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IMMUNOMODULATORY EFFECTS OF *CAMELLIA SINENSIS* AGAINST COCCIDIOSIS IN CHICKENS

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ABSTRACT

Purpose of current study was to evaluate the immunomodulatory potential of *Camellia sinensis* in broiler chickens artificially infected with mixed *Eimeria* species. A total of 108 chickens were equally divided in six groups. *Camellia sinensis* in powder form was administered in feed in three graded doses (4, 5 and 6%) containing three control groups (positive control, negative control and normal control). Cell mediated immunity was evaluated using Phytohemagglutinin-P (PHA-P) through classical toe-web assay. Humoral response was determined by micro-hemagglutination test using sheep red blood cells. Results of study demonstrated that *C. sinensis* treatment enhanced cellular and humoral immunity against coccidiosis in dose dependent manner by increasing lymohoproliferative immune response and increased immunoglobulin levels (Total immunoglobulins, IgG and IgM) in broiler chickens. It was concluded from the present study that *C. sinensis* has a potential to be used as immunomodulatory agent against coccidiosis in broiler chickens.

Key words: *Camellia sinensis***,** Coccidiosis, *Eimeria*, Immunomodulation

INTRODUCTION

Coccidiosis is recognized as major diseases of poultry which is caused by single-celled protozoon belonging to genus *Eimeria* having different species (Abbas *et al*., 2012; Chapman, 2014). The main species responsible for coccidoisis in poultry are *E. acervulina, E. tenella, E. praecox, E. mitis, E. brunetti, E. maxima* and *E. necatrix* (Chapman, 2014). Major noticeable characteristics are bloody diarrhea, poor feed conversion ratio, low productivity, and increase in morbidity and mortality of affected chickens (Masood *et al*., 2013). Coccidiosis causes heavy economic losses to commercial poultry farming and is thought to be the one of the most expensive infectious diseases of poultry all over the world including Pakistan (Abbas *et al.*, 2012; Blake and Tomley, 2014; Bachaya *et al*., 2015). According to an estimate, coccidiosis causes about $127 million losses to US poultry industry annually and likewise similar losses may occur worldwide (Chapman, 2014).

Anticoccidial drugs have been effectively administrated for the control of avian coccidiosis (Tewari and Maharana, 2011) but, their frequent and irrational use caused development of anticoccidial drug resistance to different *Eimeria* species. With this reason, the alternative methods are required to overcome this threat. Another effective way to control coccidiosis is the use of vaccines available in both live as well as attenuated forms. Vaccination is not appealing approach because of some constraints like geographical screening before their use, high cost of production and outbreak of diseases in poorly managed poultry production systems. Therefore, there is need to find out the safe alternatives to prevent avian coccidiosis (Zaman *et al*., 2012, 2015; Abbas *et al.*, 2015).

In this context, many plants and herbal products have been found to have chemotherapeutic effect against coccidiosis in poultry and are being commercialized after a series of experimental trials for their validation (Abbas *et al*., 2012). Among herbal anticoccidials, *Camellia sinensis* (green tea) has received a great attention especially due to its polyphenolic contents having strong antioxidant properties (Izzreen and Mohd-Fadzelly, 2013). *Camellia sinensis* is an important plant which contains natural flavonoids. These flavonoids have antioxidant properties due to which they have excellent anticoccidial properties (Jang *et al*., 2007). It has been used as anticarcinogenic and anti-inflammatory agent. Dried leaves of *C. sinensis* contain 30% flavonoids by its dry weight (Perva-Uzunalic ´*et al*., 2006). Plants, rich in antioxidant compounds, having the justification to be explored for their anticoccidial potential because *Eimeria spp.* result in high oxidative stress during coccidiosis disease. Therefore, keeping in view the above mentioned valuable antioxidant properties and positive effects of *C. sinensis*, current study was planned to evaluate immunomodulatory potential of *C. sinensis* in broiler chickens.

MATERIALS AND METHODS

Plant materials: Leaves of *Camellia sinensis* were purchased from local market in Faisalabad (Pakistan). The plant material was identified and authenticated by a botanist in the Department of Botany, University of Agriculture Faisalabad, Pakistan. Powdered plant material was prepared following method described by Abbas *et al*. (2010). Briefly, plant material was dried under shade and ground finely to powder in an electric mill in the department of Parasitology, University of Agriculture, Faisalabad.

