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Study of Phytochemical and Immunomodulatory Activity of Methanolic Extract of Andrographis Paniculata in Broiler Birds

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ABSTRACT

This study was conducted to investigate the phytochemical screening and immunomodulatory action of methanolic extract of *Andrographis Paniculata* in experimental model of immunity as alternative to antibiotic growth promoter in broiler chicks. Extracts of plant were obtained by soxhlets extraction in solvent methanol. In this study, a total 60 day old Ven Cobb broiler chicks were used. For cell mediated immunity study, 30 chicks were divided into 3 treatment groups (T1, T2 and T3) 10 chicks in each replicates. Similarly, for humoral immunity study, 30 chicks were divided into another 3 treatment groups of 10 chicks in each replicates. Each bird of different groups was individually identified by using leg band. T1 (Control diet), T2 (Standard growth promoter; BMD @ 0.05% in feed), T3 (APE @ 0.4g/L) in drinking water daily for consecutive 42 days. The preliminary phytochemical tests were carried out by using standard methods. Cellular immunity assessed by DNFB skin sensitization test. Whereas, humoral immunity was analyzed by indirect micro haemagglutination test. Phytochemical analysis revealed the presence of alkaloids, glycosides, phytosterols, diterpenoid, tannin, saponin, flavonoids and phenols. *Andrographis paniculta* extract in group T3 showed highest antibody titer and significantly higher skin thickness in DNFB skin sensitization test both at 24 hours and 48 hours after sensitization as compared to group T1 and group T2. Phytochemical compounds phenols and flavonoids are known to have beneficial importance in immunomodulation. It can be concluded that the *Andrographis paniculata* extract posses potent immunomodulatory activity.

Keywords: Cell mediated immunity, APE- *Andrographis paniculata* extract, humoral immunity, 2, 4-Dinitro-fluorobenzene (DNFB)

A global reliance on alternative system of medicine for chronic and acute ailments resulted in an intense area of research and discovery of a number of herbs with potential to curb diseases and Ayurvedic formulation either alone or in combinations has been exploited for modulation of immune system Patel *et al*. (2010). Herbal feed additives confer birds with greater general immunity from various diseases and as an alternative to antibiotic growth promoters (Meenachisundaram *et al.,* 2009; Sunder *et al*., 2016). Dietary supplementation of amla powder ameliorate adverse effects of summer stress on humoral immunity in murrah buffaloes due to presence of vitamin-C, tannins and flavonoids (Lakhani *et al.,* 2016). The mechanism of action of herbs in the animal for growth promotion includes changes in intestinal microbiota, increased digestibility and nutrient absorption in addition to their pharmacological effect like stimulation of immune system, antibacterial activity, coccidiostatic, antihelmenthic, antiviral and anti-inflammatory properties (Costa *et al*., 2007).

Kalmegh, (*Andrographis paniculata*) belongs to the family of *Acanthaceae* and is popular worldwide with the name of "King of Bitters". Puri *et al*. (1996) experimentally founded that *Andrographis paniculata* is useful in the treatment of wounds, ulcers, leprosy, sore throat. Whereas, in "Kalmegh" hypertension, anti-inflammatory and anti-snake venom properties was evaluated by Samy *et al*. (2008) due to inhibition of lipid peroxidation and free radical activities. The active constituents of plant derivatives such as alkaloids, lectins, peptides, flavonoids and tannins have been reported to modulate the immune system in different experimental models (Shivaprasad *et al.,* 2006). Hence, the present study was undertaken to screen the phytochemical and immunomodulatory activity of *Andrographis paniculata* extract (stem and leaves) and to evaluate its potential beneficial effects in broiler diet.

MATERIALS AND METHODS

Plant material

Certified dried stem powder of *Andrographis paniculata* were procured from Sanjeevani Ayurved, Chhattishgarh Herbals Quality Forest Products, Durg (C.G).

Preparation of plant extracts

The dried leaf powder of *Andrographis paniculata* were extracted with methanol by using Soxhlet's apparatus at 50-60 °C for about 18 hours. About 50 gm of each powder was extracted with 250 ml methanol. The extracts were concentrated to dryness in hot water bath to yield crude residue. The extract was weighed and the percentage of yield was calculated. Each obtained extract was stored in a sterile screw cap bottle and preserved in a refrigerator for further use.

Experimental animals

For immunological studies a clinically, healthy Sixty (60) day- old broiler chicks were procured from Simran hatcheries, Raipur. For study of cellular immunity 30 chicks were divided into 3 treatment groups (T1 Control, T2-BMD @.05% and T3 APE @ 0.4g/L in water) of 10 chicks in each. Similarly, for humoral immunity, 30 chicks were divided into another 3 treatment groups 10 chicks in each group. Each bird of different groups was individually identified by using leg bands (Table 1).

