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Research Article
Flaxseed Oil Alleviates Toxic Effects of Subacute Exposure to Acephate on Liver and Kidney of Broiler Chicks

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Abstract

Objective: The present study was designed to investigate the hepatic and renal toxicity of acephate (ACE) insecticide in broiler chicks by studying the hematological, biochemical, oxido-inflammatory and pathological changes after subacute exposure to ACE in diet and to evaluate the modulatory role of flaxseed oil (FSO) on these changes. **Materials and Methods:** Two hundred and forty day-old un-sexed broiler chicks (average body weight of 45.52±0.25 g) were randomly assigned into 4 groups with 4 replicates of 15 chicks based on a completely randomized design. The first group was fed a basal diet, 2nd group fed basal diet supplemented with 85.2 ppm acephate, while the 3rd group fed basal diet supplemented with 1000 ppm flaxseed oil, while the 4th group fed basal diet supplemented with 85.2 ppm ACE+1000 ppm FSO for 4 weeks. **Results:** The ACE significantly ($p<0.05$) decreased hemoglobin content, erythrocytic count and packed cell volume while increased white blood cells, lymphocyte and granulocyte counts. Moreover, there was a significant increase in lipid profile (total cholesterol, LDL and triglyceride) and serum biomarkers related to hepatic and renal functions including aspartate transaminase (AST), alanine aminotransferase (ALT), urea and creatinine with no change in alkaline phosphatase (ALP) and albumin while, serum total protein, globulin and HDL-cholesterol levels were significantly reduced with ACE group in comparison with other treatment groups. The ACE significantly ($p<0.05$) decreased the antioxidant capacity of liver and kidney and increased lipid peroxidation, interleukine-2 (IL-2) tumor and necrosis factor-alpha (TNF-α) in the two organs while increased the activity of cytochrome P_{450} in liver only. Diet supplemented with FSO showed promising modulatory effects on these undesirable changes however, some did not restored to normal levels. **Conclusion:** It is recommended to use FSO regularly as a dietary supplement for broiler chicks to provide them powerful antioxidants required to protect these birds against environmental pollution.

Key words: Acephate, flaxseed oil, performance, blood profile, hematology, antioxidant status, broilers

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pesticides have been a major cause of environmental pollution and different toxicities in humans, animals, birds and even plants in the modern era especially with the absence of regulatory measures for use and their applications over a wide range of urban regions and agricultural landscapes[1]. Insecticides alone represent about 80% of the total pesticides used across the globe to control pests particularly, organophosphrous insecticides (OPI) as they are relatively less toxic and with low persistency in environment and biological system of mammals than organochlorines[2]. Long-term exposures to OPI could result in inhibiting the activity of acetylcholine esterase (AChE) leading to nervous manifestations[3]. They also could interfere with the functions of lipid membrane bilayers due to their lipophilic nature[4]. Moreover, exposure to OPI has been reported to cause variable types of toxicities like reproductive toxicity, oxidative stress, immunotoxicity, genotoxicity, enzyme alterations and pathological lesions[5] in addition to carcinogenic and mutagenic potentials[6].

Acephate (O,S-dimethyl N-acetylphosphoramidothiolate; ACE) is one of the most important OPI used in agricultural purposes all over the world with both systemic and contact actions on many different kinds of insects[7]. The toxicity of ACE has been studied on both *in vivo* and *in vitro* assays and it is believed to be returned to bio-activation of ACE to its metabolite methamidophos, which could act as inhibitor to AChE and inducer of delayed neurotoxicity[8]. Acephate showed cytogeneotoxic, mutagenic and carcinogenic activities in prokaryotic, eukaryotic and mammalian cells[9].

Flaxseed (*Linum usitatissimum* L.) is a member of Linaceae family that is also called linseed. Flaxseed is rich in oil which represents about 45% of its mass. Flaxseed oil (FSO) is used worldwide in animal and human nutrition owing to its beneficial components and probiotic activity[10]. Omega-3 fatty acids like alpha linolenic acid, plant lignans and dietary fibers are the most important and major constituents in FSO[11]. Omega-3 fatty acids have been reported to enhance immune system, promote the growth and productive performance as well as treat heart diseases, inflammatory conditions, cancer and diabetes[12]. Additionally, plant lignans showed a promising antioxidant activity. Flaxseed as a dietary supplement could enhance the antioxidant capacity in liver of rats exposed to carbon tetrachloride and in mammary gland of cows[13].

