

Efficacy of Guava Leaves (*Psidiumguajava*) Suspension on Growth, Antibacterial and Hematological Performances in *E. coli* Included Japanese Quail

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Abstract: The present study was designed to determine the growth, antibacterial and hematological effects of guava leaf (*Psidiumguajava*) suspension in *E. coli* infected Japanese quail under the Department of Physiology and Pharmacology, HSTU, Dinajpur, Bangladesh, during September to October, 2017. A total of 40 quails of 10 days old were randomly assigned into four treatment groups named T_0 , T_1 , T_2 , T_3 and each group contained 10 birds. Group T_0 and T_1 were considered as negative and positive control, respectively. Group T_2 and T_3 were treated with guava leaf (*Psidiumguajava*) suspension and Doxycycline respectively. At 5 days interval, live body weights were recorded, bacterial loads in feces were counted and blood parameters were determined. Mortality was also observed in different treatment groups throughout the experiment. Body weights were significantly ($P < 0.05$) increased in T_2 and T_3 group compared to T_0 and T_1 where as the bacterial load counts were significantly ($P < 0.05$) decreased in T_2 and T_3 compared to T_0 and T_1 groups. The present study was revealed that the mortality rate was significantly ($P < 0.05$) higher in T_1 group. There were significant ($P < 0.05$) variation of total leucocytes count neutrophil, lymphocyte and eosinophil among the different treatment groups. But, the eosinophil count was insignificantly ($P > 0.05$) varied among the treatment groups on the day 22th and 32th.

Keywords: Guava leaves, quail, *E. coli*, antibiotics

1. Introduction

Quail is a small avian species belongs to the Pheasant family. In 1595 first domesticated in Japan. Two species of quail found in India i.e. The Black breasted quail found in Jungle (*Coturnix Coromandelica*) other species is the Brown color Japanese Quail (*Coturnix Coturnix Japonica*) which is bred for meat or the one used for commercial Quail production. Quail is fast growing bird with a short generation gap. Quail were first introduced in India in 1974 from California (Mishra Priti and Shukla Satish, 2014). Require minimum space for rearing. Require small capital. Quails are robust bird. Birds can sale at the early age of five weeks. It becomes mature at the age of six to seven weeks then start laying eggs. High rate of clutch up to 280. Quail meat is tasty other than chicken and has low fat content. It promotes body and brain development in young ones. Quail farming is a cheap enterprise compare to chicken farming. It is useful as choice of food. Quail is the important bird for scientific research. This species can be reared at interior places. It does not require the vaccination and medication. Quail litter has high fertilizer value and can be used for increasing yield of crops. Quail weighs up to 100 gm and lays 100 eggs a year, the Japanese quail weighs up to 250 gm and lays 250 eggs a year.

As per the nutritional criteria, the quail eggs are far better compare to that of chicken eggs. It has low cholesterol percentage. Quail meat and eggs are good for the pregnant women and infant feeding women (Mishra Priti and Shukla Satish, 2014). Japanese quail (*Coturnix Coturnix japonica*) is a recent addition to the poultry farming in Bangladesh. Quail farming for egg and meat is quite popular in Japan,

Hongkong, Korea, China, Singapore, India, Thailand, Malaysia, Indonesia, France, Italy, Germany, Britain and Russia. In Bangladesh it was introduced for the first time in 1990. There are about 131 species of wild quail found all over the world (Goetz, 1987). Only Bobwhite quail (*Colinusvirginianus*) and Japanese quail have been domesticated for commercial purposes. Japanese quail has several breeds and varieties of which Pharaoh (wild type), British Range, English White, Manchurian Golden, Tuxedo are most popular (Singh, et al., 1982; Panda, et al., 1987; Panda, 1990). Among these, Pharaoh is widely raised all over the world. It has two popular colour strains, wild colour and brown colour (Rahman, 1995).

In Bangladesh only these two are commercially available (Siddique and Mandal, 1996). The climate and natural condition of Bangladesh is also very suitable for quail rearing. Quail can be reared in this country throughout the year and shows a good performance in meat and egg production. It has a shorter life cycle and its production requires less capital and land. Quail may be a source of income in addition to chicken and ducks for its immense potentiality for meat and egg production (Paul and Sarker, 1992).

Escherichia coli is a part of the common microbial flora of the intestine of poultry and most isolates are nonpathogenic. About 10 to 15% of intestinal coliforms are pathogenic serotypes (Barnes and Gross, 1997). Pathogenic *E. coli* are also present in the poultry environment. *Escherichia coli* causes a variety of lesions in poultry, including yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis (Gross, 1994).

