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Research Article

EFFECT OF SUPPLEMENTATION OF ASHWAGANDHA (*WITHANIA SOMNIFERA*) ON HAEMATO-BIOCHEMICAL PARAMETERS OF *SALMONELLA* GALLINARUM INFECTED BROILER CHICKENS

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ABSTRACT

An experiment was conducted to study the effects of Ashwagandha (*Withania somnifera*) root powder on hemato-biochemical parameters of *Salmonella* Gallinarum infected broiler chicks. One hundred and sixty, day-old broiler chicks were divided into four groups (group A, B, C and D) with 40 chickes in each group. Groups A and B were given basal diet whereas dried Ashwagandha root powder @ 0.5% was given in feed in groups C and D. After 7 days, chicks in groups B and D were infected with 10^7 CFU/ml of *S.* Gallinarum *via* the subcutaneous route. Overall mortality recorded was considerably reduced in Ashwagandha supplemented group D (8) as compared to group B chicks (15). There was a significant decrease in body weight in both the infected groups (B and D) as compared to uninfected groups (A and C) from 14 DPI onwards till the end of the experiment. A significant ($p<0.05$) decrease in haematological parameters in the infected groups compared to non-infected groups were suggestive of microcytic hypochromic anaemia. The intensity of anaemia was lesser in group D as compared to group B. Leucocytois with absolute heterophilia and absolute lymphocytosis was observed in Ashwagandha fed groups. There was a decrease in total serum protein and alkaline phosphatase activity along with hypoalbuminemia in the Salmonella infected groups; the values of these parameters were higher in group D as compared to group B. Alanine transaminase, aspartate transaminase and lactate dehydrogenase activities were significantly higher in Salmonella infected groups, which were lower and attained early recovery in Ashwagandha fed group. It was concluded that supplementation of Ashwagandha root powder @ 0.5% in feed may reduce the severity of *Salmonella* Gallinarum infection in broiler chicken.

Key words: Haemato-biochemical changes, *Salmonella* Gallinarum, Ashwagandha, broiler chicken

Fowl typhoid (FT) is an acute septicaemic disease affecting all ages of chickens. The disease is caused by *Salmonella* Gallinarum, a Gram negative and non-motile bacteria. Poultry industry is facing great setback due to frequent outbreaks of FT. The disease can be controlled by use of prophylactic antibiotics and vaccination. Frequent use of antibiotics as feed additive in poultry feed may result in development of resistance pathogenic microorganisms. In ethnoveterinary practice, various herbal and medicinal plants have been used to control diseases in poultry (Waihenya *et al.,* 2002). Ashwagandha (*Withania somnifera*) has long been used in traditional medicine in India (Singh *et al.,* 2001). It has immunomodulatory (Lokhande *et al*., 2009), antioxidant (Kaur *et al.,* 2003), hepatoprotective (Harikrishnan *et al.*, 2008) and antibacterial (Owais *et al*., 2005) effects. Therefore, the present study was undertaken to study the effects of Ashwagandha root powder in broiler chicks experimentally infected with *S.* Gallinarum.

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MATERIALS AND METHODS

A total of 160, day-old broiler chicks were randomly divided into four groups A, B, C and D; each comprising 40 chicks. Groups A and B were given normal autoclaved feed while chicks in groups C and D were given dried Ashwagandha root powder @ 0.5% of feed. After 7 days, chicks in groups B and D were infected with 10^7 CFU/ml of *S.* Gallinarum via the subcutaneous route. Mortality after *S.* Gallinarum infection was recorded. Blood samples were collected from randomly selected five chicks from each group at 0, 3, 7, 14, 21, 28 and 35 days post infection. Body weight of five chicks from each group was recorded at the above said intervals. The estimation of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC) was done as per standard methods (Benjamin, 1978). The erythrocyte indices as mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated as per the standard formulae (Benjamin, 1978). The serum samples were analyzed for total serum

proteins (TSP), albumin and globulin fractions, aspartate aminotransferase (AST), alanine aminotransferase (ALT) activity, lactate dehydrogenase (LDH), alkaline phosphatase (AKP) by semi-automatic biochemistry analyzer using single step reagent kits procured from Siemens Healthcare. The Institutional Animal Ethics Committee approval was obtained prior to conduct of this study.

