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Effect of *Withania somnifera* on Monocrotophos toxicity in commercial Poultry

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Abstract
The present study was conducted to investigate the protective efficacy of aqueous extracts of root and leaves of *W.somnifera* (Ashwagandha) against monocrtophos toxicity in broilers. Seventy two day old broiler chicks, were divided randomly and equally into six groups viz. Group I (Untreated Control), group II(MCP Control), *W. somnifera* root extract was given daily@ 100 and 300(mg/kg b.wt., orally) to group III and IV respectively in drinking water, *W. somnifera* leaf extract was given daily@ 100 and 300(mg/kg b.wt., orally)to group V and VI respectively in drinking water. On 21 day group II,III, IV, V andVI were administered single oral dose of MCP @ 1.34mg/Kg b.wt.($1/5^{th}$ of LD_{50}). There was no significant difference in haematological parameters in any of the experimental groups of birds after 1 day of single monocrotophos treatment except TEC which was significantly reduced in group II in comparison to group I birds. After 7 days there was a significant ($P<0.05$) decrease in the values of Hb, TEC and % lymphocytes in group II birds in comparison to group I birds. After 7 days *W. somnifera* root and leaf extract could not change the altered value of Hb to normal in groups III to VI, however leaf extract increased the depleted values of TEC and of % lymphocytes in group V and VI.
Monocrotophos significantly increased the activities of ALT, AST and ALP after 1 day of monocrotophos exposure in group II in comparison to group I birds. *W. somnifera* root and leaf extract significantly decreased ($P<0.05$) the values of these enzymes in the birds of group III to VI. In addition to that, root extract also reduced the value of serum creatinine, total protein and albumin in group IV in comparison to group II. Other parameters like globulin level, A:G ratio and activity of LDH remain unchanged. After 7 days of monocrotophos treatment activities of ALT, LDH and creatinine level were increased significantly in group II as compared to group I birds. The AST and ALP activities were unaltered in group II as compared to group I birds. Root and leaf extract showed their potential by decreasing the activity of ALT significantly in group III to VI birds in comparison to group II. However, the extract could not ameliorate the toxic effect of monocrotophos on LDH activity and creatinine level. The other biochemical parameters were unaltered.

Keywords: Monocrotophos, Ashwagandha (*Withania somnifera*) root and leaf extract

1. Introduction
Monocrotophos is an organic ester of phosphorus, which has the ability to inhibit the AChE. It is widely used to control insects and pests of rice and other crops and ectoparasiticide in animal husbandry practices (WHO 1993) [16]. Exposure of poultry to monocrotophos causes health consequences to poultry culminating in great economic loss, while also posing a potential threat to public health due to presence of pesticide residue in poultry meat (Pal and Kushwah, 1998, 2000) [8, 9]. Chronic exposure of chicks to small amount of organophosphates leads to deleterious effects on metabolism and immune system of birds (Garg *et al*. 2004) [2, 3]. Pharmacological screening of *W. somnifera* reveals that it is used as a tonic, stimulant, alterative, aphrodisiac, diuretic and abortifacient. Prolonged medication with *W. somnifera* caused a positive effect on weight, feed consumption, general resistance, liver weight and antistress effects in men and animals (Rao *et al*., 1999, Archana and Namasivyam, 1999) [10, 1]. The present study was conducted to investigate the protective efficacy of aqueous extracts of root and leaves extract of *W. somnifera* in poultry exposed to monocrotophos.

Materials and Methods
Preparation of root and leaves extracts
After collection the plant materials were shade dried and powdered. The powders were further processed for aqueous extraction. The powder was soaked in distilled water for 24h with intermittent stirring at 40 ^{o}Cwith the help of magnetic stirrer. The infusions were filtered through muslin cloth and centrifuged at 400g for 15 minutes to get the supernatant. The filtrates were dried with the help of incubator with fan and lypholizer (37 ^{0}C) to get the final extracts.

