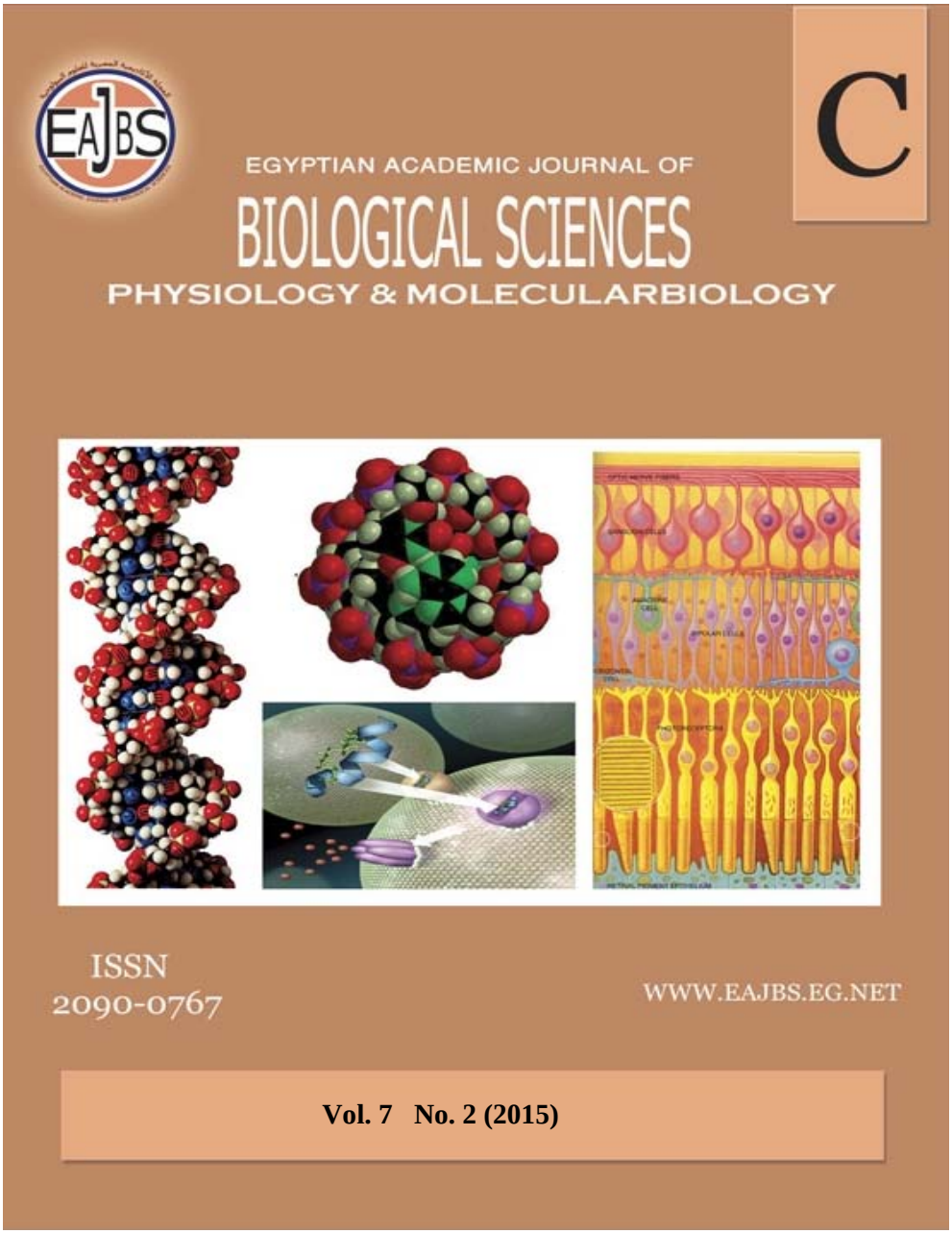

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Ameliorative Effect of Oregano Essential Oil on Mycotoxins-Induced Immune Impairments in Growing Japanese Quail

