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Effect of *Nigella Sativa* supplementation on some hematological, biochemical and histopathological studies in broiler chicks

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ABSTRACT

This study was designed to evaluate the effect of *Nigella sativa* oil (NS) in broiler chicks as a natural feed additive on some hematological and biochemical parameters as well as histopathological examination. The study was conducted on a total sixty, day old (Cobb strain) broiler chicks that were randomly divided into 3 equals groups (each of 20) as follow, 1st group (control) non-supplemented, 2nd group (NS_2) supplemented with *Nigella sativa* oil 2g /kg diet and 3rd group (NS_4) supplemented with *Nigella sativa* oil 4g /kg diet daily for three weeks. Whole blood, serum samples and tissue specimens were collected at the end of 3rdweek post supplementation for hematological, some serum biochemical analysis and histopathological examination respectively Dietary supplementation with *Nigella sativa* oil had in significant effects on the hematological parameters except total leukocytic and lymphocytic counts were markedly increased. Moreover, the serum levels of CHO, TG and LDL-C were significantly declined, while the level of HDL-C was markedly elevated in NS_2 and NS_4. In conclusion, it was cleared that *Nigella sativa* had an immunomodulating, hepatorenal protective and hypolipidimic effects in broilers.

Keywords: *Nigella sativa*; hematological parameters; hepatorenal; lipid profile; histopathological examination.

INTRODUCTION

Poultry production plays in particular an important socio-economic role in developing countries. Also, its products have a vital role in the cultural life of rural people as gifts to strengthen social relationships and provide an accessible good source of protein to combat a food insecurity (Alemayehu, 2018) *N.sativa* as natural feed additives improved the health and immunological status of the birds which could be counteract the development of microorganisms and enhance their resistance against diseases (Guler et al., 2006). *N.sativa* is a phytobiotic advised by Prophet Mohammad (PBUH) and hence due to Muslim religious belief, these ingredients had been utilized since many decays. It has a healing power due to its active principles especially thymoquinone which is the major active chemical component of the essential oil, mainly give the curative properties of the oil (Tembhurne et al., 2014).

Phytogenic feed additives are often called as phytobiotics or botanicals, such as black cumin seeds (*Nigella sativa* L.) (Akhtar et al., 2003; Guler et al., 2006), which may be used as complete plant substances or seeds or as essential oils and plants extracts (Windisch et al., 2009). It is used in food like flavoring additive in the breads and pickles because it has very low level of toxicity (Al-Ali et al., 2008). Also, they are used as alternative to antimicrobial feed additives in broiler (Alçiçek et al., 2004). *Nigella sativa* is one of the natural compounds is known as black seed (seed of capsulated plant), black cumins, Love-in-a-mist, Habatut, Sidadanah, Barakah, Habatut Sonez, Sauda, Kalonji, Krishana and Jiraka (Sultan, et al., 2009; Ismail, 2009).

Thymoquinone (TQ) has anti-hyperlipidemic (Ahmad et al., 2013), hypoglycemic (Kaleem et al., 2006), anti-inflammatory (Al-Logmani and Zari, 2011) and anti-schistosomiasis effects (Shenawy et al., 2008). It has nephroprotective (Yildiz, et al., 2010), neuroprotective effects (Mehri et al., 2014), gastro- protective (Abdel-Sater, 2009), immune-modulatory (Al-Hothaify and Al-Sanabani, 2016), antimicrobial activity

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(Rasouli-Hiq et al., 2016) and bronchodilator (Boskabady et al., 2010). Previously, Nasir (2009) and Alimohamadi et al. (2014) supplemented the broiler chick diet with NSS, resulted in no significant difference in the erythrocytes count, Hb, hematocrit values, serum total protein, albumin and globulin, respectively. Moreover, Toghyani et al. (2010) recorded no significant effect in the serum level of ALT and AST activities of broiler chick dietary supplemented with *Nigella sativa* seeds. Furthermore, Ghasemi et al. (2014) recorded a significant decrease in the TC, TG and LDL-C levels by dietary addition of *Nigella sativa* seeds in broiler chick.

Our study aimed to evaluate the effect of *Nigella sativa* oil as a natural feed additive on some hematological parameters, liver and kidney function biomarkers, lipid profile and histopathological studies in broiler chicks.