Collection of coccidian oocysts: Chicken guts susceptible to coccidian infection were collected from outbreak cases of poultry farms and different poultry sale points of Faisalabad. Guts were opened and contents thus collected from intestines were examined microscopically. The contents were placed in separate desiccators containing 25% laboratory grade sodium hypochlorite @ 4:1 for 25 minutes to discard debris. To remove the chemical, about four times more water was added to the desiccators and sediment was obtained. Coccidian oocysts were extracted following the method described by Ryley *et al*. (1976).

Sporulation of oocysts: Positive samples were placed for sporulation in 2.5% potassium dichromate solution in petri dishes and sporulation of oocysts was done by using method of Ryley *et al*. (1976). Examination of coccidian oocysts was done by making slide and examining under light microscope at 40X to confirm sporulation of oocysts. The oocysts with 4 sporocysts were considered sporulated regardless the shape and size of the sporocysts. The oocysts were slightly flattened under the pressure of a cover slip to better illustrate morphology (Molan *et al*., 2009).

Isolation of the sporulated oocysts: The sporulated oocysts were separated by Zinc sulphate floatation technique (Ryley *et al*., 1976). The counting of washed sporulated oocysts was done by McMaster technique (MAFF, 1986).

Experimental design: The experimental design used in the present study was authenticated and approved by department of parasitology in accordance with approved published research ethics guidelines. Research trial was conducted at experimental station of department of parasitology, University of Agriculture Faisalabad. For *in vivo* trial, 108 (day-old) broiler chicks were procured from local market. Chicks were reared under standard management practices. All the chicks were kept on mash feed ration. At 15th day of age, the chickens were randomly divided into six groups and at the same day first three groups were offered with graded doses of plant material (Table 1) till end of experiment (42 days). Half chickens (n=9) from each group were reserved for evaluation of cell mediated and half (n=9) were reserved for evaluation of humoral immunity. At 18th day of age, the chickens of all groups except group VI were inoculated orally with sporulated oocysts (60,000/chick) with mixed *Eimeria* species.

Table 1. Experimental plan for *in vivo* trial (n=108).

Sr. No.	Treatment groups	Number of birds	Oocyst challenge
1	*Camellia sinensis 4%*	18	60,000
2	*Camellia sinensis 5%*	18	60,000
3	*Camellia sinensis 6%*	18	60,000
4	IM	18	60,000
5	INM	18	60,000
6	NN	18	60,000

IM: Infected medicated group (treated with Toltrazuril), served as positive control
INM: Infected non-medicated group served as negative control
NN: Non-infected, non-medicated group

Immunological evaluation

(a) Cell mediated immunity: Classical Toe-web assay by using Phytohemagglutinin-P (PHA-P) was used to assess the cell-mediated immunity or lymphoblastogenic response in infected chickens following Corrier (1990). For this purpose, on day 14th post-administration of plant material (29th day of experiment), experimental and control chickens were injected PHA-P (Sigma®, USA) (100µg/500µl/ chicken) intradermally between the third and fourth digits of the right foot. The left foot injected with PBS (100µl) served as negative control. The thickness of the interdigital skin was measured with a pressure sensitive micrometer screw gauge at 24, 48 and 72 hours post injection.
Lymphoproliferative response to PHA-P was calculated by the formula:

Lymphoproliferative response = (PHA-P response, right foot) – (PBS response, left foot)

(b) Humoral immunity: Sheep red blood cells **(**SRBCs) were used to demonstrate antibody titers which were detected by using micro plate haemagglutination test (Qureshi and Havenstein, 1994). For this purpose, on day 7th post oral administration of *C. sinensis*, chickens were injected SRBCs (5%) via intramuscular route (1 ml/chicken) followed by a booster at day 7th post primary injection. Blood was collected at day 7 and 14 post primary and secondary injections to separate the serum. Antibody titers were calculated by following procedure.

Haemagglutination test: Serum obtained from the blood samples at 7th and 14th days post first and booster injections to detect the anti-SRBCs antibody titers by haemagglutination test, carried out in 96 well round bottom micro-titration plates (Flow Lab. UK).