RESULTS AND DISCUSSION

Mortality Pattern and Body Weight: There was no mortality in groups A and C. However, a total of 15 chicks died in group B while only eight chicks died in group D after *Salmonella* infection. Overall mortality was considerably reduced in Ashwagandha supplemented group D (8) as compared to group B chicks (15) indicating protective effect of feeding Ashwagandha. Owais *et al.* (2005) also observed similar findings in Balb/C mice due to experimental salmonellosis and simultaneously treated orally with *W. somnifera*. There was a significant decrease in body weight in both the infected groups (B and D) as compared to uninfected groups (A and C) from 14 DPI onwards till the end of the experiment (Table 1). The decrease in body weight in infected groups is in accordance with earlier report of Madhuri and Sadana (2000). However, the mean body weights in group D were slightly higher than group B but significant ($p<0.05$) difference was observed only on 3 and 28 DPI which was suggestive of improved feed conversion and early recovery from *S.* Gallinarum infection due to adaptogenic and antistress activity of withanolides present in roots of *W. somnifera* (Singh *et al.*, 2003).

Haematological Parameters: The Hb concentration (Fig. 1) was significantly higher in group C than group A at 3, 21 and 28 DPI. In group B, lower Hb values were observed throughout the experiment after infection with *S.* Gallinarum. Mean Hb values in group D showed similar pattern to group B. There was a decrease in PCV in group B (Fig. 1) which was significant ($p<0.05$) from 3 DPI till the end of the experiment. A similar decreasing pattern was observed in mean PCV values in group D and these values were significantly ($p<0.05$) lower as compared to group C from 3 DPI onwards till the end of the experiment. The average PCV values were higher in group D as compared to group B and but significant ($p<0.05$) difference was observed from 7 DPI to 35 DPI (except on 28 DPI). Mean TEC values in group B were significantly ($p<0.05$) lower than that of control groups (A and C) throughout the experiment (Fig. 1). The TEC values in group D were higher than group B throughout the experiment. The average MCV and MCHC values in different groups revealed no significant ($p<0.05$) difference between uninfected groups (A and C). In infected groups (B and D), a decrease in MCV was observed and it became the lowest at 14 DPI from 95.16±3.14 fl to 79.56±4.95 fl and 90.53±2.62 fl to 89.83±3.11 fl, respectively as compared to respective control groups. Thereafter, it increased up to 35 DPI. Significant ($p<0.05$) difference in MCV was observed between groups B and D only at 21 DPI. No significant ($p<0.05$) difference was observed in average MCHC values between both uninfected groups (A and C) at any interval of the experiment. In groups B and D, MCHC value first decreased up to 14 DPI, reached to its minimum level (21.26±0.396 % and 25.56±0.527 %, respectively) and thereafter values increased gradually up to 35 DPI.

The significant decrease of haematological parameters in both infected groups (B and D) were indicative of anaemia in *S.* Gallinarum infected chicks. Progressive decline in RBC count and a hypochromic nature of anaemia was probably due to the effect of lipopolysaccharide of *S.* Gallinarum on RBCs thus provoking a sudden loss of red cells in the circulation, and perhaps membrane damage in the circulating red cells (Kokosharov, 2002). The erythrocytic indices in the present study were suggestive of microcytic hypochromic type of anaemia similar to the observations of Benjamin (1978) and Kokosharov and Todorova (1987) but different from macrocytic normochromic as reported by Assoku *et al.* (1970). The intensity of anaemia was less in group D. The chicks in this group attained normal values earlier than that in group B suggesting that Ashwagandha root powder accorded protection against development of anaemia and induced faster recovery of chicks from the disease. Haemoprotective effect of *W. somnifera* in broiler chicks might have been due to its positive influence on haemopoiesis through stimulation of stem cell proliferation and increase in bone marrow cellularity (Aphale *et al.*, 1998; Mishra *et al.*, 2000). The haemoprotective effect of *W. somnifera* root powder may be due to its antioxidant activity protecting RBC from oxidative stress and improving erythrocytic enzyme activity (Sujatha *et al.*, 2010).

