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Protective effect of (Ashwagandha) *Withania somnifera* root powder on general performance and hematological profile against fenvalerate induced toxicity in cockerels

Priyanka Tikore, Pravin Rathod, RS Ingole, KK Khose and MV Khodke

Abstract

Day old 80 cockerels after acclimatization for a period of one week were randomly divided into four equal groups comprising 20 birds in each. During experimental study, birds of fenvalerate treated group showed clinical signs of paralysis, tremors and convulsion observed which indicated neurotoxic effect of fenvalerate after second week of experiment and at 6th week of age birds showed milder clinical signs. Significant reduction in body weight, weight gain, feed consumption and FCR were observed in fenvalerate treated group throughout experimental period but numerical improvement due to the effect of Ashwagandha. At the end of 5th and 6th week significant decrease ($P \leq 0.05$) in Hb (hemoglobin), TEC (Total erythrocyte count), PCV (Packed cell volume), TLC (Total Leucocytes Counts) and lymphocyte count was observed in fenvalerate group but progressive improvement seen in Ashwagandha treated group. It was concluded that supplementation of Ashwagandha root powder through feed may reduce the severity of fenvalerate toxicity in cockerels.

Keywords: haematological changes, fenvalerate, ashwagandha, cockerels

Introduction

Demand of food and fibre has increased which lead to the chemicalization of agriculture and now a day's modern agriculture is dependent on high yielding varieties, which can only be grown under the influence of fertilizers and pesticides [6]. But excessive use may cause toxicity to fauna, flora and soil also an increase in residue creates burden in the food chain. Poultry feed substances are exposed to number of pesticides of residual potential Fenvalerate is second generation pyrethroids contain α -cyano group and also one of the most persistent synthetic pyrethroids in soil and has no specific antidote [17]. Among the diverse problems related to its use, there may be the opportunity of its biological accumulation that produces problems like diverse scientific and sub-scientific effects leading to loss in animal performance or subsequent intake of animal merchandise like milk with pesticide residues with the aid of human beings. As per the EPA (Environmental Protection Agency), 1989 residues of fenvalerate in a variety of raw commodities includes corn grain (0.02 ppm), corn fodder, forage (50 ppm), soyabean (0.05 ppm), wheat grain (0.5 ppm), barley grain (0.01 ppm), cotton seed (0.2 ppm), meat, meat byproduct, fat (1.5 ppm), milk (0.3 ppm), milk fat (7.0 ppm) and alfalfa (30 ppm) respectively. Ayurveda is traditional systems of medicine which is most popular in recent years. 'Ashwagandha' botanically known as *Withania somnifera* belongs to solanaceae family [10]. The root has a strong pungent smell especially bitter to taste and contains several alkaloids (0.13 to 4.30%) which offer medicinal usages. In addition, it also contains amino acids like proline, tyrosine, tryptophan, alanine and lysine. *Withania somnifera* play an important role in pesticides toxicity protection especially in hematological profile, as an antioxidant that prevents the effect of free radicals on vital organs. Keeping in view of the above, the present study was carried out to elucidate the general performance and hematological profile effect by fenvalerate and to evaluate the protective effect of *Withania somnifera*.

Materials and Methods

Approval from IAEC

Experiment was carried out as per the national guidelines of the Committee for Purpose of

Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, forest and climate changes, Government of India. Before start of the experiment the protocol of the experiment was approved from the Institutional Animal Ethical Committee (IAEC) of PGIVAS, Akola.

Chemicals

Fenvalerate (technical grade- purity 97%) insecticide was commercially obtained from Maharashtra Insecticide Pvt. Ltd., Akola having batch no. M-1610161. Dry root powder of ashwagandha was procured from Nagarjuna Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Commercial feed as per BIS (2007) guidelines was procured from M/s Shrikrupa poultry feeds, MIDC-38, Amravati, Maharashtra. All the birds were maintained under identical managerial and hygienic conditions.

