

AMELIORATIVE EFFECT OF *WITHANIA SOMNIFERA* ON GROWTH PERFORMANCE AND PATHOMORPHOLOGICAL ALTERATIONS IN EXPERIMENTALLY INDUCED AFLATOXICOSIS IN BROILERS

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Abstract: An experimental study was carried out on the ameliorative effect of *Withania somnifera* against experimental aflatoxicosis in broilers. The study was evaluated through weekly body weights and patho-morphological changes at the end of trial for 30 days of study period.

Aflatoxicated birds showed reduction in body weight, higher FCR and pathomorphological changes showed increase in absolute weight of liver, kidney and grossly liver was enlarged, pale yellow colored with fragile consistency, occasional necrotic foci and kidneys were enlarged, pale, haemorrhagic with distinct lobulations. Microscopically liver showed vacuolar degeneration, dilated central vein and hepatic sinusoids, fatty changes, bile duct hyperplasia and mononuclear cell infiltration, kidney showed cellular swelling, lungs were congested with inflammatory changes and thickening of alveolar septa, bursa of Fabricius showed depletion of lymphocytes, spleen showed thickening of splenic artery. Intestine revealed desquamation. Findings were suggestive of *W. somnifera* @0.5% of feed ameliorate aflatoxicosis in poultry.

Keywords: Broiler birds, Aflatoxicosis, *Withania somnifera*.

Introduction

Aflatoxin is a secondary metabolite produced predominantly by *Aspergillus flavus* and *A. parasiticus*. This toxin is present worldwide in feed and causes severe economic losses in poultry and livestock industries. Aflatoxin is associated with liver damage, mutagenicity, carcinogenicity, haemorrhages and growth inhibitory effects. In the liver, Aflatoxin molecules are exposed to complex metabolic processes occurring via diverse cytochrome P450-dependent pathways (CYP) (detoxification or bioactivation processes) and lead to form the reactive AFB₁-8,9-epoxide (AFBO) that binds to DNA and other vital macromolecules, causing toxicity, mutation and cancer. It may be due to derangement in DNA transcription which results in the inhibition of protein synthesis. AFB₁ is exclusively likely to cause dose-dependent induction or inhibition of liver mixed-functions, oxygenize activities, which may affect the liver metabolism of endogenous and exogenous substrates. In liver, fat

degeneration and proliferation of biliary ducts induced changes generally seen as the increase in hepatic enzyme activity, coagulopathies and reduction in protein production. In last few decades several studies have been performed using hepatoprotective plants (*Curcuma longa*, *Trifala*, *Thyme Essence*, *Satawari*, *Picrorhiza kurroa*, *Swertia chirata*) for their hepatoprotective action against aflatoxicosis.

Withania somnifera possesses thyroid stimulant, anti-stress, anti-tumor, immunomodulatory, hepatoprotective, anticonvulsant, radio-sensitizing, cardioprotective, hypoglycemic, diuretic, hypocholesterolemic, and anti-oxidant properties. The active principle present in *W. somnifera* is known to alter the oxidative stress markers of the body by reducing the lipid peroxidation and increase the superoxide dismutase (SOD) and catalase activities significantly, thus carrying free radical scavenging property. Considering the current status of aflatoxicosis in particular and also the increasing trend of using herbal medication, the present experimental trial has been conducted to study the ameliorative effect of indigenous herbal plant, *Withania somnifera* root powder @0.5% of feed on experimentally induced aflatoxicosis in broilers.

Materials and Methods

The present study was conducted on ninety six broiler birds. The birds were equally divided into six group's viz. Group I (Healthy control), Group II (Aflatoxin @100 ppb), Group III (Aflatoxin @200 ppb), Group IV (Aflatoxin @100 ppb +*Withania somnifera* @0.5% of feed), Group V (Aflatoxin @200 ppb +*Withania somnifera* @0.5% of feed) and Group VI (*Withania somnifera* @0.5% of feed). After subculturing pure fungal culture of *Aspergillus flavus* on Czapek yeast extract agar medium, it was then inoculated in the partially grinded grains for extraction of Aflatoxin as per the standard method. Then this substrate was grounded to a fine powdered form and this powdered mouldy substrate was then sent to AFAQAL, Namakkal for quantitation of Aflatoxin and the same was mixed with feed as per dose. The birds were sacrificed as per routine procedure by using halal method.

i. Growth performance study

Ten birds from each treatment group were weighed individually at weekly intervals. Also, feed offered to birds and feed that was left uneaten, was recorded weekly in order to calculate FCR.

ii. Absolute organ weight

At 15th and 30th day of age, Liver and kidney were excised, weighed individually.

iii. Gross and Histopathological study

At the time of organ weighing, gross changes, if any, were recorded. Representative tissues were collected in 10% formal saline from above organs and processed for histopathological studies.