Acephate toxicity could be more hazardous on developing organisms and may affect the functions and structures of different organs during their growth at low doses than adults However toxic studies on broilers during the fattening period are scanty and need more investigations especially on birds of nutritional and economic importance like commercial chicks. Moreover, the FSO as a dietary supplement for growing chicks still needs more search, therefore, the present study was designed to evaluate the structural and functional alterations induced by ACE in liver and kidney tissues and their antioxidant status in Hubbard chicks and to evaluate the effectiveness of FSO in reducing such effects.

MATERIALS AND METHODS

Chemicals: Technical grade acephate (O,S-dimethyl N-acetylphosphoramidothiolate; ACE) ($C_4H_{10}NO_3PS$) (purity, 97.3%) was obtained from (Meghmani Industries Agro chemical, R and D Chemist, India). Flaxseed oil was purchased from (El-Captain Co., Egypt). Kits of Total Protein (TP), albumin (ALB), total cholesterol (TCHO), LDL-cholesterol, HDL-cholesterol, triglyceride (TRG), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were obtained from (Bio Med Diagnostic, Egypt). Inc., USA). Kits for catalase (CAT100-1KT), superoxide dismutase (SOD 19160-1KT-F), reduced glutathione (GSH, CS0260-1KT), glutathione-S-transferase (GST 14015-1KT), lipid peroxidation (Malondialdehyde; MDA, MAK085-1KT), ELISA kits of interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α) and cytochrome P_{450} and all other chemicals were purchased from Sigma (St., Louis, MO, USA). All other reagents used were of analytical grade.

Birds and experimental diets: A total of 240 day-old un-sexed Hubbard broilers with initial body weight of 45.52±0.25 g were purchased from a local hatchery. Chicks were weighed and randomly allotted into 4 treatments with 4 replicates of 15 chicks based on a completely randomized design. Birds were housed in traditional cages with feed and water provided for *ad libitum* consumption. Lighting program was 23 h light+1 h darkness. Veterinary program was done under supervision of veterinarians.

The basal diet was formulated to meet nutritional requirements of commercial broiler chickens which meet NRC[14] recommendations as shown in Table 1. Four experimental diets were formulated as follow: The 1st group was fed a basal diet, the 2nd group fed basal diet supplemented with 85.2 ppm acephate (ACE group), while the 3rd group fed basal diet supplemented with 1000 ppm flaxseed oil (FSO group) while the 4th group fed basal diet supplemented with 85.2 ppm ACE+1000 ppm FSO for

Table 1: Ingredient (g) and nutrient content of experimental diets (g kg^{-1})

Experimental diets	Starter diet (0-14 days)	Grower diet (15-28 days)
Ingredient		
Maize	605.10	666.20
Soybean meal 44%	260.00	203.10
Maize gluten meal 62%	80.00	80.00
Cotton seed oil	15.00	12.00
Limestone	11.20	11.80
Di-calcium phosphate	17.50	16.00
NaCl	3.00	3.00
Premix*	3.00	3.00
L-lysine	3.60	3.50
Dl-methionine	1.60	1.40
Total	100.00	100.00
Nutrient composition#		
ME (kcal kg^{-1})	3050.00	3101.00
Crude protein	220.00	200.00
Calcium	9.50	9.20
Nonphytate phosphorus	4.50	4.10
Lysine	13.00	11.50
TSAA	9.50	8.80

*Provides per kg of diet: Vitamin A: 12,000 IU, Vitamin D3: 5000 IU, Vitamin E: 130.0 mg, Vitamin K3: 3.605 mg, Vitamin B1 (thiamin): 3.0 mg, Vitamin B2 (riboflavin): 8.0 mg, Vitamin B6: 4.950 mg, Vitamin B12: 17.0 mg, Niacin: 60.0 mg, D-Biotin: 200.0 mg, Calcium D-pantothenate: 18.333 mg, Folic acid: 2.083 mg, Manganese: 100.0 mg, Iron: 80.0 mg, Zinc: 80.0 mg, Copper: 8.0 mg, Iodine: 2.0 mg, Cobalt: 500.0 mg and selenium, 150.0 mg, #Calculated according to NRC[14]

4 weeks. The Ethics of Animal Use in Research Committee (EAURC) of Zagazig University, Egypt approved all protocols involving animals here. All experimental procedures were performed according to the Directive 2010/63/EU of the European Parliament and of the Council (22 September, 2010) on the protection of animals used for scientific purposes.

