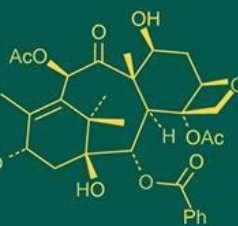
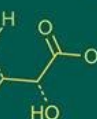
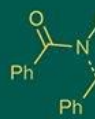
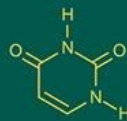
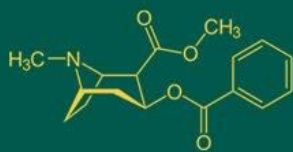


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Evaluation of immunomodulatory activities of cinnamon oil in broilers

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Abstract

The goal of this study was to assess cinnamon oil's immunomodulatory effects in broiler chickens. A total of 60 chicks were split up into five groups at random. As a control group, Group I was fed a baseline diet devoid of vitamin E, selenium, and cinnamon oil. Group II was given a proprietary product comprising vitamin E and selenium in water as a standard control. For 35 days, cinnamon oil was administered to Groups III, IV, and V at doses of 200, 400, and 800 mg/kg feed, respectively. On the fourteenth day of life, the cell-mediated immunity was evaluated using the cutaneous basophil hypersensitivity (CBH) response. In order to estimate antibody titers against the Newcastle Disease Virus vaccine using the hemagglutination inhibition (HI) test and biochemical parameters like serum total protein, serum albumin, serum globulin, and albumin to globulin ratio (A/G), blood was drawn on days 7, 21, and 35. Thin blood smears were made on the 35th day in order to microscopically assess differential leucocyte counts. At the experiment's conclusion, for histopathology analyses, birds were slaughtered. Cinnamon oil supplements (400 and 800 mg/kg diet) significantly increased the cutaneous basophil hypersensitivity (CBH) response in broiler chickens. When compared to control birds, birds fed with 200, 400, and 800 mg/kg of cinnamon oil, vitamin E, and selenium exhibited a significantly higher antibody titre. The findings imply that adding cinnamon oil to broiler diets has a positive impact on both the humoral and cell-mediated immune responses. Supplementing with cinnamon oil significantly raised serum globulin and total protein levels while significantly lowering the albumin to globulin ratio. It demonstrates how cinnamon oil stimulates the humoral immune response in broiler chickens. Birds treated with cinnamon oil showed a significant decrease in the heterophil to lymphocyte ratio, indicating its positive impact on cell-mediated immunity. In birds fed 400 and 800 mg/kg of cinnamon oil, histopathological analysis of the thymus and bursa of Fabricius revealed lymphoid cell growth, indicating the oil's immunopotentiating properties. The current study's findings showed that cinnamon oil is a safe, natural substitute that can be added to broiler diets to produce immunostimulant effects without endangering their health.

Keywords: Immunomodulatory activity, Broilers, cell mediated immune response, cinnamon oil, humoral immune response

Introduction

In India, poultry industry is one of the fastest growing segments of the agricultural sector today. In 20th livestock census, the total poultry has increased by 16.81% and the total poultry population is 851.81 million. Although poultry industry is growing rapidly and constantly, its growth is hampered by unavoidable physiological stress and immunodeficiency resulting in altered gut health and overall production leads to major economic loss to the broiler industry. The immune system plays a classical role in host defence mechanism and to maintain the homeostasis. There are numerous factors which can lead to immunodeficiency such as immunosuppressive diseases, vaccination failure, irrational use of antibiotics and physiological & environmental stressors. To overcome immunosuppression and stress conditions, it is very important to enhance immunity to prevent infectious diseases, improve overall health and performance in poultry production. Recent trend in the feed additive is directed toward the use of naturally acquired ingredients from botanical origin, which include medicinal herbs, spices and their essential oils ^[1]. In alternative strategies, use of Essential Oils (EOs) is one of the choices to modulate immune system and performance in birds.

Essential oils positively influence the avian immune system, since they promote production of immunoglobulins, enhance lymphocytic activity and boost interferon- γ release. Supplementing diets with essential oils containing herbal mixtures positively are influenced the activity of the intestinal lymphatic system, modulation of the immune system, production and secretion of endogenous digestive enzymes [2]. In recent years, many studies have been carried out on the use of essential oils in conjunction with of vaccination programs in poultry and the results of the experiments showed that essential oils promote the production of antibodies, thus enhancing the efficacy of vaccination [3, 4].

