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Original Research Article

Effect of Emblica officinalis on Monocrotophos Toxicity in Commercial Poultry

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A B S T R A C T

The present study was conducted to investigate the protective efficacy of hydroalcohalic extracts of fruit, leaf and bark of *E. officinalis* (Amla) against monocrtophos toxicity in broilers. Ninety six day old broiler chicks, were divided randomly and equally into eight groups viz. Group I(Untreated Control), group II (Monocrotophos Control), *E. officinalis* fruit extract was given daily@ 100 and 300(mg/kg b.wt., orally) to group III and IV respectively in drinking water, *E. officinalis* leaf extract was given daily@ 100 and 300(mg/kg b.wt., orally) to group V and VI respectively in drinking water and *E. officinalis* bark extract was given daily@ 100 and 300(mg/kg b wt, orally) to group VII and VIII respectively in drinking water. On 21 day group II, III, IV, V VI, VII and VIII were administered single oral dose of monocrotophos @ 1.34mg/kg b.wt. ($1/5^{th}$ of LD_{50}). There was no overt clinical sign recorded in any of the experimental groups of birds till 7 days of single monocrotophos treatment. There was a significant ($P<0.05$) decrease in the values of Hb, TEC and % lymphocytes in monocrotophos control group of birds in comparison to untreated control. *E. officinalis* fruit extract significantly brought the level of % lymphocytes to normal in monocrtophos-intoxicated birds after 1 day of exposure. In all the *E.officinalis* extract treated birds, the values of TEC and Hb were significantly ($P<0.05$) increased in MCP-treated birds. Monocrotophos significantly increased the activities of ALT, AST and ALP after 1 day of monocrotophos exposure. *E. officinalis* fruit, leaf and bark extracts reduced the activities of ALT and AST significantly ($P<0.05$) in monocrotophos-treated birds, whereas, the leaf and bark extracts of the plant could significantly change the ALP to normal in MCP-treated birds. After 7 days of monocrotophos exposure, the augmented activities of ALT and LDH and increased level of creatinine were found in serum of monocrotophos-treated birds. *E. officinalis* fruit, leaf and bark extracts reduced the values of creatinine and ALT significantly in monocrotophos-treated birds. There was a significant increase in the value of LPO in the brain of monocrotophos -treated birds. *E. officinalis* bark extract reduced the level of elevated LPO in monocrotophos -treated birds.

Keywords

Monocrotophos, Amla (*E. officinalis*) fruit, leaf and bark extract

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). 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). Chronic exposure of chicks to small amount of organophosphates leads to deleterious effects on metabolism and immune system of birds (Garg *et al.*, 2004). Pharmacological screening of *E. officinalis* reveals that it has antioxidative, immunomodulatory, hepatoprotective, antistress, antimicrobial, antipyretic, antitussive and antianemic properties (Bhattacharya *et al.*, 1999). The present study was conducted to investigate the protective efficacy of hydroalcohalic extracts of fruit, leaf and bark of *E. officinalis* against monocrtophos toxicity in broilers.

Materials and Methods

Preparation of fruit, leaves and bark extracts

After collection the plant materials were shade dried and powdered. The powders were further processed for hydroalcohalic extraction. The powder was soaked in 50% ethanol 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 Rotary Vacuum Extraction Evaporator to get the final extracts.

Experimental animals and design

Eighty day old broiler chicks procured from standard hatchery, were divided randomly and equally into eight groups viz. Group I (Untreated Control), group II (monocrotophos Control). *E. officinalis* fruit extract was given daily@ 100 and 300(mg/kg b.wt., orally) to group III and IV respectively in drinking water, *E. officinalis* leaf extract was given daily@ 100 and 300(mg/kg b.wt., orally) to group V and VI respectively in drinking water and *E. officinalis* bark extract was given daily@ 100 and 300(mg/kg b wt, orally) to group VII and VIII respectively in drinking water. On 21 day group II, III, IV, V VI, VII and VIII were administered single oral dose of monocrotophos @ 1.34mg/Kg b.wt. (1/5th 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, half of the birds from each group were sacrificed and blood samples were collected 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 remaining half birds from each group were sacrificed and blood samples were collected to evaluate haematological and biochemical parameters.