Sample collection and preparation: At day 28, chicks were sacrificed followed by collection of blood, the first part taken on anticoagulant (EDTA) for determination of hematological indices, while the second part collected without EDTA then centrifuged at 3500 rpm for 15 min to obtain serum which kept at -20°C till analysis. The kidney and liver were taken and washed in physiological saline then divided into two parts, the first part was homogenized in phosphate buffer saline and the homogenate was stored at -80°C until used. The second part was fixed in 10% buffered formalin used for histopathological examination in treatment groups.

Performance and some relative organ weights: Body weight and feed intake were recorded to compute body weight gain and Feed Conversion Ratio (FCR) (g feed intake g^{-1} weight gain), respectively. Three chicks of each treatment were taken randomly for carcass measurements at day 28. Liver and kidney were excised and weighed individually. Both organs were calculated as a percentage of the pre-slaughter weights of broiler chicks.

Hematological parameters: Hematological measurements were performed at the end of experiment. Blood picture including hemoglobin (Hb), Red Blood Cells (RBCs), Packed Cell Volume (PCV), White Blood Cells (WBCs) and Differential Leukocyte Counts (DLC) were measured in the blood collected at 10% EDTA through automatic cell counter (Hospitex Hemascreen 18-Italy). The MCV and MCHC were computed according to the following equation:

$$MCV = \frac{\text{Hematocrit (\%)} \times 10}{\text{RBCs count (millions mm}^{-3}\text{ blood)}}$$

$$MCHC = \frac{\text{Hemoglobin (g/100} \times 100)}{\text{Hematocrit (\%)}}$$

Biochemical parameters

Lipid profile and hepato-renal functions: Serum total protein (TP), albumin (ALB), globulin GLB (TP-ALB), triglyceride (TRG), total cholesterol, LDL-cholesterol and HDL-cholesterol, urea and creatinine levels as well as alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined spectrophotometrically using commercial diagnostic kits obtained from Biodiagnostic Co. (Giza, Egypt) according to Akiba *et al.*[15].

Antioxidants and inflammatory biomarkers in liver and kidney: The samples of liver and kidney were homogenized (10% w/v) in potassium phosphate buffer solution (pH 7.4) followed by centrifugation for 15 min at 3500 rpm. The obtained supernatant was used to measure SOD and CAT activities and concentrations of GSH, GST and MDA were determined by using the specified kits according to the manufacturer instructions. Concentrations of tumor necrosis factor-alpha (TNF-α), interleukin-2 (IL-2) and cytochrome P_{450} were estimated using ELISA kits[16-18].

Histopathological investigation: Liver and kidney specimens were taken from birds of tested groups, weighed and fixed in 10% buffered neutral formalin solution. Five-micron thick paraffin sections were prepared, stained by hematoxylin and eosin for histopathological investigation[19].

Statistical analysis: Data were subjected to ANOVA procedure using a completely randomized design using the GLM procedures of SPSS[20]. The differences among means were determined using the *post hoc* Newman-Keuls test ($p<0.05$).

RESULTS

Performance and carcass components: The effects of ACE and FSO and their combination on growth performance are presented in Table 2. The dietary treatments had significant ($p<0.01$) effects on the live body weights and body weight gain during the experimental period. The chicks fed FSO in diet had the highest values of live body weight and weight gain compared to other groups while the opposite occurred in ACE group. The final body weight and weight gain showed significant improvement in FSO+ACE group compared to ACE diet.

Feed intake and FCR were statistically ($p<0.05$) influenced by treatments. The largest amounts FI were recorded by birds fed FSO-diet compared to other treatments. On the contrary, the smallest amounts of FI were observed for ACE group. Supplementation of FSO-ACE contained diet lead to an observable improvement of FI. The birds receiving ACE alone had a significantly ($p = 0.023$) higher FCR compared to other groups.

In the present study, relative liver and kidney weights of broilers were statistically ($p<0.05$) influenced by the dietary treatments. The lowest values of liver and kidney percentages were achieved by birds fed ACE in diet. Conversely, the birds fed FSO supplemented diet had the highest values in comparison with control.

Hematological findings: Hematological parameters of control and other treated groups are represented in Table 3. There is no significant change between hematological parameters of control and FSO groups. The ACE supplementation to diet showed a significant ($p<0.05$) reducing effect on Hb contents, RBCs count and PCV percent, while significantly increased the WBCs, lymphocytes and granulocyte counts of chicks compared to control and other groups. The MCV and MCHC were not significantly changed by the different treatments in comparison with control. Co-exposure of birds to ACE and FSO in diet showed a significant improvement in the hematological parameters however some values did not return to normal.