MATERIALS AND METHODS

Nigella sativa oil

Soft gelatin capsule contains *Nigella sativa* seed oil 450 mg produced by Pharco Pharmaceutical Products, Alexandria, Egypt. Capsules were stored at temperature not exceeding 30°C in dry place. M.O.H Reg. No. : 831/2009.

Experimental birds and experimental design:

Sixty apparent healthy mixed sexes Cobb broiler chicks one-day-old were obtained from local commercial hatchery at Mansoura city. On arrival, chicks were weighed (43 ±0.7 g) and randomly assigned to equal three dietary supplemented groups each of 2 replicates (10 chicks/replicate) based on a completely randomized design. The chicks were allowed *ad libitum* access to feed and water. The first group served as a control non supplemented group fed on basal diet (formulated to meet the nutritional requirements as recommended by NRC, 1994), the 2nd and 3rd groups were fed on diets supplemented with 2g (NS_2) and 4g (NS_4) of *Nigella sativa* oil/kg diet, respectively (Awad et al., 2013) for 3 consecutive weeks. The diets had been obtained kindly free from antibiotic and anticoccidial drugs (Herman Company) (table 1). The chicks were fed on their formulated diets from one-day old to three weeks of age. The broilers chicks were exposed to 24 h continuous photoperiod and checked daily for food, water and mortality.

Blood Samples

At the end of 3rd week post supplementation, 5 birds of each group randomly selected, two blood samples were collected from the wing vein of each one. First one as a whole blood mixed with dipotassium salt of EDTA (0.5mg/ ml blood) as anticoagulant for hematological examination. The second blood sample was collected without anticoagulant and kept for coagulation, then left in the refrigerator at 4°C for 4 h. The clotted blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum, that collected in eppendorf tubes carefully and stored at -20°C until biochemical estimation. Additionally, after slaughtering, tissue specimens were collected from liver and kidney for histopathological examination.

Hematological parameters

Erythrocyte (RBCs) and total leukocytes (TLC) were manually counted using Natt and Herrick diluting fluid (Natt and Herrick, 1952) and improved Neubauer double hemocytometer. Furthermore, the blood hemoglobin (Hb) was determined by cyanomethemoglobin method after centrifugation (Dein, 1984). The packed cell volume (PCV) was calculated by special scale (Barbara, 1988). Blood indices (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)) values were calculated from measured PCV%, Hb concentration (g/dl) and RBCs count by standard formulae (Feldman et al., 2000) Also, two blood films were manually prepared, as soon as after collection of the blood sample, stained by

Table (1): Experimental diets

Ingredients (%)	**Control**	**N_2**	**N_4**
Corn grain (8.5%)	55	55	55
Soybean meal (44%)	35	35	35
Corn glutein	4	4	4
Soybean oil	2.2	2	1.8
Nigella sativa oil	0	0.2	0.4
limestone	1.07	1.07	1.07
Dicalcium phosphate	1.79	1.79	1.79
Vitamins and minerals premix*	0.25	0.25	0.25
Salt	0.3	0.3	0.3
DL-methionine	0.11	0.11	0.11
DL-Lysine	0.31	0.31	0.31
Chemical composition			
Cude protein %	22.97	22.97	22.97
Metabolizable energy (Kcal/kg)	3026	3026	3026
Calcium %	0.92	0.92	0.92
Available phosphorus %	0.45	0.45	0.45

Control (-ve control), **NS_2** (*Nigella sativa* oil 2g/kg) and **NS_4** (*Nigella sativa* oil 4g/kg).
*****Vitamins and minerals premixused to cover the required vitamins and minerals per each kilogram diet (Vit. A,10000 I.U.; Vit. D3, 1500 I.U.; Vit. E, 10 mg; Vit. K3, 2 mg; Vit. B1, 2 mg; Vit. B2, 5 mg; Vit. B6, 3 mg; Vit. B12, 0.01 mg; Niacin, 27 mg;Folic acid, 1 mg; Biotin, 0.05 mg; Pantothenic acid, 10 mg; Mn, 60 mg; Zn, 50 mg; Cu, 10 mg; I, 0.1 mg; Se, 0.1 mg; Co, 0.1 mg; Fe, 50).