Table 1: Experimental design for immunological studies in broiler birds

Particulars	Groups/ Treatments		
	Control	Standard	Test
	T_1	T_2	T_3
Cell mediated immunity study	10	10	10
Humoral immunity study	10	10	10
Basal feed	+	+	+
Bacitracin Methylene Disalicylate (% in Basal feed)	–	0.05	–
Andrographis paniculata Extract (APE) in drinking water (g/L)	–	–	0.4

Preliminary phytochemical screening of methanolic extract of *Andrographis paniculata*

Preliminary phytochemical analysis was carried out to check and identify the active constituents of the methanolic extract of *Andrographis paniculata* leaves such as alkaloids, carbohydrates, flavonoids, terpenes, steroids, saponins and tannins by using test methods of Draggendroff's, Mayer's test, Molisch's , Fehling's test, lead acetate, Liebermann-Burchard test, foam formation test, ferric chloride test and Alkaline Reagent Test (Raaman, 2006).

Table 2: Phytochemical Screening of methanolic extract of *Andrographis paniculata*

Phytochemicals	Phytochemical test	*Andrographis paniculata* extract
Alkaloids	Dragendroff's test	+
Saponins	Frothing test	+
Phytosterols	Liebermann-Burchardt's test	+
Phenolic compounds	Ferric chloride test	+
Tannins	Bremer's test	+
Flavonoids	Alkaline Reagent Test	+
Detection of Free sugar	Fehling's Reagent test	–
Detection of Glycosides	Kellar - Kiliani test	+

Anthraquinone	Borntrager's test	–
Terpenoid	Salkowski test	+
Volatile oil	NaOH test	–

Acute toxicity studies

Limit test was performed as per OECD guideline for testing of chemicals (OECD, 1998) to evaluate the acute oral toxicity of APE in female albino rats with the upper limit dose of 2000 mg/kg. The mortality, behavioral abnormality, signs and symptoms of toxicity, if any, were recorded for a period of 14 days of post administration**.**

Preparation of antigen

Fresh Sheep blood was collected in sterile Alsever's solution in 1:1 proportion and Kept in, refrigerator. Sheep red blood cells (SRBCs) for immunization were prepared by spinning sheep blood at 2000 rpm for 10 minutes, residue obtained after centrifugation was washed thrice in Normal saline solution (NSS) and finally a 7% suspension of SRBC was prepared (Patel *et al*., 2010).

Determination of humoral immune response

Humoral immune response was assessed by micro haemagglutination test according to the method of Thaxton *et al.* (1974) with minor modification. Each pretreated birds including the control group was immunized with 1ml suspension was injected intravenously to six birds (two from each replicate) from each group on 32 day of age and the birds were bled on day 10^{th} following injection. The blood was allowed to clot at 37 ºC for few hours and refrigerated and serum was collected. Serum was heated in a water bath to inactivate the complement fraction of the serum and Antibody production in response to the immunization was examined visually by micro-haemagglutination test. The highest serum dilution showing complete haemagglutination was noted and expressed as HA titer in $\log_2$ values.

Determination of cell mediated immunity

Cell mediated immune response was studied based on delayed hypersensitivity or the different experimental groups was measured by contact sensitivity test with 2,4-Dinitro-fluorobenzene (DNFB) test was described by Phanuphak *et al.,* (1974) and modified by Tamang *et al.,* (1988). The featherless area on 20 cm^2 on both sides of abdomen was marked for inoculation. The sites were properly sterilized. For this test, six chicks from each group were sensitized with DNFB by single percutaneous application of 0.1 ml of DNFB (2000 µg/ml) in a vehicle consisting of acetone and olive oil in the ratio of (4:1) on 28^{th} day of experiment. Sensitized birds were challenged with 50 µg of DNFB in 0.1 ml of acetone and olive oil (4:1) after 14 days. Right lateral side of abdomen was used for DNFB application whereas left side served as control. The skin thickness was measured using engineer's micrometer on day 0, 24 and 48 hours of post challenge with three readings and the overall mean skin thickness was calculated.

Statistical analysis

All the recorded data were subjected to statistical analysis as per standard methods and techniques described by Snedecor and Cochran (1994) followed by comparison of mean values were further compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Phytochemical screening provide an empirical basis for the use of plants in traditional medicinal practices the biological or therapeutic activities of medicinal are closely related to their chemical compound. Phytochemical analysis of methanolic extract of *Andrographis paniculata* revealed the presence of alkaloids, glycosides, phytosterols, diterpenoid, tannin, saponin and phenols. The findings of this study is in agreement with the result of (Bajpai *et al*., 2014; Shirisha and Mastan, 2013) in the whole plant extract of *Andrographis paniculata* (Table 2). Immunomodulatory effect of the extract on humoral immune are presented in (Fig. 1). The HA titers of groups T1, T2 and T3 were 3.33±0.86, 3.87±0.12 and 6.16±0.12 respectively. The T3 groups (APE) showed significantly ($p< 0.001$) higher HA titer values as compared to group T1 and T2. However, no significant difference was found between the groups of T1 and T2. The significantly ($P< 0.001$) higher antibody titers against sheep RBC in serum of APE supplemented broiler birds indicated immunostimulant property of extract. This finding is in accordance with the findings of (Sunder *et al.,* 2016) who reported the high B cell and T cell response

in broiler and Japanese quail by feeding of *andrographis paniculata* extract (APE). (Singh *et al.,* 2008), concluded that alkaloids were responsible for overall elicitation of immune response.