Serum biochemistry

Lipid profile: Lipid profile of control and treated groups as affected by ACE and FSO is summarized in Table 4. Total cholesterol, LDL and HDL were significantly ($p<0.001$) affected by different dietary supplements. The ACE group showed the highest values of total cholesterol and LDL followed by ACE+FSO group then control group, while showed the lowest HDL value. The FSO fed group showed the lowest values for total cholesterol, LDL and triglyceride but highest HDL. On the other hand, triglycerides were significantly ($p = 0.002$) increased in ACE and ACE+FSO groups compared to control and FSO groups.

Table 2: Effect of acephate (ACE) and flaxseed oil (FSO) supplementation on performance and tested relative organ weights of broiler chicks

	Treatments					
Items	Control	ACE	FSO	ACE+FSO	SEM	p-value
Performance indices						
Final body weight (g)	1272.00^{b}	1098.00^{d}	1290.00^{a}	1185.00^{c}	3.56	<0.001
Weight gain (g day^{-1})	45.47^{b}	39.93^{c}	50.12^{a}	46.90^{b}	0.52	<0.001
Feed intake (g day^{-1})	56.29^{a}	52.07^{b}	58.98^{a}	55.67^{a}	0.31	0.045
FCR (g feed gay^{-1} gain)	1.23^{b}	1.30^{a}	1.20^{b}	1.21^{b}	0.12	0.023
Relative organ weights (%)						
Liver	1.34^{b}	0.91^{c}	1.47^{a}	1.35^{b}	0.02	0.033
Kidney	0.77^{b}	0.63^{c}	0.83^{a}	0.75^{b}	0.02	0.049

Means in the same row within each classification bearing different letters are significantly different ($p\leq0.05$), SEM: Standard error mean, FCR: Feed conversion ratio

Table 3: Effect of acephate (ACE) and flaxseed oil (FSO) supplementation on the hematological parameters of broiler chicks

	Treatments					
Hematological parameters	Control	ACE	FSO	FSO+ACE	SEM	p-value
Hb (g dL^{-1})	14.01^{a}	11.01^{c}	13.70^{a}	12.80^{b}	0.17	0.047
RBCs (10^{6} mm^{-3})	6.83^{a}	5.24^{c}	6.72^{a}	6.19^{b}	0.03	0.029
PCV (%)	41.18^{a}	32.80^{b}	40.03^{a}	37.60^{a}	1.40	0.031
MCV/FI	55.56	53.33	54.01	52.60	0.14	0.127
MCHC (%)	29.46	29.15	29.21	28.90	1.45	0.224
WBCs (10^{3} mm^{-3})	13.42^{b}	16.35^{a}	13.31^{b}	13.10^{b}	0.37	0.031
Lymphocyte (10^{3} mm^{-3})	10.98^{c}	16.86^{a}	10.86^{c}	12.86^{b}	1.01	0.039
granulocyte (10^{3} mm^{-3})	1.36^{b}	3.30^{a}	1.47^{b}	1.42^{b}	0.66	0.025

Means in the same row within each classification bearing different letters are significantly different ($p\leq0.05$), SEM: Standard error mean, Hb: Hemoglobin, RBCs: Red blood cell, PCV: Packed cell volume, MCV: Mean cell volume, MCHC: Mean corpuscular hemoglobin concentration and WBCs: White blood cell

Table 4: Effect of acephate (ACE) and flaxseed oil (FSO) supplementation on serum lipid profile of broiler chicks

	Treatments					
Lipid profile (mg/100 mL)	Control	ACE	FSO	FSO+ACE	SEM	p-value
Total cholesterol	74.04^{c}	151.20^{a}	62.67^{d}	121.34^{b}	2.55	<0.001
LDL-cholesterol	20.20^{c}	39.20^{a}	17.30^{d}	28.44^{b}	0.03	0.004
HDL-cholesterol	47.17^{c}	40.23^{d}	100.60^{a}	85.12^{b}	0.32	<0.001
Triglyceride	65.27^{b}	127.73^{a}	64.28^{b}	121.43^{a}	0.78	0.002

Means in the same row within each classification bearing different letters are significantly different ($p \leq 0.05$), SEM: Standard error mean, LDL: Low density lipoprotein and HDL: High density lipoprotein

