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### Pruthvi S. Suthar

M.V.Sc. Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A. H., Kamdhenu University, Anand- 388001, Gujarat, India

### Kamlesh A Sadariya

Associate Professor & Head, Department of Veterinary Pharmacology and Toxicology, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A. H., Kamdhenu University, Anand- 388001, Gujarat, India

### Sanjay H. Vaghela

Ph.D. Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A. H., Kamdhenu University, Anand- 388001, Gujarat, India

### Shailesh K Bhavsar

Professor & Head, Department of Pharmacology and Toxicology, College of Veterinary Science and A. H., Kamdhenu University, Anand- 388001, Gujarat, India

### Correspondence:

#### Dr. Kamlesh A Sadariya

Associate Professor & Head, Department of Veterinary Pharmacology and Toxicology, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A. H., Kamdhenu University, Anand- 388001, Gujarat, India  
Email: kasadariya@kamdhenuuni.edu.in

## Evaluation of immunomodulatory and antioxidant activities of *Azadirachta indica* and *Tinospora cordifolia* alone and in combination in broilers

Pruthvi S. Suthar, Kamlesh A Sadariya, Sanjay H. Vaghela, Shailesh K Bhavsar

### ABSTRACT

**Background:** Phytogetic feed additives, including various herbs and spices in powder forms, are employed in poultry diet to enhance flavour, palatability and overall productive performance along with pharmacological beneficial effects, such as antioxidative, immunomodulatory and antimicrobial. It is a matter of great global concern to take effective strategies to reduce antibiotic use in chicken production to limit the spread of antimicrobial resistant bacteria and find some safe, natural alternatives to antibiotics. **Objective:** This study was conducted to evaluate the immunomodulatory and antioxidant activities of *Azadirachta indica* and *Tinospora cordifolia* alone and its combinations in broilers. **Materials and Methods:** Total of 96 chicks were divided randomly to 8 groups (n=12). Group I received Basal diet (control); Group II received Basal diet + vitamin E & selenium supplementation (standard control); Group III & IV received Basal diet + *Azadirachta indica* @ 5 and 10 g/kg feed, respectively; Group V & VI received Basal diet + *Tinospora cordifolia* @ 5 and 10 g/kg feed, respectively; Group VII & VIII received Basal diet + *Azadirachta indica* and *Tinospora cordifolia* in combination @ 5 and 10 g/kg feed, respectively. The experiment was conducted for 35 days. Various parameters like CBH, antibody titre against new castle disease vaccine and biochemical parameters like serum total protein, serum albumin, serum globulin and albumin to globulin (A/G) ratio were evaluated. On 35th day, haematological investigation (DLC and H/L ratio) and malondialdehyde (MDA) were measured. Histopathological examination of thymus, spleen and bursa of fabricius were carried out at the end of the experiment. **Results:** Dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations, showed significantly ( $p<0.05$ ) higher CBH response and HI antibody titre indicated its beneficial effect in terms of augmenting the cell mediated as well as humoral immune response in broiler. It also significantly improved the serum total protein, serum globulin and significantly decreased the A/G ratio along with significantly decreased heterophils, H/L ratio and significantly increases lymphocytes. Histopathological evaluation of bursa of fabricius, spleen and thymus revealed no histopathological alteration in powder supplemented birds. Malondialdehyde was significantly reduced in birds supplemented with *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations evinced its antioxidant activity. *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations had a similar effect to that produced by standard vitamin E & selenium supplementation. **Conclusion:** Findings from this study revealed that supplementation of *Azadirachta indica* and *Tinospora cordifolia* at given doses in feed possesses immunostimulants and antioxidant effects in broilers.

**Keywords:** *Azadirachta indica*, *Tinospora cordifolia*, Immunomodulatory, Antioxidant activities, Broilers.

### INTRODUCTION

The immune system plays a vital role in the defense against various infectious diseases. The factors which trigger immunity a body's natural defense system include previous infection, various external stimuli and immunization. Once a foreign body enters into body, the collective and coordinated response of specific cells and mediators to the foreign body forms an immune response [1]. The immune system maintains homeostasis in the healthy organisms. The function and efficiency of the immune system is influenced by a variety of extrinsic and intrinsic factors, leading to either immunosuppression or immune stimulation [2]. Clinically used most immunostimulants and immunosuppressants are cytotoxic drugs with serious side effects [3]. There is growing interest in using herbal medicines as multi-component agents to regulate the complex immune system in the prevention of infectious diseases than the treatment of immune-related diseases. It has been reported that phytochemicals such as flavonoids, polysaccharides, lactones, alkaloids, diterpenoids and glycosides present in some plants are involved in the immunomodulatory properties of plants. Therefore, the search for plant-derived natural products as a new guide for the development of effective and safe immunosuppressive and immunostimulatory agents

has received enormous research interest [4].

*Azadirachta indica* is a large, fast-growing, evergreen medicinal tree. It is called Limado in Gujarati, Neem in Hindi, Indian lilac in English and Nimba in Sanskrit. It is a member of the Meliaceae family. Neem is native to the Indian subcontinent and has been grown in tropical and subtropical regions around the world. It has great medicinal values and is used from the ancient times by many cultures for medicinal purposes. It is used in traditional remedies because of many biological active constituents [5]. Every component of the neem tree, including the leaves, roots, trunk, bark, flowers, fruits, seeds and gum has known to have some pharmacological value in the traditional medicine. Bioactive compounds isolated from different parts of the plant include azadirachtin, nimbin, nimbidin, meliacin, gedunin, salanin, quercetin, polysaccharides and many other derivatives of these principles [6]. These compounds can be classified into two major sections isoprenoids and non-isoprenoids. Isoprenoids are classified into diterpenoids and triterpenoids. Diterpenoids are further sub-classified into protomeliacin, azadirone, gedunin, amoorstatin with vepinin, vilasinin, and C-seco meliacins. Non-isoprenoids contain amino acids, polysaccharides and polyphenolic compounds, coumarin, sulphurous compound, dihydrochalcone, aliphatic compounds and tannins [7-9]. The neem tree has been demonstrated to exhibit immunomodulatory, antioxidant, antibacterial, antiviral, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antimutagenic, antidiabetic and anticarcinogenic properties [10]. *Tinospora cordifolia* is an herbaceous climbing shrub found on the roots of Neem and Mango trees. It is known as Gado in Gujarati, Giloy in Hindi, Heart-leaved moonseed in English and Guduchi/Amrita in Sanskrit. It belongs to family Menispermaceae, grows in the tropical and subtropical regions of India [11]. It is an essential medicinal plant and is well known for its medicinal properties in Indian system of medicine. The starch obtained from the stem known as "Guduchi-satva" has high nutritive value [12]. *T. cordifolia* contains many bioactive compounds, such as steroids, alkaloids, glycosides, diterpenoid lactones, sesquiterpenoids, flavonoids, furano diterpenoids and aliphatic compounds like tinosporide,  $\beta$ -sitosterol, tinosporin, berberine, magnoflorine, tinosporidine, 2 Introduction columbin, arabinogalactan polysaccharide, giloin and tinosporic acid [13-15]. *T. cordifolia* plants and their extracts exert many favorable pharmacological effects, such as immunomodulatory, antimicrobial, antioxidant, antineoplastic, hypoglycemic, antipyretic, hepatoprotective, diuretic, anti-stress, antihyperglycemic, antidiabetic and anti-tuberculous properties have also been reported [16]. Many scientific reports on combined dietary supplementation of vitamin E and selenium shows improved immune response and performance in broilers [17]. Therefore, in present study we used vitamin E and selenium supplementation in positive control birds. There are limited scientific reports on the immunomodulatory and antioxidant effects *Azadirachta indica* and *Tinospora cordifolia* powder individually and there is no study reported on their combined effect, particularly in broiler chicks till date. Hence, the present study was undertaken to evaluate the immunomodulatory and antioxidant effect of the *Azadirachta indica* and *Tinospora cordifolia* alone and its combinations for 35 days in broiler.

