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RESEARCH ARTICLE

Immunomodulatory Effects of *Artemisia brevifolia* Extract against Experimentally Induced Coccidiosis in Broiler Chicken

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ABSTRACT

Current study reports the immunomodulatory effects of *Artemisia brevifolia* (leaves extract) against experimental coccidiosis in broiler chicken. For evaluation of immunomodulatory potential, a total of 175 day-old broiler chicks were distributed into 5 groups each group having 35 birds. Mixed *Eimeria* infection (50,000 sporulated oocysts) was given orally at one week of age. At same age, *A. brevifolia* extract was orally given at 100, 200 and 300 mg/kg of body weight in first three groups respectively. Vitamin E and PBS treated chicks served as control groups. Cell mediated immunity was inquired through PHA-P, Carbon Clearance Assay, Concanavalin-A and DNCB assays. Sheep RBCs were used to check humoral immunity through hemagglutination test. Results showed that *A. brevifolia* extract induced cellular and humoral immunity against coccidiosis. Immunological response of groups administered with *A. brevifolia* extract at highest dose i.e., 300 mg was higher as compared to lower doses and caused immunomodulatory effect on infected chicks.

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INTRODUCTION

Coccidiosis is an infectious disease of poultry and the etiological agent of disease is *Eimeria* (protozoa) having many species (Abbas *et al.,* 2019; Khater *et al.,* 2020). Different species of *Eimeria* affect poultry industry at large scale in world in terms of reduced weight gain, mortality, and blood loss through feces (Bachaya *et al*., 2012, 2015). Like other diseases, poultry coccidiosis causes about $127 million losses to US poultry industry annually and similar type of losses are occurring worldwide (Abbas *et al.,* 2017a, 2017b, 2017c; Alshamiri *et al.,* 2021; Mohsin *et al.,* 2021; Nadeem *et al.,* 2022; Hamza *et al.,* 2022). Sporulation of *Eimeria* oocysts takes place outside the body and its multiplication is very high in the soil, therefore it is difficult to minimize the infection after its emergence (Lillehoj and Lillehoj, 2000). Coccidiosis is controlled by using anticoccidial drugs available in the market, but their efficacy is being lowered because resistance has been developed against most of available drugs, therefore these drugs are not fully dependable source (Abbas *et al*., 2011a; Hussain *et al.,* 2022; Tahir *et al.,* 2022).

Due to resistance issue on globe level, alternative options are being sought to fight against most of the diseases of poultry and livestock (Abbas *et al*., 2010, 2011b, c, 2012a, b; Masood *et al*., 2013; Zaman *et al*., 2015; Ashraf *et al.,* 2021; Hussain *et al.,* 2021; Ahmad *et al*., 2022; Muneer *et al.,* 2022; Akhtar *et al*., 2023). Among these options novel botanicals showed better results against coccidiosis (Wajiha and Qureshi, 2021; Hussain *et al.,* 2022; Imran and Alsayeqh, 2022; Degla *et al.,* 2022). Botanicals which are rich in antioxidant compounds such as *Camellia sinensis* (Jang *et al.,* 2007), *Ageratum conyzoides* (Nweze and Obiwulu, 2009), *Vitis vinifera* (Wang *et al*., 2008, Abbas *et al.,* 2020), *Artemisia amygdalina* (Mubashir *et al*., 2013), *Saccharum officinarum* (Awais *et al*., 2011), *Glycyrrhiza glabra* (Hussain *et al.,* 2017), *Carica papaya* (Bashir *et al.,* 2020)

and many other botanicals have been reported to have excellent immunomodulatory activity against coccidiosis.

Plants of *Artemisia* genus are well renowned plants having various antioxidant properties and are used as therapeutic agents in the treatment of various diseases of animals, birds and humans (Messaï *et al.,* 2014). Due to remarkable biological properties of *Artemisia* plants, current research was performed to check immunomodulatory effects of *Artemisia brevifolia* extract against experimentally induced coccidiosis in broiler chicken.

