad breath Aneja, *et al.*, 2009; Tyagi, *et al.*, 2011). Cinnamon also have antioxidant activity, act as a lipid-lowering agent on Hypercholesterolemic cases and have been shown to reduce oxidative stress in a dose-dependent manner

through inhibition of 5-lipoxygenase enzyme, improves glucose metabolism and diabetes not only by hypoglycemic effect but also by improving lipid metabolism and antioxidant status (Koochaksaraie, *et al.*, 2011). In poultry farms the most used lipids are those lipids which previously subjected to heating and potential oxidative processes before being used in poultry diets for cost saving (Canakci, 2007), Lipids serve as a cheap form of energy because lipids supply about 2.25 times more energy than carbohydrates and proteins (Azain, 2001). Lipids also improve the absorption of fat-soluble vitamins and increases the efficiency of the consumed energy (lower caloric increment) (Baião, & Lara, 2005). Lipids help in Supplying fat-soluble vitamins and essential fatty acids, reduce dust in the facilities, attenuating growth reduction in heat stress conditions, improve the pellet quality, and improve the diet palatability (Pettigrew, and Moser, 1991). In fast-food restaurants, fat is heated in fryers about 18 h daily, at temperatures close to 180°C. For cost-saving, heated fats are used for up to 1 week before it is discarded and replaced with a fresh one. These fats have high concentrations of lipid peroxides (Sülzle, *et al.*, 2004). Several studies reported that the consumption of oxidized fats affects metabolism in several ways (Corcos, *et al.*, 1987; Blanc, *et al.*, 1992; Hochgraf, *et al.*, 1997; Skufca, *et al.*, 2003). The concentrations of various lipid peroxidation products in heated fats depend on their thermal treatment. (Kubow, 1992) reported that oxidized fat heated at a relatively low temperature over a long period containing high concentrations of primary lipid peroxidation products affected the lipid metabolism of rats more than an oxidized fat heated at a high temperature for a shorter period (Skufca, *et al.*, 2003). Heating of oils

at high temperatures and in the presence of oxygen results in their oxidative deterioration. Oxygen from air and water from food being fried when mixed with heated oil accelerating the rate of its oxidation. The cooked food absorbs this oxidized oil so it becomes part of our diet (Ammu, *et al.*, 2000). In developing countries, the intake of highly oxidized fat through the intake of deep-fried food is high. Because lipid peroxidation is a free radical producing reaction, consumption of lipid peroxidation products can cause oxidative stress by straining the antioxidant defense system creating an imbalance of free radicals in vivo (Lindblom, 2017). Oxidative stress is caused by the imbalance between prooxidants and antioxidants at the cellular or individual level (Voljc, *et al.*, 2011). Oxidative stress constitutes an important factor of biological damage and is regarded as the cause of several pathological conditions that affect poultry growth and development (Avanzo, *et al.*, 2001; Iqbal, *et al.*, 2002). In poultry, oxidative stress may occur due to several factors such as: 1) feed (high concentration of polyunsaturated fatty acids [PUFA], contamination with fungal toxins, prolonged storage, antioxidant deficiency) (Chung, *et al.*, 2005), 2) environmental (heat, high stocking density, transportation, vaccination) (Sahin, *et al.*, 2003; Panda, *et al.*, 2008), and 3) pathological conditions (ascites, fatty liver hemorrhagic disease syndrome, arthritis, coccidiosis) (Papas, 1999; Iqbal, *et al.*, 2002).

MATERIALS AND METHODS

Birds and Management:

A total number of 150 one-day-old Cobb 500 broiler chicks of both sexes, weighing 48- 53 g were purchased from Ismailia-Misr Poultry Company, Egypt. Chicks were left in a good ventilated clean place with temperature range (32- 35° C). Electric bulbs were used as a source of light

and electrical heaters were used to adjust the temperature. The light was provided to chicks around the whole day's length (24 hours). All birds were treated in accordance with the bird's use protocol approved by the Faculty of Veterinary Medicine, Suez Canal University.

