



ALLEVIATIVE EFFECT OF MANNAN OLIGOSACCHARIDE AGAINST PENICILLIC ACID TOXICITY IN BROILER CHICKENS

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ABSTRACT

Penicillic acid (PA) is α , β -unsaturated conjugated lactone produced by *Penicillium* and *Aspergillus* species occurs in feed and foodstuffs. Mannan oligosaccharide (MOS) present in the cell wall of *S. cerevisiae*, was found to have beneficial effects in poultry against mycotoxicosis. To find out the alleviative effect of MOS against PA toxicity in broiler chickens. Feeding of broiler chickens with different diets (T1- Control, T2- MOS (0.05%), T3- PA (20 ppm) and T4-PA (20 ppm) + MOS (0.05%) of 12 chicks each. Eight chicks were allotted to no toxin-no binder-no vaccine group (T5). Two birds from each group were sacrificed on 7th, 14th, 21st and 28th day of age to study the cell mediated immunity of the birds. On the 28th day of trial, remaining birds were sacrificed to study the haematobiochemical alterations, pathological changes in different organs and immune status. The addition of MOS (0.05%) to PA (20 ppm) diet had no impact in alleviating the toxic effects of PA in broiler chicken when fed for four weeks. MOS alone induced hypoalbuminaemia, elevated BUN values and pathological changes in the liver, kidneys, spleen and bursa of Fabricius.

Key words: Penicillic acid toxicity, mannan oligosaccharide, haematobiochemistry, pathology, immunity.

INTRODUCTION

Penicillic acid (PA), a mycotoxin, was originally isolated from the cultures of *Penicillium puberulum* (Alsberg CL and Black OF, 1913). Later, it was found that *P. cyclopium* Westling produced relatively larger amounts of penicillic acid (Bentley R and Keil JG, 1962; Birkinshaw JH et al.1936). Penicillic acid occurred in high concentrations in corn (LeBars J. 1980) and was also produced concomitantly with other mycotoxins in poultry feed (Bacon CW et al.1973). The natural occurrence of penicillic acid has been detected in the poultry feed, corn, dried beans, cheese, salami and tobacco products (LeBars J. 1980). The penicillic acid toxins interfered with protein formation by the action on nucleic acids. The increased glycogen level observed during toxicosis was attributed to the interaction of penicillic acid with the enzymes of carbohydrate metabolism. It also affected the lipid

metabolism leading to lower levels of total lipids (Pandiyana V et al., 1987). The penicillic acid toxin has been shown to have antibacterial, antiviral, antitumour, antidiuretic, cytotoxic, hepatotoxic and carcinogenic properties in the mice and rats (Chan PK et al., 1980; Kawaski I et al.1972; Phillips TD et al., 1980; Suzuki S et al., 1971). Extensive research has been conducted to counter mycotoxicoses by physical, chemical, nutritional or biological approaches. The use of binding agents, which can adsorb mycotoxin molecule by means of ion exchange and thereby preclude their absorption from the gut, has gained considerable attention in recent times. Spent canola oil bleaching clays (Smith TK. 1984), hydrated sodium, calcium aluminosilicate (Kubena LF et al. 1990), zeolite ore compounds (Harvey RB et al. 1993), activated charcoal (Edrington T.S et al., 1997), bentonite (Santurio JM et al. 1999) and a

blend of organic acids and alumino silicates (Mahesh BK and Devegowda G, 1996) have shown considerable promise in countering aflatoxins. However, many of the agents lack a similar effect against other mycotoxins of practical importance (Rotter RG et al. 1989; Edrington TS et al. 1997).

The search is still on for a comprehensive tool to counter the problem of mycotoxicosis of multiple origins. Recent biotechnological progress has opened new avenues for tackling this problem. *Saccharomyces cerevisiae* was found to have beneficial effects in poultry against mycotoxicosis (Stanley VG et al. 1993; Devegowda G et al. 1996). Mannan oligosaccharide (MOS), present in the cell wall of *S. cerevisiae*, is believed to be responsible for the beneficial effects observed with *S. cerevisiae*. MOS is a combination of several molecules of mannose. Mannans (Mannose) found in the cell wall of yeast *Saccharomyces cerevisiae* has been shown to induce an antigenic response in humans. The cell wall of the yeast organism consists of carbohydrates and protein in the form of chained and branched structures of glucose, mannose and N-acetyl glucosamine (Ballou CE. 1970). It has been shown that mycotoxins are adsorbed by the polysaccharides (glucan and mannan) and not by the proteins or fatty acids of yeast cell walls (Schmitt M and Radler F, 1987). The cell wall containing polysaccharides, proteins and lipids exhibited numerous adsorption mechanisms such as hydrogen bonding, ionic bonding or hydrophobic interaction (Karlovsy P. 1999). The present study was undertaken to elucidate the effect of toxin binder- mannan oligosaccharide (MOS) against penicillic acid mycotoxicosis in broiler chicken.

MATERIALS AND METHODS

1. FUNGAL CULTURE

The *Penicillium cyclopium* NRRL 1888 culture was obtained from National Center for Agricultural Utilization Research, Microbial Genomics and Bioprocessing Research Unit, 1815 N University Street, Peoria, Illinois 61604, USA.

1.1. Maintenance of culture

The *P. cyclopium* NRRL 1888 was subcultured on potato dextrose agar at 10 days interval (Ziegler et al., 1972).

1.2. Penicillin acid production

The penicillic acid toxin was produced on maize (LeBars, 1980). The maize samples were pre-tested for the presence of mycotoxins. The penicillic acid from ground maize culture samples were quantified by using thin layer chromatography at the Central Animal Feed and Food Residue Laboratory, Directorate of Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051.