## MATERIALS AND METHODS

### Location

The experiment was carried out at the Department of Veterinary Pharmacology and Toxicology in collaboration with the Poultry Research Station (PRS), Department of Veterinary Pathology, Department of Veterinary Microbiology and Department of Veterinary Physiology & Biochemistry at College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat.

### Experimental Birds

The study was conducted on day old Ven-cobb broiler chicks. Broiler chicks were procured from Venky's (India) Limited, Anand, Gujarat and maintained under standard management conditions. The experimental protocol was approved by Institutional Animal Ethical Committee (No. IAEC/413/VPT/2023) of College of Veterinary Science and A.H., Anand.

### Procurement of experimental materials

*Azadirachta indica* leaves were collected locally and authentication were done at Department of Botany, B. A. College of Agriculture, Anand Agricultural University, Anand. *Tinospora cordifolia* stem were procured from ICAR-Directorate of Medicinal & Aromatic Plants Research, Boriavi, Anand, Gujarat. A 96 well cell culture plates (flat bottom with lid) and phytohemagglutinin PHA-P lyophilized powder were purchased from Sigma-Aldrich Chemicals Private Limited, Bengaluru. Avilamycin antibiotic was procured from local market, Anand, Gujarat. Newcastle disease vaccine and vitamin E and selenium-containing product (E-Care Se by Vetcare company) were given by poultry research station, Kamdhenu University, Gujarat. Reagents used for estimation of serum total protein and albumin were purchased from Q-line Biotech Private Limited, New Delhi, India. Kits used for estimation of antioxidant enzyme - Malonaldehyde were purchased from Genetix Biotech Asia Private Limited, New Delhi, India.

### Housing and management of birds

The birds were maintained by the standard management protocol at the brooder house of the Poultry Research Station (PRS), College of Veterinary Science and Animal Husbandry, Anand. On the day of arrival, chicks were wing-banded and individually weighed. Following weighing, the chicks were randomly allocated to eight treatment groups for immunomodulatory and antioxidant study. After distribution, chicks were supplied fresh jaggery water. A deep litter system was used for the rearing of chicks with a well-ventilated brooder house using standard management and health care practices. The birds were protected against various diseases like Ranikhet disease and Infectious Bursal Disease by vaccination as followed by PRS, Anand. During night hours, adequate artificial light was provided by using electric bulbs to keep a warm environment inside the house as the experiment was carried out during monsoon season.

### Management of Feeding and Watering

Broiler pre-starter, starter and finisher feed were prepared at the feed manufacturing unit of PRS, Anand. On the very first day, ground maize was offered to all chicks by scattering on papers and in plastic feed trays. All the birds received broiler feed according to the age of birds i.e., 1-7 days (pre-starter), 8-21 days (starter) and 22-42 days (finisher) during the entire experimental period. The powder of *Azadirachta indica* and *Tinospora cordifolia* alone and in combination at the different doses mixed with basal feed to make different treatment feeds. Treatment feeds were offered twice a day during whole experimental period. Mixing and stirring of feed in the feeder was carried out two times a day. Clean, cool and fresh drinking water was given to all experimental birds ad libitum throughout the experimental period.

### Experimental Design

A total of 96 chicks were divided randomly in 8 groups each of 12 chicks. Group I served as control and given basal feed without *Azadirachta indica* and *Tinospora cordifolia* powder, vitamin E and selenium. Chicks of group II were given vitamin E and selenium containing proprietary product in water as a standard control at the dose rate of 1.5 gm per 100 birds for first two weeks and 5 gm per 100 birds for next 3 weeks. Chicks of group III and group IV were given basal diet plus *Azadirachta indica* powder at the dose rate of 5 and 10

g/kg feed, respectively. Chicks of group V and group VI were given basal diet plus *Tinospora cordifolia* powder at the dose rate of 5 and 10 g/kg feed, respectively. Chicks of group VII were given basal diet plus *Azadirachta indica* and *Tinospora cordifolia* powders in combination at the dose rate of 5 and 5 g/kg feed, respectively and Chicks of group VIII were given basal diet plus *Azadirachta indica* and *Tinospora cordifolia* powders in combination at the dose rate of 10 and 10 g/kg feed, respectively. The study was conducted for 35 days. Various parameters were studied to evaluate immunomodulatory and antioxidant activities of clove powder in broilers.

### Cutaneous basophil hypersensitivity (CBH) response

Cell mediated immunity was assessed by conducting cutaneous basophil hypersensitivity response on 14<sup>th</sup> day by using classical toe web assay method<sup>[18]</sup>.

### Determination of antibody titer against ND virus

Blood sample (1-2 ml) was collected using 2 ml syringe equipped with 26 G needle in plain vials from wing veins of birds from each group on 7<sup>th</sup>, 21<sup>st</sup> and 35<sup>th</sup> day. Samples were centrifuged at 3000 rpm for 5-6 minutes. Serum was separated from blood, transferred to 2 ml centrifuge tubes and stored at -55 °C until analysis. Antibody titers against ND virus were determined by haemagglutination inhibition (HI) test as described by Buxton and Fraser (1977) with slight modifications<sup>[19]</sup>. The HI titre was expressed as the reciprocal of the highest dilution of serum, inhibiting agglutination of the RBC. The data of antibody titre was converted into log<sub>2</sub> value and these converted values were subjected to statistical analysis.

### Biochemical investigations

Serum was separated from blood samples collected in plain vials on 7<sup>th</sup>, 21<sup>st</sup> and 35<sup>th</sup> day and stored at -55°C till further biochemical estimations. Total protein (g/dl) and albumin (g/dl) were analyzed using total protein kit (Biuret method) and albumin kit (BCG method), respectively. Globulin (g/dl) and Albumin to globulin ratio were calculated.

### Hematological investigation

On 35<sup>th</sup> day, blood samples were collected from birds of each group and immediately after collection thin blood smears were prepared on grease free clean slides, dried at room temperature and fixed in alcohol. Later on, blood smear was then stained in field's stain, allowed to dry and examined microscopically for differential leucocyte counts under oil immersion objective (100X). Heterophil to lymphocyte ratio was calculated manually from DLC obtained data.

### Histopathological examination

At the end of the experimental period, birds of all groups were sacrificed at chicken shop by butcher under supervision. The birds were given ad-libitum water for 24 hours and kept off feed 6 hours prior to slaughter. Tissues like thymus, spleen and bursa of fabricius were collected in tissue collection bottles containing 10% formalin solution and processed by paraffin embedding technique for histopathological examination. Sections of tissues were cut at 4-5-micron thickness with the help of automatic section cutting machine. Tissues were stained using Hematoxylin and Eosin (H & E) stains and slides were observed under the light microscope.

### Antioxidant Activity

An oxidative stress marker malondialdehyde (MDA) was measured from serum sample of various groups. Serum was separated from blood samples collected in plain vials on 35<sup>th</sup> day and thiobarbituric acid reactive substance (TBARS) were estimated using standard assay kits and test protocol was followed as provided by Genetix Biotech Asia Pvt. Ltd., New Delhi.

### Statistical Analysis

Completely randomized design and one-way-analysis of variance (ANOVA) were used to compare the means of various parameters of immunomodulatory and antioxidant effect by using SPSS statistics software (version 25.0). Significant differences ( $p < 0.05$ ) between different experimental groups were analyzed by Duncan's multiple range test<sup>[20]</sup>. Preliminary calculations of data and preparation of graphs were made using Microsoft Excel Worksheet. All the data have been presented as mean  $\pm$  S. E.

### RESULTS

All birds were observed daily throughout the period of study. All the birds were found active during experimental period and did not reveal any abnormal symptoms attributable to oral administration of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations at various dose rate for 35 days in broiler. There was no mortality during study period.

