Study the efficacy of *Andrographis paniculata* (Kalomegh) leaf extract as a growth promoter in Broiler and determination of its antibacterial activity

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ABSTRACT: This experiment was conducted to evaluate the efficacy of Kalomegh *(Andrographis paniculata*) leaves extract supplementation in drinking water as a growth promoter in broiler chickens. A total of 40 day-old Chicks were purchased from local hatchery and after 7 days of acclimatization chicks were randomly divided into two groups, A and B. The group A was kept as a control and not treated. The group B was supplemented with 2% Kalomegh leaf extract with drinking water. Weekly observations were recorded for live body weight gain up to 5th weeks between A and B groups. The initial body weight of groups A and B on 7th day of this experiment were 150±5.76gm and 155±6.32gm, respectively and after 35th day of experiment final body weight were 1500±19.90gm and 1600±20.87gm, respectively; the net body weight gain were 1350±17.98gm and 1455±18.98gm, respectively. The treatment group B was recorded statistically significant (at 1% level) increase for live body weight than that of control group A. The results suggest that better growth performance could be achieved in broilers supplemented with Kalomegh leaves extract. Other hand antibiotic disks made of this extract were produced significant zone of inhibition in case of *E.coli* and *Staphylococcus aureus* organisms.

Keywords: Kalomegh, *Andrographis paniculata*, *E.coli*, *Staphylococcus aureus*

1. INTRODUCTION

In Bangladesh, broiler production plays an important role in income generation of farmers. To improve broiler production, some farmers use antibiotics. The excessive use of these antibiotics has led to contamination of Bangladeshi broiler meat. Moreover, there is now a world tendency to produce "natural" food which is free from chemicals, the so called "Green Products". Growth promoters are chemical and biological substances which are added to livestock food with the aim to improve the growth of chickens in fattening improve the utilization of food and in this way realize better production and financial results. Attempts to use medicinal plants in broiler production instead of antibiotics have been made by both farmers and researchers. However, only few trials have been carried out so far (Bunyapraphatsara, 2000). One of the medicinal plants that seem promising is *Andrographis paniculata* (AP), a shrub found throughout Southeast Asia. It is a well known medicinal plant commonly used in humans as an immune system booster. Also, it is very widely used in China, for healing common colds, inflammations and diarrhea (MPRI, 1999). The plant has some medicinal properties against diarrhea and dysentery in humans (Thanangkul and Chaichantipyuth, 1985). Its main active compound is thought to be andrographolide, a diterpenoid lactone (Tang and Eisenbrandt, 1992). More recently, medicinal plant extracts were developed and proposed for use in food as natural antimicrobials (Hsieh *et al*., 2001). However, little or no work has been done on the effects of plant extracts on body weight and performance in poultry. The present study was conducted to determine the effect of *Andrographis paniculata* (Kalomegh) extracts in broiler diets as a possible alternative to antibiotic feed additives and also determine the antibacterial activity of aqueous extracts of Kalomegh leaf powder using disk agar diffusion method.

2. MATERIALS AND METHODS

The experiment was conducted at the Department of Pharmacology and Microbiology & Hygiene, Bangladesh Agricultural University, Mymensingh, during the period from 21 April 13 to 25 May 2013.

I. Selection and collection of plants and their processing

The *Andrographis paniculata* (Kalomegh) plant selected for study was mature and free from disease condition or other deformities. Kalomegh leaves were collected from local area.

II. Preparation of Kalomegh *(Andrographis paniculata*)

After collection *Andrographis paniculata* (Kalomegh) leaves were weighted separately by electric balance for preparation of 2% (20gm fresh leaves in 1000ml water each) water extract. For the preparation of *Andrographis paniculata* leaves extract, the leaves were thoroughly washed. Then the leaves were made paste by mortar and pestle and water was added for preparation. Sodium chloride added 1% as preservative.

III. Preparation of the experimental house and equipment

An open sided house which was partitioned into 6 pens of equal size by using expanded wire net, wood, rod and bamboo materials. A service area was running along the middle of the pens. It was brushed, swiped properly and cleaned with tap water. After washing with clean water, the pens were disinfected by using chlorine solution (50ppm). The room was left vacant for 14 days. Later, it was again disinfected with Finis solution (1 gm/liter water) left to dry up properly. During this time, all the feeders waters and other necessary equipment were properly cleaned, washed and disinfected with phenol solution and dried before use.

