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# Immunomodulatory effect of *Withania somnifera*, *Boerhaavia diffusa* and *Emblica officinalis* in broilers

**Rahul Singh Tomar, RPS Baghel, S Nayak, Ankur Khare and Pramod Sharma**

**Abstract**
This study was carried out to see the immunomodulatory effect of varying levels of *Withania somnifera* (ashwagandha*), Boerhaavia diffusa* (punarnava*) and Emblica officinalis* (Amla) powders in broilers. Two thirty four day old chicks were randomly distributed into 10 dietary treatments each having 3 replicates of six chicks. Out of 10 dietary treatments, treatment one ($T_1$) acted as control, remaining 9 treatments were formulated by supplementing various herbal powders (ashwagandha, punarnava and amla) in varied levels (0.5, 1.0 and 1.5%) to $T_1$. Thus, $T_2$, $T_3$ and $T_4$ were supplemented with ashwagandha powder, $T_5$, $T_6$ and $T_7$ were supplemented with punarnava powder and $T_8$, $T_9$, and $T_{10}$ diets had amla powder, respectively. Thus, $T_2$ group produced significantly higher ($p<0.05$) weight of lymphoid organs. HI titre was found to increase with the increase in the level of ashwagandha powder ($T_3$) except at $T_4$. Cell mediated immunity (CMI) in terms of skin thickness increased in $T_2$. In Punarnava powder, $T_7$ group produced higher weight of lymphoid organs. CMI response on 15$^{th}$ day of challenge, after 24 hrs. Showed lower thickness in $T_7$ group. Thus, up to 1.0% level of punarnava powder ($T_6$) immune system was more active. $T_8$ (0.5% amla powder) group produced maximum weight of lymphoid organs and the mean value of HI titre was found higher in amla supplemented groups. CMI response when challenged with sensitization on 15$^{th}$ day, after 24hrs. Indicated no significant difference ($p<0.05$) in the skin thickness.

**Keywords:** ashwagandha, punarnava, amla, lymphoid organ, HI titre, skin thickness

## Introduction
Ashwagandha (*Withania somnifera*) or Indian ginseng is a medicinal plant belonging to family *Solanaceae*. Ashwagandha possess antistress, adaptogenic, immunomodulatory and performance enhancing properties. This plant is cultivated and also found as natural herb in many dry and hilly areas in India and Pakistan. The main constituents of this plant are alkaloids and steroidal lactonee, but withanine, the main alkaloid found in its roots and leaves is thought to be responsible for its biological activity. The extract of this plant is a potent immune-stimulator, antioxidant and anticarcinogenic. The use of *W. somnifera* has been mainly associated to its immunomodulatory effects (Tiwari and Sahni, 2011) [13].
*Boerhaavia diffusa* is herbaceous plant of the family *Nyctaginaceae.* The whole plant or its specific parts (leaves, stem, and roots) are known to have medicinal properties and have a long history of use by indigenous and tribal people in India. Pharmacological studies have demonstrated that *B. diffusa* exhibits a wide range of properties such as diuretic, anti-inflammatory, anticonvulsant, immunomodulatory and hepatoprotective activity. *B. diffusa* has shown antibacterial activity, mainly against gram-negative bacteria. It contains alkaloids known as punarnavine an anticancer, antiestrogenic, antiamoebic and immunomodulatory agent and punernavoside an antifibrinolytic agent (Manu and Kuttan, 2009) [7].
*Emblica officinalis* (Amla) fruit contains ellagic acid, gallic acid, quercetin, kaempferol, emblicanin, flavonoids, glycosides and proanthocynidin, vitamin C (Ascorbic acid or ascorbate), tannin (eg. Emblicanins A and B) and flavonoids present in amla have very powerful immunomodulatory, antioxidant and anticancer activity (Madhuri *et al*., 2011) [6].
The present study was undertaken to find out the Immunomodulatory effect of *Withania somnifera, Boerhaavia diffusa* and *Emblica officinalis* in broilers birds.