In present study, the cell mediated immune response was assessed by conducting CBH response (delayed type hypersensitivity) test using phytohemagglutinin-P (PHA-P). The CBH response was assessed with two different doses of phytohemagglutinin-P (100  $\mu$ g and 200  $\mu$ g) where six birds treated with 100  $\mu$ g and other with 200  $\mu$ g dose of PHA-P powder of same group. The CBH response in mm (Mean  $\pm$  S.E., mm) of different experimental groups have been presented in Table 1.

The results of the present study revealed that the CBH response in form of toe web skin thickness was significantly improved in chicks supplemented with *Azadirachta indica* powder (5.0 and 10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed), *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower and higher dose (5.0 and 10 g/kg feed) in comparison to birds of control group at both the doses (100 and 200  $\mu$ g) of PHA-P at 12 and 24 hours after injection. After 12 hours of PHA-P injection (100  $\mu$ g), mean toe web skin thickness of chicks supplemented with *Azadirachta indica* and *Tinospora cordifolia* alone and its combination powder (groups III, V, VI, VII and VIII) was similar to the toe web skin thickness of vitamin E and selenium supplemented chicks (group II). After 12 hours of PHA-P injection (200  $\mu$ g), mean toe web skin thickness of chicks supplemented with *Tinospora cordifolia* alone and its combination with *Azadirachta indica* powder at higher dose (10 g/kg feed) (groups V, VI and VIII) was similar to the toe web skin thickness of vitamin E and selenium supplemented chicks (group II). After 24 hours of PHA-P injection (100  $\mu$ g), mean toe web skin thickness of chicks supplemented with *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combination (groups III, VI, V, VI, VII and VIII) was similar to the toe web skin thickness of vitamin E and selenium supplemented chicks (group II). After 24 hours of PHA-P injection (200  $\mu$ g), toe web skin thickness of chicks supplemented with *Azadirachta indica* and *Tinospora cordifolia* and its combinations powder (group III, IV, V, VI and VII) was similar to the toe web skin thickness of vitamin E and selenium supplemented chicks (group II). It indicates that dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations at different doses stimulates cell mediated immune response in broiler.

Antibodies are produced from the plasma cells and B lymphocytes which plays main role in humoral immune response, where major immunoglobulins are IgG and IgM. They are involved in the opsonization, complement activation and toxin neutralization<sup>[21]</sup>. In this study, Haemagglutination inhibition (HI) test was carried out to assess humoral immune response against NDV vaccine and HI antibody titer was expressed as log<sub>2</sub> values. The mean log<sub>2</sub> values of HI antibody titer against NDV vaccinated broiler birds in different experimental groups on 7<sup>th</sup>, 21<sup>st</sup> and 35<sup>th</sup> day of experiment have been presented in Table 2.

At the first week of age, non-significant changes in antibody titer were found among the various groups. However, at third week of age, the antibody titre was significantly increased in birds supplemented with *Azadirachta indica* powder (5.0 and 10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed), *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower and higher dose (5.0 and 10 g/kg feed) in comparison to birds of control group. At fifth week of age, the antibody titre was significantly increased in chicks supplemented with *Azadirachta indica* powder (10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed), *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower and higher dose (5.0 and 10 g/kg feed) in comparison to birds of control group. Furthermore, at third week of age, the log<sub>2</sub> values of HI antibody titer in chicks supplemented with *Azadirachta indica* and *Tinospora cordifolia* alone and their combination powder supplemented chicks (group III, IV, V, VII and VIII) was similar to the log<sub>2</sub> values of HI antibody titer of vitamin E and selenium supplemented chicks (group II). At fifth week of age, the log<sub>2</sub> values of HI antibody titer in chicks supplemented with *Azadirachta indica* and *Tinospora cordifolia* alone and their combination powder supplemented chicks (group IV, V, VI, VII and VIII) was also found similar to the log<sub>2</sub> values of HI antibody titer of vitamin E and selenium supplemented chicks (group II) suggesting that *Azadirachta indica* and *Tinospora cordifolia* powder alone and their combination have similar effect as of standard supplement. It indicates that dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and their combination stimulates humoral immune response in broilers.

The result of biochemical estimations as mean values of total protein (g/dl), albumin (g/dl), globulin (g/dl) and albumin to globulin ratio in different experimental groups on 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> week of experiment have been presented in Table 3 to 6. At first and third week of age, total protein level did not differ significantly among the various groups. The total protein level at fifth week of age in birds supplemented with *Azadirachta indica* powder (5.0 and 10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed), *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower and higher dose (5.0 and 10 g/kg feed) was significantly higher in comparison to birds of control group. Total protein level in birds supplemented with *Azadirachta indica* and *Tinospora cordifolia* alone and their combination at lower and higher doses (groups III, IV, V, VI, VII and VIII) was found similar to that of vitamin E and selenium supplemented bird (group II) suggesting that *Azadirachta indica* and *Tinospora cordifolia* alone and their combination had similar effect as of standard supplement. The serum albumin level at 1<sup>st</sup> and 2<sup>nd</sup> week, did not differ significantly among the various groups. At 3<sup>rd</sup> week, the serum albumin level in birds supplemented with *Azadirachta indica* powder (10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed) was significantly higher in comparison to birds of control group. At first and third week of age, serum globulin level did not differ significantly among the various groups. However, at 5<sup>th</sup> week significant increase in serum globulin level in birds supplemented with *Azadirachta indica* powder (5.0 and 10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed), *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower and higher dose (5.0 and 10 g/kg feed) was significantly higher in comparison to birds of control group. Moreover, serum globulin level of *Azadirachta indica* and *Tinospora cordifolia* alone and their combination at lower and higher doses (groups III, IV, V, VI, VII and VIII) was similar to vitamin E and selenium supplemented birds (group II) suggesting that *Azadirachta indica* and *Tinospora cordifolia* alone and their combination had similar effect as of standard supplement. Albumin to globulin ratio at 1<sup>st</sup> and 2<sup>nd</sup> week, did not differ significantly among the various groups. At 5<sup>th</sup> week, A/G ratio was significantly decreased in birds supplemented with *Azadirachta indica* powder (5.0 and 10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed), *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower and higher dose (5.0 and 10 g/kg feed) and in vitamin E and selenium supplemented birds as compared to birds of control group.

Moreover, A/G ratio in *Azadirachta indica* and *Tinospora cordifolia* powder alone and their combination at lower and higher doses (groups III, IV, V, VI, VII and VIII) was similar to A/G ratio in vitamin E and selenium supplemented birds suggesting that *Azadirachta indica* and *Tinospora cordifolia* powder alone and their combination had similar effect to standard vitamin E and selenium supplements.

Differential Leucocyte Counts was estimated on 5<sup>th</sup> week of experiment. The results of mean ± S.E. values of differential leucocyte counts in different groups have been presented in Table 5. Heterophil counts were significantly decreased while lymphocyte counts were significantly increased in birds supplemented with *Azadirachta indica* powder (5.0 and 10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed), *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower and higher dose (5.0 and 10 g/kg feed) and in vitamin E and selenium as compared to birds of control group. H/L ratio was also significantly decreased in birds supplemented with *Azadirachta indica* powder (5.0 and 10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed), *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower and higher dose (5.0 and 10 g/kg feed) and in vitamin E and selenium supplemented birds as compared to birds of control group. Moreover, *Azadirachta indica* and *Tinospora cordifolia* alone and their combination at lower and higher doses (groups III, IV, V, VI, VII and VIII) showed similar heterophil, lymphocyte and H/L ratio to vitamin E and selenium supplemented birds suggesting that *Azadirachta indica* and *Tinospora cordifolia* powder alone and their combination had similar effect to standard vitamin E and selenium supplements.

