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Protective effect of *Andrographis paniculata* on haematobiochemical profile during chlorpyrifos induced subacute toxicity in Japanese quails

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Abstract

The present study evaluated the protective effect of *Andrographis paniculata* on haematobiochemical profile during chlorpyrifos induced subacute toxicity in quails. Seventy five, two week old broilers quails after acclimatization period of one week were randomly divided into five equal groups. Group I served as control where as group II was treated with chlorpyrifos @ 15 mg/kg in feed. Group III, IV and V were treated with dried leaves powder of *Andrographis paniculata* @ 5 gm/kg of feed. In addition to dried leaves powder, group IV and V were treated with chlorpyrifos @ 15 mg/kg and 20 mg/kg of feed, respectively. At the end of 4th week of experiment haematobiochemical profiles were recorded. Results indicated significant increase in Hb, PCV and TEC in group II and non significant increase in group IV and V compared to control group. MCV, MCH and MCHC were found to be statistically non-significant. TLC, absolute lymphocyte and neutrophil count showed significant decrease in group II while group IV and V showed significant increase in heterophil and non significant increase in lymphocyte. Serum total protein, albumin and globulin differ non significantly. Serum creatinine, AST, ALT and GGT decreased significantly in group II but group IV and V showed improvement towards control group. It is thus concluded that chlorpyrifos @ 15 mg/kg of feed has adverse effect on haematobiochemical profile but co-administration of dried leaves powder of *Andrographis paniculata* along with chlorpyrifos @ 15 mg/kg and 20 mg/kg of feed showed dose dependant putative protective effect in Japanese quails.

Keywords: Chlorpyrifos, *Andrographis paniculata*, Haematobiochemical profile, Quails

Introduction

Chlorpyrifos (O, O-diethylO-3, 5, 6-trichloro-2-pyridyl phosphorothioate) is one of the most widely used broad spectrum organophosphate for controlling a variety of insects, fleas, termites, lice etc. It is also widely used as an insecticide for control of pests in various agricultural and animal husbandry practices. Its continuous and indiscriminate use in agro-production sector, grain storage and public health management causes accumulation of the residues in different daily consumable food materials such as vegetables, cereal crops, natural water systems and act as the major source of toxicity [1]. The continuous presence of chlorpyrifos even in low dosages in the diet has deleterious effects on chicken body metabolism [2]. Chlorpyrifos has very narrow margin of safety and is highly toxic to certain bird species including Japanese quail [3]. It induces toxicity by generating free radicals and by altering the levels of enzymatic and non-enzymatic antioxidant defenses [4] causing an overload of free radicals which cannot gradually destroyed and their accumulation in the body generates an oxidative stress. This oxidative stress may be prevented by protective mechanism *in vivo*, such as an enzymatic defense system (antioxidant enzymes) and free radical scavengers (antioxidants).

Andrographis paniculata having Andrographolide as an active constituent is known to possess number of medicinal properties viz. hepatoprotective, anti-inflammatory, antibacterial, antimicrobial, antifungal, anti-HIV, antiparasitic, antioxidant, antituberculosis and anti-cancer properties [5, 6]. The presence of flavonoids in the leaves of *Andrographis paniculata* enables them to have strongest antioxidant property and holds great promise for the use as a source of strong antioxidant compounds [7]. Literature revealed number of studies to use it as an antioxidant but its efficiency against chlorpyrifos induced toxicity in broilers has not been recorded. Hence the present investigation was carried out to evaluate the protective effect of *Andrographis paniculata* on haematobiochemical profile during chlorpyrifos induced subacute toxicity in Japanese quails.

Materials and Methods

The study was conducted in Japanese quails after necessary approval from the Institutional Animal Ethics Committee.

Clinically healthy, seventy five, two week old broiler quails were procured from Venketashwara Hatchery Pvt. Ltd., Pune and were acclimatized for a period of one week. The diet was provided to birds as per norms (Bureau of Indian Standard, 2007). All the experimental birds were maintained under identical managemental and hygienic conditions throughout the experimental period of 4 weeks (i.e. 28 days).

