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ROLE OF *TINOSPORA CARDIOFOLIA* FOR THE CONTROL OF MYCOTOXICOSIS IN BROILER CHICKENS

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ABSTRACT

Tinospora cardiofolia (herbal extract) was used to control the experimentally induced mycotoxicosis in broiler chickens. For this purpose experimental chicks were fed on mycotoxins contaminated feed. Where as 30 chicks (group Z) were kept as control. Half of the mycotoxicated chicks (Group X) were given *Tinospora cardiofolia* in feed @ 100gm/50kg feed for 11 days while others were kept untreated (Group Y). In Group X and Z dropping was normal and in Group Y there was loose dropping and chickens were sluggish. During post-mortem examination congestion in intestine and hemorrhages on various parts of intestine were observed. There were also changes in color of liver. In Group Y the color of livers was yellow while in Group X and Z the livers were brown. Blood sample of 5 chicks from each group were collected at the interval of 5 days. Hematological parameters (ESR, Hb. %, TLC and DLC) and weight of internal organs (liver, heart and spleen) of chickens were determined and differences were observed in mycotoxicated untreated chicks (Group Y).

Key-words: Poultry, mycotoxicosis, aflatoxicosis, *Tinospora cardiofolia*, broilers

INTRODUCTION

The humid and hot weather conditions in Pakistan favor the fungus growth, which released the metabolites known as mycotoxins, in most of the cereal crops and stored ingredients used in poultry feed. This contaminated feed affects the commercial poultry by lowering growth rate, feed conversion efficiency, suppressing reproductive potential and immune system. Ingestion of high level toxins results in acute mortality (Shane, 1999). The presence of any of the mycotoxin in feed, even in trace amounts should be considered as a sign of defectiveness. Further more, the residues of these toxins accumulates in meat and eggs, creating serious human health problems (Khan *et al*., 1989).

Economic losses in livestock industry due to mycotoxin contamination of feed supply have been estimated in millions of dollars (Barney, 1998). Mycotoxicosis cause heavy losses to poultry by a 10% drop in egg production. It is experimentally proved that the addition of 7.5 ppm aflatoxin in broiler starter reduces the growth rate by 38% from 450 gm to 278 gm at 21 days of age. Similarly the dietary level of 4 ppm aflatoxin also causes up to 5% mortality through the first 3 weeks of brooding period (Shane, 1999). So utilization of herbal extracts is of considerable interest, as they denature the mycotoxins and reduce their absorption. In the present experiment *Tinospora cardiofolia*, a herb was used against mycotoxicosis @ 2 gm/ kg feed.

MATERIALS AND METHODS

Leaves, shoot & root of *Tinospora cardiofolia* were collected and biomass was cut into small pieces then dried them in shady place. When the material was dried completely, it was added in 70% ethanol and 30% water solution and mixed it in rotary shaker up to 12 hrs. After shaking the solution was filtered and filtrate was stored while the debris were again added in 70% ethanol and 30% water solution and mixed in rotary shaker up to 12 hrs, this process was repeated thrice. At the end collected filtrate was evaporated overnight at room temperature on rotary shaker. Moisture contents of the extract were evaporated in hot air oven slowly and collected the extract in dry form. A total of ninety chicks were used in this experiment. The temperature of the shed was kept at 37 ºC and than gradually decreased weekly. The chicks were kept under standard and hygienic experimental conditions and were vaccinated against ND, IBD and Hydropericardium Syndrome as per schedule. When the chicks were of 21 days old, they were randomly divided into three groups X, Y and Z comprising of 30 chicks in each group. The birds of group X and Y were kept on mycotoxins contaminated feed having known level of toxins for 12 days. From the next day of intoxication group X was treated with extract of *Tinospora cardiofolia* @ 2 gm/kg of feed for 11 days. While group Z was kept as control. Before the start of experiment contaminated and normal feed samples were collected from field and were sent to Bio-Min laboratory Singapure for mycotoxin analysis, objective was to feed the known

levels of mycotoxins to all groups during experiment. Sampling was done by slaughtering the 5 chicks from each group at interval of five days to study the following parameters.