2. VACCINE

The D58 live thermostable Newcastle disease (ND) vaccine was obtained from the Department of Veterinary Microbiology, Madras Veterinary College, Chennai, for immunizing birds against ND. The ND antibody titre was determined by using indirect ELISA developed by the Department of Veterinary Microbiology, Madras Veterinary College, Chennai. The birds were vaccinated against ND at the seventh day of age with D58 live thermostable ND vaccine through oronasal route

3. TOXIN BINDER

Mannan oligosaccharide (MOS) obtained from M/s. Exotic Mushrooms, Vile Parle (East), Mumbai, was used as toxin binder. Powdered maize culture material containing known amounts of penicillic acid was incorporated into the toxin free diet, so that the diet contained 20 ppm of penicillic acid. MOS was added at the level of 0.05 per cent in the diet.

4. EXPERIMENTAL DESIGN

Out of 56 day-old broiler chicks obtained, 48 were randomly allotted to four groups of 12 chicks each. Remaining eight chicks were allotted to no toxin-no binder-no vaccine group. Two birds from each group were sacrificed on 7th, 14th, 21st and 28th day of age to study the CMI of the birds. On the 28th day of trial, remaining birds were sacrificed to study the haematobiochemical alterations, pathological changes in different organs and immune status.

Groups	No. of birds
Control	12
MOS (0.05%)	12
Penicillic acid (20 ppm)	12
Penicillic acid (20 ppm) + MOS (0.05%)	12
No toxin, no binder, no vaccine	8

5. GROWTH STUDY

Body weights (g) were recorded at weekly intervals. Average weekly feed consumption (g) and feed conversion were arrived at as follows.

$$\text{Feed consumption} = \frac{\text{Total feed consumed during the week (g)}}{\text{Number of birds fed during the week}}$$

$$\text{Feed conversion} = \frac{\text{Average feed consumption per bird during the week (g)}}{\text{Average weight gain per bird during the week (g)}}$$

6. HAEMATOLOGY

Blood samples were collected by intracardiac puncture in Heller and Paul double oxalate anticoagulant mixture. Haematological studies included the estimation of PCV by microhaematocrit method, Hb by acid haematin method and TEC using Hayem's fluid (Coles EH, 1986).

7. SERUM BIOCHEMISTRY

Another set of blood samples collected were allowed to clot and centrifuged at 1500 rpm for 20 min to separate the sera. Serum total protein and albumin were estimated by modified Biuret and Dumas method (Varley H et al. 1980), glucose by glucose oxidase method, total cholesterol (TC) by cholesterol dehydrogenase peroxidase method, AST, ALT and ALP by IFCC (International Federation of Clinical Chemistry) method, BUN by glutamyl dehydrogenase method, creatinine by Jaffe's kinetic method, uric acid by enzymatic photometric test by IFCC method (Burtis CA and Ashwood ER. 1996), calcium by O-cresolphthalein complexone method, phosphorus by modified metol method, sodium and potassium by colorimetric method, amylase by colorimetric method (Coles EH, 1986), serum lipase by turbidimetric UV method (Burtis CA and Ashwood ER. 1996), HDL-Cholesterol by precipitation method and triglycerides (TG) by colorimetric enzymatic method using semi-automatic analyser (Mispa excel, Agappe Diagnostic, India). VLDL was arrived by using the formula TG/5 and LDL using the formula TC-HDL-VLDL and TC/HDL was also found out.

8. LIVER LIPID PEROXIDATION AND ANTIOXIDANTS

8.1. Sample collection

Liver tissue samples collected from control and toxin fed birds were stored at - 20°C till the required assays were carried out.

8.2. Estimation of liver lipid peroxidation

Liver lipid peroxidation was estimated by the formation of thiobarbituric acid (TBARS) following the method of Yagi K. (1976).

8.3. Estimation of liver antioxidants

The protein content was estimated by the method of Lowry OH et al. (1951). Glutathione peroxidase (GPx) was estimated by the method of Rotruck *et al.* (1973), glutathione-S-transferase (GST) by the method of Habig WH et al. (1974), superoxide dismutase (SOD) by the method of Marklund SL and Marklund G, (1974) and catalase (CAT) by the method of Caliborne AL. (1985). Reduced glutathione (GSH) was estimated by the method of Moron MS et al. (1979).

9. PATHOLOGY

After collection of blood, the birds were sacrificed by cervical dislocation and a detailed post mortem examination was conducted on sacrificed birds. Representative samples of tissues from liver, kidney, crop, proventriculus, gizzard, duodenum, pancreas, spleen, bursa of Fabricius, thymus, caecal tonsils and Harderian gland were collected in 10 per cent formal saline. Paraffin embedded

tissues were sectioned to 5 μ m thickness and stained by haematoxylin and eosin H&E) for histopathological examination (Bancroft JD and Gamble G. 2008).

10. HUMORAL IMMUNITY

The antibody titre against NDV was determined by indirect ELISA method at 28th day the trial as per the procedure described by John Kirubakaran J.(2008).

11. CELL MEDIATED IMMUNITY

Cell mediated immunity was assessed by colorimetric blastogenesis assay as described by Reynolds DL and Maraqa AD, (2000) was followed with slight modifications.

12. Statistical analysis

The data generated from different experimental trials were subjected to one-way analysis of variance (ANOVA) using SPSS version 10 software for windows.

RESULTS

The *P. cyclopium* NRRL 1888 subcultured on potato dextrose agar revealed more or less circular colonies after 48 h. The whitish and fluffy aerial mycelium turned to dull blue green velvety growth. On inoculation of spores into maize, whitish spots began to appear within two or three days and then turned to dull blue and green. The culture material yielded 20–80 ppm penicillic acid.