Experimental design

After acclimatization period, birds were randomly divided into five equal groups, each group thus comprising of 15 birds. Group I served as control where as group II was treated with chlorpyrifos @ 15 mg/kg of feed. Group III, IV and V were treated with dried leaves powder of *Andrographis paniculata* @ 5 gm/kg of feed. In addition to dried leaves powder of *Andrographis paniculata* group IV and V were treated with chlorpyrifos @ 15 mg/kg and 20 mg/kg of feed, respectively. All the birds were maintained for four weeks i.e. up to 7th week of age and were given adlib respective dietary treatments and water under identical managemental conditions. At the end of experiment (i.e. 4th week of experiment), six birds from each group were randomly selected and blood was collected aseptically from the jugular vein separately in the EDTA for hematological examination and in vacutainer for separation of serum for estimation of biochemical parameters. The biochemical parameters were estimated using Diagnostic kits supplied by M/s. Span Diagnostic Pvt. Ltd., Surat, India.

Plant and Insecticide

The dried leaves powder of *Andrographis paniculata* was procured from Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra) India, whereas Chlorpyrifos (Technical grade) was procured from Maharashtra Insecticide Pvt. Ltd., Akola.

Statistical analysis

Data obtained for different parameters under hematology and biochemical parameters were statistically analyzed by using Complete Randomized Design [8].

Results

Hematological observations

Hematological observations recorded on 28th day of experiment are presented in Table 1 and 2. The average mean Hb, PCV and TEC values were found to be significantly ($P < 0.05$) increased in group II birds when compared with group I and group III birds. However, group IV and V birds who received chlorpyrifos @ 15 and 20 mg/kg of feed respectively along with *Andrographis paniculata* @ 5 gm/kg feed showed non-significant increase in Hb, PCV and TEC values when compared with group I and group III. The values of erythrocyte indices viz. MCV, MCH and MCHC were found to be statistically non-significant between control and different treatment group birds. The marked non-significant decrease in MCV was recorded in group II birds.

The average mean value for TLC in different groups showed significantly ($P < 0.05$) lowest value in group II birds given chlorpyrifos @ 15 mg/kg of feed. However, administration of *Andrographis paniculata* revealed significant increase for TLC in group IV and non significant increase in group V birds. Absolute leucocytes count revealed significant decrease in absolute heterophil and absolute lymphocyte count in group II. However, absolute eosinophil, monocyte and basophil count showed non significant differences in control and other

treatment groups. Significant increase in absolute heterophil and non significant increase in absolute lymphocyte count in group IV and V suggested beneficial protective effect of *Andrographis paniculata* @ 5 mg/kg of feed during chlorpyrifos toxicity in Japanese quails.

Biochemical observations

The average mean values for serum total protein, albumin, globulin, A/G ratio, creatinine, ALT, AST and GGT is depicted in Tables 3.

The average mean serum total protein, albumin, globulin and A:G ratio did not reveal significant differences among different treatment groups whereas serum creatinine, ALT, AST and GGT were significantly increased in only chlorpyrifos treated group (group II) when compared with control group birds. However, serum creatinine, ALT, AST and GGT values in group III birds were comparable with that of control group birds. Serum ALT, AST and GGT values in group IV and V were found to be restored towards values of control group birds indicating putative protective effect of *Andrographis paniculata* (@ 5 gm/kg of feed) during chlorpyrifos toxicity @ 15 and 20 mg/kg in Japanese quails.

