

BIOCHEMICAL AND PATHOLOGICAL STUDIES ON EXPERIMENTAL MADURAMICIN TOXICITY IN CHICKENS

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ABSTRACT

Experimental studies were carried out in broiler chicken to know the biochemical and pathological changes after giving maduramicin medicated feed at 5 and 10 ppm for 21 days. Biochemical studies revealed increase in activities of aspartate transaminase and alanine transaminase in both the medicated groups but the increase was of higher magnitude in chicken given 10 ppm dose. There was significant increase in the activity of lactate dehydrogenase only in 10 ppm dose group from day 14 of the medication. Histopathological studies revealed necrotic enteritis, lymphocytic depletion in bursa of Fabricius, haemorrhages and myocarditis in heart and nephrosis from day 14 in both the medicated groups, though the lesions were more severe in chicken fed 10 ppm maduramicin. Maduramicin at the rate of 10 ppm also caused hepatitis, lymphocytic depletion in spleen, myositis, and haemorrhages in lungs from day 14. The lesions in both medicated groups are suggestive of immunosuppressive effect in the toxicated broiler chicken.

Key words: Chickens, clinical chemistry, pathology, maduramicin

Control of coccidiosis chiefly depends upon the prophylactic chemotherapy with anticoccidial drugs (McDougald and Reid, 1991). Polyether ionophore antibiotics viz. monensin, salinomycin and maduramicin are most widely used anticoccidial drugs in broiler industry throughout the world. Of these ionophore compounds, maduramicin, a product of *Actinomadura yumaensis* (Folz *et al.*, 1988) is comparatively a new anticoccidial drug which has been found to be effective at a dose rate of 5 ppm to prevent the infection of various species of *Eimeria* (Folz *et al.*, 1988, Logan *et al.*, 1993). The literature on maduramicin toxicity is scanty. No report could be traced on experimental maduramicin toxicity in poultry with respect to pathological changes. The present study, describes biochemical and pathological changes in maduramicin medicated broiler chicks to assess its toxicity.

MATERIALS AND METHODS

Chicks and feed: Thirty three, day-old broiler chicks (Ross strain) were procured from a

local hatchery. The chicks were reared in well-ventilated rooms under hygienic conditions. All the birds were given coccidiostat free standard chick feed and provided clean drinking water *ad libitum* throughout the experiment. Maduramicin was added in the feed of chicks on day 14 @ 5 and 10 ppm for testing its toxicity.

Experimental design: At day 14, the chicks were divided randomly into three groups viz. groups A, B and C having 11 birds in each. The birds of groups A and B were given maduramicin medicated feed @ 5 and 10 ppm, respectively for 21 days whereas the birds of group C were given non-medicated feed which served as control. Blood was collected aseptically from randomly selected five birds prior to the grouping on day 14 and subsequently from each group on days 7, 14 and 21 post-treatment in sterile tubes for serum separation and stored at -20°C.

Serum studies: Serum samples were analyzed for total proteins, albumin, aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenase (LDH) using single step reagent kits (Trans Asia Bio

Medical Ltd.) employing Auto Chemistry Blood Analyzer.

Pathological studies: Three birds were sacrificed at 0 day prior to groupings and then subsequently from each group on 7, 14 and 21 days post-treatment of the experiment. Thorough post-mortem examination was conducted on the sacrificed birds. Small pieces of intestine, liver, bursa of Fabricius, heart, muscles, lung, spleen and kidney were collected in 10% buffered formalin for histopathological studies. The formalin fixed tissues were processed for paraffin embedding technique. Sections of 3-4 μ m thickness were cut and stained with haematoxylin and eosin (Luna, 1968).

Statistical analysis: The data for various parameters were subjected to two-way analysis of variance (Snedecor and Cochran 1967). Individual means were compared for statistical significance using least significance difference ($p < 0.05$).

RESULTS AND DISCUSSION

There was no significant difference in serum total protein and albumin concentrations

among groups at different intervals (Table 1). However, a significant increase in serum AST and ALT activities was observed in both the medicated groups (groups A and B) on day 21 post-treatment as compared to the control (group C). The increase in AST activity in group B was significantly more than group A. The significant increase in activity of serum AST and ALT might be due to myocarditis and hepatic degenerative changes, respectively (Benjamin, 1978). A significant increase in serum LDH activity was observed only in group B as compared to control from day 14 (Table 1) which might be due to the lesions observed in heart, skeletal muscles, liver and lungs as this enzyme is present in the cells of these organs (Benjamin, 1978). No report could be traced on these enzymes in relation to maduramicin toxicity in chickens but higher activities of AST and LDH in serum due to maduramicin toxicity have been reported in calves (Fourie *et al.*, 1991) and rabbits (Ho *et al.*, 1997).

Small intestine of chicks in both the medicated groups on days 14 and 21 of medication revealed enteritis characterized by necrosis of mucosal epithelium, congestion and

Table 1
Mean serum concentrations of total protein (TP), albumin (Alb) and activities of aspartate transaminase (AST), alanine transaminase (ALT) and lactate dehydrogenate (LDH) in chickens given maduramicin medicated feed (mean \pm S.E.)

