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Effects of Olive, Milk Thistle, and Artichoke Extracts on Performance, Biochemical and Enzymatic Changes in Carbon Tetrachloride-intoxicated Broiler Chickens

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Abstract
This study was conducted to examine the effects of olive, milk thistle, and artichoke extracts on growth performance, biochemical and enzymatic changes in birds intoxicated with CCl_4 (at 0.5, 0.75, and 1 mL/kg body weight in days 14, 21, and 28 of age, respectively). Two hundred twenty-five 10-day-old Ross 308 female broiler chicks were used in a completely randomized block design to evaluate the effects of five treatments consisting 1) a basal diet given to the birds injected intraperitoneally with 0.5 mL saline (control-), 2) The same basal diet given to birds injected intraperitoneally with CCl_4 (control+), 3, 4, and 5) The basal diet supplemented with either 400 mg/kg milk thistle, olive extracts, or artichoke extract given to the birds injected with CCl_4. Dietary Supplementation of each herbal extract increased feed intake compared with the control birds ($P < 0.05$). The broilers fed on the artichoke supplemented diet showed a greater serum concentration of HDL-c than other birds on day 28 of age ($P < 0.05$). Feeding the control diet to the CCl_4 injected birds and the olive extract-included diet with no injection resulted in a greater serum alanine aminotransferase concentration at day 42 of age ($P < 0.05$). Serum malondialdehyde concentration decreased in the birds given the control diets ($P < 0.05$). The supplementary olive extract increased serum concentrations of malondialdehyde and total anti-oxidant on day 42 of age ($P < 0.05$). Liver health score improved in the broilers grown on the herbal extract-added diets ($P < 0.05$). In conclusion, administration of olive, milk thistle, and artichoke extracts promoted feed intake and exerted a potentially positive role as an antioxidant in liver health as evidenced by certain promising changes in serum biochemistry and enzyme activity as well as liver health score.

Introduction

The liver plays a key role in the detoxification of xenobiotics, environmental pollutants, and chemo-therapeutic agents in birds (Singh *et al.,* 2011). However, the liver may undergo pathologic changes including swelling, degeneration, necrosis, and apoptosis of hepatic cells while challenging xenobiotics (Xue *et al*., 2009). The xenobiotic-induced liver injuries may lead to liver dysfunction, suppressed performance, and even death, a phenomenon with great economic loss in the poultry industry. At present, due to the inadequate hepatoprotective pharmaceuticals in the clinic and risks concerning public health, research is intensified in the development of plant-derived hepatoprotective remedies to improve bird's health (Wang *et al.,* 2013). Therefore, the establishment of a liver damage model concomitant with the abovementioned research should also receive a high priority because a reliable source of data for the efficacy assessment of any novel hepato-tonic preparation is a must. We reconsidered carbon tetrachloride (CCl_4) to provide a model for quantifying the cure of liver injuries using different therapeutics.

Carbon tetrachloride is a well know industrial solvent (Guven and Gumez, 2003) triggering liver damage in animal models. Many reports confirmed that CCl_4 toxicity occurs with the bioactivation of CCl_4 into trichloromethyl free radical through cytochrome P_{450} system activity in liver microsomes. Trichloromethyl rapidly reacts with molecular oxygen to form the trichloromethyl peroxyl radical, which in turn, instigates lipid peroxidation and protein oxidation (Alalmalki, 2010).

Currently, many researchers are exploring the plant kingdom in search of novel medicinal agents to alleviate and prevent liver diseases (Lopez *et al.,* 2017). Several investigators have postulated that herbal extracts and plant-derived active ingredients can protect the liver against oxidative stress generated by CCl_4, a fact that may realize by modifying the antioxidant enzyme activities (Mylonaki *et al.,* 2008; Tedesco *et al.,* 2004). It was shown that milk thistle extract (MTE) could protect the hepatocellular damage induced by CCl_4 due to its antioxidant activity (Lien *et al.,* 2016). The hepatoprotective and antioxidant activity of silymarin is caused by its possibility to inhibit the free radicals production in the metabolism of toxic substances (Dietrich *et al*., 2005). It has been shown that milk thistle seed protects birds against adverse effects of aflatoxin B1 (Kalorey *et al.,* 2005) and effectively stimulates the immune function and growth performance in the presence of immunosuppressant aflatoxin B1 in the feed (Chand *et al.* 2011). Silymarin stabilizes hepatocyte and other cell membranes and stimulates macromolecular and protein synthesis.

