Immunomodulatory effect of *Withania somnifera* in broilers treated with high doses of enrofloxacin

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Abstract

The effect of supplementation of *Withania somnifera* extract on immunomodulatory response in broilers treated with high doses of enrofloxacin in poultry was investigated. Ninety day old broiler chicks of either sex were randomly divided into nine groups of 10 each. Enrofloxacin was administered at 30 and 40 mg/kg orally for seven days during the third week and *W. somnifera* crude extract was added in the feed at 1 and 2 % from second week onwards and the effect of their combinations was evaluated. The results of the study revealed that enrofloxacin treatment produced highly significant reduction in serum total protein, albumin and globulin and numerical decrease in HI titre against Newcastle vaccine. The groups treated with *W. somnifera* alone did not differ significantly from the control in all above parameters. Dose dependent increase in all the parameters were noticed in the combination groups which clearly supports the immunoprotective effect of *W. somnifera.*

Keywords: Broilers, Immunomodulatory, Withania somnifera, enrofloxacin, HI titre.

1. Introduction

Antibacterials especially enrofloxacin is used frequently by poultry farmers at very high doses from the first week to culling/ marketing. The impact analysis of this practice revealed many negative effects namely failure of treatment, escalation of treatment cost and development of resistance. Many medicinal plants showing immunomodulatory activity have been used instead of drugs because of their low toxicity for the host system, adequate absorption and capability to reach the target organ without much degradation by host enzymes. *Withania somnifera* is an herb that grows naturally in diverse areas ranging from Africa, the Mediteranean East and India. It has earned the nickname "Indian Ginseng". The chemistry of *Withania somnifera* has been extensively studied and over 35 chemical constituents have been identified. The biologically active constituents are withanolides G and D, withaferin A, isopelletierine, anaferine, withasomnine etc. (Mishra *et al*., 2000). These are believed to be responsible for its diverse pharmacological actions *viz*., anticonvulsant, anti-inflammatory, antioxidant, antitumor, anxiolytic actions (Bhattacharya *et al*., 2001). Hence the present study was planned to explore the possible immunomodulatory effect of *Withania somnifera* in broilers treated with high doses of enrofloxacin.

2. Materials and methods

Commercial day old, unsexed broiler straight run chicks (Vencob strain) belonging to a single hatch, obtained from a commercial hatchery at Namakkal were used for the experimental study. All the chicks were reared under standard and uniform managemental conditions throughout the experimental period of six weeks.

Broiler starter and finisher mash, free of toxins and pesticide residues purchased from a feed manufacturing unit at Veterinary College and Research Institute, Namakkal was used as a basal diet for formulating the experimental diet. The broiler starter and finisher mashes were fed *ad libitum* to the birds from 1 to 28 and 29 to 42 days of age respectively. The crude extracts of the herbal plant *Withania somnifera* (root) obtained from Natural Remedies, Bangalore and commercially available enrofloxacin (Enrocin) was used for the experimental study.

A survey was conducted to fix the dose of enrofloxacin. Inclusion levels of plant extract were fixed as per the literature. Experimental diets containing *W. somnifera* root extract (crude) were prepared and mixed with feed and fed to the following treatment groups.

On first day, all ninety broiler chicks were weighed with banded wing and reared in battery brooders. On eighth day, the chicks were randomly divided into nine treatment groups of ten each. Commercial enrofloxacin was given during third week for seven days (i.e., 15th to 21st day) through drinking water as pulsed dosing. The birds were subjected to respective treatment from eighth day to

forty second day at various dose levels of the drug and different inclusion levels of the plant as per table 1

Table 1. *Experimental Design*

S. No.	Treatment	Experimental groups
1	T1	Normal control
2	T2	Enrofloxacin - 30 mg/kg
3	T3	Enrofloxacin - 40 mg/kg
4	T4	Withania somnifera - 1% level
5	T5	Withania somnifera - 2% level
6	T6	Enrofloxacin - 30 mg/kg + Withania somnifera -1 % level
7	T7	Enrofloxacin - 40 mg/kg + Withania somnifera -1% level
8	T8	Enrofloxacin - 30mg/kg + Withania somnifera - 2% level
9	T9	Enrofloxacin - 40mg/kg + Withania somnifera - 2 % level

All the nine groups were administered with Newcastle disease vaccine (Lasota strain) at 7th and 21st day of age. Blood samples were collected at the end of fourth week and sixth week. Serum was separated and HI titre against Newcastle disease (Alexander, 1988), serum total protein, albumin and globulin (Varley *et al.,* 1980) were estimated. The data were statistically analysed by completely randomized block design (Snedecor and Cochran, 1989).

3. Results and Discussion

The HI titre values were estimated against Newcastle disease virus in all the groups at the end of fourth week and sixth week and presented in the table 1. At the end of fourth week the mean HI titre level did not differ significantly between the groups. But the titre value was numerically higher in *W. somnifera* control groups (T4 and T5) and the group treated with low dose of enrofloxacin and high dose of *W. somnifera (*T8).

At the end of sixth week there was no significant difference between control group and enrofloxacin control groups (T2 and T3) but the HI titre was higher in both the *W. somnifera* control groups. The group administered with 2% *W. somnifera* recorded highly significant increase in HI titre value compared to control which denotes the immunostimulating potential of the plant. The other groups did not differ from control and the groups treated with 2% *W. somnifera* performed better.

Tokarzewaki (2002) concluded that enrofloxacin and ciprofloxacin administered to hens after immunostimulation with salmonella antigen had suppressive effect on immune system. This is in accordance with the findings of the present study wherein enrofloxacin caused non significant decrease in titre value. *W. somnifera* is known to positively modulate the immune system of man and animals (Kuttan, 1996). Davis and Kuttan (1998) also reported the immunomodulatory effect of glycowithanolides from *W. somnifera* which reduced the leucopenia induced by cyclophosphamide.

