

Biochemical and Histopathological Effects of Dietary Supplementation of *Nigella sativa* and *Mentha piperita* Oils to Broilers

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ABSTRACT

This study was carried out to evaluate the biochemical and histopathological effects of dietary supplementation of *Nigella sativa* and *Mentha piperita* oils to broilers for 6 weeks. A total 80 unsexed one day old Cobb broiler chicks was obtained from Cairo Company with average body weight 55g were used. The chicks were taken and randomly allocated into 4 equal groups (20 each) named control, *Nigella sativa* oil treated group, *Mentha piperita* oil treated group and *Nigella sativa* + *Mentha piperita* oils treated group. Serum samples and tissue samples (liver and kidney) were obtained at 21 and 42 days old for some serum biochemical analysis (ALT, AST and ALP activities; creatinine, Urea, Total protein and Albumin levels) and histopathological examination (Liver and Kidney processed slide sections). The obtained results indicated that dietary supplementation of broilers diet with *Nigella sativa*, *Mentha piperita* and their combination for long period (6 weeks) couldn't alter liver and kidney functions as cleared from biochemical findings but could caused slight hepatic and renal histopathological changes as showed from histopathological examination.

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Introduction

For several decades, the use of sub-therapeutic levels of antibiotics in animal feeds has been a common practice in many countries in order to improve growth performance and prevent from the adverse effects of pathogenic and non-pathogenic enteric microorganisms. However, there are increasing concerns for the public health about the consequences from the use of antibiotics in livestock (Phillips, 1999). The risk of developing cross-resistance and multiple-antibiotic resistance in pathogenic bacteria both in human and farm animals, has been strongly linked to the therapeutic, metaphylactic or prophylactic uses of antibiotics in human and veterinary medicine, as well as growth promoters in animal feed (Gibson and Roberfroid, 1995).

The use of antibiotics as growth promoters has been complete banned by the European Union (EU) since 2006, based on their possible negative consequences for animal health and food safety (EFSA: European Food Safety Authority, 2009 and Fernando *et al.*, 2007). This ban has led to animal performance problems and a rise in the incidence of certain diseases

(Wierup, 2001; Dibner and Richards, 2005). Thus, there is an urgent need to develop alternatives to antibiotics. As a consequence of the public health concerns and the demand of the farmers to prevent the economic losses, non-antibiotic additives have been developed for prophylactic use against pathogens or as growth promoters.

Phytogenics or Phytobiotics or Botanicals which are feed additives derived from plants, have been developed alternative to antimicrobial feed additives for prophylactic use or as growth promoters for animals. Among these natural additives, aromatic plants, their extracts and their essential oils have been examined due to their advantages over the antibiotics as growth promoters. They are residue free and generally recognized as safe diets to improve their productivity and the properties of the resulting feed and animal products (Windisch *et al.*, 2009).

Essential oils derived from herbs and spices considered one of most alternative to antimicrobial feed additives. Today, this practice is receiving much attention particularly in broiler chickens (Alçiçek *et al.*, 2003, 2004; García *et al.*, 2007) and laying hens (Çabuk *et al.*, 2006). Herbal essential oils assist in colonization of the beneficial microbial population within the gastrointestinal tract to more balanced levels (Zaika, 1988; Jang *et al.*, 2007). Besides their antimicrobial properties (Ultee

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et al., 2002), they also exhibit antioxidant (Basmacioğlu et al., 2004), antifungal (Bang et al., 2000; Shin and Lim, 2004), digestion-stimulating, and enzymatic (Jamroz et al., 2003, 2005; Hernandez et al., 2004) activities. The benefits of essential oils from herbs and species in poultry diets have been recently demonstrated, not only in terms of improving performance traits but also in inhibiting pathogenic bacteria and reducing residue hazard of meat and egg products (Bassett, 2000; Gill, 2001; Hertrampf, 2001). These nutrient-sparing and health-promoting effects are most likely attributable to the effects of essential oils within the gastrointestinal track on improving the balance of gut microflora and improving nutrient digestion and absorption (Mitsch et al., 2004; Jamroz et al., 2005). However, experimental studies indicated that essential oils, either individually or in specific blends, were able to produce benefits comparable to traditional growth promoters including antibiotic, organic acid, prebiotic, and probiotic in maintaining general health status and performance of broilers (Alçiçek et al., 2003; Bozkurt et al., 2005; Zhang et al., 2005) and laying hens (Çabuk et al., 2006).