IV. Collection of birds

Day old chicks marketed by Nourish Farms Ltd. were purchased from local market for this experiment. The chickens were acclimatized for 7 days. The experiment was carried in poultry shed in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh. The body weight of all selected day-old chicks ranged from 30gm to 80gm respectively. The chicks were supplied with normal diet and water.

V. Experimental diets

The commercial broiler starter and pre-starter diets were purchased from the local agent in Mymensingh.

VI. Managements

Fresh and dried rice husk was used as a litter at a depth of 3cm. The litter was stirred three times a week from 14 days to prevent cake formation. Litter material when found damp was replaced by new litter. Each pen was 2.5ft x 2ft which was for ten birds. Therefore, the space given for each bird was 1 square ft. The bird was brooded with one 100 watt electric bulb in each pen from day old to 21 days. The bulb was hanged just above the bird's level at the center of each pen. Brooding temperature was kept 32°C at the beginning of the first week of age and decreased gradually in subsequent week until adjusted to the normal environmental temperature. Increasing or decreasing of temperatures were done by lowering or raising the bulbs according to the temperature prevailed and the birds behavior. The birds were exposed to 12 hours of lighting and a dark period of 12 hours per day throughout the experimental period. After 21 days only one 60 watt electric bulb was set at a height of 240cm which provide sufficient lighting up to the end of experiment. The dark provision was practiced to make broilers familiar with possible darkness due to electricity failure. For the first 2 days, feeds were given on tray feeders and water was supplied in a ground. After two days of age, one trough feeder and one round water bucket were provided for each replicate pen. Feeders were cleaned everyday at morning and afternoon and fresh clean drinking water was supplied for all the times. The rearing of chicks was done without vaccination. Proper hygienic and sanitation programs were followed during the experimental period. To prevent the outbreak of disease strict biosecurity was maintained during the experimental period.

VII. Feeding and drinking

Immediately after distribution of chicks in the pens electrolyte and vitamin solutions were provided to drinking water for four hours. Then dietary treatment was applied to the chicks. Control (group A) - Normal feed &water. Treatment (group B) provided 1ml Kalomegh leaf extract/liter drinking water. Feed was supplied three times daily for the first 7 days and gradually reduced to two times. Initially feed was given on tray feeder and thereafter through feeder was used to feed the birds. Leftover feeds were mixed with fresh feed into the feeder in the morning and spoiled feed was excluded by taking weight of the waste feed. In each week, birds were weighed early morning prior to feeding.

VIII. Statistical analysis

The data were analyzed statistically between control and treated groups of broilers by the well know student's test ('t' test) with SPSS.

IX. Bacterial Sensitivity Test

Leaf samples were thoroughly washed and dried in shadow and ground to powder. 10g of the powder was macerated in 200ml boiling distilled water for 20mins (Hosseini *et a*l., 2010). The macerate was first filtered through filter paper and centrifuged at 3500g for 15mins. The supernatant was removed by evaporation (Hosseini *et a*l., 2010). *Antibacterial activity Staphylococcus aureus* and *E. coli* were grown in Mueller Hinton broth (Merck, Germany) at 37°C for 24h. Final cell concentrations were 108cfu/ml according to the McFarland turbidometry. 100µl of the inoculum was added to each plate containing Mueller Hinton agar (Merck, Germany). Four different concentrations of the extract (5, 10, 15 and 20% equal to 0.05, 0.1, 0.15 and 0.2mg/ml, respectively) were prepared. The sterile filter paper disks (6mm in diameter) were saturated with 50µl of each concentration of the extract. After which this was placed in an oven and dried at 50°C overnight. *E. coli* and *Staphylococcus aureus* were supplied from the Laboratory of Microbiology, Department of Microbiology and Hygiene, BAU. The bacteria were cultured in Nutrient Agar, Blood agar, EMB agar and finally in Nutrient broth. After that the bacteria were spread over the surface of the Mueller Hinton agar then disk (dissolved with *Andrographis paniculata* leaves extract) were inoculated in the culture media. The plates were incubated at 37°C for 24h and the diameters of inhibitory zones were measured. The assay was carried out three times for each extract. Disks containing different concentrations of antibiotics were used as reference to compare the sensitivity of each tested bacterial species (Hsieh *et a*l., 2010). Antibiotics disks contain doxycyclin, streptomycin, chloramphenicol, penicillin, gentamicin, vancomycin, trimethoprim-sulfamethoxazole and tetracycline (Oxoid, Basing Stoke, UK).

