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RESEARCH ARTICLE

Immunomodulatory Potential of Sugar Beet *(Beta vulgaris)* Against Coccidiosis in Broiler Chickens

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Abstract

Recent research reports the immunomodulatory capability of *Beta vulgaris* extract against coccidiosis disease in broiler chickens. Immunomodulatory potential of medicinal plant was evaluated by *in vivo* trial. For this purpose, broiler chicks (n=175) were alienated into five groups. At one week of age chicks all groups were orally infected with parasite (oocysts) of mixed *Eimeria* species. At same age group, A, B and C were cured with Beta vulgaris extract at three doses (100, 200 and 300 mg/kg) of body weight. Group D was treated with Vitamin E and served as positive control and Group E was treated with PBS and served as negative control groups. Cellular Immune response was inquired through four classical assays including Dinitrochlorobenzene (DNCB), PHA-P, CON-A and Carbon Clearance test. Humoral immunity (antibodies levels) was evaluated by hemagglutination test. Results of study showed that *B. vulgaris* extract treated groups showed maximum immune response in terms of increasing both cellular and humoral immunity against *Eimeria* parasite. However, the immunomodulatory response of groups cured with *Beta vulgaris* at 300 mg/kg of body weight was higher ($P<0.05$) as compared to negative control group.

Keywords: Sugar Beet, *Eimeria*, Poultry, Medicinal Plant

INTRODUCTION

The poultry industry is experiencing pressure since parasitic disorders referred to as "hidden enemies" cause chronic and sometimes severe losses without visible signs. The poultry sector is facing difficulties in progress due to parasitic diseases [1]. Among them, poultry coccidiosis is affecting poultry industry at larger scale and each year, huge cost is invested a for prevention, cure and productivity reduction arising from coccidiosis disease in poultry. For coccidiosis in United States, the approximate annual cost has been estimated to be more than USD $ 127 million [2], while in China the cost has been estimated to be over USD $ 73 million [3].

Coccidiosis is caused by *Eimeria* parasite having many species. *Eimeria* species are localized in certain regions of the broiler digestive system [4-6]. Seven *Eimeria* species are known to cause coccidiosis in chickens but *E. tenella* and *E. necatrix* are is the most pathogenic, cataclysmic in the production of broiler chickens and all these species are dissimilar concerning their virulence and every one of them impacts various areas of the intestines [7,8]. Infection with *Eimeria* disrupts host mucosal cells resulting in disruption of mucosal integrity, cell permeability and loss of nutrients and proteins from the infected cells. This further leads to poor digestion and assimilation of proteins and other nutrients both major influential factors in subclinical and clinical signs of coccidiosis [9].

Some of the factors promoting the occurrence of coccidiosis include a direct cycle whereby the parasite is spread through direct contact, coprophagic transmission, the occurrence of resistant oocysts, non-susceptibility of a different species of *Eimeria*, a high rate of reproduction of oocysts, high stocking density and environmental factors (sporulation) [10]. The largest cost is the subclinical coccidiosis, which made up to three fourths of the overall costs. This is defined by low flock efficiency resulting from high feed consumption and reduced BWG [11]. Since 1939, synthetic anticoccidial drugs and ionophore drugs are used against coccidiosis. However, drug resistance impacts consumers through drug relatives in poultry products and presently it is not dependable for control [12-14].

Fortunately, the immune response generated is long-lived and strong after *Eimeria* infections and therefore vaccination becomes an option for control since treatment requires anticoccidial drugs. However, vaccines are not effective against all *Eimeria* species and poorly managed can reduce flock's performance [15].

Among novel compounds medicinal plants have shown therapeutics effects against different diseases of poultry and are appealing researchers to explore them [16-18]. Among alternatives, botanicals and herbal substances have shown better anticoccidial impacts among these choices [19,20]. Botanicals that are known to be rich in antioxidant compounds including *Camellia sinensis*, *Ageratum conyzoides*, *Vitis vinifera, Sideritis scardica and* Saccharum *officinarum* have been accounted to show excellent anticoccidial and immunomodulatory impacts counter to avian coccidiosis. *Beta vulgaris* is a well-renowned plant that has various antioxidant properties and have medicinal properties [21,22].

Beta vulgaris is well known plant for its therapeutic and medicinal effects in poultry and livestock. While *Beta vulgaris* contains betaine as its main active, other important compounds include flavonoids, alkaloids, terpenoids, steroids, tannins, saponins which have diverse antioxidant and therapeutic potential against disease of animal and public health concern [23]. In view of the possible restorative impacts current research was conducted to investigate its immunomodulatory potential of *Beta vulgaris* against *Eimeria* infection in experimental broiler birds.