Table (2): Some selective hematological parameters (Mean ± S.E) post dietary supplementation with *Nigella sativa* oil in broiler chicks.

Prameters	Treatment		
	Control	NS_2	NS_4
RBCs $10^6/\mu L$	2.72±0.10	2.65±0.06	2.72±0.11
Hb g/dl	7.12±0.04	7.21±0.17	7.24±0.10
PCV %	25.75±0.47	26.00±0.85	26.00±0.40
MCV fl	95.05±4.54	98.27±2.80	90.67±2.29
MCH pg	26.29±1.05	25.22±0.25	26.70±1.3
MCHC %	27.82±0.45	24.70±0.50	23.77±0.37
TLC $10^3/\mu L$	$22.75±1.10^b$	$24.50±0.64^a$	$24.50±0.50^a$
Heterophil $10^3/$ L	4.14±0.25	5.06±0.43	4.61±0.60
Lymphocyte $10^3/\mu L$	$13.74±0.61^b$	$13.44±0.38^b$	$15.33±0.6^a$
Monocyte $10^3/\mu L$	2.62±0.05	2.76±0.14	2.51±0.06
Eosinophil $10^3/\mu L$	0.74±0.12	0.72±0.07	0.73±0.09

Control (-ve control), **NS_2** (*Nigella sativa* oil 2g/kg) and **NS_4** (*Nigella sativa* oil 4g/kg), **RBCs** (Red blood cells), **Hb** (Hemoglobin), **PCV** (Packed cell volume), **MCV** (Mean corpuscular volume), **MCH** (Mean corpuscular hemoglobin), **MCHC** (Mean corpuscular hemoglobin concentration) and **TLC** (Total leukocyte count).
The different letters in the same row indicatesignificant difference between groups (P<0.05).

Gimsa for differential leukocytic count (Feldman et al., 2000).

Serum Biochemical Analysis

The serum alanine aminotransferase (ALT), aspartate aminotransferase and (AST) Alkaline phosphatase (ALP) were determined by colorimetric test by using ready-made kit according to Young (2001), Total cholesterol, triglyceride, HDL-Cholesterol (HDL-C) were determined by enzymatic colorimetric test by using ready-made kits. Furthermore, uric acid was estimated by a colorimetric enzymatic method (Uricase-POD) according to Young (2001), also serum creatinine was measured by photometric colorimetric test for kinetic measurement, method without deproteinization (Tietz et al., 1995). Moreover, total protein and albumin were determined by colorimetric method by using ready-made kit according to (Burtis et al., 1999) then serum globulin was calculated by

Table (3): Some selective serum biochemical parameters (Mean ± S.E) post dietary supplementation with *Nigella sativa* oil in broiler chicks.

Prameters	Treatment		
	Control	NS_2	NS_4
ALT U/L	25.17±0.70	24.45±0.39	25.37±0.34
AST U/L	65.60±0.67	65.07±0.48	65.22±0.30
ALP U/L	201.77±4.26[a]	198.12±0.96[b]	215.52±1.82[a]
Total protein g/dl	6.66±0.19	6.67±0.24	6.85±0.21
Albumin g/dl	4.00±0.14	3.92±0.04	3.82±0.14
Globulin g/dl	2.69±0.11	2.65±0.06	2.92±0.08
A/G Ratio	1.50±0.10	1.47±0.04	1.30±0.04
Creatinine mg/dl	0.65±0.01	0.64±0.01	0.64±0.00
Uric acid mg/dl	2.60±0.27	2.54±0.13	2.60±0.18

Control (-ve control), **NS_2** (*Nigella sativa* oil 2g/kg) and **NS_4** (*Nigella sativa* oil 4g/kg). **ALT** (Alanine aminotransferase), **AST** (Aspartate aminotransferase), **ALP** (Alkaline phosphatase) and **A/G** (Albumin to globulin ratio).
The different letters in the same row indicate significant difference between groups (P<0.05).

