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Phytochemical Screening of *Punica granatum* Peels Powder and Hemato-Biochemical Assessment of Induced *E. coli* Infection in Kaveri Birds Treated with *Punica granatum* Peels Powder and Antibiotic (Doxycycline + Neomycin)

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A B S T R A C T

The present experimental trial was conducted to assess the effect of *Punica granatum* peel powder (PPP) and antibiotic (Doxycycline + Neomycin) against induced *E.coli* infection in Kaveri birds. 120 day old Kaveri birds were randomly divided into 6 groups (T0, T1, T2, T3, T4 and T5). The Kaveri birds in Group-T0 were kept as healthy control. At the age of 8th day, Group-T1 birds were given oral dose of *E. coli* broth containing 0.3-0.5ml (1x10^{9}CFU/ml), Group-T2 were given broth of *E. coli* @ 0.3-0.5ml and PPP @ 1% of feed daily for 29 days, Group-T3 were given broth of *E. coli* @ 0.3-0.5ml and antibiotic @ 1gm/10 kg B.W through drinking water for 7 days. Group-T4 were given broth of *E. coli* @ 0.3-0.5ml and antibiotic @ 1gm/10kg B.W for 7 days through water and PPP @ 1% of feed for 7 days while, Group-T5 were given PPP @ 1% of feed daily. At day 0, 7th, 14th and 28th day intervals, haematobiochemical parameters were recorded at weekly intervals. The *E.coli* infected Kaveri birds showed significant alterations in haematobiochemical parameter at 7th and 14th day post infection intervals of trial. The treatment of PPP through feed against induced *E.coli* infection given in Kaveri birds played partial protective role. The antibiotic (Doxycycline + Neomycin) treatment found to be efficacious against induced *E.coli* infection at 7th and 14th post infection interval. The combination of antibiotic through water with PPP through feed had significant efficacious role than individual treatment at 7th and 14th post infection interval.

Introduction

India is primarily an agricultural based country where farmers mostly are engaged in agriculture and animal husbandry. Among the agriculture-based sectors in India, the poultry sector is regarded to be the most dynamic and rapidly developing industry. The rapid growth in poultry industry is owing to significant genetic improvement and intense nutritional feeding management which has involved poultry birds with the fastest growth and highest production potential. Poultry is domesticated species of bird that is reared for the purpose of producing eggs, meat, and other products for human use. India ranks 3rd in egg production and 4th in meat production which indicating a need to increase

output (Rajkumar *et al.*, 2021). According to the 20th livestock census, total poultry in the country is 851.81 million between 2012 and 2019 poultry population grew at an exponential 16.8 percent. Total egg production is around 103.32 billion per year with increased in egg production of 8.5%. The per capita availability of egg is estimated at 79/person/year ((BAHS, 2019).The poultry meat production in India was found to be over 3.9 million metric tons with increase in poultry meat production by 7.8%. In India, the total birds in backyard poultry and commercial poultry are 317.07 and 534.74 million respectively. The backyard poultry and commercial poultry are increased around 46% and 4.5%, respectively (20th livestock census).

Avian colibacillosis, is an infectious disease of birds, which is regarded as one of the main causes of morbidity and mortality, associated with heavy economic losses to poultry industry by its association with various disease conditions, either as primary pathogen or as a secondary pathogen. It causes variety of disease manifestations in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, coligranuloma, enteritis, cellulitis and salpingitis. Colibacillosis of poultry is characterized in its acute form by septicemia resulting death and in its sub acute form by pericarditis, airsacculitis and perihepatitis (Calnek *et al.*, 1997).

Backyard poultry is a manageable and encouraging enterprise to improve the socioeconomic and nutritional status of rural people, especially landless or poor families with low initial investment and high economic return (Chakravarti *et al.*, 2014). Backyard poultry is a potent tool for upliftment of poor because it requires hardly any infrastructure set-up. Besides income generation and poverty reduction, rural backyard poultry can provide nutrition supplementation in the form of valuable animal protein (Rath *et al.*, 2015).

Kaveri a new poultry strain identified by KVK from Central Poultry Development Organization (CPDO), Bhubaneswar in 2015 and 2016 (Khordha *et al.*, 2015). These birds have characteristic features like low early chick and laying mortality, excellent flock uniformity, early sexual maturity, withstanding predators, laying brown colour eggs etc, as reported by CPDO, 2014. Predation is one of the serious constraints in backyard poultry and withstanding to any predator is the key feature of this strain to be considered for adding in to the backyard poultry production system (Banja *et al.*, 2017).