1. Weekly weight gain. 2. Condition of dropping.
3. Hematological parameters (ESR, Hb %, TLC & DLC).
4. Gross lesions & weight of internal organs (Liver, Spleen & Heart).

The data of experimental groups was tested by analysis of variance and differences in various treatment groups were worked out by using LSD test at ($P<0.05$).

RESULTS AND DISCUSSION

Level of mycotoxins in the feed samples

The birds of group X and Y were intoxicated with mycotoxins contaminated feed. Almost all the major toxins were given to the chicks of group X&Y in high doses as shown in the sample No. 2 of the Table 1, while sample No.1 was served to control birds. It was also found that mycotoxins were present in trace amounts in normal feed sample.

Table 1. Levels of Mycotoxins in feed (ug/kg) of the experimental chickens.

Mycotoxins	Sample. No. 1	Sample. No. 2
Aflatoxin B1	12	70
Aflatoxin B2	6	65
T-2 toxin	60	600
HT –2 toxin	70	540
Diacetoxyscripenol	124	515
Deoxynivalenol	165	1250
Acetyle-deoxynivalenol	240	1460
Nivalenol	239	1230
Fumonisin	1149	>4000
Zearalenon	95	426
Ochratoxin-A	14	93

Weekly weight gain:

From the table No. 2 it is clear that before the administration of mycotoxins, no significant difference was observed in the live body weight of all the three experimental groups up to the 22 days. After mycotoxicosis, the growth rate was significantly ($P<0.05$) decreased in the birds of group Y (Table 2). The decrease in body weight was resulted due to the decreased feed utilization and feed conversion efficiency (Rizvi *et al*., 1992).

No significant difference in the body weight of group X and Z was observed during the whole experimental period.

Table 2. Weekly weight gain (grams).

Age	Group X (g)	Group Y (g)	Group Z (g)	Prob.
8 days	151.0 ± 1.73	153.7 ± 3.21	155.0 ± 5.00	NS
15 days	356.7 ± 5.77	356.0 ± 10.15	356.3 ± 5.51	NS
22 days	755.0 ± 5.00	754.0 ± 6.93	751.3 ± 3.21	NS
29 days	1048.3 ±27.54	983.7 ± 7.77	1035.0 ±21.79	0.0045**
36 days	1410.7 ±10.07	1373.3 ±10.42	1398.3 ± 7.64	0.0078**
43 days	1920.0 ± 12.11	1850.0 ± 3.21	1910 ± 11.89	0.0054**

NS = Non significant; * = Significant; ** = Highly significant

Condition of Droppings

Condition of droppings in each group was noted and shown in Table 3. Khan *et al*., (1989) also noted six percent less feed intake for every milligram (ppm) of toxins. Abnormal feathering in the birds was due to disturbance in protein synthesis (Shane, 1999). Enteritis due to mycotoxins was also reported by Balachandran *et al*., (1998). In contrast, mycotoxin induced birds that were treated with herbal extract were healthy, having few loose dropping.

Table 3. Condition of Droppings.

Group X	Group Y	Group Z
In this group very little loose dropping was observed. Birds were looking healthy and feathers were neat and clean. Feed intake was normal and birds were active.	In this group loose dropping was at its peak level, feed intake was very slow, although they eat complete feed but very late. Birds were sleepy, feathers were dirty and ruffled and comb was pale-yellow.	In this group no loose dropping was observed. Birds were healthy, comb was reddish and feathers were shining. Birds were normal and feed intake was normal.

Table 4. ESR, Hb and TLC.