Histopathological examination of bursa of fabricius, thymus and spleen from birds of control group and birds supplemented with *Azadirachta indica* and *Tinospora cordifolia* powder alone and their combination were depicted in figure 1 to 6 respectively. During necropsy there was no appreciable gross changes were observed in bursa of fabricius, thymus and spleen of any experimental broiler birds. On histopathological examination, normal architecture was observed in the sections of bursa of fabricius, thymus and spleen from birds of control group, vitamin E and selenium as well as clove powder supplemented birds.

Antioxidant enzymes are proteins involved in the catalytic transformation of reactive species (free radicals) into stable nontoxic molecules therefore representing the most important defence mechanism against oxidative stress-induced cell damage. An oxidative stress marker malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation. The serum malondialdehyde enzyme (µmol/L) was assessed at the end of 5<sup>th</sup> week (Day 35) of age in different birds of same group. The serum MDA (µmol/L) (Mean ± S.E.) of different experimental groups have been presented in Table 8. Malondialdehyde (µmol/L) was significantly reduced in birds supplemented with *Azadirachta indica* powder (5.0 and 10 g/kg feed), *Tinospora cordifolia* powder (5.0 and 10 g/kg feed), *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower dose (5.0 g/kg feed) and *Azadirachta indica* and *Tinospora cordifolia* powder in combination at higher dose (10 g/kg feed) and in birds supplemented with vitamin E and selenium as compared to birds of control group. However, mean value of MDA in birds supplemented with *Tinospora cordifolia* powder (groups V and VI) was significantly reduced as compared to birds of control group and positive control (group II). Group V and VI showed dose dependent reduction in mean value of MDA. Furthermore, mean value of MDA in birds supplemented with *Azadirachta indica* powder, *Azadirachta indica* and *Tinospora cordifolia* powder in combination at lower and higher dose (groups III, IV, VII and VIII) was similar to the mean value of MDA in vitamin E and selenium (group II) supplemented birds suggesting that *Azadirachta indica* powder, *Azadirachta indica* and *Tinospora cordifolia* powder in combination has similar antioxidant effects as of standard supplement.

**Table 1:** Effect of dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations on CBH response against phytohemagglutinin-P in broilers (Mean ± SE, n=6)

Groups	Mean toe web skin thickness (mm)					
	Pre-injection (PHA-P @ 100 µg)	Post injection (PHA-P @ 100 µg)		Pre-injection (PHA-P @ 200 µg)	Post injection (PHA-P @ 200 µg)	
		12 h	24 h		12 h	24 h
Group I	1.09 ±0.01	1.42 ±0.02 <sup>a</sup>	1.53 ±0.02 <sup>a</sup>	1.15 ±0.01	1.45 ±0.03 <sup>a</sup>	1.58 ±0.03 <sup>a</sup>
Group II	1.07 ±0.01	1.70 ±0.07 <sup>c</sup>	1.97 ±0.05 <sup>b</sup>	1.18 ±0.02	1.76 ±0.07 <sup>c</sup>	2.03 ±0.06 <sup>b</sup>
Group III	1.08 ±0.02	1.63 ±0.02 <sup>bc</sup>	2.00 ±0.10 <sup>b</sup>	1.16 ±0.03	1.60 ±0.04 <sup>b</sup>	2.24 ±0.03 <sup>bc</sup>
Group IV	1.12 ±0.02	1.54 ±0.03 <sup>b</sup>	2.04 ±0.06 <sup>b</sup>	1.16 ±0.01	1.62 ±0.02 <sup>b</sup>	2.23 ±0.09 <sup>bc</sup>
Group V	1.07 ±0.02	1.62 ±0.05 <sup>bc</sup>	2.01 ±0.14 <sup>b</sup>	1.12 ±0.02	1.65 ±0.03 <sup>bc</sup>	2.13 ±0.06 <sup>bc</sup>
Group VI	1.07 ±0.02	1.66 ±0.03 <sup>bc</sup>	2.09 ±0.10 <sup>b</sup>	1.14 ±0.02	1.65 ±0.03 <sup>bc</sup>	2.25 ±0.09 <sup>bc</sup>
Group VII	1.11 ±0.02	1.59 ±0.05 <sup>bc</sup>	2.00 ±0.08 <sup>b</sup>	1.12 ±0.02	1.62 ±0.07 <sup>b</sup>	2.12 ±0.10 <sup>bc</sup>
Group VIII	1.06 ±0.01	1.64 ±0.02 <sup>bc</sup>	2.00 ±0.03 <sup>b</sup>	1.13 ±0.02	1.66 ±0.02 <sup>bc</sup>	2.28 ±0.06 <sup>c</sup>

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

**Table 2:** Effect of dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations on HI antibody titre against ND vaccine in broiler (Mean ± SE, n=6)

Groups	HI Antibody titre (log <sub>2</sub> values)		
	1 <sup>st</sup> week (Day 7)	3 <sup>rd</sup> week (Day 21)	5 <sup>th</sup> week (Day 35)
Group I	5.00±0.26 <sup>ab</sup>	5.17±0.60 <sup>a</sup>	5.33±0.33 <sup>a</sup>
Group II	5.50±0.43 <sup>ab</sup>	7.00±0.73 <sup>b</sup>	7.50±0.56 <sup>b</sup>
Group III	4.33±0.2 <sup>a</sup>	7.33±0.42 <sup>bc</sup>	6.67±0.71 <sup>ab</sup>
Group IV	5.00±0.3 <sup>ab</sup>	7.50±0.43 <sup>bc</sup>	7.67±0.61 <sup>b</sup>
Group V	5.50±0.43 <sup>ab</sup>	7.67±0.67 <sup>bc</sup>	8.33±0.84 <sup>b</sup>
Group VI	6.17±0.48 <sup>b</sup>	8.83±0.3 <sup>c</sup>	8.50±0.76 <sup>b</sup>
Group VII	5.67±0.49 <sup>b</sup>	7.67±0.49 <sup>bc</sup>	7.67±0.71 <sup>b</sup>
Group VIII	6.00±0.26 <sup>b</sup>	7.50±0.67 <sup>bc</sup>	7.83±0.91 <sup>b</sup>

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

**Table 3:** Effect of dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations on serum total protein in broiler

Groups	Serum total protein (g/dl)		
	1 <sup>st</sup> week (Day 7)	3 <sup>rd</sup> week (Day 21)	5 <sup>th</sup> week (Day 35)
Group I	2.68±0.14	2.88±0.12 <sup>a</sup>	3.20±0.25 <sup>a</sup>
Group II	3.03±0.10	3.49±0.10 <sup>b</sup>	4.47±0.16 <sup>b</sup>
Group III	3.11±0.22	2.97±0.27 <sup>ab</sup>	4.24±0.25 <sup>b</sup>
Group IV	2.77±0.11	3.02±0.15 <sup>ab</sup>	4.38±0.23 <sup>b</sup>
Group V	2.75±0.13	2.97±0.20 <sup>ab</sup>	4.58±0.18 <sup>b</sup>
Group VI	3.09±0.17	3.19±0.22 <sup>ab</sup>	4.60±0.31 <sup>b</sup>
Group VII	2.97±0.16	3.10±0.17 <sup>ab</sup>	4.46±0.28 <sup>b</sup>
Group VIII	2.88±0.16	2.94±0.15 <sup>ab</sup>	4.68±0.46 <sup>b</sup>

Values (Mean ± S.E., n=6) bearing different superscripts (a, b) within a same column differ significantly from each other (P<0.05).