Material and Methods

Ethical Statement

This research was initiated with the approval of the Ethics Committee of Agriculture Faisalabad under PSF, Project No. 185, and PARB, Project No. 358 (No. 628/6-08-2013).

Preparation of Plant Extract

Beta vulgaris roots were purchased from local market of Faisalabad and were authenticated by botanist of Department of Botany and extraction of plant material was done following method [24] using Soxhelet Apparatus and then methanolic extract was stored at 4°C for further experimental use.

Collection and Preservation of Parasite

Guts infected with *Eimeria* parasite were collected from various outbreak places in field and afterward were examined in department of Parasitology. *Eimeria* oocysts were isolated and preserved in 2.5% potassium dichromate arrangement involving the standard protocols as described by [25].

Experimental Design

One hundred and seventy five Hubbard day old broiler chicks (Big Bird®) were bought from local hatchery and were raised under well managed system. Adequate ventilation and water were provided. Temperature, during the first week of age, was maintained at 85-90°F; however, it was reduced on weekly basis by 5°F. Light was provided for 24 h throughout the experimental period. Humidity level of environment was 60-70% as required for rearing of birds. Vaccination against Newcastle Disease, Infectious Bronchitis and Infectious Bursal Disease was done as per schedule in broiler chickens. Standard feed excluding anticoccidial additives were offered to birds *Table 1*. Feed and water was provided *ad libitum*.

Table 1. *Composition of feed offered to experimental chicks*

Ingredients	Percent/ Level
Corn	50.00
Rice	12.00
Rice polishing	3.00
Soybean meal	12.00
Canola meal	12.00
Fish meal	3.00
Corn gluten meal 60%	3.00
Molasses	4.00
DCP	1.00
Premix	0.68
DL-methionine	0.12
L–lysine	0.20

For *in vivo* trial, chicks (n=175) assigned to immunomodulatory experiment were subdivided into five equal (n=35) subgroups, i.e., A, B, C, D and E. Of the total 35 infected chicks in each group, 20 and 15 were used for investigations on cell mediated and humoral immunity respectively. At one week of age all groups were orally infected with 50.000 sporulated oocysts of mixed *Eimeria* species. At two weeks of age (14th day), A, B and C were treated with *Beta vulgaris* extract at three doses (100, 200 and 300 mg/kg) of body weight. All doses of plant extracts were dissolved in PBS and administered orally by using soft plastic tube attached with 05 mL sterile syringe. Chicks in subgroups D treated with commercially available preparation of Vitamin E at 87 mg/kg of body weight in diet. Chicks in group D were treated with PBS (1 ml/bird) which was injected in intra-digital space of chicks.

Group D treated with Vitamin E served as positive control and Group E treated with PBS served as negative control groups. The respective treatments were continued for three consecutive days. The experiment was completed in 40 days and all birds were decapitated at the end of the experiment.

Immunological Evaluation

Evaluation of Cell Mediated Immunity: Cellular Immune response was inquired through four classical assays including Dinitrochlorobenzene (DNCB), PHA-P, CON-A and Carbon Clearance tests and their detail is described as below.

Dinitrochlorobenzene Test: It was used to examine the delayed-type hypersensitivity reaction following [26]. Briefly, on day 14 of the experiment, a primary dose (0.1 mL) of 2% DNCB in acetone was applied on 4 cm^2 area on the skin of each of the five chicks followed by a secondary dose on day 21 of the experiment (7 days post primary dose). Skin thickness (mm) was measured using a vernier caliper pre and 24 h post-application of DNCB both after the primary (day 14) and secondary (day 21) exposure to DNCB.

Phytohemagglutinin-P Test: It intradermally was infused in the chick's foot in internal spaces where as a similar treaty was followed for infusion of PBS in left foot (control group). The screw Guage was utilized to estimate the skin depth at various time spans (hours) post PHA-P infusion.

Cocanavalin-A Test: Cell mediated response to CON-A was evaluated by using standard protocols as described by [27]. For this purpose, blood was collected for separation of peripheral blood lymphocytes for Concanvalin-A (CON-A) test on day 14 and 21 of experiment from experimental chicks (n=5).

Carbon Clearance Assay: Carbon clearance indexed was performed in infected chicks by method as reported by [28]. Carbon readers were used in various groups by involving standard protocols. Optical Density (OD) values were measured at 460 nm in ELISA reader.

Evaluation of Humoral Immunity

Microplate Hemagglutination test was utilized for estimation of antibodies by following [29]. Total antibodies Titers (Igs) and immunoglobulins levels of IgG and IgM were also monitored in birds.

Statistical Analysis

ANOVA and DMR tests were used for calculation of statistical significance among different groups using SAS software.