Cinnamon powder and its essential oil has gained considerable attention as natural and safe feed additive in poultry feed. Up to 4% of volatile essential oil is present in cinnamon bark and the primarily active components includes cinnamaldehyde (60 to 75%), eugenol (1 to 10%), cinnamyl acetate (1 to 5%), β -caryophyllene (1 to 4%), linalool (1 to 3%) and 1,8-cineole (1 to 2%) [5].

In folk medicine, cinnamon is used for the treatment of impotence, dyspnea, frigidity, leukorrhea, eye inflammation, rheumatism, vaginitis and neuralgia, as well as wounds and toothaches [5]. It has carminative, antidiarrheal and expectorant effects and also useful to treat bronchitis, itching and urinary disease [6]. In recent studies many health promoting and pharmacological properties have been reported to be associated with its dietary use. For instance, cinnamon oil can be used as a hypoglycemic and cholesterol-lowering, anti-inflammatory, wound pro-healing agent [7, 8, 9]. Several studies have reported the anti-inflammatory activity of cinnamon essential oil [10, 11, 12].

On the other hand, the reports on the value of cinnamon oil extracts particularly on the immunomodulatory effects in broiler nutrition are scarce. Hence, considering immunomodulatory properties of cinnamon oil, the present study was planned to evaluate the influence of cinnamon oil at the dose rate of 200, 400 and 800 mg/kg feed for 35 days in broiler chickens.

Materials and Methods

Experimental birds

Day-old Vencobb broiler chicks purchased from Shakti Hatcheries Pvt. Ltd., Anand, were used in the investigation. Broiler chicks were kept under conventional management conditions in the brooder house of the Poultry Research Station (PRS), College of Veterinary Science and Animal Husbandry, Anand. Chicks were weighed individually and had their wings banded on the day of arrival. The chicks were weighed and then divided into five treatment groups at random. Chicks were raised in a well-ventilated brooder house utilizing normal management and health care procedures in a deep litter system. Electric lights and bukharies were employed to produce enough artificial light at night. The Veterinary College, Anand's Institutional Animal Ethical Committee (No. IAEC/2019/VPT/307) authorized the experiment. The birds were protected against various diseases including Infectious Bursal Disease and Ranikhet disease by vaccination followed at PRS, Anand.

Feeding and Watering Management

Feed (broiler starter and finisher) was made at feed manufacturing facility of PRS, Anand. Every bird was fed broiler feed based on their age, which was 1–28 days for

starters and 29–42 days for finishers. The cinnamon oil was first mixed in the vegetable oil and then mixed with basal diet to make different treatment diets. For the entire duration of the experiment, weighed amounts of various treatment diets were offered twice a day. 3 to 4 times a day, the feed was mixed and stirred in the feeder and every safety precaution was taken to reduce feed waste. For the entire duration of the trial, all experimental birds were provided with unlimited access to clean, fresh, and cool drinking water.

Experimental Design

Five groups of 12 chicks each were randomly selected from a total of 60 chicks. As a control group, group I was fed a baseline diet devoid of vitamin E, selenium and cinnamon oil. As the standard control, group II received a proprietary product containing vitamin E and selenium in water at a dose rate of 1.5 grams per 100 birds for the first two weeks and 5 grams per 100 birds for the next three weeks. Cinnamon oil was administered to the remaining groups III, IV, and V at doses of 200, 400, and 800 mg/kg diet, respectively. When preparing broiler diets, cinnamon oil was added to the basal diet using a mixer in accordance with the dose rates specified for treatment groups. The duration of this trial was 35 days. The immunomodulatory effect of cinnamon oil in broilers was assessed using a number of different metrics.

Cutaneous basophil hypersensitivity (CBH) response

Using the traditional toe web assay approach as outlined by Corrier and Deloach (1990) [13], cell-mediated immunity was evaluated by measuring the cutaneous basophil hypersensitivity reaction on the 14th day.

Antibody titer against the ND vaccination

On days 7, 21, and 35, around 1.5 ml of blood was drawn from the wing veins of birds in each group using a 2 ml syringe fitted with a 26 G needle and placed in plain vials. For five minutes, samples were centrifuged at 3000 rpm. After being separated, the serum was put into 2 ml centrifuge tubes and kept at -60 °C until analysis. With minor changes, the Haemagglutination Inhibition (HI) assay, as outlined by Buxton and Frazer (1977) [14], was used to detect antibody titers against the ND vaccine. The reciprocal of the maximum serum dilution that prevented RBC agglutination was used to express the HI titre. The antibody titre data was transformed into log₂ values, which were then statistically analyzed.