One of the world's oldest medicinal herbs, and is used in Eastern and Western traditions, Peppermint (*Mentha piperita*) or mint which is a member of the Labiatae family. It is widely used in herbal medicine and believed to be particularly beneficial in building of the immune system and fighting secondary infections (Nanekarani et al., 2012). *Mentha* has been used as a carminative, antispasmodic, diuretic, and used as flavorings in breath fresheners, drinks, antiseptic mouth rinses, toothpaste, chewing gum, desserts and candies. The main medicinal action of the leaves and flowers of the mint depend on the abundant menthol which is the main phenolic component which has antibacterial activities (Schuhmacher et al., 2003). Also, peppermint could possess strong antioxidant properties as it contains polyphenolic compounds (Dorman et al., 2003). Peppermint has been investigated by several researchers as poultry feed additive (Al-Kassie, 2010; Sharifi et al., 2013) and proposed as a potential alternative to antibiotic growth promoters (AGPs). Peppermint essential oil has biological activities, such as antibacterial, antifungal and antioxidant properties. It stimulated the immune system in broiler chicks. (Akbari et al., 2005) and has beneficial influence on broilers productive performance (Al-Ankari et al., 2004).

Another one of the historically famous medicinal herb, Black seed (*Nigella sativa*) is an aromatic plant of Ranunculaceae family growing in countries, bordering Mediterranean region, South and Central Asia and is now also cultivated in Asia and Europe. Black seeds contain alkaloids, fixed and volatile oils, pharmacologically active substances (thymoquinone, dithymoquinone, thymol, carvacrol, nigellidine-N-oxide, nigellidine and alpha-hedrin), and antioxidants (selenium, DL- α -tocopherol, DL- β -tocopherol, and transretinol) (Nasir et al., 2005; Al-Saleh et al., 2006). *Nigella sativa* (black cumin) seed could be the most suitable alternative to antibiotics in poultry nutrition as it not only promote bird's health and production performance, but also plays a significant role as a natural antioxidant and immuno-stimulant (Azeem et al., 2014). Better dressing percentage along with improved feed intake and weight gain was observed by dietary supplementation of 4 % black seeds to broilers (Durrani et al., 2007). Black seeds or black cumin and its oil extract positively affected feed intake and body weight in the broilers (Halle et al., 1999; Guler et al., 2006; Ziad and Mohammad, 2008; Erener et al., 2010).

However, although there have been a number of studies associated with the effect of dietary supplementation with peppermint (*Mentha piperita*) and black seed or black cumin (*Nigella sativa*) as alternatives to AGPs in broiler diets on broiler chicken performance, immune-responsiveness and lymphoid organs but, the results of many of them were con-

troversial, some of which proved effective role in increasing performances (EL-ghammry et al., 2002; AL-Beitawi et al., 2008 ; Ziad and Mohammad, 2008) and improving the immune system efficiency (Akhtar et al., 2003; Nasir and Grashorn, 2010), on contrary others proved ineffective (Abbas and Ahmed, 2010; Nasir and Grashorn, 2010 and Toghyani et al., 2010) and therefore there is a pressing need for many studies in this area and that was the purpose of performing this study.

The objective of this study was to evaluate the effects of dietary supplementation *Nigella sativa* and *Mentha piperita* oils on broiler chicken biochemical and histopathological investigation of some visceral organs (liver and kidney).

Materials and methods

Black seed oil (*Nigella sativa* oil)

It was obtained from El-Captain Company (CAPPHARM) For Extracting Natural Herbs and Cosmetics, Egypt.

Peppermint oil (*Mentha piperita* oil)

It was obtained from El-Gomhorya Company, Egypt (It is manufactured by: M/S Bhagat Aromatics Limited).

Experimental chicks, housing and management

A total 80 unsexed one day old Cobb broiler chicks was obtained from Cairo Company with average body weight 55g. The chicks were taken and randomly housed in floor pens with wood shaving and reared up to 6 weeks at the rent room beside to Animal Health Institute, Asyut, Egypt.

All chamber partitions, feeders, drinkers and heaters were cleaned and disinfected before the study. Environmental temperature was adjusted according to the age. It was set at 32°C for the first week of age and then decreased by 2°C per week till reach 22°C at the 6th week of age.

