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RESEARCH ARTICLE

Immunomodulatory Effects of *Carica papaya* Extract against Experimentally Induced Coccidiosis in Broiler Chickens

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A B S T R A C T

The current study demonstrates the immunomodulatory properties of *Carica papaya* extract to counter experimental coccidiosis. For this purpose, one hundred and seventy-five chicks were placed into 05 groups to evaluate immunomodulatory effects of *C. papaya* extract. At 7th day, chicks of all groups were fed with sporulated oocysts of different *Eimeria* species. Members of groups A, B and C were treated with three doses of *C. papaya* extract (100, 200, and 300 mg/kg of body weight) distinctly. Members of group D were fed with Vitamin-E and served as positive control while Group E was treated with PBS and served as negative Control Group. PHA-P, Carbon Clearance Assay, CON-A and Dinitrochlorobenzene tests were performed for the evaluation of cell mediated immune response. Humoral immune response was evaluated by performing hemagglutination test. Statistical analyses revealed that groups treated with *C. papaya* extract had a stronger cellular and humoral response against coccidiosis. However, the immunological response was excellent in the group treated with *C. papaya* extract at dose rate of 300mg. This shows the potential of *C. papaya* to be used in coccidiosis control programs, but further studies are required to evaluate its complete potential as anticoccidial agent.

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INTRODUCTION

Coccidiosis is an important parasitic disease of poultry caused by *Eimeria* (protozoa) having different species (Abbas *et al*., 2015, 2019; Bachaya *et al.,* 2015). *Eimeria* species cause critical business hinderance to poultry industry in Pakistan (Abbas *et al.*, 2017a, 2017b; Khater *et al.,* 2020). Clinical signs of disease include bloody diarrhea, low FCR and loss of appetite. *Eimeria* oocysts are released in fecal droppings of birds where they sporulate and multiply quickly, making it difficult to control once it has been transmitted to birds (Shivaramaiah *et al*., 2014).

In the past, coccidiosis outbreaks have caused havoc in the poultry industry. Preventive measures and synthetic anticoccidials (synthetic chemicals and ionophores) have helped farmers to avoid severe outbreaks of this disease (Grandi *et al*., 2016). Synthetic anticoccidials have been a popular and effective tool to control coccidiosis in commercial poultry flocks, but some issues are being mentioned by practitioners and scientists which put serious concerns about the future use of these drugs (Hussain *et al.,* 2023). Anticoccidial drug resistance (Abbas *et al*., 2009; 2011a) is among the major problems which are being faced by scientists currently as *Eimeria* parasite is showing resistance to synthetic drugs. Alternatively, vaccines have been developed, but vaccine failures and high costs are minimizing the use of anticoccidial vaccines. These scenarios demand a suitable alternative that may control this devastating disease (Hussain *et al.,* 2017). Synthetic anticoccidial prescriptions are being used for disease prevention, but their viability has been decreased due to resistance, cost and in the field medication restriction issues (Alzahrani *et al*., 2016).

Thus, to make progress in controlling coccidiosis alternative treatments and techniques are being applied by different scientists (Abbas *et al*., 2010; 2011b, c; Bajwa *et al.,* 2022; Hussain *et al.*, 2022, 2023; Jamil *et al.,* 2022). Accordingly, different natural and pseudo natural therapeutic agents including botanicals are executed against infectious diseases in different areas of world

(Abbas *et al*., 2012; Ahmad *et al*., 2022; Kandeel *et al.*, 2022; Nuriyasa *et al.*, 2022; Akhtar *et al*., 2023). Botanical preparations are among the most prominent alternatives which are researched globally for the control of coccidiosis. Among plant products, essential oils are widely popular among researchers. Essential oil-based anticoccidial products like Orego-stim® products have been commercialized. Botanical extracts, their essential oils are environmentally safe, non-toxic and consumer-friendly. Moreover, plant extracts have low risk of resistance development and have exhibited anticoccidial activity by preventing infection or parasite dispersion (Abbas *et al.*, 2017a, 2017b).