Experimental Diet:

Experimental birds offered 2 rations (starter, from 1- 3 weeks of age and grower, from 3 – 6 weeks of age). Both diets were formulated to meet the nutrient requirements of broiler chicks according to NRC (1994).

Experimental Design:

Chicks were randomly divided into 3 random groups; each group contains 50 Cobb 500 chicks. The first group: Fed on basal ration without any additives and act as a control group (G1). The 2nd group: Fed on basal ration supplied with 2g cinnamon/kg diet (G2). The 3rd group: Fed on basal ration supplied with 5% thermally oxidized palm oil in combination with 2 g cinnamon/kg diet (G3).

Preparation of Thermally Oxidized Oil:

Palm oil purchased from the local market. The thermal oxidation of the palm oil was done in an uncovered stainless-steel pan fryer. The thermal oxidation processes were repeated 15 times at 175 ± 5 °C (15 minutes each) twice daily for 8 successive days. No renewal of oil was done. At the end of the experiment, oil was taken out until it reaches the room temperature then placed in a bottle in the refrigerator (4°C), and then thoroughly mixed with the basal diet freshly day by day (Izaki, & Uchiyama, 1984).

Bodyweight and body weight gain:

Body weight and body weight gain of each bird were determined weekly according to Brady, (1968). The live body weight changes were taken as a measure for growth. Bodyweight gain was determined by subtraction of 2 successive weights.

Feed Consumption g/ week:

The feed consumption (g/week) was calculated per group by obtaining sum difference between the weight of offered feed and the remained portion for 7 days.

Feed Conversion Ratio (FCR):

Feed conversion ratio FCR (g) /bird/week was obtained by dividing food consumption (g)/ week by the number of birds in each group. Bodyweight gain was calculated by subtracting 2 weekly successive weights. The feed conversion ratio (FCR) was calculated weekly.

$$FCR = \frac{\text{Feed consumption}(g)/\text{bird}/\text{week}}{\text{Body weight gain}(g)/\text{bird}/\text{week}}$$

Blood & Tissue Sampling:

At the age of 3 and 6 weeks 15 chicks from each group were taken and fasted overnight and then blood samples were collected by slaughtering into plain tubes (nonheparinized tubes) for serum separation. Blood was left for 15 min to clot then kept in the refrigerator for 3 hours then centrifuged at 3000 rpm for 20 min to obtain serum which is stored at -20°C for biochemical analysis. Liver samples were taken immediately and kept at RNA-Later Stabilization Solution which stabilizes and protects cellular RNA, and stored at -20°C for PPAR- α gene expression analysis.

Determination of Serum Lipid Profile (mg/dl):

Serum levels of total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C), were measured using enzymatic calorimetric kits (Cat. No. 0599, Stanbio Laboratory, USA, Cat. No. 304710050, ELITech Diagnostic, France and (Cat. No. 303113050, ELITech Diagnostic, France) following the instructions of the corresponding reagent kit., respectively, Serum low-density lipoprotein cholesterol (LDL-C) was measured using enzymatic calorimetric kits (lot no. 990610, QCA Co., Spain), following the

instructions of the corresponding reagent kit.

Determination of Liver total Cholesterol:

Total lipids from the liver were extracted using the modified method of Folch, *et al.* (1957). Briefly, 250 mg of frozen liver tissue from the same region of the liver was weighed and transferred into a 2-mL flat-bottom centrifuge tube containing 0.5 mL methanol. After homogenization, 0.5 mL of chloroform and 0.4 mL of dist. water were added to the liver homogenate and mixed by vortexing. The lipid fraction in chloroform was separated from the aqueous fraction and liver debris by centrifuging for 10 min at 14,000 rpm at 20°C and was then transferred to a new glass tube. After drying the lipid fraction was reconstituted in n-butanol for further analysis of TC. TC concentrations were determined enzymatically by conducting colorimetric assays (Pointe Scientific, Canton, MI) in a 96-well plate reader (SpectraMAX 250, Molecular Devices, Sunnyvale, CA).

