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The protective effects of *Withania somnifera* on biochemical profile of hepato-renal and endocrine system in fenvalerate exposed cockerels

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Abstract
After acclimatization for a period of one week, a day old 80 cockerels were randomly divided into four equal groups comprising 20 birds in each. Group T1 (control); group T2 was treated with fenvalerate @ 300 mg/kg of feed; group T3 was treated with *Withania somnifera* @ 200 mg/kg of feed and group T4 was given fenvalerate @ 300 mg/kg of feed with *Withania somnifera* @ 200 mg/kg of feed for four weeks and withdrawal of treatment for seven days. At 5th week as well as at 6th week serum AST and ALT showed significant (P≤0.05) increase in fenvalerate treated (T2) group while T4 group showed improvement. At the end of 5th week T2 group showed significant (P≤0.05) increase in BUN, creatinine, GGT and cholesterol but significant (P≤0.05) decrease in serum total protein, albumin, globulin, triiodothyronine, thyroxine and triiodothyronine: thyroxine ratio when compared with control and T4 group. From the present results it is concluded that *Withania somnifera* has protective effects on the various biochemical parameters against fenvalerate toxicity.

Keywords: Biochemical, cockerels, fenvalerate, *Withania somnifera*

Introduction
The pyrethroid class of insecticides was derived from natural compounds isolated from flower head of plant Chrysanthemum cincrariaefolium of pyrethrum species. Synthetic pyrethroid are chemically more potent and environmentally stable than natural pyrethrins [17]. Fenvalerate is a potent pyrethroid being used to kill the insect by spraying on food crops and food grain, its residual effect on agricultural product, food chain and consequently in poultry feed cannot be denied which may cause adverse effect on the health of poultry itself and finally its user [19]. Presentation of poultry to such lethal substances causes wellbeing perils and financial loses. Biochemical biomarkers are progressively utilized in natural hazard evaluation to distinguish the occurrence and impacts of ecological poisons. These chemicals interfere in the defence mechanisms of the host. Fenvalerate toxic effect showed severe neurotoxicity and estrogenic activity which further leads to neuroendocrine disturbing chemical. In several studies suggest that exposure to pyrethroids including fenvalerate may lead to developmental impairment, reproductive dysfunction and cancer through hormonal pathways [4].
Withania somnifera, otherwise called ashwagandha, Indian ginseng, and winter cherry, has been a significant herb in the Ayurvedic and indigenous medicinal frameworks for more than 3000 years. In Withania somnifera principal bioactive compounds are withanolides which derivatives of highly oxygenated C-28 steroid. More than 40 withanolides have been isolated and identified from *Withania somnifera.* [3] Withanoides are exceptionally oxygenated phytoconstituents and the oxidation at different site of skeleton is in charge of the auxiliary varieties in various classes of withanoides. Ashwagandha is believed to be amphoteric for example it can help manage significant physiologic procedures. The hypothesis is that when there is an overabundance of a specific hormone, the plant-based hormone antecedent involves cell layer receptor destinations so the genuine hormone can't join and apply its impact.[16] *Withania somnifera* possesses anti-oxidant, anti-stress, anti-tumor, immune-modulatory, hemopoietic and rejuvenating properties and exerts a positive influence on endocrine. Keeping in view of above, the present study was carried out to elucidate the biochemical alterations induced by fenvalerate and to evaluate the protective effect of *Withania somnifera.*

Materials and Methods

IAEC approval

The experiment was conducted as per Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and approved by the Institutional Animal Ethics Committee (IAEC) of PGIVAS, Akola.

Test chemical

The technical grade fenvalerate (purity- 97%) was procured from Maharashtra Insecticide Pvt. Ltd., Akola having batch no. M-1610161. The dry root powder of *Withania Somnifera* was procured from Nagarjuna Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Animals

Eighty, a day old chicks of cockerels was procured from M/s. Venketeshwara hatcheries Pvt. Ltd., Hyderabad, Telangana. 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 managemental and hygienic conditions.

Experimental animals

Following an acclimatization period of one week, these chicks were divided into four groups consisting of twenty birds in each group. From 2nd week group T1, T2, T3 and T4 were given respective dietary treatments up to 5th week of age, group T1 served as the control, group T2 was treated with fenvalerate @ 300 mg/kg of feed, group T3 was treated with *Withania somnifera* @ 200 mg/kg of feed and group T4 was treated with fenvalerate @ 300 mg/kg of feed with *Withania somnifera* @ 200 mg/kg of feed for four weeks. However at the start of 6th week respective dietary treatment was withdraw and all groups were fed with normal control diet for one week.