Results and Discussion

Body weight and FCR

There was no statistically significant effect of aflatoxin on body weight of broilers up to 4th week of age. Subsequently, there was a non-significant reduction in body weight in aflatoxicated groups as compared to control group. Treatment with Ashwagandha could protect the effects of toxins on body weight. Feed conversion ratio was higher in Group II and III, followed by Groups IV, V, I and VI. In general, herbal feed supplement improved the FCR of broilers better than was the case in their respective control groups. Dietary aflatoxin affects body weight and feed conversion ratio of broilers. Similar, Khan *et al.*(2005); Watts *et al.*,(2003); Denli *et al.*,(2004) and Arab Abousadi *et al.*,(2007) observed minimal to severe growth depressing effect of dietary aflatoxin even at very low level of inclusion which supports the present observation. There was a slight improvement in the body weight of *Withania somnifera* treated birds

Mechanism of decreased body weight effect includes inhibition of RNA, DNA synthesis as well as RNA polymerase activity resulting into reduced protein synthesis which ultimately would reduce the growth. Increased average body wt. and better FCR in Ashwagandha treated group was due to its health restorative activity and general tonic property.

Organ weight

There was a significant increase in absolute weight of liver of broilers receiving aflatoxin whereas feeding of aflatoxin to broilers non-significantly reduced absolute weight of kidney in both intervals of study, *W. somnifera* protects this adverse effect in the aflatoxin fed groups. In this study absolute organ weight was altered due to dietary aflatoxin. The data indicate significant increased in the weight of liver and kidney respectively due to feeding of aflatoxin. The results of experimental aflatoxicosis are in accordance with Maurice *et al.*,(1983), Raju and Devegowda (2000), Arvind *et al.*(2003), Denli *et al.*(2004), Abousadi *et.al.*, (2007). This increase in liver weight could be attributed to increased lipid deposits in liver due to impaired fat metabolism. However, the increase in the weights of kidney might have been attributed to induced nephrotoxicity.

Gross pathology

In aflatoxin-fed Group II and III, had enlarged, pale yellow colored liver with fragile consistency and occasional necrotic foci. Kidneys of birds belonging to aflatoxicated groups were enlarged, pale and haemorrhagic with distinct lobulations, similar with findings of Moregaonkar (2002), Bedre (2008) and Mubarak *et.al.*,(2009). Intensity of gross pathological changes was less in *W. somnifera* aflatoxin treated groups.

Histopathological examination

In birds of group I and VI, sections of liver, kidneys, spleen, lungs, intestine and bursa of Fabricius did not reveal any appreciable histopathological changes on 15th and 30th day of study.

The liver of birds of toxicated groups on microscopic examination showed varied degree of alterations, degenerative changes and vacuolar degeneration along with necrobiosis. Central vein, hepatic sinusoids found to be dilated, fatty changes in hepatic parenchyma, bile duct hyperplasia and mononuclear cell infiltration were noted at places. The liver section in Groups IV and V revealed similar types of change. However, the changes were mild in degree. Similar lesions were noted by Fernandez *et.al.*,(1994), Leudox *et.al.*, (1998) and Moregaonkar (2002) in their studies.

Kidneys of group II and III birds showed vacuolar degeneration, necrobiotic changes and cellular swelling. Kidneys also showed focal lymphoid aggregation along with presence of varying sized cysts at places. There was mild to moderate congestion, and focal to multifocal MNC infiltration in sections of kidneys. Kidneys lesions were mild and represented by degenerative change and mild congestion in group IV and V indicative of restoration of kidney architecture due to addition of *Withania somnifera*. (Khan 2005 and Rathod 2006)

Lungs from aflatoxicated birds revealed congestion and acute inflammatory changes with thickening of alveolar septa. (Chahota 2000).