Average TLC values revealed a significant leucocytosis in both infected groups (B and D) as presented in Fig 2. An increase in TLC values was observed from 3 DPI in groups B and D. Leucocytosis was due to absolute heterophilia as well as absolute lymphocytosis as revealed by absolute leucocyte count in these groups. However, heterophilia contributed more

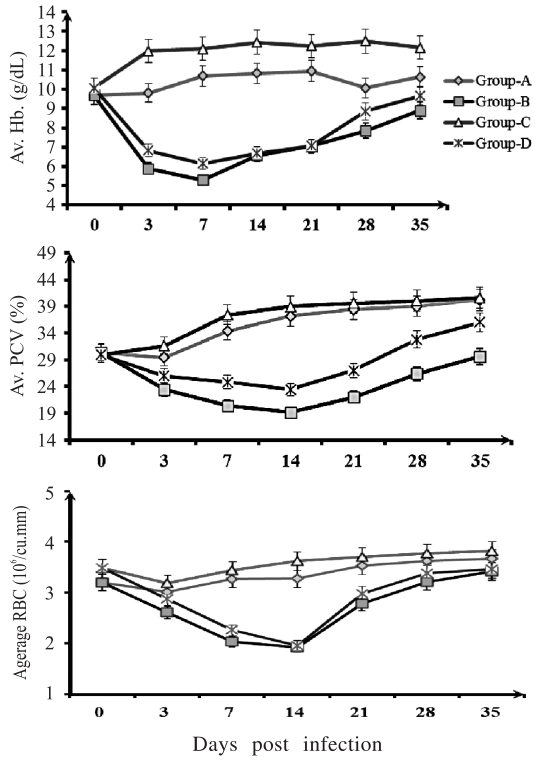

Fig 1. Total erythrocytes (RBC), haemoglobin (Hb), packed cell volume (PCV) in broiler chickens fed Ashwagandha and experimentally infected with *S.* Gallinarum

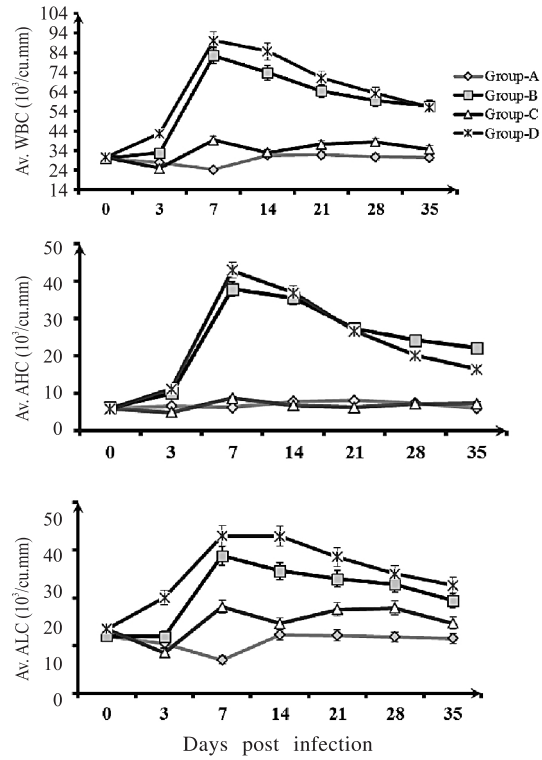

Fig 2. Total leucocyte count (WBC), absolute heterophils (AHC) and absolute lymphocytes (ALC) in broiler chickens fed Ashwagandha and experimentally infected with *S.* Gallinarum

than lymphocytosis in the development of leucocytosis. A significant leucocytosis found in both infected groups is in accordance to Madhuri and Sadana (2000) and Kokosharov (2003). The results in absolute counts of leucocytes corresponded with the findings of Allan and Duffus (1971) who reported more increase in number of heterophils in comparison to lymphocytes in *S.* Gallinarum infected chicks. The leucocytosis was attributed to bone marrow hyperplasia (Assoku *et al.,* 1970). Higher values noticed in group D might be due to stimulating effect of *W. somnifera* on the bone marrow cells as reported by Davis and Kuttan (2000). Heterophilia could be due to acute inflammatory and degenerative/ necrotic changes in internal organs. Heterophil percent was lower in group D as compared to group B which might be due to lesser degenerative/ necrotic changes in different visceral organs due to effect of Ashwagandha. Percent lymphocytes and absolute lymphocytes were higher in group D which might be due to immunopotentiative effect of Ashwagandha mediated through varying degree of lympho-proliferative changes in lymphoid organs thereby increasing phagocytic activity of cells and improving cell mediated immunity (Gatne *et al.,* 2010).

