RESEARCH ARTICLE

Altered biochemical composition in testis of rabbits during aflatoxicosis

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ABSTRACT

Feeding Aflatoxin-contaminated diet (7.5mg/kg) to young adult New Zealand strain of rabbits for 90 days caused a significant reduction in body weight and weight of testis. Reduction in glycogen (P<0.02) and protein contents (P <0.05). were followed with elevation in lipid (P< 0.001) while sodium, Potassium, magnesium and inorganic Phosphorus contents reduced, calcium concentration was increased in the the testis of aflatoxin fed rabbits. Percent ash content increased in aflatoxin fed rabbits indicating accumulation of inorganic constituents. Among monovalent cations, sodium concentration was comparatively higher than potassium (Na^+/K^+ = 1.62). Ingestion of aflatoxin contaminated diet for 90 days caused increase in sodium and reduction in potassium contents, as a result the ratio between sodium and potassium was increased (2.78). Glycogenolysis and water loss can be accounted for alterations in K^+ and Na^+ concentrations respectively. It indicates a situation of dehydration. Among divalent cations, calcium concentration was comparatively much less than magnesium (Ca^{+2}/Mg^{+2} = 0.29). Feeding aflatoxin contaminated diet to rabbits caused an increase in both calcium and magnesium concentrations, resulting in increased calcium and magnesium ratio (0.30). Some of the key enzyme activities were estimated 1n testis of rabbits during aflatoxicosis. Activity of SDH showed a decrease suggesting decreased operation of mitochondrial TCA cycle. LDH and G-5-pase activities showed significant increase (P < 0.00l).

Keywords: Aflatoxin, Aflatoxicosis, Testis.

INTRODUCTION

Aflaxtoxins are secondary toxic fungal metabolites produced by *Aspergillus flavus*. Ingestion of aflatoxin contaminated food/feed stuffs causes occurrence of a serious toxic disease called aflatoxicosis. aflatoxin is potent hepatotoxin (Busby and Wogan,1984) and hepatocarcinogenesis to several animals PC and suspected to be linked to liver cancer in men (Groopman et. al.,1988; Stoloff,1977; Verma and Raval,1992a; Kolhe and Ingale, 2011) in addition dietary aflatoxicosis also caused biochemical changes in liver, heart and kidney (Verma and Kolhe,1997; Kolhe *et al*., 2006) causing hyperglycemia, hypercalcaemina

(Verma and Chaudhari.,1995) as well as reduced RBC count hemoglobin contained PCV (Verma and Raval,1992b). Marvan and his colleagues (1983) studied distribution patterns of AFBI residues in goslings and chicken. Results revealed that highest concentrations of AFB_1 residues are present in gonads. Testis is one of the important reproductive organs which perform functions like spermatogenesis. Male sexual act and regulation of male reproductive functions by various hormones. Hence the present investigation was undertaken.

MATERIAL AND METHOD

Aspergillus parasiticus (NRRL3240 obtained from the Indian Agricultural Research Institute. New Delhi, India) was grown on sucrose, magnesium sulfate - potassium nitrate – yeast extract **(SMK**Y) liquid medium at 28 ± 2 ^{0}C for 10 days (Diener and Davis, 1966). Culture filtrate, were pooled and extracted with analytical grade chloroform (1:2. v/v) and passed through the bed of anhydrous sodium sulfate. The chloroform extract was evaporated to dryness and the residue dissolved in fresh chloroform, transferred to vials and labelled. For quantitative analysis, 100 µl aflatoxin extract was spotted on silica gel G coated TLC plates along with an aflatoxin standard (a gift from the International Agency for Research on Cancer, Lyon, France) and developed in a solvent comprising toluene: iso- amyl alcohol: methanol (90:32:2. v/v) (Reddy *et al.*, 1970) The air-dried plates were observed under long -wave UV light (360 nm) for aflatoxins. Aflatoxins were confirmed chemically by spraying trifluoro acetic acid and 25 % sulfuric acid. Each spot was scraped separately, dissolved in chilled methanol and subjected to spectrophotometric analysis at 360 nm using a Shimadzu UV- 160A spectrophotometer (Nabney and Nesbitt, 1965). Crude aflatoxin concentrate in chloroform was thoroughly mixed with feed to obtain a concentration of 7.5 mg/kg. This toxin mixed feed was left overnight for complete evaporation of the chloroform. Food for control animals was similarly treated with chloroform alone and analysed for the absence of toxins.