Results

Cellular Immune Response

Higher cellular immune response to PHA-P antigen was seen in chicks treated with *B. vulgaris* (P<0.05) extract as compared to infected group and higher immune response was observed to CON-A antigen (P<0.05) in *(Fig.1)*. Carbon clearance index was same to that of Vitamin E (P>0.05) and was significantly different to infected groups (P<0.05) and cell mediated response was recorded at various time periods of DNCB and was comparable to Vitamin E (P>0.05) in *(Fig. 2)*.

Humoral Immune Response

Prominent antibody response was detected in chick's treated with *B. vulgaris* extract at highest dose and immune response was same to that of Vitamin E (>0.05)

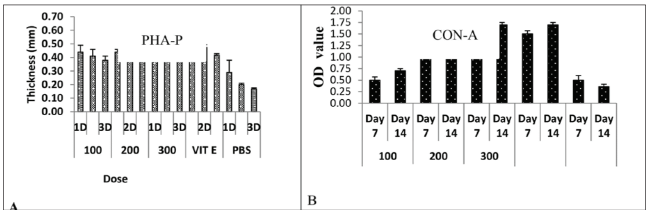

Fig 1. A: Cellular immune response to PHA-P antigen, **B:** Cellular immune response to CON-A antigen. ***VIT E:*** Vitamin E, ***PBS:*** Phosphate-Buffered Saline, ***OD:*** Optical Density

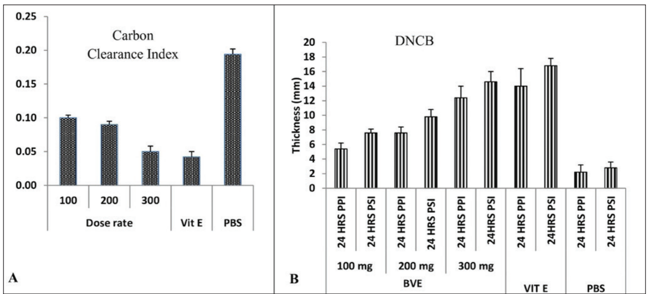

Fig 2. A: Carbon Clearance Index, **B:** Cellular immune response to DNCB. ***VIT E:*** Vitamin E, ***PBS:*** Phosphate-Buffered Saline

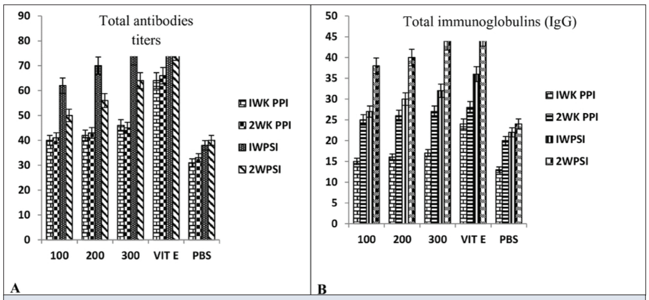

Fig 3. A: Total antibody titers, **B:** Total immunoglobulins (IgG). ***VIT E:*** Vitamin E, ***PBS:*** Phosphate-Buffered Saline

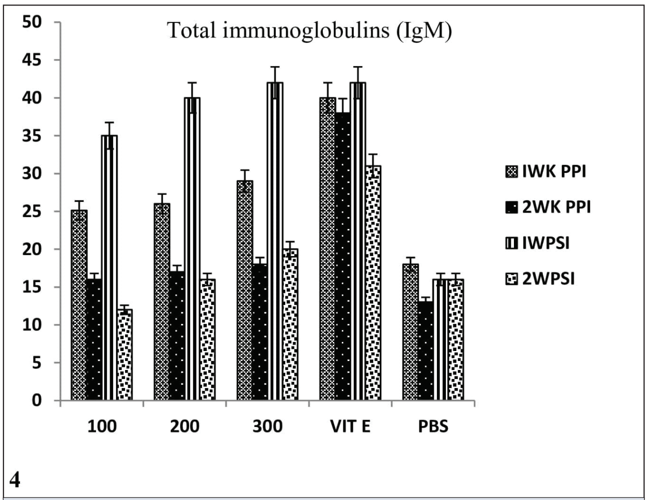

Fig 4. Total immunoglobulins (IgM). ***VIT E:*** Vitamin E, ***PBS:*** Phosphate-Buffered Saline

and elevated Immunoglobulins-G antibody levels were detected in chick's accomplishment the at highest dose of *B. vulgaris* extract and immune response was same to that of Vitamin E in *(Fig. 3)*. Elevated Immunoglobulins-M antibody levels were seen in chick's accomplishment the *B. vulgaris* extract at highest dose and immune response was similar with Vitamin E treated group in *(Fig. 4)*.