Biochemical Parameters: The TSP (Fig. 3) decreased significantly ($p<0.05$) from 3 DPI till 7 DPI in both the infected groups (B and D). A similar decreasing trend was also observed for serum albumin in both the infected groups. However, the decline in albumin was severe in group B as compared to group D with significant differences between these two groups from 3 DPI onwards (Fig. 3). A significantly higher globulin concentration was observed in group B from 14 to 35 DPI while in group D it was significantly higher from 3 to 35 DPI when compared to uninfected groups (A and C). The lowering of TSP in groups B and D was in accordance with Kokosharov (2006) which may be due to degenerative and necrotic changes in liver caused by *S.* Gallinarum infection. The observed hypo-albuminaemia might be due to liver damage caused by *S.* Gallinarum infection, as liver is the only organ where albumin is synthesized (Kokosharov, 2006). According to Kaneko (1997), acute phase proteins are preferentially produced in liver during sepsis and thus albumin synthesis is inhibited. A similar increase in globulin concentration was observed by Mamta and Mishra (2007) in experimental *S.* Gallinarum infection. Total proteins, albumin and globulin contents were higher in groups C and D which could be due to increased availability of protein to the birds because of supplementation of Ashwagandha (Wanjari, 2004; Choudhari *et al.,* 2006).

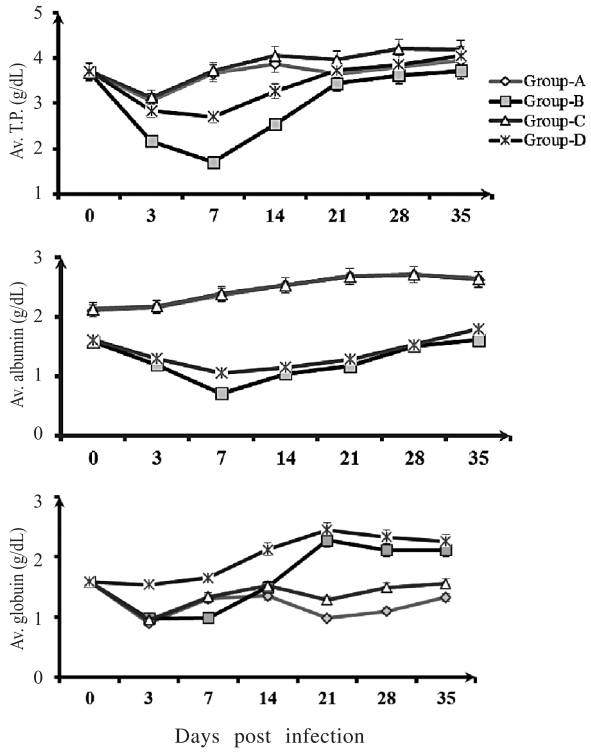

Fig 3. Total serum protein (TP), albumin and globulins in broiler chickens fed Ashwagandha and experimentally infected with *S.* Gallinarum

Serum ALT activity was significantly ($p<0.05$) higher in group B throughout the experiment (Table 2). Whereas, the ALT activity in group D was significantly lower as compared to group B throughout the experiment. A similar pattern in AST activity was observed in both the infected groups (Table 2). However, the increase in values in group D was less as compared to that in group B and it returned close to normal on 35 DPI, when it was significantly lower on comparison to group B. Increased serum ALT and AST activities has also been reported by Kokosharov and Goranov (1997) during experimental *S.* Gallinarum infection in broilers chicks which could be attributed to hepatic necrosis, degeneration in heart and skeletal muscles. Less increase in these enzyme activities in group D as compared to group B suggested lesser degenerative and necrotic changes in liver and heart in group D, which was evident during histopathological examination and lesion scores (Divya, 2012). Hepatoprotective and cardioprotective properties of Ashwagandha have been reported by Mohanty *et al.* (2004), Hamza *et al.* (2008) and Harikrishnan *et al.* (2008). Ashwagandha offers hepatoprotection by influencing the levels of lipid peroxidation products and