Determination of Oxidative Stress Markers in Serum:

Harvested sera was used for the determination of serum oxidative stress markers. Serum Catalase (CAT) activity was assessed by measuring catalase degradation of H₂O₂ using a redox dye (ELISA Kit: QuantiChrom™, BioAssay Systems, USA, Catalog No. ECAT-100) according to Cowell, *et al.*, (1994). Superoxide dismutase (SOD) activity was measured by the xanthine oxidase method (ELISA Kit: Cayman Chemical Company, USA, Catalog No. 706002), which monitors the inhibition of nitro blue tetrazolium reduction by the sample (Sun, *et al.*, 1988). Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) forming the MDA-TBA product in acidic conditions and high temperatures (90-100°C) and

measured colorimetrically at 540 nm. Sample malonaldehyde concentration was compared to a MDA standard curve (Fernández-Dueñas, 2010).

Gene Expression Analysis:

The oligonucleotide primers and probes used in SYBR Green real time pcr are demonstrated at (Table 1)

Extraction of RNA (according to RNeasy Mini Kit instructions) :

Thirty mg of organ sample was weighed and put in 2 ml screw-capped tubes. 2) 600 µl of the Buffer RLT (with 10 µl β-Mercaptoethanol/ml Buffer RLT) was added into the tubes. 3) For the homogenization of samples, tubes were placed into the adaptor sets, which are fixed into the clamps of the TissueLyser. Disruption was performed in 2 minutes high-speed (30 Hz) shaking step. 4) The lysate was centrifugated for 3 min at 14000 rpm. 5) One volume of 70% ethanol was added to the cleared lysate and mixed immediately by pipetting. 6) 700 µl of the sample, including any precipitate that may have formed, was transferred to an RNeasy spin column placed in a 2 ml collection tube. Centrifugation was done for 1 min. at 14000 rpm. The flow-through was discarded. 7) Step 6 was repeated again for the excess

volume. 8) 700 µl of Buffer RW1 was added. Centrifugation was done for 1 min. at 10000 rpm. The flow-through was discarded. 9) 500 µl of Buffer RPE was added. Centrifugation was done for 1 min. at 10000 rpm. The flow-through was discarded. 10) Step 9 was repeated again, but Centrifugation was done for 2 min. at 10000 rpm. 11) RNA was eluted by adding 50 µl RNase-free water. Centrifugation was done for 1 min. at 10000 rpm. Cycling conditions for SYBR green real time PCR according to Quantitect SYBR green PCR kit are demonstrated at (Table 2)

Analysis of the SYBR Green rt-PCR Results:

Amplification curves and ct values were determined by the Stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the control group according to the "ΔΔCt" method stated by Yuan, *et al.*, (2006) Whereas $\Delta\Delta Ct = \Delta Ct_{reference} - \Delta Ct_{target} - \Delta Ct_{target} = Ct_{control} - Ct_{treatment}$ and $\Delta Ct_{reference} = Ct_{control} - Ct_{treatment}$

Table 1: Oligonucleotide primers and probes used in SYBR Green real-time PCR

Gene	Primer sequence (5'-3')	Reference
PPAR-α	TGGACGAATGCCAAGGTC	Zhou, <i>et al.</i> 2016
	GATTTCCTGCAGTAAAGGGTG	
β. actin	CCACCGCAAATGCTTCTAAAC	Yuan, <i>et al.</i> , 2007
	AAGACTGCTGCTGACACCTTC	

Table 2: Cycling Conditions for SYBR green real-time PCR according to Quantitect SYBR green PCR kit

Gene	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
			Secondary denaturation	Annealing (Optics on)	Extension	econdary denaturation	Annealing	Final denaturation
PPARα	50°C 30 min	94°C 15 min.	94°C 15 sec.	60.3°C 30 sec.	72°C 30 sec.	94°C 1 min.	60.3°C 1 min.	94°C 1 min.
β. actin	50°C 30 min.	94°C 15 min.	94°C 15 sec.	51°C 30 sec.	72°C 30 sec.	94°C 1 min.	51°C 1 min.	94°C 1 min.