Ration

The chicks were fed on formulated broiler starter basal rations from one day old to three weeks of age and then formulated grower finisher ration was used until the end of the experiment at the six weeks of age. The diet was formulated to meet the nutritional requirements as recommended by the (NRC, 1994).

Vaccination

The birds were routinely vaccinated against Newcastle Disease (ND), Gumboro (IBD) and Avian Influenza (AI).

Experimental design

At one day-old, all chicks were randomly allocated into 4 equal groups (each of 20 chicks) as following:

Group 1 (Control group)

The birds in this group were fed on the basal diet only without any additives all over the entire experimental period.

Group 2 (*Nigella sativa* oil treated group)

The birds in this group were fed on the basal diet provided with black seed oil at dosage rate of 2ml/Kg basal diet (Awad et al., 2013).

Group 3 (*Mentha piperita* oil treated group)

The birds in this group were fed on the basal diet provided with peppermint oil at dosage rate of 300mg/Kg basal diet (Nanekarani *et al.*, 2012 for dose) and (Emami *et al.*, 2012 for route of administration).

Group 4 (*Nigella sativa* and *Mentha piperita* oils treated group)

The birds in this group were fed on the basal diet provided with a combination of both black seed oil at dosage rate of 1ml/kg basal diet (Hermes *et al.*, 2010) and peppermint oil at dosage rate of 200mg/kg basal diet (Emami *et al.*, 2012).

Blood sampling

Samples were collected at 21 and 42 day from all dietary groups, five birds were selected from each group. About 10ml of blood was collected from each bird and allowed to flow freely and gently into clean dry sterile two sets of sterilized labeled sample tubes. The sera were collected without anti-coagulants through, leaving to clot at room temperature, then centrifuged for about 10 minutes at 3000 r.p.m. The sera were collected carefully by micropipette to clean dry and sterile Ependorf tubes and stored at -200C to be used in the evaluation of different biochemical parameters.

Serum biochemical analysis

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were estimated according to Reitman and Frankel (1975). Serum alkaline phosphatase (ALP) activity was determined according to Marsh *et al.* (1959). The total protein level was evaluated according to Schultze and Heremans (1966), albumin was determined according to Doumas *et al.* (1971). Serum creatinine level was measured according to Bowers and Wong (1980) and serum urea level was estimated according to Batton and Crouch (1977).

Histopathology Examination

At 21 and 42 day, 5 birds from each group were slaughtered; tissue samples (liver and kidney) were collected from each bird and washed by neutral saline then, preserved in formalin saline 10%. Tissue specimens from liver and kidney were fixed in 10% neutral buffered formalin, then dehydrated in a graded alcohol series, then, cleared with methyl benzoate and

embedded in paraffin wax. Thin sections of 5 microns thickness were cut. Harris hematoxylin and eosin stain was carried out routinely for general histopathological examination (Bancroft *et al.* 1996).

Statistical analysis

The obtained data were statistically analyzed by variance method (ANOVA) considering $P < 0.05$ using the General Linear Model (GLM) procedure of SAS® software (SAS Institute Inc., 1998) and significant between groups were differentiated by Multiple Range Test (Duncan, 1955) compare to the means.

Results

Effects of *Nigella sativa* and *Mentha piperita* oils on liver and kidney functions

The current study showed that total protein, urea, creatinine, ALT and AST levels provoked no significant changes between all treated groups in comparison with control (Table 1). Moreover, at day 21 and day 42, total protein showed that no significant change among all groups as compared with each other and with control (Table 2). Additionally, albumin level in *Nigella sativa* oil treated group was decrease significantly ($P < 0.05$) in comparison with control group, but there was no significant change in *Mentha piperita* oil and *Nigella sativa* oil + *Mentha piperita* oil treated groups as compared to control (Table 1). At day 21 and day 42, albumin level in *Nigella sativa* oil treated group, represented a significant decrease ($P < 0.05$) in comparison with control but there was no significant change in comparison with *Mentha piperita* oil and *Nigella sativa* oil + *Mentha piperita* oil treated groups (Table 2). Both *Mentha piperita* oil and *Nigella sativa* oil + *Mentha piperita* oil treated groups provoked no significant change as compared with control (Table 2). Urea and creatinine at day 21 and day 42 cleared no significant change among all groups (Table 2). ALT level at day 21 in *Mentha piperita* oil and *Nigella sativa* oil + *Mentha piperita* oil treated groups showed a significant increase ($P < 0.05$) in comparison with control. There was no significant change in ALT level at day 42 among all groups (Table 2). AST at day 21 and day 42 reflected no significant changes among all groups (Table 2). ALP level in *Mentha piperita* oil treated group significantly increased ($P < 0.05$) in comparison with *Nigella sativa* oil treated group and control (Table 1). ALP at day 21 in *Mentha piperita* oil treated group showed significant increased ($P < 0.05$) in comparison with other treated and control. However, at day 42, there was insignificant change