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ABSTRACT

Mycotoxins contaminated feed is one of the most serious problems in poultry and animal production sectors. Therefore, the current study aimed to investigate the effects of dietary oregano essential oil (OEO) for 4 weeks against the adverse effect of aflatoxins (AFs) on immunity, oxidative stress, Total and differential leucocytes counts (TLC and DLC), interleukin 1 (IL-1), nitric oxide (NO), lysozyme enzyme activity, serum malondialdehyde (MDA), catalase, superoxide dismutase (SOD) and reduced glutathione (GSH). Beside growth performance [Weekly body weight gain and feed conversion ratio (FCR)] were also determined. A total of 132 male, 2 weeks old Japanese quails chicks (*Coturnix japonica*) were used in this study and equally divided into 4 groups. Group I is considered as negative control at which birds fed only basal diet, group II served as positive control and were fed AFs 2.5 mg /Kg supplemented basal diet, Groups III and IV were fed basal diet with 2.5 mg AFs /Kg and treated with OEO at a rate of 200 and 400 mg/Kg , respectively. Regarding growth performance, there was a significant ($P<0.05$) reduction in weight gain and FCR at 4th week of the experiment in group II, additionally different pattern was noticed in group IV which represented a significant ($P<0.05$) improvement as compared to the other AFs treated groups and was nearly similar to that of group I. Concerning Lipid peroxidation, oxidative stress enzyme and immune parameters at 4th week of the experiment, MDA was significantly ($P<0.05$) increased in AFs treated quails (II and III) but when AFs co-administered with 400 mg OEO, MDA was nearly similar to that of group I. The SOD, catalase, and GSH were decreased significantly ($P<0.05$) in groups (II and III) and was remarkably improved in group IV. The NO level was increased significantly ($P<0.05$) while lysozyme activity was decreased significantly ($P<0.05$) in group II, while they were decreased and increased in group IV, respectively. IL-1 and globulin as well as TLC were declined significantly ($P<0.05$) in groups II and III and improved nearly similar to control one in group IV. In conclusion, OEO has a potential and a protective effect against AFs B_1 immuno-deteriorating effect in Japanese quails especially at 400 mg/ kg dose.

INTRODUCTION

Mycotoxins are metabolites produced from filamentous fungi causing a toxic response when ingested by animals.

There are variety of toxins that are produced by several fungi (Glenn, 2007). Cereal plants, which are main ingredient in poultry feed, may be contaminated by mycotoxins due to growth of fungi on plants or stored cereals (Krnjaja *et al*., 2008). Aspergillus species are the main contaminants of feed. Aflatoxins (AFs) is produced mainly by *Aspergillus parasiticus* and *Aspergillus flavus* (Ghiasian and Maghsood, 2011). There are about twenty different AFs have been identified, with the major ones being B_1, B_2, G_1 and G_2, (Morgavi and Riley, 2007). AFs B_1 is the most prevalent toxin in cereals and induce the greatest toxigenic threat (Leeson *et al*., 1995). They are incorporated into membrane structures causes various adverse changes including fatty acid oxidation and lipid peroxidation (Surai *et al*., 2008). Consequently, physiological functions like development, growth, immunity and reproduction are altered. These changes involve reduction in efficiency of feed utilization, body weight gain, reproductive capacities and immune suppression (Pestka, 2007) which leads to economic losses (Wu, 2004; Wu, 2006)

Interest in naturally occurring antioxidants for feed is emerging due to their positive impacts on animals' health including poultry (Dhama *et al*., 2015; Quiroz-Castañeda and Dantán-González, 2015). Many plants have been identified as excellent poultry antioxidants among which are oregano (Botsoglou *et al*., 2007; Botsoglou *et al*., 2002; Rahal *et al*., 2014). Oregano (*Origanu-mvulgare* subsp. *Hirtum*) is a spice that belongs to family Labiatae, that is famous in Mediterranean countries (Kintzios, 2002). Steam distillation of oregano plant yields the oregano essential oil (OEO) that contains more than 30 ingredients, most of which are phenolic compounds that exert various activities (Botsoglou *et al*., 2002; Yesilbag *et al*., 2013). The major components of OEO are thymol and carvacrol that constitutes about 78–82% of the total oil and are principally responsible for OEO antioxidant activity (Botsoglou *et al*., 2002; Yesilbag *et al*., 2013). Oregano was proven to have antioxidant (Bampidis *et al*., 2006; Botsoglou *et al*., 2007; Botsoglou *et al*., 2002; Park *et al*., 2015), antibacterial (Hernández-Hernández *et al*., 2014), anti-inflammatory (Khaki *et al*., 2013) and antifungal (Cleff *et al*., 2010) activities. Moreover, OEO has been shown to have positive effects on poultry performance, production and immunity (Çabuk *et al*., 2014; Christaki *et al*., 2011; Yesilbag *et al*., 2013).

Contamination of mixed feed and feed ingredients with mycotoxins is a worldwide issue and due to their ubiquitous nature, it is hard to eliminate them totally from feed and feed ingredients (Azarakhsh *et al*., 2011). Several dietary strategies to cover the toxic effects of mycotoxins especially AFs have been proposed with antioxidants (Galvano *et al*., 2001). Consequently, the current study aimed to investigate the effect of dietary OEO at different levels of 200 and 400 mg/Kg on performance and immunosuppression caused by dietary AFs in growing Japanese quails.