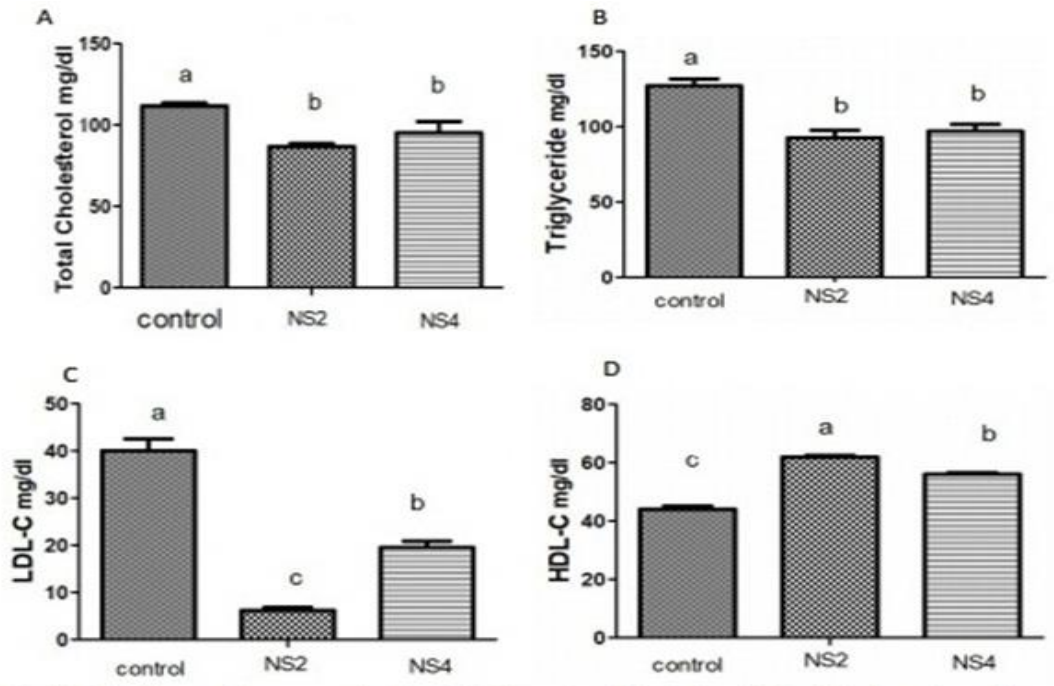

Fig.1 (A-D): **Control** (-ve control), **NS_2** (*Nigella sativa* oil 2g/kg) and **NS_4** (*Nigella sativa* oil 4g /kg). The serum levels of **(A) total cholesterol**, **(B)** triglyceride, **(C)** LDL-C (Low-density lipoprotein cholesterol) and **(D) HDL-C** (High-density lipoprotein cholesterol) at the 3rd week post dietary supplementation with *Nigella sativa* oil in broiler chicks.

subtracting albumin from total protein and A/G ratio determined by dividing albumin to globulin (Kaneko et al., 1997).

Histopathological study

Specimens were collected from the liver, kidney and intestine, fixed in neutral buffered formalin 10% and processed to be 5 micron in its thickness then stained with Hematoxyline and Eosin (Bancrrof et al., 1996).

Statistical analysis

Statistical software program (SPSS for Windows, version 20, USA) was used for analyzed the data. For each variable, means and standard error were done to be evaluated. One way ANOVA was used with Duncan multiple comparison tests to carried out the differences between means of different groups. Dissimilar superscript letters in the same row show a significance ($P<0.05$) and the graphed was prepared by Graph Pad Prism5.

RESULTS

Hematological results

As presented in table (1), our findings

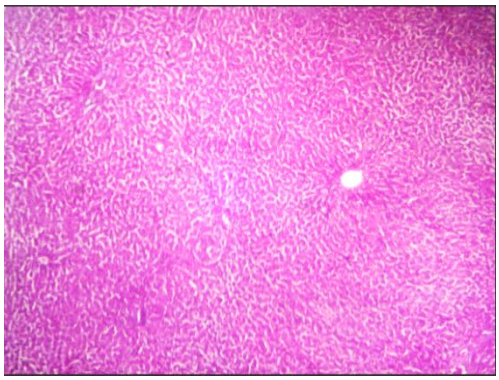

Fig. (2): Photomicrograph of the liver of the control group showing normal tissue architecture and cellular details (H & E x 200)

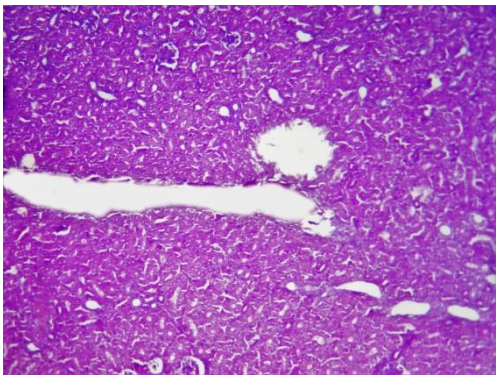

Fig. (3): Photomicrograph of kidney of the control group showing normal renal cortex with normal glomeruli and renal tubules (H & E x 200).

