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ALLEVIATIVE EFFECT OF GINGEROL ON CELL MEDIATED AND HUMORAL IMMUNITY AND IMMUNE ORGANS AGAINST PENICILLIC ACID MYCOTOXICOSIS IN BROILER CHICKENS

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ABSTRACT

80 days-old broiler chicks obtained, 72 were randomly allotted to six groups of 12 chicks each with diets of [(T1-control, T2- Gingerol (0.1%), T3- Gingerol (0.2%), T4- Penicillic acid (15 ppm), T5- Penicillic acid (15 ppm) + gingerol (0.1%), T6- Penicillic acid (15ppm) + gingerol (0.2%)] from 0 to 28 days of age. Remaining eight chicks were used as no toxin, no gingerol and no vaccine group (T7). Two birds from each group were sacrificed on 7th, 14th, 21st and 28th day of age for Cell Mediated Immunity (CMI) assay. Remaining birds were sacrificed (28th day) to study the effect on growth, pathological changes in lymphoid organs and immune status. There was a highly significant ($P<0.01$) decrease in the splenic lymphocyte stimulation index values of T4 and T5. Highly significant ($P<0.01$) increase in the CMI was observed in T6 group when compared to T4 group. Histopathological examination in the T4 group revealed mild to moderate lymphoid cell depletion and mild to moderate lymphoid cell depletion in the bursa of Fabricius. T2 group revealed mild medullary lymphoid cell depletion in a few follicles, lymphocytolysis and apoptosis in the bursa of Fabricius, thinning of cortex in thymus and plasma cell depletion in Harderian gland. T5 group revealed moderate lymphoid cell depletion in the bursa of Fabricius. T6 group revealed cystic changes in the follicles of the bursa of Fabricius. Inclusion of gingerol at 0.2% level alleviated the effect on cell mediated immunity against penicillic acid mycotoxicosis (15 ppm) in broiler chickens.

KEYWORDS: *Gingerol, Penicillic acid toxicity, Cell mediated immunity, Humoral 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, 1967; 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). 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 lowered levels of total lipids (Pandiyan 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).*Zingiber officinale Roscoe* commonly known as ginger (*Zingiberaceae*) is cultivated commercially in India, China, South East Asia, West Indies, Mexico and other parts of the world. It is consumed world wide as a spice and flavouring agent and is attributed to have many medicinal properties (Nazam Ansari M et al., 2006). The British herbal compendium reported its actions as carminative, anti-emetic, spasmolytic, peripheral circulatory stimulant, anti-inflammatory (Bhandari U et al., 2003) and antioxidant (Bradley PR.1992; Jitoe A et al.1992; Krishnakanth TP and Lokesh BR, 1990; Reddy AC and Lokesh BR, 1992). [7, 11, 14, 20]. Limited information is available on the hepatoprotective activity of ginger rhizome (Hikino H et al.1985; Shirwaikar A et al. 1992; Sohini YR and Bhatt RM,1996; Sohini YR et al. 1995). Ginger prevents hepatotoxicity by reducing hepatic injury, exhibiting membrane stabilizing and antioxidant properties (Bhandari U et al., 2003).The objective of the present study to find out the alleviative effect of gingerol on immuniy against penicillic acid mycotoxicosis in broiler chickens.

MATERIALS AND METHODS

Preparation of fungal culture

The *Penicillium cyclopium* NRRL 1888 culture was obtained from the National Center for Agricultural Utilization Research, Microbial Genomics and Bioprocessing Research Unit, 1815 N University Street, Peoria, Illinois 61604, USA. The *P. cyclopium* NRRL 1888 was subcultured on potato dextrose agar at 10 days interval (Ciegler A et al. 1972). The penicillic acid toxin was produced on maize (LeBars J. 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 Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), Directorate of Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai–600 051,India. The *P. cyclopium* NRRL 1888 subcultured on potato dextrose agar and the culture material yielded 20–80 ppm penicillic acid.

Vaccination of the birds

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

Preparation of gingerol

Ginger soft SCF (Super Critical Fluid) extracts 20 per cent contains 6-gingerol (65-70%), 8-gingerol (22-25%) and 10-gingerol (7-10%) was obtained from M/s. Sami Labs, 19/1 &19/2, I main, II Phase, Peenya Industrial Estate, Bengaluru, India. Powdered maize culture material containing known amounts of penicillic acid were incorporated into the toxin free diet, so that the diet contained 15 ppm of penicillic acid. Gingerol was added at the levels of 0.1 and 0.2 per cent in the diet.