MATERIALS AND METHODS

Plant material: *Artemisia brevifolia* leaves were obtained locally from Faisalabad market and extracted in methanol using Soxhelet Apparatus and obtained plant material was stored at 4°C for further experimental protocols. Extraction of plant materials was done following Abbas *et al*. (2017).

Parasite: *Eimeria* oocysts of mixed species (*E. tenella, E. necatrix, E. brunetii, E. mitis)* were collected from the caeca of infected chickens from outbreak cases in Faisalabad. Oocysts were preserved and sporulated in Potassium dichromate solution (2.5% concentration) following method of Ryley *et al*. (1976).

Experimental design: 175-day old broiler chicks were acquired from local hatchery and were reared under best management protocols (Zaman *et al*., 2012). At seven days of age, all chicks were divided into five equal groups. First three groups were given *Artemisia brevifolia* extract (ABE) at 100, 200 and 300mg/kg for three continuous days by oral route. Fourth group was treated with Vitamin E and Group five treated with PBS served as control group. Vitamin E (Known Immunomodulatory) treated served as positive control while PBS treated served as negative control group. Infection of *Eimeria* (50,000 sporulated) oocysts of mixed species was given at two weeks of age. Cell mediated immunity and humoral immunity were evaluated by using standard procedures post infection of *Eimeria.* Water and adequate ventilation were provided. In each group, 20 chicks were reserved for cell mediated immunity and 15 chicks were reserved for evaluation of humoral immune response. Standard vaccination against Newcastle Disease, Infectious Bronchitis and Infectious Bursal Disease was done. Trial continued for 40 days.

Immunological Assessment

Cell mediated immunity: Evaluation of cell mediated immunity was done by using four different assays as described below:

Phytohemagglutinin-P test: Phytohemagglutinin-P test was conducted following method as described by Corrier (1990). Phytohemagglutinin-P (100µg/100ml/chick) was injected in the chick's right foot in inter digital spaces whereas the same protocol was followed for injection of PBS in left foot (control group). The screw gauge was used to measure the skin thickness at different time intervals (hours) post PHA-P injection.

Carbon Clearance Assay: Carbon clearance was checked in different groups by using standard protocols as reported by Zhang *et al*. (2004). ELISA reader was used for assessment of optical density values.

Dinitrochlorobenzene and Concanvalin-A (CON-A): Dinitrochlorobenzene (DNCB) test was used to examine cellular response following the steps described by Blumink *et al*. (1974). CON-A test was carried out to determine *in vitro* lymphoblastogenic response of chicken lymphocytes to Con-A by following Qureshi *et al.* (2000).

Evaluation of humoral immunity: Microplate hemagglutination test was used for calculation of antibodies by following method of Qureshi and Havenstein (1994).

Statistical analysis: Duncan's multiple range (DMR) and ANOVA were used for evaluation of statistical significance. Data was analyzed using SAS software. Data was considered significant at $P<0.05$.

RESULTS

Cell mediated immunity: Higher cellular immune response (PHA-P) was observed in chicks receiving the *Artemisia brevifolia* extract (ABE). Results of all treatment groups were observed in dose dependent manners, but the outstanding results were seen at 300 mg/kg which were comparable to Vitamin-E treated group ($P>0.05$) and were significantly higher than PBS treated group ($P<0.05$) (Fig. 1).

Higher carbon clearance index was observed in chicks receiving the ABE. Results of all treatment groups were observed in dose dependent manners, but the maximum results were noticed at 300 mg/kg of body weight which were comparable to Vitamin-E treated ($P>0.05$) and were significantly higher than PBS treated group ($P<0.05$) (Fig. 2).

Results show that improved cell mediated immunity was recorded at different intervals of post application of DNCB in chicks receiving the ABE. Results of all treatment groups were observed in dose dependent manners, but the outstanding results were observed at 300 mg/kg of body weight which were comparable to Vitamin-E treated ($P>0.05$) and were significantly higher than PBS treated group ($P<0.05$) (Fig. 3).

