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Immunomodulatory and growth promoting potentials of *Artocarpus heterophyllus* lam herb in immunised and immunosuppressed broilers

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Abstract

In poultry, utilization of immunomodulatory drugs are considered as an important solution and accepted widely, since they improve the immunity and decrease the susceptibility to pathogenic diseases along with good management practices including proper vaccination. Vaccination is an alternate, but not an effective measure at all times. Also, search on our traditional herbal medicine has increased in recent times to find out alternatives as immunomodulators against infectious diseases and stressor agents.

Artocarpus heterophyllus Lam. (Jackfruit) used traditionally for having immunomodulatory, antioxidant and antibacterial properties. The crude leaf powder of *Artocarpus heterophyllus* Lam at 1% inclusion level in feed was studied to explore the immunomodulatory and growth promoting potentials in immunized and immunosuppressed broilers. The trial was conducted with four control groups as normal, positive (levamisole treated), negative (cyclophosphamide treated) and combined (levamisole and cyclophosphamide) and two treatment groups as *Artocarpus heterophyllus* Lam. leaf powder at 1% level in both immunized and immunosuppressed birds. Weekly assessment of cumulative feed intake, body weight gain, feed Conversion Ratio and HI titre against La Sota antigen was done to assess growth promoting and humoral mediated immunity in broilers.

In immunized broilers, this herb showed significant growth promoting and immunomodulatory effect than normal birds and equal to the standard immunostimulant drug – levamisole and even in immunosuppressed group the herb showed better results than immunosuppressant control birds.

Significant level of immunomodulatory phytochemicals like isophytol, squalene and cyclotrisiloxane in *Artocarpus heterophyllus* Lam. might be the reason for its promising immunomodulating potentials in turn for its growth promoting potentials in broilers and this study recommends the usage of this herb with further detailed studies.

Keywords: Feed conversion ratio, hemagglutination inhibition, immunomodulation and *Artocarpus heterophyllus* Lam

Introduction

The world population is getting multiplied rapidly in the past 50 years than ever before, and more rapidly than it is assumed to increase in the future (Byarugaba, 2007) [4]. Because of this anticipated growth, the demand for high-protein diet of animal source as meat and egg products is expected to increase from 70 to 100%. Among all other classes of livestock farming, the poultry sector is being considered as an important source to meet out the demand throughout the world (Godfray *et. al.*, 2010) [5].

In broiler farming, genotype with higher body weight, intensive systems of rearing and high density of stocks normally expose the birds to different kind of infections (by pathogenic bacteria, viruses, fungi and parasites) and many stressors. Factors such as vaccination failure, indiscriminate use of drugs, adverse environmental conditions and climatic changes have increased the chance of reduced immune potential, disturbed growth and decreased production in broilers. Currently there are many proven proprietary immunomodulatory drugs available. But they are poor cost effective, toxic, causing drug interaction and having the chance to get deposited in tissues as residues. So, the search turned back to our traditional medicine to find out an alternative for the prevention, treatment and control of infectious diseases in broilers. Many medicinal herbs have been proved for growth promoting and immunomodulatory effects with limited data available on veterinary usage. *Artocarpus heterophyllus* Lam. (Jack fruit tree) used traditionally for having immunomodulatory, antioxidant and antibacterial properties has not yet been studied in broilers. Hence, the present study was under taken to explore the growth promoting and immunomodulatory effects of crude leaves powder of this herb on immunized and immunosuppressed broilers.

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Materials and methods

A. Collection of plant and preparation of *Artocarpus heterophyllus* Lam Crude Leaves Powder

Leaves of *Artocarpus heterophyllus* Lam. was collected from different locations of Coimbatore, Tamilnadu and authenticated. The collected plant leaves were washed, shade dried, finely powdered and used for further studies.

B. Identification of bioactive components by Gas Chromatography – Mass Spectrum (GC - MS) analysis

Methanolic extract of *Artocarpus heterophyllus* Lam leaves

was prepared and used for the identification of bioactive phytochemicals. GC-MS (Gas-Chromatography Mass-Spectrometry) of *Artocarpus heterophyllus* Lam. was carried out at M/s. Sargam Laboratory, Chennai, Tamilnadu using GC-MS 5975 C Agilent System and Gas Chromatograph interfaced to a mass spectrometer. Interpretation of mass spectrum was conducted for the pharmacological actions of the herb using Dr. Duke's phytochemical and ethno botanical database which is having more patterns on phytochemicals (Table. 1).