Table 5: Effect of acephate (ACE) and flaxseed oil (FSO) supplementation on hepato-renal functions of broiler chicks

	Treatments					
Item	Control	ACE	FSO	FSO+ACE	SEM	p-value
Liver function biomarkers						
AST (μL L^{-1})	7.21^{b}	35.90^{a}	7.23^{b}	6.96^{b}	3.49	<0.001
ALT (μL L^{-1})	8.33^{b}	16.35^{a}	8.50^{b}	15.78^{a}	2.12	<0.001
ALP (μL L^{-1})	126.00	133.00	131.00	128.00	10.35	0.421
TP (mg/100 mL)	6.55^{a}	5.60^{b}	6.39^{a}	6.48^{a}	0.13	0.113
ALB (mg/100 mL)	3.70	3.23	3.61	3.64	0.08	0.123
GLB (mg/100 mL)	2.88^{a}	2.47^{b}	2.78^{a}	2.84^{a}	0.10	0.041
Kidney function biomarkers						
Urea (mg dL^{-1})	20.22^{c}	67.40^{a}	19.94^{c}	52.72^{b}	0.75	0.001
Creatinine (mg dL^{-1})	1.13^{c}	1.86^{a}	1.09^{c}	1.41^{b}	0.03	0.022

Means in the same row within each classification bearing different letters are significantly different ($p \leq 0.05$), SEM: Standard error mean, TP: Total protein, ALB: Albumin, GLB: Globulin, AST: Aspartate amino transferase, ALT: Alanine amino transferase, ALP: Alkaline phosphatase and G6P: Glucose-6-phosphate

Table 6: Effect of acephate (ACE) and flaxseed oil (FSO) supplementation on hepatic antioxidant parameters of broiler chicks

	Treatments					
Antioxidant indices	Control	ACE	FSO	ACE+FSO	SEM	p-value
SOD (U g^{-1} tissue)	27.50^{b}	12.00^{d}	29.36^{a}	19.07^{c}	0.15	<0.001
CAT (nmol g^{-1} tissue)	76.01^{b}	42.01^{d}	79.18^{a}	53.01^{c}	0.35	<0.001
GSH (ng g^{-1} tissue)	15.60^{a}	3.50^{c}	15.23^{a}	7.01^{b}	0.13	<0.001
GST (ng g^{-1} tissue)	0.33^{a}	0.15^{b}	0.37^{a}	0.33^{a}	0.04	0.023
MDA (ng g^{-1} tissue)	55.60^{c}	156.90^{a}	43.25^{d}	80.16^{b}	13.20	<0.001
IL-2 (pg g^{-1} tissue)	132.93^{c}	162.27^{a}	130.17^{c}	159.13^{b}	1.78	0.002
TNF-α (pg g^{-1} tissue)	40.08^{c}	75.69^{a}	43.50^{bc}	67.40^{b}	2.33	0.023
CYP_{450} (ng g^{-1} tissue)	1.30^{b}	2.82^{a}	1.24^{b}	2.73^{a}	0.29	0.021

Means in the same row within each classification bearing different letters are significantly different ($p \leq 0.05$), SEM: Standard error mean, SOD: Superoxide dismutase, CAT: Catalase, GSH: Reduced glutathione, GST: Glutathione-S-transferase, MDA: Malondialdehyde, IL-2: Interleukin 2, TNF-α: Tumor necrosis factor-α, CYP_{450}: Cytochrome b-450

Liver and kidney functions: The effect of dietary supplements on liver and kidney function biomarkers of broiler chicks is illustrated in Table 5. Serum ALT and AST activities were significantly ($p<0.001$) increased in birds fed on diet supplemented with ACE, while ALP was not affected compared to control group. The FSO significantly decreased ALT and AST activities to control values when supplemented to ACE diet. Supplementation of diet with FSO did not change the TP, ALB or GLB contents compared to control. On the contrary, ACE significantly decreased TP and GLB than control, while supplementation of FSO-ACE diet improved their values to be comparable with control.

Urea and creatinine concentrations were significantly ($p<0.001$ or $p = 0.022$) affected in response to dietary supplements, where higher values were obtained by ACE, followed by ACE+FSO then FSO group which did not changed than control.