Evaluation of biochemical parameters

At the end of experiment (at 5th week and at 6th week (7th day PWP) withdrawal period) six birds from each group were selected for blood collection, serum was separated and stored at -20 ^{0}C until further use. Biochemical parameters included estimation of serum Total Protein, Albumin, Globulin, A/G ratio, BUN, Creatinine, Cholesterol, ALT, AST and GGT using diagnostic kits supplied by AGD Biomedicals (P) Ltd. Andheri (E) Mumbai India, by using autoanalyzer (Make AGD Biomedical Model No.AGD2020) as per standard method.

The thyroid hormones i.e. triiodothyronine (T_3) and thyroxin (T_4) were estimated by radioimmunoassay technique using the kits supplied by Board of Radioisotope Technology (BRIT), Bhabha Atomic Research Centre, Mumbai. Work carried out at Department of Veterinary nuclear Medicine, Bombay Veterinary College, Parel, Mumbai.

Statistical analysis

The data obtained during present investigations was analyzed by applying equal Completely Randomized Design (CRD) as described by Snedecor and Cochran [15].

Results

At 5th week of age average values of serum total protein, albumin, globulin in different treatment and control groups revealed significant differences (Tables 1). There was significantly (P≤0.05) decrease in serum total protein, albumin and globulin content in fenvalerate treated (T2) group and significantly improvement was observed in fenvalerate toxicity combine with *Withania somnifera* treated (T4) group as compared to control and fenvalerate treated group. At 6th week of age (7th day PWP) revealed non significant differences among control and different treatment groups. At the end of 5th and 6th week of age (7th day PWP), serum A:G ratio revealed non-significant differences between control and different treatment group birds (Table 1).

Table 1: Serum total protein (gm/dL), Albumin (gm/dL), Globulin (gm/dL) and A/G Ratio in different treatments groups at the end of 5th and 6th week age of experiment (n=6)

Groups	Total Protein (gm/dL)		Albumin (gm/dL)		Globulin (gm/dL)		A:G Ratio	
	5thwk of age	6thwk of age	5thwk of age	6thwk of age	5thwk of age	6thwk of age	5thwk of age	6thwk of age
T_1	3.23±0.14^a	3.19±0.18	1.69±0.08^a	1.77±0.09	1.54±0.12^a	1.42±0.24	1.12±0.10	1.47±0.30
T_2	1.85±0.06^c	2.80±0.04	0.99±0.10^c	1.79±0.03	0.88±0.13^b	1.02±0.05	1.50±0.55	1.79±0.10
T_3	3.45±0.13^a	3.19±0.07	1.88±0.05^a	1.78±0.05	1.57±0.12^a	1.42±0.11	1.23±0.11	1.30±0.13
T_4	2.39±0.14^b	2.83±0.15	1.28±0.07^b	1.77±0.03	1.11±0.15^b	1.06±0.14	1.27±0.18	2.04±0.56
CD (0.05)	0.356	NS	0.232	NS	0.378	NS	NS	NS

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

At the end of 5th and 6th week of age (7th day PWP) average values of serum asparate aminotransferase and alanine transaminase was significantly (P≤0.05) increased in T2 group when compared with control group indicating severe hepatotoxicity by fenvalerate @ 300 mg/kg in feed. However, values in group T4 was found to be restored towards the values of control group indicating protective effect of *Withania somnifera* @ 200 mg/kg in feed during fenvalerate toxicity given @ 300 mg/kg feed in cockerels (Tables 2). At 5th week, significant (P≤0.05) increase in serum GGT was observed in fenvalerate treated T2 group when compared to control group but showed improvement in T4 group due to hepatoprotective effect of *Withania somnifera*. At 6th week of age (7th day PWP) mean values of GGT were found to be non significant indicated mild restoration towards control group after withdrawal of toxicant (Tables 2).