Experimental design

Out of 80 days-old broiler chicks obtained, 72 were randomly allotted to six groups of 12 chicks each. Remaining eight chicks were used as no toxin, no gingerol and no vaccine group. The birds were fed with following diets from 0 to 28 days of age. Two birds from each group were sacrificed on 7^{th}, 14^{th}, 21^{st} and 28^{th} day to study the CMI of the birds. On 28^{th} day of trial, remaining birds were sacrificed to study the haematobiochemical alternations, pathological changes in different organs and immune status.

Groups	No. of birds
Control	12
Gingerol (0.1%)	*12*
Gingerol (0.2%)	12
Penicillic acid (15 ppm)	12
Penicillic acid (15 ppm) + gingerol (0.1%)	12
Penicillic acid (15ppm) + gingerol (0.2%)	12
No toxin, no gingerol, no vaccine	8

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 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 μm thickness and stained by haematoxylin and eosin (H&E) for histopathological examination (Bancroft JD, Gamble G, 2008).

Humoral immunity

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

Cell mediated immunity

Cell mediated immunity Reynolds DL, Maraqa AD, 2000 was followed with slight modifications

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

Fungal culture

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 velvetty 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.

Pathology

Grossly, no changes were observed in the lymphoid organs. Penicillic acid toxin (15 ppm) fed birds showed pale and enlarged or yellowish discolouration of the liver.

Histopathology

Histopathological examination in penicillic acid fed group revealed mild to moderate lymphoid cell depletion as shown in Fig.1a and reticulum cell hyperplasia in the spleen and mild to moderate lymphoid cell depletion and occasionally lymphocytolysis in the bursa of Fabricius as shown in Fig.1b. Gingerol (0.1%) group revealed mild medullary lymphoid cell depletion in a few follicles, lymphocytolysis and apoptosis in the bursa of Fabricius as shown in Fig.1c, thinning of cortex in thymus as shown in Fig.1d and plasma cell depletion in the Harderian gland as shown in Fig.1e. Gingerol (0.2%) group revealed no microscopical changes. Penicillic acid (15ppm) + gingerol (0.1%) fed group revealed moderate lymphoid cell depletion in the bursa of Fabricius as shown in Fig. 1f. Penicillic acid (15ppm)+gingerol (0.2%) fed group revealed cystic changes in the follicles of the bursa of Fabricius as shown in Fig.1g and other lymphoid organs showed no change.