It was reported that the hydroxyl groups in the structure of the well-known phenolic compounds such as oleuropein, hydroxytyrosol, and luteolin are responsible for the antioxidant feature of olive extract (OE; Al-Azzawie and Alhamdani, 2006). The antioxidant properties of caffeoylquinic acids present in artichoke extract (AE) are also verified to be mainly responsible for their hepatoprotective action (Speroni et al., 2003). Therefore, this study aimed at examining the potential protective effects of olive, milk thistle, and artichoke extracts in hepatic damages in broiler chickens subjected to the oxidative stress and toxicity induced by repeated CCl_4 injection.

Materials and Methods

Animals and Diets

A total of 225 Ross 308 female broiler chicks (10-d-old with an average weight of 300 ± 5 g) were distributed in 74 wire cages where they experienced a period of 3-day acclimatization in advance of the experimentation period. The birds were selected from a flock brooded in a force ventilated grow-out house during the first 10 days of age, following the protocols of the Animal Care Committee of the Lorestan University, Iran. During the same period, the ambient temperature and relative humidity were set to 32 ± 1°C and 60 ± 5%, respectively. The birds were also provided with an 18: 6 hours lighting to darkness lightening regimen, except for the first three days when they offered with 24-h illumination. Throughout the same period, the birds had free access to feed (Table 1) and water and immunized against Newcastle and Infectious Bronchitis viruses on day 4 of age. On day 10, the experimental birds were transferred to the battery cages (45 × 50 × 40 cm for length, width, and attitude, respectively) arranging in seven rows (blocks) perpendicular to the airflow direction.

On day 14, the experiment was started by assigning 15 replicate cages of three birds each to any of the five experimental treatments consisting; 1) a basal pelleted diet given to the birds injected intraperitoneally (I.P.) with 0.5 mL saline (control -), 2) the basal pelleted diet given to birds injected i.p. with CCl_4, 3) The basal diet + 400 mg/kg milk thistle extract given to the birds injected with CCl_4, 4) The basal diet + 400 mg/kg artichoke extract was given to the birds injected with CCl_4, and 5) The basal diet + 400 mg/kg olive extract given to the birds injected with CCl_4. All CCl_4 injections were applied at 0.5, 0.75, and 1 mL/kg body weight (BW) in olive oil at a ratio of 1:1, v/v on days 14, 21, and 28 of age, respectively. The lightning schedule persevered identical to the pre-experimentation period. The environmental temperature during the first week of the experiment was set at 29°C and then slowly decreased by 2 to 3°C weekly to reach 24°C at the end of the fourth week when it was kept constant.

Composition of herbal extracts

Milk thistle extract provided from Zardband Pharmaceuticals Co, Tehran-Iran. The product was a commercially available extract which contained silybins A and, the isosilybins A and B, silychristin A, isosilychristin, silydianin, and silymarin. Artichoke extract was supplied by Soha Jissa Co, Mazandaran-Iran, and its analysis revealed that it comprises caffeoylquinic acids, luteolin-7-glucoside, luteolin, apigenin-7-glucoside, caffeic acid, chlorogenic acid, eugenol, and rosmarinic acid (trace amount). Olive extract (freeze-dried) provided from Dana Kasian Co, Khorammabad, Lorestan, Iran, and contained oleuropein as the major polyphenolic compound, followed by oleic acid, palmitic acid, linoleic acid, octadecadienoic acid, stearic acid, palmitoleic acid, and tridecanoic acid.

Performance data

Individual live body weight and cage-wise feed intake (FI) were recorded weekly and the collected data were used to generate daily weight gain (DWG), daily feed intake (DFI), and feed conversion ratio (FCR) data in days 28, 35 and 42 of age. No mortality was observed during the experimentation period.

Table 1. Ingredients and nutrients composition of the basal diet.

Ingredients (%)	Starter (1-10 day)	Grower (11-25 day)	Finisher (25-42 day)
Yellow maize	57.56	60.96	63.96
Soybean meal (44 % Protein)	36.28	33.80	30.40
Soybean oil	1.50	1.00	1.50
Dicalcium phosphate	1.24	1.30	1.10
CaCo3	1.80	1.33	1.20
DL-Methionine	0.20	0.27	0.20
L-Lysine HCL	0.28	0.20	0.50
Salt	0.14	0.14	0.14
Mineral Premix[1]	0.50	0.50	0.50
Vitamin Premix [2]	0.50	0.50	0.50
Nutrient composition			
ME (Kcal/kg)	3000	2942	3054
Crude protein (%)	21.50	20.43	18.62
Lysine (%)	1.44	1.09	0.97
Methionine	0.56	0.60	0.50
Methionine + Cystine (%)	1.08	0.59	0.54
L-Threonine (%)	0.97	0.79	0.72
Calcium (%)	0.96	0.85	0.75
Available P (%)	0.48	0.42	0.37
Na (%)	0.20	0.20	0.20
Chlorine (%)	0.20	0.04	0.04