Pomegranate (*Punica granatum* L.) is an ancient favorite table-fruit of the tropical and subtropical regions of the world, belonging to the family *Punicaceae*. Pomegranate peels are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, galic and ellagic acid). These compounds are concentrated in pomegranate peel and juice which account for 92 per cent of the antioxidant activity associated with the fruit. Gallic acid, ellagic acid and punicalagin, in addition to their free radical scavenging properties, also posses antibacterial activities against intestinal flora, particularly enteric pathogens i.e. *Escherichia coli*, *Salmonella spp*. *Shigella spp.* as well as *Vibrio cholera* (Negi *et al.*, 2003). Moreover, pharmacological properties includes antiatherosclerotic, antimicrobial, antidiabetic, anti-inflammatory, analgesic, antidiarrheal, antimutagenic, antioxidant, antiparasitic, antiviral, astringent, abortifacient, hemorrhoids, cancer-chemopreventive, improve fertility, neuronal activity, gastroprotective, hepatoprotective, nephroprotective and skin whitening activity (Jacinto *et al.*, 2018).

Considering the prevalence of *E.coli* infection as one of the potent infectious disease targeting poultry and the current rearing pattern of Kaveri birds the present trial have been conducted to study *E.coli* infection experimentally in Kaveri birds. Also, with the intense use of herbal medication as current trend, the experimental trial was studied with the objective of assessing the effect of *Punica granatum* peel powder against induced *E.coli* infection.

Materials and Methods

Experimental birds

For the present study 120 Healthy day old Kaveri chicks were purchased and housed in poultry shed of College of Veterinary and Animal Sciences Parbhani, Maharashtra. The Kaveri birds were randomly divided into 6 groups (T_0, T_1, T_2, T_3, T_4 and T_5), each group containing 20 birds. Birds were randomly assigned to the control and treatment groups and were kept in separate pen. Birds were given *ad-libitum* feed according to age group with ample quantity of fresh drinking water.

A proper scientific vaccination schedule was prepared and birds were vaccinated according to that schedule for different diseases. Specially prepared pre-starter, starter and finisher feed (without antibiotic) was purchased from M/s VRK Nutritional Solutions poultry feed manufacture, Miraj, Sangli Maharashtra. The antibiotic (Doxycycline + Neomycin) was purchased from the local medical store of Parbhani.

E.coli culture, Dose and Route of administration

Pure culture of known pathogenic strain of *E.coli* having MTCC No (1610) was obtained from Microbial Type Culture Collection (MTCC), Chandigarh.

This culture was maintained, propagated (as per the manufactures guide line) and used to infect Kaveri chicks for the experimental trial. The infection was given to the Kaveri chicks at 8^{th} day by oral route with a single dose of 0.3 to 0.5 ml of nutrient broth (approximately bacterial concentration of 1x 10^9 CFU/ml).

Preparation, Dose and route of *Punica granatum* peels powder

Fresh *Punica granatum* peels were collected locally, from area in and around Parbhani. The *Punica granatum* plant was identified and authenticated by the Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences Parbhani, Maharashtra. Fresh *Punica granatum* peels were collected locally, from area in and around Parbhani.

The peels were sun dried for 4 to 5 days and were kept in gunny bags. The powder was made by using mixer and grinder. Dried powder of *Punica granatum* peel was given to three different group's visually (Group-T1, Group-T2 and Group-T_5) @ of 1 % of feed.

Antibiotic used for treatment

The antibiotic (Doxycycline + Neomycin) was purchased from the local medical store of Parbhani.

Parameter studied

Hematological studies

Serum biochemical studies

The individual blood samples were tested for Total serum protein (gm/dl), Albumin (gm/dl), Globulin (gm/dl), ALT (IU/L), AST (IU/L), and Creatinine (mg/dl). The biochemical values were estimated using an Automatic Biochemical Analyzer. For each parameter, the procedure and combination of reagents employed were in accordance with the analyser's suggested criteria.

From each group six Kaveri birds were sacrificed at Pre-infection (day 0) and Post-infection (7^{th}, 14^{th} and 28^{th} day) intervals of experimental trial to evaluate the haematological and biochemical parameter

Proximate analysis

Proximate analysis was carried out for dried powder of *Punica granatum* peels by sending sample to AFAQAL, Namakkal. Determinations of Moisture, Crude protein, Crude fiber, Ether extract, Total ash, and Gross energy were performed.

Phytochemical Analysis

Qualitative Phytochemical Screening

The different qualitative chemical tests were performed for establishing profile of dried powder of *Punica granatum* peels for its chemical composition. Chemical tests for the screening and identification of chemical constituents in the *Punica granatum* peels by using cold water extract were carried out using the standard protocol.