Table 1. Effect of *Nigella sativa* oil, *Mentha piperita* oil and their combination on some biochemical parameters of broiler chickens

Bl. para.	Control	<i>Nigella sativa</i>	<i>Mentha piperita</i>	<i>N. s. + M. p.</i>	Significant
Total protein (g/dl)	3.73 ± 0.24 ^A	3.41 ± 0.24 ^A	3.75 ± 0.19 ^A	3.89 ± 0.18 ^A	N.S.
Albumin (g/dl)	2.01 ± 0.12 ^A	1.55 ± 0.06 ^B	1.79 ± 0.07 ^{AB}	1.78 ± 0.08 ^{AB}	*
Urea (mg/dl)	14.74 ± 1.77 ^A	11.05 ± 0.41 ^B	14.20 ± 1.20 ^{AB}	11.30 ± 0.54 ^{AB}	N.S.
Creatinine (mg/dl)	0.52 ± 0.06 ^A	0.39 ± 0.02 ^B	0.47 ± 0.04 ^{AB}	0.44 ± 0.03 ^{AB}	N.S.
ALT (U/l)	5.10 ± 0.48 ^A	4.90 ± 0.53 ^A	5.90 ± 0.64 ^A	6.10 ± 0.59 ^A	N.S.
AST (U/l)	7.50 ± 0.31 ^A	7.40 ± 0.31 ^A	8.20 ± 0.49 ^A	8.00 ± 0.45 ^A	N.S.
ALP (U/l)	4210 ± 1258.3 ^B	4145.4 ± 1262.7 ^B	4640 ± 1401.18 ^A	4371.7 ± 1319.64 ^{AB}	*

N.s.: *Nigella sativa* M.p: *Mentha piperita*

Each group consist 20 chicken, samples taken at day 21 and day 42 (5 sample / group), In each row, value followed with different letter superscript is significant ($P < 0.05$)

Table 2. Effect of *Nigella sativa* oil, *Mentha piperita* oil and their combination on some biochemical parameters of broiler chickens at day 21 and day 42

Bl. Para.	Group			42 nd day sampling			Sign.
	Cont.	NS	MP	NS+MP	MP.	NS+MP	
	21 st day sampling						
Total Protein (g/dl)	3.19 ± 0.21 ^B	3.29 ± 0.35 ^B	4.03 ± 0.09 ^{AB}	3.92 ± 0.33 ^{AB}	4.26 ± 0.28 ^A	3.54 ± 0.34 ^{AB}	3.86 ± 0.18 ^{AB} *
Albumin (g/dl)	2.02 ± 0.13 ^A	1.55 ± 0.08 ^B	1.79 ± 0.05 ^{AB}	1.76 ± 0.02 ^{AB}	1.99 ± 0.22 ^A	1.54 ± 0.10 ^B	1.81 ± 0.16 ^{AB} N.S.
Urea (mg/dl)	13.88 ± 3.23 ^A	10.60 ± 0.60 ^A	13.80 ± 1.62 ^A	11.60 ± 0.98 ^A	15.60 ± 1.81 ^A	11.50 ± 0.55 ^A	11.00 ± 0.55 ^A N.S.
Creatinine (mg/dl)	0.54 ± 0.07 ^A	0.40 ± 0.03 ^A	0.48 ± 0.06 ^A	0.41 ± 0.04 ^A	0.50 ± 0.09 ^A	0.38 ± 0.04 ^A	0.46 ± 0.05 ^A N.S.
ALT (U/l)	4.80 ± 0.80 ^B	5.60 ± 0.98 ^{AB}	7.20 ± 0.80 ^A	7.20 ± 0.80 ^A	5.40 ± 0.60 ^{AB}	4.20 ± 0.20 ^B	5.00 ± 0.55 ^{AB} N.S.
AST (U/l)	7.00 ± 0.00 ^A	7.00 ± 0.00 ^A	8.80 ± 0.73 ^A	8.20 ± 0.73 ^A	8.00 ± 0.55 ^A	7.80 ± 0.58 ^A	7.80 ± 0.58 ^A N.S.
ALP (U/l)	7984.0 ± 51.15 ^B	7931.0 ± 83.28 ^B	8820.0 ± 312.09 ^A	8320.0 ± 198.49 ^B	436.0 ± 27.94 ^C	359.8 ± 49.90 ^C	423.40 ± 50.64 ^C *