[1] Each kilogram contains Vitamin A, 12000 IU; vitamin D3; 5000 IU; vitamin E 80 IU; vitamin K3; 3.2 mg; vitamin B1; 3.2 mg; vitamin B2; 8.6 mg; vitamin B3; 20 mg; vitamin B5; 65 mg; vitamin B6; 4.3 mg; vitamin B9; 2.2 mg; vitamin B12; 0.017 mg; vitamin H2; 0.30 mg; choline chloride; 1700 mg

[2] Each kilogram contains 1000 mg manganese; Zinc; 110000 mg; copper; 16,000 mg; selenium; 300 mg; iodine; 1250 mg; iron; 20,000 mg.

Blood Parameters

At days 28, 35, and 42 of age, blood samples were collected from one bird of each cage (15 birds per treatment) via jugular vein puncture in a 10-mL anticoagulant-free tube. After stewing for about 90 minutes, blood samples were centrifuged at 2000 × g for 10 min. Sera samples were analyzed for concentration of triglycerides (TG), high-density lipoprotein-Cholesterol (HDL-c), total cholesterol (CHO), total protein (TP), urea, albumin (ALB), Creatinine (CRA), aspartate aminotransferase (AST) as well as alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) enzymatic activities using an automated biochemical analyzer (Selects E Autoanalyzer, Sr. No. 8-7140, Vital Pharma BV, Maarheeze, TheNetherlands). The analyzer employed enzymatic procedures that have been described by Elliott (1984) using SEPPIM Diagnostic Kits, in two replicates at 25°C.

Lipid oxidation was measured by the Thiobarbituric Acid Reactive Substances (TBARS) test. This method assesses oxidative stress by quantifying malondialdehyde (MDA), the main eventual product of lipid breakdown caused by oxidative stress. Plasma TAS in whole blood were assayed by an RX Daytona automatic clinical chemistry analyzer (Randox, Crumlin, UK) using a commercially available kit and following kit instructions (Miller *et al.*, 1993).

Liver and internal organs

At 28, 35, and 42 days, 15 birds per treatment were weighted and killed. Immune organs (bursa, spleen, and thymus) and liver, abdominal fat (AF), neck fat (NF), and pericardial fat (HF) were dissected, weighed, and were expressed as a percentage of BW. Livers from the slaughtered birds (42 days) were weighed and then macroscopically appraised for color and apparent health. Liver scores were assigned as follows: the most severe lesion and color alteration was given score 4 on a 4-point scale where normal received score 0, as described by Trott *et al*. (2014) with slight modifications. Then, specimens of the livers were kept at -4 °C pending fat extraction according to the method of Folch *et al*. (1956). Briefly, about 1 g of liver tissue was weighed, added to chloroform/methanol (2/1) to a final volume 20 times the volume of the tissue sample vortexed for one minute, allowed to stand with agitation for 2 h. The separated liquid was filtered through Whatman #1 filter paper into a 100-mL 54 graduated cylinder, and 5 mL of 7.3% Potassium chloride solution was included and mixed. After phase separation, the top layer was entirely drained off. Total lipids were measured gravimetrically after evaporating the solvent. The samples were then dried and weighed, and the total lipid weight was expressed as the percentage of liver fat against the total liver weight.

Statistical analysis

A complete randomized block design was used to evaluate the response of broiler chickens to five experimental treatments. The blocks were 7 rows of cages perpendicular to the airflow direction in the experimental house. All data were analyzed using the GLM procedures of SAS 9.1 (SAS Institute, 2003). The Tukey test was used for multiple treatment comparisons (Kramer, 1956). The data collected as liver health scores were subjected to frequency analysis using PROC FERQ in the same statistical analysis software (SAS Institute, 2003). For all tests, significance was set at $P < 0.05$.

Results

Administration of CCl_4 irrespective of the supplementary OE, MTE, and AE had no significant effect on the DWG of the birds on days 14 to 42 of age (Table 2). Mean DFI was greater in the birds receiving the plant extract-added diets than those fed with the un-supplemented diet with and without CCl_4 injection on days 14 to 42 of age ($P < 0.05$). Birds who received the un-supplemented diet with CCl_4 injection showed an improved FCR (1.31) compared with the birds fed on the diet supplemented with AE (1.58) on days 14 to 28 of age ($P < 0.05$). Mean FCR decreased in the broilers fed with the un-supplemented diet along with and without CCl_4 injection compared with those receiving other diets on day 14 to 35 of age ($P < 0.05$; Table 2).

Table 2. Weight gain (BWG, g), average feed intake (DFI, g) and feed conversion ratio (FCR) in broiler chickens fed on the experimental diets[1] during Days 28, 35, and 42 of age.