Biochemical

Serum was separated from nonheparinised blood samples. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated by the method of Reitman and Frankel (1957), 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), 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 *Emblica officinalis* on haematological profile

The haematological profile of broiler chicks exposed to extracts of fruit, leaves and bark of *E. officinalis* after one day of monocrotophos treatment is presented in table 1.There was no significant change in haematological parameters except %lymphocytes in birds of MCP control group (group II) as compared to untreated control (group I).The values of TEC were altered significantly in birds of groupV, VII and VIII, as compared to monocrotophos control birds. There was a significant ($P<0.05$) decrease in value of % lymphocytes in MCP control group in comparison to group I birds. Both doses (100 and 300mg/kg) of *E. officinalis* fruit extract significantly reversed the level of lymphocytes near to normal in group III and IV birds. The other haematological parameters (Hb, PCV and TLC) remained unchanged in all extract treated birds (group III to VIII), when compared to control. Effect of *E. officinalis* extracts after seven days of monocrotophos treatment is presented in table 2. The values of TEC, Hb and % lymphocytes were significantly ($P<0.05$) decreased in birds of group II in comparison to group I birds. In extract treated birds extracts of *E. officinalis* fruit, leaf and bark extracts significantly increased the value of Hb, TEC and %lymphocytes in monocrotophos treated birds.

Effect of *Emblica officinalis* on biochemical profile

The level of AST,ALT, ALP and LDH, total protein, total albumin, globulin and creatinine were estimated in serum of birds after one and seven days of monocrotophos treatment. Monocrotophos significantly increased the activities of ALT, AST and ALP after one day of monocrotophos exposure in group II birds as compared to group I birds (Table 3).No other biochemical parameter was found altered after one day of monocrotophos treatment. *E. officinalis* fruit, leaf and bark extracts reduced the activities of ALT and AST significantly ($P<0.05$) in monocrotophos treated birds, whereas the leaf and bark extracts significantly brought the activity of ALP near to normal in group V, VII and VIII birds. The serum globulin level was significantly increased by *E. officinalis* leaves and bark extracts in group VI and VIII birds, in comparison t group I and II birds. The albumin level and A:G ratio were found to be significantly lower in group IV and VI birds, as compared to group I and II birds. After seven days of monocrotophos exposure, augmented activities of ALT and LDH and increased level of creatinine were found in serum of group II birds, as compared to birds of control group (Table 4).There was no significant difference in other biochemical parameters among group I and II birds. Emblica fruit, leaf and bark extracts reduced the values of creatinine and ALT significantly in monocrotophos treated birds of group III to VIII.

Table.1 Effect of 50% ethanol extract of *E.officinalis* on haematological profile in broiler chickens after 1 day of MCP toxicity (mean ± S.E., n=6)

Group	I	II	III	IV	V	VI	VII	VIII
Extract/ Drug	Control	MCP control	EFE + MCP	EFE+ MCP	ELE + MCP	ELE + MCP	EBE + MCP	EBE + MCP
Dose (mg/kg)	-	1.34	100+1.34	300+1.34	100+1.34	300+1.34	100+1.34	300+1.34
Hb (g/dl)	13.05 ±0.81	11.75 ± 0.62	13.2 ± 0.54	13.0 ± 0.40	13.1 ±0.47	13.1 ±0.46	12.75 ±0.85	11.6 ±1.02
PCV (%)	30.0 ±0.40	29.25 ±1.79	32.5 ±2.72	32.25 ±1.70	28.25 ±2.28	30.75 ±2.21	29.5 ±2.17	32.25 ±2.49
TEC (×10^9 /ml))	2.26 ±0.25	2.31 ±0.03	2.56 ±0.13	2.51 ±0.07	1.72[b] ±0.08	2.13 ±0.03	2.96[b] ±0.22	2.89[b] ±0.14
TLC (×10^6 /ml)	35.5 ±1.55	32.75 ±1.65	33.25 ±1.31	34.5 ±1.25	32.25 ±2.68	33.25 ±2.05	32.75 ±1.10	34.0 ±2.08
Lymphocytes (%)	67.5 ±1.55	59.2[a] ±1.70	66.7[b] ±2.49	68.5[b] ±2.02	64.4 ±2.27	64.7 ±1.37	64.7 ±2.68	64.0 ±2.54
Monocytes (%)	3.75 ±0.85	3.5 ±0.64	3.5 ±0.64	3.25 ±0.47	3.5 ±0.64	2.0 ±0.40	2.75 ±0.47	4.25 ±0.47
Neutrophils (%)	22.5 ±0.64	22.5 ±1.19	23.75 ±1.25	22.75 ±0.47	23.25 ±0.47	23.5 ±1.65	23.5 ±1.04	23.75 ±0.75
Eosinophils (%)	2.0 ±0.40	2.5 ±0.64	1.5 ±0.28	1.75 ±0.47	2.5 ±0.64	1.7 ±0.47	1.75 ±0.47	2.0 ±0.70
Basophils (%)	1.0 ±0.40	1.5 ±0.28	1.5 ±0.28	1.75 ±0.25	1.75 ±0.47	2.0 ±0.40	1.25 ±0.25	1.5 ±0.28