GROWTH STUDY

Body weight

Mean \pm SE weekly body weights (g) of broiler chicken fed MOS against penicillic acid toxin are presented in Table 1. There was a highly significant ($P < 0.01$) difference between the control and penicillic acid toxin fed groups from the third week onwards. No significant differences were observed between the control and MOS groups. Highly significant ($P < 0.01$) decrease in the body weight gain was observed in the penicillic acid and PA+MOS fed groups when compared to the control and MOS groups from the third week onwards.

Table 1
Mean (\pm SE) weekly body weights (g) of broiler chicks fed MOS against penicillic acid

Groups	Hatch weight (g)	Age in weeks							
		1		2		3		4	
		BW (n=12)	RBW	BW (n=10)	RBW	BW (n=8)	RBW	BW (n=6)	RBW
Control (0 ppm)	48.42 \pm 0.70	114.13 \pm 3.61	100	232.90 \pm 14.61	100	451.88 ^x \pm 31.11	100	814.17 ^x \pm 41.84	100
MOS (0.05%)	48.52 \pm 0.88	118.58 \pm 3.95	104	223.00 \pm 8.35	100	400.00 ^x \pm 9.16	89	732.50 ^x \pm 30.10	90
Penicillic acid (20 ppm)	47.44 \pm 0.80	112.33 \pm 2.93	98	199.00 \pm 8.23	89	295.00 ^y \pm 15.03	65	415.00 ^y \pm 26.89	51
Penicillic acid (20 ppm) +MOS (0.05%)	47.35 \pm 0.85	116.26 \pm 4.38	102	205.00 \pm 7.42	91	325.00 ^y \pm 14.39	72	465.00 ^y \pm 22.29	57

BW - Body weight; RBW - Relative body weight

Means with same superscripts within a column do not differ from each other ($P > 0.01$)

FEED CONSUMPTION AND FEED CONVERSION

Mean \pm SE weekly feed consumption (g) and feed conversion values of broiler chicken fed MOS against penicillic acid toxin showed that there was no significant difference among the different treatment groups for feed consumption and feed conversion.

CLINICAL SIGNS

The clinical sign observed in penicillic acid toxin treated birds was depression without any mortality.

HAEMATOLOGY

Mean \pm SE haematological values of broiler chicken fed MOS against penicillic acid are presented in Table 2. There was no significant difference in the PCV, Hb and TEC values between the control and other treatment groups.

Table 2
Mean (\pm SE) haematological values of broiler chicks fed MOS against penicillic acid (n=6)

Groups	PCV (%)	Hb (g/dL)	TEC (millions/cmm)
Control (0 ppm)	34.17 \pm 3.45	11.33 \pm 1.05	2.89 \pm 0.20
MOS (0.05%)	31.00 \pm 1.18	11.17 \pm 0.48	2.90 \pm 0.10
Penicillic acid (20 ppm)	31.50 \pm 1.12	9.69 \pm 0.76	2.57 \pm 0.05
Penicillic acid (20 ppm) + MOS (0.05%)	32.67 \pm 1.52	10.33 \pm 0.61	3.00 \pm 0.04

SERUM BIOCHEMISTRY

Serum glucose

Mean \pm SE serum glucose values (mg/dL) of broiler chicken fed MOS against penicillic acid are presented in Table 3. The glucose value of penicillic acid toxin fed group differed significantly ($P < 0.05$) from that of control and MOS groups. Significant ($P < 0.05$) increase in the serum glucose value was observed in the penicillic acid toxin treated birds when compared to the other groups except PA+ MOS group.

Table 3
Mean (\pm SE) serum glucose values (mg/dL) of broiler chicks fed MOS against penicillic acid (n=6)

Groups	Glucose (mg/dL)
Control (0 ppm)	136.38 ^b \pm 13.47
MOS (0.05%)	151.63 ^b \pm 24.25
Penicillic acid (20 ppm)	260.06 ^a \pm 32.32
Penicillic acid (20 ppm) + MOS (0.05%)	202.83 ^{ab} \pm 34.34

Means with same superscripts within a column do not differ from each other ($P > 0.05$)

Serum total protein, albumin, globulin and albumin to globulin ratio values

Mean \pm SE serum total protein, albumin, globulin and albumin to globulin ratio values (g/dL) of broiler chicken fed MOS against penicillic acid are presented in Table 4. There was no significant difference between the control and other groups except for albumin values. No significant differences were observed in the albumin values among the control, penicillic acid and PA+MOS groups. Significant ($P < 0.05$) decrease in the serum albumin value was observed in the MOS group when compared to the other groups.

Table 4
Mean (\pm SE) serum total protein, albumin, globulin and albumin to globulin values (g/dL) of broiler chicks fed MOS against penicillic acid (n=6)

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Albumin to globulin
Control (0 ppm)	3.70 \pm 0.23	1.89 ^a \pm 0.12	1.58 \pm 0.17	1.24 \pm 0.13
MOS (0.05%)	3.78 \pm 0.56	1.37 ^b \pm 0.15	2.41 \pm 0.47	0.76 \pm 0.08
Penicillic acid (20 ppm)	3.36 \pm 0.33	1.78 ^a \pm 0.06	2.15 \pm 0.68	0.95 \pm 0.19
Penicillic acid (20 ppm) + MOS (0.05%)	3.26 \pm 0.44	1.76 ^a \pm 0.16	1.85 \pm 0.24	1.02 \pm 0.15

Means with same superscripts within a column do not differ from each other ($P>0.05$)

Serum enzymes

Mean \pm SE serum enzyme values (U/L) of broiler chicken fed MOS against penicillic acid are presented in Table 5. There was no significant difference in the serum enzyme values of different groups except for ALT ($P<0.01$). Comparison of means revealed highly significant ($P<0.01$) differences in the ALT value between the penicillic acid and other groups. There was highly significant ($P<0.01$) increase in the serum ALT value in the penicillic acid group when compared to the other groups.