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Colibacillosis is an economically important disease, which is prevalent throughout the world (Margie and Lawrence, 1999).

Several serotypes of *Escherichia coli* have been associated with disease conditions in poultry, the most common manifestation being colisepticemia (Sojka, 1965). *E. coli* O78:K80 is one of the serogroups most commonly isolated from affected birds (Hemsley et al., 1967 and Sojka, 1965). *Escherichia coli* (*E. coli*) infection includes colibacillosis, Hjarre's disease coligranuloma, peritonitis, salpingitis, synovitis, omphalitis, air sac disease etc. Colibacillosis occurs as an acute fatal septicemia or subacute pericarditis and airsacculitis. It is a common systemic disease of economic importance in poultry and is seen worldwide. *E. coli* is a normal inhabitant of the intestinal tracts of animals and birds and is harmless as long as it is kept in check by other intestinal bacteria (Barnes et al., 2003) although most are nonpathogenic, a limited number produce extra-intestinal infections and its presence in drinking water is considered indicative of faecal contamination. *E. coli* persist for long period of time, particularly when dry rodents droppings often contain pathogenic coliforms.

Guava is a small tropical tree that grows up to 35 feet tall; it is widely grown for its fruit in tropics. It is a member of the Myrtaceae family, with about 133 genera and more than 3,800 species. The leaves and bark of *P. guajava* tree have a long history of medicinal uses that are still employed today (Nwinyi et al., 2008).

According to our socio-economic situation, the knowledge of our farmer is very little because most of them are not properly trained for poultry production, but unemployed young generation is coming in this business for short return of value and profit. Pharmaceutical companies take this advantage. They are convincing farmers for using antibiotics as a growth promoter or life savings for poultry. As a result, each and every poultry is a depot of antibiotics. When these poultry are consumed by human this antibiotic residue enters into human body and causing serious human health hazards with drug residues. Due to the prohibition of most of antimicrobial growth promoters (AGP), plant extracts have gained interest in animal feed strategies. Medicinal plants are cheap and renewable sources of pharmacologically active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basile et al., 1999).

Guava (*Psidium guajava* Linn.) commonly known for its food and nutritional values throughout the world. The medicinal properties of guava fruit, leaf and other parts of the plant are also well known in traditional system of medicine. Since, each part of guava tree possesses economic value, it is grown on commercial scale. Guava plant is considerable process has been achieved regarding the biological activity and medicinal application of guava and the fruit considered as poor man apple of tropics. The guava plant parts are used for the development of various industrial and pharmaceutical products (Priya, 2011).

Psidium guajava L. (guava), a fruit plant belonging to the family Myrtaceae, is found all over the world. Guava leaves, roots, and fruits have been used for the prevention and

treatment of diarrhea (Lutterrodt, 1989; Alnieida et al., 1995). In several studies, guava showed significant antibacterial activity against common food borne diarrhea-causing bacteria such as *Staphylococcus spp.*, *Shigella spp.*, *Salmonella spp.*, *Bacillus spp.*, *E. coli*, *Clostridium spp.*, and food spoilage bacteria such as *Pseudomonas spp* (Alnieida et al., 1995; Jaiarj et al., 1999; Farhana et al., 2017).

Guava leaves have long been recognized for their antimicrobial activity (Bansode and Chavan, 2014). Guava leaves have several chemical constituents such as comarins, essential oils, flavonoids, triterpenes and ellagitannins which are known to have antimicrobial properties (Sapkota et al., 2012).

In view of above facts, the present study was undertaken with a view to fulfilling the following objectives:

- 1) To know the effect of Guava leaf suspension on body weight, blood parameters in quail infected with *E. coli*.
- 2) To know the effect of Guava leaves suspension against *E. coli*.
- 3) To differentiate the antibacterial effect of Guava leaf from synthetic drug.

2. Materials and Methods

2.1 Study area and study period

The present study was conducted during September to October, 2017 in the research unit under the Department of Physiology & Pharmacology at Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

2.2 Study birds

A total number of 40 quails were collected from Bahadur Bazar, Dinajpur, Bangladesh.