Preparation of plant extracts

Extraction of aqueous component

Cold water extraction: A total of 50 grams of dried powder of *Punica granatum* peels was soaked in 250 ml of cold water in a conical flask for 24 hour s and then filtered off using sterile Whatman No. 1 filter paper into a sterile conical flask and evaporated by using solvent distillation apparatus. The extract was got with the help of muslin cloth and centrifuged at 10 000 rpm for 5 min. The supernatant was obtained and stored at 4^0C for further use. (Al-Alak *et al.*, 2015).

Results and Discussion

In present study, the mean values of Kaveri birds of healthy control group-T0 remained comparable with each other for haematological and biochemical parameters at day 0 (Pre-infection) and 21st day (Post-infection) of experimental trial.

Group-T1 (*E. coli* infected)

The kaveri birds of group-T1 which were infected with known strain of *E.coli* showed reduction in haematological (Hb, PCV, TEC) and biochemical parameter (serum total protein, serum albumin and serum globulin) at 7th and 14th day post infection intervals of trial. The reduction in mean hemoglobin concentration and PCV values in the birds of group-T1 might have resulted due to acute bacterial infection concequencing decrease absorption of nutrients leading to anemia Verma *et al.,* (2013), The observations of present study also corroborates with result reported of Sharma *et al.,* (2016), Sonwane *et al., (*2017), Godbole *et al.,* (2018) and Patil *et al.,* (2018). The reduced feed consumption and declined nutrient absorption in gut due to *E.coli* infection and oxidative stress inducing lysis of RBC's might have resulted into reduction in TEC in group-T1 at 7th and 14th day post infection interval. Verma *et al.,* (2013) and Tandale *et al.,* (2019) also observed similar findings in their respective studies.

However, at 7th and 14th day post infection interval, the increase in TLC and heterophil counts might be resulted due to inflammatory response induced by *E. coli* inidicated leucocytosis with heterophilia in *E.coli* infected Kaveri birds. In acute bacterial infection heterophil being a first line of defense attends infection their by which causes heterophilia. The present observation goes well with the findings of Gangane *et al.,* (1996), Sharma *et al.,* (2016), Sonwane *et al.,* (2017), Patil *et al.,* (2018), Hashem *et al.,* (2019) and Tandale *et al.,* (2019).

The significant decrease in mean values of serum total protein, serum albumin and serum globulin indicated hypoproteinemia in experimental group-T1 Kaveri birds. Sharma *et al.,* (2015) reported decrease in total serum protein, serum albumin and serum globulin which might be due to liver and kidney damage leading to failure in plasma protein synthesis. The *E.coli* organisms had multiple organ involvement in its pathogenesis, in general and liver in particular. The wide ranged pathological conditions are induced in liver by *E.coli* infections. Similarly in present study, the hepatopathy was noticed in *E.coli* infected birds as evidenced by related biochemical parameters and histomorphological studies. These observations of present experimental study are in close approximation with findings of Ghany *et al.,* (2013), Verma *et al.,* (2013), Rath *et al.,* (2017), Godbole *et al.,* (2018) and Kumari *et al.,* (2020).

There was increase in serum creatinine, serum AST and serum ALT in Kaveri birds of group-T1 at 7th

and 14th day post infection study period. The increase in mean values of serum creatinine noted in present study might be consequence due to *E.coli* induced nephropathy in Kaveri birds. The present findings in respect to increase in mean creatinine values goes well with the reports of Verma *et al.,* (2013) and Ghany *et al.,* (2013). The hepatocyte membrane distortion is associated with membrane leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of the serum marker enzymes namely ALT and AST as a marker for hepatobiliary damage, Sonwane *et al.,* (2017). The increase in serum AST is indicative of cellular injury to cardiac muscle and hepatocyte. Verma *et al.,* (2013), Tabatabei *et al.,* (2015) and Rath *et al.,* (2017) in their respective studies noted similar observations.

Group-T2 (*E. coli* infected and PP powder treated)

The mean values of Kaveri birds of PPP treated group-T2 showed improvement in haematolgical and biochemical parameters as compared to group-T1 at 7th and 14th day post infection intervals of study. The findings of present study indicated that PPP in the diet improved the level of hemoglobin concentration, PCV and TEC value partially. Iron is a mineral which is required for synthesis of haemoglobin. Sharma *et al.,* (2018) reported that PPP contains iron which would have been helped for hemoglobin formation. In addition, oxidative stress caused by *E.coli* infection attributing to haemolysis might have been reduced by antioxidant potential of PPP.