Higher cell mediated response was recorded at different intervals of post application of CON-A in chicks receiving the ABE. Results of all treatment groups were observed in dose dependent manners, but good results were observed at 300 mg/kg of body weight which were comparable to positive control or Vitamin- E treated group ($P>0.05$) and were significantly higher than PBS treated group ($P<0.05$) (Fig. 4).

Humoral immunity: Results of overall elevated antibody levels were observed in chicks getting the ABE in dose dependent manners, but the outstanding results were seen at 300 mg/kg which were comparable to Vitamin- E treated group ($P>0.05$) and were significantly higher than PBS treated group ($P<0.05$) (Fig. 5).

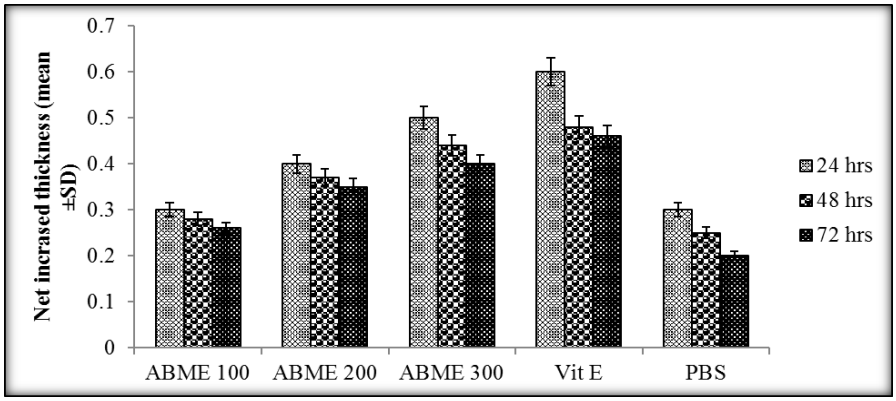

Fig. 1: PHA-P response in different treated groups: ABME (*Artemisia brevifolia* Methanolic Extract), Vit E (Vitamin E), PBS (Phosphate Buffer Saline).

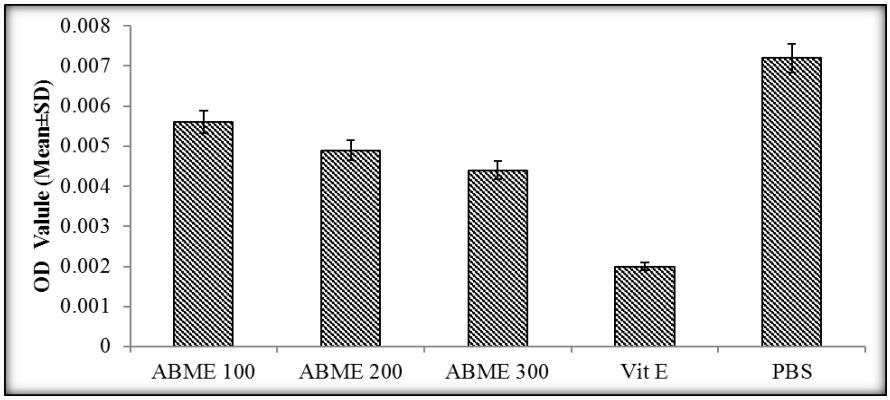

Fig. 2: Carbon clearance index in different treated groups: ABME (*Artemisia brevifolia* Methanolic Extract), Vit E (Vitamin E), PBS (Phosphate Buffer Saline).

