AMELIORATIVE EFFECT OF VITAMIN E AGAINST THE TOXICITY OF AFLATOXIN B1 ON RATS WITH SPECIAL REFERENCE TO ITS EFFECT ON MALE FERTILITY

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ABSTRACT

Aflatoxins are secondary toxic fungal metabolites produced by *A. flavus* and *A. parasiticus*. They not only contaminate our foodstuffs, but also found in edible tissues, milk, and eggs after consumption of contaminated feed by farm animals. In utero, exposure of aflatoxin through mother’s blood has also been reported in human beings. To assess the ameliorative effects of vitamin E on AFB1 toxicity on rats, 30 male albino rats ranged from 125 to 145 gm were classified into 3 groups each of 10 rats; group (1) was kept as control; Group (2) was given AFB1 7.5 ug/200 g body weight orally for 3 successive weeks and Group (3) was given vit.E 0.9 mg/200 g b.wt. orally 1 week before dosing AFB1, then continue orally dosing AFB1 plus Vit.E in a dose similar to group (2) . AFB1 lowered body weight; growth rate; total protein; albumin; globulin; testis weight; relative testicular weight; percentage of morphologically normal sperm; serum testosterone concentration; RBCs; WBCs; Hb; PCV and MCHC, while increased serum levels of GOT; GPT; creatinine; urea; sperm abnormalities; MCV and MCH concentration. The liver; testis; kidney; heart and lungs showed sever histopathological changes in group (2), but these histopathological lesions were mild or nearly absent in group (3). Vit.E. treatment significantly ameliorated the above mentioned AFB1-induced changes.

Abbreviations:

AFB1 = Aflatoxin B1 - GOT = glutamic oxaloacetic transaminase
GPT = glutamic pyrovate transaminase RBCs =Red blood cells Vit.E. = vitamin E
WBCs =White blood cells Hb=Hemoglobin PCV =Packed cell volume
MCH=Mean corpuscular haemoglobin MCHC=Mean Corpuscular haemoglobin concentration
MCV= Mean corpuscular volume
INTRODUCTION

Aflatoxin is an environmental toxicant which frequently contaminate foodstuffs in different parts of the world. Literatures have shown that complete eradication of aflatoxins from foodstuffs is difficult to attain because of a combination of factors such as climatic conditions that favour easy growth, proliferation and toxin production by fungi (19).

Aflatoxins are well known for their hepatotoxic and hepatocarcinogenic effects (49). AFB1 is activated to AFB1-8,9-epoxide and forms adduct primarily at N-7 position of guanine and is responsible for its mutagenic and carcinogenic affects (9).

In goslings and chickens, Marvan (29) experimentally studied the distribution of AFB1 where according to AFB1 concentration, the organs and tissues were categorized in the order from high to low concentrations as follows: the gonades, the parenchymatous organs (Liver and kidney), the lymphopoietic organs (spleen, bursa and thymus), the endocrine glands, the muscles and the lungs, while the brain had the lowest concentration. Also, it has been reported that aflatoxins have a deleterious effect on the reproductive systems of a wide spectrum of domestic animals (10). Naidu et al (31) observed multifocal hepatic necrosis, bile ductular proliferation, areas of altered hepatocytes, neoplastic nodules and hepatocellular carcinoma constituted the total spectrum in both adult and newborn rats exposed to AFB1. Meanwhile, progressive hepatic degeneration, necrosis and bile duct hyperplasia were the constant pathological changes observed in rats and chickens (39). In addition, AF administration induced degenerative changes in the hepatic and renal tissues of rats (1). Moreover, AFB1 induced mononuclear cell infiltration and / or focal lymphoid cell accumulation in the intertubular areas of the tests and epididymis; degeneration and desquamation in the epithelium and decrease in the size and thickness of the germinative layer of the seminiferous tubules and lowered plasma testosterone levels in adults roosters (33).

Abdel-Wahhab et al. (1) observed that AFs treatment significantly reduced blood haemoglobin, erythrocytes, leukocytes, cholesterol, triglycerides, cholinesterase, total protein, albumin; zinc and copper concentrations. On the other hand, it significantly increased creatinine, bilirubin, urea nitrogen, alkaline phosphatase and transaminase concentrations.

AFB1 in bull’s feed greatly reduced sperm motility with high percentage of serum abnormalities such as broken and looped tails and separation of the acrosomal cap from the sperm head. During the period of poor semen quality, plasma testosterone and oestrodial-17 concentrations were elevated (26). Moreover, Nair and Verma (32)
observed reduced weight of testis scattered and disorganized cell population in the seminiferous tubules in mice orally administered with AFB₁.

