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Effect of Andrographis paniculata on hematobiochemical profile during monensin induced toxicity in Japanese quails

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Abstract

The present investigation was carried out to study the ameliorative effect of *Andrographis paniculata* on hematobiochemical profile during monensin toxicity in Japanese quails. At the end of 28 day quails fed with monensin @ 150 mg/kg of feed showed adverse effect on haematological profile of Hb, PCV, TEC, TLC, heterophil and lymphocyte count, whereas birds given monensin along with dried leaves powder of *Andrographis paniculata* showed dose dependant (@ 3g/kg and 5 g/ kg feed) beneficial effect. Monensin only toxicated birds showed significant increase in serum AST, creatinine, and phosphorus whereas birds given monensin along with dried leaves powder of *Andrographis paniculata* showed significant increase in serum AST, creatinine, and phosphorus whereas birds given monensin along with dried leaves powder of *Andrographis paniculata* showed significant decreased values and suggested beneficial property of plant during monensin toxicity. Increased serum calcium level in monensin treated birds showed restoration when given *Andrographis paniculata* along with it. Thus it is concluded that dried leaves powder of *Andrographis paniculata* showed hematinic, hepatoprotective and renoprotective effect @ 3g/kg and 5 g/ kg feed during monensin sodium toxicity in Japanese quails given @ 150 mg/kg in feed.

Keywords: Andrographis paniculata, biochemical, hematology, monensin

Introduction

Monensin is a fermentation product of *Streptomyces cinnamonensis* and is the 1st antibiotic used as an anticoccidials. Due to its broad spectrum activity, it acts on trophozoites and 1st generation schizonts. Its activity is generally within first 2 days of life cycle of coccidian. It gives protection against all species of coccidia at 0.01 - 0.121% concentration in the feed. In commercial poultry farming monensin is used in commercially prepared feed @ 125 mg/kg in broiler chicken, @ 100 mg/kg in turkey and @ 73 mg/kg in quails as prophylactic dose. Monensin given at 20 to 50 percent over dosage may cause the first evidence of toxicity. Simultaneous applications of monensin and tiamulin (a pleuromutilin derivative used against mycoplasma infections) results in increased toxicity ^[1]. The monensin induced growth depression was recorded when diets contained lower amounts of crude protein ^[2]. The occurrences of monensin toxicity due to contaminated or improperly prepared feed have been reported in the chickens, turkeys and guinea fowl ^[3, 4, 5, 6]. Monensin Na⁺ specific ionophore easily forms lipophilic complexes with other ions (monovalent cations) and results in an influx of Na⁺ with corresponding efflux of H⁺ and K⁺ leading to an increase in intracellular Ca⁺. Oxidative stress produced due to monensin causes monensin toxicity [7]. The monensin toxicity primarily manifest as a neuromuscular dysfunction which can result in paralysis and death of toxicated birds with intermyofibrilar vacuolization, vacuolization in the epicardium, myofibrilar degeneration and necrosis of skeletal and myocardial muscle.

The aerial parts, roots and whole plant of *Andrographis paniculata* have been used for centuries in Asia as traditional medicine for the treatment of various ailments. The leaf extract is a traditional remedy for the treatment of infectious diseases, fever-causing diseases, colic pain, loss of appetite and diarrhoea ^[8]. The chemical constituents of *Andrographis paniculata* are diterpene lactones, andrographolides, neoandrographolides and kalmeghin, several flavones and flavonoids ^[9]. The two main diterpenoids isolated from *Andrographis paniculata* are 14-deoxy-11, 12-didehydroandrographiloid ^[10]. Presence of these constituents could attribute to cardioprotective activity of *Andrographis paniculata* by antioxidant, free radical scavenging and antilipid peroxidation property. Literature reviewed however did not reveal information relating to experimental studies of use of *Andrographis paniculata* plant during monensin toxicity in Japanese quail. Considering antioxidant and cardioprotective activity of *Andrographis paniculata* is enclosed to evaluate the ameliorative

effect of *Andrographis paniculata on* hematobiochemical parameters during subacute monensin toxicity in Japanese quails.