Young inbred New Zealand strain rabbits (*Oryctolagus cuniculus)* weighing approximately 1.2 kg were fed with food and water *ad libitum* and were maintained under laboratory conditions. 10 such rabbits five in each group) were fed with aflatoxin-contaminated meal (7.5 mg/kg) for 90 days. Group 2animals received non –toxic feed and served as the controls.

On completion of the treatment, rabbits were sacrificed by cervical dislocation; testis was isolated weighed and processed for biochemical analysis of glycogen (Seifter *et al*., 1950), protein (Lowry *et al*., 1951), lipid (Folch *et al.,*1957) and water (by drying the tissue in anhydric air oven) contents.

A known amount of nonfat dry tissue (NFDT) was ashed in a muffle furnace at 600 degree centigrade for overnight and ash content measured gravimetrically samples were digested in 1 N HCL and analyzed for their Sodium and potassium contents by flame photometry calcium and magnesium by titration (Jackson.1973) and phosphorus by photo colorimetric method (Jackson, 1973).

Student's t-test was used for statistical analysis of data.

RESULTS

Rabbits fed with aflatoxin contaminated diet showed behavioral alterations like. reduced food and water consumption and weight loss. The rabbits became dull and lethargic with the signs of respiratory disorders. staggering. ataxia of nervous system. Falling of hairs; ear twitching and grinding of teeth were also noted reduced body weight was noted. Results revealed a significant decrease in body weight from 30 days onwards after the treatment. Relative increase in weight also showed a decrease.

The data presented in table biochemical profile of testis indicates altered during aflatoxicosis. Glycogen. protein and water contents were significantly decreased after feeding aflatoxin contaminated diet to rabbits ($P< 0.02$. $P< 0.05$. $P<0.01$ respectively). Lipid content was significantly increased ($P< 0 .001$). The concentration of various inorganic components in testis. It is apparent that the ash content showed an increase in aflatoxin fed rabbits Indicating an accumulation of inorganic constituents.

Among monovalent cations Na+ concentration was comparatively higher than K+ (Na+/K+ = 1.62).

Table 1: Effect of feeding aflatoxin contaminated diet on the testis of rabbits

Parameters		Control	Treated
Initial body weight (gm)		1168.80 ± 34.12	1175.60 ± 35.09
Final body weight (gm)		1768.10 ± 21.56	1383.60[a] ± 23.29
Relative Increase in body weight		51.27	17.69
Testis weight (gm)		7.0950± 0.2320	4.9560[a]± 0.2638
Testis weight (gm/100gm body wt)		0.4390± 0.1381	0.3225± 0.2147
Biochemical composition	Glycogen[1]	0.6421 ± 0.0458	0.4110[c]± 0.0632
	Protein[1]	7.1743 ± 0.2120	5.9572[d]± 0.4210
	Lipid[2]	4.9300 ± 0.0934	6.8100[a]± 0.1270
	Water[2]	79.9500 ± 0.9620	74.8900[b]± 0.7390
Inorganic constituents	Ash[3]	8.34 ± 0.09	8.97 ± 0.13
	Sodium[4]	76.08± 2.85	121.74[a] ±1.95
	Potassium[4]	46.85 ± 0.57	43.67[c] ±0.66
	Sodium/Potassium	1.62	2.78
	Ca1cium[4]	153.67 ±1 .47	159.00 ±3.34
	Magnesium[4]	514.02 ± 2.21	522.38[b] ± 0.66
	Calcium/Magnesium	0.29	0.30
	Inorganicphosphorus[4]	826.67 ± 12.88	826.53 ± 10.16

Values are mean ± S.E.M.; n = 5.