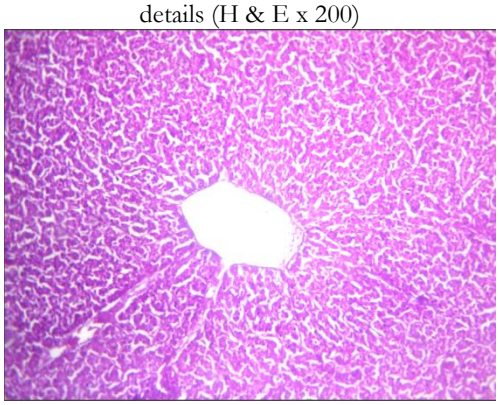

Fig. (4): Photomicrograph of chicken liver of NS_2 group showing normal hepatic cells and blood vessels with slight dilatation of the hepatic sinusoids (H & E x 400).

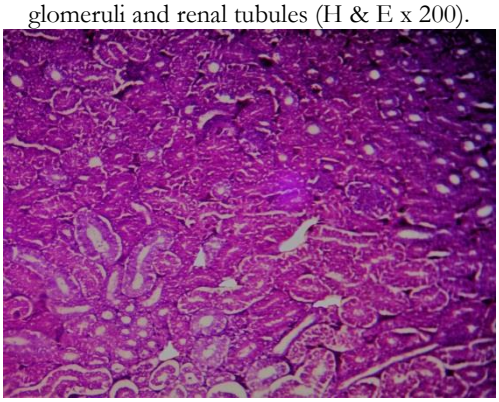

Fig. (5): Photomicrograph of kidney of chickens of NS_4 group showing the renal cortex with normal tubules and glomerulari (H & E x 200)

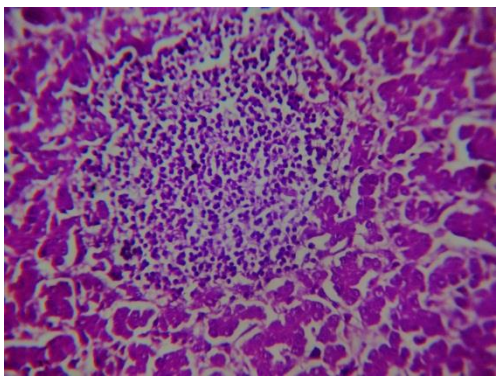

Fig. (6): Photomicrograph of the liver of NS_4 group showing focal lymphocytic cells infiltration in the hepatic parenchyma (H & E x 400).

revealed that dietary supplementation with *Nigella sativa* oil either with 2 or 4 g/kg diet had no effects on the erythrogram results, as the erythrocytes count, haemoglobin concentration, PCV% and blood indices (MCV, MCH and MCHC) had no significant change compared to the control group. Also, the leukogram findings showed a significant increase in the total leukocytic in both *Nigella sativa* oil supplemented groups, furthermore, the significant increase of the lymphocytes count was observed in NS_4 group, compared to control group. Meanwhile, the heterophiles, monocytes and eosinophils counts had no significant differences, compared to control group.

Liver function markers

The serum activities of liver enzymes (ALT and AST) were insignificantly affected in all groups. Otherwise, the serum activity of ALP was significantly decreased by dietary supplementation with low dose of *Nigella sativa* oil. Furthermore, serum levels of total protein, albumin, globulin and albumin/ globulin ratio were not changed, compared to control birds (table. 2).

Kidney function markers

The results of serum levels of uric acid and creatinine did not show any significant alteration in the *Nigella sativa* supplemented groups, compared to control group, as displayed in table (2).