## Material and Methods
The proposed experiment was conducted in the Department of Animal Nutrition, College of Veterinary Science and AH, Jabalpur (M.P). The study was planned to see immuno-modulatory effects of different herbs like ashwagandha (*Withania somnifera*), punarnava

*(Boerhaavia diffusa)* and amla *(Emblica officinalis)*. Experiment was conducted for a period of five weeks.

The feed ingredients were analyzed for proximate composition before formulation of diet. The experimental diets were formulated as per ICAR (1998) [5] specifications and mineral mixture was added @ 3.0 % of the diet. All the diets were iso-nitrogenous containing 22% CP and iso-caloric containing 2800 kcal ME per kg for broilers. Composition of experimental broiler diets used in the study is given in Table1.

**Table 1:** Composition of experimental broiler diet (control)

| Ingredients | (%) |
|---|---|
| Maize | 59.50 |
| Soybean meal | 37.00 |
| Mineral mixture | 3.00 |
| Methionine | 0.50 |
| Vitamin ($AD_3EK$) | + |
| Vitamin (B complex) | + |
| Total | 100.00 |

Two thirty four day old chicks were randomly distributed into 10 dietary treatments each having 3 replicates of six chicks. Different dietary treatments which were used in the study were $T_1$: standard broiler diet as per ICAR (1998) [5] specifications (Control), $T_2$: $T_1$+ (ARP) Ashwagandha root powder 0.5 %, $T_3$: $T_1$ + ARP 1.0 %, $T_4$: $T_1$ + ARP 1.5 %; $T_5$: $T_1$+Punarnava powder (PP) 0.5 %, $T_6$: $T_1$ + PP 1.0 %, $T_7$: $T_1$ + PP 1.5 %; $T_8$: $T_1$ + Amla powder (AP) 0.5 %, $T_9$: $T_1$ + AP 1.0 %, $T_{10}$: $T_1$ + AP 1.5 %. Design of the experiment was completely randomized design as per Steel and Torrie (1980) [11].

## Results and Discussion

### Weight of lymphoid organs:

### Varying levels of ashwagandha root powder (ARP)

Effect of varying levels of ARP on lymphoid organ weight is given in Table 2. Data revealed that maximum weight of bursa, spleen and thymus was observed in broilers assigned $T_2$ diet. The results were in agreement with the study conducted by Rama Rao *et al*. (2003) [10].

**Table 2:** Effect of varying levels of ashwagandha root powder on weight of lymphoid organs (% live weight) in broilers

| Treatments | Bursa (%) | Spleen (%) | Thymus (%) |
|---|---|---|---|
| $T_1$ | $0.07^{a}$±0.01 | $0.09^{a}$±0.01 | $0.17^{a}$±0.02 |
| $T_2$ | $0.08^{a}$±0.01 | $0.15^{a}$±0.02 | $0.18^{a}$±0.02 |
| $T_3$ | $0.03^{b}$±0.02 | $0.09^{b}$±0.02 | $0.06^{c}$±0.01 |
| $T_4$ | $0.05^{b}$±0.02 | $0.07^{c}$±0.01 | $0.10^{b}$±0.01 |

Mean values with different superscripts differ significantly ($p<0.05$)

### Varying levels of punarnava powder (PP)

Effect of varying levels of PP on lymphoid organ weight is furnished in Table 3. Treatment means of the organs weight indicated that $T_6$ diet was having maximum weight of bursa and thymus while, spleen weight was noted with $T_7$ diet.

**Table 3:** Effect of varying levels of punarnava powder on weight of lymphoid organs (% live weight) in broilers

| Treatments | Bursa (%) | Spleen (%) | Thymus (%) |
|---|---|---|---|
| $T_1$ | 0.07±0.00 | $0.09^{c}$±0.01 | $0.17^{b}$±0.02 |
| $T_5$ | 0.08±0.01 | $0.10^{b}$±0.01 | $0.18^{a}$±0.01 |
| $T_6$ | 0.09±0.00 | $0.11^{a}$±0.01 | $0.19^{a}$±0.01 |
| $T_7$ | 0.08±0.01 | $0.13^{a}$±0.02 | $0.18^{a}$±0.01 |

Mean values with different superscripts differ significantly ($p<0.05$)

### Varying levels of amla powder (AP)

Effect of varying levels of AP on weight of lymphoid organs is shown in Table 4. Treatment means of the data related to organs weight indicated that $T_8$ diet was found maximum and significantly ($p<0.05$) higher weight of bursa and spleen and of thymus was noted in $T_9$ diet. These results were also in agreement with McKee and Harrison (1995) [8].