Biochemical investigations

Blood samples were collected in plain vials on the 7th, 21st, and 35th days, and serum was extracted. The samples were then refrigerated at -60 °C until further biochemical analyses. The total protein kit (Biuret method) and albumin kit (BCG method) were used to measure total protein (g/dl) and albumin (g/dl), respectively. Albumin (g/dl) levels were subtracted from total protein (g/dl) to determine globulin (g/dl). The A/G ratio or albumin to globulin ratio, was computed.

Haematological investigation

Blood samples were taken from birds in each group on the 35th day. Immediately after collection, thin blood smears were made on clean, grease-free slides, dried at room

temperature and fixed in alcohol. Blood smears were subsequently stained with Field's stain, dried and viewed under an oil immersion objective (100X) under a microscope to check for differential leucocyte counts. The ratio of heterophils to lymphocytes was then computed.

Histopathological analysis

All groups of chickens were sacrificed in the chicken shop by a butcher under supervision at the end of the trial period. Before being killed, the birds received no food for six hours and given unlimited access to water for a full 24 hours. For histological analysis, tissues such as the thymus, spleen, and bursa of Fabricius were collected in tissue collection vials filled with a 10% formalin solution and processed using the paraffin embedding procedure. Haematoxylin and Eosin (H&E) stains were used to stain the tissues, and slides were examined under a light microscope.

Statistical analysis

Using SPSS statistical software (version 20.0), the means of different immunomodulatory effect parameters were compared using a completely randomized design test and one-way analysis of variance (ANOVA). Duncan's multiple range test was used to examine significant differences

($p < 0.05$) between the various experimental groups [15]. Every data has been displayed as mean $\pm$ S. E.

Results

Every day throughout the entire duration of the study, every bird was observed. The oral administration of cinnamon oil at doses of 200, 400, and 800 mg/kg feed for 35 days in broiler chickens did not cause any aberrant symptoms, and all the birds were reported to be active during the trial period. During the study period, there was no fatality.

Cell mediated immunity (CMI) is an immune response that involves effector mechanisms which are carried out by particularly T lymphocytes and their products like lymphokines. In order to defend against foreign antigens, pathogenic organisms, and delayed type hypersensitivity reactions, cell-mediated immune responses are crucial [16]. The cutaneous basophil hypersensitivity (CBH) response (delayed type hypersensitivity) test was used in this investigation to measure the cell-mediated immune response using phytohemagglutinin-P (PHA-P). Two distinct dosages of phytohemagglutinin-P (100 μ g and 200 μ g) were administered to birds in the same group before, after 12 hours, and after 24 hours of PHA-P injection in order to measure the CBH response. Table 1 displays the CBH response (Mean $\pm$ S. E., mm) for each experimental group.

Table 1: Effect of cinnamon oil supplementation on broiler chickens' cutaneous basophil hypersensitivity reaction to phytohemagglutinin-P (Mean $\pm$ S. E., mm).

Group	Mean toe web skin thickness (mm)					
	Pre- injection (PHA-P @ 100 μ g)		Post-injection (PHA-P @ 100 μ g)		Pre- injection (PHA-P @ 200 μ g)	
			12 h	24 h		
I	1.06 $\pm$ 0.04		1.43 $\pm$ 0.04 ^a	1.46 $\pm$ 0.07 ^a	1.11 $\pm$ 0.05	1.54 $\pm$ 0.11 ^a
II	1.04 $\pm$ 0.01		1.86 $\pm$ 0.13 ^{bc}	1.88 $\pm$ 0.06 ^b	1.17 $\pm$ 0.04	2.23 $\pm$ 0.09 ^b
III	1.13 $\pm$ 0.03		1.61 $\pm$ 0.05 ^{ab}	1.65 $\pm$ 0.06 ^{ab}	1.10 $\pm$ 0.05	1.73 $\pm$ 0.09 ^a
IV	1.08 $\pm$ 0.05		1.81 $\pm$ 0.16 ^{bc}	1.86 $\pm$ 0.12 ^b	1.13 $\pm$ 0.04	2.14 $\pm$ 0.18 ^b
V	1.07 $\pm$ 0.03		1.96 $\pm$ 0.11 ^c	1.92 $\pm$ 0.14 ^b	1.10 $\pm$ 0.03	2.16 $\pm$ 0.10 ^b

a, b, c values (Mean $\pm$ S. E., n=6) bearing different superscripts differ greatly from one another within the same column. ($P < 0.05$).