MATERIALS AND METHODS

Experimental birds and management

A total of 132 male, 2 weeks old Japanese quails chicks (*Coturnix japonica*) weighing 35-40 g were purchased from Agricultural Technological Centre, Faculty of Agriculture, Cairo University, Egypt. They were kept in wire battery cages and allowed *ad libitum* access to feed and water. Ventilation and temperature were controlled to provide comfortable growing of the birds. Room were electrically heated and provided with 23 h

lighting. Chicks were checked three times daily (6 am, 2 pm and 10 pm) for feed, water and mortality. All the protocols regarding the study were conducted according to the ethical guidelines for the use of animals in the Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Experimental diet

The experimental diet (Table 1) was formed to fulfil the nutritional requirements for Japanese quail (NRC, 1994). It contained 24% CP and 2900 kcal ME/Kg. After preparation of the experimental diets, a sample of 1 Kg was collected and screened by HPLC according to Nabney and Nesbit (1965) and it was found to be mycotoxins free.

Table 1: Ingredients of Japanese quails' basal experimental diet

Ingredient	Concentration %
Ground yellow corn	55.780
Soya bean meal	31.960
Fish meal	1.000
Corn gluten	7.450
Bran	1.000
Dicalcium Phosphate (22%Ca&19%P)	0.710
Limeston (38% Ca)	1.300
Lysine (purity 98%)	0.160
DL – Methionine (purity 98%)	0.080
Iodized sodium chloride	0.300
Vitamin & Mineral premix*	0.300

*supplied by Misr Feed Additives Co., Egypt

Aflatoxins

The AFs used in the experiment was produced at Laboratory of Animal Health Research Centre, El-Dokki, Egypt by fermenting parboiled rice with toxigenic fungus strain of *Aspergillus parasiticus* NRRL 2999 according to Shotwell *et al*. (1966). Mouldy rice was dried and ground to fine powder. AFs levels in rice powder were measured by HPLC at Mycotoxins Central Laboratory and Food Safety of the National Research Centre, El Dokki, Giza, Egypt. The analysis was performed according to Nabney and Nesbit (1965) using AFs B_1, standard (Biopure Referenzsubstanzen GmbH, Austria). The level of AFs B_1 in rice was 6.5 mg/Kg.

Experimental design

The experimental birds were randomly divided into 4 experimental groups; each group was subdivided into 3 replicates (11 chicks per each). Group I, was a negative control quails fed basal diet only. Group II was fed basal diet with 2.5 mg AFs /Kg diet according to Eraslan *et al*. (2004). Group III was fed basal diet with 2.5 mg AFs /Kg diet and 200 mg/Kg OEO (NBT Company, Turkey). Group IV fed basal diet with 2.5 mg AFs /Kg diet and 400 mg/Kg OEO. Treatments were continued for 4 weeks.

Weight gain and feed conversion ratio

Body weight gain of each bird was determined weekly according to Brady (1968). Feed intake (g/week) was calculated per group by obtaining the difference between the weight of offered feed and the remained portion daily for 7 days. Feed conversion ratio (FCR) was calculated weekly.

FCR = (Feed consumption (g) /bird/week)/ (body weight gain (g) /bird/week).

Blood and tissue sampling

Twelve birds of each group were slaughtered at the 2nd week and the end of experimental period (4th week). One blood sample was collected in EDTA sterilized tubes for total and differential leucocytes count (TLC & DLC). Other blood sample was collected on sterilized plain tubes for serum separation. Serum was stored at -20ºC for determination of

malondialdehyde (MDA), catalase, superoxide dismutase (SOD), reduced glutathione (GSH), nitric oxide (NO), lysozyme enzyme activity, interleukin1 (IL-1), serum globulin. Thymus and spleen were obtained and fixed in 10% formalin solution for 48 h for histopathology.

Serum MDA

Serum MDA, a measure of the intensity of lipid peroxidation, was calorimetrically assayed according to the method of Ohkawa *et al*. (1979) by commercial kit (Biodiagnostic, Egypt).

Serum oxidative stress assay

Serum SOD, GSH and catalase activity were determined from all experimental and control groups using commercial kits (Biodiagnostic, Egypt) according to Aebi (1984), Nishikimi *et al*. (1972) and Tietez (1969), respectively.

Nitric oxide assay

Serum NO level was measured using the method described by Rajaraman *et al*. (1998). A total volume of 100 µl of each serum sample was incubated with an equal Griess reagent volume (1% sulphanilamide, 0.1% N-Naphthyl-ethylenediamine, 2.5% phosphoric acid) in a 96 plate well with flat bottom and incubated for 10 min. at 27 ºC. Then the optical density was measured at 570 nm using ELISA reader. A standard curve of $NaNO_2$ was done for molar NO_2 concentrations determination.