Experimental Design

Eighty, a day old chicks of cockerels (n=80) was procured from M/s. Venketeshwara hatcheries Pvt. Ltd., Hyderabad and were acclimatized for a period of one week. These chicks were then divided into four equal groups comprising of twenty birds in each group. From 2nd week group A, B, C and D were given respective dietary treatments up to 5th week of age, Group A (control), group B was treated with fenvalerate @ 300 mg/kg of feed, whereas dried ashwagandha root powder @ 200mg/kg feed was given in groups C and group D was given fenvalerate @ 300 mg/kg of feed with ashwagandha @ 200 mg/kg of feed for four weeks however at the start of 6th week respective dietary treatment was withdrawn and all groups were fed with normal control diet for one week.

General Performance

During experimental period of 5 weeks (2nd to 6th week) birds of each group were kept under close observations for clinical symptoms and mortality. Average weekly feed consumption, FCR, Average weekly body weights (g), Average weekly body weight gain (g) were recorded for each group during 2nd to 6th week age of birds during experimental period.

Relative organ weight (g/100 g b.wt.)

At the end of 5th and 6th week, after gross pathological observations liver, spleen, and heart were separated out and weighed on digital weighing balance. Calculated relative organ weight by using following formula:

$$\text{Organ weight factor} = \frac{\text{Organ weight}}{\text{Whole body weight}} \times 100$$

Intestine length (cm)

At the end of 5th and 6th week, after gross pathological observations intestine was separated out and measured by using measuring tape.

Hematological observations

For hematological examination, six birds from each group were randomly selected for collection of blood sample at the end of experiment (i.e. at 5th and 6th week (7 day PWP)). About 2 ml of blood was collected aseptically from the jugular vein with 2 ml disposal syringe in the vial containing EDTA as an anticoagulant. Hematological parameter studied

included Hemoglobin (Hb, g/dL), Packed cell volume (PCV,%), Total erythrocyte count (TEC, x 10⁶ /cumm), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Total leucocytes count (TLC, x 10³ cumm) described by Natt and Herrick^[14] and Differential leucocytes count (DLC) were estimated as per the standard methods described as under.

Statistical analysis

The data pertaining to various parameters were analyzed by applying equal Completely Randomized Design (CRD) as described by Snedecor and Cochran^[23].

Results and Discussion

Clinical symptoms and Mortality

During experimental period of five weeks, birds from control and ashwagandha treated C group did not show any clinical symptoms but B group birds feeding fenvalerate @ 300 mg/kg in feed exhibited nervous symptoms of closed eyes/dropped eyes, paralysis, tremors, convulsion and incoordination in movement. While combine toxicity with plant D group birds were showed milder clinical symptom as compared to fenvalerate treated B group. During withdrawal period, birds of group B showed milder signs and in D group no symptoms were observed. During fenvalerate toxicity these clinical symptoms might be attributed due to fenvalerate keeps the sodium channels open for prolonged periods of time leading to hyperexcitation of the entire nervous system^[25]. Present symptoms during fenvalerate toxicity in broilers were also observed by Rangachar *et al.*^[18] and Shriwas^[21]. In Wistar rat similar findings were also reported by Saxena and Sharma^[20] and Patel^[17]. Present findings of D group were supported by Varma *et al.*^[25] and Sudani^[24]. Mishra *et al.*^[12] also reported anticonvulsive, neuroprotective, antiepileptic activity of *Withania somnifera*. No mortality was recorded in any of the group during experiment.

Relative organ weight (g/100 g b.wt.) and Intestine length (cm)

The relative organ weight of liver, heart, spleen and length of intestine differ non-significantly at 5th week as well as at 6th week of age during experimental period (Table 1). But relative organ weight of liver, heart, spleen numerical lower values were recorded in fenvalerate treated group due to production of reactive oxygen species causing membrane damage of cells resulting into degeneration to necrotic changes might be the reason for reduction in relative organ weight.

Similar finding of no alteration in liver weight during fenvalerate treated broiler was also recorded by Shriwas^[21], Roy *et al.*^[19] and Narayani^[13]. Vasanthakumar *et al.*^[26] recorded relatively more intestinal length in ashwagandha fed groups and suggested better gut health might be the reason.