Lipid Profile result

Fig. 1 represented the effects induced by *Nigella sativa* oil supplementation on lipid profile, as *Nigella sativa* significantly decreased the serum total cholesterol (TC), triglyceride and LDL-C, compared to control group. In contrast, the serum level of HDL-C significantly increased especially in the low dose of *Nigella sativa* supplemented group (NS_2) compared to control.

Histopathological finding

The histopathological finding of the liver and renal tissues revealed that *Nigella sativa* had no serious pathological effects on broiler except minimal changes such mild degenerative changes in both liver and kidneys, as shown in Fig. (2, 3, 4, and 5).

DISCUSSION

Nigella sativa has been used as alternative treatments; it has numerous applications in the refractory diseases treatment due to its satisfactory clinical efficacy and low toxicity. The erythrogram results showed no significant change induced by dietary supplementation with *Nigella sativa* oil, as oil has no toxic effect on the health of birds or did not affect the blood system negatively. Our results were agreed with Al-Homidan et al. (2002) who stated no significant differences in RBCs, Hb and PCV between the control and broiler chicks dietary supplemented with 20 or 100g *Nigella sativa* seed (NSS)/kg for 7 weeks. Also, the similar findings were recorded by Nasir (2009) who investigated the effect of different doses of grounded *Nigella sativa* seeds for 35 days in broiler chick. Furthermore, Toghyani et al. (2010) did not find any changes in the blood indices in broiler chicks fed on 2 and 4 g black seed /kg diet for 42 days. In contrast to our findings, Shewita and Taha (2011) observed a marked elevation in the RBCs count in chick dietary supplemented with 4, 6 and 10g black seed /kg for 42 days, this may be related to the difference in the dose, duration or bird strain. In addition, a significant increase in the RBCs, Hb, PCV, MCV, MCH and MCHC were recorded in broiler chicks vaccinated against Newcastle disease and supplemented with 1.25, 2.5 or 5.0% black cumin seed (BCS) for 42 days (Khan et al., 2012).

The results of leukogram cleared that, *Nigella sativa* induced a significant increase in the total leukocytic and lymphocytes counts which may be attributed to the immune-modulating effects of *Nigella sativa* and its active components as nigllone, thymoquinone and thymohydroquinone which activated T lymphocytes to secrete IL-3 and enhanced IL-B_1 production (Haq et al., 1995). Also, it stimulated the T cell and natural killer **cell-mediated immune responses** (Salem, 2005). Our results were

in harmony with, Al-Beitawi et al. (2009) who revealed that 0.2% of oil mixture (*Nigella sativa* seeds, *Pimpinella anisum,* and *Thymus vulgaris* leaves) (1:1:1) in broiler vaccinated against Newcastle disease, significantly increased the TLC, heterophilis, lymphocytes and monocytes counts, in vaccinated and non-vaccinated supplemented groups. Furthermore, Ali et al. (2014) recorded a significant increase of heterophil to lymphocyte ratio in broiler dietary supplemented with 0.25% or 0.5% BCS + 500 ppm vitamin C. Also, TLC was increased in broiler chicks were supplemented with *Nigella sativa seed* at dose of 1.25, 2.5 or 5.0%, (Khan et al., 2012).

The present study showed that the serum activities of the hepatic enzymes ALT and AST were not affected by supplementation with *Nigella sativa* oil, while, ALP level was decreased significantly, this may be attributed to the hepatobiliary protective effect of *Nigella sativa* oil. Our result confirmed histopathologically where the liver tissues did not show sever toxic effects as degenerative or necrotic changes by *Nigella sativa* supplementation. These hepatoprotective effect may be referred to the thymoquinone (the active component of *Nigella sativa* oil) and other oil compounds such as p-cymene, m-cymene, α- thujene and carvacrol which have scavenging activity of the free radicals and protect the liver enzymes leakage (Abdel-Wahhab and Aly, 2005). Our results were in harmony with El-Sayed et al. (2005) who reported a significant decrease in the ALP activity, while, the serum ALT and AST were insignificantly varied in the male chicks supplemented with 1, 3 or 10 % crushed *Nigella sativa* seed (NSS).