Different botanicals like *Camellia sinensis* (Abbas *et al.*, 2017c), *Argyreia speciosa* (Gokhale *et al.*, 2003), *Piper sarmentosum* (Wang *et al*., 2016), *Bidens pilosa* (Chang *et al*., 2016), *Saccharum officinarum* (Abbas *et al*., 2015) and *Beta vulgaris* (Abbas *et al.*, 2017b) have been proved to have brilliant therapeutic and immunomodulatory action against coccidiosis. Different botanicals are well known for their therapeutic and immunomodulatory properties against various disorders of animals and humans (Abbas *et al*., 2012; Shalaby *et al.*, 2022).

Carica papaya is a notable plant having cell reinforcement capacities that has been demonstrated to be helpful in the treatment of a number of diseases of animals and human. It also has anti-inflammatory qualities, protects caecal epithelial cells and inhibits coccidial reproduction. *C. papaya* belongs to the *Caricaceae* family, which has been revealed to be active against poultry coccidiosis in Africa due to its antioxidant and polyphenolic compounds (Nghonjuyi *et al.*, 2015). It can also improve feed palatability, which in turn develops chick growth performance. In light of the various positive possessions of *C. papaya*, the current study was carried out to evaluate its immunomodulatory proficiency of *C. papaya* against experimental *Eimeria* infection.

MATERIALS AND METHODS

Plant material: *Carica papaya* seeds were purchased from local plant markets of Faisalabad, Pakistan and were extracted in methanol utilizing the Soxhlet Apparatus. Plant extract was prepared following Abbas *et al*. (2019). The plant extract was stored at 40°C to be used in *in vivo* trial.

Parasite: *Eimeria* infected guts and intestine were collected from disease cases and poultry shops. Oocysts of *Eimeri*a were harvested and preserved in 2.5% potassium dichromate solution following Ryley *et al*. (1976).

Experimental design: One seventy-five (n=175) broiler chicks were purchased from Al-Jadeed Company Pakistan and were reared under good practices. Water and suitable ventilation were made accessible. Keeping guidelines, immunization against Newcastle Infection and other viral diseases was done following Zaman *et al*. (2012). At 7th day, chicks were divided into 05 equal groups having 35 chicks in each for immunomodulatory testing. All groups were orally infected with 60,000 sporulated oocysts of mixed *Eimeria* species at day 14 of experiment. Groups A, B and C were treated with *C. papaya* extract orally at dosages of 100, 200, and 300 mg/kg body weight distinctly. Group D was served with Vitamin-E and served as positive control group and Group E was treated with PBS and served as negative Control Group. In each group (n=35), 20 chicks were reserved for evaluation of cellular immune response and 15 chicks were reserved for evaluation of humoral immune response. At 2 weeks of age, cellular immune response was evaluated by four assays including Carbon Clearance Assay, PHA-P, CON-A and Dinitrochlorobenzene tests. Humoral immune response was evaluated by Hemagglutination test.

Immunological evaluation

A-Evaluation of cell mediated immunity: Cellular immune response was tested by following four parameters.

Phytohemagglutinin-P and CON-A test: Phytohemagglutinin-P (100g/100ml/chick) was pervaded intradermally in the chick's right foot in interdigital areas, while PBS was managed in the left foot utilizing a similar system (control group). The screw gauge was utilized to evaluate skin thickness at unalike time spans (hours) following injection. Cocanvalin-A Test was applied following method as described by Qureshi *et al.* (2000).

Carbon Clearance Assay (CCA): Carbon assay was applied in different assemblies utilizing laid out techniques, as depicted by Zhang *et al*. (2004).

Dinitrochlorobenzene (DNCB) test: Dinitrochlorobenzene (DNCB) test was utilized to inspect the on-time type extreme touchiness response following Bluminks *et al*. (1974).

B-Evaluation of humoral immunity: The Microplate Hemagglutination Test was done to check the antibodies levels in different groups as described by Qureshi and Havenstein (1994).

Statistical analysis: For Statistical analysis ANOVA and DMR Tests were applied on data to determine significance. P<0.05 was used to determine significance among groups.

RESULTS

Cell mediated immune response: *C. papaya* extract (CPE) enhanced cell mediated immune response in infected chickens. Cellular immune response to PHA-P was significantly (P<0.05) higher in groups treated with *C. papaya* extract (CPE) and prominent cell mediated immune response was observed. However, at higher dose (300mg) CPE showed excellent response as compared to control group than lower doses (Fig. 1).