Table 2: Serum AST (IU/L), ALT (IU/L), GGT (IU), Creatinine (mg/dl), BUN (mg/dl) and Cholesterol (mg/dl) in different treatments groups at the end of 5th and 6th week age of experiment (n=6)

Group	SGOT/AST (IU/L)		SGPT/ALT (IU/L)		GGT (IU/L)		Creatinine (mg/dl)		BUN (mg/dl)		Cholesterol (mg/dl)	
	5th week of age	6th week of age	5th week of age	6th week of age	5th week of age	6th week of age	5th week of age	6th week of age	5th week of age	6th week of age	5th week of age	6th week of age
T1	153.33± 0.48^{c}	151.88± 0.66^{b}	16.08± 0.17^{c}	14.97± 0.45^{b}	16.50± 0.24^{c}	16.00± 0.10	0.35± 0.02^{c}	0.44± 0.01	11.98± 0.35^{b}	12.07± 0.26	117.25± 0.55^{c}	117.55± 0.48
T2	162.57± 0.61^{a}	156.38± 1.78^{a}	21.92± 0.11^{a}	16.35± 0.19^{a}	23.07± 0.66^{a}	16.63± 0.16	0.63± 0.03^{a}	0.47± 0.02	14.00± 0.18^{a}	12.82± 0.21	123.93± 0.40^{a}	118.12± 0.48
T3	153.52± 0.43^{c}	153.27± 0.41^{b}	16.50± 0.27^{c}	15.33± 0.31^{b}	17.08± 0.08^{c}	16.10± 0.39	0.37± 0.01^{c}	0.45± 0.04	12.07± 0.26^{b}	12.00± 0.30	117.67± 0.34^{c}	117.58± 0.57
T4	157.57± 1.65^{b}	153.78± 1.10^{b}	17.53± 0.26^{b}	15.17± 0.07^{b}	21.63± 0.47^{b}	16.30± 0.41	0.50± 0.04^{b}	0.42± 0.03	13.32± 0.20^{a}	12.43± 0.31	119.98± 0.43^{b}	117.82± 0.23
CD (0.05)	2.767	3.276	0.637	0.853	1.249	NS	0.038	NS	0.755	NS	1.283	NS

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

At 5th week, significant (P≤0.05) increase in serum creatinine and BUN were observed in fenvalerate treated T2 group when compared to control group but showed improvement in T4 group due to nephrorotective effect of *Withania somnifera*. At 6th week of age (7th day PWP) mean values of Creatinine and BUN were found to be non significant indicated mild restoration towards control group after withdrawal of toxicant (Tables 2).

At 5th week, serum cholesterol was found to be significantly (P≤0.05) increased in fenvalerate treated T2 group when compared with control group indicating severe hepatotoxicity and nephrotoxicity along with nutritional imbalances by fenvalerate @ 300 mg/kg in feed. However, value in group T4 was found to be restored towards values of control group indicating protective effect of *Withania somnifera* during fenvalerate toxicity in cockerels (Tables 2). At the end of 5th week, significant (P≤0.05) decrease in serum triiodothyronine (T_3), thyroxine (T_4) and triiodothyronine: thyroxine ratio was observed in fenvalerate treated T2 group when compared to control group but showed improvement in T4 group due to protective effect of *Withania somnifera*. At 6th week (7th day PWP) average serum triiodothyronine (T_3), thyroxine (T_4) and T_3:T_4 ratio in different groups revealed non significant differences between control and treatment group (Tables 3).

Table 3: Serum triiodothyronine (T_3), thyroxine (T_4) and T_3:T_4 ratio in different treatments groups at the end of 5th and 6th week age of experiment (n=6)

Groups	Triiodothyronine (T_3) (ng/mL)		Thyroxine (T_4) (ng/mL)		T_3:T_4 Ratio	
	5thwk of age	6thwk of age	5thwk of age	6thwk of age	5thwk of age	6thwk of age
T1	2.52±0.10^{a}	2.45±0.02	26.17±0.60^{a}	25.83±0.31	0.096±0.002^{a}	0.095±0.002
T2	1.70±0.06^{c}	2.35±0.04	22.00±0.58^{c}	24.83±0.48	0.078±0.003^{b}	0.095±0.003
T3	2.60±0.04^{a}	2.52±0.08	26.50±0.43^{a}	26.17±0.95	0.098±0.003^{a}	0.097±0.005
T4	2.30±0.09^{b}	2.40±0.05	24.00±0.37^{b}	25.00±0.37	0.096±0.003^{a}	0.096±0.002
CD (0.05)	0.110	NS	1.483	NS	0.008	NS