The sperm count and motility were significantly reduced and a large number of non-viable spermatozoa probably due to loss of membrane integrity was also observed in aflatoxin-treated mice. Similarly, Agnes and Akbarsha (2) recorded that the fertility of the treated mice with AFB₁ was reduced drastically. Sperm concentration in the epididymis and sperm motility decreased whereas sperm abnormalities increased. In particular, sperm abnormalities like two axonemes in a common cytoplasm, sticking together of head/tails, ect. were noted. While in roosters, Ortatali et. al. (33) observed significantly atrophid testis in birds treated with aflatoxin when compared to control birds; no spermatogenesis in the testis. Furthermore, abnormal spermatozoa were observed.

Ibeh and Saxena (22) observed a protective influence of alpha-tocopherol (Vit.E) on AFB₁ toxicity in rats. Also, Verma and Nair a,b and (44,45, 46) recorded that vitamin E pretreatment significantly ameliorates aflatoxin induced lipid peroxidation; aflatoxin-induced changes in testis of mice and aflatoxin-induced changes in steriodogenesis in mice , respectively.

With the background that the foodborne mycotoxin AFB₁ could be toxic to the male reproductive mechanism in man as well as wild and domestic animals; the antioxidant vitamin E plays an important role in various physiological processes in the body including detoxification of different toxic compounds. The present study aimed to investigate the influence of vitamin E co-administration on the toxicity of AFB₁ on the male reproductive system. Such a study could be of significance to understand the mechanism(s) of aflatoxin toxicity, especially on the reproductive system and to predict possible benefits to animal health and fertility problems due to co-administration of vitamin E during exposure to AFB₁.
MATERIAL AND METHODS

Chemicals:
1) Toxin: AFB$_1$ was obtained from Sigma Chemical Company (Sigma Aldrich Corporation, P.O. Box 14508, St. Louis, Missouri, 63178, USA).
2) Vitamin E: was obtained from Vetafarm Company, Garden City, Cairo, Fax:7950645. Where each 100 gm contains 2000 mg Vit. E.

Experimental animals: Thirty male albino rats ranged from 125 to 145 gm were kept with a source of light, and was given a balanced diet.

Dosing and grouping: The animals were randomly divided into 3 equal groups each of 10 rats.

Group I (Control): Were given 1.00 ml normal saline through oral intubation daily for 3 successive weeks.

Group II: Were given AFB$_1$ 7.5 ug/200 g. b.wt. adopted after Ibeh and Saxena (22) in normal saline in identical manner as group I.

Group III: Were given vit.E 0.9 mg/200 g b.wt adopted after Ibeh and Saxena (22) in normal saline 1 week before dosing AFB$_1$ (as dose given in group 2 ) then vit.E plus AFB$_1$ were continued per os daily for 3 successive weeks.

At the termination of experiment, body weight were recorded, then all animals were sacrificed, blood was collected in Apendorfe tubes containing EDTA for counting RBSc, WBCs, PCV and Hb. Serum samples were stored at -20°c for serum profile.

Immediately after slaughter, rat epididymis was taken, minced and extended in 1 ml physiological saline. Specimens from liver, kidney, brain, heart, lungs and testis were preserved in 10% formaline.

Methods: RBCs and WBCs were counted according to Miller and Sewaed (30). PCV was determined in microhematocrit tubes. Hb was assayed by the method of Suedecor et al. (42). MCV, MCH and MCHC were calculated according to Lea and Febige (27).

Total protein, albumin and globulins were estimated according to Henry (20), Doumas et al. (11) and Coles (7), respectively. GOT; GPT; urea and creatinine were determined after Reitman and Frankel (36), Basdhandy et al. (3) and Henry (20), respectively.

Histopathological investigation was carried out after Drury and Willington (12).
The percentage of morphologically normal spermatozoa was estimated. At the same time, the total sperm concentration in rat epididymis was estimated by using the haemocytometric technique according to Bearden and Fluquary (4). Testosterone in serum was evaluated according to Yalow and Berson (50). Data were tabulated and statistically analyzed according to Snedecor and Cochran (41).