The mean values of TLC, heterophil and lymphocyte count were partially improved than values in birds of group-T1 at 7th day post infection interval. The mean values of TLC, heterophil and lymphocyte count in Kaveri birds of group-T2 statistically remained comparable with group-T0 at 14th day post infection interval. The findings of present study revealed that, improvement in TLC, heterophil and lymphocyte counts in Kaveri birds of group-T2 as compared to respective mean values of group-T1 might have resulted due to antibacterial activity of PPP. Bhandari *et al.,* (2015); Hamday *et al.,* (2015) evaluated the antibacterial activity of *Punia granatum* peel and observed positive results.

There was significant increase in mean values of total serum protein, serum albumin and serum globulin in Kaveri birds of group-T2 than the respective value of group-T1 at 7th and 14th day post infection intervals of study respectively.

However, these mean values didn't attended to the level of respective control group mean values and remained significantly lower than that of healthy control group-T0 at 7th and 14th day intervals of study respectively. The treatment of PPP given against *E.coli* infection might have elicited this beneficial effect of increase in total serum protein, serum albumin and serum globulin levels in Kaveri birds.

Mean values of serum creatinine, serum AST and serum ALT in Kaveri birds of group-T2 were numerically decreased as compared to mean value in birds of group-T1 at 7th and 14th day post infection intervals. However, the mean values of serum creatinine, serum AST and serum ALT were significantly higher as compared to mean values of healthy control group-T0 at 7th and 14th day post infection intervals of study respectively.

The Results indicated that, there was improvement in these mean values in Kaveri birds of group-T2 as compared to *E.coli* infected group-T1. This might have resulted due to nephroprotective and hepatoprootective effect of PPP. Yassein *et al.,* (2015) observed similar findings with respect to mean values of serum creatinine in Japanese quails.

Jahromi *et al.,* (2015) stated that the administration of pomegranate peel extract in BALB/c mice did not showed any alteration in serum AST values which was attempted to study its toxic effect. The present results in respect to improvement in serum ALT level are in agreement with the findings of Yassein *et al.,* (2015) and Rath *et al.,* (2017).

Group-T3 (*E. coli* infected and Doxycycline + Neomycin treated)

In experimental Kaveri birds of group-T3, while comparing the mean value of hemoglobin, PCV and TEC in Kaveri birds of group-T1 and group-T2 with group-T3, it was found that there was statistically significant increase in mean value of hemoglobin, PCV and TEC in Kaveri birds of group-T3 than the mean value of group-T1 at 7^{th} and 14^{th} day post infection. The significant increase mean value of hemoglobin, PCV and TEC in group-T3 as compared to group-T1 indicated that antibiotic tried was effective against experimentally induced *E.coli* infection in Kaveri birds. The observations of hemoglobin, PCV and TEC values of present experimental study are in close approximation with findings of Sonwane *et al.,* (2017) and Tandale *et al.,* (2019). The present results on improvement in hemoglobin, PCV and TEC level goes well with the findings of Godbole *et al.,* (2018) who assessed the efficacy of antibiotic cephalexine against *E.coli* infection in broilers.

The numerically and statistically significant reduction in mean values of Total Leucocyte Count and heterophil counts in the Kaveri birds of group-T3 was noticed when comparison was made with the mean values of group-T1 and group-T2 at 7^{th} and 14^{th} day of study intervals except, the mean values of group-T3 at 14^{th} day was statistically comparable with group-T2. Also, these values were statistically comparable with respective control group-T0 values. The mean value of TLC and heterophil counts in Kaveri birds of group-T3 showed considerable improvement which might be due to antibiotic (Doxycycline + Neomycin) used. As a fact, the bacterial infections are being treated with antibiotics which help in reducing the induced leucocyte counts and heterophil counts. Feyzi *et al.,* (2017) reported decrease in total leucocyte counts and heterophil counts in broiler birds treated with doxycycline. The mean values of lymphocyte counts were statistically comparable amongst themselves with non-significant variations. The findings of present experimental study are in close approximation with findings Tandale *et al.,* (2019). The mean values of serum total protein, serum albumin and serum globulin in Kaveri birds of group-T3 were improved significantly as compared to group-T1 and group-T2 at 7^{th} and 14^{th} day post infection interval of study, respectively. The results indicated that, the treatment given to *E.coli* infected Kaveri birds with targeted antibiotic significantly improved the level of serum albumin as compared to *E.coli* infected group. Tandale *et al.,* (2019) assessed the efficacy of Leofloxacin as antibiotic in *E.coli* infection in broilers and noted the targeted antibiotic as effective.