Cont. = Control group; NS = *Nigella sativa* group; MP = *Mentha piperita* group; NS+MP = *Nigella sativa* + *Mentha piperita* group
 Each group consist 20 chicken, samples taken at day 21 and day 42 (5 sample / group)
 In each row, value followed with different letter superscript is significant (P<0.05)

ALP level among all groups (Table 2).

The effect of Nigella sativa and Mentha piperita oils on liver tissue

Chicken treated with *Nigella sativa* oil at day 21 showed mild vacuolar degeneration of hepatocytes (arrow) and congestion of central vein (Fig. 1). While chicken treated with *Nigella sativa* oil at day 42 showed congestion in all hepatic

blood vessels of (star) (Fig.4). Chicken treated with *Mentha piperita* oil at day 21 showed vacuolar degeneration of hepatocytes (arrow) (Fig. 2). While chicken treated with *Mentha piperita* oil at day 42 showed sever vacuolar degeneration of hepatocytes (arrow) (Fig. 5). Chicken treated with *Nigella sativa* +*Mentha piperita* oil at day 21 showed lymphocytic reaction between hepatic cords (Fig. 3). While chicken treated with *Nigella sativa* +*Mentha piperita* oil at day 42 showed sever lymphocytic reaction in portal area (Fig. 6)