Table 1: Activity of the phytochemicals identified in GC-MS analysis of methanolic leaves extract of *Artocarpus heterophyllus* Lam. Herb

S. No	Name of the Compound	Nature of Compound	Activity
1.	2-Pentadecanone, 6,10,14 trimethyl,	Volatile Oil	Antioxidant, antibacterial and allelopathic activity.
2.	Hexadecanoic acid, ethyl ester,	Palmitic acid ethyl ester	Antioxidant, Hypocholesterolemic, Nematicide and Pesticide
3.	Isophytol	Diterpene	Antioxidant, anticancer, diuretic, anti-inflammatory and precursor for Vitamins E and K ₁
4.	Linoleic acid ethyl ester	Linoleic acid ethyl ester	Hypocholesterolemic, nematicide, antiarthritic, hepatoprotective and antihistaminic
5.	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	Linoleic acid ester	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective analgesic, antipyretic, anticonvulsant and antiseptic
6.	Octadecanoic acid, ethyl ester	Stearic acid, ethyl ester	No report on activity available
7.	Squalene	Triterpene	Antibacterial, antioxidant, pesticide, anticancer, immunostimulant, chemo preventive, lipoxygenase-inhibitor, antiulcerogenic, antispasmodic and antibronchitic
8.	Cyclotrisiloxane, hexamethyl	Cyclic siloxane	Antioxidant

C. Experimental design

In this experiment, straight run day old broiler chicks belonging to a single hatch of "Cobb" strain procured from local commercial breeder was used. Chicks were randomly divided into six groups of ten birds each with two replicates (Table. 2). The trial was conducted with four control groups as normal (T₁), positive (T₂ - levamisole treated), negative (T₃ - cyclophosphamide treated) and combined (T₄ - levamisole and cyclophosphamide) and two treatment groups as *Artocarpus heterophyllus* Lam. leaf powder at 1% level in

immunized (T₅) and immunosuppressed birds (T₆). The birds of groups T₃, T₄ and T₆ were injected with cyclophosphamide, a proven immunosuppressive drug @ 150 mg/kg b.wt., i/v (Mode *et al.*, 2009)^[10] on day 21, as a single dose and birds of T₂ group were provided with levamisole, an immunomodulatory drug @ 30 mg/kg b.wt. in drinking water for 2 days before and 2 days after each vaccination (Mostafa Habibi *et al.*, 2012)^[11]. All the treatment groups were immunized against Newcastle disease virus with B₁ on 7th day and La Sota on 21st day.

Table 2: Experimental design for the study on immunopharmacological and growth promoting potentials of *Artocarpus heterophyllus* Lam herb in broilers

Groups		Treatment	Name of the Groups	Total No. of Birds
T ₁	Control	Basal Diet alone	Normal control group	20
T ₂	Positive Control	Basal Diet + Levamisole (Immunostimulant) @ 30 mg / kg b. wt in drinking water 2 days before and after vaccination.	Positive control group	20
T ₃	Negative Control	Basal Diet + Cyclophosphamide (Immunosuppressant) injected on day 21 @ 150 mg/kg b.wt, i/v single dose.	Negative control group	20
T ₄	Positive and Negative Control	Basal Diet + Levamisole (Immunostimulant) @ 30 mg / kg b. wt in drinking water 2 days before and after vaccination + Cyclophosphamide injected on day 21 @ 150 mg/kg b. wt, i/v single dose.	Combined control group	20
T ₅	Immunized Broilers	Basal Diet + <i>Artocarpus heterophyllus</i> Lam (Leaves crude powder) @ 1% inclusion level	Herbal treated group I	20
T ₆	Immunized & Immunosuppressed Broilers	Basal Diet + <i>Artocarpus heterophyllus</i> Lam (Leaves crude powder) @ 1% inclusion level + Cyclophosphamide injected on day 21 @ 150 mg/kg b. wt, i/v single dose.	Herbal treated group II	20

D. Growth Parameters

The growth performance of the birds of all groups was assessed by assessing the weekly cumulative feed intake

(Table. 3), body weight gain (Table. 4), and feed conversion efficiency (Table. 5) during the study period.