Cellular response to CON-A was significantly higher in chickens treated with CPE (P<0.05) than PBS treated control group (Fig. 2). Carbon Clearance Index was also significantly higher (P<0.05) in CPE treated groups than PBS treated control group and was similar to Vitamin-E treated group P>0.05) (Fig. 3). Cell mediated response following DNCB administration in CPE treated groups was similar to Vitamin-E treated group (P>0.05) and was significantly higher (P<0.05) than PBS treated control group (Fig. 4).

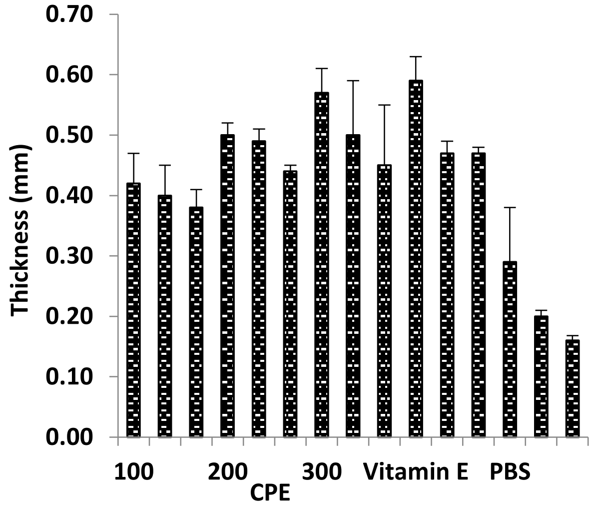

Fig. 1: Cellular immune (PHA-P) response of *C. papaya* extract.

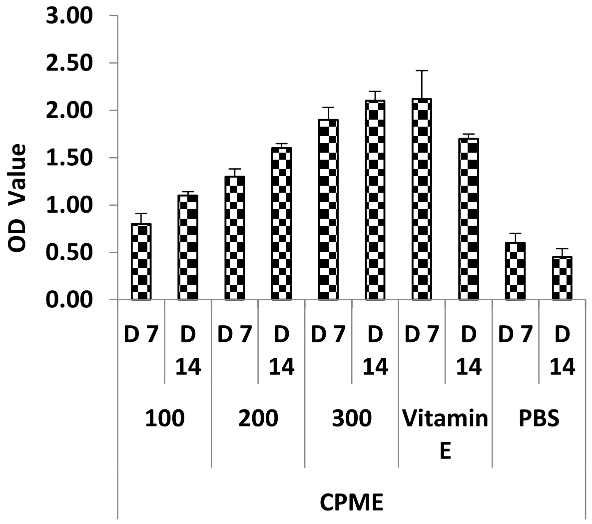

Fig. 2: Cellular Immune Response (CON-A) of *C. papaya* Extract.

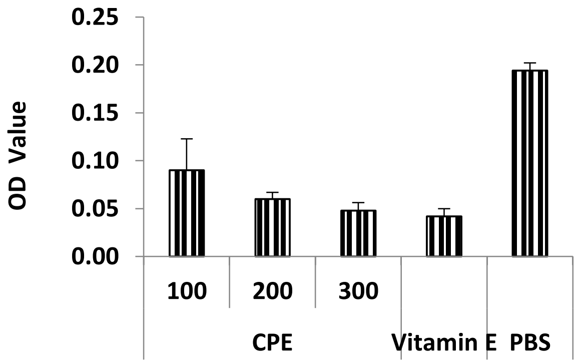

Fig. 3: Cellular immune response (carbon clearance) of *C. Papaya* extract.

Humoral immunity: *C. Papaya* extract (CPE) enhanced total antibody levels that were significantly higher than negative control group ($P<0.05$) (Fig. 5). Higher Immunoglobulins-G levels were observed in CPE treated groups ($P<0.05$) than PBS treated control group (Fig. 6). CPE treated groups demonstrated enhanced immunoglobulins-M levels and at dose of 300mg of CPE, immunoglobulins-M levels were similar to those of Vitamin-E ($P>0.05$) and significantly higher than negative control group ($P<0.05$) (Fig. 7).