Statistical Analysis:

Data collected from treated groups were statistically analyzed in comparison to the control group and each other for the mean and standard error. Data were expressed as means \pm SE. Differences between means of different groups were carried out using one-way ANOVA followed by Duncan multiple comparison tests using a statistical software program (SPSS for Windows, version 16, USA). Differences were to be significant at ($P < 0.05$) and highly significant at ($P < 0.01$) according to (Coakes, *et al.*, 2010). In addition, relationships between measures of the TC level in both serum and liver tissue variables were evaluated by simple linear correlation (Pearson correlation coefficients) analysis using a statistical software program (SPSS for Windows, version 16, USA). Treatment effects were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Table (3) demonstrated that LBW was non-significantly altered in 1st and 2nd week of treatments. While in the 3rd week there was a significant increase in the G2 group than both G1, G3 groups. In the 4th week, there was a significant increase in (G2) group LBW than G3 group, while in the 5th and 6th weeks of treatment (G2) showed a significant increase in LBW than the other treatment groups. Our study results agree with the results of (Lee, *et al.*, 2003; Hussein, 2015) who reported that the addition of cinnamon to the diet of broilers improved their growth performance, body weight and body weight gain, (Chang *et al.* 2008; Park, 2008) reported that cinnamon extract supplementation had significantly higher daily body weight gain. Al-Kassie (2009) also found positive effect of cinnamon on the live weight gain and improvement of the health of broiler chickens. Singh *et al.* (2014) reported that the dietary inclusion of cinnamon might improve the growth performance of broilers.

Shirzadegan (2014) observed that supplementing different concentrations of cinnamon powder in the diet (especially at a level of 0.50%) increased the final body weight of broiler chickens. While in contrast with our results (Koochaksaraie, *et al.*, 2011; Toghyani, *et al.*, 2011; Sampath, and Atapattu, 2013; Najafi, and Taherpour, 2014; Symeon, *et al.*, 2014; Hussein, 2015) they reported that dietary supplementation of cinnamon has no significant effect in improving the body weight and body weight gain. Symeon *et al.* (2014) reported that body weight, feed intake and feed conversion ratio of broiler chicken had no significant change with cinnamon oil supplementation.

Feed consumption at the first week show no significant difference in chicks among different groups, while in the 2nd week of treatment, feed consumption showed a significant ($p < 0.05$) decrease in (G2) and (G3) groups than the control. At the 3rd, 4th, 5th and 6th week of treatment, group (G2) revealed a significant ($P < 0.05$) increase in food consumption than other groups (Table 3). Our study results agree with the results of Sampath and Atapattu (2013) found that supplementation of dietary of cinnamon tends to increase the feed intake and feed conversion ratio (FCR) but had no effects on final live weight. In contrast with our results (Hussein, *et al.*, 2015) showed no significant effect ($p \leq 0.05$) on feed intake between different groups, while (Najafi and Taherpour, 2014) reported that the broiler diets supplemented with cinnamon decreased ($P < 0.05$) feed intake and body weight gain.

Feed conversion ratio demonstrated a significant ($P < 0.05$) reduction in G2 groups as compared with G1 and G3 after the 1st week of treatments. However, at 2nd, 3rd, 4th, and 5th week, there was a significant ($P < 0.05$) increase in G3 group than other treatment groups (Table 3). The

decrease in FCR can be expressed as Improved FCR and growth performance. The results of our study agree with the previous results of (Jamroz and Kamel, 2002; Al-Kassie, 2009; Sampath and Atapattu, 2013; Najafi and Taherpour, 2014). In contrast to our study findings, (Toghyani, *et al.* 2011) reported that there was no significant difference between the different groups.