**Table 4:** Effect of varying levels of amla powder on lymphoid organ (% live weight) in broilers

| Treatments | Bursa (%) | Spleen (%) | Thymus (%) |
|---|---|---|---|
| $T_1$ | $0.07^{c}$±0.01 | 0.09±0.00 | $0.17^{a}$±0.01 |
| $T_8$ | $0.17^{a}$±0.03 | 0.12±0.01 | $0.17^{a}$±0.01 |
| $T_9$ | $0.15^{b}$±0.04 | 0.10±0.01 | $0.18^{a}$±0.01 |
| $T_{10}$ | $0.07^{c}$±0.02 | 0.10±0.02 | $0.10^{b}$±0.01 |

Mean values with different superscripts differ significantly ($p<0.05$)

### Humoral Immune response

### Ashwagandha root powder (ARP) on HI titer (F strain) at different intervals

Effect of varying levels of ARP on the HI titer in broilers is furnished in Table 5. Titer level recorded at day 14th indicated linear increase in the level of HI titer in relation to increase in the level of powder. But at day 28th increase was recorded only up to supplementation of 1.0% level ($T_3$). However, there was no significant ($p>0.05$) difference between the level of $T_3$ and $T_4$. While, Akotkar *et al.* (2007) [1] observed significantly more HI titre and skin thickness in 1.25% ashwagandha supplemented group.

**Table 5:** ARP on HI titer (F strain) in broilers at different intervals

| Treatments | Day 14th | Day 28th |
|---|---|---|
| $T_1$ | $1.61^{c}$±0.10 | $1.41^{c}$±0.10 |
| $T_2$ | $2.21^{b}$±0.10 | $2.01^{b}$±0.20 |
| $T_3$ | $2.61^{a}$±0.10 | $2.41^{a}$±0.00 |
| $T_4$ | $2.31^{a}$±0.20 | $2.11^{a}$±0.30 |

Mean values with different superscripts differ significantly ($p<0.05$)

### Varying levels of punarnava powder on HI titer (F strain) at different intervals

Effect of varying levels of punarnava powder on the HI titer is given in Table 6. Titer level recorded at day 14th and 28th both indicated linear increase in the level of HI titer in relation to increase in the level of powder but only up to 1.0% level ($T_6$). Similar, findings were also observed by Goyal *et al.* (2010) [4].

**Table 6:** Punarnava powder on HI titer (F strain) at different intervals

| Treatments | Day 14th | Day 28th |
|---|---|---|
| $T_1$ | $1.61^{b}$±0.10 | $1.41^{c}$±0.10 |
| $T_5$ | $2.11^{a}$±0.17 | $1.91^{b}$±0.10 |
| $T_6$ | $2.41^{a}$±0.00 | $2.21^{a}$±0.10 |
| $T_7$ | $2.21^{a}$±0.20 | $1.91^{b}$±0.10 |

Mean values with different superscripts differ significantly ($p<0.05$)

### Effect of varying levels of amla powder on HI titer (F strain) at different intervals

Effect of varying levels of amla powder on the HI titer in broilers is shown in Table 7. Titer level recorded at day 14th and 28th both indicated that use of 0.5% level of amla powder increased the level of HI titer. But higher increase in the level of powder (1.0% and 1.5%) reduced the titer level, although differences were non significant ($p>0.05$). Suja *et al.* (2009) [12] was in agreement with the results of present study.