Table 5
Mean (\pm SE) serum enzyme values (U/L) of broiler chicks fed MOS against penicillic acid (n=6)

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Amylase (U/L)	Lipase (U/L)
Control (0 ppm)	5.94 ^y \pm 1.23	160.58 \pm 15.27	3792.00 \pm 776.71	696.35 \pm 174.19	50.67 \pm 13.92
MOS (0.05%)	4.34 ^y \pm 0.66	150.99 \pm 16.40	4886.90 \pm 1438.66	691.31 \pm 200.41	89.12 \pm 22.44
Penicillic acid (20 ppm)	14.63 ^x \pm 1.59	169.74 \pm 16.88	8402.07 \pm 1200.57	297.13 \pm 20.19	57.16 \pm 5.35
Penicillic acid (20 ppm) + MOS (0.05%)	4.66 ^y \pm 0.69	154.74 \pm 23.77	8250.40 \pm 1731.66	480.23 \pm 266.41	67.30 \pm 7.41

Means with same superscripts within a column do not differ from each other ($P>0.01$)

Kidney function tests

Mean \pm SE BUN and serum creatinine and uric acid values (mg/dL) of broiler chicken fed MOS against penicillic acid are presented in Table 6. There was highly significant ($P<0.01$) difference in the BUN and serum uric acid values among the different treatment groups. Comparison of means revealed highly significant ($P<0.01$) differences in the BUN values of MOS and PA+MOS groups when compared to the control and penicillic acid

groups. Serum uric acid value of penicillic acid and PA+MOS groups differed highly significantly ($P<0.01$) and also from control and MOS fed groups. There was no significant difference in the creatinine values between the control and treated groups. Highly significant ($P<0.01$) increase in the BUN values of MOS and PA+MOS groups and in the uric acid values of penicillic acid and PA+MOS groups when compared to the control and MOS groups were observed.

Table 6
Mean (\pm SE) BUN and serum creatinine and uric acid values (mg/dL) of broiler chicks fed MOS against penicillic acid (n=6)

Groups	BUN (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)
Control (0 ppm)	6.03 ^z \pm 0.76	0.22 \pm 0.06	5.76 ^z \pm 0.66
MOS (0.05%)	10.99 ^y \pm 0.90	0.49 \pm 0.12	5.69 ^z \pm 0.79
Penicillic acid (20 ppm)	7.80 ^z \pm 0.65	0.49 \pm 0.11	14.24 ^x \pm 1.23
Penicillic acid (20 ppm) + MOS (0.05%)	15.78 ^x \pm 0.75	0.65 \pm 0.17	8.91 ^y \pm 0.38

Means with same superscripts within a column do not differ from each other ($P>0.01$)

Lipid profile

Mean \pm SE serum lipid values (mg/dL) of broiler chicken fed MOS against penicillic acid are presented in Table 7. Significant ($P<0.05$) differences in the total cholesterol, triglycerides, VLDL ($P<0.01$) and LDL ($P<0.05$) values were observed in different treatment groups when compared to the control group. No significant differences were observed for the HDL-cholesterol and TC/HDL ratio values. Comparison of means revealed no significant differences between the control and MOS, MOS and penicillic acid and penicillic acid and PA+MOS groups for serum total cholesterol. Comparison of means revealed that serum triglycerides value of penicillic acid fed group differed highly significantly ($P<0.01$) from

that of all other groups. The LDL value of PA+MOS group differed significantly ($P<0.05$) from that of all other groups. The VLDL value of penicillic acid group differed highly significantly ($P<0.01$) from that of all other groups. Compared to the control group, there was highly significant ($P<0.01$) increase in the serum total cholesterol values of penicillic acid and PA+MOS groups. The serum triglycerides value of penicillic acid fed group showed a highly significant ($P<0.01$) increase when compared to the other groups. The LDL value of PA+MOS group increased significantly ($P<0.05$) from that of other groups. The VLDL value of penicillic acid fed group increased highly significantly ($P<0.01$) when compared to the other groups.

Table 7

Mean (\pm SE) serum lipid profile values (mg/dL) of broiler chicks fed MOS against penicillic acid (n=6)

Groups	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	TC/HDL ratio
Control (0 ppm)	85.34 ^z \pm 3.71	71.12 ^y \pm 11.05	19.68 \pm 1.70	51.47 ^b \pm 6.12	14.22 ^y \pm 2.21	4.57 \pm 0.56
MOS (0.05%)	92.48 ^{yz} \pm 6.84	61.59 ^y \pm 4.97	17.56 \pm 1.43	62.60 ^b \pm 6.70	12.32 ^y \pm 0.10	5.51 \pm 0.67
Penicillic acid (20 ppm)	115.72 ^{xy} \pm 5.76	153.95 ^x \pm 11.82	18.69 \pm 1.33	64.96 ^b \pm 7.23	30.79 ^x \pm 2.36	6.32 \pm 0.48
Penicillic acid (20 ppm) + MOS (0.05%)	140.88 ^x \pm 12.53	83.44 ^y \pm 9.14	21.34 \pm 1.27	86.17 ^a \pm 9.60	16.69 ^y \pm 1.83	6.71 \pm 0.67

Means with same superscripts within a column (a,b/x,y,z) do not differ from each other ($P>0.05/P>0.01$)

Serum electrolytes and minerals

Mean \pm SE serum sodium, potassium (mEq/L), calcium, phosphorus (mg/dL) and Ca:P levels of broiler chicken fed MOS against penicillic acid showed that there was no significant difference between the control and treated groups for serum electrolytes and minerals.