Materials and Methods:

The necessary prior approval was granted from the Institutional Animal Ethics Committee for conduct of the experiment. The experimental protocol met the national guidelines as per the guidelines of Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The dry leaves powder of *Andrographis paniculata* was used during experiment was procured from Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Experimental design:

Two week old seventy five broiler chicks were acclimatized for a period of one week under identical management and hygienic condition. After acclimatization period, birds were randomly divided into five equal groups, each group thus comprising of 15 birds. Group T1 was served as control, where as Group T2, T4 and T5 were fed with monensin sodium (C₃₆H₆₂O₁₁) @ 150 mg/kg of feed for a period of 4 weeks. Groups T3 birds were fed with dry leaves powder of Andrographis paniculata @ 5gm/kg of feed. Group T4 and T-5 were additionally fed with dry leaves powder of Andrographis paniculata @ 3 gm/kg and 5 gm/kg of feed, respectively to evaluate its protective effect at two different dose levels for a period of 4 weeks. All the respective treatment feed were fed adlib for 28 days. All the birds were maintained under similar managemental and hygienic conditions during experimental period of 28 days.

At the end of experiment, six birds from each group were selected randomly and blood was collected aseptically from jugular vein separately in EDTA (Ethylene di-anime tetra acetic acid) vial for hematology and in vacutainer for serum separation for estimation of biochemical parameters using Auto analyzer (Make- Span Autochem, 2001).

Statistical analysis

The data generated during the experiment for hematology and biochemical parameters were analyzed by using Completely Randomized Design using WASP ICAR Goa, Version 2. (http://www.ccari.res.in/waspnew.htm).

Results

Hematological observations

The haematological observations recorded at the end 28th day (4th week) are presented in Table 1 and 2. The mean haemoglobin values revealed non significant decrease, whereas, PCV and TEC values were significantly decreased in T2 group birds when compared with control and other treatment groups. Group T4 and T5 given monensin along with plant showed dose dependant improvement in Hb, PCV and TEC when compared with group T2 fed with monensin sodium alone. The values of MCV, MCH and MCHC differ non significantly within treatment groups as well as with control group. Average TLC values differ significantly among control and different treatment groups and were found to be 9.22 ±0.210, 10.62 ±0.439, 10.32 ±0.177, 10.19 ±0.304 and 10.95 ±0.371 in T1, T2, T3, T4 and T5 group, respectively. The significantly lower TLC was recorded in group T1 and significantly higher TLC was observed in group T5. The increase in average TLC value in all treatment groups reflected reaction to mild to moderate stress induced by monensin sodium. The significant increase in absolute heterophil count along with lymphocytopenia was observed in T2 group whereas, group T3 showed significant increase in absolute lymphocyte count. Improvement in absolute lymphocyte and decrease in absolute heterophil count was observed in T4 and T5 groups. Absolute monocyte, eosinophil and basophil count showed non significant differences.

Biochemical observations

The mean values of serum total protein, albumin, globulin, AST, ALT, GGT, creatinine, calcium and phosphorus are presented in Table 3 and 4.

Biochemical observations revealed significant increase in serum total protein and non significant increase in serum albumin and globulin in T2 group when compared with control and other treatment groups. Serum A:G ration differ non significantly among control and treatment groups and was found to be numerically lower in T2 group. Serum AST, creatinine and phosphorus revealed significant increase and serum ALT and GGT revealed non significant increase in group T2 given monensin alone, whereas group T4 and T5 showed significant decrease in these parameters indicated beneficial hepatoptrotective and nephroprotective effect of plant during monensin toxicity in Japanese quails. Serum calcium was significantly decreased in T2 group, however the serum calcium level was found to be significantly increased in birds dried leaves powder of Andrographis paniculata along with monensin.

Group	Hb (g/dl)	PCV (%)	TEC (10 ⁶ /cumm)	MCV (fL)	MCH (pg)	MCHC (g/dl)
T1	10.79 ± 0.657	38.66 ^b ±3.040	$5.37^{ab} \pm 0.465$	73.02 ±4.463	20.86 ± 1.979	28.66 ± 2.270
T2	10.38 ± 0.459	36.00 ^b ±1.460	4.38°±0.382	84.14 ±5.067	24.55 ±2.303	29.03 ±1.598
T3	11.70 ± 0.656	48.00 ^a ±2.463	$7.30^{a}\pm0.508$	78.03 ±4.847	19.32 ± 1.962	24.94 ±2.516
T4	10.68 ± 0.512	42.00 ^{ab} ±2.366	5.69 ^b ±0.141	74.02 ±4.493	18.86 ± 1.109	25.54 ±0.773
T5	11.71 ± 0.324	47.00 ^a ±2.633	6.23 ^{ab} ±0.450	66.86 ± 7.748	16.53 ± 1.500	25.11 ±0.754
	NS	CD for treatment= 7.133 (Significant at 5% level)	CD for treatment= 1.197 (Significant at 5% level)	NS	NS	NS