	Con_+	Con-	Olive	Artichoke	Silymarin	*SEM*[2]	*P*-value
				Day 28			
BWG	889.00	851.47	910.00	883.20	863.07	30.065	0.6799
DFI	1164.62^b	1187.42^b	1322.42^a	1397.89^a	1300.89^a	34.314	0.0001
FCR	1.31^d	1.40^{dc}	1.46^{bc}	1.58^a	1.53^{ab}	0.034	0.0001
				Day 35			
BWG	1290.53	1326.67	1275.73	1335.87	1330.60	39.993	0.7688
DFI	1935.46^b	2020.25^b	2257.09^a	2281.52^a	2234.72^a	48.057	0.0001
FCR	1.52^b	1.52^b	1.80^a	1.72^a	1.68^a	0.042	0.0001
				Day 42			
BWG	1566.1	1687.1	1679.7	1659.6	1745.7	75.093	0.5608
DFI	2661.2^b	2829.9^b	3139.1^a	3108.2^a	3143.0^a	81.030	0.0001
FCR	1.73	1.69	1.89	1.89	1.86	0.071	0.1393

[a,b] Means in the same column without the same superscript differ significantly ($P < 0.05$).

[1]Experimental diets; a pelletized diet with no intoxication or medication (Control negative; Con-) or grown on the same pelletized diet with CCl_4 injection (Control positive; Con+) and CCl_4 injection with the administration of olive, artichoke, or silymarin extract. Con+: CCl_4 were intraperitoneally injected at 0.5, 0.75, and 1 mL/kg body weight dosages in olive oil at a ratio of 1: 1, v/v in Days 14, 21, and 28 of age.

[2]Standard error of the mean.

Mean abdominal fat, neck fat, and para-cardiac fat reduced in the birds receiving the MTE -added diet compared to the other birds on day 28 of age ($P < 0.05$; Table 3). The birds fed with the un-supplemented pelleted diet with CCl_4 injection showed a greater liver fat percentage than those who received the same diet and CCl_4 injection on day 28 of age ($P < 0.05$). The birds maintained on the diet supplemented with OE had a greater proportional thymus and heart weight than those fed control diets on day 35 of age ($P < 0.05$). At the end of the experimentation period, no significant difference was found in internal organs and fat percentage among birds subjected to the different treatments ($P > 0.05$).

The serum concentration of HDL-c was greater in the birds fed on the diet supplemented with AE compared with those receiving the olive extract-added diet on day 28 of age ($P < 0.05$, Table 4). Mean serum concentration of CHO, TG, HDL-c, Urea, ALB, TP, and CRA was not affected by the experimental treatments at day 35 of age (Table 4, $P > 0.05$). Serum activity of CHO decreased in the birds receiving the AE-supplemented diet than those fed with the positive control and the MTE supplemented diets at the end of the experiment ($P < 0.05$). The mean concentration of HDL-c was lesser in the birds grown on the AE supplemented- diet compared to other birds except for negative control birds ($P < 0.05$). The broilers in the positive control group showed a greater serum TG level compared with the negative control birds ($P < 0.05$).

Feeding the AE-supplemented diet increased serum activity of AST compared with those receiving the diet containing MTE on day 28 of age ($P < 0.05$, Table 5). In the birds grown on the diet supplemented with AE and the negative control diet, serum activity of ALT was lesser than the birds fed with the diet supplemented with OE on day 28 of age ($P < 0.05$). Serum ALT activity decreased in the birds maintained on the OE supplemented diet than those fed with diets supplemented with AE on day 35 of age ($P < 0.05$). At the end of the experimentation period, the broilers fed with a non-supplemented diet along without CCl_4 injection, and those fed on the diet containing OE showed a greater ALT activity than other treatments (P.<.0.05). Serum LDH activity was lesser in the control negative birds on day 42 of age ($P < 0.05$), however, no difference was observed among the birds on days 28 and 35 of age ($P > 0.05$).

Table 3. Mean liver, thymus, bursa of Fabricius (BF), Abdominal Fat (AF), Neck Fat (NF), Heart Fat (HF), and Liver Fat (LF) percentage (as % of BW) in broiler chickens fed on the experimental diets[1] during in Day 28, 35 and 42 of age.