Test Procedure: A 50 µl phosphate buffer saline was added in each well of the microtitration plate. A 50 µl of test sera was added in first well and after proper mixing again 50 µl was taken out from 1st well and transferred to the next one and so on up to 11th well to make the two fold serial dilutions whereas the last well was kept as control. Then, 50 µl suspensions of 5% sheep red blood cells was added in each well of the microtitration plate and mixed gently. The plates were incubated at 25°C for half an hour and results were recorded. The titer of the well containing 50% agglutination and 50% reticulum settling (clumping) was considered as the total anti-SRBC antibody titer of the test sera. Results were expressed in terms of geometric mean titers (Burg, 1978).

Determination of IgG Levels: Same protocol was adopted for IgG titers but in the first step 50 µl of 0.01M 2-mercaptoethanol (Riedel-de Haen, Germany) was added in phosphate buffered saline in each well that destroyed the IgM immunoglobulins and the remaining titer was of IgG.

Determination of IgM Levels: Total IgM titer was calculated by subtracting the IgG titer from total antibody titer of the respective samples or by using following formula:

Total IgM titers= Total antibody titer-IgG titer

Statistical Analysis: One way analysis of variance (ANOVA) and Duncan's multiple range tests were used for the determination of statistical significance using SAS statistical analysis software version 2004 (SAS, 2004). Difference were considered statistically significant at (P>0.05).

RESULTS

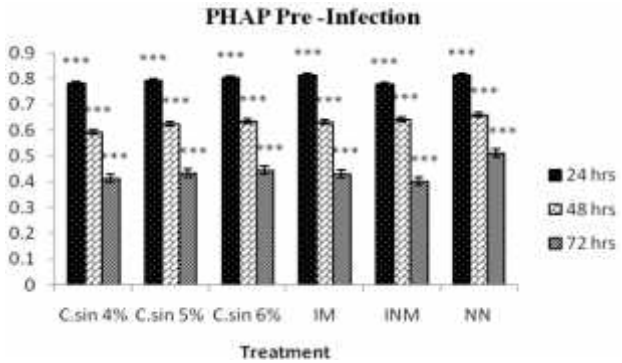
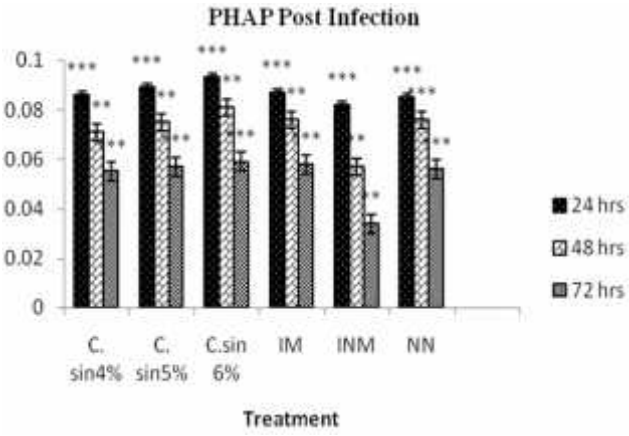


Figure 1: Lympho-Proliferative response to PHAP-Pre infection in *C. sinensis* 4%, 5% and 6% treated broiler chickens artificially infected with mixed *Eimeria* species

IM: Infected medicated group
INM: Infected non-medicated group
NN: Non-infected non- medicated group

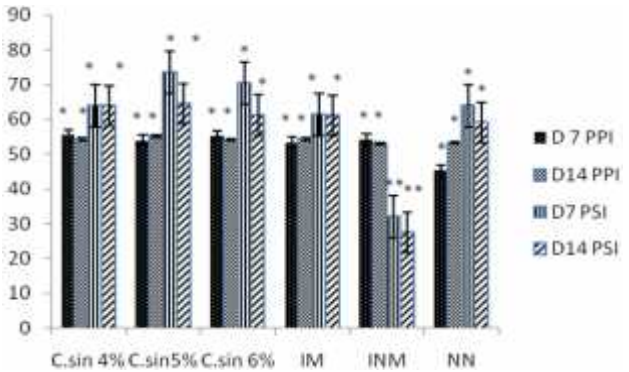
There was no significant difference in pre-PHAP cellular response in all groups at 24, 48 and 72hrs.*** and ***show that there is no significant difference (P>0.05) in all groups. The results are the means and standard error of means.