The gene transcripts (mRNAs) of the PPAR- α gene were successfully detected in all liver tissues within all treated groups. The gene expression was normalized with the expression values of the β -Actin gene. At 3rd and 6th w the results revealed PPAR- α mRNA expression in the liver tissues of the (G2), (G3) groups were significantly higher ($P < 0.05$) than control (Table 4). The active compounds of cinnamon include water-soluble polyphenol type-A polymers (Anderson, *et al.*, 2004; Cao, *et al.*, 2007), CA (Babu *et al.*, 2007; Zhang *et al.*, 2008; Anand *et al.*, 2010; Chao *et al.*, 2010) and procyanidin oligomers (Lu, *et al.*, 2011). As a major effective compound isolated from cinnamon (Chang *et al.*, 2001; cheng, *et al.*, 2004), CA produces hypoglycemic and hypolipidemic effects in both mice (Huang *et al.*, 2011) and streptozotocin-induced rats (Khan *et al.*, 1990; Jarvill-Taylor *et al.*, 2001) and improves the function of pancreatic islets (Anand *et al.*, 2010). PPARs have emerged as key

coordinators of both lipid and glucose homeostasis. In addition to beneficial effects on lipid and lipoprotein metabolism, PPAR activation reduces adiposity and improves glucose tolerance and insulin sensitivity in different obese mouse models (Tanaka *et al.*, 2003). PPAR- α is abundantly expressed in adipocytes and plays a pivotal role in adipocyte differentiation (Tontonoz *et al.*, 1993) (Forman *et al.*, 1995). The activation of PPAR and PPAR- α improves insulin sensitivity and glucose tolerance. Sheng, *et al.*, (2008) reported that cinnamon can act as a dual activator of PPAR γ and α , and may be an alternative to PPAR γ activator in managing obesity-related diabetes and hyperlipidemia. As cinnamon not only elevated the expression of PPAR- γ and its target genes CD36, LPL, FAS, and GLUT4 significantly, but also increased the expression of PPAR- α and its target gene ACO markedly. The gene expression of PPAR γ and its target genes CD36, LDL in white fat tissue, and PPAR α and its target gene ACO in liver were also elevated in cinnamon treated mice indicating that cinnamon may act as a dual activator of PPAR γ and PPAR α resulting in improved insulin resistance and lowered serum lipids (Sheng, *et al.*, 2008), cinnamon also played similar roles in hypoglycemia and hypolipidemia. The results of our present study indicate that cinnamon activated PPAR- α .

Table 3: Effect of cinnamon oil on LBW (g), feed consumption (g) and FCR of Cobb broiler chicks

	Age/ w	G1	G2	G3
Live body weight (LBW)	1 st w	77.00 ± 5.14 ^a	84.00 ± 2.92 ^a	79.00 ± 4.10 ^a
	2 nd w	301.00 ± 2.70 ^a	246.00 ± 2.35 ^a	278.00 ± 2.91 ^a
	3 rd w	526.00 ± 3.40 ^b	667.00 ± 3.96 ^a	576.00 ± 3.79 ^{ab}
	4 th w	1305.00 ± 6.79 ^a	1319.00 ± 6.34 ^a	1102.00 ± 5.73 ^b
	5 th w	2062.00 ± 9.91 ^b	2352.00 ± 7.72 ^a	2052.00 ± 8.57 ^b
	6 th w	2473.00 ± 7.12 ^{ab}	2779.00 ± 7.52 ^a	2349.00 ± 7.54 ^b
Feed Consumption (g/week)	1 st w	163.00 ± 0.29 ^a	158.00 ± 0.29 ^b	156.70 ± 0.17 ^b
	2 nd w	365.00 ± 0.29 ^c	158.00 ± 0.29 ^b	156.70 ± 0.17 ^b
	3 rd w	670.30 ± 2.05 ^a	700.00 ± 2.88 ^a	676.70 ± 2.60 ^a
	4 th w	1061.6 ± 5.74 ^a	1042.0 ± 6.67 ^a	1000 ± 4.82 ^a
	5 th w	1200 ± 6.87 ^a	1220 ± 5.28 ^a	1230 ± 7.64 ^a
	6 th w	1399.70 ± 6.06 ^c	1428.30 ± 6.93 ^a	1383.30 ± 3.33 ^c
Feed conversion ratio (FCR)	1 st w	1.33 ± 0.21 ^a	1.05 ± 0.05 ^c	1.14 ± 0.11 ^b
	2 nd w	1.05 ± 0.16 ^b	1.03 ± 0.02 ^c	1.17 ± 0.03 ^a
	3 rd w	1.29 ± 0.02 ^b	1.23 ± 0.08 ^c	2.03 ± 0.31 ^a
	4 th w	1.29 ± 0.02 ^b	1.24 ± 0.07 ^c	2.03 ± 0.30 ^a
	5 th w	1.96 ± 0.06 ^a	1.65 ± 0.14 ^c	1.86 ± 0.06 ^b