In Kaveri birds, the mean values of serum creatinine in Kaveri birds of group-T3 were significantly reduced as compared to group-T1 at 7^{th} and 14^{th} day post infection intervals respectively. Also, there was significant decrease in mean values of serum creatinine in Kaveri birds of group-T3 as compared to mean values of PPP treated group- T2 at 7^{th} day post infection. These mean values in Kaveri birds of group-T3 remained statistically comparable with group-T0 and group-T2 but these values were significantly lower than that of mean values of serum creatinine at 14^{th} day post infection interval during experimental trial.

On screening the data in respect to serum creatinine, it was observed that treatment of *E.coli* infected Kaveri birds with given antibiotic might have protected kidney function. Godbole *et al.,* (2018) observed similar results in broilers which were infected with *E.coli* and treated with antibiotic cephalixine.

In Kaveri birds, the mean values of serum AST and ALT in Kaveri birds of group-T3 were significantly reduced as compared to group T1 at 7^{th} and 14^{th} day post infection intervals respectively. Also, significant and non significant decrease in mean values of serum AST and ALT in Kaveri birds of group-T3 as compared to mean values of PPP treated group-T2 was noticed at 7^{th} and 14^{th} post infection intervals during experimental trial. However, mean values of serum AST and ALT in

birds of group-T3 were statistically comparable to group-T0, group-T4 at 7th and 14th day post infection intervals. The improvement in serum AST and ALT level in present study might have resulted due to antibacterial effects of antibiotic (Doxycycline + Neomycin) given against *E.coli* infection in Kaveri birds. The treatment given might have protected the induced hepatopathy. The present findings in respect to mean AST and ALT values goes well with the reports of Sonwane *et al.,* (2017) and Tandale *et al.,* (2019).

Group-T4 (*E. coli* infected, PPP and Doxycycline + Neomycin treated)

In present study the mean values of hemoglobin, PCV and TEC in Kaveri birds of group-T4 were statistically significant increase when compared with the mean values in birds of group-T1 and group-T2 at 7th and 14th day post infection interval.

However, these values appeared to be comparable with the mean values group-T5 and group-T0 at scheduled intervals of experimental study in Kaveri birds. The hemoglobin, PCV and TEC appeared to be regained in the *E.coli* infected Kaveri birds of group-T4 which were when treated with antibiotic (Doxycycline + Neomycin) through drinking water and PPP through feed might have played protective role in mitigating the induced infection. Tandale *et al.,* (2019) also reported concurrent observations in his experimental study in broilers.

At 7th and 14th day post infection interval of study, the mean values of Total Leucocyte Count, lymphocyte and heterophil counts in Kaveri birds of group-T4 were found to be reduced significantly than the mean values of group-T1.. Moreover, the mean values of TLC and heterophil counts in Kaveri birds of group-T4 showed much improvement than that of group-T2 and group-T3 at 7th and 14th day post infection intervals. The treatment given in present study against *E.coli* infection in Kaveri birds might have controlled the increase in Total Leucocyte Count and heterophil counts. The present results on improvement in Total Leucocyte Count and heterophil counts are in close approximation with the findings of Tandale *et al.,* (2019) who assessed the efficacy of antibiotic Leofloxacin against *E.coli* infection in broilers and noted favourable results.

The mean values of serum total protein, albumin and globulin in Kaveri birds of group-T4 were significantly improved as compared to mean values of group-T1 and group-T2, at 7th and 14th day intervals of study period respectively. However, the mean values in Kaveri birds of group-T4 were non-significantly higher as compared to group-T3 at 7th and 14th day post infection intervals of study, but this mean values in Kaveri birds of group-T4 were statistically comparable with mean values of healthy control group-T0, at 7th and 14th day post infection intervals. The positive improvement in total serum protein, albumin and globulin level after addition of antibiotic through water and PPP in feed might have improved the liver function. This was well supported with the improvement in growth performance of experimental Kaveri birds in present study. These observations are in close approximation with Godbole *et al.,* (2018) and Tandale *et al.,* (2019).

The mean values of serum creatinine, AST and ALT in Kaveri birds of group-T4 were statistically decreased as compared to *E.coli* infected group-T1, group-T2 and group-T3 at 7th and 14th day post infection intervals of study. However, the mean values of serum creatinine, AST and ALT values did not differ significantly when it was compared with the mean values of group-T0 at 7th and 14th day post infection intervals of trial.

The antibacterial effect of antibiotic (Doxycycline + Neomycin) and nephroprotective, hepatoprotecitve effect of PPP might have protected from developing pathological changes and hence, could have contributed in maintaining the serum creatinine, AST and ALT level. The findings of Tandale *et al.*, (2019) are identical with present observations.