Average weekly feed consumption and Feed Conversion Ratio (FCR)

The average feed consumption and FCR was decreased in fenvalerate treated group as compare with control and D group from 3rd to 5th weeks of experiment which might be due to toxic action of fenvalerate on intestine (Table 2, 3). Numerical increase in feed consumption and FCR were observed in D group birds treated with *Withania somnifera* during fenvalerate toxicity compared to B group suggested

beneficial effect on feed consumption. At 6th week of the experiment feed consumption and FCR revealed marginally improved in B and D group. Present findings of decreased feed intake also corroborated with Patel ^[17], Bhelonde and Ghosh ^[4] in Wistar rat and Shriwas ^[21] also reported gradual decrease in body weight gain and FCR in subacute toxicity of fenvalerate toxicity in broiler chicks. However, numerical increase in feed consumption and FCR were observed in C and D group of birds treated with *Withania somnifera* during fenvalerate toxicity when compared with fenvalerate treated B group of birds suggest its beneficial effect on feed consumption during fenvalerate toxicity ^[31].

Average weekly body weights (g) and Average weekly body weight gain (g)

The non significant differences was recorded in average weekly body weight at 2nd week and average body weight gain at 2nd, 3rd and 6th week of experiment. However, average weekly body weight at 3rd, 4th, 5th and 6th week of age and average body weight gain at 4th and 5th week of age showed significant ($P \leq 0.05$) decrease in body weight of B group birds treated with fenvalerate @ 300 mg/kg of feed (Table 4, 5). Significantly improvement in average weekly body weight in D group birds showed protective effect of *Withania somnifera*. At the end of 6th week (7th day PWP) significant ($p < 0.05$) decrease in mean body weight but improve body weight gain was observed in B group birds. Bhardwaj *et al.* ^[3] reported improved average body weight with better FCR due to the supplementation of *Withania somnifera* due to general tonic, health restorative activity, adaptogenic, anti-stress activity of withanolides present in roots of *Withania somnifera*. Similar findings of decreased body weight during fenvalerate toxicity in broiler were also recorded by Roy *et al.* ^[19], Shriwas ^[21] and Singh *et al.* ^[22] and suggested reflection of direct toxicity of insecticide on gastrointestinal tract which causes weight loss and decreased appetite and absorption. Parker *et al.* ^[15], Patel ^[17], Caglayan *et al.* ^[5] recorded similar observations in rats. Sudani ^[24] described that *Withania somnifera* extract having ability to protect body weight loss during cypermethrin toxicity in male albino rats which might be the reason for observation in group D.

Hematological Parameters

At the end of 5th and 6th week significantly ($P \leq 0.05$) decrease in Hb and TLC was observed in fenvalerate treated B group but progressive improvement in D group birds receiving *Withania somnifera* with fenvalerate suggested its haematinic and leucopoiesis property (Table 6, 7). The administration of fenvalerate resulted in suppression of erythropoiesis, hemoglobin synthesis and reduction in RBC and Hb content could be probably due to the blockage of protein synthesis and histogenesis ^[1]. Present result of decreased mean hemoglobin and TLC values in broiler during fenvalerate toxicity is in accordance with Abd El-Hamid ^[1], Shriwas ^[21], Verma and Pathak ^[29] and Gill ^[8]. Patel ^[17] and Bhelonde and Ghosh ^[4] recorded similar findings in rats. On the contrary, Garg *et al.* ^[7] and Verma ^[27] reported no change in the values of hemoglobin in chicks fed with fenvalerate @ 20 ppm and 3mg/kg body weight respectively in broilers.