2.3 Collection and management of quails

At 10 days of age, forty (40) Japanese quails were used for study. They were collected from Bhadur Bazar, Dinajpur, Bangladesh and fed with quail commercial (Power Feed Ltd., Gagipur, Bangladesh) and water ad libitum. The quails were allowed to acclimatize in their new environment for 6 days before the commencement of the experiment. After collection, glucose and vitamin C were supplied with drinking water for three days. They were divided into 4 Groups (Groups T₀, T₁, T₂, & T₃) and were kept in separate quail cages. The body weights of assigned quails were taken with digital weight balance and the data were recorded. Also feces was collected for the examination of bacteria colony count.

2.4 Experimental designs

The quails were randomly divided into 4 equal Groups (T₀, T₁, T₂, T₃) in which each group consisted of 10 birds for assessing the efficacy of guava leaves extracted juice and antibiotic as mortality, hematological parameter, postmortem, bacterial load and growth performance of quail.

Group T₀ was kept on -ve control (no supply *E. coli* & Guava leaves).

Group T₁ was +ve control (supply *E. coli* bacteria but no Guava leaves).

Group T₂ was supplied *E. coli* & were treated with Guava leaves juice @ 1.5 ml per 100 ml drinking water for 10 days.

Group T₃ was supplied *E. coli* & were treated with Antibiotic (Doxycycline) @ 1 g per 2L drinking water for 10 days.

Layout of the Experiment

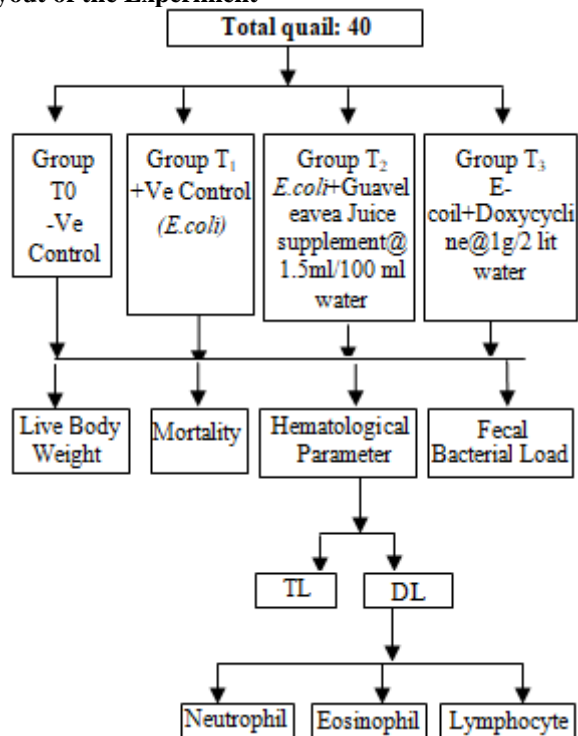


Figure 1: Layout of the experimental design (each group consisting of ten quails)

2.5 Test organisms collection and preparation

The test organism (*E. coli*) was collected from the laboratory under the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

Nutrient broth (NB) was used to grow the organisms from the collected samples before feeding the quails orally (Cheesebrough, 1985).



Figure 2: Japanese quail in experimental shed

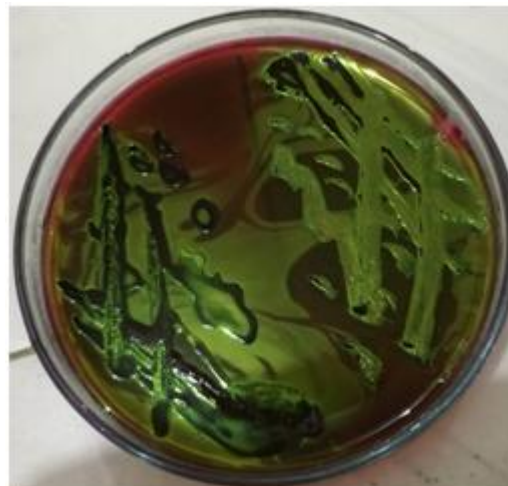


Figure 3: Test organism metallic sheen product by *E. coli* on EMB agar

2.5.1 Preparation of nutrient broth

2.5.1.1 Requirements

Bacto-nutrient broth (Difco)..... 13.0 g
 Distilled water..... 1000 ml

2.5.1.2 Procedure

13.0 grams of Bacto-nutrient broth (Difco) was dissolved in 1000 ml of cold distilled water and heated up to boiling to dissolve it completely. The solution was then distributed in tubes, stoppered with cotton plugs and sterilized in the autoclave machine at 121°C and 15 pounds pressure per square inch for 15 minutes. The sterility of the medium was judged by incubating overnight at 37°C and used for cultural characterization (Carter, 1979).