It was observed that chicks supplemented with cinnamon oil at 400 and 800 mg per kg in diet (groups IV and V) and vitamin E & selenium (group II) showed significantly increased CBH response in both the doses (100 and 200 μ g) of PHA-P at 12 and 24 hours after injection in chicks. Moreover, the magnitude of CBH response increased gradually up to 24 hours and high in birds treated with 200 μ g dose of PHA-P.

The primary immunoglobulins in the humoral immune response are IgG and IgM, and antibodies are produced by B lymphocytes and plasma cells. They are involved in toxin neutralization, complement activation, and opsonization [16]. The humoral immune response to the ND vaccine was evaluated in this study using the Haemagglutination Inhibition (HI) test, and the HI titre was represented as log₂ values. In different experimental groups, the HI log₂ values (Mean $\pm$ S. E.) against ND vaccine on 1st, 3rd and 5th week of experiment have been displayed in Table 2.

Appreciable amount of maternal antibody titre was observed in chicks before vaccination at first week. This was explained by either the transfer of natural passive immunity from the hen to the chicks or the vertical transfer of maternal antibodies in the form of immunoglobulin from the immunized hen to the egg yolk. [22, 23].

On 14th day post vaccination (3rd week), antibody titre decreased in birds of control group while significantly increased in cinnamon oil (group IV and V) and vitamin E

& selenium (group II) supplemented birds as compared to birds of control group. Similarly, on 14th day post booster vaccination (5th week), antibody titre was significantly increased in cinnamon oil (group III, IV and V) and vitamin E & selenium (group II) supplemented birds as compared to birds of control group. Additionally, the HI titre of birds supplemented with cinnamon oil (800 mg/kg diet) was similar to that of birds fed with vitamin E and selenium (group II), indicating that the effects of cinnamon oil (800 mg/kg diet) are comparable to those of standard supplements.

Table 2: Effect of dietary supplementation of cinnamon oil on haemagglutination inhibition (HI) antibody titre against ND vaccine in broiler birds (Mean $\pm$ S. E., HI log₂ titre)

Group	HI log ₂ antibody titre		
	1 st week (7 th Day)	3 rd week (21 st Day)	5 th week (35 th Day)
I	4.83 $\pm$ 0.31	4.33 $\pm$ 0.42 ^a	4.33 $\pm$ 0.21 ^a
II	5.67 $\pm$ 0.42	7.00 $\pm$ 0.73 ^c	7.67 $\pm$ 0.21 ^d
III	5.33 $\pm$ 0.33	5.50 $\pm$ 0.43 ^{ab}	5.33 $\pm$ 0.3 ^b
IV	5.50 $\pm$ 0.34	6.67 $\pm$ 0.42 ^{bc}	6.67 $\pm$ 0.42 ^c
V	5.50 $\pm$ 0.62	7.83 $\pm$ 0.31 ^c	8.00 $\pm$ 0.37 ^d

a, b, c, d values (Mean $\pm$ S. E., n=6) bearing different superscripts differ greatly from one another within the same column. ($P < 0.05$).

Tables 3 and 4 show the findings of biochemical estimations as mean values of total protein (g/dl), albumin (g/dl),

globulin (g/dl), and albumin to globulin ratio in various experimental groups for the 1st, 3rd and 5th weeks of the experiment. Total protein, albumin, globulin, and A/G ratio results showed that birds supplemented with cinnamon oil (groups III, IV, and V) and vitamin E & selenium (group II) had significantly higher total protein and globulin concentrations and a significantly lower A/G ratio than birds in the control group. The birds supplied with vitamin E and

selenium (group II) and those supplemented with cinnamon oil (groups III, IV, and V) had similar amounts of total protein. Similarly, the globulin level and A/G ratio in cinnamon oil (800 mg/kg diet, group V) supplemented birds was similar to the birds supplemented with vitamin E & selenium (group II) suggesting cinnamon oil (800 mg/kg diet) has similar effect as of standard supplement in improving total protein, globulin and A/G ratio.