Discussion

The present investigation determined the protective role of *Andrographis paniculata* on hematobiochemical parameters during experimentally induced chlorpyrifos toxicity in quails. Present findings of increased Hb, PCV and TEC values in chlorpyrifos treated birds corroborated with earlier workers in cockler chickens [9], in mice [10] in indigenous chickens [11] suggested hemoconcentration might be the possible cause. Contrary to the present findings dose dependant low Hb level in chlorpyrifos treated broilers were also the findings in previous research work [12, 13]. Recently subacute toxicity study of chlorpyrifos @ 50 ppm in feed in broilers did not reveal significant difference in Hb and PCV values [14]. Increase in TEC in chlorpyrifos toxicity in birds suggested indicator of hypoxic condition. This might be because of a stress-linked release of new erythrocytes and synthesis of more hemoglobin in older to improve the ability of exposed birds in carrying oxygen [9]. The comparable values in group I and III and non significant increase in TEC level in group IV and V indicated partial restoration and beneficial effect of *Andrographis paniculata* during chlorpyrifos toxicity in broilers. The erythrocyte building capacity of *Andrographis paniculata* might be due to level of iron present in plant [15]. The present findings of marked non-significant decrease in MCV in group II was supported by earlier work [16]. A lower MCV value indicates decreased size of erythrocyte indicating microcytic anemia and restoration of MCV values in group IV and V and overall hematology parameters indicated beneficial hematonic property of *Andrographis paniculata* during chlorpyrifos subacute toxicity up to 20mg/kg of feed. Present finding of decrease in TLC in group II also supported by earlier research work [12, 13, 10] during chlorpyrifos toxicity while group IV and V suggested leucopoiesis effect of *Andrographis paniculata* during chlorpyrifos toxicity even at 15 and 20 mg/kg of feed in Japanese quails. The decrease in absolute heterophil and lymphocyte count in chlorpyrifos treated group was in consistent with previous findings [9]. The decrease in neutrophils in chlorpyrifos toxicity indicated involvement of neutrophils in phagocytosis during xenobiotic intoxication [16]. Leucopenia in the present study may be due to cytotoxic effects of chlorpyrifos since the lymphocyte are the main cells to play the role in defence mechanism and

reduction in lymphocyte is an indication of immunosuppression [17]. Significant increase in absolute heterophil count and non-significant increase in absolute lymphocyte count in group IV and V birds indicated restoration and regenerating effect of *Andrographis paniculata* on heterophil and lymphocyte count during chlorpyrifos toxicity in broilers.

The non significant decrease in serum total protein, albumin, globulin and A:G ratio might be due to oxidative injury caused to liver by chlorpyrifos which ultimately reduced capacity of liver to synthesis of protein [18]. Somewhat similar changes were also recorded in layer birds during chlorpyrifos induced toxicity [17]. Reversible changes of liver protein synthesis due to prevention of tissues damaging through the oxidation by *Andrographis paniculata* during chlorpyrifos toxicity might be the reason for numerical increase in serum total protein, albumin and globulin level in group IV.

However, *Andrographis paniculata* @ 5 gm/kg of feed during chlorpyrifos toxicity @ 20 mg/kg of feed could not showed that much beneficial effect.

Present finding of increase in serum creatinine, AST, ALT and GGT values due to experimental chlorpyrifos toxicity in group II are confirmed with previous findings [19, 20, 21, 22] suggested acute hepatic and renal injury by generating free radicals and by altering the levels of enzymatic and non-enzymatic antioxidant defences caused by chlorpyrifos [4]. The restoration of serum creatinine, AST, ALT and GGT in group IV and V towards group I and III could be due to the antioxidant effect of andrographiloides of *Andrographis paniculata* that catalyze the reaction of chlorpyrifos and might be the reason for putative protective activity against hepatic and renal tissue injury [23, 24] during chlorpyrifos toxicity @ 15 and 20 mg/kg of feed in Japanese quails.

Table 1: Hematological value of different groups at the end of 4th week of experiment

Group	Hb (gm/dl)	PCV (%)	TEC (10 ⁶ /cumm)	TLC (10 ³ /cumm)	MCV (fl)	MCH (pg)	MCHC (gm/dl)
I	10.21 ^b ± 0.70	38.00 ^{bc} ± 2.12	4.88 ^{bc} ± 0.14	11.58 ^a ± 0.31	87.70 ± 7.25	20.97 ± 1.43	24.34 ± 1.42
II	12.00 ^a ± 0.29	43.00 ^a ± 1.23	5.86 ^a ± 0.24	8.28 ^d ± 0.49	68.62 ± 5.39	20.72 ± 1.29	30.74 ± 1.89
III	9.96 ^b ± 0.47	37.33 ^c ± 1.68	4.26 ^c ± 0.28	11.00 ^{ab} ± 0.31	90.62 ± 7.05	23.96 ± 1.95	26.81 ± 2.09
IV	10.92 ^{ab} ± 0.41	42.33 ^{ab} ± 1.49	5.24 ^{ab} ± 0.31	10.10 ^{bc} ± 0.37	76.28 ± 7.29	21.32 ± 1.87	28.52 ± 1.81
V	11.00 ^{ab} ± 0.31	42.66 ^{ab} ± 1.33	5.15 ^{ab} ± 0.31	9.49 ^{cd} ± 0.66	79.56 ± 6.64	21.62 ± 1.08	27.60 ± 1.40
	CD for treatment = 1.350 (significant at 5% level)	CD for treatment = 4.680 (significant at 5% level)	CD for treatment = 0.780 (significant at 5% level)	CD for treatment = 1.312 (significant at 5% level)	NS	NS	NS