**Table 4:** Effect of dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations on serum albumin in broiler

Groups	Serum albumin (g/dl)		
	1 <sup>st</sup> week (Day 7)	3 <sup>rd</sup> week (Day 21)	5 <sup>th</sup> week (Day 35)
Group I	1.19±0.06	1.21±0.04	1.23±0.04 <sup>a</sup>
Group II	1.23±0.03	1.28±0.05	1.43±0.05 <sup>b</sup>
Group III	1.31±0.11	1.30±0.08	1.38±0.03 <sup>ab</sup>
Group IV	1.32±0.08	1.34±0.05	1.44±0.04 <sup>b</sup>

Group V	1.37±0.06	1.34±0.04	1.41±0.08 <sup>b</sup>
Group VI	1.37±0.07	1.41±0.07	1.40±0.03 <sup>b</sup>
Group VII	1.36±0.14	1.44±0.09	1.31±0.05 <sup>ab</sup>
Group VIII	1.38±0.05	1.37±0.06	1.31±0.06 <sup>ab</sup>

Values (Mean ± S.E., n=6) bearing different superscripts (a, b) within a same column differ significantly from each other (P<0.05).

**Table 5:** Effect of dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations on serum globulin in broiler

Groups	Serum globulin (g/dl)		
	1 <sup>st</sup> week (Day 7)	3 <sup>rd</sup> week (Day 21)	5 <sup>th</sup> week (Day 35)
Group I	1.49±0.09	1.68±0.11	1.97±0.26 <sup>a</sup>
Group II	1.80±0.10	2.21±0.13	3.04±0.19 <sup>b</sup>
Group III	1.80±0.13	1.67±0.26	2.86±0.26 <sup>b</sup>
Group IV	1.44±0.09	1.68±0.11	2.94±0.24 <sup>b</sup>
Group V	1.38±0.10	1.63±0.19	3.17±0.19 <sup>b</sup>
Group VI	1.73±0.16	1.79±0.27	3.20±0.30 <sup>b</sup>
Group VII	1.60±0.08	1.65±0.18	3.14±0.26 <sup>b</sup>
Group VIII	1.50±0.16	1.58±0.19	3.37±0.42 <sup>b</sup>

Values (Mean ± S.E., n=6) bearing different superscripts (a, b) within a same column differ significantly from each other (P<0.05).

**Table 6:** Effect of dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations on albumin to globulin ratio (A/G ratio) in broiler

Groups	Albumin/Globulin ratio		
	1 <sup>st</sup> week (Day 7)	3 <sup>rd</sup> week (Day 21)	5 <sup>th</sup> week (Day 35)
Group I	0.80±0.04	0.73±0.04	0.68±0.09 <sup>b</sup>
Group II	0.70±0.06	0.60±0.06	0.48±0.05 <sup>a</sup>
Group III	0.73±0.05	0.84±0.10	0.51±0.06 <sup>a</sup>
Group IV	0.93±0.07	0.81±0.04	0.51±0.04 <sup>a</sup>
Group V	1.01±0.07	0.87±0.10	0.45±0.04 <sup>a</sup>
Group VI	0.83±0.08	0.91±0.16	0.45±0.03 <sup>a</sup>
Group VII	0.86±0.10	0.93±0.12	0.44±0.05 <sup>a</sup>
Group VIII	0.97±0.11	0.94±0.12	0.42±0.05 <sup>a</sup>

Values (Mean ± S.E., n=6) bearing different superscripts (a, b) within a same column differ significantly from each other (P<0.05).

**Table 7:** Effect of dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations on differential leukocyte counts and heterophil to lymphocyte (H/L) ratio in broiler

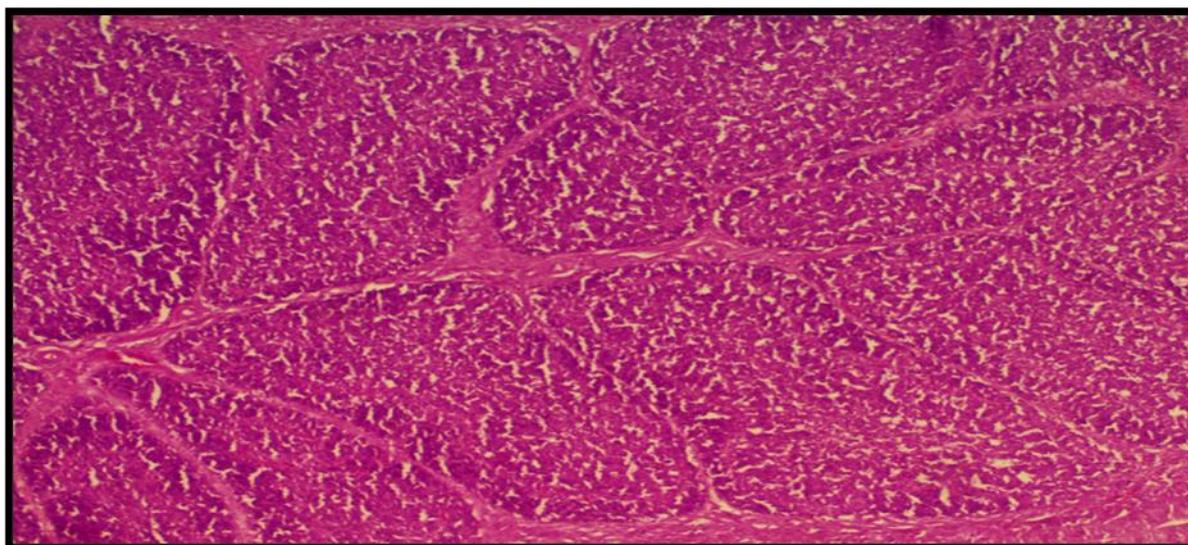
Groups	Differential leukocyte counts (%)				H/L Ratio
	Heterophils	Lymphocytes	Monocytes	Eosinophils	
Group I	40.33±1.31 <sup>b</sup>	51.17±1.01 <sup>a</sup>	4.83±0.31	3.67±0.33	0.79±0.04 <sup>b</sup>
Group II	31.50±1.93 <sup>a</sup>	60.50±2.03 <sup>b</sup>	4.67±0.33	3.33±0.21	0.53±0.05 <sup>a</sup>
Group III	32.00±0.82 <sup>a</sup>	60.17±0.87 <sup>b</sup>	5.00±0.37	2.83±0.31	0.53±0.02 <sup>a</sup>
Group IV	30.83±1.14 <sup>a</sup>	59.67±1.80 <sup>b</sup>	6.00±0.45	3.50±0.72	0.52±0.04 <sup>a</sup>
Group V	30.00±0.63 <sup>a</sup>	61.67±0.71 <sup>b</sup>	5.67±0.61	2.67±0.33	0.49±0.01 <sup>a</sup>
Group VI	30.33±0.61 <sup>a</sup>	60.67±0.84 <sup>b</sup>	5.67±0.56	3.33±0.42	0.50±0.01 <sup>a</sup>
Group VII	32.83±1.38 <sup>a</sup>	58.00±1.88 <sup>b</sup>	6.00±0.89	3.17±0.48	0.57±0.05 <sup>a</sup>
Group VIII	30.50±0.50 <sup>a</sup>	60.83±0.48 <sup>b</sup>	6.17±0.48	2.50±0.34	0.50±0.01 <sup>a</sup>

Values (Mean ± S.E., n=6) bearing different superscripts (a, b) within a same column differ significantly from each other (P<0.05).