The decrease in TLC values may directly related with either their decreased production in the lymphoid organ or their increased lysis due to presence of fenvalerate in the bone

marrow as evident from the microscopic picture of spleen observed during present study Garg *et al.* ^[7]. Bhardwaj *et al.* ^[3] reported that supplementation of *Withania somnifera* in feed enhances immune status of bird. Varma *et al.* ^[25] in cockerels also recorded significant increase values of hemoglobin and TLC with supplementation of *Withania somnifera* during fenvalerate toxicity. So *Withania somnifera* has protective role on fenvalerate toxicity in cockerels. At the end of 5th week, fenvalerate treated B group showed significant ($P \leq 0.05$) increase in heterophil count whereas significant ($P \leq 0.05$) decrease (Table 6, 7) was recorded in PCV, TEC and lymphocyte count indicated systemic stress to birds while in D group improvement due to protective effect of *Withania somnifera* on fenvalerate toxicity in cockerels was observed. Also non-significant values were observed at 6th week (7th day Post withdraw period Similar findings of increase in heterophil count and decrease in lymphocyte count during fenvalerate toxicity in broiler chicks were observed by Garg *et al.* ^[7], Shriwas ^[21] and Gill ^[8] in buffalo calves.

Similar finding of reduction of PCV, TEC during fenvalerate toxicity was noted by Abd El-Hamid (2004) in chicken embryos, Gill ^[8] in buffalo calf and Amaravathi *et al.* ^[2], Bhelonde and Ghosh ^[4] in Albino rats. Reduction of TEC in fenvalerate due to induced free radicals in plasma and its metabolite have the propensity to cause significant oxidative damage in erythrocytes in which is associated with marked damage to membrane proteins ^[13]. Decreased percent of lymphocyte and TLC in fenvalerate treated group indicated a risk of lymphopenia and immunomodulation.

At the end of 5th week D group showed improved value of PCV, TEC and lymphocyte count when compared with fenvalerate treated B group indicating protective effect of *Withania somnifera*. Similarly plant effect was observed by Bhardwaj *et al.* ^[3], Amaravathi *et al.* ^[2] and Varma *et al.* ^[25] observed significant restore values of in PCV, TEC and lymphocyte count values in *Withania somnifera* combine with fenvalerate treated group suggested immunomodulatory effect of plant.

Non-significant differences were observed in MCV, MCH, MCHC, eosinophil, monocyte and basophil count at end of 5th and 6th week (7th day PWP) in control and treatment groups (Table 6, 7). Birds treated with fenvalerate either alone or in combination with *Withania somnifera* showed numerical decrease in mean values of MCV, MCH and MCHC as compared to control and *Withania somnifera* treated group. Lower MCV values indicate decrease in size of erythrocyte suggesting possibility of iron deficiency anemia as described by Benjamin (2001). Present observation is supported by the reports of Institoris *et al.* ^[9] and Patel ^[17] in Wistar rat, Narayani ^[13] and Kumari *et al.* ^[10] in broilers.

Similar findings of no variation in eosinophil, monocyte and basophil count percentage during fenvalerate toxicity in broiler chicks also corroborates with Garg *et al.* ^[7], Shriwas ^[21] and Narayani ^[13] in alpha-cypermethrin toxicity. However, Varma *et al.* ^[25] found significantly increases the basophil and eosinophil count in pesticides intoxicated cockerel at 12 and 24 weeks interval and simultaneously feeding of *Withania somnifera* subside the levels in comparison to control. Fenvalerate feeding for a period of four weeks affects minimum on eosinophil, monocyte and basophil count whereas *Withania somnifera* feeding group could maintain eosinophil, monocyte and basophil count normal.

Table 1: Average organ weight (g) and length of intestine (cm) in control and different treatment groups at 5th and 6th week age of experiment

Groups	Liver weight (gm)		Spleen weight (gm)		Heart weight (gm)		Intestine length (cm)	
	5 th wk of age	6 th wk of age	5 th wk of age	6 th wk of age	5 th wk of age	6 th wk of age	5 th wk of age	6 th wk of age
T1	2.71± 0.01	2.75± 0.01	0.23± 0.09	0.24± 0.05	0.65± 0.03	0.66± 0.01	33.48± 0.81	35.36± 0.32
T2	2.69± 0.05	2.77± 0.03	0.21± 0.01	0.23± 0.02	0.64± 0.02	0.65± 0.02	32.27± 1.13	34.59± 0.23
T3	2.70± 0.02	2.75± 0.04	0.23± 0.04	0.24± 0.04	0.65± 0.01	0.66± 0.01	34.27± 1.28	35.44± 0.34
T4	2.70± 0.03	2.76± 0.02	0.24± 0.08	0.23± 0.07	0.65± 0.02	0.67± 0.03	32.98± 2.01	34.70± 0.32
CD	NS	NS	NS	NS	NS	NS	NS	NS