Age	Hematological tests	Group X	Group Y	Group Z	Prob.
25 days (1st sampling)	ESR (mm/h)	1.5 ± 0.16	1.3 ± 0.28	1.6 ± 0.32	0.0238*
	Hb %	13.9 ± 0.85	12.64 ± 0.63	13.5 ± 0.32	0.0252*
	TLC (1000/mm^3)	10.2 ± 0.96	7.04 ± 0.72	9.76 ± 1.19	0.0005**
30 days (2nd sampling)	ESR	1.7 ± 0.44	1.4 ± 0.28	1.8 ± 0.26	0.0221*
	Hb %	13.6± 0.38	12.14 ± 1.19	13.42 ± 0.29	0.0176*
	TLC (1000/mm^3)	10.5 ± 0.66	9.24 ± 0.81	10.72 ± 0.65	0.0132*
35 days (3rd sampling)	ESR (mm/h)	1.7 ± 0.52	1.3 ± 0.19	1.5 ± 0.17	0.1573^{NS}
	Hb %	13.54 ± 0.9	12.42 ± 1.01	13.54 ± 0.22	0.0703^{N}
	TLC (1000/mm^3)	10.42 ±2.89	7.90 ± 1.99	10.64 ± 1.84	0.0425*
39 days (4th sampling)	ESR (mm/h)	1.8 ± 0.26	1.3 ± 0.21	1.5 ± 0.07	0.0086**
	Hb %	13.38 ±0.33	12.24 ± 1.03	13.46 ± 0.33	0.0210*
	TLC (1000/mm^3)	12.46 ±2.40	9.46 ± 1.84	11.1 ± 3.27	0.2230^{NS}

NS = Non significant; * = Significant; ** = Highly significant

Haematology

Results of hematology are shown in Table 4, which are similar to Haq *et al*. (1994), who also observed low ESR level during aflatoxicosis in broiler chickens. The results showed that toxins considerably reduced the level of hemoglobin in diseased birds. Mycotoxins especially aflatoxin B_1 cause significant reduction in hemoglobin values so it matches with the findings of Singh *et al*. (1992). Leukocytes are a source of local immunity that becomes active at the time of infection. The mean leukocyte values in diseased birds were also low than the normal values as studied by Athar and Ahmad (1996). The decrease in leukocyte values was due to decrease in lymphocyte and monocyte numbers, caused by the ingestion of zearalenene and ochratoxins, this was also observed by Frederic *et al*. (1991).

Results of Differential Leukocytic Count (DLC %) are shown in Table 5, in which basophil number was significantly ($P<0.05$) decreased in Group Y throughout the experimental period except on the day 35. Decreased number of basophil was also observed by Haq *et al*. (1994) in the chickens suffering from aflatoxicosis. Neutrophils are the first cells to arrive at the site of inflammation and their number increase in the presence of infections (Kuby, 1996). Increased number of neutrophils was also observed in diseased birds during the experiment. Mycotoxicosis, especially the aflatoxicosis directly hit the immune system (Frederic *et al*., 1991). During the experiment decreased number of lymphocyte was found in diseased birds, which is aligned with the findings of Haq *et al*. (1994). Monocyte number decreased in the birds suffering from mycotoxicosis as compared to treated and control groups. Our findings are in line with the findings of Haq *et al*. (1994), he also observed decreased monocyte number during aflatoxicosis in broilers.

Weight of Internal Organs:

Weights of internal organs are shown in Table 6, which showed that due to Mycotoxins the weight of liver increased. An increasing pattern in the liver weight was in line with the findings of Kubena *et al*., (1997), who also observed increased liver weight in broiler chickens, as a result of T-2 toxin, fumonisin B1 and deoxynivalenol intoxication. During mycotoxicosis we found that the size and hence the weight of spleen increased. Increased

spleen weight was also reported by Edrington *et al*., (1997). Heart weight was significantly increased in diseased birds while control and treated birds had normal heart weight. Increased heart weight was also reported in broiler chickens during mycotoxicosis by Ledoux *et al*., (1995). Increased in size and weight of heart was also found by Giroir *et al*., (1991). So our findings are in line with their reports.

Table 5. Differential Leukocytic Count (%).