Table.1 Experimental design and protocol

Group	Number of birds	Treatment and Dose	Period of experiment
T_0	20	Healthy control	29 days
T_1	20	*E.coli* @ 0.3-0.5ml broth culture ($1x10^9$CFU/ml) by oral route at the age of 8^{th} day.	29 days
T_2	20	*E.coli* @ 0.3-0.5ml broth culture ($1x10^9$CFU/ml) + Pomegranate peels powder @ 1% of feed daily.	29 days
T_3	20	*E.coli* @ 0.3-0.5ml broth culture ($1x10^9$CFU/ml) by oral route at the age of 8^{th} day + (Doxycycline + Neomycin) @ 1gm/10 kg B.W through water for 7 days.	29 days
T_4	20	*E.coli* @ 0.3-0.5ml broth culture ($1x10^9$CFU/ml) by oral route at the age of 8^{th} day+ (Doxycycline + Neomycin) @ 1gm/10 kg B.W through water for 7 days+ Pomegranate peels powder @ 1% of feed daily for 7 days.	29 days
T_5	20	Pomegranate peels powder @ 1% of feed daily from 8^{th} day till end of trial.	29 days

Table.2 The following hematological investigations were carried out as per the methods described below

Sr. No.	Parameter	Method of estimation	Reference
1	Hemoglobin	Acid haematin	Jain (1986)
2	Packed cell volume	Microhaematocrit	Jain (1986)
3	Total erythrocyte count	Haemocytometer	Natt and Herrick's (1952) and Nambiar (1960)
4	Total leukocyte count	Haemocytometer	Natt and Herrick's (1952) and Nambiar (1960)
5	Differential leukocyte count	Wright's stain	Lucas and Jamroz (1974)

Table.3 Mean values of Hb, PCV, TEC, TLC, Heterophil and Lymphocyte in Kaveri birds

Group	Hb (gm/dl)	PCV (%)	TEC (10^6/mm^3)	TLC (10^3/mm^3)	Heterophil (%)	Lymphocyte (%)
7th day post infection interval						
T0	11.01^{a} ±0.39	33.20^{a} ± 0.86	3.30^{a} ± 0.06	25.30^{c} ± 0.27	26.50^{c} ± 0.42	63.50^{a} ± 1.38
T1	9.61^{c} ± 0.25	28.53^{c} ± 0.89	2.78^{c} ± 0.09	31.18^{a} ± 0.63	35.50^{a} ± 2.20	54.66^{b} ± 2.20
T2	9.97^{bc} ± 0.14	29.98^{bc} ±0.98	3.08^{b} ± 0.07	28.06^{b} ± 0.40	30.33^{b} ± 1.62	60.33^{a} ± 1.38
T3	10.71^{ab} ± 0.42	31.72^{ab} ± 1.45	3.21^{ab} ±0.05	27.14^{bc} ±1.01	28.83^{bc} ±1.13	61.83^{a} ± 1.19
T4	10.98^{a} ± 0.24	32.33^{ab} ± 0.67	3.27^{ab} ±0.05	26.09^{c} ± 0.71	27.50^{bc} ±0.34	62.66^{a} ± 0.49
T5	11.08^{a} ± 0.30	33.34^{a} ± 0.93	3.33^{a} ± 0.07	25.50^{c} ± 0.66	26.66^{c} ± 0.49	63.16^{a} ± 0.65
	HS	**HS**	**HS**	**HS**	**HS**	**HS**
14th day post infection interval						
T0	11.15^{ab} ± 0.28	34.06^{a} ± 0.99	3.47^{a} ± 0.05	27.75^{abc} ±0.49	28.16^{b} ± 0.87	62.16^{a} ± 0.79
T1	9.99^{c} ± 0.38	29.60^{c} ± 1.01	3.01^{c} ± 0.04	29.39^{a} ± 0.85	33.00^{a} ± 1.39	57.66^{b} ± 1.81
T2	10.22^{bc} ± 0.27	30.44^{bc} ± 0.69	3.11^{c} ± 0.06	28.89^{ab} ± 0.44	29.16^{b} ± 1.24	62.00^{a} ± 1.29
T3	10.97^{ab} ± 0.21	31.86^{abc}± 1.08	3.30^{b} ± 0.03	28.23^{abc} ±0.56	28.50^{b} ± 1.23	62.33^{a} ± 1.22
T4	11.20^{ab} ± 0.43	33.66^{ab} ± 1.63	3.40^{ab} ±0.04	27.54^{bc} ± 0.62	27.66^{b} ± 0.74	63.83^{a} ± 0.47
T5	11.21^{a} ± 0.37	34.33^{a} ± 1.41	3.43^{ab} ±0.05	26.52^{c} ± 0.65	28.33^{b} ± 0.80	63.00 ^{a} ± 0.63
	S	**S**	**HS**	**S**	**S**	**S**
21st day post infection interval						
T0	11.42 ± 0.22	33.66 ± 0.45	3.50 ± 0.08	28.12 ± 0.68	28.83 ± 0.74	62.00 ± 0.89
T1	10.85 ± 0.61	32.98 ± 1.49	3.29 ± 0.03	27.23 ± 0.70	29.33 ± 1.02	61.16 ± 1.30
T2	11.01 ± 0.27	33.15 ± 0.64	3.33 ± 0.06	27.06 ± 0.47	28.50 ± 1.33	62.00 ± 1.15
T3	11.18 ± 0.24	33.20 ± 0.73	3.37 ± 0.03	27.00 ± 0.40	28.33 ± 0.61	62.33 ± 0.95
T4	11.28 ± 0.47	33.36 ± 0.96	3.44 ± 0.06	26.97 ± 0.12	28.16 ± 1.13	63.00 ± 1.21
T5	11.35 ± 0.39	33.53 ± 0.89	3.51 ± 0.06	26.90 ± 0.39	28.00 ± 0.63	62.66 ± 0.66
	NS	**NS**	**NS**	**NS**	**NS**	**NS**