3. RESULTS

A total of 40 day-old chicks randomly were divided into 2 groups (A and B) for assessing the efficacy of plants leaves extract as growth promoter on broilers. In Group A (Control group) live weight were measured and found as initial live wt 150g, final live wt 1500g and weight gain 1350g. In Group B initial live wt 155g, final live wt 1600g and weight gain 1455g. The observations for live body weight (g) means of A, and B groups after 5 weeks of the experimental period were 1500g and 1600.0g, respectively. It is observed from the results in Table 1, that supplementation of Kalomegh leaves extract in A and B groups of broilers effected significant ($P<0.01$) increase in mean live body weights as compared to control group.

Table 1: Initial and final live weight, weight gain of broilers fed different levels of Kalomegh leaves extract from 1 to 5 weeks of age

Variables	Treatment		P Value
	$A_{(n=20)}$ Control Mean±SEM	$B_{(n=20)}$ Kalomegh Mean±SEM	
Initial live weight (g) on 7th day	150±5.76	155±6.32	0.034*
Final live weight (g) on 35th day	1500±19.90	1600±20.87	0.041*
Weight gain (g)	1350±17.98	1455±18.98	0.042*

N. B. - Mean values within the same row, which have different superscripts, were significantly different. In this and other tables, A = control, B = 1ml Kalomegh *(Andrographis paniculata*) extract.

Supplementation of Kalomegh leaves extract was found to be more profitable than control group of broiler rearing. However, dietary inclusion of Kalomegh extract fetched the maximum profit as compared to the control groups. Increase in the profit margin of the birds fed rations containing herbal growth promoters may be attributed to

the better efficiency of feed utilization, which resulted in more growth and better feed to gain ratio, ultimately leading to higher profit margin in the broilers reared on Kalomegh supplemented rations.

Table 2: Inhibition zone (mm) *Kalomegh* aqueous extract at different concentration (Mean ±SD)

Bacterial species	Concentration of extract			
	5%	10%	15%	20%
S. aureus	10.33±0.57	13.33±2.30	6±1	21±1
E. coli	13.33±2.88	15.66±1.15	18± 1.73	19.33±1.52

Antibacterial activity of aqueous extract of *Kalomegh* is presented in table 2. The maximum antibacterial activity in 20% concentration of the plant was 21mm for *S. aureus* and 19.33mm for *E. coli*. Other hand *S. aureus* was resistant to penicillin and tetracycline. *E. coli* was resistant to doxycyclin, streptomycin, penicillin, vancomycin, trimethoprim-sulfamethoxazole and tetracycline.

4. DISCUSSSION

Kalomegh leaves extract has effects as alternative growth promoter and no mortality without any antibiotic, vaccination taking proper biosecurity. This result may be due to antibacterial, anti-inflammatory, antistress, antifungal, insecticidal and liver tonic properties of *Andrographis paniculata* (Kalomegh) leaves which help to ensure the microbial load of birds and improve the feed consumption and feed efficiency. Care should be taken to ensure its safe use for medicinal references. Manwar *et.al.* (2005) Supplemented *Andrographis paniculata* (Kalomegh) leaves extract @ 1 ml drinking water and reported significant increase in the live body weight of broilers in the *Andrographis paniculata* (Kalomegh) fed groups when compared with control group.