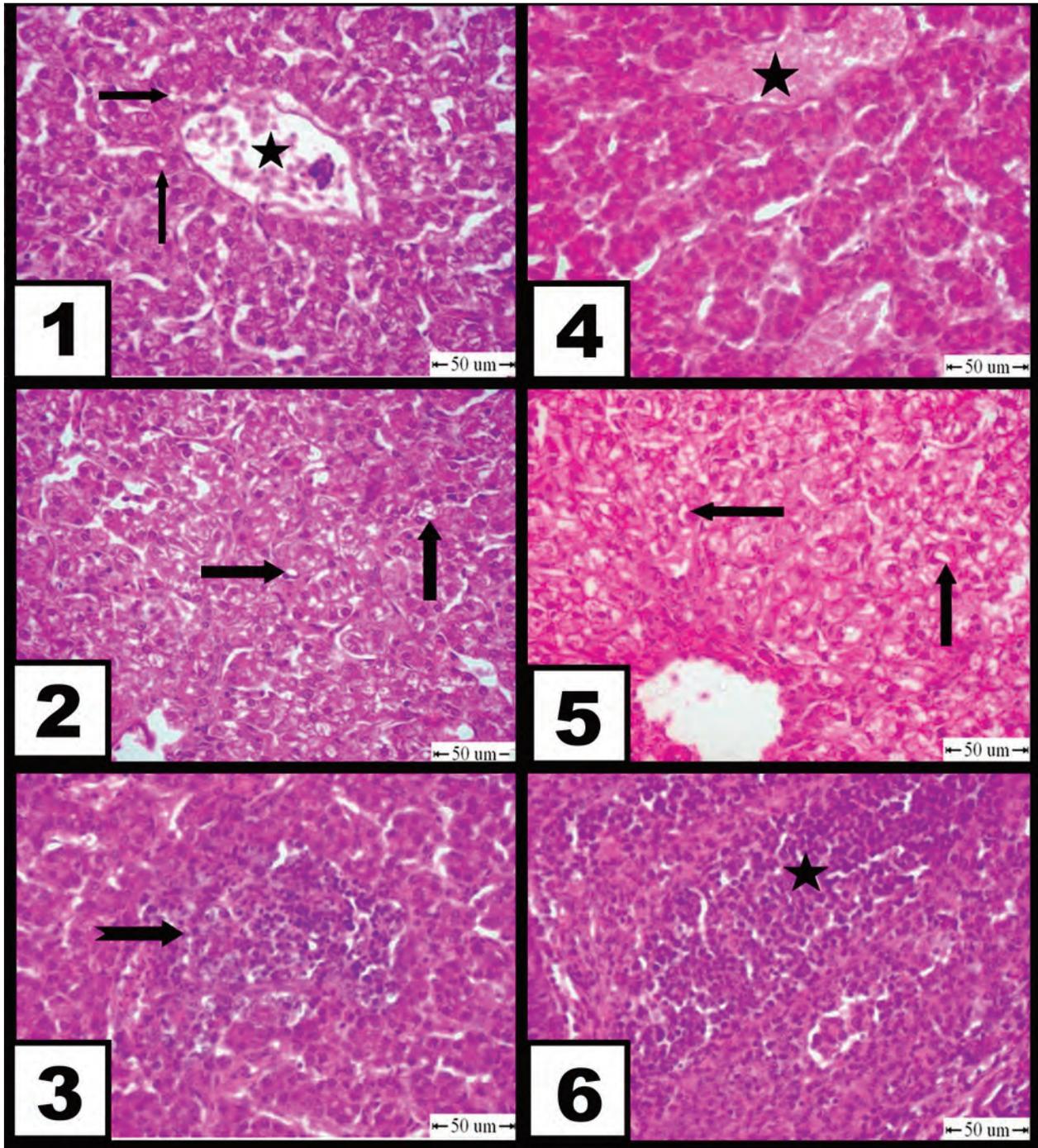


Fig.1. Liver, chicken treated with *Nigella sativa* oil, and examined after twenty one days. Bar=50. Fig. 2. Liver, chicken treated with *Mentha piperita* oil, and examined after twenty one days. Bar=50. Fig. 3. Liver, chicken treated with *Nigella sativa* + *Mentha piperita* oils, and examined after twenty one days. Bar=50. Fig. 4. Liver, chicken treated with *Nigella sativa* oil, and examined after forty two days. Bar=50. Fig. 5. Liver, chicken treated with *Mentha piperita* oil, and examined after forty two days. Bar=50. Fig. 6. Liver, chicken treated with *Nigella sativa* +*Mentha piperita* oils, and examined after forty two days. Bar=50.

The effect of *Nigella sativa* and *Mentha piperita* oils on kidney tissue

Chicken treated with *Nigella sativa* oil at day 21 showed sever lymphocytic reaction in between renal tubules (Fig. 7). While chicken treated with *Nigella sativa* oil at day 42 showed large area of inflammatory reaction filled with mononuclear cells and neutrophils (Fig.10).Chicken treated with *Mentha piperita* oil at day 21 revealed vacuolar degeneration of epithelium of renal tubules (arrow), other vascular changes ap-

peared as red thrombus consist of RBCs (notched arrow) and fibrin network (star) (Fig. 8).Chicken treated with *Mentha piperita* oil at day 42 revealed small area of inflammatory reaction in (star) (Fig.11). Chicken treated with *Nigella sativa* +*Mentha piperita* oils at day 21 showed higher magnification of the inflammatory reaction filled with mononuclear cellular reaction (Fig.9).While chicken treated with *Nigella sativa* +*Mentha piperita* oil at day 42 heavily populated areas filled with lymphoblasts, lymphocytes and macrophages (star) (Fig. 12).