[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 50% ethanol extract of *E.officinalis* on haematological profile in broiler chickens after 7 day of MCP toxicity (mean ± S.E., n=6)

Group	I	II	III	IV	V	VI	VII	VIII
Extract/ Drug	Control	MCP control	EFE + MCP	EFE+ MCP	ELE + MCP	ELE + MCP	EBE + MCP	EBE + MCP
Dose (mg/kg)	-	1.34	100+1.34	300+1.34	100+1.34	300+1.34	100+1.34	300+1.34
Hb (g/dl)	12.1 ±0.29	10.83[a]±0 .28	11.86[b] ± 0.19	11.53 ±0.30	10.93 ±0.48	11.96[b] ±0.36	11.96[b] ±0.37	12.3[b] ±0.30
PCV (%)	32.5 ±1.52	30.0 ±2.87	30.16 ±1.97	29.5 ±2.59	29.33 ±2.23	30.00 ±2.23	29.66 ±2.17	27.16 ±2.62
TEC (×10^9 /ml))	2.36±0.0 6	2.1[a]±0.1 8	2.53[b]±0.03	2.39[b]±0.04	2.23±0.06	2.38[b]±0.06	2.44[b]±0.03	2.43[b]±0.03
TLC (×10^6 /ml)	33.83±1. 66	30.16±1. 07	30.33±0.84	34.16±2.27	29.33±1.28	34.0±1.36	32.66±0.98	31.83±1.01
Lymphocytes (%)	65.2 ±2.49	59.3[a] ±1.33	61.33 ±1.11	61.33 ±1.60	62.0 ±1.18	63.0 ±1.43	64.7[b] ±1.78	60.5 ±2.48
Monocytes (%)	3.5±0.61	3.16±0.4 7	2.83±0.60	2.66±0.49	2.83±0.60	2.00±0.36	2.66±0.33	3.33±0.66
Neutrophils (%)	24.33±0. 88	24.33±1. 20	23.66±0.88	23.0±0.51	23.16±0.87	24.66±0.95	24.16±0.79	24.16±0.76
Eosinophils (%)	1.83±0.3 0	1.66±0.2 1	1.5±0.34	2.33±0.42	2.0±0.36	1.66±0.33	1.83±0.47	2.0±0.36
Basophils (%)	1.33±0.4 2	1.66±0.3 3	1.5±0.42	1.5±0.42	1.0±0.25	1.33±0.42	1.33±0.42	1.66±0.30

[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 50% ethanol extract of *E.officinalis* on biochemical profile profile in broiler chickens after 1 day of MCP toxicity (mean ± S.E., n=6)