Supplementation of *Andrographis paniculata* (Kalomegh) was found to be more profitable than control group of broiler rearing. However, dietary inclusion of *Andrographis paniculata* (Kalomegh) at 0.5% fetched the maximum profit as compared to the control groups. The results of the present study are in line with the findings of Ahmad (1993), who reported that dietary inclusion of Andrographis paniculata (Kalomegh) at 0.5% in the rations was more beneficial in broiler production. The results of this study (Table 2) showed that aqueous extract of *Kalomegh* effectively inhibited the growth of *E. coli* and *S. aureus*. The antibacterial activity was enhanced with increase of the extract concentration. Antibacterial activity of the plant was considerable in comparison with the other reports. Therefore, in this experiment the agar diffusion test was chosen. Yin and Guo (1993) found that a dose of 500 mg per day for six day of Kalomegh was effective on acute bacterial diarrhea in human patients. Thanangkul and Chaichantipyuth (1985) found that Kalomegh had effect on curing diarrhea and bacillary dysentery in the group of patients in Ramatipbodee Hospital in Bangkok, Thailand. Dhamma-Upakorn et al. (1992) and Sawasdimongkol et al. (1990) found that the Kalomegh extract has an effect to reduce the movements of the smooth muscle in stomach and intestinal tract in human.

5. CONCLUSION

This research work shows that continuous treatment with *Andrographis paniculata* (Kalomegh) extract produced a significant ($p<0.05$) increase in live body weight and also exhibited strong antibacterial activity and antibacterial activity was enhanced with the increase of the extract concentration. In Bangladesh broiler farming is very difficult for small scale farmer and they are getting looser.

For this reason in our experiment we observed that production cost can be reduced by eliminating vaccines and antibacterial. This is a preliminary work and the technology is very simple. Farmers could adopt this technology without any specialized technical knowledge and medicinal ingredients are available. As a result by using Kalomegh extract small scale farmers would be able to sustain in their farming business and produce broilers without any drug residues.

REFERENCES

1. Ahmad, M. and Asmawi, M.Z. 1993. Some pharmacological effects of aqueous extract of *Andrographis paniculata* Nees. The International Conference on the use of Traditional Medicine and other natural Products in Health-Care (Abstracts). pp. 122.

2. Bunyapraphatsara, N. 2000. Research strategies for the use of medicinal plants in animal production. A paper presented at an International Conference on Tropical Agriculture for better health and environment at Kasetsart University, Kampaengsaen, Nakornpathom, 29 Nov.-1 Dec 2000. Thailand. pp. 4.

3. Dhamma-Upakorn; Chaichantipyuth, P.C; Pongjunyakul, P.; Sangalungkarn, V. and Chaimongkol, S. 1992. Spasmolytic activity of some active substances from *Andrographis paniculata.* J. SPSNRCT Seminar: Pharmacological Active Substance from natural source. Faculty of Pharmacy, Chulalongkorn University, Bangkok, Thailand. pp. 21

4. Hosseini, H.; Handali, S.; Parishani, M.R.; Ghezelbash, G.H.R. and Ameri, A. 2010. A comparative study of antibacterial effects of aqueous extract of *Oxalis corniculata* L. with antibacterial effects of common antibiotic in *S. aureus* and *E. coli* infections. J Med Plants; 9: 103-7.

5. Hsieh, P.C.; Mau, J.L. and Huang, S.H. 2001. Antimicrobial effect of various combinations of plant extracts. Food Microbiol; 18: 35-43.

6. Manwar, S.J.; Thirumurgan, P.; Konwar, D.; Chidanandaiah and Karna, D.K. 2005. Effect of *Azadirachta indica* leaf powder supplementation on broiler performance. Indian Vet. J.; 84: 159-162.

7. MPRI. 1999. Division of Medical Plants Research and Development,Department of Medical Science, Ministry of Public Health. Standard of Thai Herbal Medicine: *Andrographis paniculata* (Burm. f.) Nees. The War Veterans Organization Press, Bangkok. pp. 66.

8. Sawasdimongkol, k.; Permpipat U. and Kiatyingungsulee, N. 1990. Pharmacological study of *Andrographis Paniculata* Nees. National Institute of Health, Bangkok, Thailand.

9. Tang, W. and Eisenbrandt, G. 1992. Chinese Drugs of Plant Origin. Springer-Verlag, Berlin. pp. 1056.

10. Thanangkul, P. and Chaichantipyuth, C. 1985. Clinical studies of *Andrographis paniculata* on diarrhea and dysentery. J. Ramatipbodee Med. Sci.; 8: 57-62.

11. Yin, J. and Guo, L. 1993. Contemporary traditional Chinese medicine. Beijing: Xie Yuan. Yearly Statistic Reports. Planning Division Department of Livestock Development. Bangkok, Thailand.