Experimental animals and design

Seventy two day old broiler chicks procured from standard hatchery, were divided randomly and equally into six groups viz. Group I (Untreated Control), group II (MCP Control). *W. somnifera* root extract was given daily@ 100 and 300(mg/kg b.wt., orally) to group III and IV respectively in drinking water, *W. somnifera* leaf extract was given daily@ 100 and 300(mg/kg b.wt., orally) to group V and VI respectively in drinking water. On 21 day group II, III, IV, V and VI were administered single oral dose of MCP @ 1.34mg/Kg b.wt. ($1/5^{th}$ of LD_{50}). The chicks were maintained in the experimental poultry shed of College of Veterinary and Animal Sciences, Pantnagar under standard managemental and husbandry conditions. Following parameters were recorded

Haematological

After 24 hrs of monocrotophos treatment, blood samples were collected from wing vein of half of the birds in sterilized disposable syringe (24 gauze needle) from each group to evaluate haematological and biochemical parameters.TEC and TLC were determined by the method of Natt and Herric (1952) using poultry diluting fluid, DLC was done by Zig-Zag method as described by Lucas and Jamroz (1961), PCV was estimated using method of Jain (1986), Hb% was estimated using Sahli's haemoglobinometer. Heparin was used as anticoagulant. Similarly after seven days of monocrotophos treatment blood samples were collected from remaining half birds from each group to evaluate haematological and biochemical parameters.

Biochemical

Serum was separated from non heparinised blood samples. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated by the method of Reitman and Frankel (1957) [12], Alkaline phosphatise (ALP) and Lactate dehydrogenase (LDH) were estimated by the method described by (Wotten, 1964), total protein and total albumin were estimated by the method described by Reinhold (1953) [11], globulin was obtained simply by subtracting albumin from total protein, creatinine was estimated by the method described by Oser (1971). Seven days post monocrotophos treatment, serum was separated from non-heparinised blood samples collected from remaining half birds from each group and all the above mentioned biochemical parameters (AST,ALT,ALP and LDH, total protein, total albumin, globulin and creatinine) were again estimated.

Results and Discussion

Effect of *W. somnifera* on haematological profile

The haematological profile of broiler chicks exposed to extracts of root and leaves of *W. somnifera* after one day of monocrotophos treatment is presented in table 1. There was no significant difference in haematological parameters in any of the experimental groups of birds after 1 day of single monocrotophos treatment except TEC which was significantly reduced in group II in comparison to group I birds. The haematological profile of broiler chicks exposed to extracts of root and leaves of *W. somnifera* after one day of monocrotophos treatment is presented in table 1. After 7 days there was a significant (P<0.05) decrease in the values of Hb, TEC and % lymphocytes in group II birds in comparison to group I birds. After 7 days *W. somnifera* root and leaf extract could not change the altered value of Hb to normal in groups III to VI, however leaf extract increased the depleted values of TEC and of % lymphocytes in group V and VI. Monocrotophos has been reported to change in haematological parametersin different animals including fish (Shantakumar., *et al* 1999), rats (Siddiqui etal., 1991) [14], mice (Gupta *et al*., 1982) [4] and (Garg *et al*., 2004a) [2]**.** The pesticide(single doses of 0.8-3.0mg/kg,p.o.) was found to decrease the values of Hb, TEC, Lymphocytes, ESR, platelet count and haematocrit and to increase total WBCcount(TLC),neutrophils and basophils significantly in rats and mice as compared to control (Gupta *et al*.,1982; Siddiqui *et al*., 1991) [4, 14]. Inthe present study, the significant decrease in Hb, Tec and %lymphocytes by monocrotphos was in agreement with the above findings. Haemolysis caused by monocrotophos was probably the cause of reduced Hb and TEC in this study (Singh *et al*., 2004) [15]. Lymphocytopenia produced by monocrotophos was an indication of immunosuppression in this study. Similar haematological findings in toxicity studies of other organophosphorus insecticides have also been reported in poultry (Singh *et al*., 1988; Kumar *et al*., 2006) [6]. The stimulation of stem cell proliferation was investigated following administration of methanolic extract of Ashwagandha in gama–radiated mice. It caused increase in bone marrow cellularity and normalized the ratio of normochromic erythrocytes and polychromic erythrocytes (Girija *et al*., 1996). Ashwagandha extract significantly reduced leucopenia induced by cyclophosphamide in Swiss albino mice (Davis and Kuttan, 1998; Gupta *et al*., 2001). In present investigation, all the observations are not in accordance with earlier findings. Although the values of TEC and percent lymphocytes were significantly increased by *W. somnifera* in monocrotophos treated birds in group V and VI after 7 days, other parameters remained unaffected by the treatment with Ashwagandha root or leaf extract. Hence, it may be assumed from the present investigation that Ashwagandha was unable to improve the haemogram of poultry to a greater extent in acute monocrotophos toxicity with higher single dose (1.34mg/kgp.o.).