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

Discussion

Reduction of protein synthesis might be due to fenvalerate toxicity attributed due to increased proteolytic activity and destruction of hepatic protein synthesizing subcellular structures. Which result liver damage and increased transaminase activity may be associated with the rapid utilization of reserved food material, i.e. carbohydrates and proteins. Present finding of decrease total protein, albumin, globulin values during fenvalerate toxicity in broilers was also observed by Garg *et al.* [6], Shriwas [14], Verma [17], Verma and Pathak [18]. In Wistar rat similar findings were also reported by Demerdash *et al.* [5] and Waheed and Mohammed [19]. Restored these values in *Withania somnifera* fed cockerels along with pesticides was also reported by Varma *et al.* [16] and Amaravathi *et al.* [3] in Wistar rats.

Degenerative changes in hepatocytes causes increased permeability of cell membrane resulting in release of transaminases in the blood stream [17] might be the reason for present observation in fenvalerate treated group birds. Similar findings of increase in AST, ALT and GGT during fenvalerate toxicity in broiler chicks were also observed by Majumder *et al.* [10], Abd El-Hamid [1], Shriwas [14], Roy *et al.* [13], Verma [17], Verma and Pathak [18], Gill *et al.* [7] in buffalo calves and Mongi *et al.* [11] in male Wistar rats. Amaravathi *et al.* [3] in rat and Varma *et al.* [16] in cockerels reported significant improvement in AST, ALT and GGT in combine group of *Withania somnifera* along with fenvalerate toxicity and suggested that *Withania somnifera* exhibited antioxidant and hepatoprotective property which might be the reason for decrease level of serum AST, ALT and GGT in T4 group.

The increase in creatinine and BUN level during fenvalerate toxicity was also reports by Shriwas [14] in broiler and Demerdash *et al.* [5] in male Wistar rats. The antioxidant effect of withanolides could be due its ability to activate antioxidant enzyme that catalyze the reaction of oxidants and are effective in renal damage which might be the reason for significant decrease in creatinine level in T4 group. Varma *et al.* [16] in cockerels and Amaravathi *et al.* [3] in Wistar rats reported restoration of creatinine and BUN level in fenvalerate group by addition of *Withania somnifera* @ 100 mg/kg feed and 200 mg/kg feed, respectively.

Cholesterol is used for the synthesis of bile acids and steroid

hormones. Esterification of cholesterol is less during severe liver dysfunctions which increased cholesterol levels [7]. Plasma cholesterol concentration increase could be due to decrease utilization of cholesterol for steriodogenesis during insecticide administration. These findings of the present study are in accordance with the reports of previous workers in broiler chick by Majumder *et al.* [10]; Shriwas [14]; Roy *et al.* [13] and Verma [17]. Similar findings of restored cholesterol values were also reported by Varma *et al.*[16] and Amaravathi *et al.* [3] during pesticides toxicity in cockerels and Wistar rat respectively. At 6th week average serum cholesterol in different groups revealed non-significant differences.

It was reported that fenvalerate toxicity decreased serum triiodothyronine concentration due to inhibits the conversion of serum thyroxine to triiodothyronine, which is major pathway of serum triiodothyronine generation. Serum thyroxine concentration in fenvalerate treated group indicated that the fenvalerate inhibits synthesis or release of T_4 at the glandular level. The inverse correlation between serum levels of cholesterol and thyroid hormone. The mechanism of its disruption may involve the effects on steroidogenesis signaling cascades and steroidogenic enzyme's activity [7]. Most of the circulating thyroid hormones bind to plasma proteins, albumin and α-globulin and only free hormones are responsible for biological activity. Due to decrease level of serum protein, albumin and globulin in blood there is less binding site available to thyroid hormone so decreases level in serum. Present findings of decrease in serum triiodothyronine (T_3), thyroxine (T_4) and triiodothyronine: thyroxine ratio during fenvalerate toxicity was also reported by Maiti and Kar [9] in male mice, Patel [12], Akhtar *et al.* [2] and Ibrahim *et al.* [8] in neonatal Wister rats, Gill (2014) in buffalo calves.

Conclusion

The present study has demonstrated the protective effect of *Withania somnifera* on serum biochemical parameter, altered by repeated fenvalerate toxicity administration in cockerels. This protective effect may be due to pharmacological activity of *Withania somnifera* has been attributed to two main withanolides, withaferin A and withanolide D.

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