Antioxidant indices in liver: The data in Table 6 showed that hepatic SOD and CAT activities as well as GSH and GST concentrations were significantly ($p<0.05$) decreased in ACE fed groups than control. On the other hand, MDA, IL-2 and TNF-α concentrations were significantly ($p<0.05$) elevated in ACE group in comparison with control. Similarly, ACE

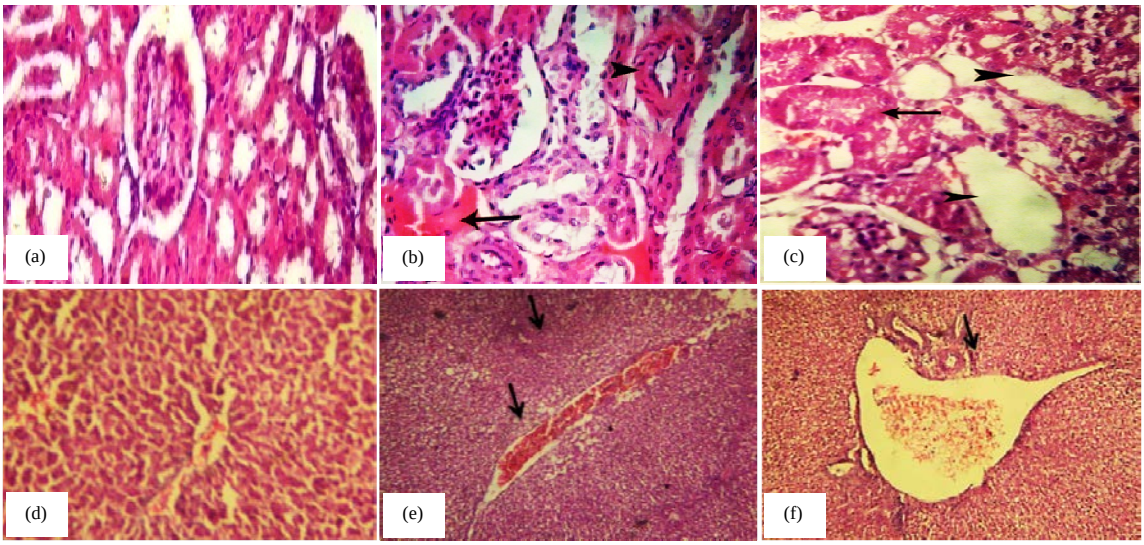

Fig. 1(a-f): (a) Kidney from both control and FSO control showed normal tubular and glomerular structures of nephrons, (b) Kidney from ACE group showed congestion of the renal blood vessels (arrows), (c) Kidney form ACE+FSO group showed coagulative necrosis, diltation and flattening of tubular epithelium (arrows) H and E x 400, (d) Liver of control and FSO groups showed normal hepatic architectures, (e) Liver of ACE showed and extravasation of erythrocytes with congested hepatoportal blood vessels (arrows) and (f) Liver of ACE+FSO group showed pyknotic nuclei and focal aggregations of mononuclear cells (arrows) (HE × Scale bar = 20 μm)

Table 7: Effect of acephate (ACE) and flaxseed oil (FSO) supplementation on renal antioxidant parameters of broiler chicks

	Treatments					
Antioxidant indices	Control	ACE	FSO	ACE+FSO	SEM	p-value
SOD (U g^{-1} tissue)	29.50	26.96	31.32	28.04	1.23	0.213
CAT (nmol g^{-1} tissue)	66.38^{a}	36.70^{b}	70.74^{a}	39.36^{b}	0.39	0.023
GSH (ng g^{-1} tissue)	14.33^{b}	2.88^{d}	17.28^{a}	7.96^{c}	0.15	0.002
GST (ng g^{-1} tissue)	0.35^{a}	0.19^{b}	0.37^{a}	0.31^{a}	0.02	0.043
MDA (ng g^{-1} tissue)	45.60^{b}	125.73^{a}	37.12^{c}	47.98^{b}	25.01	0.001
IL-2 (pg g^{-1} tissue)	129.00^{c}	153.00^{a}	123.56^{d}	142.00^{b}	2.01	0.042
TNF-α (pg g^{-1} tissue)	42.13^{c}	77.20^{a}	40.48^{c}	61.84^{b}	2.03	0.034
CYP_{450} (ng g^{-1} tissue)	1.21	1.38	1.27	1.34	0.16	0.089

Means in the same row within each classification bearing different letters are significantly different ($p \leq 0.05$), SEM: Standard error mean, SOD: Superoxide dismutase, CAT: Catalase, GSH: Reduced glutathione, GST: Glutathione-S-transferase, MDA: Malondialdehyde, IL-2: Interleukin 2, TNF-α: Tumor necrosis factor-α, CYP_{450}: Cytochrome b-450

significantly ($p = 0.021$) increased the CYP_{450} in hepatic tissue than control. These effects were improved in ACE+FSO, however not reach control values. Supplementation of FSO alone significantly improved the hepatic antioxidant defense by increasing the antioxidant reserve and decreased the MDA content than control, while it has no significant effect on IL-2 and TNF-α.