LIVER TBARS ASSAY

Mean \pm SE liver TBARS values of broiler chicken fed MOS against penicillic acid showed that there was no significant difference between the control and other treatment groups for liver TBARS.

LIVER ANTIOXIDANT ASSAY

Mean \pm SE SOD, CAT, GPx, GST and GSH values of broiler chicken fed MOS against penicillic acid are presented in Table 8. No significant differences were observed for the antioxidants between the control and treated birds except for GPx. Comparison of means revealed highly significant ($P<0.01$) differences between the penicillic acid and other groups for GPx except control group. There was highly significant ($P<0.01$) increase in the GPx values of MOS and PA+MOS groups when compared to the penicillic acid group.

Table 8
Mean (\pm SE) liver TBARS and antioxidant values in MOS fed against penicillic acid in broiler chicks (n=6)

Groups	TBARS ¹	SOD ²	CAT ³	GPx ⁴	GST ⁵	GSH ⁶
Control (0 ppm)	194.93 \pm 26.87	0.15 \pm 0.02	0.50 \pm 0.09	583.10 ^{xy} \pm 12.41	2.83 \pm 0.99	560.99 \pm 26.30
MOS (0.05%)	170.90 \pm 27.03	0.22 \pm 0.09	0.51 \pm 0.11	634.49 ^x \pm 16.03	3.23 \pm 0.51	619.97 \pm 38.82
Penicillic acid (20 ppm)	283.48 \pm 52.02	0.09 \pm 0.01	0.42 \pm 0.05	518.86 ^y \pm 37.78	4.15 \pm 0.84	490.43 \pm 39.48
Penicillic acid (20 ppm) + MOS (0.05%)	206.87 \pm 29.01	0.13 \pm 0.03	0.51 \pm 0.09	639.22 ^x \pm 14.14	2.44 \pm 0.45	502.28 \pm 59.41

Means with same superscripts within a column do not differ from each other ($P>0.01$)

¹TBARS level in mg/g of tissue

²Enzyme required for inhibiting 50% pyrogallol autooxidation/min/mg protein

³Catalase required for decomposing μ m of H₂O₂/min/mg protein

⁴GPx expressed as μ m of glutathione utilized/min/mg protein

⁵GST expressed as μ m CDNB-GSH conjugate formed /min/mg protein

⁶GSH level in mg/g of tissue

HUMORAL IMMUNITY

Mean \pm SE NDV antibody titre values of broiler chicken fed MOS against penicillic acid showed that there was no significant difference between the control and other treated groups for NDV titres.

CELL MEDIATED IMMUNITY

Mean \pm SE splenic lymphocyte stimulation index values of broiler chicken fed MOS against penicillic acid are presented in Table 9. Highly significant ($P<0.01$) differences were observed

among the various treatment groups for splenic lymphocyte stimulation index values. Comparison of means revealed highly significant ($P<0.01$) differences between the MOS and other groups except vaccine+no toxin group and between penicillic acid and other groups except PA+MOS. No significant difference was observed between the vaccine+no toxin group and PA+MOS groups. There was highly significant ($P<0.01$) decrease in the splenic lymphocyte stimulation index values of no vaccine+no toxin and penicillic acid groups when compared to the vaccine+no toxin and MOS groups.

Table 9
Mean (\pm SE) splenic lymphocyte stimulation index of broiler chicks fed MOS against penicillic acid (n=6)

Groups	I week	II week	III week	IV week	Overall means
No vaccine+ No toxin	-0.191 \pm 0.031	-1.383 \pm 0.875	-0.533 \pm 0.013	-0.142 \pm 0.038	-0.562 ^z \pm 0.501
Vaccine+ No toxin	0.488 \pm 0.048	0.428 \pm 0.323	0.679 \pm 0.029	0.217 \pm 0.007	0.453 ^{wx} \pm 0.176
MOS (0.05%)	1.366 \pm 0.333	0.064 \pm 0.037	0.353 \pm 0.186	0.286 \pm 0.018	0.517 ^w \pm 0.406
Penicillic acid (20 ppm)	0.501 \pm 0.243	0.058 \pm 0.020	-0.070 \pm 0.022	-0.340 \pm 0.121	0.037 ^y \pm 0.252
Penicillic acid (20 ppm)+ MOS (0.05%)	0.078 \pm 0.073	0.080 \pm 0.026	0.277 \pm 0.007	0.085 \pm 0.059	0.130 ^{xy} \pm 0.074

Overall means with same superscript within a column do not differ from each other ($P>0.01$)

LIVER WEIGHT

Respective mean \pm SE liver weights (g) of control, MOS, penicillic acid and PA+MOS groups were 3.02 ± 0.23 , 2.51 ± 0.07 , 3.26 ± 0.53 and 2.88 ± 0.19 and revealed that there was no significant difference between the control and other treatment groups for liver weights.

PATHOLOGY

Gross pathology

The penicillic acid toxin (20 ppm) treated liver showed pale discolouration in three birds. Congestion, mild enlargement and pale to yellowish discolouration (Figure 1) were observed in the remaining birds.



Figure 1
Liver -Pale to yellowish discolouration

Histopathology

The MOS (0.05%) fed birds showed mild focal to diffuse vacuolar degeneration of hepatocytes (Figure 2) and mild perivenous (periportal) fibrosis in the liver in three cases. Kidneys revealed mild degeneration and necrosis of tubular epithelial cells. In spleen mild to moderate lymphoid cell depletion and reticulum cell hyperplasia (Figure 3) were seen. Bursa of Fabricius showed mild lymphoid cell depletion in a few follicles.