Mean values with common alphabet as superscript do not differ significantly

NS= Non Significant

Table 2: Average weekly feed consumption (g) in different treatment groups during experimental period from 2th to 6th week (n=20)

Groups	Experimental period from 2 nd to 6 th week						Pooled mean	NS
	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week		
T1	1510	2920	3830	4190	4420	3690	3426.67±437.10	NS
T2	1485	2710	3500	3850	3880	3450	3145.83±374.22	
T3	1495	3115	3910	4330	4430	3760	3506.67±445.63	
T4	1490	2820	3620	4100	4150	3610	3298.33±410.99	

NS= Non Significant

Table 3: Average weekly feed conversion ratio (FCR) in different treatment groups during experimental period from 2th to 6th week

Groups	Experimental period from 2 nd to 6 th week						Pooled mean	NS
	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week		
T1	1.00	1.11	1.00	0.81	0.67	0.68	0.88±0.08	NS
T2	1.03	1.11	0.97	0.82	0.66	0.67	0.87±0.08	
T3	1.04	1.25	1.06	0.88	0.70	0.69	0.94±0.09	
T4	1.04	1.14	1.00	0.85	0.67	0.67	0.90±0.08	

NS= Non Significant

Table 4: Average weekly body weights (g) per bird in different groups during experimental period from 2nd to 6th week (n=20)

Group	0 day wt.	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	Pooled mean
T1	40.25 ±0.39	72.00 ±0.43	124.75 ±0.45	182.35 ±0.39 ^{ab}	244.95 ±2.09 ^{ab}	312.25 ±1.20 ^b	386.50 ±1.87 ^{ab}	194.72 ±48.08
T2	39.95 ±0.43	71.80 ±0.34	122.15 ±1.80	180.35 ±0.94 ^b	232.90 ±2.11 ^c	292.75 ±1.21 ^c	366.43 ±2.34 ^c	186.62 ±44.89
T3	40.00 ±0.40	71.95 ±0.32	124.55 ±0.88	183.75 ±0.78 ^a	247.05 ±1.03 ^a	316.60 ±1.33 ^a	389.79 ±1.05 ^a	196.24 ±48.72
T4	40.15 ±0.36	71.65 ±0.36	123.70 ±0.81	180.20 ±1.07 ^b	242.50 ±0.65 ^b	308.80 ±1.49 ^b	382.07 ±1.57 ^b	192.72 ±47.47
CD (0.05)	NS	NS	NS	2.348	4.520	3.702	5.023	NS

Mean values with common alphabet as superscript do not differ significantly

NS= Non Significant

Table 5: Average weekly body weight gain (g) per bird in different groups during experimental period from 2th to 6th week

Group	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	Pooled mean
T1	31.75± 0.48	52.75± 0.64	57.60± 0.55	62.60± 2.22 ^a	67.30± 1.91 ^a	73.29± 2.07	57.55± 5.94
T2	31.85± 0.47	50.35± 1.97	58.20± 1.73	52.55± 2.51 ^b	59.85± 2.44 ^b	72.21± 2.54	54.17 ±5.44
T3	31.95± 0.47	52.60± 0.97	59.20± 0.93	63.30± 1.32 ^a	69.55± 1.70 ^a	73.86± 1.44	58.41± 6.11
T4	31.50± 0.49	52.05± 0.79	56.50± 1.24	62.45± 1.53 ^a	66.30± 1.52 ^a	72.64± 2.06	56.91± 5.88
CD (0.05)	NS	NS	NS	5.509	5.866	NS	NS

Mean values with common alphabet as superscript do not differ significantly

NS= Non Significant

Table 6: Haematological values related to erythrocytes in different groups at the end of 5th and 6th week age of experiment (n=6)