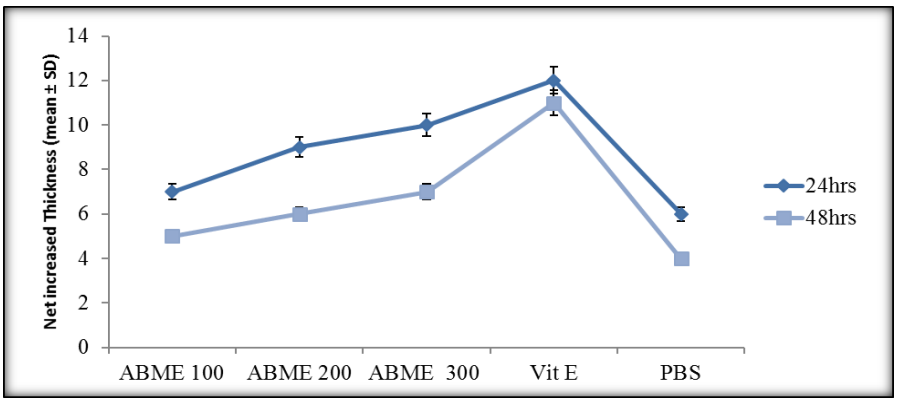

Fig. 3: Cell mediated response to DNCB in different treated groups: ABME (*Artemisia brevifolia* Methanolic Extract), Vit E (Vitamin E), PBS (Phosphate Buffer Saline).

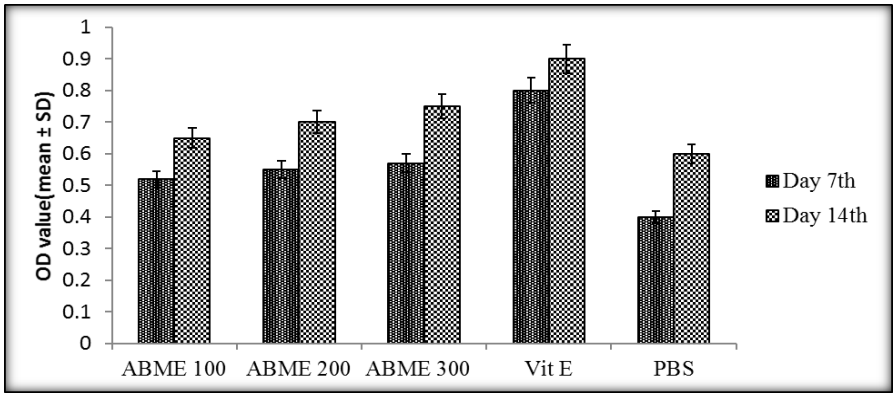

Fig. 4: Cell mediated response to CON-A in different treated groups: ABME (*Artemisia brevifolia* Methanolic Extract), Vit E (Vitamin E), PBS (Phosphate Buffer Saline).

Elevated immunoglobulins-G antibody levels were observed in all treatment groups of ABE like 100 mg/kg, 200 mg/kg and 300 mg/kg but the outstanding results were seen at 300 mg/kg which were comparable to Vitamin- E treated group ($P>0.05$) and were significantly higher than PBS treated group ($P<0.05$) (Fig. 6).

Elevated immunoglobulins-M antibody levels were observed in all treatment groups of ABE like 100 mg/kg, 200 mg/kg and 300 mg/kg but the outstanding results were seen at 300 mg/kg which were comparable to Vitamin- E treated group ($P>0.05$) and were significantly higher than PBS treated group ($P<0.05$) (Fig. 7).

DISCUSSION

Different botanicals rich in antioxidants compounds have shown excellent immunomodulatory and anticoccidial effects in poultry (Abbas *et al*., 2017a, 2017b). According