Age	Type of WBC	Group X	Group Y	Group Z	P
25 days	Eosinophils	01.50 ± 0.64	02.18 ± 0.49	01.84 ± 0.23	0.1273^{NS}
	Basophils	01.82 ± 0.40	01.00 ± 0.68	1.62± 0.33	0.0132**
	Neutrophils	26.40 ± 2.13	30.76 ± 2.01	27.12 ± 0.63	0.0039**
	Lymphocytes	60.30 ± 1.38	56.10 ± 1.32	59.18 ± 1.27	0.0009**
	Monocytes	10.58 ± 0.81	09.04 ± 1.22	10.44 ± 1.08	0.0731^{NS}
30 days	Eosinophils	1.18 ± 0.43	2.18 ±0.50	1.86 ±0.18	0.0055**
	Basophils	1.88 ± 0.26	0.54 ± 0.48	1.64 ±0.23	0.0000**
	Neutrophils	26.32 ±1.78	30.02 ± 2.75	26.33 ±1.11	0.3360^{NS}
	Lymphocytes	59.52± 1.52	58.16 ± 2.79	59.72 ±0.64	0.1709^{NS}
	Monocytes	11.1 ± 0.91	09.10 ± 1.62	10.44 ± 1.25	0.0826^{NS}
35 days	Eosinophils	0.92 ±0.15	2.3 ± 0.44	01.86 ± 0.11	0.0000**
	Basophils	1.80 ±0.34	1.72 ± 0.53	01.76 ± 0.27	NS
	Neutrophils	28.0 ±2.67	30.72 ± 2.82	26.94 ± 1.01	NS
	Lymphocytes	60.18 ±2.35	57.2 ± 1.59	59.08 ± 1.16	0.0592*
	Monocytes	9.10 ± 0.56	8.06 ± 1.84	10.34 ± 0.52	0.0053**
39 days	Eosinophils	1.06 ±0.30	2.14 ± 0.45	01.80 ± 0.25	0.0010**
	Basophils	1.0 ±0.41	1.98 ± 0.31	1.18 ± 0.40	0.0032**
	Neutrophils	27.68 ±2.08	26.46 ± 0.54	27.32 ± 1.16	NS
	Lymphocytes	60.54 ± 1.65	58.00 ±1.50	59.22 ± 1.06	0.0474*
	Monocytes	9.72 ± 0.87	11.42 ± 1.51	10.48 ± 1.11	0.1202^{NS}

NS = Non significant; * = Significant; ** = Highly significant

Table 6. Weight of internal organs (grams).

Age	Organ	Group X (g)	Group Y (g)	Group Z (g)	Prob.
25 days (1st sampling)	**Liver**	25.54 ± 1.17	27.93 ± 1.68	25.84 ± 1.16	0.0050**
	Spleen	0.61 ± 0.16	0.74 ± 0.22	0.49 ± 0.16	0.1171^{NS}
	Heart	3.99 ± 0.36	4.59 ± 0.82	4.40 ± 0.94	NS
30 days (2nd sampling)	**Liver**	33.83 ± 3.70	36.02 ± 6.39	29.50 ± 4.54	0.1544^{NS}
	Spleen	0.87 ± 0.15	1.07 ± 0.33	0.99 ± 0.18	NS
	Heart	5.56 ± 0.79	7.43 ± 1.36	6.11 ± 1.01	0.0481*
35 days (3rd sampling)	**Liver**	39.51 ± 5.66	40.79 ± 3.32	32.98 ± 5.96	0.0440*
	Spleen	1.56 ± 0.49	2.18 ± 0.31	1.69 ± 0.35	0.0433*
	Heart	6.62 ± 1.51	7.65 ± 1.00	6.43 ± 0.99	0.0894^{NS}
39 days (4th sampling)	**Liver**	41.80 ± 7.25	45.18 ± 1.67	41.84 ± 7.20	0.0052**
	Spleen	1.88 ± 0.49	2.18 ± 0.91	1.96 ± 0.77	NS
	Heart	8.28 ± 0.93	9.75 ± 0.89	8.42 ± 1.20	0.0787^{NS}

NS = Non significant; * = Significant; ** = Highly significant

Gross lesions of the internal organs

In the present experiment the liver of mycotoxicosis induced birds showed extreme pale color through out the experiment. In contrast the liver of Group X & Z retained the normal reddish brown color. Mycotoxins especially

the aflatoxins interfere with the lipid metabolism, due to which the liver becomes more friable and pale in color with fatty changes (Younus, 1994). During postmortem examination hemorrhages on proventriculus of group Y were observed through out the experiment. Similar pale discoloration of liver and hemorrhages of proventriculus was also observed by Ghosh *et al*., (1991). The intestine of intoxicated birds also had hemorrhages. Uneven thickness on various parts of small intestine was observed in the birds of Group Y at all the sampling days, which might be because of change in pH & proliferation of normal microorganisms in gut (Stephen, 1997).

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