Effect of *W. somnifera* on biochemical profile

Monocrotophos (1.34mg/kgp.o.) did not change the values of total protein, albumin and globumin significantly after 1 and 7 days of exposure in broiler chicks in the present study (Tables3, 4). There was a significant (P< 0.05) increase in activities of ALP,ALT and AST after 1 day of monocrotophos exposure in group II birds in comparison to group I birds(Tables3).Withania root and leaf extracts decreased the activities of these enzymes significantly (P< 0.05) in monocrotophos treated birds of group III to VI. In addition to that, Ashwagandha root extract also reduced the values of serum creatinine, total protein and albumin in birds of group IVwhen compared to birds of group II. Ashwagandha root and leaf extracts did not alter the levels of globulin, A: G ratio and activity of LDH in monocrotophos treated birds after 1-day.

After 7 days of monocrotophos treatment there was a significant increase in the activities of ALT and LDH and serum creatinine level in group II birdsas compared to groupI birds. The AST and ALP activities remained unaffected by monocrotophos in these test birds. Ashwagandha root and leaf extracts decreased the activities of ALT significantly in III to VI groups when compared to group I birds.However, the extracts could not ameliorate the toxic effect of monocrotophos on LDH activity and creatinine level in birds. Other biochemical parameters were remained unaltered in the

presence of Ashwagandha extracts in experimental birds. Exposure of broiler chicks to Monocrotophos(2ppm in feed) for 2 months caused significant decrease in serum globulin, however, no significant change was observed in the levels of serum calcium, inorganic Phosphorus and blood urea nitrogen(Garg., *et al* 2004a) [2]. In the present study, however, we could not record significant alteration in the level of serum globulin in monocrotophos-treated birds of groupie. This may be probably because the single dose of pesticide was not sufficient to reduce the globulin level. Monocrotophos administration at different intervals increased the activities of ALT, AST and ALP in liver and plasma of rats (Kushwah. *et al*., 2000) [9]. An increase in serum ALP activity and decrease in serum total protein have been noted in broiler chicks treated with monocrotophos(2ppm) for 8 weeks (Garg., *et al* 2004b) [3]. In the present investigation also, an increase in activities of serum ALT,AST and ALP after1 day and serum ALT and LDH activities after 7 days of single dose monocrotophos (1.34mg/kgp.o.) treatment were observed (Table 3 and 4). These findings suggest that monocrotophos may cause hepato and cardio-toxicity in chickens. Ashwagandha incorporation into the feed significantly influences the haematobiochemical parameters of broiler chicks.It increases Hb concentration, haematocrit value, total serum protein, albumin, globulin calcium and phosphorus levels (Samarth *et al*., 2003) [13]. A decrease in serum Creatinine phosphokinase(CPK), LDH, serum corticosterone and lipid peroxidation(LPO) levels in Wistar rats has been observed following treatment with 1-oxo-5beta,6-beta-epoxywitha-2-ene-27-etoxyoxide isolated from the root of *W. Somnifera* (Kaur *et al*.,2003) [5]. In the present investigation, the root and leaf extracts of *W. Somnifera*reduced the elevated activities of ALT,AST and ALP after1 day and ALT after 7 days in group III,IV, V and VI, as compared to monocrotophos treated group. The observations of this study suggest hepatoprotective potential of Ashwagandha in monocrotophos treated commercial poultry.