	Con+	Con-	Olive	Artichoke	Silymarin	*SEM*[2]	*P*-value
				Day 28			
Liver	2.62	2.65	2.33	2.52	2.37	0.110	0.1591
Thymus	0.15	0.14	0.17	10.11	0.11	0.017	0.0903
Spleen	0.08	0.09	0.08	0.08	0.08	0.006	0.0903
BF	0.15	0.14	0.14	0.14	0.13	0.011	0.9404
AF	1.22^{a}	1.25^{a}	1.28^{a}	1.25^{a}	0.98^{b}	0.079	0.0695
NF	0.35^{ab}	0.46^{a}	0.44^{a}	0.33^{ab}	0.25^{b}	0.055	0.0728
HF	0.05^{a}	0.06^{a}	0.05^{a}	0.05^{a}	0.04^{b}	0.005	0.0287
LF	5.63^{a}	3.54^{b}	4.61^{ab}	4.52^{ab}	5.25^{ab}	0.681	0.0196
				Day 35			
Liver	2.18	2.26	2.27	2.13	2.12	0.106	0.7883
Thymus	0.17^{b}	0.16^{b}	0.27^{a}	0.20^{ab}	0.20^{ab}	0.023	0.0238
Spleen	0.10	0.09	0.11	0.10	0.09	0.007	0.5084
BF	0.11	0.13	0.14	0.13	0.13	0.009	0.4024
Heart	0.32^{b}	0.32^{b}	0.36^{a}	0.35^{ab}	0.32^{b}	0.012	0.0454
AF	1.39	1.35	1.36	1.32	0.36	0.084	0.9841
NF	0.46	0.42	0.51	0.47	0.48	0.082	0.9673
HF	0.06	0.06	0.07	0.05	0.06	0.010	0.4700
LF	7.09	6.88	6.18	6.99	6.86	0.867	0.9526
				Day 42			
Liver	2.21	2.10	1.95	2.05	1.94	0.103	0.3296
Thymus	0.15	0.18	0.17	0.18	0.18	0.024	0.8696
Spleen	0.09	0.09	0.09	0.10	0.09	0.010	0.9460
BF	0.08	0.10	0.07	0.09	0.09	0.011	0.5856
Heart	0.31	0.31	0.30	0.32	0.32	0.0173	0.9695
AF	1.24	1.26	1.27	1.32	0.30	0.107	0.9860
NF	0.21	0.30	0.29	0.31	0.28	0.050	0.6644
HF	0.04	0.06	0.04	0.05	0.06	0.007	0.2917
LF	5.73	4.70	4.63	6.17	5.16	0.554	0.2541

[a,b] Means in the same column without the same superscript differ significantly ($P < 0.05$).

[1]Experimental diets; a pelletized diet with no intoxication or medication (Control negative; Con-) or grown on the same pelletized diet with CCl_4 injection (Control positive; Con+) and CCl_4 injection with the administration of olive, artichoke, or silymarin extract. Con+: CCl_4 were intraperitoneally injected at 0.5, 0.75, and 1 ml/kg body weight dosages in olive oil at a ratio of 1: 1, v/v in Days 14, 21, and 28 of age.

[2]Standard error of the mean.

Serum malondialdehyde concentration increased in the positive control birds and those fed with the olive supplemented diet on day 28 of age ($P < 0.05$, Table 6). The broilers grown on the diet supplemented with OE had a greater serum MDA level than those fed with the negative control diet on day 42 of age ($P < 0.05$). No difference was found in serum total anti-oxidant (TA) and MDL concentration at day 35 of age (P.>.0.05). The broiler fed with the diet supplemented with AE had a greater serum TA concentration compared with those receiving the olive supplemented diet ($P < 0.05$), while the reverse results were observed in the same birds at 42 days of age (P.<.0.05).

Liver health score was appraised based on a 4-grade scale, influenced by the experimental treatments ($P < 0.05$; Table 7). The frequency of scores 1 and 3, was greater (66.67% and 24.39) in birds receiving the positive control diet. The birds receiving the AE-supplemented diet showed higher frequency for score 2 compared with the birds receiving other treatment combinations on days 35 and 42 of age ($P < 0.05$).

Table 4. Mean Total Cholesterol (TC), Triglycerides (TG), High-density lipoprotein-cholesterol (HDL-c), Urea, Albumin (ALB), Total protein (TP), and Creatinine (CRA) concentrations (mg/dL) in broiler chickens fed on the experimental diets[1] during Days 28, 35 and 42 of age.