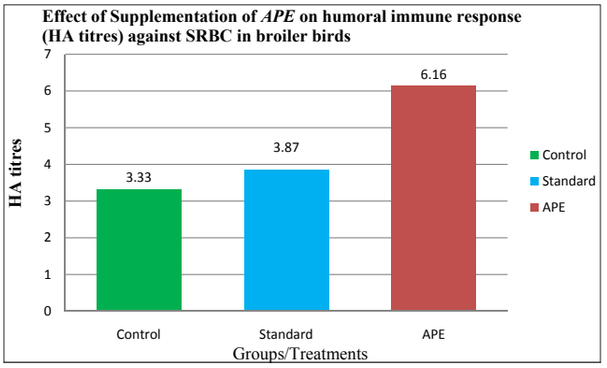

Fig. 1: Effect of Supplementation of *Andrographis paniculata* extract on humoral immune response (HA titers) against SRBC in broiler bird

Supplementation of kalmegh in the present study influenced significantly higher cell mediated immunity (P<0.01) (Fig. 2a) as compared to control.

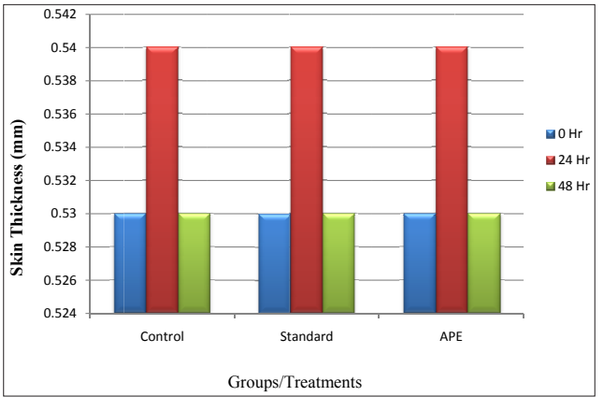

Fig. 2(a): Effect of supplementation of *Andrographis paniculata* extract on Cell Mediated Immune Response (skin thickness without DNFB swabbing on left control side) of broiler birds

Cell mediated immune response of groups T1, T2 and T3 were **2**.14 ± 0.05,^{b} 2.12 ± 0.05^{b} and 3.2 ± 0.05^{a} at 24 hours, 2.63 ± 0.07^{c}, 2.50± 0.07^{c} and 3.63±0.0^{a} at 48 hours respectively. The mean increase in abdominal skin thickness of broiler chicks of different groups at different hours post challenge is presented in (Fig. 2b).

Broiler chicks exposed to the challenge dose of DNFB revealed erythema, oedema, vesiculation and scab formation. The birds of groups T3 showed significant (P<0.001) increase in abdominal skin thickness at both 24 and 48 hours post challenge as compared to group T1 and T2 which indicated that APE individually caused enhancement of cellular immunity as well as humoral immunity.

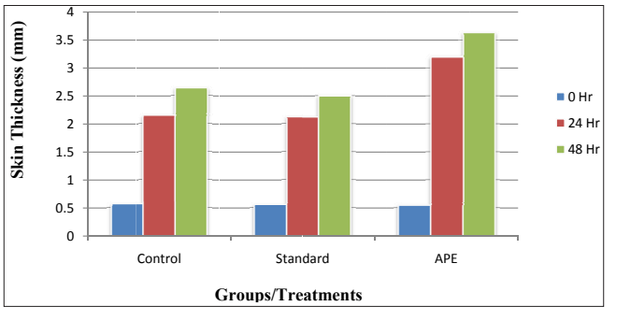

Fig. 2 (b): Effect of supplementation of *Andrographis paniculata* extract on Cell Mediated Immune Response (skin thickness without DNFB swabbing on right control side) of broiler birds

CONCLUSION

It may be concluded that the humoral immunity status of the group T3 (APE) broiler birds showed significantly higher HA titer value as compared to the T1 and T2. In cell mediated immune study, group T3 showed significantly higher skin thickness in DNFB skin sensitization test both at 24 hours and 48 hours after sensitization. These indicated that supplementation of APE individually stimulated both CMI and humoral immune responses in broiler birds. Kalmegh might be promising alternative medicinal plant for antibiotic growth promoters as well as commercial immune boosters in the platform of production of antibiotic residue free poultry production. Further studies will need to isolate and characterize the active principles to elucidate their immunodulation mechanism.

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