IM: Infected medicated group
INM: Infected non-medicated group
NN: Non-infected non- medicated group

Figure 2: Lympho-Proliferative response to PHAP-Post infection in *C. sinensis* 4%, 5% and 6% treated broiler chickens artificially infected with mixed *Eimeria* species

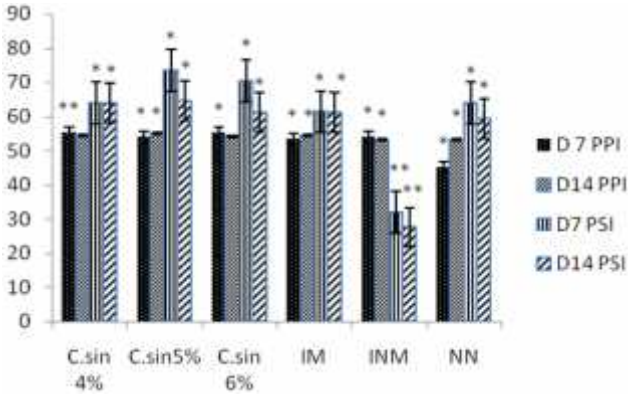
There was significant difference (P<0.05) in post PHA-P cellular response of *C. sinensis* 4, 5 and 6% treated groups as compared to INM group. *** and ** show significant difference (P<0.05) among each other. *C. sinensis* treated chickens showed significant increase in the cellular response as compared to INM group. The results are the means and standard error of means.



PPI; Post primary injection
PSI; Post-secondary injection
IM; infected medicated group
INM; infected non medicated group
NN; non-infected non medicated group

Fig 3: Total anti-SRBCs (sheep red blood cells) antibody titer in *C. sinensis* 4, 5 and 6% treated broiler chickens artificially infected with *Eimeria* species.

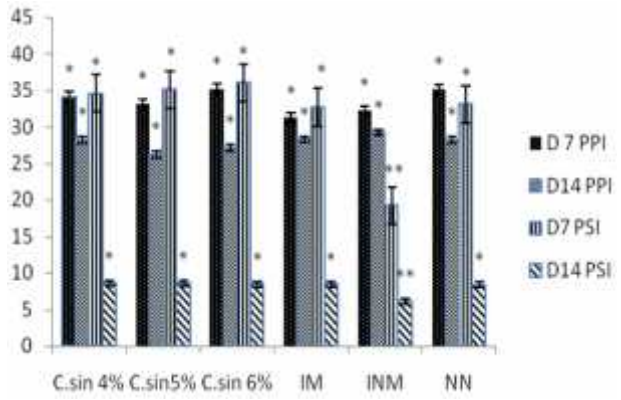
Total anti-body titers at 7 days of PPI were non significantly different (P >0.05) in all groups. At 7 and 14 days of PSI these titers were non significantly different (P>0.05) in *C. sinensis* treated chickens but, were significantly different (P<0.05) to INM group as shown in fig. 3.



PPI; Post primary injection
PSI; Post-secondary injection
IM; infected medicated
INM; infected non medicated
NN; non-infected non medicated

Figure 4: Immunoglobulin- G levels (IgG) in *C. sinensis* 4, 5 and 6% treated broiler chickens artificially infected with *Eimeria* species

IgG titers at 7 days of PPI were non significantly different (P >0.05) in all group. While IgG titers at 7 and 14 days of PSI were nons ignificantly different (P>0.05) in *C. sinensis* treated chickens but, were significantly different (P<0.05) to INM groups as shown in fig. 4.



PPI; Post primary injection
PSI; Post-secondary injection
IM; infected medicated group
INM; infected non medicated group
NN; non-infected non medicated group

Figure 5: Immunoglobulin- M levels (IgM) in *C. sinensis* 4, 5 and 6% treated broiler chickens artificially infected with *Eimeria* species.

IgM titers at 7 days PPI were non significantly different (P >0.05) in all groups. While IgM titres at 7 and 14 days PSI were non significantly different (P>0.05) in *C. sinensis* treated chickens but, were significantly different (P<0.05) to INM group as shown in fig 5.