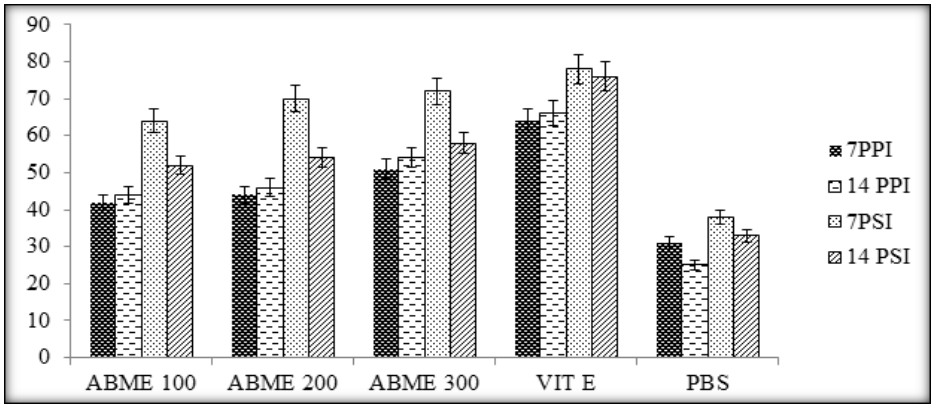

Fig. 5: The overall higher (GMT) levels in different treated groups: ABME (*Artemisia brevifolia* Methanolic Extract), Vit E (Vitamin E), PBS (Phosphate Buffer Saline).

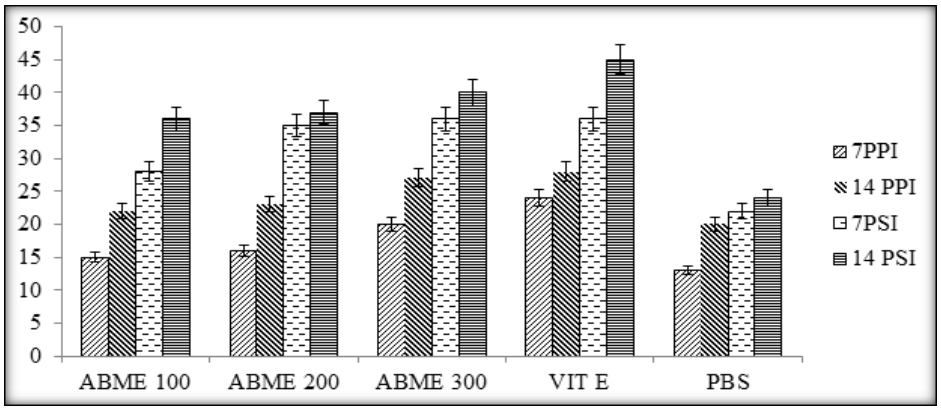

Fig. 6: Total Immunoglobulins (IgG) levels in different treated groups: ABME (*Artemisia brevifolia* Methanolic Extract), Vit E (Vitamin E), PBS (Phosphate Buffer Saline).

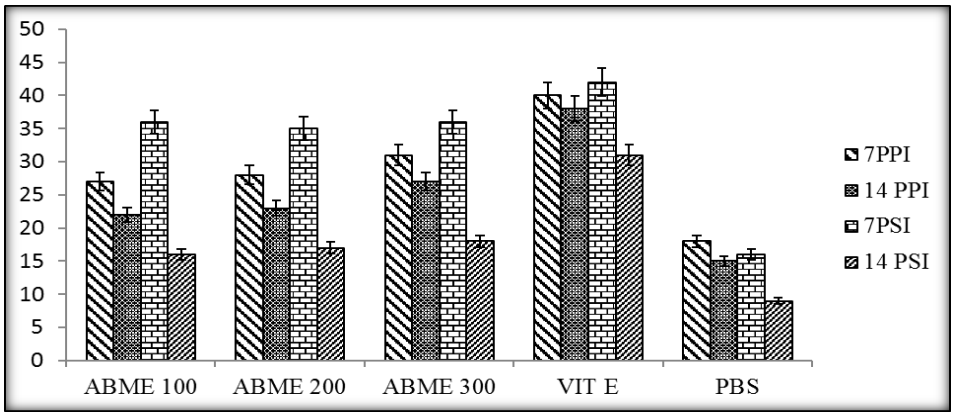

Fig. 7: Total Immunoglobulins (IgM) levels in different treated groups: ABME (*Artemisia brevifolia* Methanolic Extract), Vit E (Vitamin E), PBS (Phosphate Buffer Saline).

to the researchers, these antioxidant compounds showed better immune responses against *Eimeria* infection (Akhtar *et al*., 2012; Masood *et al*., 2013; Idris *et al*., 2017).

In current experiment, *Artemisia brevifolia* extract showed immunomodulatory activity against coccidiosis disease. Recent studies have elaborated role of *Artemisia* in immunomodulation that is due to actions of its compounds including flavonoids, terpenoids and among these main phytochemicals is artemisinin and its derivatives which possess antiparasitic and immunomodulatory activity.