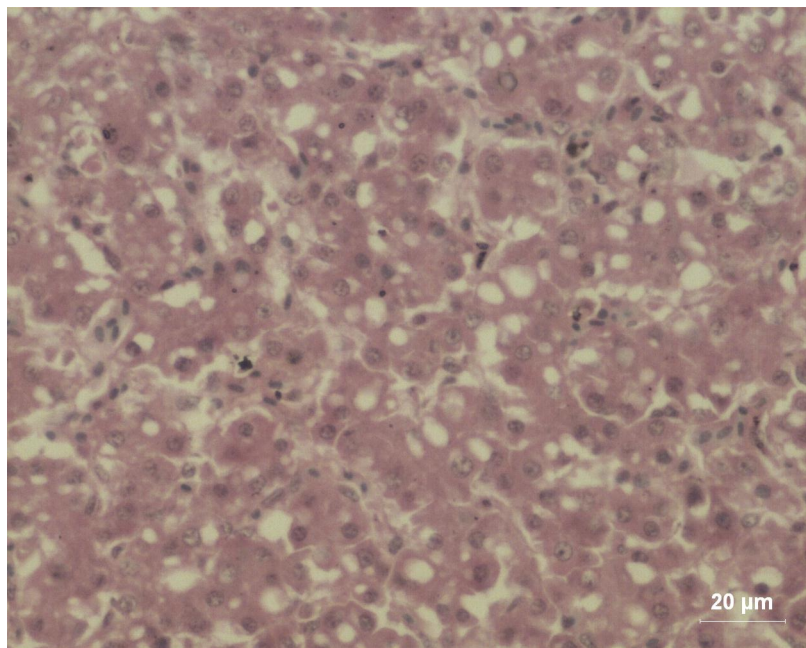


Figure 2
MOS (0.05%) Group- Liver- mild focal to diffuse vacuolar degeneration of hepatocytes Scale Bar H&E 20μm

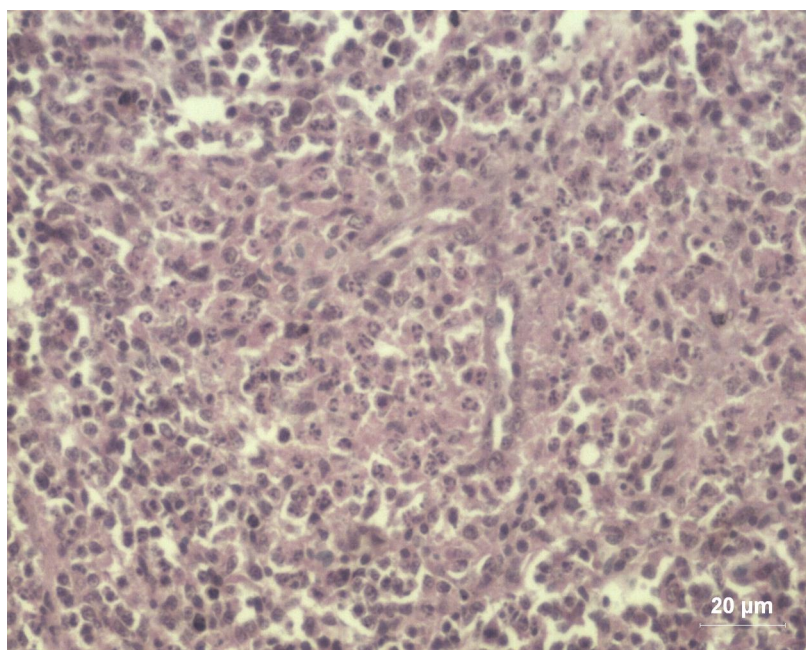
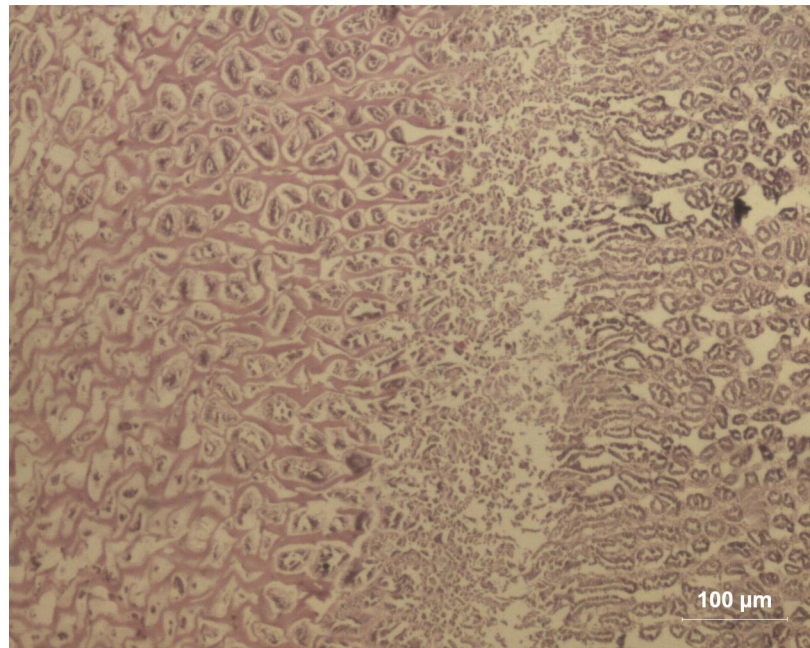


Figure 3
MOS (0.05%) Group- Spleen-Mild to moderate lymphoid cell depletion Scale Bar H&E 20μm