**Table 7:** Effect of varying levels of amla powder on HI titer (F strain) in broilers at different intervals

| Treatments | Day 14th | Day 28th |
|---|---|---|
| $T_1$ | $1.61^{b}$±0.10 | $1.41^{b}$±0.10 |
| $T_8$ | $2.31^{a}$±0.20 | $2.11^{a}$±0.00 |
| $T_9$ | $2.21^{a}$±0.10 | $2.01^{a}$±0.10 |
| $T_{10}$ | $2.11^{a}$±0.00 | $1.91^{a}$±0.10 |

Mean values with different superscripts differ significantly ($p<0.05$)

**Cell mediated immunity (CMI) response:-**

**Varying levels of ARP on mean value of skin thickness (mm)**

Mean values of skin thickness by varying levels of the ARP at different intervals is presented in Table 8. Before sensitization (0 day), there was no significant ($p>0.05$) difference in the skin thickness. However, after 24 hrs., skin thickness increased in all the groups but, within as well as between the groups, it was significantly ($p<0.05$) higher only in $T_2$ group. After challenging the sensitization (15th day and after 24 hrs.) between the groups, significantly ($p<0.05$) lower thickness was noted in $T_3$ group and higher thickness was measured in $T_2$ group. Similar reports have been reported by Ghosal *et al.* (1989) [3] and Akotkar *et al.* (2007) [1].

**Varying levels of punarnava powder on mean value of skin thickness (mm)**

Treatment means of the varying levels of the punarnava powder on the mean value of skin thickness in broilers at different intervals is furnished in Table 9. Before sensitization (0 day) no significant difference in the skin thickness. However, after 24 hrs., skin thickness increased in all the groups but, when it was compared within the groups, it was significantly ($p<0.05$) higher in $T_6$ group. After challenging the sensitization (after 15th day), between the groups, significantly lower skin thickness was measured in $T_7$ and higher thickness was measured in $T_6$ group. When it was compared within the groups (after 24 hrs.) significantly ($p<0.05$) higher thickness was measured in $T_5$ group. When comparison was made between the groups challenge without sensitization (15th day), it was significantly ($p<0.05$) higher in $T_1$. Bhattacharya *et al.* (2013) also observed similar findings in his study.

**Varying levels of amla powder on mean value of skin thickness (mm)**

Mean values of skin thickness in broilers at different intervals is given in Table 10. Before sensitization (0 day and 24 hrs.) no significant difference was observed. But, when it was compared within the groups, significantly ($p<0.05$) higher thickness was noted in $T_9$ and $T_{10}$ groups. After challenging the sensitization (after 15th day), between the groups, significantly higher skin thickness was recorded in $T_1$ and lower in $T_9$ group. But within the groups (after 24 hrs.), significantly ($p<0.05$) higher thickness was measured in $T_8$ and $T_9$ groups. When comparison was made between the groups challenge without sensitization (15th day), it was significantly ($p<0.05$) higher in $T_1$ as compared to other groups. Within the groups (after 24 hrs.), significantly higher thickness was noted in $T_{10}$ group. Results of the present study were in agreement with Mode *et al.* (2009) [9].

**Table 8:** Varying levels of ARP on mean value of skin thickness (mm) in broilers at different intervals

| Treatments | Sensitization | | Challenge With sensitization | | Challenge without sensitization | |
|---|---|---|---|---|---|---|
| | Skin Thickness (mm) | | Skin Thickness (mm) | | Skin Thickness (mm) | |
| | 0 day | 24 Hrs after | 15th day | After 24 Hrs | 15th day | After 24 Hrs |
| $T_1$ | $0.66^{2}$±0.06 | $0.91^{b;1,2}$±0.03 | $0.93^{ab;\ 1,2}$ ±0.11 | $1.13^{ab;\ 1,2}$±0.15 | $0.80^{1,2}$±0.04 | $0.83^{1,2}$±0.04 |
| $T_2$ | $0.74^{2}$±0.01 | $1.37^{a;1}$±0.38 | $0.77^{ab;2}$±0.24 | $1.34^{a;1}$±0.08 | $0.65^{2}$±0.05 | $0.98^{2}$±0.10 |
| $T_3$ | 0.72±0.04 | $0.89^{b}$±0.04 | $0.67^{b}$±0.08 | $0.91^{b}$±0.09 | 0.55±0.08 | 0.73±0.10 |
| $T_4$ | $0.60^{2}$±0.04 | $0.99^{b;1}$±0.10 | $1.02^{a;1}$±0.05 | $1.09^{ab;1}$±0.10 | $0.52^{2}$±0.01 | $0.90^{1,2}$±0.07 |