Lysozyme activity

The lysozyme activity was estimated via agarose gel cell lysis assay. lysoplates were prepared by dissolving 0.01% agarose in 0.0067 M PBS (pH 6.3) at 100°C, when agarose temperature reached 60-70°C, 500 mg of *Micro-coccus lysodeikticus* suspension in 5 ml saline was added to 1L of agrose and mixed well, then the plates were poured, 25µl of each of the serum samples and standard lysozyme solutions were put in each poured well. The plates were incubated at 28-30 °C for 12-18 h. and the clear zone ring diameters were measured. The diameters of clear zones were plotted against the standards for obtaining lysozyme concentration in the samples (Schultz, 1987).

Interleukins assay

IL- 1 levels were determined using commercial ELISA kits (CUSABIO, China) according to manufacturer's protocol.

Serum globulin

Serum total protein was determined using commercial kits (No. 025418, Diamond diagnostics Co., Egypt) by Biuret reaction described by Koller and Kaplan (1984). Serum albumin was determined using commercial calorimetric kits (No. 52027, Biodiagnostic Co., Egypt) according to Titez (1995). Serum globulins were calculated by subtracting of serum albumin value from the serum total proteins value (Omran *et al*., 2008).

Total and differential leucocytic counts

The TLC was performed using manual haemocytometer according to Campbell (1995). Blood films were stained by 10% Giemsa stain. One hundred leucocytes were counted on each slide for DLC, the preparation of blood films and staining procedure are according to Houwen (2002). Two slides were counted/ bird and mean was calculated for each bird (Gross and Siegel, 1983).

Histopathology

Formalin fixed thymus and spleen were embedded in paraffin blocks and standard procedures for Hematoxylin and Eosin stain were performed as described by Bancroft *et al*. (1996).

Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM). The differences were determined by One-way analysis of variance. The inter-group comparisons were done by ANOVA and fisher's least protected significance test. A significant *P* value was considered at < 0.05. The analysis was done by IBM

SPSS software computer program version 16, NY, USA (Inc., 1989-2010).

RESULTS

Body weight gain and food conversion

Body weight gain at 4th week of the experiment was significantly (P<0.05) reduced in quails of group II and III than control. There was no difference in body weight gain of control quails and that treated with 2.5 mg AFS and 400 mg OEO. Moreover, the feed conversion ratio was much higher (P<0.05) in AFs treated quails compared to other treated ones (Table 2).

Table 2: Body weight gain and feed conversion ratio in control, aflatoxins and aflatoxins-oregano oil treated quails

Parameters	Weeks	Group I	Group II	Group III	Group IV
Body weight gain (g)	1st	50.26^{a}±1.40	25.00^{b}±2.962	33.05^{b}±3.21	46.57^{a}±3.45
	2nd	26.17^{a}±1.32	11.867^{c}±1.96	11.875^{c}±1.19	17.66^{b}±1.81
	3rd	63.33^{a}±4.89	36.66^{c}±2.90	44.16^{bc}±5.53	50.90^{ab}±4.20
	4th	38.16^{a}±4.18	29.16^{ab}±3.52	22.91^{b}±2.497	37.91^{a}±4.05
Feed conversion ratio	1st	2.04^{b}±0.14	2.57^{a}±0.17	2.362^{b}±0.16	2.235^{a}±0.15
	2nd	3.248^{c}±0.11	3.76^{ab}±0.22	3.58^{bc}±0.17	3.39^{bc}±0.16
	3rd	3.42^{c}±0.17	4.94^{a}±0.17	4.00^{b}±.20	3.76^{bc}±0.22
	4th	2.97^{b}±0.25	4.24^{a}±0.32	3.82^{ab}±0.38	3.24^{b}±0.24

Different superscripts within the same row indicated significant differences at $P < 0.05$.

Lipid peroxidation and oxidative stress enzyme

At 4th week of the experiment, MDA was significantly increased in quails treated with AFs than control. When AFs co-administered with 400 mg OEO, MDA was nearly similar to that of control quails. The catalase, SOD and GSH were significantly (P<0.05) decreased in AFs treated quails than control and was remarkably improved when AFs co-administered with 400 mg OEO than group II (Table 3).