Table 3: Effect of dietary supplementation of cinnamon oil on serum total protein and albumin in broiler birds (Mean $\pm$ S. E., g/dl)

Groups	Serum total protein (g/dl)			Serum albumin (g/dl)		
	1st week (7 th Day)	3rd week (21 st Day)	5th week (35 th Day)	1st week (7 th Day)	3rd week (21 st Day)	5th week (35 th Day)
I	3.74 $\pm$ 0.08 ^{ab}	2.91 $\pm$ 0.04 ^a	3.20 $\pm$ 0.04 ^a	2.31 $\pm$ 0.07 ^{ab}	1.86 $\pm$ 0.05 ^{ab}	2.11 $\pm$ 0.02
II	3.50 $\pm$ 0.12 ^{ab}	4.22 $\pm$ 0.19 ^c	4.23 $\pm$ 0.04 ^b	2.09 $\pm$ 0.12 ^{ab}	2.14 $\pm$ 0.07 ^b	2.19 $\pm$ 0.10
III	3.37 $\pm$ 0.02 ^a	3.73 $\pm$ 0.12 ^b	4.43 $\pm$ 0.30 ^b	1.92 $\pm$ 0.04 ^a	1.67 $\pm$ 0.22 ^a	2.69 $\pm$ 0.31
IV	3.68 $\pm$ 0.19 ^{ab}	3.66 $\pm$ 0.15 ^b	4.28 $\pm$ 0.26 ^b	2.18 $\pm$ 0.20 ^{ab}	1.64 $\pm$ 0.12 ^a	2.40 $\pm$ 0.25
V	3.86 $\pm$ 0.16 ^b	3.91 $\pm$ 0.12 ^{bc}	4.51 $\pm$ 0.21 ^b	2.38 $\pm$ 0.17 ^b	1.89 $\pm$ 0.11 ^{ab}	2.44 $\pm$ 0.17

a, b, c values (Mean $\pm$ S. E., n=6) bearing different superscripts differ greatly from one another within the same column. ($P < 0.05$).

Table 4: Effect of dietary supplementation of cinnamon oil on serum globulin and albumin to globulin ratio (A/G ratio) in broiler birds (Mean $\pm$ S. E., g/dl)

Groups	Serum globulin (g/dl)			Albumin to globulin ratio		
	1st week (7 th Day)	3rd week (21 st Day)	5th week (35 th Day)	1st week (7 th Day)	3rd week (21 st Day)	5th week (35 th Day)
I	1.44 $\pm$ 0.01 ^{ab}	1.05 $\pm$ 0.01 ^a	1.09 $\pm$ 0.02 ^a	1.61 $\pm$ 0.05	1.77 $\pm$ 0.06 ^b	1.94 $\pm$ 0.02 ^c
II	1.41 $\pm$ 0.01 ^a	2.09 $\pm$ 0.18 ^b	2.04 $\pm$ 0.06 ^d	1.49 $\pm$ 0.09	1.06 $\pm$ 0.09 ^a	1.08 $\pm$ 0.08 ^a
III	1.45 $\pm$ 0.02 ^{abc}	2.06 $\pm$ 0.31 ^b	1.74 $\pm$ 0.01 ^b	1.31 $\pm$ 0.04	0.99 $\pm$ 0.25 ^a	1.55 $\pm$ 0.19 ^b
IV	1.50 $\pm$ 0.01 ^c	2.02 $\pm$ 0.20 ^b	1.87 $\pm$ 0.05 ^c	1.46 $\pm$ 0.14	0.88 $\pm$ 0.15 ^a	1.28 $\pm$ 0.13 ^{ab}
V	1.48 $\pm$ 0.03 ^{bc}	2.01 $\pm$ 0.03 ^b	2.07 $\pm$ 0.05 ^d	1.62 $\pm$ 0.14	0.94 $\pm$ 0.05 ^a	1.17 $\pm$ 0.06 ^a

a, b, c, d values (Mean $\pm$ S. E., n=6) bearing different superscripts within a same column differ significantly from each other ($P < 0.05$).