DISCUSSION

Recently, a significant number of scientific publications have demonstrated the potential benefit of different chemicals of plant origin against avian coccidiosis (Jang *et al*., 2007; Gandi *et al.*, 2016; Idris *et al*., 2017). Use of botanicals and natural products has been proved safe, effective and economically cheap alternatives to anticoccidial drugs for the control of coccidiosis in poultry. Several experiments have confirmed that supplementation of natural herbal preparations in animal feed enhances immune response and causes reduction in severity of various kinds of infections (Jang *et al*., 2007; Awais *et al*., 2011; Aslam *et al*., 2016; Awaad *et al*., 2016). Natural plants contain several useful compounds such as tannins, flavonoids, natural polyphenols and essential oils which show remarkable biological, physiological and therapeutic properties (Ishaq *et al*., 2015; Javed *et al*., 2015; Yue *et al*., 2016; Rehman *et al*., 2016; Chen *et al*., 2016; Liaqat *et al*., 2016).

Wheat bran derived polysaccharides which are named as arabinoxylans were tested against mixed *Eimeria* infections in chickens. It was concluded that organ body weights and production of immune cells by the thymus elevated in groups treated with wheat bran derived polysaccharides which ultimately showed immunostimulatry response against *Eimeria i*nfection. So, it was confirmed that Wheat (*Triticum aestivum*) bran derived polysaccharides arabinoxylans have excellent ability to enhance immunity level against poultry coccidiosis (Akhtar *et al*., 2012a). Awais *et al*. (2011) reported that aqueous and ethanolic extracts of *Saccharum officinarum* which is commonly known as sugar cane have immunotherapeutic effects against the coccidiosis in broiler chickens. The extracts of *Saccharum officinarum* induced the immune response, improved body weight and reduced the oocyst shedding in chickens infected with *E. tenella*. Moreover, the ethanolic extracts showed higher anticoccidial index as compared to aqueous extracts of *Saccharum officinarum*. Akhtar *et al*. (2012b) conducted a study to evaluate the anticoccidial and immunomodulatory effects of *Aloe vera* extracts against coccidiosis in broiler birds and reported excellent results of *Aloe vera* in terms of improved immune response and increased weight gain in birds against mixed *Eimeria* infection. So, it can also be used as an immunomodulatory agent against avian coccidosis. Dalloul *et al*. (2006) evaluated the immunostimulatory and anticoccidial effect of a lectin obtained from the

mushroom *Fomitella fraxinea*. Both the *in vivo* and *in vitro* activity of *F. fraxinea* lectins (FFrL) was determined. FFrL was injected into embryos of 18 day old chicken embryos and mixed *Eimeria* infection was given orally after hatch. Results showed that FFrL treatment resulted in favorable results in terms of weight gain and reduced expulsion of oocyst in feces. Experiment results demonstrated that FFrL extract can be used as an excellent agent for promotion of growth and immunity in broiler birds against coccidiosis.

Recently, similar type of dose dependant immunomodulatory efficacy of *Carica papaya* aqueous extract was observed in rats (Ramesh *et al*., 2016). In another study, *Carthamus tinctorius* (sunflower) leaves have also showed immunomodulatory effects against avian cocccidiosis in dose dependent manner (Lee *et al*., 2009). In another study, methanolic extract of *Beta vulgaris* caused significant inhibition of growth of tumor cells and showed immunomodulatory effects against cancer (Tripathy and Pradhan, 2013).

Neyestani *et al*. (2007) evaluated antimicrobiological effects of tea extract against *Escherichia coli* by *in vitro* method. Results showed that it has antimicrobial effect in dose dependent manner. At high concentration, it caused more antimicrobial activity. Engelhardt (2010) reported that *C. sinensis* also contains some important compounds including alkaloids, carotenoids, minerals, amino acids, carbohydrates, lipids and volatiles compounds. Plants containing such type of antioxidant compounds can be used as an effective anticoccidial agent in birds as similar immunomdulatory results are reported in current study.

Previously, Jang *et al*. (2007) have also reported the anticoccidial effects of *C. sinensis* against *Eimeria maxima* infection in chickens. Immunomodulatory or anticoccidial potential of *C. sinensis* might be attributed due to action of its antioxidant compounds like catechins and their derivatives such as epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (Jang *et al*., 2007). Epigallocatechin gallate was used as an antioxidative (Fraga *et al.*, 1987), anti-inflammatory (Lin and Lin, 1997), antiproliferative (Shammas *et al*., 2006), antibacterial (Mabe *et al.*, 1999), antiviral (Williamson *et al.*, 2006; Mahmood *et al*., 2016), trypanocidal agent (Paveto *et al*., 2004) and also showed inhibitory effect against ovine *Babesia* (Abou-Laila *et al.*, 2010). Furthermore, *C. sinensis* has also been reported very effective in terms of its *in vitro* anthelmintic activity against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Ryu, 1982). Most of the above mentioned studies regarding the antiparasitic and especially anticoccidial activity of *C. sinensis* suggest its activity because of having antioxidant compounds; however, the exact mode of action is still unknown.