Antioxidant indices in kidney: The results represented in Table 7 showed that ACE has significant decreasing effects on renal hepatic CAT activity as well as GSH and GST concentrations with no significant change in the SOD activity compared to control. While, MDA, IL-2 and TNF-α concentrations were significantly ($p<0.05$) increased by ACE in comparison with control group. On the other hand, the renal content of CYP_{450} was not significantly ($p>0.05$) affected by ACE compared to control. Co-exposure of birds to ACE and FSO resulted in improving the antioxidant status in kidney than ACE alone group, while the best values were obtained by supplementation of FSO alone.

Histopathological findings

Kidney: The histopathological changes in kidney of control and treated groups are described in Fig. 1. Kidney from control and FSO fed chicks revealed normal tubular and glomerular histoarchitecture of the nephrons. While kidney from ACE group appeared with congestion of the renal blood vessels and thickened and hyalinized wall of the renal arteriole.

Kidney of chickens form ACE+FSO group showed coagulative necrosis in the tubular epithelium and dilated tubules with flattened epithelium.

Liver: Liver of control and FSO fed chicks showed normal architectures of hepatocytes and hepatic sinusoides. Liver of ACE showed and extravasated erythrocytes and severe congestion in the hepatoportal blood vessels in addition to hydropic degeneration and lymphocytes aggregations. The liver of ACE+FSO fed chicks revealed pyknotic nuclei with focal aggregations of mononuclear cells.

DISCUSSION

Spreading of the environmental pollution represents a threat for different kinds of life around the world especially avian species which has a great importance as alternative meat sources in developing countries. The results of the present study showed that exposure of birds to ACE during the fattening period decreased the body weight, weight gain, feed intake, feed efficiency and relative organ weights. On the other hand supplementation of diet with FSO in presence of ACE significantly improved these values and enhance growth performance of birds could be due to improving the feed consumption and FCR of birds.

The results of the present study also revealed that ACE significantly decreased the RBCs count, Hb content and PCV. Our results came on line with Rajini *et al.*[21] who found that exposure to insecticides showed a destructive effect on RBCs manifested by hemorrhage and anemia. On the other hand, leukocytosis was observed in ACE-treated birds; in addition to increasing the lymphocyte and granulocyte counts it could be resulted from the enhancement of immune system by inflammatory and necrotic effects of insecticide suggesting its immunotoxic potential[22]. On the contrary, exposure to ACE for 14 or 28 days did not affect the leukocytic count in white leghorn cockerels[23]. While, leukocyte and lymphocyte counts were significantly decreased highly in mice exposed to ACE in a dose of (17.55 g kg^{-1} b.wt.) for the same period with no changes in granulocyte or monocyte counts[24].

Flaxseed oil could ameliorate the hematological disorders induced by ACE possibly due to its high content of Poly Unsaturated Fatty Acids (PUFA) such as n-3 PUFA which could protect cell membrane of RBCs by increasing its content from α- and γ-tocopherol[25]. In another study on rats, flaxseed oil could restore the different hematological parameters to normal values and improve the lipid profile and antioxidant status probably due to the presence of Linolenic Acid (LA) and its metabolities docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) which act as free radical scavengers and protect erythrocytic membrane from lipid peroxidation[26].

Concerning hepatic functions, ACE intoxication increased the activities of hepatic enzymes ALT and AST but not ALP, possibly due to the ability of pesticides to generate Reactive Oxygen Species (ROS) which involved in toxic aldehydes induction and lipid peroxidation enhancement consequently elevating the release of intracellular hepatic enzymes. Histopathological alterations of the liver tissue of ACE-exposed group confirmed these observations. Undesirable effects of pesticides on hepatic enzymes were also reported by Toor *et al.*[27].

The ACE significantly decreased the serum total proteins and globulin as compared to control, indicating significant damage to hepatocytes consequently, disturbance of protein synthesis and metabolism. Similarly, subacute exposure to ACE decreased the total protein level in white leghorn birds[23] and decreased both total protein and globulin in mice[24]. These changes could be attributed to the stress and general toxic impact of ACE on birds which also reflected on the decreased body weight gain.