	Con+	Con-	Olive	Artichoke	Silymarin	*SEM*[2]	*P*-value
				Day 28			
CHO	117.70	120.00	116.60	125.30	119.90	5.717	0.4064
TG	83.90	75.40	77.40	73.90	92.10	7.119	0.4811
HDL-c	63.80^{ab}	63.40^{ab}	60.20^{b}	71.60^{a}	66.20^{ab}	3.514	0.0341
Urea	3.85	3.57	3.21	4.40	3.49	0.433	0.3005
ALB	1.02	1.11	0.96	1.05	1.04	0.054	0.2112
TP	2.89	3.06	2.78	2.97	2.87	0.099	0.3147
CRA	0.26	0.16	0.21	0.16	0.20	0.057	0.9351
				Day 35			
CHO	142.00	121.80	118.80	122.40	124.50	9.815	0.6010
TG	65.50	59.90	57.90	55.40	60.70	5.514	0.1850
HDL-c	79.20	67.20	66.40	71.00	73.00	5.159	0.5375
Urea	2.48	2.37	1.87	1.56	1.69	0.359	0.2283
ALB	1.31	1.11	1.21	1.22	1.13	0.121	0.5765
TP	3.73	3.07	3.31	3.61	3.33	0.267	0.4531
CRA	0.16	0.13	0.14	0.13	0.09	0.028	0.1759
				Day 42			
CHO	126.70^{a}	105.80^{ab}	116.50^{ab}	87.30^{b}	127.80^{a}	13.620	0.3121
TG	101.90^{a}	65.90^{b}	79.70^{ab}	70.40^{ab}	78.80^{ab}	11.820	0.0207
HDL-c	69.20^{a}	58.20^{ab}	69.60^{a}	51.40^{b}	69.00^{a}	5.846	0.0562
Urea	3.50	2.89	2.71	2.61	3.53	0.585	0.6500
ALB	1.25	0.95	1.30	0.90	1.34	0.170	0.3132
TP	3.49	2.82	3.47	2.94	3.81	0.401	0.5200
CRA	0.22	0.15	0.21	0.23	0.22	0.043	0.2561

^{a,b} Means in the same column without the same superscript differ significantly ($P < 0.05$).

[1]Experimental diets; a pelletized diet with no intoxication or medication (Control negative; Con-) or grown on the same pelletized diet with CCl_4 injection (Control positive; Con+) and CCl_4 injection with the administration of olive, artichoke, or silymarin extract. Con+: CCl_4 were intraperitoneally injected at 0.5, 0.75, and 1 ml/kg body weight dosages in olive oil at a ratio of 1: 1, v/v in Days 14, 21, and 28 of age.

[2]Standard error of the mean.

Table 5. Means Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Lactate dehydrogenase (LDH) concentrations (U/liter) in broiler chickens fed on the experimental diets[1] during Days 28, 35, and 42 of age.

	Con+	Con-	Olive	Artichoke	Silymarin	*SEM*[2]	*P*-value
				Day 28			
AST	247.80^{ab}	265.20^{ab}	243.00^{ab}	284.60^{a}	238.80^{b}	15.863	0.2992
ALT	8.30^{ab}	6.60^{b}	9.40^{a}	6.70^{b}	7.80^{ab}	0.863	0.1007
LDH	5.83	6.18	6.56	5.66	6.52	0.627	0.6721
				Day 35			
AST	351.90	312.20	294.10	291.20	299.60	24.234	0.7276
ALT	5.40^{bc}	9.10^{ab}	4.00^{c}	10.40^{a}	8.70^{ab}	1.470	0.0187
LDH	4.70	5.26	6.80	5.33	5.57	0.753	0.5640
				Day 42			
AST	301.20	313.40	32.20	262.10	305.60	27.640	0.8307
ALT	7.70^{b}	21.30^{a}	25.70^{a}	6.70^{b}	7.60^{b}	1.750	0.0001
LDH	8.98^{a}	6.52^{b}	8.73^{a}	9.64^{a}	8.60^{a}	0.694	0.0124

^{a,b,c} Means in the same column without the same superscript differ significantly ($P < 0.05$).

[1]Experimental diets; a pelletized diet with no intoxication or medication (Control negative; Con-) or grown on the same pelletized diet with CCl_4 injection (Control positive; Con+) and CCl_4 injection with the administration of olive, artichoke, or silymarin extract. Con+: CCl_4 were intraperitoneally injected at 0.5, 0.75, and 1 ml/kg body weight dosages in olive oil at a ratio of 1: 1, v/v in Days 14, 21, and 28 of age.

[2]Standard error of the mean.

Table 6. Means MDA and Total anti-oxidant concentrations (μmol/L) in broiler chickens fed on the experimental diets[1] during Days 28, 35, and 42 of age.

	Con+	Con-	Olive	Artichoke	Silymarin	*SEM*[2]	*P*-value
				Day 28			
MDA	1.61[a]	1.22[b]	1.49[a]	1.47[b]	1.22[b]	0.088	0.0224
TA	1.57[ab]	1.52[ab]	1.34[b]	1.72[a]	1.54[ab]	0.109	0.0456
				Day 35			
MDA	1.49	1.37	1.50	1.89	1.48	0.187	0.6725
TA	1.18	1.12	1.07	1.02	1.02	0.118	0.7360
				Day 42			
MDA	1.60[ab]	1.26[b]	1.85[a]	1.37[ab]	1.39[ab]	0.175	0.0139
TA	1.13[ab]	1.20[ab]	1.29[a]	0.88[b]	1.26[ab]	0.0294	0.0325

[a,b] Means in the same column without the same superscript differ significantly ($P < 0.05$).