**RESULTS**

**Table 1.** Effect of AFB$_1$ alone or plus Vitamin E on body weight (in grams) and growth rate of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial weight (gm)</th>
<th>Final weight (gm)</th>
<th>% of growth rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (I) Control</td>
<td>134.8 ± 1.90</td>
<td>209.6 ± 2.006</td>
<td>53.55%</td>
</tr>
<tr>
<td>Group (II) AFB$_1$</td>
<td>135.6 ± 2.06</td>
<td>172.1 ± 1.68**</td>
<td>26.84%</td>
</tr>
<tr>
<td>Group (III) AFB$_1$ + Vit.E</td>
<td>135.4 ± 2.32</td>
<td>188.1 ± 1.88**</td>
<td>38.92%</td>
</tr>
</tbody>
</table>

* significant at < 0.05 ** Highly significant at P<0.01

**Table 2.** Effect of AFB$_1$ alone or plus vitamin E. on serum levels of total protein, albumin and globulin (g/dl) of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (I) Control</td>
<td>8.94 ± 0.56</td>
<td>5.54 ± 0.67</td>
<td>3.40 ± 0.35</td>
</tr>
<tr>
<td>Group (II) AFB$_1$</td>
<td>6.03 ± 0.09**</td>
<td>4.84 ± 0.05</td>
<td>1.196 ± 0.05**</td>
</tr>
<tr>
<td>Group(III) AFB$_1$ + Vit. E</td>
<td>7.09 ± 0.18**</td>
<td>4.66 ± 0.08</td>
<td>2.43 ± 0.18**</td>
</tr>
</tbody>
</table>

* significant at < 0.05 ** Highly significant at P<0.01
Table 3. Effect of AFB$_1$ alone or plus vitamin E on serum levels of GOT (u/L); GPT (u/L); creatinine (mg/dL) and urea (mg/dl) of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GOT (u/L)</th>
<th>GPT (u/L)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.44 ± 1.83</td>
<td>8.67 ± 0.31</td>
<td>0.80 ± 0.05</td>
<td>26.38 ± 0.33</td>
</tr>
<tr>
<td>AFB$_1$</td>
<td>19.35 ± 1.53*</td>
<td>13.79 ± 1.78**</td>
<td>0.97 ± 0.15</td>
<td>38.02 ± 0.33**</td>
</tr>
<tr>
<td>AFB$_1$ + Vit. E</td>
<td>15.29 ± 1.39*</td>
<td>9.36 ± 1.38*</td>
<td>0.85 ± 0.05</td>
<td>31.91 ± 0.056**</td>
</tr>
</tbody>
</table>

* significant at < 0.05 ** Highly significant at P<0.01

Table 4. Effect of AFB$_1$ alone or plus vitamin E on some male reproductive parameters of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tests weight (gm)</th>
<th>Relative testicular weight</th>
<th>Testosterone (ng/ml)</th>
<th>% morphologically normal sperm</th>
<th>Sperm count (x10$^6$/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.1 ± 0.14</td>
<td>3.39 %</td>
<td>8.013 ± 0.382</td>
<td>97.40 ± 0.72</td>
<td>1.40 ± 0.16</td>
</tr>
<tr>
<td>AFB$_1$</td>
<td>5.37±0.34*</td>
<td>3.12%</td>
<td>6.02 ± 0.281**</td>
<td>91.20±0.83**</td>
<td>0.80±0.13**</td>
</tr>
<tr>
<td>AFB$_1$ + Vit. E</td>
<td>6.13±0.12*</td>
<td>3.26%</td>
<td>7.047 ± 0.143**</td>
<td>94.40±0.81**</td>
<td>1.00±0.19</td>
</tr>
</tbody>
</table>

* significant at < 0.05 ** Highly significant at P<0.01
Table 5. Effect of AFB_1 alone or plus vitamin E on some haematological parameters of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>RBCs (x10^6/mm^3)</th>
<th>WBCs (x10^3/mm^3)</th>
<th>Hb (gm%)</th>
<th>PCV (%)</th>
<th>MCV (cm^3)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.11x10^6 ±0.24</td>
<td>11.44x10^3 ±0.196</td>
<td>18.35 ±0.24</td>
<td>33.33 ±0.72</td>
<td>32.97</td>
<td>18.15</td>
<td>55.06</td>
</tr>
<tr>
<td>Group (II) AFB_1</td>
<td>6.11x10^6 ±0.11**</td>
<td>6.03x10^3 ±0.31**</td>
<td>13.85±0.64**</td>
<td>28.66 ±1.96*</td>
<td>46.91</td>
<td>22.67</td>
<td>48.33</td>
</tr>
<tr>
<td>Group (III) AFB_1 + Vit. E.</td>
<td>8.196x10^6±0.13**</td>
<td>9.95x10^3±0.12**</td>
<td>15.08 ±1.08</td>
<td>30.00 ±1.06</td>
<td>36.60</td>
<td>18.39</td>
<td>50.27</td>
</tr>
</tbody>
</table>