The ACE significantly increased the levels of urea and creatinine in serum may be as a result of perfusion and excretion of the insecticide. The obtained histological changes in the kidney tissue corroborate these suggestions. Total cholesterol, LDL and triglycerides were significantly elevated in ACE treated birds reflecting the impairment in lipid synthesis and metabolism as a result of hepatic injury exerted by ACE.

Flaxseed oil improved the hepatic and renal functions biomarkers and lipid profile in serum, however, still under control values. Similarly, flaxseed improved the lipid profile, antioxidant status and histological structure of rat liver[11] and has a hypocholesterolemic effect in hens[28]. These improvements could be returned to the relieving effects of flaxseed oil on hepatic and renal architectures which are important for metabolism and excretion of both toxic and beneficial materials.

Superoxide dismutase, catalase, GSH and lipid peroxidation are important biomarkers for evaluating the degree of oxidative damage in different body systems. The ACE intoxication produces oxidative stress in both liver and kidney tissues as evidenced by increased MDA and decreased antioxidants (SOD, CAT, GSH and GST) however, SOD did not affect in kidney tissue. Similar results on the effect of ACE on the lipid peroxidation and antioxidant profile were obtained in rats[29]. These changes suggest the ability of ACE to produce

of ROS which accumulate in the different tissues causing their inflammation and depletion of antioxidant defense inside them and could explain the impairment of liver functions obtained in this study.

Our results showed that flaxseed oil has positive impacts on restoring the antioxidant capacity and decreasing lipid peroxidation in liver and kidney[30]. Similarly, flaxseed oil decreased lipid peroxidation and oxidized glutathione contents in RBCs[25] and normalized the antioxidant status in kidney exposed to toxic agents[31]. Flaxseeds also decreased MDA level, increased the activities of SOD and CAT and prevented liver damage[32] as well as enhanced hepatic functions[33]. These effects could be returned to the high content of n-3 PUFA which contains double bonds act as membrane protectant against oxidative injury. In addition to omega-3 fatty acids and plant lignans which act as powerful scavengers of free radicals and quencher of singlet oxygen[34]. The cytokine IL-2 from T-helper cells is of importance for cellular immunity, while TNF-α is produced from T-lymphocytes, leukocytes and parenchymal tissue to protect cells by promoting their responses in presence of different pathogens. In the present study, ACE treated birds showed a significant increase in TNF-α and IL-2 suggesting that ACE could induce inflammatory cascades in liver and kidney tissues and could modulate the response of immune system . Similar responses following exposure to pesticides were reported[35]. These results agreed with those of non-specific immunity parameters (total protein and WBCs and lymphocyte counts) in addition to the pathological alterations in liver and kidney tissues.

Flaxseed oil was found to considerably reduce the deleterious impacts of ACE on co-exposed birds may be due to LA in the oil which could diminish the production of pro-inflammatory cytokines as reported by James *et al.*[36].

Metabolism of pesticides is regulated by CYP_{450} enzymes[37]. The ACE enhanced the activity of CYP_{450} in liver only and not in kidney. Similarly, ACE caused CYP super family induction in different tissues of CD1 mice[38], this induction produce huge quantities of oxygen free radicals involved in the carcinogenic activity of ACE[39]. These results are in harmony with the effects of ACE on the antioxidant status of liver and kidney and confirm the ability of ACE to generate ROS. The excess of ROS could produce oxidation of macromolecules inside the cells as DNA, lipids and proteins[40]. Flaxseed oil in the present study restored the hepatic CYP_{450} activity to normal levels thereby enhancing the metabolism and destruction of ACE, preventing its accumulation and lowering its toxicity.

CONCLUSION

From the results of the present study we could conclude that, the organophosphrous insecticide, ACE could disturb the growth performance and blood picture of growing broiler chicks, alter the histological structure and function biomarkers of liver and kidney as well as inhibit their antioxidant defense systems. The ACE also showed hypercholesterolemic effects in addition to inflammatory and immunotoxic potential. These results suggest the ability of ACE to induce oxidative damage and generation of ROS. Flaxseed oil showed good antioxidant effects and hypocholesterolemic activities probably via the radical scavenger effects of its beneficial constituents that could modulate antioxidant enzymes and reduced lipid oxidation and proinflammatory cytokines as well as repair the liver and kidney tissue damage after ACE exposure suggesting FSO as a good dietary supplement for growing broiler chicks even in the presence of environmental pollutants.

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