On day 35 (5th week) of the trial, Differential Leucocyte Counts (DLC) were carried out. Table 5 displays the average values of the H/L ratio and differential leucocyte count for each group. In comparison to birds in the control group, birds supplemented with vitamin E and selenium (group II) and cinnamon oil at 200, 400, and 800 mg/kg diet (groups III, IV, and V) showed significantly higher lymphocyte counts and significantly lower heterophils and

heterophil to lymphocyte ratios, suggesting an improved immune response. Moreover, the results with respect to lymphocytes, heterophils and heterophil to lymphocyte ratio in birds supplemented with cinnamon oil (group III, IV and V) was almost similar to the birds supplemented with vitamin E & selenium (group II) suggesting that cinnamon oil has similar beneficial effects as of standard supplement.

Table 5: Effect of dietary supplementation of cinnamon oil on Differential Leucocyte Counts (DLC) and heterophil/lymphocyte (H/L) ratio in broiler birds (Mean $\pm$ S. E.)

Groups	Differential leukocyte counts (%)				H/L ratio
	Heterophils	Lymphocytes	Monocytes	Eosinophils	
I	38.17 $\pm$ 1.08 ^c	53.83 $\pm$ 1.47 ^a	4.67 $\pm$ 0.42	3.33 $\pm$ 0.42	0.71 $\pm$ 0.04 ^c
II	28.33 $\pm$ 1.69 ^{ab}	63.17 $\pm$ 1.99 ^{bc}	4.83 $\pm$ 0.31	3.67 $\pm$ 0.33	0.45 $\pm$ 0.04 ^{ab}
III	33.00 $\pm$ 2.80 ^{bc}	60.50 $\pm$ 2.77 ^b	3.83 $\pm$ 0.17	2.67 $\pm$ 0.21	0.56 $\pm$ 0.08 ^b
IV	30.50 $\pm$ 1.67 ^b	62.17 $\pm$ 1.97 ^{bc}	4.17 $\pm$ 0.31	3.17 $\pm$ 0.40	0.50 $\pm$ 0.05 ^{ab}
V	25.00 $\pm$ 1.15 ^a	67.17 $\pm$ 1.17 ^c	4.67 $\pm$ 0.33	3.17 $\pm$ 0.17	0.37 $\pm$ 0.02 ^a

a, b, c values (Mean $\pm$ S. E., n=6) bearing different superscripts within a same column differ significantly from each other ($P < 0.05$).

Histopathological examination revealed normal architecture in the sections of bursa of Fabricius from birds of control group. As compared to birds of control group, sections of bursa of Fabricius from birds supplemented with cinnamon oil (400 and 800 mg/kg diet) revealed mild to moderate enlarged bursal follicle and lymphocytic hyperplasia at 35th day of experiment. Normal architecture was also observed in the sections of thymus from birds of control group. As compared to birds of control group, sections of thymus from birds supplemented with cinnamon oil (400 and 800 mg/kg

diet) showed slight lymphocytic hyperplasia at 35th day of experiment. Sections of the spleen did not show any histopathological changes in all the experiment groups at 35th day of experiment. Histopathological examination findings of thymus, bursa of Fabricius and spleen has depicted in Figure 1A-F. The histopathological findings in bursa of Fabricius and thymus showed improvement in lymphoid cell proliferation in birds supplemented with cinnamon oil and it further confirmed the view that cinnamon oil has immunopotentiating activity.