Table 3: MDA, catalase, SOD and GSH in control, aflatoxins and aflatoxins-oregano oil treated quails

		Group I	Group II	Group III	Group IV
MDA (µmol/L)	2nd week	2.49±0.2	2.8±0.26	2.38±0.23	2.62±0.19
	4th week	2.65^{bc}±0.25	3.47^{a}±0.26	3.14^{ab}±0.24	2.39^{c}±0.24
Catalase (U/ml)	2nd week	21.94±2.12	18.7±2.23	19.47±1.75	20.85±1.03
	4th week	20.53^{a}±1.86	14.53^{b}±2.35	14.75^{b}±1.6	17.96^{ab}±1.07
SOD (U/ml)	2nd week	212.45±16.91	197.14±12.37	189.55±8.72	196.59±13.48
	4th week	186.35^{ab} ±13	161.61^{ab} ± 10.23	158.82 ^{b}±9.91	188.61^{a} ±5.41
GSH (U/ml)	2nd week	14.02^{a}±0.68	12.48^{ab}±0.54	12.06^{b}±0.62	12.57^{ab}±0.53
	4th week	18.82^{a}±1.53	11.31^{d}±1.1	12.2^{cd}±0.77	14.86^{bc}±0.96

Different superscripts within the same row indicated significant differences at $P < 0.05$

NO, interleukins, globulin and lysozyme activity

Both groups treated with AFs (II and III) at 2nd week of the experiment showed fluctuation in the level of NO, while, at 4th week it was increased significantly (P<0.05) in group II and non significantly in group III., On the other hand, NO level was decreased when AFs co-administered with 400 mg OEO. IL-1, lysozyme activity and globulin was declined significantly (P<0.05) in AFs treated quails (group II), while their levels were improved nearly similar to control one when 400 mg OEO was co-administrated to AFs treatment at the 4th week (Table 4).

Total and differential leucocytic count

TLC was decreased significantly (P<0.05) in quails treated with AFs then it restored to control values when AFs co-administered with 400 mg OEO at 4th week of experiment. AFs treatment (group II and III) resulted in a significant

(P<0.05) increment in heterophils % than control that begin to be restored toward control values when AFs co-administered with OEO in a dose depending manner. However, during the experimental period lymphocytes, monocytes and eosinophils % were significantly or non-significantly (P<0.05) declinedin both groups II and III, respectively, than control. However, OEO co-administration at dose 400 mg/ kg with AFs significantly (P<0.05) improved their values toward control (Table 5).

Table 4: Nitric oxide, lysozyme activity, interleukins and globulin in control, aflatoxins and aflatoxins-oregano oil treated quails

		Group I	II	III	IV
NO (µM)	**2nd week**	11.97±1.17	13.33±1.26	12.41±1.27	10.28±0.84
	4th week	14.64^{bc}±0.54	20.93^{a}±2.40	17.17^{ab}±2.32	11.95^{c}±0.6
Lysozyme activity (µg/ ml)	**2nd week**	152.71^{a}±7.51	123.57^{b}±7.90	136.57^{ab}±7.17	139.29^{ab}±6.89
	4th week	127.01^{a}±4.12	93.96^{b}±5.80	116.43^{a} ±7.86	112.19^{ab} ±8.91
IL-1 (Pg/ml)	**2nd week**	41.66^{a} ±2.22	35.22^{b} ±2.61	41.03^{a} ±1.44	41.81^{a}±1.06
	4th week	37.80^{a}±2.13	32.00^{b}±1.38	35.97^{ab}±1.72	36.82^{ab}±1.99
Globulin (mg/dl)	**2nd week**	2.36^{ab}±0.02	2.07^{ab}±0.14	1.90^{b}±0.17	2.56^{a}±0.27
	4th week	3.94^{a}±0.68	2.02^{b}±0.11	2.47^{b}±0.37	2.58^{b}±0.22

Different superscripts within the same row indicated significant differences at $P < 0.05$

Table 5: Total leucocytes and differential leucocytes counts in control, aflatoxins and aflatoxins-oregano oil treated quails.

			Group I	Group II	Group III	Group IV
TLC/µl	**2nd week**		18.90±2.30	16.60±1.30	15.90±1.30	17.30±1.10
	4th week		33.10^{a} ±2.70	22.10^{b} ±1.10	23.10^{b} ±1.10	29.60^{a} ±1.60
DLC	**2nd week**	Heterophils %	31.90^{c} ±0.90	38.20^{a} ±0.90	36.70^{ab} ±0.70	35.50^{b}±0.90
		Lymphocytes%	62.30^{a} ±0.90	57.10^{b} ±0.70	58.20^{b} ±0.50	59.20^{b}±0.70
		Monocytes %	3.10^{a}±0.10	2.30^{b}±0.30	2.60^{ab}±0.20	2.80^{ab} ±0.20
		Eosinophils%	2.70±0.20	1.90±0.20	2.10±0.30	2.30±0.30
		Basophils%	0.20±0.20	0.50±0.22	0.55±0.34	0.70±0.30
	4th week	Heterophils%	31.90^{d}±0.60	51.00^{a}±1.1	41.30^{b}±1.3	36.50^{c}±1.30
		Lymphocytes%	61.20^{a}±0.40	46.20^{d}±0.80	54.80^{c}±1.31	58.50^{b}±0.91
		Monocytes%	3.50^{a}±0.3	1.10^{c}±0.20	2.30^{b}±0.30	2.50^{b}±0.20
		Eosinophils%	3.10^{a}±0.20	1.40^{c}±0.30	1.50^{bc}±0.2	2.30^{ab}±0.40
		Basophils%	0.30±0.20	0.30±0.20	0.11±0.11	0.20±0.20