Values are means ± standard error (SE); Values within the same row with different superscripts (a, b & c) indicate significant difference at (P<0.05)

Table 4: Estimation of the effect of cinnamon on the PPAR- α gene expression in hepatic tissue at 3rd and 6th week of age.

PARAMETERS		G2	G3
PPAR- α	3 rd W	3.37 ± 0.13 ^a	4.06 ± 0.29 ^a
	6 th W	3.31 ± 0.07 ^a	3.21 ± 0.34 ^a

Values are means ± standard error (SE); Values within the same row with different superscripts (a, b & c) indicate significant difference at (P<0.05)

At 3rd w of treatment, the total cholesterol (TC) revealed non-significant change, while at the 6th w of treatment (G2) group revealed a significant (P<0.05) decrease in TC concentration in comparison with other groups of treatment (Table 5). Triglycerides (TG) showed non-significant (P>0.05) changes between the different groups at the 3rd w. While at the 6th week of treatment the (G3) group showed a significant (p<0.05) decrease than all other groups (Table 5). High-density lipoprotein cholesterol (HDL-c) showed non-significant changes in all experimental groups when compared to the control at both 3rd and 6th w of treatment. Low-density lipoprotein cholesterol (LDL-c) revealed a significant decrease in

the G2 at the 3rd W of treatment. While at the 6th W the LDL was significantly increased in G3 group than the other groups (Table 5). Our study results come in agreement with the results of (Elson, *et al.* 1989; Yu, *et al.* 1994; Case, *et al.* 1995; Ciftci, 2010). This may be related with cinnamon added to the diet and its inhibition mechanism on HMG-CoA reductase activity. Two key enzymes involved in regulating cholesterol metabolism are HMG-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway, and ACAT, the cholesterol-esterifying enzyme in tissue. The inhibition of HMG-CoA reductase decreases cholesterol synthesis and its inhibitors are very effective in lowering plasma

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cholesterol in most animal species, including humans (Alberts, 1988). Cinnamic acid (0.02%, w/w) and its synthetic derivatives (HPP304, HPP305) significantly inhibit hepatic HMG-CoA reductase activity and

decrease serum total cholesterol level (Lee, *et al.* 2001; Lee, *et al.* 2007). Unlike these findings, Lee, *et al.*, (2003) failed to show any hypocholesterolemic Effects of the cinnamon on the treated groups.

Table 5: Effect of cinnamon on the serum Lipid profile parameters at 3rd and 6th week of treatment

PARAMETERS		G1	G2	G3
TC MG/DL	3W	143.33 ± 7.17 ^{ab}	127.00 ± 6.50 ^b	150.00 ± 8.08 ^{ab}
	6W	137.00 ± 5.50 ^b	113.67 ± 4.26 ^c	170.33 ± 5.70 ^a
TG MG/DL	3W	144.42 ± 3.38 ^a	142.42 ± 5.80 ^a	144.42 ± 8.72 ^a
	6W	137.00 ± 8.50 ^b	112.67 ± 4.26 ^c	170.0 ± 3.76 ^a
HDL-C MG/DL	3W	69.00 ± 4.00 ^a	78.67 ± 4.10 ^a	81.67 ± 3.87 ^a
	6W	78.67 ± 4.10 ^a	68.32 ± 13.48 ^a	60.00 ± 9.53 ^a
LDL-C MG/DL	3W	42.38 ± 15.26 ^a	26.79 ± 3.11 ^b	51.44 ± 23.84 ^a
	6W	36.93 ± 2.12 ^b	28.45 ± 3.13 ^b	95.61 ± 3.16 ^a