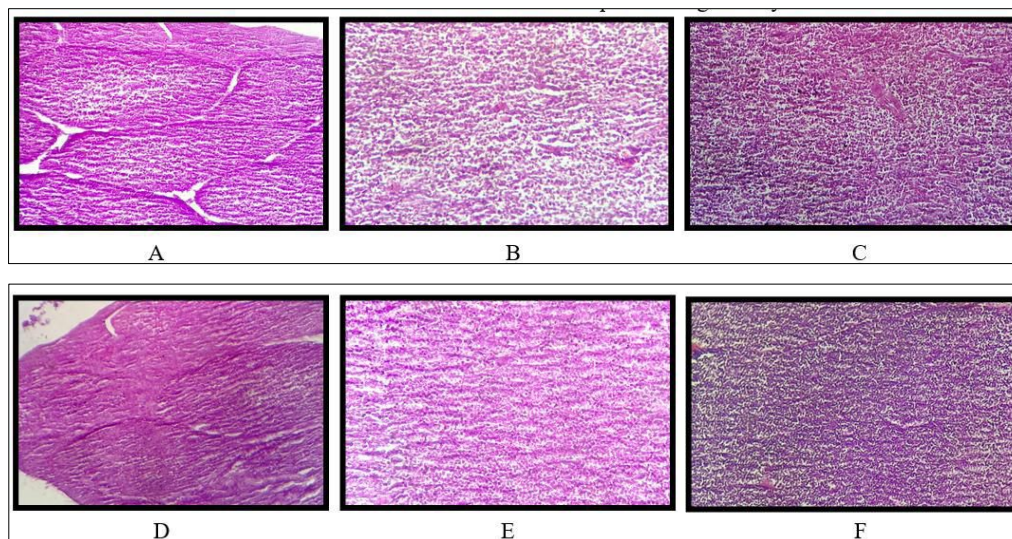


Fig 1: (A) Section of bursa of Fabricius from bird of control group showing normal architecture. (D) Section of bursa of Fabricius from bird of cinnamon oil supplemented group showing mild to moderate lymphocytic hyperplasia. (B) Section of thymus from bird of control group showing normal architecture. (E) Section of thymus from bird of cinnamon oil supplemented group showing slight lymphocytic hyperplasia. (C) Section of spleen from control group bird showing normal architecture and no histopathological changes. (F) Section of spleen from bird of cinnamon oil supplemented group showing normal architecture and no histopathological changes.

Discussion

In present study, higher CBH response against PHA-P were supported by Khaksar *et al.* (2012) ^[17] who observed significant change in CBH response in group supplemented with thyme essential oil. Unfortunately, reports on CBH response of essential oil supplementation in broilers are limited. However, Farhath *et al.* (2013) ^[18] and Balekar *et al.* (2014) ^[19] performed delayed type hypersensitivity (DTH) response to assess cell mediated immunity in rats treated with mix essential oil and cinnamon oil. They also reported dose dependent significant increase in DTH response. In present study, overall highest cell mediated immune response in terms of CBH response was observed in positive control group supplemented with vitamin E & selenium and this finding supported by Moriguchi *et al.* (1993) ^[20] who stated that vitamin E is an important factor in T-cell differentiation in thymus. Additionally, increased cell-mediated immunity in broilers treated with vitamin E and selenium has been reported by Swain *et al.* (2000) ^[21]. In present study, higher CBH response against PHA-P in cinnamon oil and vitamin E & selenium supplemented groups might be due to enhanced cell mediated immunity in birds.

The results of antibody titre against NDV align with the conclusions of Sadeghi *et al.* (2012) ^[24] who found that broilers given an infusion of cinnamon (5 grams per liter of water) had a markedly higher antibody titre against NDV. Sang-Oh *et al.* (2013) ^[25] reported significant increase in immunoglobulin levels in broilers supplemented with 3, 4 and 5% cinnamon powder at the end of experiment after 5 weeks. Hossain *et al.* (2014) ^[26] observed significant increase in antibody level in broilers supplemented with 1% cinnamon powder. Faghani *et al.* (2014) ^[27] reported significant increase in antibody titre against ND vaccine in broilers supplemented with 0.02% cinnamon powder. Beg *et al.* (2016) ^[28] reported significant increase in antibody level (sample to positive control ratio for ND) in broilers supplemented with 1% cinnamon powder. Abdel-Hafez and Mohamed (2016) ^[29] showed significant increase in antibody titre against ND vaccine in broilers supplemented with oregano essential oil and vitamin E plus selenium.

Pathak *et al.* (2017) ^[30] observed significantly higher antibody titers against ND vaccine on day 28 and 35 in broilers supplemented with combination of cinnamaldehyde and calcium formate. According to Chowdhury *et al.* (2018) ^[3], broilers fed 300 mg/kg of cinnamon oil showed a notable rise in antibody titre against the ND vaccination. On the contrary, Toghyani *et al.* (2011) ^[31] who reported no significant difference in antibody titre against NDV at 18 and 28 days in broilers supplemented with cinnamon powder at 2 and 4 g/kg in diet. Similarly, Naderi *et al.* (2014) ^[32] also observed no significant difference in antibody titre against ND vaccine at 28 day of age in broilers supplemented with cinnamon powder at 2.5 and 7.5 g/kg in diet.

The mechanism by which essential oil enhances immune response in birds is largely unknown. It is stated that principle active components of essential oil act as additional ligands to Fc receptors of antibodies which stimulate the immune response ^[33]. Najafi and Torki (2010) ^[34] reported an enhanced antibody titer as a result of the antioxidant properties of aromatic plants while Liu *et al.* (2014) ^[35] stated that bioactive components of aromatic plant regulate the expressions of various genes which are involved in immunomodulation. The results of this investigation suggest that cinnamon oil improves the humoral immune response in broilers.