(Mean values bearing different superscripts in alphabet in a column differ significantly in between groups)
(Mean value bearing different superscripts in numeric in a row differ significantly in between intervals)

**Table 9:** Varying levels of punarnava powder on mean value of skin thickness (mm) in broilers at different intervals

| Treatments | Sensitization | | Challenge With sensitization | | Challenge without sensitization | |
|---|---|---|---|---|---|---|
| | Skin Thickness (mm) | | Skin Thickness (mm) | | Skin Thickness (mm) | |
| | 0 day | 24 Hrs after | 15th day | After 24 Hrs | 15th day | After 24 Hrs |
| $T_1$ | $0.66^{2}$±0.06 | $0.91^{b;1,2}$±0.03 | $0.93^{ab;1,2}$±0.11 | $1.13^{a;1}$±0.15 | $0.80^{a;1,2}$±0.04 | $0.83^{1,2}$±0.04 |
| $T_5$ | $0.40^{3,4}$±0.05 | $0.71^{b;2,3}$±0.09 | $0.62^{bc;2,3,4}$±0.08 | $1.21^{a;1}$±0.09 | $0.30^{b;4}$±0.03 | $0.78^{2}$±0.09 |
| $T_6$ | $0.49^{2}$±0.10 | $1.27^{a;1}$±0.25 | $0.98^{a;1}$±0.23 | $1.01^{ab;1}$±0.08 | $0.47^{b;2}$±0.08 | $0.66^{2}$±0.02 |
| $T_7$ | $0.55^{1,2}$±0.05 | $0.82^{b;1}$±0.08 | $0.51^{c;1,2}$±0.08 | $0.77^{b;1}$±0.02 | $0.38^{b;2}$±0.04 | $0.69^{1,2}$±0.04 |

(Mean value bearing different superscripts in alphabet in a column differ significantly in between groups)
(Mean value bearing different superscripts in numeric in a row differ significantly in between intervals)

**Table 10:** Varying levels of amla powder on mean value of skin thickness (mm) in broilers at different intervals

| Treatments | Sensitization | | Challenge With sensitization | | Challenge without sensitization | |
|---|---|---|---|---|---|---|
| | Skin Thickness (mm) | | Skin Thickness (mm) | | Skin Thickness (mm) | |
| | 0 day | 24 Hrs after | 15th day | After 24 Hrs | 15th day | After 24 Hrs |
| $T_1$ | $0.66^{2}$±0.06 | $0.91^{1,2}$±0.03 | $0.93^{a;1,2}$±0.11 | $1.13^{1}$±0.15 | $0.80^{a;1,2}$±0.04 | $0.83^{1,2}$±0.04 |
| $T_8$ | $0.51^{2,3}$±0.02 | $0.85^{1,2}$±0.18 | $0.63^{ab;2,3}$±0.14 | $1.01^{1}$±0.19 | $0.36^{b;3}$±0.03 | $0.65^{2,3}$±0.01 |
| $T_9$ | $0.63^{2,3}$±0.05 | $1.02^{1}$±0.03 | $0.43^{b;3}$±0.03 | $0.94^{1,2}$±0.03 | $0.46^{b,3}$±0.04 | $0.73^{1,2,3}$±0.08 |
| $T_{10}$ | $0.71^{2,3}$±0.10 | $1.13^{1}$±0.07 | $0.63^{ab;2,3}$±0.21 | $0.89^{2,3}$±0.21 | $0.46^{b;3}$±0.11 | $0.81^{1,2}$±0.11 |

(Mean value bearing different superscripts in alphabet in a column differ significantly in between groups)
(Mean value bearing different superscripts in numeric in a row differ significantly in between intervals

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