E. Immunological parameters

The immunomodulatory effect of this herb was assessed by evaluating the humoral mediated immunity. Humoral mediated immunity was assessed by estimating the Haemagglutination Inhibition (HI) titre (Log 2 values) against NDV La Sota antigen (OIE, 2000) [12] weekly (Table. 6).

The data collected on parameters were analyzed statistically as per the methods of Snedecor and Cochran (2004) [17].

Results and discussion

A. On phytochemical analysis

2-pentadecanone, hexadecanoic acid, isophytol, octadecatrienoic acid, linoleic acid ethyl ester, octadecanoic acid, cyclotrisiloxane and squalene are the eight phytochemicals identified (Table. 2) in phytochemical analysis in GC-MS method. Out of these, components like isophytol, cyclotrisiloxane and squalene were identified for

having the properties as precursor for vitamin E, immunostimulant and antioxidant properties respectively based on the mass spectrum interpretation.

B. On Growth Parameters

1. Cumulative Feed Intake

The cumulative feed intake was noticed similar in all the groups except the group treated with immunosuppressant drug cyclophosphamide. *Artocarpus heterophyllus* Lam. at 1% inclusion level (T₅) showed performance in feed intake compared to normal and positive control groups which indicated the pharmacological effects of herbs as adaptogenic, appetite stimulant, antioxidant and rejuvenating properties. The results of the present study are in accordance with the study of Al-Kassie *et al.* (2011) [1] and Issa and Omar (2012) [6] in which broilers supplemented with herbs resulted in increased feed consumption due to improved efficiency of digestive organs, digestibility of crude protein and dry matter.

Table 3: Effect of supplementation of *Artocarpus heterophyllus* Lam. herb as feed additive on cumulative feed intake (g) in broilers (n=20)

	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
1 Week	120.43 ^a ± 0.75	118.50 ^a ± 0.86	120.57 ^a ± 0.72	120.52 ^a ± 0.70	119.40 ^a ± 0.92	120.69 ^a ± 0.76
2 Weeks	470.07 ^a ± 0.63	466.00 ^a ± 2.53	470.72 ^a ± 0.81	470.66 ^a ± 0.82	467.70 ^a ± 1.94	469.60 ^a ± 1.99
3 Weeks	1070.80 ^a ± 0.88	1068.35 ^a ± 0.95	1071.62 ^a ± 1.04	1069.97 ^a ± 4.55	1069.72 ^a ± 1.27	1069.62 ^a ± 0.85
4 Weeks	1841.85 ^b ± 0.87	1840.15 ^b ± 0.74	1811.62 ^a ± 4.70	1838.12 ^b ± 3.53	1841.52 ^b ± 1.10	1841.52 ^b ± 0.88
5 Weeks	2949.05 ^b ± 0.84	2947.55 ^b ± 0.75	2922.65 ^a ± 4.91	2944.87 ^b ± 4.30	2948.62 ^b ± 1.20	2949.87 ^b ± 1.10
6 Weeks	4079.15 ^c ± 0.66	4077.42 ^c ± 0.93	4045.67 ^a ± 4.46	4070.22 ^b ± 3.77	4079.12 ^c ± 1.15	4079.47 ^c ± 0.97

Columns bearing common superscript did not vary significantly at ($P < 0.05$) level.

2. Body Weight Gain

In broilers, the final body weight gain achieved was very much appreciated in the group treated with *Artocarpus heterophyllus* Lam. as feed additive at 1% inclusion level. A favourable effect on body weight gain was produced by the herb without the immunosuppressant was in ascending form from first week to sixth week. The herb showed similar results to the standard immunostimulant group and also produced some better weight gain even in immunosuppressed broilers when compared to immunosuppressant control group. From the results it is inferred that the inclusion of this herb could improve the body weight gain at 1% inclusion level.

These findings are in agreement with the findings of Tollba and Hassan (2003) [19], in their study with the effects of natural feed additives on productive performance and growth rate even under high temperature conditions in broiler chicks. The possible reasons of *Artocarpus heterophyllus* Lam. for having promising effect on body weight in treated broilers might be due to its enriched phytochemical molecules with high nutritive value (Om Prakash *et al.*, 2009) [14], broad antibacterial activity (Khan *et al.*, 2003), antioxidant activity (Ko *et al.*, 1998) [9] and hepatoprotective effect (Saxena *et al.*, 2016) [16].