Different superscripts within the same row indicated significant differences at $P < 0.05$

Histopathology

Thymus of control group revealed normal histological structure (Fig. 1-A). On the other hand, after 4 weeks aflatoxication thymus revealed atrophy of cortex and medulla besides apoptosis in thymocytes with the appearance of tingible body macrophages in (Fig. 1-B). Co-administration of AFs with 200 mg/kg OEO, thymus revealed moderate depletion of lymphocytes in cortex and medulla (Fig. 1-C). While, co-administration of AFs with 400 mg/kg OEO retained thymus normal density of lymphoid tissue in cortex and medulla (Fig. 1-D).

AFs treated spleens showed Lymphocytolysis and depletion of lymphoid cells (Fig. 2-C) as well as haemorrhage and fibrosis around sheathed arteries (Fig. 2-B). AFs co-administration with 200 mg/kg OEO, showed moderate depletion of lymphoid tissue (Fig. 2-D). While AFs treated with 400 mg/kg OEO retained normal density of splenic lymphoid tissue.

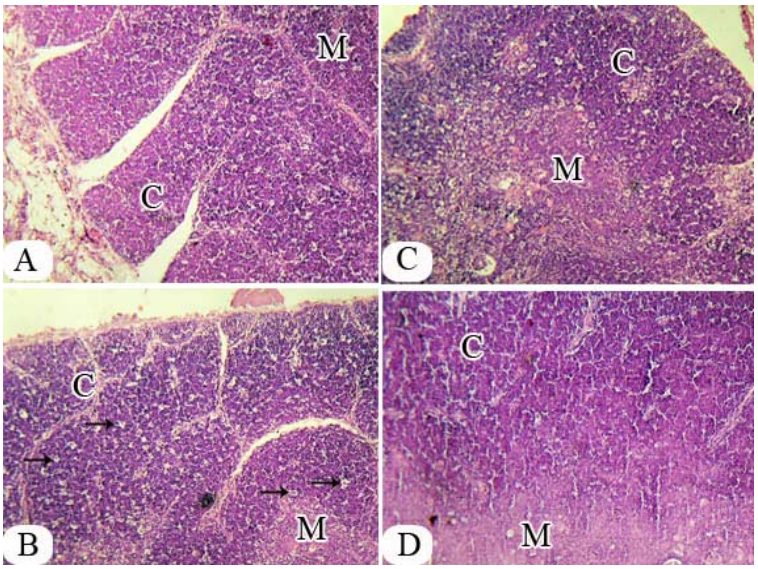

Fig. 1: Photomicrographs of hematoxylin and eosin stained quails' thymus sections. (A) shows normal histological structure of control group (group I); (B) (group II) After 4th week of AFs intoxication , arrow points to tingible body macrophages with intracytoplasmic apoptotic bodies besides medullary atrophy. (group III) AFs treated with 200mg/kg oregano oil, shows moderate depletion of cortex and medulla. (group IV) AFs treated with 400 mg/kg oregano oil retained normal density of lymphoid tissue in cortex and medulla. (C) Cortical zone (C) and Medullary zone (M) (magnification 200X).