2.5.2 Inoculation of organism

The organism (*E. coli*) was inoculated into nutrient broth media by metallic loop and was incubated for overnight in incubator at 37°C temp.

2.5.3 Feeding of organism

The nutrient broth culture was shaken properly and the quails of groups T₁, T₂ & T₃ were inoculated orally with 2-3 drops of the inoculums at 6th day after resting period.

2.6 Collection and processing of plant materials

Guava leaves were collected from the HSTU, Dinajpur, Bangladesh. The collected young guava leaves were washed in the tap water and the fleshy parts were mashed with the help of pestle and mortar. The guava leaf juice was extracted from mashed leaf.

2.7 Feeding of experimental diet



Figure 4: Guava Leave



Figure 5: Mashed of guava

The guava juice was supplied to the quails of T₂ group with drinking water @ 1.5 ml per 100 ml drinking water for 10 days.

Doxycycline antibiotic was supplied to the quails of T₃ group with drinking water @ 1g per 2L drinking water for 10 days.

2.8 Recording body weight

Body weight of each bird was recorded at five days interval with the help of digital balance.



Figure 6: Measurement of body weight

2.9 Estimation of bacterial loads

A number of total four (4) feces samples were collected directly from different groups T₀, T₁, T₂ & T₃. The samples were brought to the bacteriology laboratory, Department of

Microbiology, HSTU, Dinajpur, Bangladesh and processed for the bacteriological colony examination.

2.9.1 Preparation of culture media

2.9.1.1 Eosin Methylene Blue (EMB) agar medium

Eosin methylene blue (EMB) agar medium was used to observe the growth of *Escherichia coli* (Cheesebrough, 1985).

2.9.1.2 Requirements

EMB agar base (Hi-media, India)..... 36.0 g
Distilled water 1000 ml

2.9.1.3 Procedure

36.0 grams of EMB agar base (Hi-media, India) was added to 1000 ml of distilled water in a conical flask and heated until boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in to sterile glass petridishes. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged and used or stored at 4°C in refrigerator for future use (Carter, 1979).

2.9.2 Serial dilution of sample

The number of total four feces samples were weighted 1 g individually on digital weighting balance and diluted into 100 ml PBS solution as serial 10 fold dilution.



Figure 7: Prepared Serial dilution

Serial 10 fold dilutions of each of the feces samples in a series of dilution tubes were prepared. At first for each of the feces samples 10 sterile test tubes were placed on a test tube holder rack containing 9 ml of 2% buffered peptone water.

1 ml feces sample was mixed with 9 ml of Phosphate buffer solution in the 1st test tube in order to make 10-1 dilution. Then 1ml solution from 1st test tube mixed with 2nd test tube, then from 2nd test tube to 3rd test tube and finally 9th to 10th test tube and 1ml discard from 10th test tube by the help of pipette and in every steps mixing was done properly.

2.9.3 Enumeration of Total Viable Count (TVC)

For the determination of total viable bacterial count, 1 ml of each ten-fold dilution was transferred and spread on duplicate plate count agar using a fresh pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a cotton bud. One cotton bud was used for each plate. The plates were then kept in an incubator at 37 for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted by the digital colony counter machine. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to Icrate MSF 1998. The results of the total bacterial count were expressed as the number of organism or colony forming units per ml (CFU/ml) of feces sample.



Figure 8: Counts of Bacterial colony

2.10 Haematological parameters

0.5 ml blood from each group was collected from wing vein with the help of syringe (1 ml) and needle. The collected blood was sent to the Tavfiqve Agro Lab, Rangpur, Bangladesh for the estimation of different blood parameters such as TLC (Total Leucocytes Count) and DLC (Different Leucocytes Count). The blood parameters were determined by semi automatic haematological analyzer machine (cure inc. U.S.A.).



Figure 9: Blood Collection of Quail from wine vein

2.11 Recording mortality percentages

The quail cages were observed everyday and the number of death bird was recorded on the recording note book. The mortality percentage was calculated by the following formula:

$$\text{Mortality (\%)} = \frac{\text{No. of death bird}}{\text{Total no. of bird}} \times 100$$

2.12 Statistical analysis

Data were analyzed by analysis of variance using Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1986) using Completely Randomized Design (CRD). Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Performed with the help of SPSS 20 software to find out the difference among the treatments.