The bursa of Fabricius on histopathological assessment showed mild depletion of lymphocyte. (Bakshi 1995 and Moregaonkar 2002).

Spleen of birds in aflatoxin fed group II and III showed mild depopulation of lymphocytes and there was thickening of splenic artery, changes were similar with findings of Bakshi (1995).

The intestine of experimental birds belonging to group II and III revealed mild enteritis characterized by desquamation, exfoliation of epithelial linings and inflammatory

cell infiltration. Also, there were degenerative to necrobitotic change in epithelial cells of mucosa. Occasionally, intestinal lumen was with inflammatory exudates. The sections of intestine of experimental birds of group I, IV and V did not showed remarkable histopathological changes.

The histo-architctural studies of various organs from almost treated birds revealed varying degree of alterations. The changes noted in present study were dose dependent. The histopathological changes in birds of group IV and V were comparatively low profiled which might have resulted due to supplementation of *Withania somnifera*.

Table I: Effect of Ashwagandha on Aaverage weekly body weight in aflatoxicated birds

Group	Weeks			
	1 st	2 nd	3 rd	4 th
I	154.00 ^a ±1.08	441.40 ^a ±5.28	775.68 ^a ±14.61	1190.80±37.35
II	153.50 ^a ±0.95	420.38 ^a ±6.80	751.40 ^a ±14.00	1127.80 ^a ±24.38
III	151.00 ^a ±0.92	410.20 ^a ±3.82	741.28 ^a ±14.00	1121.50 ^a ±24.13
IV	154.40 ^a ±1.16	430.60 ^a ±4.40	759.80 ^a ±6.56	1163.90 ^a ±20.56
V	152.40 ^a ±4.23	428.20 ^a ±8.99	745.40 ^a ±18.37	1131.20 ^a ±21.06
VI	155.60 ^a ±1.53	443.89 ^a ±15.74	778.88 ^a ±10.54	1197.80 ^a ±17.99

Table II: Mean of absolute organ weights in broilers from various treatment groups

Age (days)	I	II	III	IV	V	VI	CD
Absolute weights of liver (g)							
15 th	12.23 ^d ±0.03	14.32 ^{bc} ±0.48	16.20 ^a ±0.42	13.53 ^{cd} ±0.65	15.07 ^{ab} ±0.52	12.25 ^a ±0.32	1.40
30 th	27.55 ^c ±0.69	29.35 ^b ±0.27	31.00 ^a ±0.67	28.60 ^{bc} ±0.38	30.10 ^{ab} ±0.69	27.86 ^c ±0.62	1.64
Absolute weights of kidneys (g)							
15 th	3.40 ^a ±0.11	3.59 ^a ±0.07	3.69 ^a ±0.09	3.50 ^a ±0.08	3.52 ^a ±0.10	3.42 ^a ±0.13	NS
30 th	8.00 ^a ±0.29	8.23 ^a ±0.06	8.49 ^a ±0.06	8.36 ^a ±0.11	8.20 ^a ±0.22	8.06 ^a ±0.25	NS

Summary and Conclusion

An experimental study was conducted to note ameliorative effects of *Withania somnifera* on experimentally induced aflatoxicosis in broilers at Department of Veterinary Pathology, COVAS, MAFSU, Parbhani (M.S).

All the birds were carefully examined for growth performance & pathomorphological alterations caused due to aflatoxicosis and effect of *Withania somnifera* at 15th and 30th day of experiment against induced aflatoxicosis. The experimental birds of group II & VI did not show any considerable changes in weekly body weight, FCR values & pathological alterations at study interval period.

In birds of group II & III showed non-significant reduction in weekly body weights than control group. The mean values of FCR numerically higher than the mean values of control group at all intervals of study period. In group IV & V, there was numerical reduction in the body weight & FCR as compared to control group but these values were greater than values of group II & III.

There were no appreciable gross changes noticed in any organs. Histoarchitectural changes in the organs studied were with similar histopathological changes with of comparatively less intensity indicates restoration of organ texture through *Withania somnifera* treatment.

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