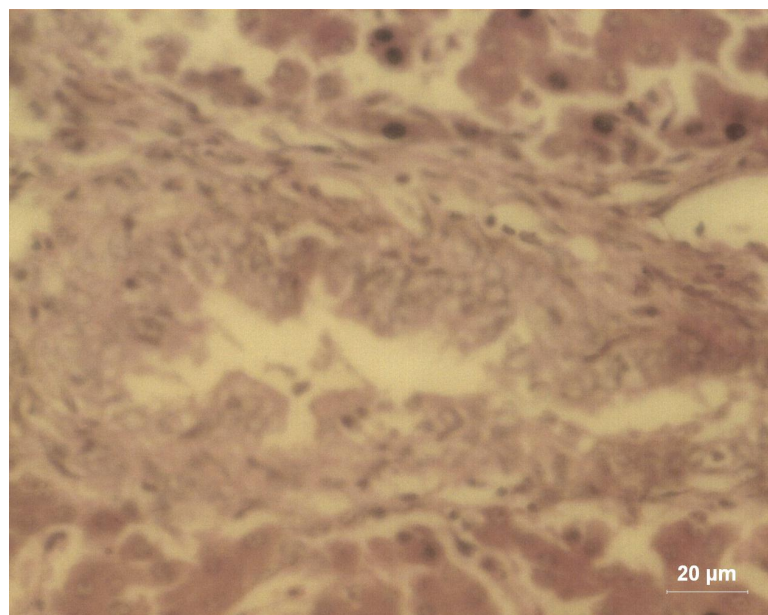
The penicillic acid toxin (20 ppm) fed birds showed moderate to diffuse vacuolar degeneration of hepatocytes in the liver. Kidneys revealed degeneration and necrosis of tubular epithelial cells. Crop showed extensive mucosal hyperplasia. Proventricular mucosa revealed necrosis and diffuse mononuclear cell infiltration. Proventricular glandular epithelium showed vacuolar degeneration and necrosis. Gizzard revealed dilatation of glands and defective kaolin

formation (Figure 4). Pancreas showed moderate degeneration and necrosis of acinar cells. Spleen showed moderate lymphoid cell depletion and reticulum cell hyperplasia. Bursa of Fabricius showed moderate lymphoid cell depletion in the cortex and medulla with cystic changes in the follicles. Thymus showed starry-sky appearance. Caecal tonsils showed moderate lymphoid cell depletion. Moderate plasma cell depletion was observed in the Harderian gland.

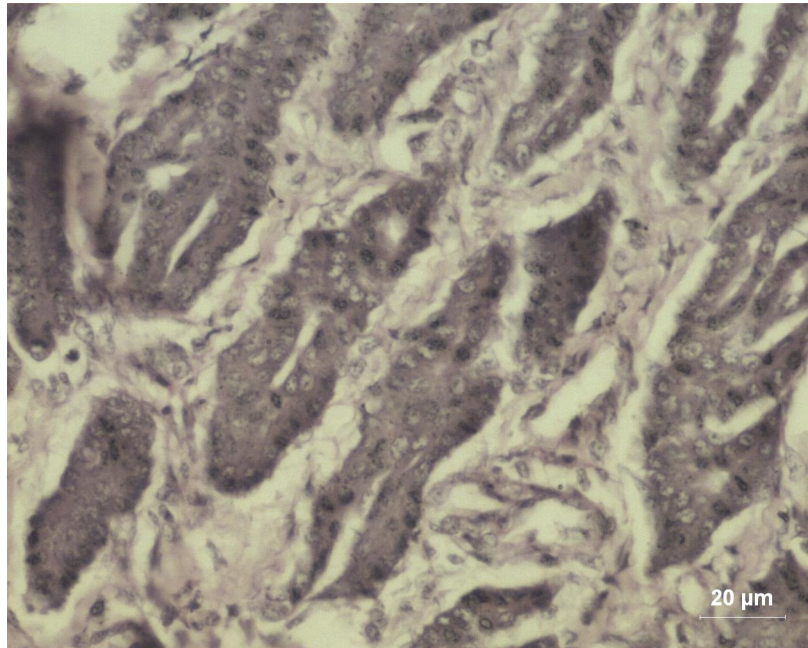
**Figure 4**

PA 20 ppm Group- Gizzard - Defective kaolin formation Scale Bar H&E 100μm

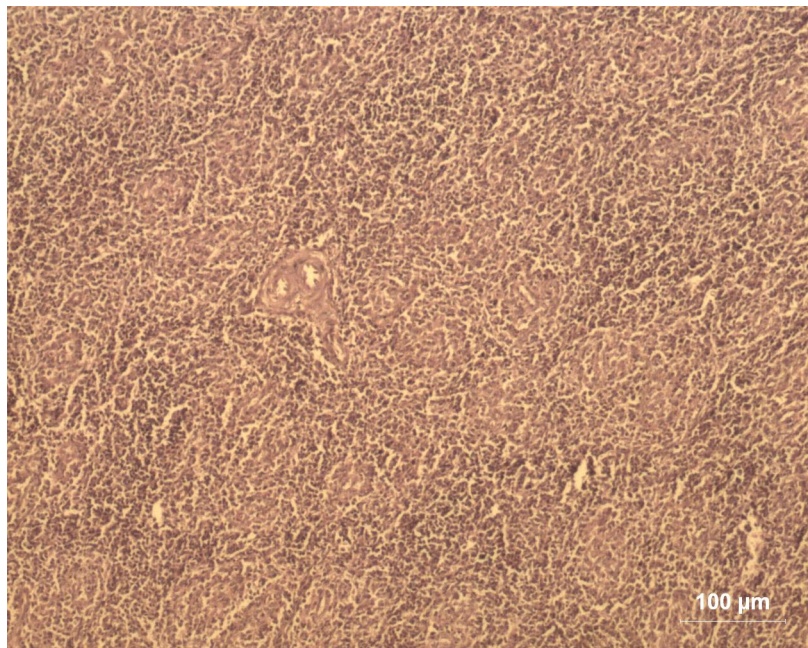
The PA+MOS fed group birds showed bile duct hyperplasia (Figure 5). Crop mucosa showed focal hyperplastic changes. Proventriculus showed mucosal necrosis with mononuclear cell infiltration. Two birds showed proventricular glandular necrosis. Gizzard showed defective kaolin formation (Figure 6) with two cases showing bacterial colonies (Plate 15a). Three cases showed hyperplastic changes in the glandular mucosal epithelium and mild interglandular fibrosis. Duodenal mucoasa revealed broader villi and partial necrosis with catarrhal changes. Spleen revealed moderate lymphoid cell depletion (Figure 7). Caecal tonsils revealed mild lymphoid cell depletion (Figure 8).