3. Results and Discussions

3.1 Effect of treatment with guava leaf suspension and Doxycycline on the body weight of quails infected with *E. coli*

Table 1 shows the various treatments on the body weight of quail. *E. coli* infection affected body weight gain of infected quail. There was significantly ($P < 0.05$) increased in body weight in T_2 (guava leaf extract) and T_3 group (Doxycycline) than T_0 (negative control) and T_1 (positive control). At the age of 32 days the highest body weight was found in T_3 group (98.30 ± 1.66) followed by T_2 (96.50 ± 1.95), T_0 (89.20 ± 1.34) and T_1 (80.80 ± 3.70). The present results are more or less similar to the study of Geidam *et al.*, (2015), who reported that body weight was found higher in guava extract and oxytetracycline than group infected with *E. coli*. El-Sayed *et al.*, (2013), also reported that guava leaves had a significant improved effect on body weight and weight gain and FRC in broiler. On the other hand, some researchers (Rattanaphol and Rattanaphol, 2009) and (Wedy, 2012) declared that use of 0.04% or 0.06% of guava leaves extract in poultry ration didn't have significant effect on body weight and weight gain.

Table 1: Average body weight (Mean±SEM) of quail infected with *E. coli* and treated with guava leaf suspension and Doxycycline

Age (Days)	T ₀	T ₁	T ₂	T ₃	Level of significance
17	30.20±1.85 ^a	30.30±1.89 ^a	29.50±1.57 ^a	29.80±1.55 ^a	NS
22	50.00±1.89 ^a	44.10±2.62 ^a	49.90±1.83 ^a	49.80±1.68 ^a	NS
27	70.56±1.61 ^b	63.40±2.39 ^a	77.30±2.37 ^c	79.00±2.45 ^c	*
32	89.20±1.34 ^b	80.80±3.70 ^a	96.50±1.95 ^c	98.30±1.66 ^c	*

Note: Values are expressed as mean± standard error of means. ^{a,b,c} Means between column are statistically significant (P<0.05) *= Significant at 5% level of significance. NS means statistically not significant.

3.2 Effect of treatment with guava leaf suspension and Doxycycline on the faecal bacterial load count of quails infected with *E. coli*

The results of average bacterial load count in the feces of quail are shown in table 2. The present results indicate that bacterial shedding load was significantly (P<0.05) increased in T₀ and T₁ but significantly (P<0.05) decrease in T₂ and T₃ group in relation to age of quail. The bacterial load count in T₀ group was 88.00±1.03, 88.00±1.03, 92.00±1.03 and 96.00±1.03 at day 17, 22, 27 and 32 respectively. In T₁ group the bacterial load was 184.00±1.03, 188.00±1.03, 200.00±1.03 and 196.00±1.03 at day 17, 22, 27 and 32 respectively. In T₂ group the bacterial load was 184.00±1.03, 196.00±1.03, 164.00±1.11 and 96.00±0.79 at day 17, 22, 27 and 32 respectively. In T₃ group the bacterial load was 184.00±1.03, 156.00±1.03, 76.00±1.03 and 72.00±1.03 at day 17, 22, 27 and 32 respectively. The present results are in the line of the observation of Geidametal., (2015), who observed that bacterial shedding load was significantly lower in groups treated with guava leaf extract and oxytetracycline than those without intervention in chickens. Vieira et al., 2001 have also reported antibacterial effect of guava leaves extract and found that they inhibited growth of bacteria. Similar results are also observed by Mohammad et al., 2012. Who reported that significant antibacterial activity against *S. aureus* and *E. coli*. Gitika and Kumar (2016). Also reported that guava leaves extract had antibacterial effect against *E. coli*.

Table 2: Mean bacterial colony count (Mean±SEM) of quail infected with *E. coli* and treated with guava leaf suspension and Doxycycline

Group	Age (Days)				Level of significance
	17	22	27	32	
T ₀	88.00±1.03 ^a	88.00±1.03 ^a	92.00±1.03 ^b	96.00±1.03 ^c	*
T ₁	184.00±1.03 ^a	188.00±1.03 ^b	200.00±1.03 ^d	196.00±1.03 ^c	*
T ₂	184.00±1.03 ^c	196.00±1.03 ^d	164.00±1.11 ^b	96.00±0.79 ^a	*
T ₃	184.00±1.03 ^d	156.00±1.03 ^c	76.00±1.03 ^b	72.00±1.03 ^a	*

Note: Values are expressed as mean± standard error of means. Means between column are statistically significant (P<0.05) *= Significant at 5% level of significance.