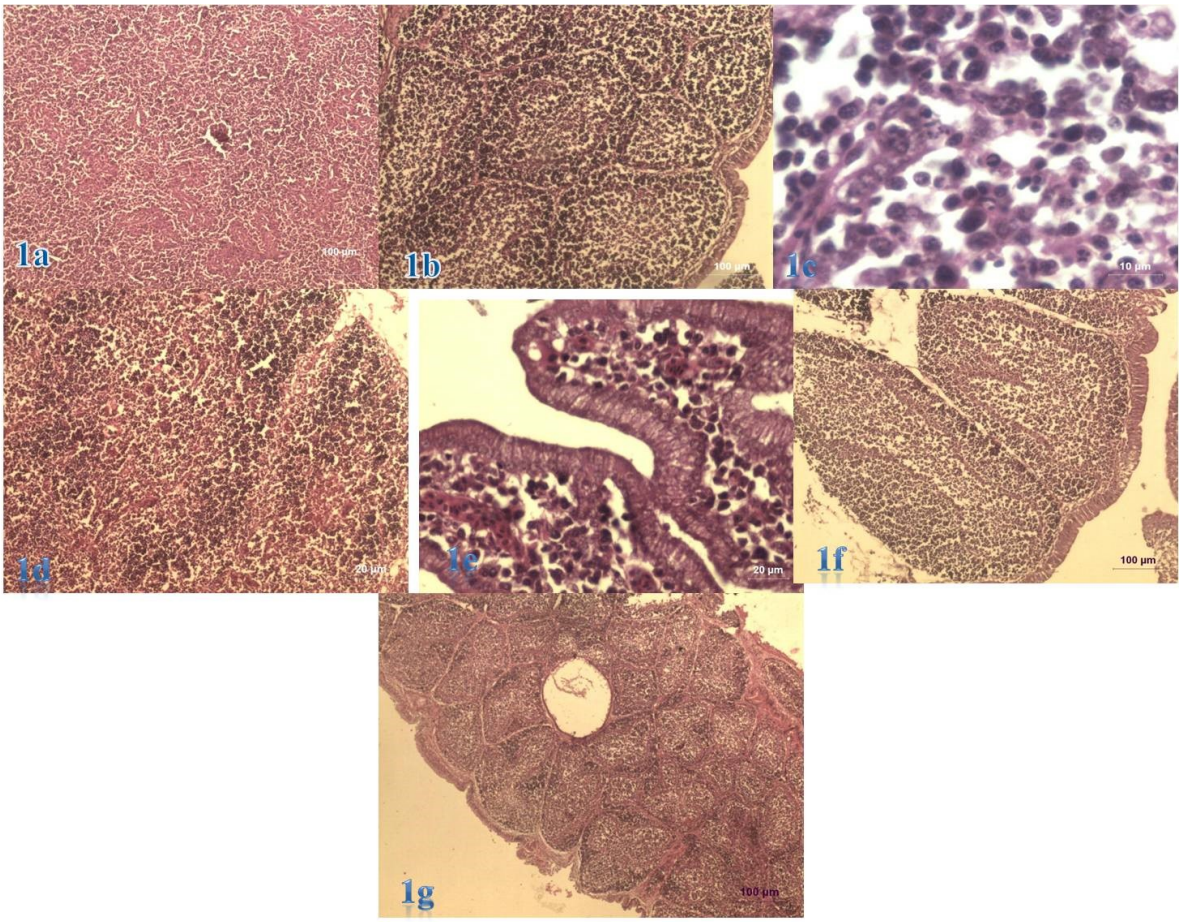

Figure.1

a. ***Broiler chicken-Penicillic acid (15 ppm)- Spleen-mild to moderate lymphoid cell depletion H&E scale Bar 20 µm*****, b.** ***Broiler chicken- Penicillic acid (15 ppm)- Bursa of Fabricius- Mild to moderate lymphoid cell depletion H&E scale Bar 100 µm*****, c.** ***Broiler chicken- Gingerol (0.1%)- Apoptosis in bursa of Fabricius H&E scale Bar 10 µm*****, d.** ***Broiler chicken-Gingerol (0.1%)- Thymus- Thinning of cortex H&E scale Bar 20 µm*****, e.** ***Broiler chicken-Gingerol (0.1%)- Harderian gland- plasma cell depletion H&E scale Bar 20 µm*****, f.** ***Broiler chicken- Penicillic acid (15ppm) + gingerol (0.1%) – Bursa of Fabricius Moderate lymphoid cell depletion H&E scale Bar 100 µm*****, g.** ***Broiler chicken- Penicillic acid (15ppm)+ gingerol (0.2%)-Bursa of Fabricius- Cystic changes in the follicles H&E scale Bar 100 µm***

Humoral immunity

No significant difference was observed between the control and other groups for NDV antibody titres as shown in Table 1.

Table 1

Mean (± SE) antibody titre of NDV of broiler chicks fed gingerol against penicillic acid

Groups	NDV (log^2)
Control (0 ppm)	2.908 ± 0.114
Gingerol (0.1%)	2.398 ± 0.127
Gingerol (0.2%)	2.836 ± 0.229
PA (15 ppm)	2.703 ± 0.164
PA (15 ppm) + Gingerol (0.1%)	2.442 ± 0.060
PA (15 ppm) + Gingerol (0.2%)	2.889 ± 0.124

Cell mediated immunity

Mean ± SE splenic lymphocyte stimulation index values of broiler chicken fed gingerol against penicillic acid are presented in as shown in Table 2. There was a highly significant decrease in the splenic lymphocyte stimulation index values of penicillic acid and PA+gingerol (0.1%) groups when compared to the vaccine+no toxin group. Highly significant ($P<0.01$) increase in the cell mediated immunity was observed in PA+gingerol (0.2%) group when compared to the PA group as shown in Fig. 2.

Table 2
Mean (± SE) splenic lymphocyte stimulation index of broiler chicks fed gingerol against penicillic acid