Table.4 Mean values of Serum Total Protein, Serum Albumin, Serum Globulin, Serum Creatinine, Serum Aspartate Transaminase, Serum Alanine Transaminase in Kaveri birds

Group	Serum Total Protein (gm/dl)	Serum Albumin (gm/dl)	Serum Globulin (gm/dl)	Serum Creatinine (gm/dl)	Serum Aspartate Transaminase (IU/L)	Serum Alanine Transaminase (IU/L)
7th day post infection interval						
T0	$4.53^{a} \pm 0.03$	$2.39^{a} \pm 0.01$	$2.13^{ab} \pm 0.04$	$0.91^{c} \pm 0.06$	$125.73^{c} \pm 1.64$	$11.02^{d} \pm 0.36$
T1	$3.42^{c} \pm 0.02$	$1.87^{c} \pm 0.05$	$1.54^{c} \pm 0.05$	$1.89^{a} \pm 0.05$	$150.33^{a} \pm 2.51$	$16.86^{a} \pm 0.20$
T2	$3.96^{b} \pm 0.07$	$2.09^{b} \pm 0.07$	$1.86^{b} \pm 0.10$	$1.69^{a} \pm 0.05$	$138.50^{b} \pm 4.53$	$14.96^{b} \pm 0.36$
T3	$4.40^{a} \pm 0.05$	$2.24^{ab} \pm 0.04$	$2.08^{ab} \pm 0.09$	$1.35^{b} \pm 0.16$	$127.38^{c} \pm 3.92$	$13.74^{c} \pm 0.11$
T4	$4.50^{a} \pm 0.10$	$2.30^{a} \pm 0.04$	$2.10^{ab} \pm 0.08$	$1.02^{c} \pm 0.12$	$126.86^{c} \pm 1.17$	$11.26^{d} \pm 0.34$
T5	$4.52^{a} \pm 0.07$	$2.37^{a} \pm 0.09$	$2.12^{ab} \pm 0.17$	$1.06^{bc} \pm 0.09$	$125.91^{c} \pm 2.25$	$11.16^{d} \pm 0.48$
	HS	**HS**	**HS**	**HS**	**HS**	**HS**
14th day post infection interval						
T0	$4.77^{a} \pm 0.07$	$2.37^{a} \pm 0.09$	$2.39^{a} \pm 0.15$	$0.89^{c} \pm 0.08$	$127.38^{bc} \pm 4.01$	$11.13^{c} \pm 0.24$
T1	$3.92^{c} \pm 0.04$	$1.99^{c} \pm 0.07$	$1.93^{b} \pm 0.11$	$1.34^{a} \pm 0.08$	$141.30^{a} \pm 1.48$	$14.91^{a} \pm 0.68$
T2	$4.38^{b} \pm 0.04$	$2.17^{b} \pm 0.05$	$2.18^{b} \pm 0.07$	$1.15^{ab} \pm 0.06$	$134.50^{ab} \pm 2.75$	$12.75^{b} \pm 0.23$
T3	$4.72^{a} \pm 0.05$	$2.35^{a} \pm 0.07$	$2.36^{a} \pm 0.10$	$0.95^{bc} \pm 0.06$	$129.68^{bc} \pm 2.01$	$11.25^{c} \pm 0.04$
T4	$4.79^{a} \pm 0.02$	$2.40^{a} \pm 0.03$	$2.38^{a} \pm 0.04$	$0.90^{c} \pm 0.06$	$127.53^{bc} \pm 2.6$	$11.08^{c} \pm 0.09$
T5	$4.74^{a} \pm 0.05$	$2.50^{a} \pm 0.02$	$2.24^{a} \pm 0.06$	$0.89^{c} \pm 0.08$	$127.25^{c} \pm 3.93$	$11.00^{c} \pm 0.15$
	S	**HS**	**S**	**HS**	**HS**	**HS**
21st day post infection interval						
T0	4.74 ± 0.02	2.49 ± 0.03	2.25 ± 0.03	0.79 ± 0.02	128.86 ± 2.52	11.35 ± 0.48
T1	4.65 ± 0.04	2.45 ± 0.02	2.19 ± 0.05	0.88 ± 0.03	134.36 ± 2.27	11.23 ± 0.34
T2	4.71 ± 0.02	2.47 ± 0.04	2.23 ± 0.03	0.87 ± 0.02	132.21 ± 3.04	11.20 ± 0.46
T3	4.73 ± 0.03	2.52 ± 0.02	2.20 ± 0.05	0.85 ± 0.02	129.87 ± 3.41	11.18 ± 0.39
T4	4.78 ± 0.01	2.55 ± 0.02	2.22 ± 0.03	0.84 ± 0.03	128.98 ± 1.33	11.13 ± 0.28
T5	4.74 ± 0.03	2.50 ± 0.01	2.24 ± 0.03	0.79 ± 0.02	128.53 ± 0.48	11.17 ±0.24
	NS	**NS**	**NS**	**NS**	**NS**	**NS**