Immunomodulatory efficacy of many other herbal plants showed same types of results in previous studies (Singh *et al*., 2015). Plants and their extracts enhance immunity by increasing antibody levels and by acting on immune cells by their proliferation and maturation in infected chicken (Chihara, 1992). *Carthamus tinctorius* (sunflower) leaves extract showed immunity against *Eimeria* infection in broiler chickens (Lee *et al*., 2009).

In an experiment, *Triticum aestivum* (wheat bran) driven polysaccharides (arabinoxylans) have been reported to have immunomodulatory potential against *Eimeria* infection in poultry birds and improved weight gain was also observed in infected chickens (Akhtar *et al*., 2012). *Saccharum officinarum* extract showed similar type of immunomodulatory effects against coccidiosis in broiler chicks (Awais *et al*., 2011).

The results of another study showed that *Beta vulgaris* extract has role in stabilizing intestinal epithelium and minimize infection against *Eimeria* in broilers (Kettunen *et al*., 2001). *B. vulgaris* helped in reducing oxidative stress in different diseases due to its antioxidant properties (Wettasinghe *et al*., 2002). *Beta vulgaris* (extract) also reduced tumor cell growth and enhanced immunomodulatory potential in mice (Tripathy and Pradhan, 2013).

Likewise, Abbas *et al.* (2017b) evaluated the immunomodulatory effects of *Pinus radiata* extract in broiler chickens. Broiler chickens were experimentally infected with mixed *Eimeria* species. *Pinus radiata* extract was given orally at three doses to infected chickens. Results of study indicated that *Pinus radiata* extract enhanced cellular and humoral immune response in broiler chicken.

In another study, dietary supplementation of *Camellia sinesis* (green tea) in feed produced immunomodulatory effects against coccidiosis in broiler chickens which were artificially infected with *Eimeria* (Abbas *et al.,* 2017c).

Hussain *et al.* (2017) also reported immunomodulatory effects of *Glycyrrhiza glabra* extract against *Eimeria* infection in broiler chicken. *Glycyrrhiza glabra* extract enhanced cellular and humoral immune response and better immune response was observed at highest dose rate at 300 mg/kg of body weight against coccidiosis disease in broiler chicks.

In one study, *Pimpinella anisum* commonly known as Aniseed in powder form enhanced cellular and humoral immune response in the broiler chickens against Newcastle and Infectious bursal disease. Supplementation of *Pimpinella anisum* in basal diet improved immunomodulatory response in chickens. However, higher dose caused adverse effects (Mahmood *et al.,* 2014).

Awais *et al.* (2011) reported the immunomodulatory effects of bagasse and sugar cane juice (*Saccharum officinarum* L.). This study concluded that ethanolic extract of sugar cane juice showed immunotherapeutic response against coccidiosis in chicken by observing the better results of some parameters like body weight gain, oocyst count and lesion score.

In another study, Bashir *et al.* (2020) observed the effects of *Carica papaya* leaf extract on blood hematology, serum biochemistry and immune response of poultry birds. The study showed that leaf extract of *Carica papaya* which is rich in vitamins and antioxidant has positive effects on immunity.

Conclusions: Current study confirmed the immunomodulatory activity and protective efficacy of *Artemisia brevifolia* against mixed *Eimeria* infection in broiler chickens. *Artemisia brevifolia* extract enhanced cellular and humoral immune response in experimental birds. However, additional research work is required to explore the antioxidant and other compounds of *Artemisia brevifolia* which are involved in enhancing the immunomodulatory potential against coccidiosis.

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Authors contribution: KH got the main idea, AA designed the study plan and helped throughout the research work. HAHA, AMAA, AAA, helped in data analysis, interpretation of results and preparation of manuscript. AR, MUW, MAR, RY, BA, NB, HUAK helped in data analysis and finalization of manuscript write up. All authors read and approved the manuscript.

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