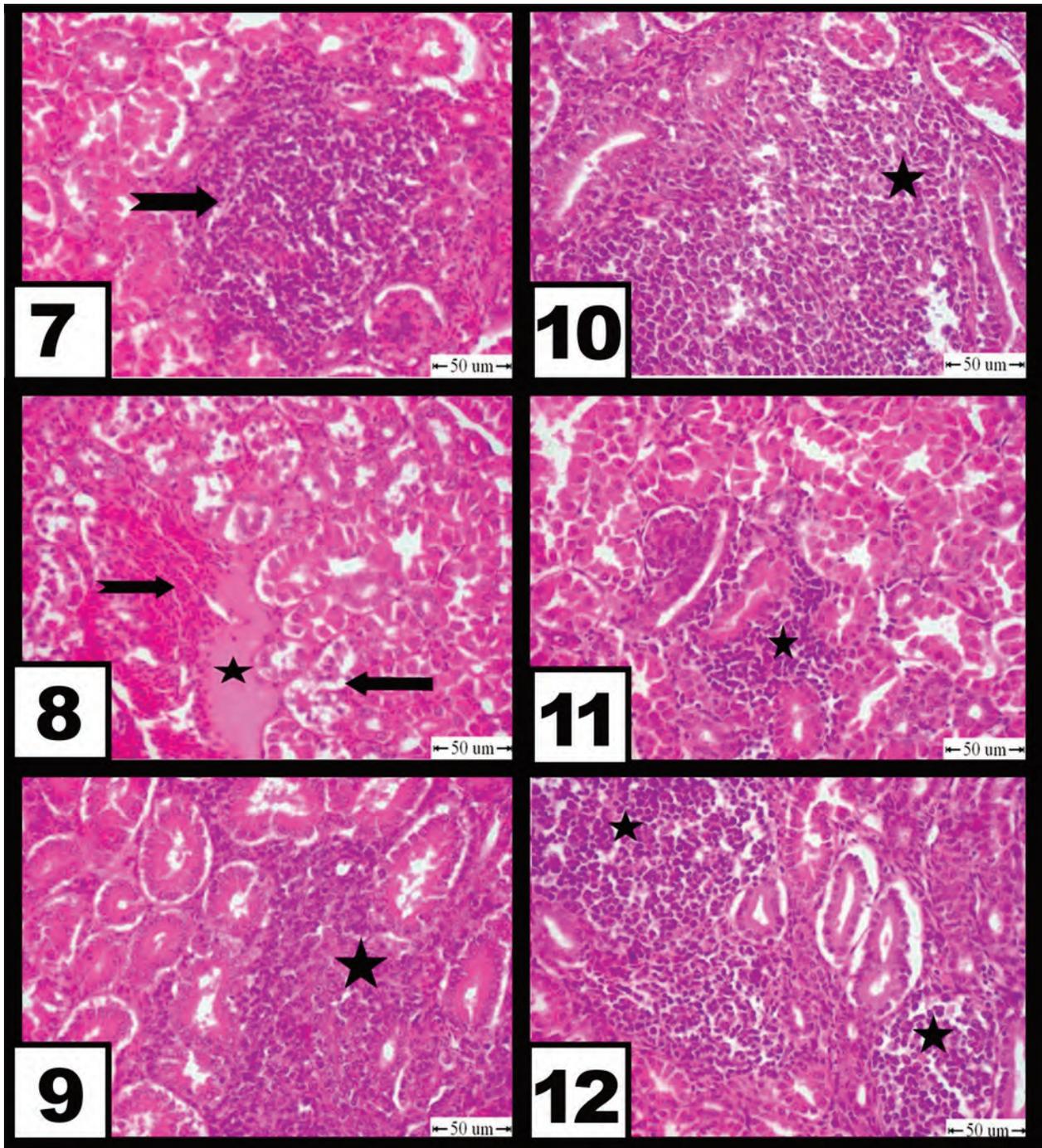


Fig. 7. Kidney, chicken treated with *Nigella sativa* oil, and examined after twenty one days. Bar=50. Fig. 8. Kidney, chicken treated with *Mentha piperita* oil, and examined after twenty one days. Bar=50. Fig. 9. Kidney, chicken treated with *Nigella sativa* + *Mentha piperita* oils, and examined after twenty one days. Bar=50. Fig. 10. Kidney, chicken treated with *Nigella sativa* oil, and examined after forty two days. Bar=50. Fig. 11. Kidney, chicken treated with *Mentha piperita* oil, and examined after forty two days. Bar=50. Fig. 12. Kidney, chicken treated with *Nigella sativa* +*Mentha piperita* oils, and examined after forty two days. Bar=50.