Conclusion: Present study confirmed the immunomodulatory effect of *Camellia sinensis* in dose dependent manner against coccidiosis in chickens. This work is helpful for making an herbal immunomodulator to prevent coccidiosis in poultry.

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REFERENCES

Abbas, R.Z., D.D. Colwell and J. Gilleard (2012). Botanicals: An alternative approach for the control of avian coccidiosis. Worlds Poult. Sci. J., 68: 203-215.

Abbas, R.Z., Z. Iqbal, M.N. Khan, M.A. Zafar and M.A. Zia (2010). Anticoccidial activity of *Curcuma longa* L. in broiler chickens. Braz. Arch. Biol. Technol., 53: 63-67.

Abbas, A., Z. Iqbal, R.Z. Abbas, M.K. Khan and J.A. Khan (2015). *In-vitro* anticoccidial potential of *Saccharum officinarum* extract against *Eimeria* oocysts. Bol. Latinoam. Caribe. Plant Med. Aromat., 14: 456-461.

Abou-Laila, M., N. Yokoyama and I. Igarashi (2010). Inhibitory effects of (-)-epigallocatechin-3-gallate from green tea on the growth of *Babesia* parasites. J Parasitol., 137: 785-791.

Akhtar, M., A. Haia, M.M. Awais, Z. Iqbal, F. Muhammad, A.U. Haq and M.I. Anwar (2012a). Immunostimulatory and protective effects of *Aloe vera* against coccidiosis in industrial broiler chickens. Vet. Parasitol., 186: 170–177.

Akhtar, M., A. Fraz, M. Tariq, M.M. Awais, Z. Iqbal, F. Muhammad, M. Shahid and E. Hiszczynska-Sawicka (2012b). Studies on wheat bran Arabinoxylan for its immunostimulatory and protective effects against avian coccidiosis. Carbohydarte Polym., 90:333-339.

Aslam, A., M. I. Shahzad, S. Parveen, H. Ashraf, N. Naz, S.S. Zehra, Z. Kamran, A. Qayyum and M. Mukhtar (2016). Evaluation of antiviral potential of different Cholistani plants against infectious bursal disease and infectious bronchitis virus. Pakistan Vet. J., 36: 302-306.

Awaad, M.H.H., M.A.A. Afify, S.A. Zoulfekar, F.F. Mohammed, M.A. Elmenawy and H.M. Hafez (2016). Modulating effect of peppermint and eucalyptus essential oils on vVND infected chickens. Pakistan Vet. J., 36: 350-355.

Awais, M.M., M. Akhtar, F. Muhammad, A.U. Haq and M.I. Anwar (2011). Immunotherapeutic effects of some sugar cane (*Saccharum officinarum* L.) extracts against coccidiosis in industrial broiler chickens. Exp. Parasitol., 128: 104–110.

Bachaya, H.A., R.Z. Abbas, M.A. Raza, Z. Iqbal, T.U. Rehman, W. Baber and R Hussain, 2015. Existence of coccidiosis and associated risk factors in broiler chickens in Southern Punjab, Pakistan. Pakistan Vet. J., 35: 81-84.

Blake, D.P. and F.M. Tomley (2014). Securing poultry production from the ever-present *Eimeria* challenge. Trends. Parasitol., 30: 12-19.

Burgh, M. A. (1978). Simple method for recording and analyzing serological data. Avian Dis., 2: 362-365.

Chapman, H.D. (2014). Milestones in avian coccidiosis research A review. Poult. Sci., 93, 501-511.

Chen, L., T. Jiang, X. Li, Q. Wang, Y. Wang and Y. Li (2016). Immunomodulatory activity of β- glucan and mannan-oligosaccharides from Saccharomyces cerevisiae on broiler chickens challenged with feed-borne Aspergillus fumigatus. Pakistan Vet. J., 36: 297-301.