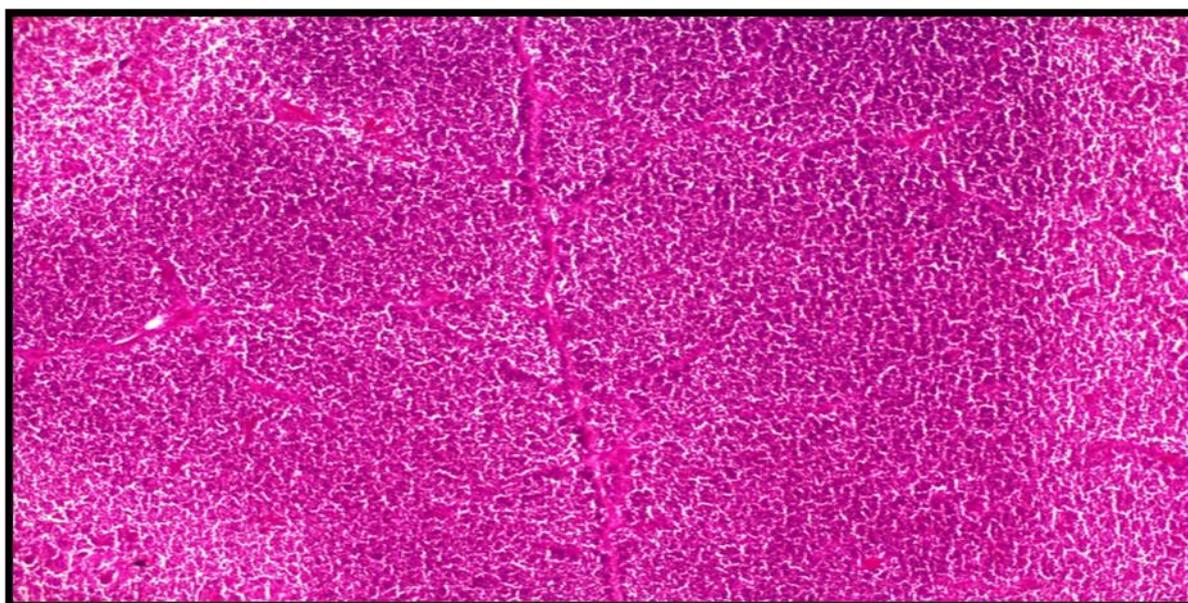
**Table 7:** Effect of dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations on serum malondialdehyde ( $\mu\text{mol/L}$ ) of broiler

Groups	Serum MDA ( $\mu\text{mol/L}$ ) (5 <sup>th</sup> Week)
Group I	11.17 $\pm$ 0.49 <sup>c</sup>
Group II	8.25 $\pm$ 0.35 <sup>b</sup>
Group III	7.51 $\pm$ 0.06 <sup>ab</sup>
Group IV	7.69 $\pm$ 0.28 <sup>ab</sup>
Group V	7.13 $\pm$ 0.28 <sup>a</sup>
Group VI	6.87 $\pm$ 0.37 <sup>a</sup>
Group VII	7.52 $\pm$ 0.17 <sup>ab</sup>
Group VIII	7.52 $\pm$ 0.31 <sup>ab</sup>

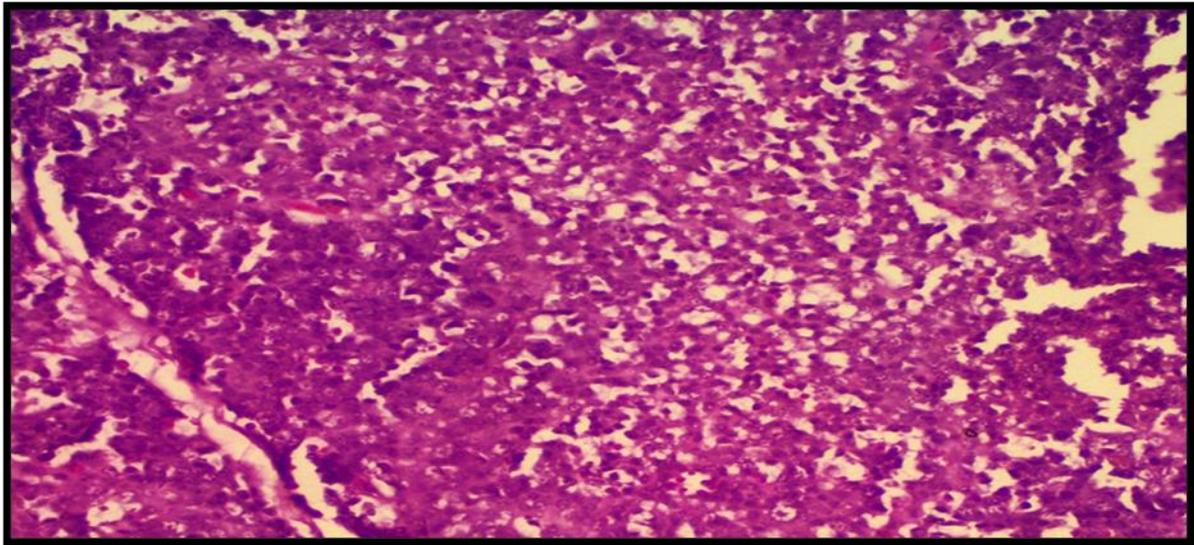
Values (Mean  $\pm$  S.E., n=6) bearing different superscripts (a, b, c) within a same column differ significantly from each other (P<0.05).



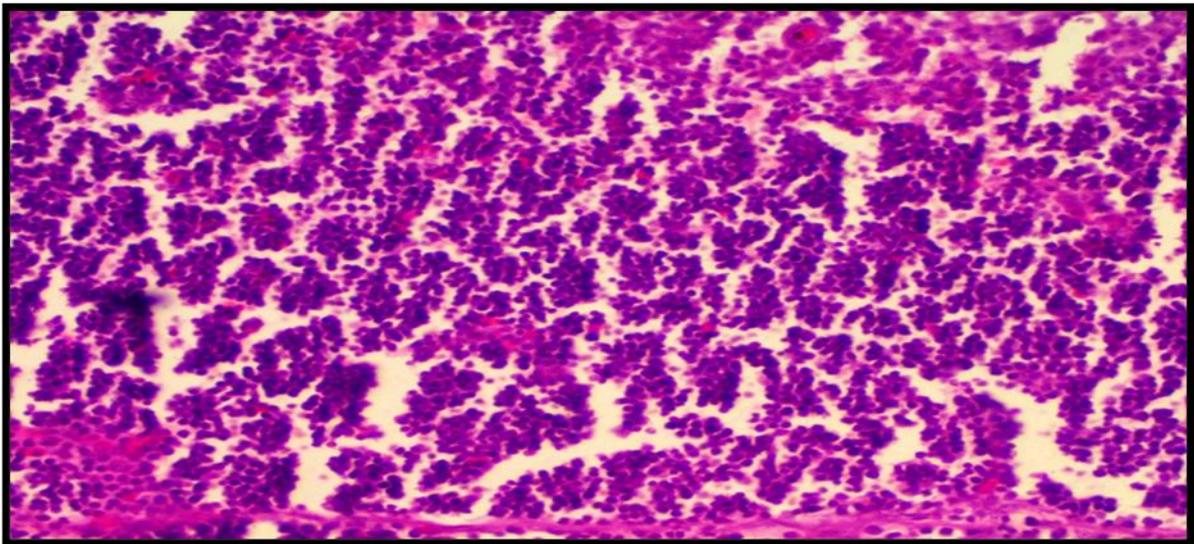
**Figure 1:** Section of bursa of fabricius from birds of control group showing normal architecture at 35<sup>th</sup> day of experiment (H &E Stain, 10X)