[1]Experimental diets; a pelletized diet with no intoxication or medication (Control negative; Con-) or grown on the same pelletized diet with CCl_4 injection (Control positive; Con+) and CCl_4 injection with the administration of olive, artichoke, or silymarin extract. Con+: CCl_4 were intraperitoneally injected at 0.5, 0.75, and 1 ml/kg body weight dosages in olive oil at a ratio of 1: 1, v/v in Days 14, 21, and 28 of age.

[2]Standard error of the mean.

Table 7. Percent of Liver color in broiler chickens fed on the experimental diets[1] during Days 28, 35, and 42 of age.

	Con+	Con-	Olive	Artichoke	Silymarin	Chi-square	*P*-value
				Day 28			
Score 0	50.00	50.00	00.00	00.00	00.00	2.00	0.158
Score 1	9.09	13.64	22.73	22.73	31.82	88.00	0.0001
Score 2	24.44	17.78	20.00	20.00	17.78	180	0.0001
Score 3	11.11	33.33	11.11	44.44	00.00	27.00	0.0014
				Day 35			
Score 0	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Score 1	66.67	00.00	00.00	00.00	33.33	3.00	0.0833
Score 2	15.63	21.88	18.75	28.13	15.63	128.00	0.0001
Score 3	22.50	20.00	22.50	12.50	22.50	160.00	0.0001
				Day 42			
Score 0	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Score 1	66.67	00.00	00.00	00.00	33.33	3.00	0.0833
Score 2	12.90	22.58	19.35	29.03	16.13	124.00	0.0001
Score 3	24.39	19.51	21.95	12.20	21.95	164.00	0.0001

[a,b] Means in the same column without the same superscript differ significantly ($P < 0.05$).

[1]Experimnatal diets; A pelletized diet with no intoxication or medication (Control negative; Con-) or grown on the same pelletized diet with CCl_4 injection (Control positive; Con+) and CCl_4 injection with the administration of olive, artichoke, or silymarin. Con+: CCl_4 were intraperitoneally injected at 0.5, 0.75, and 1 ml/kg body weight dosages in olive oil at a ratio of 1: 1, v/v in Days 14, 21, and 28 of age.

Discussion

Carbon tetrachloride is among the agents potentially able to damage the liver by inducing the production of free radicals. In the current study, intraperitoneal administration of CCl_4 demonstrated no or minimal effects on most of the evaluated parameters. We anticipated deteriorating effects of CCl_4 injection on liver function evidenced by suppressed performance and prominent elevation in liver enzymes activity in serum as well as obvious liver lesions as previously reported by Sonkusale *et al.* (2011), Khodadust *et al.* (2015), Wang *et al.* (2013), Vahed *et al.*, (2016) and Baradaran *et al.* (2019) in broiler chickens as well as Behboodi *et al.* (2017) and Khorramshahi *et al.* (2014) in Japanese quail. We chose the administrated dose and routes for CCl_4 based on the Sonkusale *et al.* (2011) and Gad *et al.* (2011) studies; however, the lack of an obvious response in the birds could be attributed to the bird's health and immune system function. Our results disagree findings of Sonkusale *et al.* (2011) and Gad *et al.* (2011) who reported intraperitoneal injection of CCl_4 to broilers significantly increased serum concentrations of total cholesterol and decreased serum concentrations of albumin, total protein, and HDL. Although, the outcome of the current study is not in line with Vahed *et al.* (2016) results who described that liver weight was lesser than that of the birds received CCl_4 (1.581 vs 1.451) and marigold oil extracts (MOE) alleviated the negative effect of CCl_4 on this trait. In this study, the relative weight of the thymus was greater in the birds who received 300 mg/kg of MOE + CCl_4. These researchers also observed that serum activity of SGOT and SGPT were increased when CCl_4 was added to the diets and MOE was able to reduce the negative effects of CCl_4 on blood metabolites of bilirubin, cholesterol, and triglyceride.

Irrespective of CCl_4 administration, supplementary plant extracts did not improve the growth performance of the birds in terms of BWG and FCR at day 42 of age, the results which confirm Behboodi *et al*. (2017) and Khodadust et al. (2015) findings. Our results also are inconsistent with the El Iraqi *et al.* (2013), Akbari and Torki (2014), and Khodadust *et al*. (2015) findings, who reported that the alcoholic extract of many herbs improved growth performance in broiler chicken. In the current study, FI increased in the positive control birds but the mean values of FI decreased in the olive oil-treated birds than that of the negative control birds in the 3rd week of the experimentation period. This outcome disagrees Al-Seeni *et al.* (2019) findings, who reported no change in FI as a result of CCl_4 induced liver hepatotoxicity.