The serum total protein, albumin and globulin were estimated at the end of fourth week and sixth week and presented in table 2, 3 and 4 respectively. At the end of fourth week serum total protein was significantly decreased in both the enrofloxacin control groups and the group treated with higher dose of enrofloxacin and lower dose of *W. somnifera.* The remaining groups did not differ significantly from control.

Table 2. *Effect of Withania somnifera on Haemagglutination Inhibition (log 2) titre in enrofloxacin treated broilers*

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
IV Week	4.83 ± 0.31	4.33 ± 0.21	4.50 ± 0.22	5.33 ± 0.42	5.33± 0.33	4.67 ± 0.21	4.67 ± 0.32	5.33 ± 0.21	5.00 ± 0.26
VI Week	5.33^{bc} ± 0.21	4.67^{c} ± 0.21	4.61^{c} ± 0.21	5.83^{ab} ± 0.17	6.17^{a} ± 0.17	4.67^{c} ± 0.21	5.00^{c} ± 0.26	5.33^{bc} ± 0.21	5.17^{bc} ± 0.17
n=6 Overall means bearing different superscripts between columns differ significantly (P<0.01)									

A significant decrease in serum albumin was noticed only in high enrofloxacin control group (T3) and decrease in globulin was noticed in low enrofloxacin control group (T2) at fourth week. The other groups did not differ significantly from control. Of the treatment groups, the group treated with low dose of enrofloxacin and high dose of *W. somnifera* (T8) performed better.

Anbalagan and Sadique (1981) reported the ability of *W. somnifera* to enhance the synthesis of certain modulator protein in rat liver. Dhenge *et al.* (2009) reported significant increase in serum total protein and globulin level and non significant increase in albumin level and significant decrease in albumin globulin ratio of broilers in their study on haematobiochemical profile of broilers supplemented with *W. somnifera* and *Andrographis paniculata*. The phytochemicals present in *W. somnifera* root extract might have

contributed to the reversal of changes in serum total protein, albumin and globulin to that of normal (Udayakumar *et al.*, 2009).

Table 3. *Effect of Withania somnifera on serum total protein level (g/dl) in enrofloxacin treated broilers*

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
II Week	5.66 ± 0.17	5.29 ± 0.10	5.63 ± 0.16	5.44 ± 0.19	5.24 ± 0.23	5.47 ± 0.16	5.37 ± 0.18	5.51 ± 0.14	5.34 ± 0.17
IV Week	5.24^{ab} ± 0.24	4.02^{d} ± 0.12	3.90^{d} ± 0.16	5.30^{ab} ± 0.20	5.37^{a} ± 0.18	4.57^{bcd} ± 0.19	4.33^{cd} ± 0.16	5.35^{a} ± 0.24	4.82^{abc} ± 0.21
VI Week	5.41 ± 0.22	4.96 ± 0.22	5.15 ± 0.24	5.21 ± 0.26	5.22 ± 0.15	5.47 ± 0.16	5.37 ± 0.18	5.51 ± 0.14	5.24 ± 0.23
n=6 Overall means bearing different superscripts between columns differ significantly (P<0.01)									

Table 4. *Effect of Withania somnifera on serum albumin level (g/dl) in enrofloxacin treated broilers*

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
II Week	1.66 ± 0.08	1.62 ± 0.08	1.74 ± 0.06	1.68 ± 0.06	1.70 ± 0.05	1.69 ± 0.04	1.68 ± 0.07	1.62 ± 0.09	1.67 ± 0.05
IV Week	1.64^{abc} ± 0.05	1.32^{cd} ± 0.10	1.07^{d} ± 0.06	1.69^{ab} ± 0.10	1.74^{a} ± 0.06	1.43^{abc} ± 0.11	1.42^{abcd} ± 0.10	1.54^{abc} ± 0.12	1.38^{bcd} ± 0.12
VI Week	1.65 ± 0.11	1.64 ± 0.05	1.64 ± 0.06	1.74 ± 0.08	1.74 ± 0.06	1.67 ± 0.07	1.69 ± 0.08	1.72 ± 0.08	1.73 ± 0.08
n=6 Overall means bearing different superscripts between columns differ significantly (P<0.01)									

Table 5. *Effect of Withania somnifera on serum globulin level (g/dl) in enrofloxacin treated broilers*

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
II Week	4.00 ± 0.23	3.67 ± 0.13	3.89 ± 0.21	3.76 ± 0.17	3.54 ± 0.28	3.78 ± 0.17	3.67 ± 0.20	3.89 ± 0.17	3.66 ± 0.18
IV Week	3.60^{ab} ± 0.24	2.70^{c} ± 0.11	2.84^{bc} ± 0.16	3.61^{ab} ± 0.22	3.63^{ab} ± 0.20	3.13^{abc} ± 0.25	2.91^{bc} ± 0.17	3.81^{a} ± 0.31	3.43^{abc} ± 0.25
VI Week	3.60 ± 0.24	2.70 ± 0.11	2.84 ± 0.16	3.68 ± 0.22	3.56 ± 0.20	3.13 ± 0.25	2.91 ± 0.17	3.81 ± 0.31	3.43 ± 0.25
n=6 Overall means bearing different superscripts between columns differ significantly (P<0.01)									

4. Conclusion

The present study confirm the immunosuppressive effect of high dose of enrofloxacin in broilers and its effective reversal by the dose dependent supplementation of crude extract of *Withania somnifera.*

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6. Reference

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