Table.5 Proximate analysis of dried powder of *Punica granatum* peels

Sr. No	Test Name	Result	Test Method
1.	Moisture	9.39%	AOAC 930.15
2.	Crude Protein	4.37%	AOAC 984.13
3.	Crude Fibre	17.81%	AOAC 962.08
4.	Ether Extract	1.39%	AOAC 2003.06
5.	Total Ash	3.65%	IS 14827-2000
6.	Gross Energy	3626 Kcal/kg	Calculation

Table.6 Phytochemical analysis of dried powder of *Punica granatum* peels

Sr. No.	Phytochemical	Result	Test Performed
1.	Carbohydrate	Positive	Benedict's Test
2.	Saponins	Positive	Foam Test
3.	Quninoes	Positive	Sodium hydroxide Test
4.	Steroid	Negative	Salkowski Test
5.	Resins	Positive	Acetone Test
6.	Alkaloid	Positive	Mayer's Test
7.	Tannins	Positive	Ferric chloride Test
8.	Flavonoids	Positive	Alkaline reagent Test

Group-T5 (PPP control)

Mean values of haematobiochemical parameters in Kaveri birds of group-T5 were significantly improved than values in birds of group-II, III and IV and mean values of all these parameters were at par with values in birds of group-T0 at 7^{th} and 14^{th} day post infection interval of study. Thus, treatment of PPP along with le significantly improved altered values of all haematobiochemical parameters in *E. coli* infected birds. However, perusal of available literature did not reveal any data related to findings of present study.

Proximate analysis

Proximate analysis of dried powder of *Punica granatum* peels are mention in Table.5.

Determinations of moisture, crude protein, crude fiber, ether extract, total ash, and gross energy were performed to know the nutritional significance (Table 4.3). Kaur *et al.,* (2018) and Salama *et al.,* (2020) also reported similar observation. Thus findings of present study are compatible with result reported by above authors.

Phytochemical analysis

Phytochemical analysis of dried powder of *Punica granatum* peels are mention in Table.6

Qualitative phytochemical screening of *punica granatum* peel powder revealed presence of carbohydrate, saponins, quninoes, resins, alkaloid, tannins, flavonoids and absence of steroid. Similar observations were recorded by Uchgaonkar *et al.*, (2011); Hasni sayyed *et al.,* (2012), Nitave *et al.*, (2014); Bhandary *et al.,* (2012) and Sharma *et al.*, (2018).

In conclusion, on induction, *E.coli* was developed infection in Kaveri birds as evidenced by alterations in haematobiochemical parameters. The treatment of PPP through feed against induced *E.coli* infection given in Kaveri birds, played partial protective role. The antibiotic (Doxycycline + Neomycin) treatment against induced *E.coli* infection in Kaveri birds found to be efficacious. The combination of antibiotic (Doxycycline + Neomycin) through water with PPP through feed in *E.coli* infected Kaveri birds had significant efficacious role as evidenced by results obtained than individual treatment. Presence of phytochemical alkaloid, tannin and flavonoids in PPP which act as primary antioxidants or free radical scavengers which protect the liver function and is effective against *E.coli* infection.

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