* significant at < 0.05  ** Highly significant at P<0.01

Table 1 and Figs. 1 and 2 showed a significant decrease in body weight and growth rate of rats dosed AFB_1 daily for 3 successive weeks, while dosing rats with vitamin E before and with AFB_1 treatment leads to an increase in body weight and growth rate, but still lower than the control group. Also, data of table 2 and fig. 3 indicate a similar results on total protein; albumin and globulin. Meanwhile, serum levels of GOT; GPT; creatinin and urea were increased due to dosing AFB_1 as shown in table 3 and figs.(4-6). Testicular weight and its relative weight; serum testosterone level (ng/ml); % of morphologically normal sperm and epididymal sperm count (x10^6/ml) were significantly decreased after dosing AFB_1, but dosing vitamin E elevate this decrease as shown in table 4 and fig. (7-9). Table 5 and fig. (10-11) showed the effect of AFB_1 and or vitamin E on some hematological parameters, fig. 12 showed normal spermatozoa, but Fig. 13 showed sperm abnormalities with double head, double middle piece and double tail with deformed head after dosing rats with AFB_1, while dosing vitamin E with AFB_1 produce double tail and deformed head of sperm as shown in fig. 14, moreover congestion of superficial testicular blood vessel was shown in fig. 15.

Concerning histopathological investigation: In group II given 7.5 mg/200 gm b.wt. of AFB_1 for three weeks:
The liver: The liver of this group showed hydropic and vacuolar degeneration of hepatocytes, mainly around the congested blood vessel (Fig. 16). Lymphocytic cellular aggregation were also seen (Fig 17). Inflammatory cellular infiltration of portal area mostly lymphocytes and congestion of portal vessels with presence of small newly formed bile ductules (Fig 18).

The testis: Degeneration of lining epithelium of seminiferous tubules and congestion of testicular blood vessels with intertubular edema were noticed in the rats of this group (Fig. 19). Moreover, coagulative necrosis of entire lining epithelium of some seminiferous tubules that replaced by haemogenous eosinophilic debris in their lumina were also seen (Figs. 20 and 21).

The kidney: The renal blood vessels and intertubular capillaries were dilated and engorged with blood. Focal areas of cloudy swelling of the lining epithelium of convoluted tubules together with presence of some eosinophilic debris in some lumen of other tubules (Fig. 22). Leucocytic cellular infiltration of renal interstitial tissues particularly lymphocytes with shrinkage of the glomerular tufts were noticed. Moreover, focal aggregation of dark brown granules of haemosidrin pigment among renal parenchyma was also found.

The brain: The microscopic examination of the brain of rats in this group revealed meningitis represented by congestion of meningeal blood vessels with inflammatory cellular infiltration mostly lymphocytes and few neutrophils (Fig. 23).

The heart: The heart showed congestion of intermuscular blood capillaries with focal areas of myocardial hyalinosis.

The lungs: The histological examination of lungs showed sever congestion of pulmonary blood vessels and perivascular lymphatic cellular cuffing. The bronchioles showed desquamation of lining epithelium with mononuclear infiltration in their lumina.

The third group in which the rats received 0.9 mg of vitamin E/ 200 gm b.wt. for one week , then 7.5 mg of AFB\textsubscript{1} for 3 weeks with vitamin E.

The liver: The liver of this group showed dilatation of central vein with normal hepatic parenchyma (Fig. 24).

The testis: The microscopic examination of testis of rats in this group showed nearly normal histological structure of seminiferous tubules.

The Kidneys: Congestion of intertubular blood capillaries was observed (Fig. 25).

The brain, the heart and lung: Showed no histopathological lesions.
Fig 1: Effect of AFB1 alone or plus vitamin E on body weight (in gram)

Groups

- Group (1) Control
- Group (2) AFB1
- Group (3) AFB1 + Vit E

Initial weight

135 135.6 134.8

Final weight

188 172 209.6
Fig. 14: Showing sperm abnormality occurred in tail and deformed head of sperms after dosing AFB1 (7.5ug/200g b.w.) with Vit.E. (0.9 mg/200g b.w.)