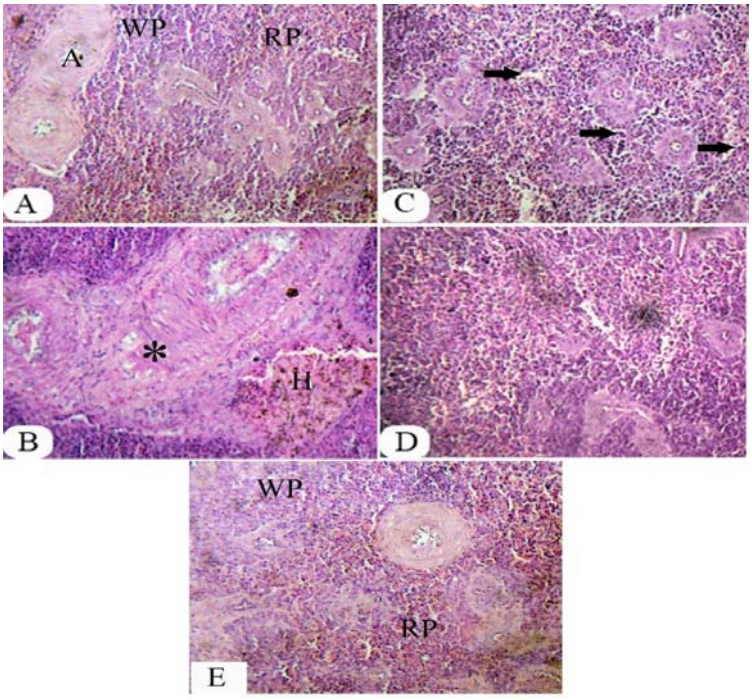

Fig. 2: Photomicrographs of hematoxylin and eosin stained quails spleen sections. (A) Shows the normal histological structure of spleen control group (group1); (B) AFs treated group shows haemorrhage (H) and fibrosis around sheathed arteries (*) (C), AFs treated group exhibits Lymphocytolysis and depletion of lymphoid cells (arrows) of white pulp. (D) (group III) AFs treated with 200 mg/kg oregano oil, shows moderate depletion of lymphoid tissue. (E) (group IV) AFs treated with 400 mg/kg oregano oil retained normal density of lymphoid tissue in spleen. (RP) red pulp, (WP) white pulp (A) artery (magnification 200X).

DISCUSSION

Recently, there is an increased interest about natural products which may counteract the detrimental effects of toxic environmental compounds and prevent several diseases in animals and human. In this concern, different types of natural products have been evaluated for their immune-protection effect against toxic compounds in animals and poultry feed. In the current study, we evaluated the ability of OEO to protect Japanese quails from the toxic effects of AFs.

The results indicated that ingestion of AFs for 4 weeks resulted in a significant ($P<0.05$) increase in FCR and a significant loss in the body weight gain. These results were in harmony with Nazar *et al*. (2012). The decreased in body weight in the quails of group II may be due to the effects of AFs on the balance between orexigenic and anorexigenic circuits in brain that regulate the homeostatic loop of body weight regulation, leading to cachexia (Rastogi *et al*., 2001). The reduction in body weight may be also due to reduction in leptin concentration (Abdel-Wahhab *et al*., 2010). The low leptin concentration is usually associated with the high levels of cortisol and IL-6 which act together on the influence the feeding response, causing weight loss (Barber *et al*., 2004). This correlation may explain the obtained reduction in body weight in quails ingested AFs. OEO co-administration with AFs improved significantly ($P<0.05$) weight gain and FCR than other treated groups. These results are in agreement with Çabuk *et al*. (2014) and Dalkilic *et al*. (2015). This effect may be related to digestion stimulating effects as well as palatability of oregano volatile oils that have been proven previously to have positive effects on poultry performance (Brenes and Roura, 2010; Mountzouris *et al*., 2011), thus it could counter-act the AFs adverse effects. On contrary, Botsoglou *et al*. (2007); Yesilbag *et al*. (2012) reported that OEO have no effect on quails body weight gain and FCR.

In the current study, quails fed AFs contaminated diet had much more lipid peroxidation and oxidative stress as indicated by the significant ($P<0.05$) increment in lipid peroxidation (MDA) and the reduction ($P<0.05$) of enzymatic antioxidants such as catalase, SOD and GSH. These results coincide with Citil *et al*. (2005); Cao and Wang (2014); Ma *et al*. (2015). AFs produced oxidative stress via producing reactive oxygen species (ROS) such that exceeds the tolerance capacity of the cellular antioxidant defense system. MDA contents in blood, as the end product of lipid peroxidation, increased following the occurrence of oxidative stress. Where the free radicals easily peroxidated the lipid membranes which are highly susceptible to peroxidation, and MDA was generated. Moreover, MDA also causes peroxidation itself, and accelerates peroxidation by synergism with free radicals (Essiz *et al*., 2006). Thus it have been suggested as one of the underlying mechanisms of the DNA damage and cellular damage (Yang *et al*., 2000), thereby causing immuno-pathologies in host immune cells (Costantini and Møller, 2009). The effect of AFs was markedly ameliorated with their co-administration with 400 mg/kg OEO than 200 mg/kg which manifested by significant ($P<0.05$) decrease in MDA and increments in catalase, SOD and GSH in group IV. These results are in agreement with Bulbul *et al*. (2014) who demonstrated the weak effect of low and higher concentrations of OEO on quail MDA and antioxidant enzymes system. The significant ($P<0.05$) increase in serum NO level than control beside the improvement with co-administration of OEO 200 and 400 mg/kg to levels comparable to control augmented the results of MDA and oxidative stress