Groups	I week	II week	III week	IV week	Overall means
No vaccine + No toxin	-0.234 ± 1.342	-0.286 ± 0.216	-0.308 ± 0.092	-0.341 ± 0.091	$-0.292^{z} \pm 0.123$
Vaccine + No toxin	0.575 ± 0.260	0.280 ± 0.110	0.794 ± 0.140	0.638 ± 0.460	$0.572^{xy} \pm 0.250$
Gingerol (0.1%)	0.225 ± 0.05	0.510 ± 0.120	0.253 ± 0.130	0.307 ± 0.020	$0.323^{yz} \pm 0.11$
Gingerol (0.2%)	0.762 ± 0.60	0.567 ± 0.540	0.338 ± 0.25	0.516 ± 0.04	$0.546^{xy} \pm 0.34$
Penicillic acid (15 ppm)	0.559 ± 0.34	-0.560 ± 0.09	-0.247 ± 0.07	-0.415 ± 0.01	$-0.166^{z} \pm 0.35$
PA (15 ppm) + Gingerol (0.1%)	-0.390 ± 0.04	-0.497 ± 0.03	0.199 ± 0.06	0.060 ± 0.04	$-0.157^{z} \pm 0.22$
PA (15 ppm) + Gingerol (0.2%)	1.715 ± 1.17	0.381 ± 0.03	1.262 ± 1.25	0.509 ± 0.38	$0.967^{x} \pm 0.78$

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

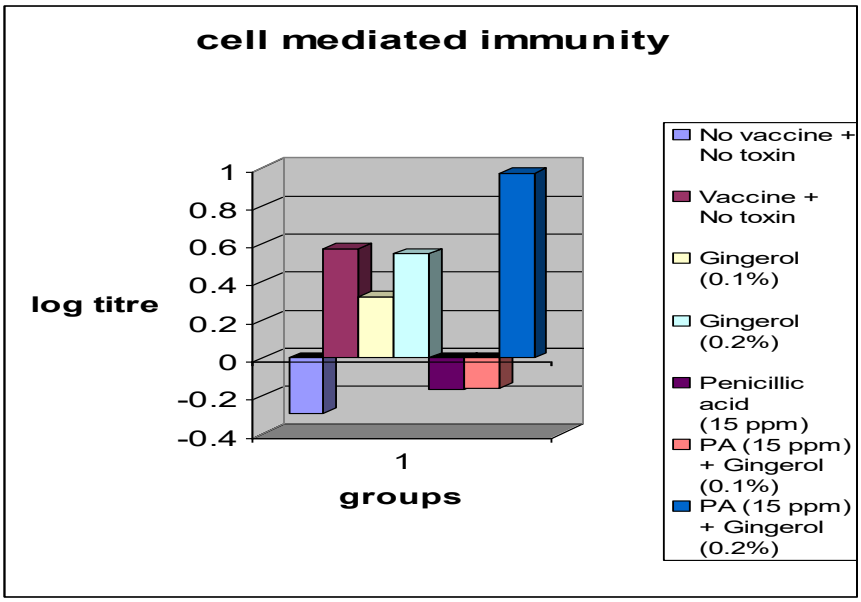

Figure.2
Mean (± SE) splenic lymphocyte stimulation index of broiler chicks fed gingerol against penicillic acid

DISCUSSION

No significant difference was observed between the control and other groups for NDV antibody titres. There was a highly significant (P<0.01) decrease in the splenic lymphocyte stimulation index values of penicillic acid and PA+gingerol (0.1%) groups when compared to the vaccine+no toxin group. Highly significant (P<0.01) increase in the cell mediated immunity was observed in PA+gingerol (0.2%) group when compared to the PA group. Penicillic acid fed group revealed mild to moderate lymphoid cell depletion and reticulum cell hyperplasia in the spleen and mild to moderate lymphoid cell depletion and occasionally lymphocytolysis were also observed in the bursa of Fabricius in 15 ppm PA fed group. Mild medullary lymphoid cell depletion in a few follicles with lymphocytolysis and apoptosis in the bursa of Fabricius, thinning of cortex in the thymus and plasma cell depletion in the Harderian gland in gingerol (0.1%) level. Gingerol (0.2.%) group revealed no microscopical changes Moderate lymphoid cell depletion in the bursa of Fabricius in PA+gingerol (0.1%) group. A cystic change in the bursa of Fabricius was observed in PA+gingerol (0.2%) group and other lymphoid organs showed no changes. Inclusion of gingerol at 0.2% level alleviated the effect on cell mediated immunity against penicillic acid mycotoxicosis (15 ppm) in broiler chickens. Inclusion of gingerol at 0.2% level alleviated the effect on pathological changes in lymphoid organs against penicillic acid mycotoxicosis (15 ppm) in broiler chickens. Limited information is available on the hepatoprotective activity of ginger rhizome (Hikino H et al.1985; Shirwaikar A et al. 1992; Sohini YR and Bhatt RM,1996; Sohini YR et al. 1995).

CONCLUSION

Inclusion of gingerol at 0.2% level alleviated the effect on cell mediated immunity against penicillic acid mycotoxicosis (15 ppm) in broiler chickens

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