Table 4: Effect of supplementation of *Artocarpus heterophyllus* Lam. herb as feed additive on body weight gain (g) in broilers (n=20)

Week	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
1	111.48 ^a ± 2.16	124.02 ^{bc} ± 1.41	107.54 ^a ± 2.29	124.00 ^{bc} ± 1.86	119.00 ^b ± 1.68	124.61 ^c ± 1.14
2	346.38 ^b ± 2.64	373.47 ^c ± 2.06	336.29 ^a ± 2.99	372.57 ^c ± 1.98	361.46 ^c ± 2.72	371.65 ^{de} ± 2.13
3	684.87 ^a ± 4.06	714.81 ^c ± 4.01	697.49 ^b ± 4.83	714.11 ^c ± 4.23	723.81 ^c ± 3.01	718.23 ^c ± 2.97
4	1210.03 ^b ± 14.90	1215.02 ^b ± 13.81	1001.20 ^a ± 8.85	1003.82 ^a ± 9.90	1235.00 ^b ± 8.98	1029.45 ^a ± 9.57
5	1689.09 ^c ± 18.37	1760.83 ^d ± 9.17	1491.30 ^a ± 18.57	1510.24 ^a ± 18.09	1766.05 ^d ± 8.20	1572.46 ^b ± 15.09
6	2242.64 ^c ± 27.14	2299.51 ^d ± 29.64	1930.24 ^a ± 16.69	1996.52 ^{ab} ± 19.02	2271.10 ^d ± 29.68	1992.17 ^{ab} ± 21.77

Columns bearing common superscript did not vary significantly at ($P < 0.05$) level.

3. Feed Conversion Ratio

In broilers, particularly at four to six weeks of age, the feed conversion ratio was noticed with significant difference between the treated groups and controls. Poor feed conversion ratio was observed in immunosuppressed group. The best feed conversion ratio was achieved in the group treated with *Artocarpus heterophyllus* Lam. at 1% inclusion level alone and positive control. The results of the present study are in accordance with the study of Allinson *et al.* (2013) [2] on

herbal extracts which enhanced the performance and decreased the feed gain ratio by significantly decreasing the pathogenic bacterial and oocyst count in the gut. The hepatoprotective (Saxena *et al.*, 2016) [16], nematocidal (Arung *et al.*, 2007) [3], anti-ulcer (Om Prakash *et al.*, 2015) [15] and antibacterial (Jitendra *et al.*, 2014) [7] activities of *Artocarpus heterophyllus* Lam. would be the reasons for the improved feed conversion efficiency of the birds in this study.

Table 5: Effect of supplementation of *Artocarpus heterophyllus* Lam. herb as feed additive on feed conversion ratio in broilers (n=20)

Week	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
1	0.74 ^b ± 0.01	0.68 ^a ± 0.01	0.76 ^c ± 0.01	0.69 ^a ± 0.01	0.70 ^a ± 0.01	0.68 ^a ± 0.00
2	1.18 ^d ± 0.00	1.10 ^a ± 0.01	1.22 ^c ± 0.01	1.11 ^{ab} ± 0.00	1.13 ^{bc} ± 0.01	1.11 ^{ab} ± 0.01
3	1.45 ^c ± 0.01	1.39 ^a ± 0.00	1.43 ^b ± 0.01	1.40 ^a ± 0.01	1.38 ^a ± 0.00	1.39 ^a ± 0.00
4	1.46 ^a ± 0.02	1.46 ^a ± 0.02	1.72 ^b ± 0.01	1.74 ^b ± 0.02	1.43 ^a ± 0.01	1.70 ^b ± 0.01
5	1.70 ^b ± 0.02	1.63 ^a ± 0.01	1.90 ^d ± 0.01	1.89 ^d ± 0.02	1.62 ^a ± 0.01	1.82 ^c ± 0.02
6	1.78 ^b ± 0.02	1.73 ^a ± 0.02	2.04 ^d ± 0.02	1.99 ^{cd} ± 0.02	1.76 ^a ± 0.02	2.01 ^{cd} ± 0.02

Columns bearing common superscript did not vary significantly at ($P < 0.05$) level.