enzymes. The usage of OEO as natural antioxidant could reverse the immunosuppressive effect of AFs (2.5 ppm) via modulation of NO level. Serum lysozyme activity was significantly ($P<0.05$) declined in AFs treated quails than control that coincides with Valtchev *et al*.(2015). This confirms the immunosuppressive effect of AFs that induced by AFs. As lysozyme enzyme has a part in an innate immunity and has a bactericidal activity (Leśnierowski, 2015) The co-treatment with OEO significantly ($P<0.05$) improved quails' lysozyme activity and subsequently the antibacterial effect (Gopi *et al*., 2014). OEO possesses antioxidant properties that can keep polymorph nuclear (PMN) leucocytes integrity that produce lysozyme (Brenes and Roura, 2010; Jolles and Jolles, 1984; Leśnierowski, 2015). Moreover, serum IL-1 followed the same trend where AFs significantly ($P<0.05$) reduced serum IL-1 levels than the control. These results are in agreement with Moon *et al*. (1999) who suggested the immuno-suppressive effect of AFs. IL-1 is produced by PMN leucocytes especially monocytes and considered an essential messenger in array signal toward infection (Berczi *et al*., 2005) via activation of macrophages and T lymphocytes that lead to production of other cytokines and chemokines (Wigley and Kaiser, 2003). PMN leucocytes were adversely affected by oxidative stress induced by AFs that was reversed by the antioxidant effect of OEO co-administration with AFs.

AFs treatment revealed significant ($P<0.05$) reduction in serum globulins that coincide with previous records of (Madheswaran *et al*., 2004; Nazar *et al*., 2012). This could be attributed to the ability of AFs to inactivate the biosynthetic enzymes of proteins including globulin (Madheswaran *et al*., 2004). Unfortunately , no literature was found concerning humoral response , So , it is worth to mention that the fall in serum globulins observed in the current study is an indicator for diminished production of the proteins family to which antibodies belong (Nazar *et al*., 2012). OEO 400 mg/ kg was significantly ($P<0.05$) improved serum globulin than AFs treated group. These results are in accordance with the results of Abd El-Ghany, and Ismail (2014) who confirmed the immunostimulatory effect of OEO in broilers as well as their effect in improving albumin globulin ratio.

Current data demonstrated that the immunodepressing effect of AFs significantly ($P<0.05$) reduced TLC with heterophilia, lymphocytopenia, monocytopenia and eosinopenia. These results coincide with Nazar *et al*. (2012) and Monson *et al*. (2015). The elevation of the heterophil percentage and the decrement in lymphocyte percentage is a hematological indicator that quails were suffering from stressful condition (Gross and Siegel, 1983) caused by AFs. Oxidative stress influence hypothalamic-pituitary-adrenal axis as well as the increased NO level that catalysed cortisol synthesis (Minka *et al*., 2012). Cortisol reduce the lymphocyte number but also decreasing eosinophils and increase the number of heterophils (Dhabhar *et al*., 1995, 1996). Moreover, oxidative stress could affect differentiation and phagocytic ability of leucocytes especially monocytes against infection (Ponnappan and Ponnappan, 2011). Consequently, the OEO 400 mg/ kg as natural antioxidant improved AFs induced oxidative stress, TLC, heterophil%, lymphocyte%, monocyte% and eosinophil % toward control values.

Quails dietary aflatoxication with 2.5 mg induced thymus atrophy with apoptosis in thymocytes. The same trend was noticed in spleens which showed Lymphocytolysis with depletion of lymphoid cells as well as haemorrhage and fibrosis around sheathed arteries. These results are in accordance with

Ortatatli (2001) and Monson (2015). Oxidative stress produced by AFs induce DNA damage that are the main cause of apoptosis processes in lymphocytes via caspase 3 (Chen *et al*., 2013; Wang *et al*., 2013). OEO mixture alleviated AFs effect through free radicals scavenging in a dose depending pattern thus ameliorated the histopathological lesions of aflatoxication in thymus and spleen.

To our knowledge, few studies inform about the effect of AFs contamination and the protective role of OEO on immune parameters of Japanese quail. According to current results, the dietary AFs had a depressing effect on the cell-mediated immunity through lysozyme activity, IL1, TLC and DLC modulation. Beside to thymic and splenic lymphoid depletions caused by oxidative stress. Moreover, humoral immune response can b also affected via reducing serum globulin. All of these were reflected on the body weight gain and FCR. Co-treatment of AFs and OEO resulted in a significant improvement in body weight gain and, immunological parameters as well as histological picture of the spleen and thymus. These improvements were doses dependant and seemed to be better in 400 mg/ kg dose. Therefore, OEO may play an ameliorative and protective role against AFs mediated immunosuppression through its antioxidant and free radical scavenging properties (Yesilbag *et al*., 2013).

CONCLUSION

It could be concluded that OEO is an immuno-protective against aflato-xicosis in Japanese quails especially when added by a rate of 400 mg/kg. The mode of action of theses extracts might be through scavenging of ROS. Therefore, this plant extract have an anti-immunodepressant effect due to its content of phenolic compounds. Thus it can be used in quails' diet as a source of natural antioxidants especially 400 mg/kg concentration.

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