Table 1

Mean body weight (g) of broiler chicks fed Ashwagandha and experimentally infected with *S.* Gallinarum

DPI	Mean body weights (g) in groups			
	A	B	C	D
0	85.00^{a}±1.67	85.00^{a}±1.67	87.60^{a}±0.81	87.60^{a}±0.81
3	159.00^{a}±4.00	151.00^{a}±4.00	185.00^{c}±5.00	172.00^{b}±1.67
7	296.00^{a}±9.27	254.00^{a}±14.00	346.00^{b}±18.33	272.00^{a}±3.74
14	596.00^{bc}±30.75	440.00^{a}±34.20	678.00^{c}±22.89	514.00^{ab}±25.41
21	1098.00^{b}±46.30	786.00^{a}±29.93	1116.00^{b}±37.49	890.00^{a} ±31.30
28	1400.00^{b}±83.66	1120.00^{a}±58.30	1506.00^{b}±84.47	1340.00^{b}±29.15
35	1970.00^{b}±122.06	1600.00^{a}±70.71	1980.00^{b}±66.33	800.00^{ab}±130.38

A=Basal diet alone and uninfected; B=Basal diet and infected with S. Gallinarum; C=Basal diet containing Ashwagandha; D=Basal diet containing Ashwagandha and infected with S. Gallinarum

Table 2

Mean serum alanine aminotransferase (ALT; IU/L) and aspartate aminotransferase (AST; IU/L) in broiler chickens fed Ashwagandha and experimentally infected with *S.* Gallinarum

DPI	Enzyme activities in groups							
	A		B		C		D	
	ALT	AST	ALT	AST	ALT	AST	ALT	AST
0	10.0^{a}±0.70	152.97^{a}±5.73	10.0^{a}±0.70	152.97^{a}±5.37	9.40^{a}±0.92	160.62^{a}±5.27	9.40^{a}±0.92	160.62^{a}±5.27
3	11.40^{a}±0.50	260.78^{ab}±6.99	22.80^{c}±0.86	298.31^{b}±20.63	10.86^{a}±0.39	231.04^{a}±15.34	18.33^{b}±0.72	252.04^{a}±10.30
7	12.05^{a}±0.82	216.08^{a}±15.36	40.60^{c}±1.20	363.08^{b}±8.30	11.70^{a}±0.53	197.46^{a}±20.71	35.80^{b}±1.15	352.84^{b}±9.12
14	10.75^{a}±0.38	195.60^{a}±7.43	59.71^{c}±0.89	362.44^{b}±3.08	10.26^{a}±0.64	195.34^{a}±14.45	46.80^{b}±1.46	334.50^{b}±16.63
21	10.78^{a}±0.17	185.00^{b}±3.95	52.80^{c}±1.15	343.92^{c} ±3.73	10.31^{a}±0.27	164.10^{a}±4.55	31.13^{b}±1.03	335.70^{c} ±9.59
28	10.66^{a}±0.47	183.72^{a}±10.79	47.17^{c}±0.78	284.02^{b}±22.87	10.00^{a}±0.31	167.20^{a}±12.12	23.60^{b}±0.92	257.74^{b}±10.75
35	9.40^{a}±0.50	223.76^{b}±6.64	34.84^{c}±1.41	318.04^{d}±1.81	9.00^{a}±0.44	153.32^{a}±7.45	18.00^{b}±0.70	243.88^{c} ±4.45

Means with unlike superscripts in the row differ significantly ($p<0.05$)

A=Basal diet alone and uninfected; B=Basal diet and infected with S. Gallinarum; C=Basal diet containing Ashwagandha; D=Basal diet containing Ashwagandha and infected with S. Gallinarum

Table 3

Mean serum lactate dehydrogenase (LDH; IU/L) and alkaline phosphatase (AKP; IU/L) in broiler chickens fed Ashwagandha and experimentally infected with *S.* Gallinarum