Corrier, D.E. (1990). Comparison of phytohemagglutinin-induced cutaneous hypersensitivity reactions in the interdigital skin of broiler and layer chicks. J. Avian. Dis., 34: 369–373.

Dalloul, R.A., H.S. Lillehoj, J.S. Lee, S.H. Lee and K.S. Chung (2006). Immunopotentiating effect of a *Fomitella fraxinea*-Derived Lectin on Chicken Immunity and Resistance to Coccidiosis. Poult. Sci., 85:446–451.

Engelhardt, U.H. (2010). Chemistry of Tea. Comprehensive Natural Products II. Chem. Biol., 9: 999-1032.

Fraga, C.G., V.S. Martino, G.E. Ferraro, J.D. Coussio and A. Boveris (1987). Flavonoids as antioxidants evaluated by in vitro and in situ liver chemiluminescence. Biochem. Pharmacol., 36: 717-720.

Grandi G., L.H. Kramer, A. Quarantelli and F. Righi (2016). Influence of oregano essential oil (OEO) on prevalence and oocyst shedding dynamics of naturally acquired *Eimeria* spp. infection in replacement dairy heifers. Ann. Anim. Sci. 16: 171–179.

Idris, M, R. Z. Abbas, S .Masood, T. Rehman, U. Farooq, W. Babar, R. Hussain, A. Raza and U. Riaz (2017). The potential of antioxidant rich essential oils against avian coccidiosis. World's Poult. Sci. J., 73: 89-104.

Ishaq, B., J.A. Khan, S. Murtaza, R.Z. Abbas, T. Khaliq, A. Khan, H.A. Arshad and H. Anwar (2015). Protective potential of *Trachyspermum ammi* seeds in gentamicin-induced nephrotoxicity in rabbit model. Bol. Latinoam. Caribe. Plant Med. Aromat., 14: 280-286.

Izzreen, M.N. and A.B. Mohd-Fadzelly (2013). Phytochemicals and antioxidant properties of different parts of *Camellia sinensis* leaves from Sabah Tea Plantation in Sabah, Malaysia. Int. Food. Res. J., 20: 307-312.

Javed, S., J. A. Khan, T. Khaliq, I. Javed and R. Z. Abbas (2015). Experimental evaluation of nephroprotective potential of *Calotropis procera* (Ait) flowers against gentamicin-induced toxicity in albino rabbits. Pakistan Vet. J., 35: 222-226.

Jang, I. J., M. Jun, H.S. Lillehog, R.A. Dalloul, I.K. Kong, S. Kim and W. Min (2007). Anticoccidial effect of green tea-based diets against *Eimeria maxima*. Vet. Parasitol., 144: 172-175.

Lee, D.Y., G. Choi, T. Yoon, M.S. Cheon, B.K. Choo and H.M. Kim (2009). Anti-inflammatory activity of *Chrysanthemum indicum* extract in acute and chronic cutaneous inflammation. J. Ethnopharmacol., 123:149-154.

Liaqat, I., Q. Pervaiz, S.J. Bukhsh, S.I. Ahmed and N. Jahan (2016). Investigation of bactericidal effects of medicinal plant extracts on clinical isolates and monitoring their biofilm forming potential. Pakistan Vet. J., 36: 159-164.

Lin, Y.L. and J.K. Lin (1997). Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappaB. Mol. Pharmacol., 52: 465-472.

Mabe, K., M. Yamada, I. Oguni and T. Takahashi (1999). *In vitro* and *in vivo* activities of tea catechins against *Helicobacter* pylori. Antimicrob. Agents. Chemother., 43: 1788-1791.

Mahmood, M.S., J.L. Mártinez, A. Aslam, A. Rafique, R. Vinet, C. Laurido, I. Hussain, R.Z. Abbas, A. Khan and S. Ali (2016). Antiviral effects of green tea (*Camellia sinensis*) against pathogenic viruses in human and animals (a mini-review). African J. Trad. Complement. Altern. Med., 13: 176-184.

Masood, S., R.Z. Abbas, Z. Iqbal, M.K. Mansoor, Z.D. Sindhu, M.A. Zia and J.A. Khan (2013). Role of natural antioxidants for the control of coccidiosis in poultry, Pakistan Vet. J., 33: 401-407.