Group	I	II	III	IV	V	VI	VII	VIII
Extract/ Drug	Control	MCP control	EFE + MCP	EFE+ MCP	ELE + MCP	ELE + MCP	EBE+ MCP	EBE + MCP
Dose (mg/kg)	-----	1.34	100+1.34	300+1.34	100+1.34	300+1.34	100+1.34	300+1.34
Total protein(g/dl)	3.45 ±0.11	3.44 ±0.20	3.22±0.06	3.51±0.14	3.43±0.07	3.74±0.20	3.38±0.11	3.78±0.19
Albumin(g/dl)	1.73 ±0.07	1.69 ±0.05	1.61±0.03	1.45[b]±0.05	1.53±0.05	1.46[b]±0.04	1.66±0.02	1.57±0.12
Globulin(g/dl)	1.72 ±0.04	1.74 ±0.21	1.62±0.08	2.08±0.16	1.90±0.07	2.28[b]±0.22	1.72±0.10	2.20[b]±0.08
A:G	1.00 ±0.03	1.01 ±0.12	0.99±0.06	0.72[b]±0.07	0.81±0.04	0.66[b]±0.09	0.97±0.06	0.71[b]±0.05
Creatinine(mg/dl)	0.52 ±0.05	0.60 ±0.01	0.44±0.04	0.59±0.01	0.57±0.05	0.61±0.01	0.37[b]±0.01	0.66±0.13
ALT(U/L)	33.75 ±1.84	111.0[a] ±9.67	85.5[b]±5.79	91.75[b]±5.80	67.0[b]±6.24	77.0[b]±4.50	74.75[b]±3.94	99.75±10.18
AST(U/L)	185.5 ±5.5	202.4[a] ±2.6	172.2[b]±4.8	184.5±6.7	187.7[b]±5.1	186.7[b]±4.1	189.5±2.9	186.7[b]±6.3
LDH(U/L)	261.81 ±45.75	332.56 ±45.27	258.56±45.70	308.34±51.80	270.41±48.23	179.40±42.5	330.42±17.90	248.97±23.66
ALP(U/L)	18.49 ±2.29	40.22[a] ±3.61	30.49±6.50	26.06±9.05	13.11[b]±2.67	18.45±5.81	8.87[b]±2.03	14.86[b]±3.28

[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 50% ethanol extract of *E.officinalis* on biochemical profile profile in broiler chickens after 7 day of MCP toxicity (mean ± S.E., n=6)

Group	I	II	III	IV	V	VI	VII	VIII
Extract/ Drug	Control	MCP control	EFE + MCP	EFE+ MCP	ELE + MCP	ELE + MCP	EBE+ MCP	EBE+ MCP
Dose (mg/kg)	-----	1.34	100+1.34	300+1.34	100+1.34	300+1.34	100+1.34	300+1.34
Total protein(g/dl)	4.39±0.12	4.29±0.11	4.38±0.08	4.40±0.12	4.21±0.05	4.22±0.08	4.17±0.04	3.57[b]±0.51
Albumin(g/dl)	1.89±0.18	1.75±0.15	1.98±0.19	1.94±0.12	1.81±0.19	1.80±0.14	1.76±0.16	1.74±0.10
Globulin(g/dl)	2.49±0.12	2.53±0.10	2.40±0.21	2.53±0.18	2.39±0.14	2.41±0.11	2.41±0.19	2.33±0.12
A:G	0.78±0.12	0.70±0.09	0.90±0.18	0.80±0.10	0.80±0.12	0.76±0.09	0.78±0.14	0.77±0.09
Creatinine(mg/dl)	0.71±0.04	1.28[a]±0.29	0.66[b]±0.07	0.52[b]±0.05	0.93±0.13	0.76[b]±0.20	0.67[b]±0.06	0.78[b]±0.16
ALT(U/L)	27.0±2.17	110.8[a]±6.93	93.16[b]±3.56	92.0[b]±2.30	88.16[b]±2.71	85.66[b]±3.27	80.5[b]±2.12	95.0[b]±3.46
AST(U/L)	191.1±4.5	197.0±3.5	199.7±2.1	203.2±3.6	198.2±2.4	200.3±1.8	197.4±3.2	197.6±3.1
LDH(U/L)	205.70±22.39	374.89[a]±51.96	416.11±22.86	328.87±25.03	362.57±33.44	247.73±12.14	353.63±46.15	294.57±16.77
ALP(U/L)	13.13±1.54	18.95±3.13	15.22±1.45	17.60±1.98	17.53±0.72	19.40±2.12	9.40[b]±1.27	17.97±3.11

[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.