Groups	Parameters	Medication period (days)			
		0	7	14	21
A	TP (g/dl)	2.83 \pm 0.08 ^a	2.56 \pm 0.14 ^a	2.75 \pm 0.02 ^a	2.76 \pm 0.04 ^a
	Alb (g/dl)	1.8 \pm 0.07 ^a	1.59 \pm 0.04 ^a	1.75 \pm 0.01 ^a	1.82 \pm 0.04 ^a
	AST (IU/L)	153.02 \pm 5.75 ^a	175.22 \pm 6.74 ^a	168.08 \pm 32.89 ^b	453.22 \pm 64.81 ^b
	ALT (IU/L)	17.50 \pm 0.94 ^a	23.17 \pm 1.16 ^a	20.35 \pm 1.25 ^a	34.66 \pm 4.82 ^a
	LDH (IU/L)	1244.6 \pm 26.6 ^a	1282.8 \pm 41.5 ^a	1155.6 \pm 64.9 ^a	1403 \pm 62.9 ^a
B	TP (g/dl)	2.83 \pm 0.08 ^a	3.02 \pm 0.08 ^a	2.92 \pm 0.08 ^a	2.94 \pm 0.06 ^a
	Alb (g/dl)	1.8 \pm 0.07 ^a	1.93 \pm 0.05 ^a	1.84 \pm 0.04 ^a	1.88 \pm 0.05 ^a
	AST (IU/L)	153.02 \pm 5.75 ^a	182.90 \pm 12.87 ^a	202.36 \pm 13.69 ^a	730.30 \pm 72.40 ^a
	ALT (IU/L)	17.50 \pm 0.94 ^a	19.36 \pm 1.85 ^a	22.47 \pm 2.76 ^a	36.77 \pm 2.88 ^a
	LDH (IU/L)	1244.6 \pm 26.6 ^a	1302.2 \pm 27.2 ^a	1447 \pm 45.8 ^b	1448.2 \pm 23.1 ^b
C	TP (g/dl)	2.83 \pm 0.08 ^a	2.81 \pm 0.16 ^a	2.58 \pm 0.08 ^a	2.76 \pm 0.02 ^a
	Alb (g/dl)	1.8 \pm 0.07 ^a	1.7 \pm 0.07 ^a	1.65 \pm 0.05 ^a	1.67 \pm 0.05 ^a
	AST (IU/L)	153.02 \pm 5.75 ^a	168.42 \pm 7.86 ^a	123.68 \pm 7.38 ^b	172.38 \pm 6.35 ^c
	ALT (IU/L)	17.50 \pm 0.94 ^a	18.03 \pm 0.61 ^a	20.35 \pm 0.63 ^a	14.56 \pm 0.41 ^b
	LDH (IU/L)	1244.6 \pm 26.6 ^a	1276 \pm 17.8 ^a	1189 \pm 32.6 ^a	1375.2 \pm 18.9 ^a

^{a,b,c}Mean in respective parameters with unlike letters in a column are significantly different, $P < 0.05$

infiltration of lymphocytes. Ren *et al.* (1996) reported an outbreak of maduramicin toxicity in broiler chickens in China in which catarrhal enteritis was noticed on gross examination.

From day 14 onwards, liver of birds in group B showed mild fatty changes in hepatocytes, hyperplasia and hypertrophy of bile duct epithelium along with haemorrhages and mononuclear cells infiltration mainly of lymphocytes in portal triads. However, liver of birds in group A did not reveal any significant alteration. Almost similar results have been reported in calves given maduramicin medicated ration (Shlosberg *et al.*, 1997). Ren *et al.* (1996) reported congestion and swelling of liver in broilers due to maduramicin toxicity in clinical cases but they did not examine the liver histopathologically. Fatty changes in liver might be due to the fact that the ionophores are compounds that form lipid soluble cation complexes (Pressman, 1976).

Bursa of Fabricius of birds in the both the medicated groups from day 14 onwards revealed focal areas of necrosis of lymphocytes forming small vesicle like structures in the cortex. On day 21, there was atrophy of few follicles and increase in interfollicular space due to reticuloendothelial cells proliferation. Spleen of birds in group B from day 14 post-treatment revealed depletion of lymphocytes (Fig 1) in white pulp along with reticular cells proliferation and haemorrhages. These alterations in spleen

and bursa revealed that maduramicin medication may cause immunosuppression. No report could be traced in the literature on effect of ionophores toxicity on the lymphoid organs.

Heart sections of birds in group B on day 14 revealed myocarditis characterized by mononuclear cell infiltration, haemorrhages and oedema in the myocardium whereas, focal areas of myocardial necrosis were noticed on day 21. In group A, aforesaid described lesions in heart were of mild nature. More or less similar cardiac lesions have been described in maduramicin toxicosis in calves (Shlosberg *et al.*, 1997) and rabbits (Ho *et al.*, 1997). Skeletal muscles in group B exhibited myositis characterized by congestion, haemorrhages and inflammatory reaction mainly of mononuclear cells and a few heterophils along with oedema from day 14 of the medication. In group B, lungs showed severe haemorrhages, congestion and mild emphysema on day 21 of medication (Fig 2). Almost similar results have also been documented in calves due to maduramicin toxicity (Shlosberg *et al.*, 1997). On days 14 and 21, kidney revealed focal areas of cloudy swelling in the renal tubules of group A whereas in group B, necrotic lesions were observed in the renal tubules.