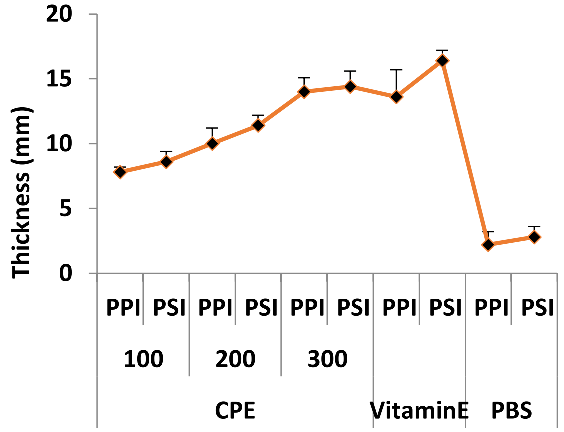

Fig. 4: Cellular immune (DNCB) of *C. papaya* extract.

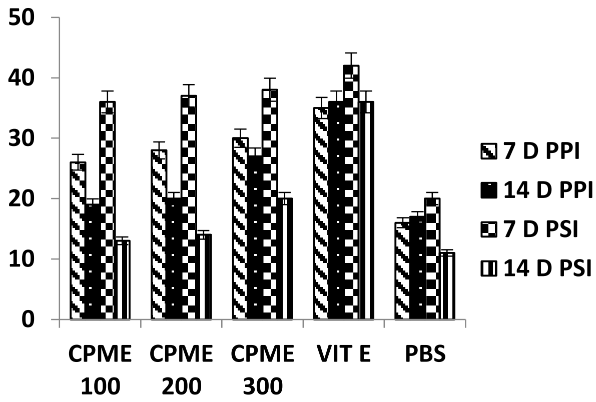

Fig. 5: Total antibody titers of *C. papaya* extract.

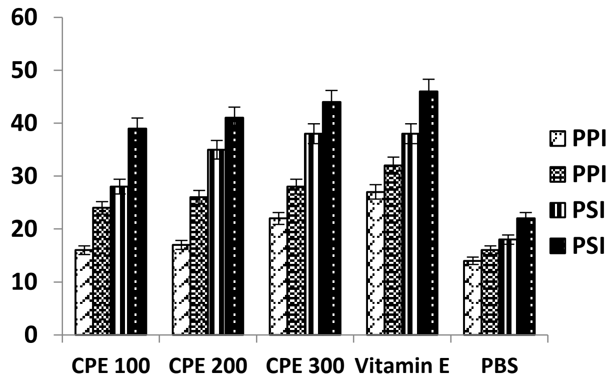

Fig. 6: Humoral immune response (IgG levels) of *C. papaya* extract.

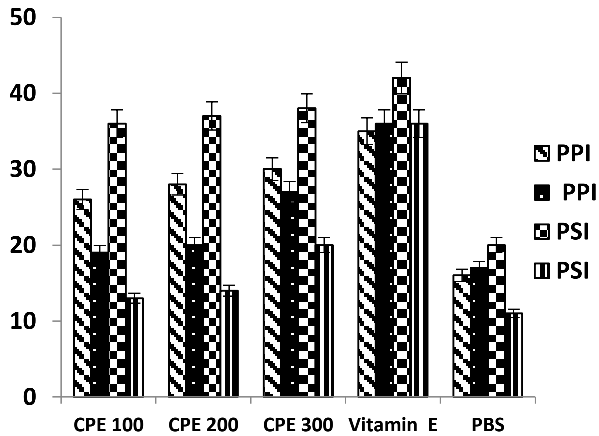

Fig. 7: Humoral immune response (IgM levels) of *C. papaya* extract.

The study's finding revealed that *C. papaya* extract treated groups had a stronger cellular and humoral response to coccidiosis. However, the immunological response was excellent in the group treated with *C. papaya* extract at dose rate of 300mg.