The values of total protein and ALP were significantly reduced by *E. officinalis* bark extract in birds of groups VIII and VII, respectively when compared to birds of group I or II. None of the extracts was found to change the activity of LDH significantly in birds of group III to VIII.

Poultry has been reported to be highly susceptible to monocrotophos toxicity. The oral LD_{50} value of this toxicant in chickens is 6.7mg/kg body weight (Hooper, www). In earlier studies, single doses(0.8-3.0mg/kg, P.O.) of monocrotophos were found to decrease Hb, TEC, lymphocytes, ESR, platelet count and haematocrit value and to increase total WBC count(TLC), neutrophils and basophils significantly in rats and mice, as compared to control(Gupta *et al.*,1982; Siddiqui *et al.,* 1991). However, when poultry were treated with monocrotophos (2 ppm in feed) for eight weeks the value of TEC,PCV, Hb, eosinophil and monocytecount did not change, but TLC and T-lymphocyte counts significantly decreased(Garg *et al.,* 2004). In the present study, a significant decrease in Hb, TEC and %lymphocytes by monocrotophos is in agreement with the above findings. Lymphocytopenia produced by monocrotophos is an indicator of immunosuppression in this study. Lymphocytopenia observed in monocrotophos treated birds coincides with haematological changes reported by other workers in rats (Cho *et al.,* 1989). Similar haematological findings following toxicity studies with other organophosphorus insecticides have also been reported in poultry (Singh *et al.,* 1988, Kumar *et al.,* 2006). Haemolysis caused by monocrotophos may be a probable cause of reduced haemoglobin and TEC (Singh *et al.,* 2004). *E. officinalis* has been reported to show antianemic potential in mice exposed to eadioactive materials (Hari Kumar *et al.,* 2004). In the present study, 50% ethanolic extract of *E. officinalis* fruit, leaf and bark significantly increased the value s of Hb, TEC and %lymphocytes in monocrotophos treated birds. These findings suggests that *E. officinalis* fruit, leaf and bark extract showed protective effect on haemopoeitic system against acute acute monocrorophos toxicity. The flavonoids present *E. officinalis* might be responsible for the improved haematological profile (Anila and Vijaylaxmi, 2000).In respect to biochemical profile monocrotophos was found to affect the biochemical parameters adversely in different experimental animals. In chickens, a long term exposure(8 weeks) of the pesticide (2ppm in feed) significantly ($P<0.01$) reduced the values of blood glucose, serum globulin and serum AChE activity in treated groups compared to control (Garg, *et al.,* 2004a). Monocrotophos administration at intervals increased the activities of ALT, AST and ALP in liver and plasma of rats (Kushwah *et al.,* 2000). An increase in serum ALP activity and decrease in total serum protein were observed in broiler chicks treated with monocrotophos (2 ppm) for 8 weeks were decreased (Garg *et al.,* 2004b). Increase in activity of serum ALT, considered as liver specific enzyme, is used as a marker of hepatocellular necrosis or increased cell membrane permeability (Traulos *et al.,* 1996). In the present study, monocrotophos (1.34mg/kg, p.o., single dose) significantly increased the activities of ALT, AST and LDH and creatinine level after 1 or 7 days of exposure. These findings are in agreement with the findings of Garg, *et al.,* (2004) and Kushwah *et al.,* (2000). *E. officinalis* is considered to have good anti stress, adaptogenic and immunomodulatory properties (Bhattacharya *et al.,* 2004). Hepatoprtective action of fruit, leaf and bark extract of *E. officinalis* was observed in this study, as evidenced by serum enzymatic

profile is in accordance with the reports made by earlier workers (Chaudhary, 1978; Jose and Kuttan, 2000). The antioxidants present in the *E. officinalis* possibly protected the birds from monocrotophos induced toxicity(Bhattacharya *et al.*,2000).The results of present study favour using *E. officinalis* fruit as hepatoprotective and cardiotonic agent.

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