The result of biochemical parameters could be correlated with findings of Al-Kassie (2009) ^[36] who observed significantly higher total protein level in cinnamon oil (200 ppm) fed birds as compared to birds of control group. Tamam *et al.* (2017) ^[37] observed significant increase in total protein and globulin concentration in broiler birds supplemented with cinnamon oil (0.1 and 0.3% in diet) and cinnamon powder (1 and 3 g/kg diet). Ghazlan *et al.* (2017) ^[38] reported the similar trend and observed significant increase in total protein and globulin concentration in broilers supplemented with rosemary essential oil (0.5, 1.0 and 1.5%) at 3rd and 5th week of the study. Similarly, Mahrous *et al.* (2017) ^[39] also observed significant increase in total protein and globulin concentration in broilers supplemented with clove powder (0.5, 1.0 and 1.5

g) at 3rd and 5th week of the study. Mudragada (2017) [40] showed significantly increase in total protein and globulin levels in Japanese quail at all levels of cinnamon powder supplementation (0.25, 0.50, 0.75 and 1.0%) in dose dependent manner. Contrary to results of the present study Toghyani *et al.* (2011) [31] reported non-significant in A/G ratio between birds supplemented with cinnamon powder (2 and 4 g/kg) and birds of control group. Chowdhury *et al.* (2018) [3] illustrated non-significant difference in total protein and albumin levels in birds fed with cinnamon oil (300 mg/kg) in comparison to birds of control group.

Serum proteins are synthesized mainly in liver and it yields amino acids which are important in growth and repair of tissues. So, increase in protein levels used as indicator of hepatoprotective and beneficial metabolic activity of liver [41, 37]. Moreover, globulins are the integral part of the immunoglobulins. So, enhancement in immunoglobulin production leads to increase in globulin content in serum and ultimately reduction in A/G ratio which is suggestive of better humoral immunity [42]. Based on the findings of the present study, it could be stated that cinnamon oil supplementation has beneficial effects on serum parameters related to immunity.

The findings of hematological investigation aligned with Al-Kassie (2009) [36] who observed significantly reduced H/L ratio in cinnamon oil (100 and 200 ppm) fed birds as compared to birds of control group. Naderi *et al.* (2014) [32] reported significantly increase in lymphocyte counts and significantly decreased H/L ratio in birds supplemented with cinnamon powder (7.5 g/kg diet) as compared to birds of control group. Najafi and Torki (2010) [34] also reported significantly decreased heterophils and increased lymphocytes in birds supplemented with cinnamon oil (200 mg/kg diet) as compared to birds of control group. Gawande (2015) [1] also observed significant decrease in H/L ratio in birds supplemented with cinnamon oil (300 ppm) compared to birds of control group. Mustafa and Wasman (2020) [43] observed significantly decreased H/L ratio in quails fed diet with cinnamon oil (1 ml/kg diet) and cinnamon powder (2 g/kg diet) and reported higher results in quails supplemented with cinnamon oil as compared to cinnamon powder. Dissimilar result was reported by Toghyani *et al.* (2011) [31] who observed non-significant difference in H/L ratio in birds supplemented with cinnamon powder (2 and 4 g/kg diet) in comparison to birds of control group.

Results of histopathological examination confirms the result obtained by Awaad *et al.*, (2010) [44] who observed lymphocytic hyperplasia in the sections of bursa and thymus while no histopathological changes in sections of spleen from birds supplemented with peppermint and eucalyptus essential oil in broiler birds and concluded that administration of volatile oil has a potent immunomodulatory effect in chickens.

Conclusions

Based on above finding the present study it is suggested that dietary supplementation of cinnamon oil (200, 400 and 800 mg/kg diet) has beneficial effect in terms of augmenting the cell mediated and humoral immune response and has similar effect produced by vitamin E & selenium supplementation in broiler birds. Furthermore, histopathological evaluation of bursa of Fabricius and thymus showed lymphoid cell proliferation in birds supplemented with cinnamon oil (400 and 800 mg/kg diet) suggesting immunopotentiating activity

of cinnamon oil. It can be stated that cinnamon oil can be used as a natural and safe alternate substance in broiler diet to get immunostimulant effects without any ill effects on the health.

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