The present study indicated that maduramicin medication @5 and 10 ppm for 7 days did not produce any lesion in the broiler chicken, however, it caused toxic lesions in various visceral organs from day 14 onwards

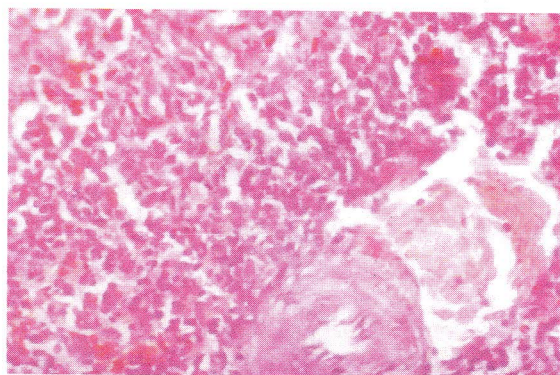


Fig 1. Spleen from 10 ppm group showing lymphocytic depletion in white pulp along with haemorrhages and reticular cells proliferation on day 14 of the medication. (H. & E. x 400)

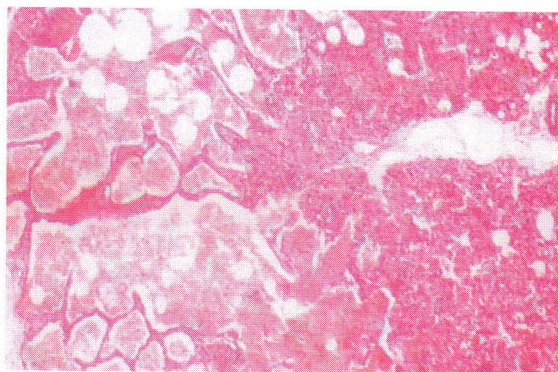


Fig 2. Lung from 10 ppm group showing severe haemorrhages and congestion on day 21 of the medication. (H. & E. x 100)

at both the levels. Lesions in lymphoid organs reflect its immunosuppressive effect.

REFERENCES

- Benjamin, M.M. (1978). Outline of Veterinary Clinical Pathology. The Iowa State University Press, Ames, Iowa, U.S.A.
- Folz, S.D., Lee, B.L., Nowakowski, L.H., and Conder, G.A. (1988). Anticoccidial evaluation of halofuginone, lasalocid, maduramicin, monensin and salinomycin. *Vet. Parasitol.* **28**: 1-9.
- Fourie, M., Batiello, S.S., Prozesky, L., Nel, P.W. Ekelle and Man, T.S. (1991). Cardiomyopathy of ruminants induced by the litter of poultry fed on rations containing the ionophore antibiotic, maduramicin 1. Epidemiology, clinical signs and clinical pathology. *Onderstepoort J. Vet. Res.* **58**: 291.
- Ho, L.L., Ho, S.P. and Wang, W.S. (1997). Toxicity of maduramicin ammonium (Cygro) in New Zealand white rabbits. *Taiwan J. Vet. Med. Anim. Husb.* **67**: 87-92.
- Logan, N.B., McKenzie, M.E. and Conway, D.P. (1993). Anticoccidial efficacy of semaduramicin. Evaluation against field isolates including comparisons with salinomycin, maduramicin and monensin in battery tests. *Poult. Sci.* **72**: 2058-2063.
- Luna, L.G. (1968). Manual of Histology Staining Methods of Armed Forces Institute of Pathology. McGraw Hill Book Co., New York.
- McDougald, L.R. and Reid, W.M. (1991). Coccidiosis. In: Diseases of Poultry, (9th ed. Calnek, B.W. ed.), Iowa State University Press, Ames, USA. pp. 780-797.
- Pressman, B.C. (1976). Biological application of ionophores. *Annual Rev. Biochem.* **45**: 501-530.
- Ren, Z., Xin, H.L., Hong, L., Limin, Z. and Meng, D.Z. (1996). Poisoning of broilers by maduramicin. *Chinese J. Vet. Med.* **22**: 18-19.
- Snedecor, W.G. and Cochran, G.W. (1967). Statistical Methods. The Iowa State University Press, Ames, Iowa, USA.
- Shlosberg, A., Perl, S., Harmalin, A., Hnaji, V, Bellaiche, M., Bogin, E., Cohen, R., Markusfeldnir, O., Shigel, N., Eisenberg, Z., Furman, M., Brosh, A., Holzer, Z. and Aharoni, Y. (1997). Acute maduramicin toxicity in calves. *Vet. Rec.* **140**: 643-646.
- Singh, T. and Gupta, R.P. (2003). Clinico-haematological and mineral studies on experimental maduramicin toxicity in chickens. *Vet. Parasitol.* **116**: 345-353.

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