Ministry of Agriculture, Fisheries and Food. (1986). Manual of Veterinary Laboratory, Parasitological Techniques, Reference Book 418, Her Majesty's stationery Office, London.

Molan, A.L., L. Zhuojian and S. De, 2009. Effect of pine bark (*Pinus radiata*) extracts on sporulation of coccidian oocysts. Folia Parasitol., 56: 1-5.

Neyestani, T.R., K. Niloufar and G. Azam (2007). Black and green teas may have selective synergistic or antagonistic effects on certain antibiotics against

Streptococcus pyogenes in *vitro*. J. Nut. Environ. Med., 16: 258-266.

Paveto, C., C. María, L. Güida , I. Mónica, G. Esteva, V. Martino, J. Coussio, M. Mirtha, D. Flawiá and N. Torres (2004). Anti-*Trypanosoma cruzi* activity of green tea (*Camellia sinensis*) catechins. Antimicrob. Agents. Chemother., 48, 69-74.

Perva-Uzunalic, A., M. Skerget, Z. Knez, B. Weinreich, F. Ottoand and S. Grucher (2006). Extraction of active ingredients from green tea (*Camellia sinensis*), extraction efficiency of major catechins and caffeine. Food Chem., 4: 597-605.

Qureshi, M.A and G.B. Havenstein (1994). A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed "typical" 1957 and 1991 broiler diets. Poult. Sci., 73: 1805-1812.

Ramesh, K.S., R.S. Kambimath and N. Venkatesan (2016). Study of immunomodulatory activity of aqueous extract of *Carica papaya* in Wistar rats. Nat. J. Physiol. Pharm. Pharmacol., 6: 1-3.

Rehman, N., N. Jahan, K.U. Rahman, K.M. Khan and F. Zafar (2016). Anti-arrhythmic potential of Coriandrum sativum seeds in salt induced arrhythmic rats. Pakistan Vet. J., 36: 465-471.

Ryley, J.F, R. Meade, J.H. Burst and T.E.Robinson (1976). Methods in coccidiosis research: Separation of oocysts from faeces. J. Parasitol., 73: 311-326.

Ryu, E. (1982). Prophylactic effect of tea on pathogenic microorganism infections to humans and animals. II. Protozoacidal effect on *Toxoplasma gondii in vitro* and mice. Int. J. Zoon., 9: 126-131.

SAS, (2004). SAS Statistical Software Version 9.1. SAS Institute Inc. Cary, NC, USA.

Shammas, M.A., P. Neri, H. Koley, R.B. Batchu, R.C. Bertheau, V. Munshi, R. Prabhala, M. Fulciniti, Y.T. Tai, S.P. Treon, R.K. Goyal, K.C. Anderson and N.C. Munshi (2006). Specific killing of multiple myeloma cells by (-)-epigallocatechin-3-gallate extracted from green tea: Biologic activity and therapeutic implications. Blood., 108: 2804-2810.

Tewari A.K. and B.R. Maharana (2011). Control of poultry coccidiosis: changing trends. J. Parasitol. Dis., 35: 10-70.

Tripathy, G. and D. Pradhan (2013). Evaluation of *in vitro* antiproliferative and *in vivo* Immunomodulatory activity of *Beta vulgaris*. Asian J. Pharm. Clin. Res., 6: 127-130.

Williamson, M.P., T.G. McCormick, C.L. Nance and W.T. Shearer (2006). Epigallocatechin gallate, the main polyphenol in green tea, binds to the T-cell receptor, CD4: Potential for HIV-1 therapy. J. Allergy. Clin. Immunol., 118: 1369-1374.

Yue, J., C.Q. Lu, H.Y. Lin, X.N. Wang, J.Q. Zheng, J.J. Chen and R. Gooneratne (2016). Effect of ultrafine pulverization of Senecio scandens on growth, immune system and faecal microorganisms in piglets. Pakistan Vet. J., 36: 425-430.

Zaman, M.A., Z. Iqbal, R.Z. Abbas, and M.N. Khan (2012). Anticoccidial activity of herbal complex in broiler chickens challenged with *Eimeria tenella*. Parasitol., 139: 237-243.

Zaman, M.A., Z. Iqbal, R.Z. Abbas and S. Ehtisham-ul-Haque (2015). *In vitro*, efficacy of herbal extracts against *Eimeria tenella.* Int. J. Agric. Biol., 17: 848-850.