In the current study, supplementary milk thistle extract reduced the proportional weight of abdominal fat, neck fat as well as the fat accumulated around the heart. Samudram *et al.* (2008) reported lower total protein levels in blood serum following the destructive effects of CCl_4 on the function of the rat liver. Fani Makki *et al*. (2014) reported that milk thistle extract had no effects on serum concentration of total protein and albumin which disagrees with our findings. Behboodi *et al.* (2017) observed that injection of CCl_4 to broilers decreased serum concentration of total protein and albumin by 28 and 18 percent, respectively. In the current study, milk thistle extract + CCl_4 treated birds showed lower serum levels of triglyceride and LDL, signifying the stimulatory effects of milk thistle extract in lipid metabolism as previously reported by Tollba *et al.* (2010) who demonstrated that using milk thistle extract can reduce blood lipids in broilers, hens and quails.

It was already shown that hepatic necrosis irrespective of pathogenesis, causes elevated serum enzyme activity and decreases the activity of antioxidant enzymes such as SOD and catalase (Eidi, 2011). Our results revealed that artichoke and olive extracts reduced ALT activity. Al-Seeni *et al.* (2016) reported that CCl_4 -induced hepatotoxicity increased ALT, AST, and ALP activity and decreased serum total protein, albumin, and total bilirubin concentration in the rat. They observed that superoxide dismutase (SOD) and glutathione reductase (GSST) activity decreased and lipid peroxide in serum and liver tissue increased as a result of the liver damage. In this study treating the CCl_4- intoxicated rats with olive oil significantly improved liver integrity and function. Nateghi *et al.* (2013) showed that artichoke leaf extract reduced ALT level in CCl_4-intoxicated broilers. Mehmetçik *et al.* (2008) and Fallah *et al.* (2013) also reported that feeding artichoke leaf extract to rats decreased liver enzyme activity compared with the birds poisoned with CCl_4. On the contrary, Abdo *et al*. (2007) observed no effect of dietary levels of artichoke leaves in broiler diets on liver enzymes.

Malondialdehyde is widely used to assess the magnitude of oxidative deterioration of lipids due to oxidative stress (Baradarana *et al.,* 2019). On day 42, supplementary artichoke extracts decreased MDA in the birds feeding the diet containing milk thistle extract. The protective and antioxidant activities of milk thistle extract on cellular mitochondrial stability and integrity have frequently been reported (Pradeep *et al.,* 2007). A previous study indicated that milk thistle extract can inhibit CCl_4 activation through inhibition of the cytochrome P_{450} system (Mikstacka et al., 2002). On the contrary to our results, Baradaran *et al.* (2019) showed that serum MDA content was greater in CCl_4-challenged birds. The greater concentration of MDA in the birds receiving the OE-supplemented diet in the present study disagree with the observations of Oke *et al*. (2017), who reported olive leaf extract reduced MAD in broiler chickens and attributed this finding to the ability of the olive extract to confer adequate antioxidant protection against lipid peroxidation. These results confirm the outcome of Gorinstein *et al.* (2002) report who demonstrated that olive oil ingredients (virgin, and to less degree lampante), possess hypolipidemic and antioxidant properties, in particular, when these oils are added to the diets of rats fed cholesterol. They attributed the positive properties of the olive oils mostly to their phenolic compounds.

In this study, we apprised liver color and apparent health based on the method described by Choi *et al.* (2012) on a 5-point scale, with 1 being a normal deep red color, and 5 being a yellow or putty-like colored liver. The birds receiving AE supplemented diet, showed higher frequency for score 2 compared with the birds receiving other treatment combinations on days 35 and 42 of age. Based on the scores assigned to each liver, no evidence was found for either increased visible liver damage by CCl_4-intoxication or improved external liver health as well as color by dietary supplementation of herbal extracts.

Conclusion

It was concluded that exposure to CCl_4 (at the doses of 0.5, 0.75, and 1 mL/kg body weight) through an intraperitoneal route did not disrupt liver function in broiler chicken indicated by no leakage of the liver enzymes into the bloodstream and no depreciated liver external appraisal scores, but it is very likely CCl_4 are highly toxic to birds at high doses. Administration of olive, milk thistle and artichoke extracts showed a potentially positive role as an antioxidant in liver health as evidenced by certain promising changes in serum biochemistry and enzyme activity as well as liver health score.

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