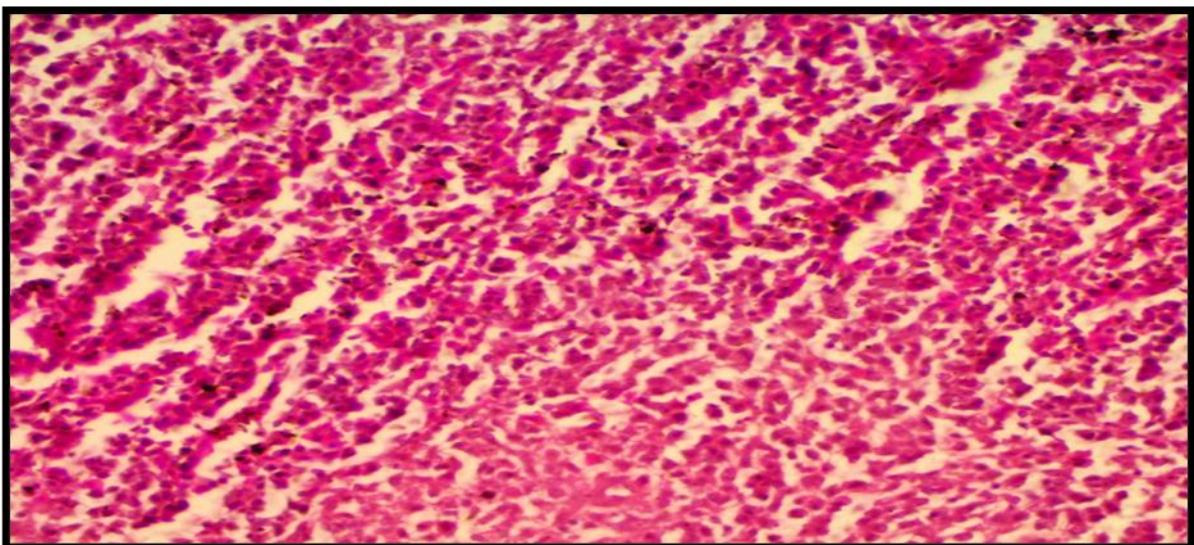
**Figure 2:** Section of bursa of fabricius from birds supplemented with *Azadirachta indica* and *Tinospora cordifolia* powder in combination at higher dose (10 g/kg feed) showing normal architecture at 35<sup>th</sup> day of experiment (H &E, 40X)



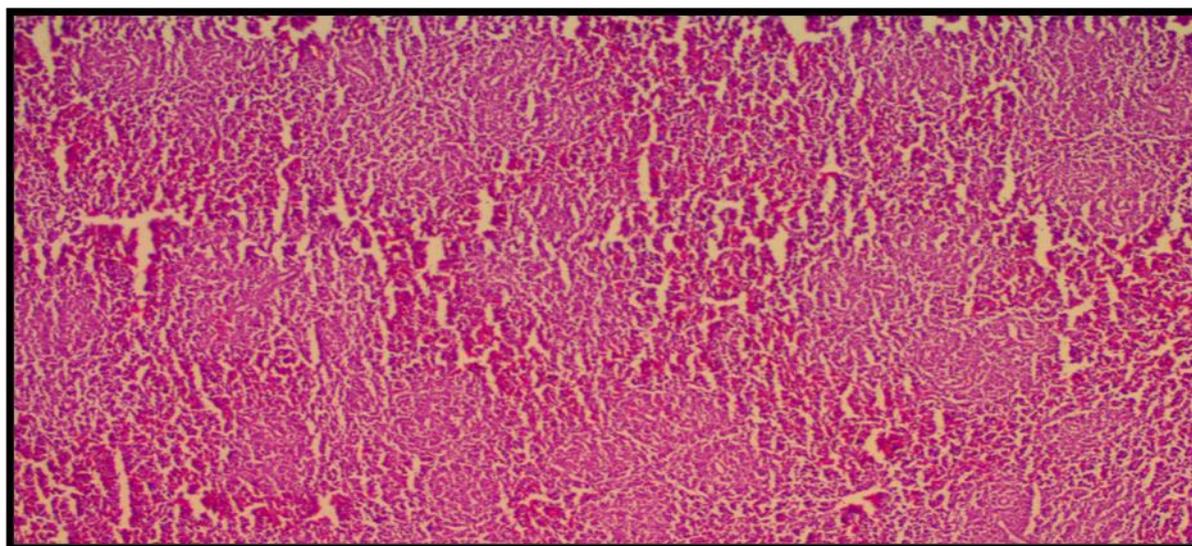
**Figure 3:** Section of thymus from birds of control group showing normal architecture at 35<sup>th</sup> day of experiment (H &E Stain, 10X)



**Figure 4:** Section of thymus from birds supplemented with *Azadirachta indica* and *Tinospora cordifolia* powder in combination at higher dose (10 g/kg feed) showing normal architecture at 35<sup>th</sup> day of experiment (H &E Stain, 40X)



**Figure 5:** Section of spleen from birds of control group showing normal architecture at 35<sup>th</sup> day of experiment (H &E Stain, 40X)



**Figure 6:** Section of spleen from birds supplemented with *Azadirachta indica* and *Tinospora cordifolia* powder in combination at higher dose (10 g/kg feed) showing normal architecture at 35<sup>th</sup> day of experiment (H & E Stain, 10X)

## DISCUSSION

The present findings in respect to CBH response are in agreement with results reported by Kumar *et al.* (2018) who observed significantly higher skin thickness (at 12, 24, 36, 48 and 72h post infection) in *Azadirachta indica* (7 g/L of water ethanolic extract) supplemented birds as compared to control groups [22]. Similarly, Sharma *et al.* (2016) observed significantly higher delayed-type hypersensitivity response of birds against dinitrochlorobenzene (DNFB) antigen in 10% neem leaf extract supplemented groups as compared to control group [23]. Likewise, Ray *et al.* (1996) observed significantly higher skin thickness in mice fed with neem meal (100 mg neem leaf extract/kg diet) than control group [24]. In accordance to our study, Nety *et al.* (2017a) illustrated that the cell-mediated immune responses of the *T. cordifolia* group in comparison to those of the control group were increased as increasing in the thickness of the abdominal skin in birds at both 24 and 48 h after challenge [25]. Bhardwaj *et al.* (2012) reported significantly high skin thickness (in mm) of birds, treated with *Tinospora cordifolia* stem (aqueous extracts) at different hours post-challenge with dinitrofluorobenzene (DNFB) [26]. CBH is localized in vivo inflammatory response to phytohaemagglutinin (PHA) and has been used to measure cell-mediated immunity. It is primarily a thymus dependent reaction mediated by T lymphocytes. Phytohaemagglutinin P is an excellent T cell mitogen that specifically stimulates T lymphocytes and induces proliferation at the injection site (Goto *et al.* 1978; Yadav *et al.* 2018) [27,28].

Also, T cells are involved in the CBH swelling response and secrete cytokines during swelling that recruits more effector cells. However, other white blood cells also affect tissue swelling and secrete additional cytokines that further promote the infiltration or proliferation of more white blood cells and this indicates that the PHA-P swelling response is a cell-mediated immune response Martin *et al.* (2006) [29].

The results of the present study with respect to antibody titre against NDV vaccine were in alignment with the findings of Asipa *et al.* (2024), who reported significantly increased HI antibody titre against Newcastle disease virus in 3% neem leaf meal supplemented chicks [30]. Omidiwura *et al.* (2023) also reported significant increase in specific antibody titre against Newcastle disease virus in broiler birds supplemented with 3% neem leaf meal [31]. The results of the present study are also in accordance to the findings with Kumar *et al.* (2018), also reported that *Azadirachta indica* (7 g/L of water ethanolic extract) treated group maintained a significantly high level of HI titres ( $1.603 \pm 0.06$ ) compared to untreated group ( $1.405 \pm 0.06$ ) [32]. Sachan *et al.* (2019) observed that the birds treated

with both *T. cordifolia* and CpG ODN (group E) prior to the vvIBDV challenge showed a highly significant increase in HI titre, than those of groups C and D at two and three weeks post-NDV vaccination ( $p < 0.05$ ) [33]. However, no significant difference was noticed at one-week post-NDV vaccination among all the groups. Similarly, Nety *et al.* (2017a) reported that supplementation with a dried stem powder of *T. cordifolia* in the amount of 0.4 g/L of drinking water significantly increased the levels of hemagglutinin titre values as compared to the control, BMD antibiotic, and dried leaf powder from *Azadirachta indica* treated groups [34]. Bhardwaj *et al.* (2012) reported that the mean hemagglutination inhibition antibody titre (log<sub>10</sub> value) of chick sera against NDV shows significant increment in *Tinospora cordifolia* extract treated groups as the age of bird increased. However [35], the present findings are not in agreement with Priya *et al.* (2020), who reported no significant impact of *Tinospora cordifolia* on antibody titre production against ND vaccine [36]. Govind *et al.* (2022) also reported non-significant difference in HI antibody titre against RD vaccination in birds supplemented with stem dry *Tinospora cordifolia* powder as compared to another group. It was observed that before vaccination at the age of day 7 (1<sup>st</sup> week), did not influence much on HI titre. It revealed considerable maternal antibody titre in chicks. This was credited to vertical transfer of maternal antibodies in the form of immunoglobulin which are transferred from immunized hen to egg yolk or transfer of natural passive immunity from hen to the chicks [37]. Heller *et al.* (1975) reported at first week of age, non-significant changes in antibody titre were found among the various groups and observed antibody titre could be from maternal part (immunized hen to egg yolk) [38].