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Table 1: Effect of aqueous extract of *W. somnifera* on haematological profile in broiler chickens after 1 day of MCP toxicity(mean ± S.E., n=6)

Group	I	II	III	IV	V	VI
Extract/ Drug	Control	MCP control	WRE +MCP	WRE+MCP	WLE+MCP	WLE+MCP
Dose (mg/kg)	-	1.34	100 + 1.34	300 +1.34	100+ 1.34	300+1.34
Hb (g/dl)	12.7 ±0.69	12.3 ±0.51	12.85 ±0.47	12.9 ±0.42	12.45 ±0.26	12.65 ±0.27
PCV (%)	28.5 ±1.04	30.0 ±1.29	30.75 ±1.10	30.25 ±1.49	30.0 ±1.47	31.75 ±1.49
TEC (×10^9/ml))	2.53 ±0.23	2.11[a] ±0.07	2.03 ±0.06	2.09 ±0.17	2.26 ±0.03	2.42 ±0.13
TLC (×10^6/ml)	33.75 ±2.01	30.75 ±1.31	33.5 ±1.44	33.5 ±1.44	33.50 ±1.65	33.50 ±1.50
Lymphocytes (%)	65.5 ±2.63	59.3 ±2.90	62.75 ±2.13	57.75 ±1.43	59.75 ±1.37	64.75 ±3.06
Monocytes (%)	2.75 ±0.47	2.50 ±0.28	4.00 ±0.40	3.75 ±0.85	2.75 ±0.85	2.25 ±0.47
Neutrophils (%)	23.5 ±1.55	23.25 ±0.85	23.25 ±1.65	25.00 ±1.05	24.75 ±1.10	25.75 ±1.37
Eosinophils (%)	1.50 ±0.28	1.75 ±0.47	2.50 ±0.64	2.25 ±0.62	2.75 ±0.47	2.50 ±0.64
Basophils (%)	1.25 ±0.25	1.50 ±0.50	1.50 ±0.28	1.75 ±0.47	1.25 ±0.25	1.75 ±0.47

[a] =$P<$ 0.05 as compared to control in the same row.
[b] =$P<$ 0.05 as compared to MCP control in the same row.

Table 2: Effect of aqueous extract of *W. somnifera* on haematological profile in broiler chickens after 7 day of MCP toxicity(mean ± S.E., n=6)

Group	I	II	III	IV	V	VI
Extract/ Drug	Control	MCP control	WRE +MCP	WRE+MCP	WLE+MCP	WLE+MCP
Dose (mg/kg)	-	1.34	100+1.34	300+1.34	100+1.34	300+1.34
Hb (g/dl)	12.67± 0.32	11.4± 0.38	10.9± 0.28	11.3± 0.23	11.26 ±0.22	11.76± 0.43
PCV (%)	32.5 ±1.52	30.0 ±2.87	29.5± 2.02	30.33±2.26	28.83± 1.49	26.66± 1.6
TEC (×10^9/ml))	2.58 ±0.14	2.10 ±0.18	2.26± 0.09	2.24 ±0.09	2.48± 0.04	2.47 ±0.04
TLC (×10^6/ml)	33.83± 1.66	30.16± 1.07	31.0 ±1.50	33.66± 1.64	31.50±1.91	33.66± 1.68
Lymphocytes (%)	65.17 ±3.37	58.67± 2.74	61.83 ±1.81	61.33 ±1.60	65.33± 1.96	62.33± 0.95
Monocytes (%)	2.33±0.61	3.00±0.57	3.16±0.60	2.50±0.76	2.50±0.61	2.33±0.49
Neutrophils (%)	23.5±0.76	24.83±1.27	24.16±1.30	23.66±1.08	23.5±0.76	25.33±0.91
Eosinophils (%)	1.66±0.33	1.83±0.30	2.00±0.36	1.83±0.30	2.16±0.47	2.00±0.51
Basophils (%)	1.00±0.25	1.16±0.30	1.50±0.34	0.83±0.30	1.00±0.25	1.00±0.25

[a] =$P<$ 0.05 as compared to control in the same row.
[b] =$P<$ 0.05 as compared to MCP control in the same row.