Values are means ± standard error (SE); Values within the same row with different superscripts (a , b &c) indicate significant difference at (P<0.05)

At the 3rd and 6th w of treatment, the cholesterol level in the liver tissue showed a significant decrease (P<0.05) in (G2) group than all other groups (Table 6) At 3rd W of age there was a significant (P<0.05) positive correlation relationship between cholesterol in both serum and liver tissue. And at 6th W of age, there was a highly significant (P<0.01) positive correlation between cholesterol in both serum and liver tissue as shown in figures 1 & 2 respectively. In previous studies to determine the effect of polyunsaturated fat feeding in man (Spritz, *et al.*, 1965; Grundy, *et al.*, 1970) non-human primates (Corey, *et al.*, 1976) and rabbits (Bieberdorf, and Wilson, 1965)

have in part led to the hypothesis that unsaturated fats cause a redistribution of cholesterol between plasma and tissue pools. Increases in liver cholesterol concentrations (Avigan, and Steinberg, 1958; Reiser, *et al.*, 1963) have been reported in rats fed unsaturated fat with minimal changes in plasma cholesterol. When the effects of cinnamon hypocholesterolemic properties were taken into consideration, the results of the present study were in agreement with the reports of the previous studies (Elson *et al.* 1989; Yu *et al.* 1994; Case *et al.* 1995). Unlike these findings, Lee *et al.* (2003) failed to show any hypocholesterolemic effects of cinnamon.

Table 6: Effect cinnamon on the cholesterol level in the liver tissue at 3rd and 6th week of treatment:

PARAMETERS		G1	G2	G3
CHOLESTEROL	3 rd W	173.33± 4.41 ^a	149.3± 3.48 ^b	160.0 ± 5.77 ^{ab}
	6 th W	197.7 ± 6.74 ^a	179.0± 2.08 ^b	198.33± 2.28 ^a

Values are means ± standard error (SE); Values within the same row with different superscripts (a , b &c) indicate significant difference at (P<0.05)

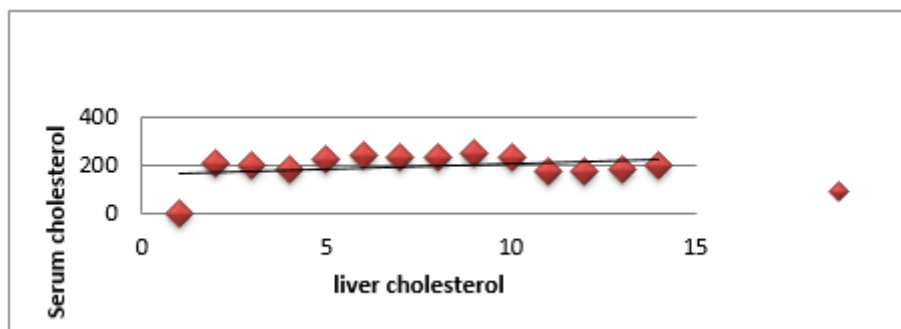


Fig. 1: Correlation between the cholesterol level in both serum and liver tissue at 3 weeks age