Table 1: Hematological observations in control and different treatment groups at 28th day of experiment

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

Table 2: Total leucocyte count (10³/ cumm) and absolute leucocytes count in control and different treatment groups at 28th day of experiment

Group	Total leucocyte count (TLC)	Absolute heretophil	Absolute lymphocyte	Absolute monocyte	Absolute eosinophil	Absolute basophil	
T1	$9.22^{b} \pm 0.210$	$3321.13^{d} \pm 119.891$	$5607.40^{b} \pm 131.088$	138.66±21.408	193.38±51.073	15.73±15.733	
T2	10.62 ^a ±0.439	6590.31ª ±229.909	$3588.48^{d} \pm 310.525$	198.16±22.721	137.06±31.087	51.31±23.265	
T3	10.32 ^a ±0.177	$3167.93^{d} \pm 130.258$	6778.23 ^a ±152.104	171.00±20.543	153.80±33.792	52.36±23.473	
T4	10.19 ^a ±0.304	$5103.78^{b} \pm 140.817$	4731.31° ±227.641	154.41±43.378	150.50±38.589	48.20±21.564	
T5	10.95 ^a ±0.371	4317.00 ^c ±208.996	6297.16 ^a ±122.654	168.00±47.237	152.28±22.655	17.33±17.333	
	CD for treatment= 0.979	CD for treatment= 500.726	CD for treatment= 587.892	NS	NS	NS	
	(Significant at 5%)	(Significant at 5%)	(Significant at 5%)	115	115	145	

Mean values with common alphabet as superscript do not differ significantly

NS= Non significant

Table 3: Serum total protein (g/dl), albumin (g/dl), globulin (g/dl) and A/G ratio in different groups at 28th day of experiment

Group	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
T1	4.26 ^c ±0.207	1.91 ±0.252	2.35 ±0.311	0.69 ±0.059
T2	6.36 ^a ±0.162	2.93 ±0.368	3.43 ±0.460	0.59 ± 0.108
T3	4.71 ^{bc} ±0.279	2.46 ±0.288	2.25 ±0.405	0.99 ±0.174
T4	5.03 ^b ±0.178	2.05 ±0.252	2.98 ±0.297	0.93 ±0.129
T5	4.56 ^{bc} ±0.213	2.06 ±0.259	2.50 ±0.230	0.66 ± 0.096
	CD for treatment= 0.619 (Significant at 5%)	NS	NS	NS

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

Table 4: Serum AST (IU/L), ALT (IU/L), GGT (IU/L), creatinine (mg/dl), calcium (mg/dl) and phosphorus (mg/dl) in different groups

Group	AST (IU/L)	ALT (IU/L)	GGT (IU/L)	Creatinine (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)
T1	217.78 ^{bc} ±12.977	23.10 ± 2.537	12.01 ± 2.569	0.91° ±0.047	11.71 ^a ±0.638	3.31 ^b ±0.284
T2	275.64 ^a ±5.566	28.28 ± 1.650	16.11 ± 1.663	1.51 ^a ±0.130	6.23° ±0.579	6.00 ^a ±0.222
T3	201.39 ^c ±4.868	20.31 ± 1.543	11.44 ± 2.081	1.21 ^b ±0.091	11.42 ^a ±0.767	3.64 ^b ±0.420
T4	229.63 ^b ±3.705	24.27 ± 1.741	13.31 ± 1.898	$1.18^{b} \pm 0.060$	8.83 ^b ±0.217	3.42 ^b ±0.298
T5	214.71 ^b ±4.530	24.33 ± 2.146	12.72 ± 1.089	1.40 ^{ab} ±0.063	$9.76^{b} \pm 0.378$	4.09 ^b ±0.525
	CD for treatment= 20.901	NS N	NS	CD for treatment= 0.244	CD for treatment= 1.608	CD for treatment= 1.066
	(significant at 5%)	Gri	CM	(significant at 5%)	(significant at 5%)	(significant at 5%)

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

Discussion:

Monensin is found to be commonly used as a prophylactic coccidiostat in commercially prepared poultry feed. However, 20 to 50 percent over dosage may cause evidence of toxicity in birds. Considering this the present investigation was planned to study hematobiochemical profile of Japanese quails during monensin sodium induced toxicity and its amelioration with Andrographis paniculata in Japanese quails. The decreased haematological values of Hb, PCV and TEC in monensin fed group birds are in agreement with earlier findings in broilers at different toxic levels of monensin^[12]. Improvement in Hb, PCV and TEC in T4 and T5 groups than T2 group suggested erythrocyte building capacity of Andrographis paniculata and this might be due to presence of iron in the plant ^[13]. These findings in T4 and T5 group corroborates with earlier findings ^[14, 15, 16] given Andrographis paniculata during different toxicities in broilers. The MCV revealed mild microcytic anaemia in group T2 given monensin while improvement in group T4 and T4 suggested hematinic property of plant might be due to iron content of plant. Total leukocyte count did not differ significantly within treatment groups but was differ significantly with control group. Contrary to the present findings leucocytosis was reported during monensin toxicity by pervious workers ^[17, 18, 19]. The significant increase in absolute heterophil count in T2 group followed by T4 and T5 group might be due to systemic stress caused by monensin sodium which may leads to endogenous release of corticosteroids and ultimately leads to non inflammatory neutrophilia [20]. Significant increase in values of absolute lymphocyte count in T3, T4 and T5 group birds indicated immunomodulatory property of *Andrographis paniculata*^[15] even during monensin toxicity @ 150 mg/kg of feed. Absolute monensin, eosinophil and basophil count revealed non significant differences between treatment and control group birds indicated no significant effect on these leukocytes.

The increase in serum total protein level during monensin toxicity was also observed by earlier workers ^[17, 19]. However, the cause could not be correlated. Contrary to present findings decrease in serum total protein was recorded at different toxic levels of monensin in broiler chicks in one of the earlier report ^[12]. The increased level of serum AST in T2 group indicated cardiac or hepatic damage by monensin sodium. The present findings are in agreement with earlier reports in Japanese quails ^[21], broilers ^[12, 22], in cattle ^[19] and in goats ^[23] during monensin sodium toxicity. The significant (p<0.05) dose dependant decreased serum AST in T4 and T5 group might be due to antioxidant property of Andrographis paniculata ^[11] which may inhibit the oxidative stress in hepatic tissues caused due to monensin toxicity. Numerically higher serum GGT in T2 group fed with monensin is suggestive of necrosis of hepatocytes that result in increased leakage of serum GGT. Reduction in serum GGT in group T4 and T5 given monensin @ 150 mg/kg along with Andrographis paniculata @ 3 g/kg and 5 g/kg of feed respectively, suggested decrease in adverse effect of monensin and dose dependant hepatoprotective effect of Andrographis paniculata. Increased concentration of creatinine in blood of T2 group issued as index of glomerular filtration rate and renal damage which confirmed nephrotoxic effect of monensin in quails ^[20]. Present findings of significantly decreased serum creatinine level in *Andrographis paniculata* fed groups suggested protective or ameliorative nephroprotective effect of plant during monensin toxicity in Japanese quails was which is also corroborates with earlier findings in broilers during lead toxicity ^[14].

Monensin is a sodium selective ionophore which causes influx of sodium in the cells and produces higher intracellular concentrations of sodium. A rise in intracellular sodium in turn increases the intracellular level of calcium due to an ATPase – driven exchange at the cell membrane and triggers the release of calcium from intracellular stores which might be the reason for decrease in serum calcium level in T2 group ^[17]. Calcium level in T4 and T5 group suggested beneficial properties of plant in calcium restoration during monensin sodium toxicity. Higher intracellular concentrations of sodium leads to shift of phosphate from the intracellular to the extracellular space and might be the reason for significant increase in serum phosphorus level in T2 group birds ^[17]. Similar findings of increase phosphorus level have been reported in previous reports in broilers during monensin toxicity ^[18, 24]. Serum phosphorus levels in T3, T4 and T5 groups were found to be significantly decreased when compared with value of T2 group birds and were comparable with control group (T1) suggested beneficial properties of Andrographis paniculata at both doses during monensin toxicity.

Conclusion

It is thus concluded that dried leaves powder of *Andrographis paniculata* showed beneficial effect on hematopiotic system and suggested hepatoprotective and renoprotective effect @ 3g/kg and 5 g/ kg feed during monensin sodium toxicity given @ 150 mg/kg in feed.

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