DPI	Enzyme activities in groups							
	A		B		C		D	
	LDH	AKP	LDH	AKP	LDH	AKP	LDH	AKP
0	328.00^{a}±8.60	306.60^{a}±1.07	328.00^{a}±8.60	306.60^{a}±1.07	350.00^{a}±7.07	310.20^{a}±1.56	350.00^{a}±7.07	310.20^{a}±1.56
3	342.00^{a}±5.83	353.20^{b}±11.90	613.80^{b}±42.32	170.40^{a}±6.08	350.00^{a}±7.07	346.20^{b}±10.92	596.00^{b}±23.79	167.60^{a}±7.60
7	319.20^{a}±12.97	389.20^{b}±9.78	792.00^{c}±39.16	177.80^{a}±3.39	304.00^{a}±9.27	410.20^{b}±12.31	672.00^{b}±26.34	184.20^{a}±5.05
14	345.00^{a}±5.00	339.60^{b}±6.21	1039.00^{c}±55.23	146.20^{a}±7.08	321.80^{a}±6.84	342.60^{b}±2.63	775.20^{b}±26.53	147.20^{a}±5.48
21	291.60^{a}±12.36	322.00^{b}±17.95	675.20^{c}±48.72	172.60^{a}±6.56	260.00^{a}±7.07	419.00^{b}±23.58	468.00^{b}±11.57	177.40^{a}±4.66
28	269.80^{a}±6.93	325.60^{a}±1.56	524.00^{c}±49.25	294.40^{a}±11.96	259.60^{a}±3.18	322.40^{a}±2.73	358.00^{b} ±18.93	287.80^{a}±18.59
35	279.40^{a}±3.78	330.00^{a}±1.7	421.40^{b}±24.59	303.20^{a}±6.40	267.60^{a}±2.50	317.20^{a}±1.77	316.00^{a} ±27.17	306.00^{a}±6.23

A=Basal diet alone and uninfected; B=Basal diet and infected with S. Gallinarum; C=Basal diet containing Ashwagandha; D=Basal diet containing Ashwagandha and infected with S. Gallinarum

this could be due to presence of alkaloids, withanolids and flavonoids, its free radical scavenging property and its antioxidant property. But the exact underlying mechanism is still unclear (Harikrishnan *et al.,* 2008).

The LDH activity also increased in both the infected groups i.e. B and D (Table 3). In group B, activity was significantly (P<0.05) elevated on 14 DPI and remained significantly higher till 35 DPI. The chicks in group D also had elevated LDH activity. However, increase in LDH activity in group D was less than group B with significant (p<0.05) difference throughout the experiment. The increase in LDH activity in group D compared to group B after *Salmonella* infection could be due to cardioprotective effect of *W. somnifera* which acts by restoring the myocardial antioxidant status and maintaining membrane integrity of myocardial muscles (Mohanty *et al.,* 2004).

The activity of AKP declined significantly (p<0.05) in group B between 3 to 14 DPI (Table 3). A similar decreasing trend was observed in group D chicks. Decrease in AKP activity has also been reported by Itoh *et al.* (1996) during experimental *S. typhimurium* infections in broilers and by Kokosharov and Goranov (1997) in *S.* Gallinarum infection. In chicks little AKP activity has been demonstrated in the liver and none has been found in the lung, skeletal muscles or heart (Bogin and Israeli, 1976). So elevation of AKP in avian species has been predominantly associated with increased osteoblastic activity (Lewandowski *et al.,* 1986) and skeletal growth. As the body weight gain was depressed in infected groups, the AKP activity was also found to decrease in infected groups (B and D). Reduction in severity of biochemical alterations induced by *S.* Gallinarum infection in group D indicated the protective effects of Ashwagandha against injury caused by *Salmonella*. Gupta *et al.* (2008) also observed an increased AKP activity in broiler chicks challenged with *S.* Gallinarum and fed ochratoxin A. From the present study it can be concluded that Ashwagandha root powder reduces mortality, improves body weight and accords protection on haematobiochemical alterations due to *S.* Gallinarum infection in broiler chickens.

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