Group	Hb (gm/dL)		PCV (%)		TEC (10 ⁶ /cumm)		MCV (fL)		MCH (pg)		MCHC (g/dL)	
	5 th week of age	6 th week of age	5 th week of age	6 th week of age	5 th week of age	6 th week of age	5 th week of age	6 th week of age	5 th week of age	6 th week of age	5 th week of age	6 th week of age
T1	13.60± 0.03 ^a	12.30± 0.24 ^b	32.83± 0.48 ^b	35.17± 1.47	3.00± 0.05 ^b	3.20± 0.17	109.52± 1.81	110.68± 4.89	41.95± 0.90	41.02± 2.79	41.43± 0.66	35.21 ±1.22
T2	11.00± 0.09 ^c	11.83± 0.14 ^b	29.17± 0.48 ^c	33.83± 0.75	2.83± 0.06 ^c	2.99± 0.19	103.25± 2.82	105.18± 7.14	38.94± 0.86	36.72± 2.27	37.75± 0.40	35.04 ±0.92
T3	13.88± 0.04 ^a	12.94± 0.24 ^a	36.33± 0.67 ^a	36.50± 0.62	3.33± 0.04 ^a	3.33± 0.14	109.23± 2.70	110.76± 5.05	41.72± 0.46	41.20± 1.88	38.28± 0.79	35.47 ±0.66
T4	11.43± 0.20 ^b	12.14± 0.14 ^b	30.00± 0.63 ^c	34.83± 1.01	2.87± 0.07 ^{bc}	3.06± 0.22	104.76± 2.79	107.80± 4.39	39.96± 1.21	37.63± 1.46	38.24± 1.34	35.02 ±1.23
CD (0.05)	0.277	0.582	1.682	NS	0.164	NS	NS	NS	NS	NS	NS	NS

Mean values with common alphabet as superscript do not differ significantly NS= Non Significant

Table 7: Hematological values related to leucocytes in different groups at the end of 5th and 6th week age of experiment (n=6)

Group	TLC (10 ³ /cumm)		Lymphocyte (%)		Heterophil (%)		Monocyte (%)		Eosinophil (%)		Basophil (%)	
	5 th week of age	6 th week of age	5 th week of age	6 th week of age	5 th week of age	6 th week of age	5 th week of age	6 th week of age	5 th week of age	6 th week of age	5 th week of age	6 th week of age
T1	12.83± 0.04 ^a	11.89± 0.30 ^b	47.67± 0.76 ^a	46.00± 0.73	44.67± 0.67 ^c	46.00± 0.37	4.00± 0.37	4.00± 0.58	2.67± 0.33	3.00± 0.37	1.00± 0.26	1.00± 0.38
T2	11.42± 0.20 ^c	11.60± 0.18 ^b	41.00± 1.29 ^c	44.83± 0.83	50.00± 1.44 ^a	45.00± 0.52	4.17± 0.31	4.33± 0.42	3.00± 0.26	3.17± 0.31	1.83± 0.31	1.00± 0.37
T3	13.18± 0.18 ^a	12.77± 0.26 ^a	46.17± 1.54 ^{ab}	45.33± 0.61	45.67± 0.56 ^{bc}	45.67± 0.21	4.00± 0.58	4.00± 0.58	2.83± 0.48	3.00± 0.37	1.33± 0.42	2.00± 0.36
T4	12.03± 0.16 ^b	11.70± 0.30 ^b	42.83± 1.17 ^{bc}	44.83± 0.31	48.33± 0.88 ^{ab}	45.00± 0.26	3.50± 0.22	4.33± 0.42	3.33± 0.33	3.17± 0.48	2.00± 0.26	1.00± 0.26
CD (0.05)	0.462	0.778	3.603	NS	2.799	NS	NS	NS	NS	NS	NS	NS

Mean values with common alphabet as superscript do not differ significantly
NS= Non Significant

Conclusion

For supply of increases food demand in market farmers use more insecticide in agriculture for better production. Due to overuse of insecticide in field caused bioaccumulation in food chain that may target flora and fauna. Present study demonstrated that exposure to fenvalerate toxicity caused suppression in growth performance, alteration in hematological parameters. Treatment of *Withania somnifera* was found effective in restoration of hematological parameters as well as to reduce the stress due to fenvalerate toxicity in cockerels.

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