**Figure 5**

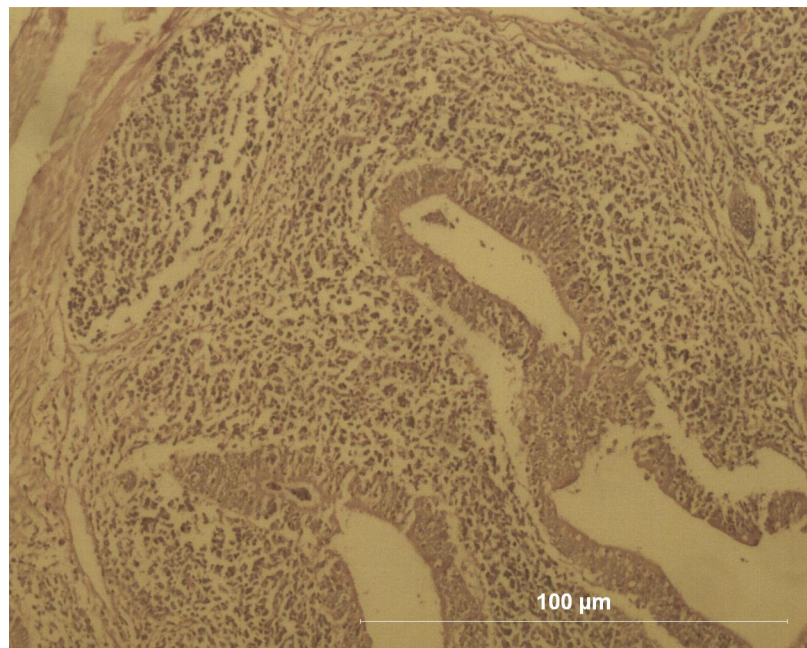
PA 20 ppm + MOS (0.05%) Group- Bile duct hyperplasia Scale Bar H&E 20μm

**Figure 6**

PA 20 ppm + MOS (0.05%) Group - Gizzard - Defective kaolin formation Scale Bar H&E 20μm

**Figure 7**

PA 20 ppm + MOS (0.05%) Group - Spleen-Moderate lymphoid cell depletion Scale Bar H&E 100μm

**Figure 8**

PA 20 ppm + MOS (0.05%) Group - Caecal tonsils -Mild lymphoid cell depletion Scale Bar H&E 100µm

DISCUSSION

MOS (0.05%)

Broiler chicken fed with MOS alone in the feed revealed toxic changes in the liver and kidneys which were reflected in the reduced serum albumin and elevated BUN values. Further, spleen and bursa of Fabricius showed mild to moderate lymphoid cell depletion in the MOS treated birds. However, there was no impairment of humoral and cell mediated immunity. No such reports were available on the pathological changes in MOS feeding. Though the body weight gain of MOS group was not significantly different from that of the control group, there was 10 per cent decrease in the body weight gain of MOS alone fed group when compared to the control group at the end of 28 days experimental period. Further, the MOS alone fed birds consumed 12 per cent more feed than that of the control group to gain 10 per cent less weight than that of the control birds. This will affect the cost: benefit ratio of the broiler chicken.

Penicillic acid (20 ppm)

The birds fed 20 ppm penicillic acid toxin showed more toxic changes than the 15 ppm fed group of the previous penicillic acid toxicity trial. The body weight gain of penicillic acid group was 49 per cent less than that of the control group. The birds consumed almost equal to that of control group to

gain 49 per cent less weight than that of the control birds.

PA+MOS

Concurrent feeding of PA and MOS could reverse the effect of penicillic acid on serum ALT, triglycerides, VLDL, GPx and partially alleviate the penicillic acid effects on serum glucose, uric acid and CMI values. Barring the above marginal beneficial effects, the addition of MOS could not alleviate other toxic effects of penicillic acid, more importantly, the economic trait of body weight gain. No significant differences were found between the penicillic acid and PA+MOS groups for body weight gain. There was a marginal improvement of six per cent body weight gain in the PA+MOS group over the penicillic acid group and to achieve this six per cent body weight gain, the birds has consumed five per cent more feed than that of control birds. Further, the LDL values of PA+MOS group significantly increased than that of other groups. Thus the study on the efficacy of MOS in alleviating the toxicity due to penicillic acid did not show any encouraging results barring improvements in the serum ALT, VLDL and GPx values. Moreover, MOS incorporation to minimize the toxic effects of penicillic acid did not improve the growth performance of the birds. The above results clearly suggested that the MOS had no impact in alleviating the toxic effects of penicillic acid which could very well be appreciated by persistent

lesions almost in all the organs studied in the PA+MOS treated group. No such studies were carried out earlier with MOS versus penicillic acid. However, beneficial effects of MOS against other mycotoxins like aflatoxin, ochratoxin and T-2 toxin have been reported (Manoj KB and Devegowda G, 2000; Raju MVLN and Devegowda G, 2000; Aravind KL et al. 2003).

CONCLUSION

Inclusion of toxin binder MOS at 0.05 per cent level did not alleviate the penicillic acid toxicity (20 ppm) in broiler chicken. MOS and gingerol alone produced toxic changes in broiler chicken.

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