DISCUSSION

Different plants, their extracts, essential oils and their products have shown remarkable effects as alternative to anticoccidial drugs regarding controlling coccidiosis in chickens (Kujawska *et al*., 2009; Yang *et al*., 2015; Grandi *et al*., 2016; Hussain *et al.*, 2023; Saeed *et al*., 2023). Different plants and their products are well known to have potential to control and treat avian coccidiosis due to their beneficial compounds contained in them. The well-known plants having anticoccidial and immunomodulatory efficacy include cereal driven polysaccharides (Wheat, Corn, Rice, Oat etc.), extracts of grape seed, *Dichroafe brifuga, Sinomenium acutum, Ulmus macrocarpa, Dictamnus dasycarpus, Pulsatilla koreana* and many other plants which contain antioxidants compounds (Akhtar *et al*., 2012; Zhang *et al.*, 2012).

Different compounds contained in plants possess antiparasitic, immunomodulatory and medicinal properties (Swantara *et al.*, 2023). Because of having proven successful and effective potential to control coccidiosis, eco-friendly and economic benefits, recently demand and use of novel botanicals have been increased in world (Idris *et al.*, 2017; Jamil *et al.*, 2022). Comparative outcomes on different botanicals have also been described and reported in earlier studies (Singh *et al*., 2015; Laxmi *et al*, 2015; Abbas *et al.*, 2017c). Abbas *et al.* (2017c) conducted a study to evaluate the immunomodulatory effects of *Camellia sinensis* (green tea) against experimental coccidiosis. The supplementation of *Camellia sinensis* in diets of chickens improved cellular and humoral immune response against infection of mixed *Eimeria* species.

Lee *et al.* (2008) reported that *Prunus domestica* commonly known as plum have immunomodulatory effects against coccidiosis as demonstrated by low oocyst shedding, enhanced cellular immune response and increased levels of cytokines in infected chickens which were offered dietary plum. The immunomodulatory effects were high at dose of 1.0% plum in the standard diet. Study concluded that plum can be used as an alternative prophylactic agent against poultry coccidiosis disease. Recently, Hussain *et al.* (2023) reported the immunomodulatory potential of *Artemisia brevifolia* plant in poultry. *Artemisia brevifolia* extract improved immunity in the birds that were experimentally infected with *Eimeria*. Cellular and humoral immunity both were enhanced in the infected birds.

Plants and their products help in increasing immunity levels through multiplication and development in birds (Chihara, 1992; Mickdam *et al.*, 2022). In one study, supplementation of leaves of *Carthamus tinctorius* (sunflower) improved immunity levels against poultry coccidiosis (Lee *et al*., 2009). *Triticum aestivum* or wheat grain polysaccharides (arabinoxylans) were also found to have immunomodulatory influences against *Eimeria* infection (Akhtar *et al*., 2012).

Likewise, *Saccharum officinarum* also showed the improvement in immunological response against coccidiosis (Awais *et al*., 2011). In Chickens, *C. papaya* extract plays a competency in gastrointestinal epithelial adjustment thus diminishing *Eimeria* disease. In another study, *Beta vulgaris* also enhanced immune response in chickens (Wettasinghe *et al*., 2002). In mice, *C. papaya* extract reduced cancer cell multiplication while enhancing immunomodulatory effects (Tripathy and Pradhan, 2013).

Hussain *et al.* (2017) evaluated the potential and effect of *Glycyrrhiza glabra* extract on immune response in broiler chickens. *G. glabra* extract was given at three doses (100, 200 and 300mg). Chicks were orally infected with mixed *Eimeria* species. The results of the study showed that *G. glabra* improved cell mediated and humoral immune response. The results of the current study of *C. papaya* plant are also in line with study conducted on *G. glabra* plant. Recently, Saeed and Alkheraije (2023) have concluded that botanicals are primarily and effectively used as anticoccidials against cecal coccidiosis due to their antioxidant and immunomodulatory activities. Because of the medicinal properties of botanicals, some commercial products have also been developed. However, further research is needed to confirm their pharmacological effects, mechanisms of action and methods of concentrated preparation.

Conclusions: The present study concludes that *Carica papaya* has immunomodulatory properties against *Eimeria* parasite. *Carica papaya* produced immunomodulatory impacts in *Eimeria* infected chickens. However, more exploration is needed to study the dynamic potential in *Carica papaya* and mechanism of action to formulate novel drug against *Eimeria* parasite of poultry.

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Authors contribution: AA and KAA conceived the idea and designed the study. AA conducted major part of research under the guidance and supervision of KAA. Both authors equally contributed in analysis of data and finally approved the manuscript for publication.

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