The result with respect to serum biochemical investigations are in agreement with the findings of Omidiwura *et al.* (2023) reported broiler chickens fed with 3% neem leaf meal supplemented diet showed the significantly higher ( $p < 0.05$ ) in TP levels while, the albumin levels increased significantly ( $p < 0.05$ ) across the groups treated with the aqueous extracts of *Azadirachta indica* and *Khaya senegalensis* at a dose ratio of 1 : 1 at 100, 200 and 400 mg/kg compared to the control [39]. Bhardwaj *et al.* (2012) also reported that the mean total protein, albumin, globulin concentrations (g/dl) and A:G ratio in different groups of broiler supplemented with *Tinospora cordifolia* extract did not differ significantly [40]. The present findings are also not in agreement with Obikaonu *et al.* (2011) reported non-significant ( $P > 0.05$ ) difference in total protein that steadily decreased with increase in dietary neem leaf meal (2.5, 5.0, 7.5, 10 %) also, serum albumin and globulin show significant ( $P < 0.05$ ) reduction at 10% dietary neem leaf meal inclusion in broilers [41]. Sarag *et al.* (2001) also reported non-significant effect on inclusion of *Tinospora*

*cordifolia* in broilers in serum albumin, serum globulin and total protein [42].

The result with respect to haematological investigation are in agreement with the Eladl *et al.* (2020) who reported that the mean Heterophil/ lymphocyte (H/L) ratio during 1<sup>st</sup> week post vaccination was reduced significantly in chicks supplemented with Mixed herbal extract a mixture of ethanolic extracts (w/v) of *Tinospora cordifolia*, *Ocimum sanctum*, *Withania somnifera*, *Embllica officinalis*, *Mangifera indica* and Asphaltum (shilajit) as compared to control group [43]. Jain *et al.* (2021) reported improvement in leucogram and H/L ratio of broilers due to supplementation *Tinospora cordifolia* (0, 0.25, 0.50, 0.75, 1 %) [44]. Okoroafor *et al.* (2015) also reported that the mean absolute lymphocyte counts increased significantly ( $p > 0.05$ ) in the birds supplemented with *Azadirachta indica* extract (200, 400, 600mg/kg) at days 49 of age till the end of the experiment [45]. The result of present study is in agreement with the Kwawukume *et al.* (2013), reported that mean lymphocyte percentage counts were significantly ( $P < 0.05$ ) differences in the values of lymphocyte, monocyte neutrophils, eosinophils and basophils count which were significantly higher in birds administered with neem powder (@ 6g) than control during 56<sup>th</sup> day of age [46]. Nety *et al.* (2017b) reported significant reduction of lymphocyte count and heterophil count were non-significant in birds supplemented with *Azadirachta indica* extract (0.4 g/L) as compared to control group [47]. Nety *et al.* (2017b) reported significant reduction of lymphocyte count in birds supplemented with *Azadirachta indica* extract (0.4 g/L) as compared to control group [47]. Nodu *et al.* (2016) also reported that hematology values analyses on broiler chickens administered *Azadirachta indica* leaf extract (@ 3, 4 and 5g) were equally not significant for all the parameters [48]. Gotep *et al.* (2016) reported non-significant ( $p > 0.05$ ) change in heterophils between the negative control and the treated groups in a bird administered with combined aqueous extracts of *Azadirachta indica* and *Khaya senegalensis* at a dose ratio of 1 : 1 at 100, 200 and 400 mg/kg [49]. Okoroafor *et al.* (2015), observed that the mean absolute heterophil counts of vaccinated-treated groups was significantly ( $p > 0.05$ ) higher in the birds supplemented with *Azadirachta indica* extract (200, 400, 600mg/kg) at days 56 and 63 of age [50]. Obikaonu *et al.* (2011) also, reported that the lymphocytes, heterophil, eosinophil, monocyte and basophil were doing not differ significantly in the birds supplemented with neem leaf meal (2.5, 5.0, 7.5, 10 %) among all the group [51]. Egbeyale *et al.* (2018) recorded non-significant difference in the monocytes, eosinophils and basophils of broilers fed neem leaf extracts [52]. Latheef *et al.* (2013) reported that the chicks supplemented with *T. cordifolia* (1%) and *A. indica* (0.2%) showed significant reduction in percentage lymphocyte counts, when compared with the virus infected group [53]. Salahuddin *et al.* observed non-significant difference in Eosinophils, Basophils, Neutrophils, Lymphocyte and Monocytes of infected birds supplemented with extract of *Tinospora cardiofolia* and Carvacol @ 2 g/kg feed at 33<sup>rd</sup> day [54].

The result with respect to the histopathological examination were in agreement with Akter *et al.* (2006), who observed immunocompetent cells were arranged scatteredly or densely and as unorganized or organized lymphatic nodules in the lymphoid tissues and the length and breadth of the thymic lobule, bursal follicle, splenic white pulp and lymphatic nodule of cecal tonsils were varied within the lymphoid tissues and even one another [55]. The present findings are not in agreement with results reported by Garba *et al.* (2018), observed mild follicular lymphoid depletion in bursa of fabricius in group C (3mg/ml Neem Extract- vaccinated and unchallenged) [56]. However, normal bursa of fabricius were observed in chickens of group D (3mg/ml Neem Extract - unvaccinated and unchallenged group). Mild to moderate follicular lymphoid depletions were observed in bursa of fabricius in group G (3mg/ml Neem Extract- vaccinated and challenged group). Follicular atrophy, moderate lymphoid depletion and haemorrhages during 28-42 day of age) after challenge of IBDV. Pathological lesions were less in thymus of treatment group C and D as compared to control infection group. Severity of pathological lesions was less in treatment group C and D supplemented with T.

*cordifolia* as compared to infection group B and also group E and F supplemented T. *cordifolia* after the challenge.

The result with respect to serum malondialdehyde (MDA) investigation are in accordance with the findings of Nakamura *et al.* (2022) who reported significant reduction in MDA concentration (nmol MDA/g tissue) in the skeletal muscle of broiler chickens supplemented with 0.5% and 2.0% dried *Azadirachta indica* leaf extracts in diet as compared to control group [57]. Onyiche *et al.* (2021) also reported that there was a significant ( $P < 0.05$ ) decreased serum MDA level in all treatment groups administered with combined aqueous extracts of *Azadirachta indica* and *Khaya senegalensis* at a dose ratio of 1 : 1 at 100, 200 and 400 mg/kg [58]. Shivananjappa *et al.* (2012) also reported that dietary *Tinospora cordifolia* (1% and 2%) supplements showed significantly decreased malondialdehyde (MDA) level in maternal organs (30%) of rat and cyto fractions of the fetal brain (34%), respectively [59].

## CONCLUSION

Based on the finding of present study, it suggests that dietary supplementation of *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations has potential to enhance the cell mediated as well as humoral immune response in broiler. It can be stated that *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations can be used as a natural and safe alternate dietary substance at given dosage in broiler diet without any ill effects on the health of broiler. *Azadirachta indica* and *Tinospora cordifolia* powder alone and its combinations has potential substance as it shows immunostimulant and antioxidant effects in broilers.

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## Conflict of interest

The authors declared no conflict of interest.

## Financial Support

None declared.

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