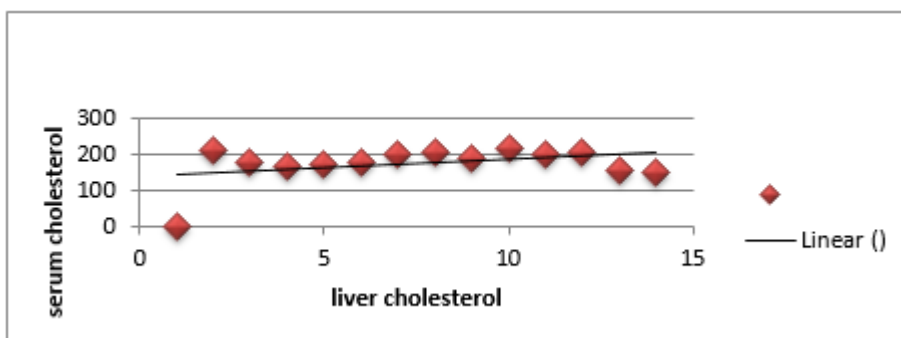


Fig.2: Correlation between the cholesterol level in both serum and liver tissue at 6 weeks age.

Table 7: Effect of cinnamon on the Oxidative stress markers at 3rd and 6th weeks of age:

PARAMETERS		G1	G2	G3
MDA (NMOL/ML)	3W	1.45 ± .038 ^b	1.72 ± .097 ^{ab}	2.00 ± .053 ^a
	6W	1.55 ± .07 ^c	2.07 ± .09 ^b	3.67 ± .049 ^a
SOD (U/ML)	3W	138.02 ± 3.26 ^b	142.82 ± 1.65 ^{ab}	150.03 ± 2.77 ^a
	6W	146.99 ± 2.02 ^c	172.69 ± 2.45 ^b	189.62 ± 2.41 ^a
CAT (U/ML)	3W	33.51 ± 1.43 ^b	41.22 ± 3.96 ^{ab}	47.49 ± 2.36 ^a
	6W	35.64 ± 1.05 ^c	53.92 ± 2.00 ^b	73.72 ± 4.85 ^a

At 3rd week of age, MDA showed a significant increase ($P < 0.05$) in (G3) group in comparison with the control group (G1), while at 6th week of age MDA showed a significant increase ($P < 0.05$) in (G3) group in comparison with (G1) & (G2) groups (Table 7). Our study results agree with the results of (Ciftci, 2010). These effects may be due to the antioxidant property of cinnamon (Lin *et al.* 2003). The protective role of cinnamon may result from its antioxidative defense mechanism through the induction of antioxidant enzyme activities (Hsu,

and Liu 2004; Choi, & Hwang, 2005; Sahib, 2016). Many previous studies reported that Cinnamon had an antioxidant property (Yu *et al.* 1994; Case *et al.* 1995; Lee *et al.* 2001; Lee *et al.* 2007). This antioxidant property of cinnamon was supported in the present study.

In the 3rd week of age SOD showed a significant increase ($P < 0.05$) in (G3) group in comparison with the control. At the 6th week of age SOD showed a significant increase ($P < 0.05$) in (G3), (G2) group in comparison with the (G1) group (Table 7).

Sahib, (2016) reported that MDA level highly significantly decreased while SOD level significantly increased, also (Rao, and Gan, 2014) reviewed that cinnamon increased GSH level, increase the activity of SOD, which indicate that cinnamon has antioxidant effect.

At 3rd week of age, CAT showed a significant increase ($P < 0.05$) in (G3) group in comparison with the (G1), (G2) groups. While at the 6th w of age CAT showed a significant increase ($P < 0.05$) in (G3) and (G2) group in comparison with (G1) (Table 7). SOD, CAT, and GPx are known as protective enzymes against free radical formation in tissues. Our study results revealed the protective role of cinnamon powder in decreasing lipid peroxidation and by normalizing antioxidant systems. In harmony with our study findings (Ciftci. *et al.*, 2009) reported that cinnamon oil (1000 ppm) reduced MDA level ($P < 0.05$) and increased GSH-Px and CAT activities. These effects are due to the antioxidant property of cinnamon oil (Lin *et al.* 2003). The protective role of cinnamon may result from its antioxidative defense mechanism through the induction of antioxidant enzyme activities (Hsu, and Liu, 2004). Choi, (2005) reported that the intake of cinnamon in rats results in an increase in antioxidant enzyme activity and a decrease in MDA

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