Our data recorded that the serum levels of total protein, albumin, globulin, albumin/globulin ratio were insignificantly affected. This in accordance with, Attia and Al-Harthi (2015) who recorded that dietary supplementation with NSO for 45 days by the doses 0.5, 1.0 or 1.5g /kg broiler chicks, did not induce any significant change in the serum albumin and globulin levels. The same result was recorded by Nasir (2009) in the broilers dietary supplemented with 0.5, 1.0, 1.5, 2.0 and 2.5% grounded *Nigella* sativa seed. Furthermore, Miraghaee et al. (2011) reported that dietary supplementation of one day-old broiler chickens with 1% *Nigella sativa* for 6weeks had not any significant effect on the serum total protein and globulin.

Supplementation with *Nigella sativa* oil has no effect on the serum creatinine and uric acid, as the *Nigella sativa* oil had a protective and curative effects on the renal tissues, (Windisch, 2009). Since, *Nigella sativa* scavenged the free radicals and protect the renal tissue from the degenerative changes, (Saleem et al., 2012; Rehman et al., 2012). These results were supported by our histopathological findings, as the kidney of *Nigella sativa* supplemented group showing normal renal cortex with normal glomerulus, renal tubules and normal architecture of kidney similar to the control group. Our results are in accordance with Rehman et al. (2012) who recorded nephrocurative and nephro-protective effects of NSO or/and Vit-C (2ml or 200mg /kg/day) dietary supplementation respectively in rabbits for 26 days. Moreover, they induced a significant reduction in the serum levels of creatinine and urea, as well as augmented the antioxidant defense mechanism of the body against gentamicin induced morphological damages in kidneys.

The results of lipid profile recorded that the level of serum cholesterol, triglyceride and LDL-C were markedly reduced, meanwhile, the serum level of HDL-C was markedly elevated, by all doses of *Nigella sativa*. The hypolipidemic effects of *N.sativa* may referred to different constituents presented in *N.sativa*, including TQ, nigellamine, sterols, flavonoids and high content of poly unsaturated fatty acids (PUFAs) (Ali and Blunden, 2003). There are multiple mechanisms which may contribute to the hypolypidimic effect of *N.sativa*, as it was able to regulate cholesterol synthesis

through regulation of HMG-CoA reductase. Additionally, TQ and other *N. sativa* constituents mediated Apo-A1, Apo-B100 and LDL-receptor genes through enhancing the LDL receptor gene expression and the sterols are probably declined the absorption of dietary cholesterol, enhanced the primary bile acid synthesis as well as stimulated and increased the biliary excretion of cholesterol and increased the fecal losses of dietary cholesterol **(**Moruisi et al., 2006; Gargari et al., 2009).

Additionally, high content of PUFAs in *N. sativa* may also lead to decreasing the serum total cholesterol level (Djousse et al., 2003). Also, flavonides have a lipid lowering effect (Talati et al., 2009) through enhance the efficiency of liver cells to remove LDL from the blood circulation and decrease the intracellular cholesterol by increasing LDL receptor densities in the liver and binding to apolipoprotein B, resulted in the rapid clearance of LDL particles from circulation (Beshbishy et al., 2006). Furthermore, falvonids have antioxidant effect as it decreases the cholesterol synthesis and suppress reactive oxygen and nitrogen species formation and protect the antioxidant defense system (Arts and Hollman, 2005). Our findings were in concept with the findings of Boka et al. (2014) who reported that the serum concentration of cholesterol and triglycerides were markedly reduced. Otherwise serum HDL-C was increased after 10 weeks from the dietary inclusion of 1%, 2% and 3% black cumin seed in laying hens. Moreover, Al-Hothaify and Al-Sanabani (2016) observed that the serum total cholesterol was significantly reduced as a result of NSS dietary supplementation for 5 weeks. In contrast to our finding, Yalcin et al. (2009) and Shirzadegan et al. (2015) noted that the dietary inclusion of 10 and 15 g BCS/kg for 12 weeks to laying hen and broiler chicks respectively, had no significant effects on the serum concentrations of triglycerides and cholesterol.

CONCLUSION

Dietary supplementation of *Nigella sativa* oil in broiler chicks had no toxic effect and had immunomodulating, hepato-renal protective and hypolipidimic effects. Further studies will be recommended for evaluation more parameters with long duration for *Nigella sativa* oil supplementation.

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

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