C. On immunological parameters

In this experiment, the herb *Artocarpus heterophyllus* Lam. at 1% inclusion level in feed showed significant results in humoral immunity (Table. 5) in immunized and also in immunosuppressed broilers.

Humoral Mediated Immunity

The HI titre against specific NDV antigen (La Sota) was very much appreciated in the groups treated with *Artocarpus heterophyllus* Lam. at 1% inclusion level. The herb treated with immunized birds alone showed equal HI titre values with levamisole treated birds for first four weeks of age and significantly higher HI titre than the levamisole treated group during fifth and sixth weeks of age.

The broilers of herb treated with immunized group showed equal HI titre values with combined control group and better HI titre value than the cyclophosphamide treated group for the first three weeks of age. After immunosuppression, broilers of

herb treated with immunized group showed better HI titre value than the cyclophosphamide treated group and showed equal titre values with combined control group.

From the results it is inferred that the herb *Artocarpus heterophyllus* Lam. showed an improvement in the humoral mediated immunity. The presence of various therapeutic phytochemicals like squalene, cyclotrisiloxane and isophytol in this could be the reason for increase in humoral mediated immunity especially in treated broilers. The previous studies of Om Prakash *et al.* (2013) [13] on the immunomodulatory effect of *Artocarpus heterophyllus* Lam. leaves extract for the treatment of opportunistic infections with the suspect of having immunomodulatory properties was proved in this study. These findings are in agreement with the findings of Tekade *et al.* (2008) [18] who studied the effect of *Asparagus racemosus*, *Sida cordifolia* and levamisole on experimentally induced immunosuppression by cyclophosphamide in broilers.

Table 6: Effect of supplementation of *Artocarpus heterophyllus* Lam. herb as feed additive on HI titre (log 2) against La Sota antigen in broilers (n=20)

Week	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
1	2.80 ^a ± 0.03	3.53 ^c ± 0.01	2.81 ^a ± 0.02	3.53 ^c ± 0.02	3.30 ^b ± 0.01	3.30 ^b ± 0.02
2	4.73 ^a ± 0.01	5.31 ^b ± 0.01	4.72 ^a ± 0.01	5.33 ^b ± 0.01	5.31 ^b ± 0.01	5.33 ^b ± 0.02
3	4.11 ^a ± 0.01	4.31 ^b ± 0.01	4.11 ^a ± 0.01	4.30 ^b ± 0.03	4.28 ^b ± 0.01	4.29 ^b ± 0.02
4	5.35 ^c ± 0.05	5.93 ^d ± 0.01	2.35 ^a ± 0.01	3.78 ^b ± 0.03	5.96 ^d ± 0.05	3.63 ^b ± 0.01
5	5.29 ^c ± 0.02	6.01 ^d ± 0.02	2.61 ^a ± 0.02	4.23 ^b ± 0.02	6.15 ^e ± 0.04	4.12 ^b ± 0.02
6	4.88 ^c ± 0.02	5.72 ^d ± 0.01	2.64 ^a ± 0.02	4.03 ^b ± 0.03	5.89 ^e ± 0.01	4.02 ^b ± 0.03

Columns bearing common superscript did not vary significantly at ($P < 0.05$) level.

Conclusion

From this study it is concluded that, the crude leaves powder of *Artocarpus heterophyllus* Lam. showed significant results on growth and humoral mediated immunity, in immunized and immunosuppressed broilers. The phytochemicals identified in this plant as mentioned above could be the possible reasons for their promising growth promoting and potent immunopharmacological properties since they are proved as cytoprotective, immunostimulatory, antioxidant, adaptogenic, free radical scavenging, rejuvenating, antibacterial, anti-inflammatory, antidepressant, wound healing, anti-ageing and hepatoprotective. This study also recommends the usage of this herb as a supplement in broiler feed at 1% level for better immunity. Further, the specific herbal constituents responsible for immunostimulation, other dosage forms of this herb, toxicological investigation with special reference to meat quality and detailed immunological investigations including molecular aspects of immunology and immuno histochemistry can be ascertained in future.

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