3.3 Effect of treatment with guava leaf suspension and Doxycycline on mortality (%) of quails infected with *E. coli*

The mortality (%) among the different treatment groups is shown in table 3. The present study revealed that mortality (%) was significantly (P<0.05) higher in T₁ (40%) group than others. But in guava leaf extracted group (T₂), there was found mortality (%) zero (0%). In T₃ group 1 (10%) quail was died before treatment. Birdi et al., (2010) and Birdi et al., (2011), reported that guava leaves have a broad spectrum of antimicrobial action. Biswas et al., (2013), also determined antibacterial potential of guava leaf extract against *E. coli* and *Salmonella enteritidis*.

Table 3: Effect of treatment with guava leaf suspension and Doxycycline on mortality (%) of quails infected with *E. coli*

Parameter	T ₀	T ₁	T ₂	T ₃	χ ²	Level of significance
Mortality (%)	1 (10%)	4 (40%)	0 (0%)	0 (0%)	9.83	*

Note: Means between column are statistically significant (P<0.05) *= Significant at 5% level of significance.

3.4 Effect of treatment with guava leaf suspension and Doxycycline on the different blood parameters of quails infected with *E. coli*

Table 4 shows the results of different blood parameters in different treatment groups. In the present study, it was found that there was significant variation of TLC, neutrophil (%), Lymphocyte (%), eosinophil (%) among the different treatment groups (T₀, T₁, T₂ and T₃) but at day 22 and day 32, the eosinophil (%) varied insignificantly among the treatment groups. Similar results are also reported by El-Sayed et al., (2013). Who found that guava leaf significantly increased TLC count.

Table 4: Effect of treatment with guava leaf suspension and Doxycycline on the different blood parameters (TLC, Neutrophil, Lymphocyte and Eosinophil) of quails infected with *E. coli*

Age (Days)	T ₀	T ₁	T ₂	T ₃	Level of significance
TLC					
22	6600.00±70.71 ^b	6000.00±70.71 ^a	6800.00±70.71 ^b	6200.00±70.71 ^a	*
27	6500.00±70.71 ^b	5800.00±70.71 ^a	6600.00±70.71 ^b	6000.00±70.71 ^a	*
32	6200.00±70.71 ^a	6700.00±70.71 ^b	6500.00±70.71 ^b	6200.00±70.71 ^a	*
Neutrophil					
22	40.00±1.70 ^b	50.00±1.70 ^c	30.00±1.70 ^a	40.00±1.70 ^b	*
27	50.00±1.70 ^b	60.00±1.70 ^c	40.00±1.70 ^a	50.00±1.70 ^b	*
32	50.00±1.70 ^b	45.00±1.70 ^{ab}	40.00±1.70 ^a	45.00±1.70 ^{ab}	*
Lymphocyte					
22	20.00±1.61 ^a	30.00±1.70 ^c	25.00±1.84 ^{ab}	25.00±1.84 ^{ab}	*
27	30.00±1.70 ^a	40.00±1.61 ^b	35.00±1.61 ^a	30.00±1.61 ^a	*
32	60.00±1.70 ^b	50.00±1.61 ^a	45.00±1.70 ^a	50.00±1.61 ^a	*
Eosinophil					

22	2.00± 0.32 ^a	2.00± 0.32 ^a	1.00± 0.32 ^a	1.20± 0.37 ^a	NS
27	1.20± 0.37 ^a	3.00± 0.32 ^b	1.20± 0.37 ^a	1.20± 0.37 ^a	*
32	1.20± 0.37 ^a	2.00± 0.32 ^a	1.20± 0.37 ^a	1.20± 0.37 ^a	NS

Note: Values are expressed as mean± standard error of means. Means between column are statistically significant (P<0.05) *= Significant at 5% level of significance. NS means statistically not significant.

4. Conclusion and Recommendation

This study was conducted to investigate the effect of guava leaf (*Psidiumguajava*) suspension on *E. coli* quail. The treatment groups T₂, T₃ had statistically increased body weight and decreased bacterial load count in relation to control group. The mortality (%) was significant lower in T₂ group (%), which was higher in T₁ group that was infected with *E. coli*. It is concluded that guava leaf (*Psidiumguajava*) suspension effectively controlled *E. coli* infection and had effective antibacterial activity. Guava leaf and Doxycycline had the similar effect on the body weight and bacterial load. Guava leaf suspension can be used instead of Doxycycline. It is recommended that further research is needed to ascertain the mechanism of action for its application in clinical practice.

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