Mean value with common alphabets as superscript do not differ significantly
NS = Non significant

Table 2: Total leucocyte count (10³/cumm) and absolute leucocyte count in different groups at 4th week of experiment

Group	Total leucocyte count (TLC)	Absolute heterophil	Absolute lymphocyte	Absolute eosinophil	Absolute monocyte	Absolute basophil
I	11.58 ^a ± 0.31	3849.66 ^a ± 168.49	7232.50 ^a ± 175.59	231.33 ± 31.81	269.83 ± 50.39	0.00 ± 0.00
II	8.28 ^d ± 0.49	1902.36 ^c ± 168.65	6053.83 ^c ± 290.09	131.23 ± 43.21	182.53 ± 25.09	13.36 ± 13.36
III	11.00 ^{ab} ± 0.31	3569.66 ^a ± 119.09	7099.10 ^{bc} ± 248.06	127.56 ± 16.91	183.33 ± 23.96	20.33 ± 20.33
IV	10.10 ^{bc} ± 0.37	3339.83 ^{ab} ± 86.42	6239.00 ^c ± 312.28	215.33 ± 44.37	305.83 ± 60.28	0.00 ± 0.00
V	9.49 ^{cd} ± 0.66	2744.70 ^b ± 411.86	6362.83 ^{bc} ± 374.30	199.06 ± 24.82	183.40 ± 43.98	13.90 ± 13.90
	CD for treatment = 1.312 (significant at 5% level)	CD for treatment = 648.977 (significant at 5% level)	CD for treatment = 838.457 (significant at 5% level)	NS	NS	NS

Mean value with common alphabets as superscript do not differ significantly
NS = Non significant

Table 3: Biochemical value in different groups at 4th week of experiment

Group	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	A:G Ratio	Creatinine (mg/dl)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
I	5.30 ± 0.88	1.45 ± 0.08	3.85 ± 0.90	0.53 ± 0.17	0.95 ^c ± 0.10	23.18 ^b ± 3.29	207.78 ^c ± 16.63	11.02 ^b ± 2.27
II	3.55 ± 0.42	1.21 ± 0.11	2.33 ± 0.43	0.61 ± 0.14	1.53 ^a ± 0.06	31.86 ^a ± 1.78	286.81 ^a ± 20.47	17.24 ^a ± 2.03
III	4.60 ± 1.27	1.35 ± 0.04	3.25 ± 1.26	0.57 ± 0.11	1.02 ^{bc} ± 0.20	22.45 ^b ± 2.56	221.98 ^{bc} ± 7.23	11.08 ^b ± 0.64
IV	3.91 ± 0.53	1.33 ± 0.10	2.58 ± 0.47	0.62 ± 0.17	1.46 ^{ab} ± 0.18	26.61 ^{ab} ± 2.13	252.76 ^{abc} ± 18.24	14.15 ^{ab} ± 1.15
V	3.65 ± 0.41	1.23 ± 0.12	2.41 ± 0.36	0.54 ± 0.08	1.50 ^a ± 0.14	28.85 ^{ab} ± 1.05	261.83 ^{ab} ± 19.07	14.77 ^{ab} ± 0.69
	NS	NS	NS	NS	CD for treatment = 0.440 (significant at 5% level)	CD for treatment = 6.675 (significant at 5% level)	CD for treatment = 49.520 (significant at 5% level)	CD for treatment = 4.424 (significant at 5% level)

Mean value with common alphabets as superscript do not differ significantly
NS = Non significant

Conclusion

From the present investigations, it is concluded that chlorpyrifos induced subacute toxicity @ 15 mg/kg of feed in Japanese quails and administration of dried leaves powder of *Andrographis paniculata* @ 5 gm/kg of feed showed beneficial protective effect on hematobiochemical profile

during experimental chlorpyrifos toxicity @ 15 and 20 mg/kg of feed.

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