Discussion

In current study, the obtained data reflected that total protein; ALT and AST levels elicited no significant change among all treated groups as compared to control group (Tables, 1, 2) indicating normal liver function and hepatoprotective effect of both black seeds and peppermint oils. The phytochemicals hepatoprotective effect was mainly attributed to the presence of essential oil mixtures in its components that possessed biological activities such as antioxidant effect (Miura *et al.*, 2002). Moreover, the essential oils able to repair hepatic cell injury or to reduce the toxic effect in hepatic toxicity and prevent enzyme leakage into blood circulation (Hernandez *et al.*, 2004). The hepatoprotective effects of *Nigella sativa* oil may be owing to the radical scavenging activity of thymoquinone and other compounds in the oil such as p-cymene, m-cymene, α -thujene and carvacrol (Abdel-Wahhab and Aly, 2005). These results were confirmed by Hermes *et al.* (2010) who recorded that feeding broiler chicken on *Nigella sativa* treated diet had a non-toxic effect on liver and did not alter the liver enzymes activity. Toghyani *et al.*, (2010) who reported that none of the serum biochemical parameters tested was significantly influenced by the dietary supplementation as 2 and 4 g/kg black seed, 4 and 8 g/kg peppermint added to the basal diet. Recently, Abdelaziz *et al.* (2015) stated that *Mentha piperita* oil supplementation provoked non-significant change in AST and ALT. Creatinine and urea levels elicited no significant change among all treated groups as compared to control group indicating normal renal function and renal protective effect. Our findings were consistent with Toghyani *et al.* (2010) who reported that AST and ALT enzymes concentrations were not statistically influenced by the dietary supplementation as 2 and 4 g/kg black seed, 4 and 8 g/kg peppermint added to the basal diet. Also, Shewita and Taha (2011) who reported that non-significant differences for AST level were observed among groups received different levels from *Nigella sativa*. Moreover, these results were further confirmed by Eleiwa *et al.* (2011); Khosravinia *et al.* (2013) and Fasanmi *et al.* (2014). On the other side, these obtained findings seemed incompatible with Al-Homidan *et al.* (2002) who found that feeding 20 and 100 g/kg *Nigella sativa* seed diets were correlated with alterations in serum aspartate transaminase (AST) and alanine transaminase (ALT) activities, Hermes *et al.* (2009) who stated that *Nigella sativa* oil at 0.5 and 1% decreasing alanine aminotransferase (ALT) and Hermes *et al.* (2010) who reported that plasma AST and ALT were reduced significantly ($P < 0.05$) by feeding 0.5% *Nigella sativa* oil. Recently, Khan *et al.* (2012) who recorded that feeding 2.5 and 5.0% black cumin seed could reduce the activities of blood enzymes activity. More recently, Saleh (2014) who reported that feeding of *Nigella* seed oil at 1ml/kg could lower the activity of plasma AST and ALT enzymes, but not significantly. Alkaline phosphatase (ALP) activity in *Mentha piperita* oil treated group was significant increased ($P < 0.05$) as compared to *Nigella sativa* oil treated and control groups. In contrast, there was no significant change appeared in comparison with *Nigella sativa* oil + *Mentha* oil treated groups these results were supported by Vo *et al.* (2003) who found that chronic treatment of rat liver and cultured human liver cells with *Mentha piperita* oil resulted in a significant increase in ALP activity.

The histopathological examination revealed that at day 21, the liver sections obtained from chicken treated with *Nigella sativa* oil showed mild vacuolar degeneration of hepatocytes and congestion of central vein. Kidney sections showed severe lymphocytic reaction in between renal tubules. These findings were in agreement with Hänsel and Sticher (2007) and Chauhan *et al.* (2014). At day 42, the liver sections showed congestion in all hepatic blood vessels. Kidney sections

showed large area of inflammatory reaction filled with mononuclear cells and neutrophils were noticed that may be due to the toxic effects of black cumin seeds as confirmed by Zaoui *et al.* (2002) and Ali and Blunden (2003).

At day 21, the liver sections from chickens treated with *Mentha piperita* oil showed vacuolar degeneration of hepatocytes. The kidney sections revealed vacuolar degeneration of epithelium of renal tubules, other vascular changes appeared as red thrombus consist of RBCs and fibrin network. At day 42 liver sections showed severe vacuolar degeneration of hepatocytes. The kidney sections revealed small area of inflammatory reaction. These findings were correlated with Thorup *et al.* (1983) and Vo *et al.* (2003).

At day 21 the liver sections obtained from chicken treated with *Nigella sativa* + *Mentha piperita* oils showed lymphocytic reaction between hepatic cords and higher magnification of the inflammatory reaction filled with mononuclear cellular reaction. At day 42, the liver sections showed severe lymphocytic reaction in portal area and heavily populated areas filled with lymphoblasts, lymphocytes and macrophages. These results were in agreement with Bassolé and Juliani, (2012).

Conclusion

Dietary supplementation of broilers diet with *Nigella sativa*, *Mentha piperita* and their combination for long period (6 weeks) couldn't alter liver and kidney functions as cleared from biochemical findings but could be responsible for slight hepatic and renal histopathological changes.

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