Table 3: Effect of aqueous extract of *W. somnifera* on biochemical profile in broiler chickens after 1 day of MCP toxicity (mean ± S.E.,n=6)

Group	I	II	III	IV	V	VI
Extract/ Drug	Control	MCPcontrol	WRE+MCP	WRE+MCP	WLE+MCP	WLE+MCP
Dose (mg/kg)	-----	1.34	100+1.34	300+1.34	100+1.34	300+ 1.34
Total protein(g/dl)	3.46±0.06	3.92±0.09	3.66±0.19	3.23[b]±0.26	3.51±0.23	3.75±0.24
Albumin(g/dl)	1.73±0.07	1.69±0.05	1.62±0.10	1.35[b] ±0.06	1.62±0.06	1.51±0.06
Globulin(g/dl)	1.73±0.10	2.23±0.15	2.04±0.21	1.87±0.29	1.89±0.28	2.24±0.30
A:G	1.02±0.10	0.77±0.08	0.83±0.11	0.77±0.11	0.92±0.14	0.71±0.10
Creatinine(mg/dl)	0.53±0.04	0.60±0.01	0.47±0.06	0.36[b] ±0.03	0.42[b] ±0.01	0.59±0.07
ALT(U/L)	33.25±1.10	115.0[a] ±4.65	66.5[b] ±7.37	78.0[b] ±7.95	54.01[b] ±1.37	76.75[b] ±12.21
AST(U/L)	188.3±3.9	202.9[a] ±1.2	186.8[b] ±1.9	181.5[b] ±5.4	185.1[b] ±5.9	187.6[b] ±5.8
LDH(U/L)	265.11±36.22	303.05±25.64	278.24±34.66	285.29±42.36	278.47±45.26	212.28±9.79
ALP(U/L)	20.36±2.48	37.88[a] ±2.91	32.00±2.29	21.38[b] ±4.72	18.46[b] ±5.02	14.92[b] ±3.09

[a] =$P<$ 0.05 as compared to control in the same row.
[b] =$P<$ 0.05 as compared to MCP control in the same row.

Table 4: Effect of aqueous extract of *W. somnifera* on biochemical profile in broiler chickens after 7 day of MCP toxicity (mean ± S.E.,n=6)

Group	I	II	III	IV	V	VI
Extract/ Drug	Control	MCPcontrol	WRE+MCP	WRE+MCP	WLE+MCP	WLE+MCP
Dose (mg/kg)	-----	1.34	100+1.34	300+1.34	100+1.34	300+ 1.34
Total protein(g/dl)	4.37±0.06	4.360.11	4.46±0.10	4.48±0.07	4.45±0.05	4.27±0.06
Albumin(g/dl)	1.67±0.08	1.65±0.10	1.84±0.13	1.57±0.31	2.03±0.14	1.62±0.23
Globulin(g/dl)	2.69±0.13	2.70±0.13	2.62±0.20	2.90±0.27	2.42±0.19	2.64±0.22
A:G	0.63±0.06	0.62±0.07	0.74±0.10	0.60±0.13	0.88±0.11	0.68±0.16
Creatinine(mg/dl)	0.72±0.04	1.23 [a] ±0.26	0.78±0.23	0.84±0.15	0.85±0.11	1.04±0.12
ALT(U/L)	28.0±2.23	111.0 [a] ±6.65	81.83[b] ±6.87	84.66[b] ±2.57	75.5 [b] ±7.96	82.66[b] ±5.78
AST(U/L)	193.5±4.0	197.4±3.5	200.1±1.5	199.5±2.3	194.4±5.7	198.1±3.7
LDH(U/L)	208.35±17.63	331.02[a] ±60.13	291.45±39.59	256.88±31.87	249.24±11.47	366.30±53.81
ALP(U/L)	13.61±1.46	19.06±2.79	24.29±1.00	22.60±2.08	13.32±1.59	22.06±3.09

[a] =$P<$ 0.05 as compared to control in the same row.

[b] =$P<$ 0.05 as compared to MCP control in the same row.

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