Discussion

Botanicals and their compounds have been promising in controlling the pathogenesis of avian coccidiosis as they possess antioxidant and other effective compounds. These compounds provide protection level against coccidiosis and improve cellular and humoral immunity [30,31]. More than 1200 plant species have been reported to possess medicinal and antiprotozoal properties. Plant and their products are included in poultry diets due to growth promoting and natural enhancer impact on bird's immunity [32,33].

Currently research has been focused on use and consumption of herbal product through noting them to be safer approaches in the control of various diseases to reduce drug resistance and toxic effects of drugs. Herbal anticoccidial agents can reduce severity of coccidiosis disease and has positive effect on avian health [34]. Systematic review evidence suggests that medicinal plants have the ability to minimize antibiotic and antiprotozoal inputs in poultry. From results obtained from one study *O. vulgare, C. sativum, A. annua* and *B. pilosa* are suitable plant species for prevention or treatment of bacterial and protozoal infections in poultry. The heterogeneous effect of plants is attributable to variations in dosage and phytochemical contents of the material used on the results of the systematic review. The researchers have already suggested almost 15 years ago a description of used herbal preparations for human clinical trials investigating medicinal plants and similar recommendations should be applied in the further trial's livestock poultry in particular. The lack of patentability for the phytogenic feed additives may be filled by the phytochemical fingerprints alongside that some general descriptions and assessments of the used plant material [35].

In this study, *B. vulgaris* extract exhibited tremendous immunomodulatory activity against *Eimeria* parasite. Similar types of dose dependent trends have also been reported in previous studies on evaluation of immuno-modulatory potential of different herbal extracts [36,37]. Plants driven compounds upgrade cellular resistance by expanding by following up on insusceptible cells by their multiplication and furthermore promote antibodies levels [38]. *Carthamus tinctorius* which is generally known as sunflower have displayed to upgrade cell and humoral insusceptibility counter to poultry coccidiosis [39]. *Triticum aestivum* (wheat bran) polysaccharides (arabinoxylans) have promoted immunomodulatory and protective effects to counter to disease due to *Eimeria* parasite in chickens. It additionally produced positive effects on organ weight gain infected birds.

In another study, *Saccharum officinarum* (sugar cane) extract also produced comparative kind of immunomodulatory impact counter to coccidiosis in chickens [40]. In recent study, it reported the immunomodulatory effects of *Carica papaya* extract and results showed that *Carica papaya* improved cellular and humoral immune response in broiler chickens [21].

B. vulgaris (sugar beet) have a role in improving intestinal health and showed excellent anticoccidial effects against experimental infection in chickens as evidenced by reduced oocyst count, lesion score and reduced mortality. It also improved the hematological and serological parameters of birds. *B. vulgaris* is well known for its antioxidant and exhibited immunomodulatory effects in mice [41].

Rosmarinus officinalis and *Thymus vulgaris* are considered as the most used plants in the Algerian pharmacopoeia and contain a great number of phytochemicals against coccidiosis. By the multi-pharmacological effects of their co-products, they can successfully prevent and treat coccidiosis and mitigate the negative impacts on the host's immune response, redox potential, and gut microbiota due to the *Eimeria* life cycle in many experimental and field trials. The effectiveness of their application in feeds may be different [2].

Furthermore, treatment with the mixture of the medicinal plants can mitigate the impact of coccidiosis in broiler chickens, but this was appreciated in comparison to sulfaclozine. More studies may be required to access the analyzed combination of herbal extracts, to determine the processes by which various compounds have an impact [42]. In another recent research reported the anticoccidial effects of *Illicium verum* (star anise) essential oil in broiler chicks. *Illicium verum* essential oil reduced oocyst count and improved intestinal health of broiler chicks infected with mixed *Eimeria* species [43]. The similar type of Immunomodulatory effects of *Artemisia brevifolia* extract against coccidiosis in broiler chicken. *Artemisia brevifolia* extract improved cellular and humoral immune response in chicks infected with mixed *Eimeria* species [1].

The current research concludes that *Beta vulgaris* has immunomodulatory potential against ccoccidiosis. However, further studies are needed to conduct research and formulate novel drug against *Eimeria* parasite as alternative to synthetic anticoccidial drugs being used in poultry.

Declarations

Availability of Data and Materials: The data will be provided by the main author (A. Abbas) on requirement.

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Ethical Statement: This research was started with the approval of the Ethical Committee of Agriculture Faisalabad under PSF, Project No. 185 and PARB, Project No. 358 (No. 628/6-08-2013).

Competing Interest: There is no dispute of interest between all authors

Generative Artificial Intelligence (AI): No AI tool is used in this manuscript.

Author Contributions: AA anticipated the study; KH, MTA, SS, HS and MMM helped in methodology, work plan, and statistical analysis writeup.

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