Significant at: a = P < 0.001; b = P <0.01; c = P <0.02; d = P <0.05

Values expressed as: 1. Mg % fresh weight; 2. Mg % dry weight ; 3. Mg % non-fat dry tissue, 4. Meq/1000 gm non-fat dry tissue

Induced aflatoxicosis caused an increase in sodium and reduction in potassium contents, as a result the ratio between sodium and potassium was increased (2.78). Glycogenolysis and water loss can be accounted for alterations in K^+ and Na^+ concentrations, respectively. It indicates a Situation of dehydration with loss of water along with salts. Among divalent cations, calcium concentration was comparatively much less than magnesium (Ca+2/Mg+2 = 0.29). Feeding aflatoxin contaminated feed to rabbits caused an increase in both calcium and magnesium concentrations. resulting in increased calcium and magnesium ratio (Ca +2/Mg +2 = 0.30). Feeding aflatoxin contaminated diet caused slight decrease in inorganic phosphorus. Table also shows activities of enzymes such as SDH. LDH and G-6-pase in the testis of the control and aflatoxin fed rabbits. SDH activity showed a decrease suggesting a decreased operation of mitochondrial TCA cycle. LDH and G-6-pase activities showed significant (P<0.001) increase.

DISCUSSION

Significantly decreased glycogen concentration during aflatoxicosis in rabbits could be due to the effect of aflatoxin on synthetic enzymes, inhibition of glycogenesis or increased glycogenolysis. Reduction in glycogen content coincidences with decreased K+ and increased ca^{+2} concentrations: later is known to enhance glycogen phosphorylase activity (Mc Gilvery. 1979). Intraperitoneal administration of aflatoxin to day-old chicks reduced UDP-glucose, glycogen trans-glucosylase activity (Shankaran *et al*.1970). Reduction in protein concentration can occur due to alteration in the mechanism of gene expression and changes in DNA dependent RNA synthesis (Clifford and Rees. 1967: Newberne and Butler, 1969; Edds. 1973). and protein synthesis (Sarasin and Moule. 1975; 1976; White and Rees. 1980; Jeffery *et al*.1984). Decreased K+ can be correlated with reduced protein synthesis (Harper, 1963, Verma *et al*, 1998). This reduction in protein content may be due to reduced synthesis as mentioned above and/or its increased utilization in gluconeogenesis in liver and kidney (Kolhe. 1994). Increased accumulation of lipid content in aflatoxin fed rabbits might be due to fatty infiltration in testis. Accumulation of lipid in the liver of chicks and ducklings fed with aflatoxin contaminated diet have been reported (Shank and Wogan. 1966; Carnaghan *et al*.1966; Newberne and Butler. 1969). Reason for this accumulation is not clearly understood. Decreased water content may be due to dehydration.

Feeding aflatoxin contaminated meal caused alteration in Na+/K+ ratio (Verma *et al*, 1998) which indicates permeability alterations. It could be due to a decreased activity of sodium pump, responsible for maintaining higher concentration of K^+ intracellularly with expulsion of Na^+ It is an energy requiring step. Reduced SDH activity during aflatoxicosis might be causing reduction in stored energy and hence activity of sodium pumps. Further, peroxidation or membrane lipids during aflatoxicosis initiates loss of membrane integrity and membrane bound enzyme activities (Younes and Seigers. 1984; Pasquali-Rouchetti *et al*. 1979) which in turn brought about a disturbance in cellular homeostasis. Increased Na+ concentration could be correlated with increased accumulation of water intracellularly. Also reduced K+ concentration in the cells could be correlated with increased glycogenolysis (Williams *et al*. 1971; Luly *et al*.1972).
Among the divalent cations. Ca^{+2} concentration was comparatively low than Mg^{+2} (Ca^{+2}/Mg^{+2} = 0.29). During aflatoxicosis calcium concentration registered a little increase while magnesium showed significant increase. Increased concentration of Ca^{+2} in cytosol, mitochondria and microsomal fraction as a result of AFB_1 treatment have been reported by Toskulkao and Glinsukon (1988). Increased accumulation inside the mitochondria causes mitochondrial dysfunction and reduction in ATP content.

SDH is a key enzyme of Krebs cycle which is primarily concerned with aerobic oxidation of Acetyl Co-A to produce energy. Activity of SDH showed decrease during aflatoxicosis in rabbits. It could be due to reduction in oxygen transport to the tissues. Aflatoxin induced alterations in mitochondrial activity (Toskulkao and Glinsukon. 1988) and mitochondrial swelling has been reported (Roy. 1968).

It is concluded that aflatoxin contaminated diet caused alterations in testis during aflatoxicosis in rabbits.

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