Fig. 15: Showing congestion of superficial testicular bl.v. after dosing AFB1 (7.5ug/200g b.w.) for 3 successive weeks

Fig. 16: Liver of rats received 7.5ug/200g b.w. AFB1 for 3 weeks showing vacuolar and hydropic degeneration of hepatocytes (H & E stain x200)

Fig. 17: Liver of rats received 7.5ug/200g b.w. AFB1 for 3 weeks showing lymphocytic cellular aggregations (H & E stain x200)

Fig. 18: Liver of rats received 7.5ug/200g b.w, AFB1 for 3 weeks showing congestion of portal vessels and inflammatory cellular infiltration of portal areas mostly lymphocytes (H & E stain x200).

Fig. 19: Testis of rats received 7.5 ug/200g b.w. AFB1 for 3 weeks showing degeneration of lining epithelium of seminiferous tubules with intratubular oedema (H & stain x 200)
Fig. 20: Testis of rats received 7.5ug / 200g b.w. AFB1 for 3 weeks showing coagulative necrosis of centric lining epithelium of semineferous Tubules (H & E stain x 200)

Fig. 21: High power of the previous figure showing homogenous eosinophilic debris within the necrosed semineferous tubules (H & E stain x 200).

Fig. 22: Kidney of rats received 7.5 ug/200 g b.w. AFB1 for 3 weeks showing cloudy swelling of some renal tubular epithelium with some eosinophilic debris in lumen of other tubules 9H&E stain x 200)

Fig. 23: Brain of rats received 7.5 ug/200 g b.w. AFB1 for 3 weeks showing mononuclear inflammatory cellular infiltration mainly lymphocytes (H & E stain x 200)

Fig. 24: Liver of rats received 0.9 mg Vit.E & 7.5 ug AFB1 /200g b.w. for 3 weeks showing dilatation of central vein with normal hepatic parenchyma (H & E stain x 200)

Fig. 25: Kidney of rats received 0.9 mg Vit. E. & 7.5ug AFB1 /200g b.w. for 3 weeks showing intratubular blood capillaries (H & E stain x 200)
DISCUSSION

The quality of food consumed in any given community has a prevailing influence on the health and general wellbeing of members of the community. Thus, foods can easily lose nutritive value to become agents for the transmission of bacterial, protozoal and fungal diseases including those associated with mycotoxins such as aflatoxins which could occur in common staple foods in most countries at biologically significant levels (5).

This study has attempted to investigate the toxicity of AFB$_1$ on rats and the impact of Vitamin E (alpha-tocopherol) on the pathology of AFB$_1$ to shed more light on the deleterious effects of AFB$_1$ on rats and possibly predict any benefits in administration of vitamin E in lowering the toxicity of AFB$_1$.

Table 1 and Fig. 1 showed that dosing AFB$_1$ (7.5 ug/200 g b.wt.) daily for 3 successive weeks significantly reduced body weights of rats (172.1 gm) when compared to control (209.6 gm). Dosing rats vitamin E (0.9 mg/200 g b.wt.) one week prior to dosing AFB$_1$ and then co-administration with it, produced an increase in body weight (188.1 gm) in relation to AFB$_1$ alone (group2). This loss of body weight may be due to improper assimilation or metabolism of feed due to the hepatotoxic effects of AFB$_1$ and this was confirmed in our histopathological study and the study of liver function or may be a reflection of reduced feed intake and poor feed conversion. Also from data of table 1 and Fig. 2 we can notice that rats fed AFB$_1$ have lower growth rate percentage (26.84 %) than that of the control (53.55%). Feeding rats with vitamin E increased growth rate (38.92%) but still lower than that of the control. Our results were agree with that of Ineh and Saxena (22) who mentioned that there were disturbed body weights of rats exposed to aflatoxin which was influenced by co-administration of alpha-tocopherol, providing a reduction in the effect of aflatoxin in this regard. This finding may be related to an enhanced metabolism in the alphatocopherol dietary supplemented group.

Dosing rats AFB$_1$ for three weeks lowered serum concentration of total protein (6.03 g/dl); albumin (4.84 g/dl) and globulin (1.19 g /dl) than that of control group (8.94; 5.54 and 3.40 g /dl, respectively). Moreover feeding rats vitamin E one week prior to AFB$_1$ and then co-administration of both produce a somewhat increase in the serum levels of total protein (7.09 g/dl) and globulin (2.43 g/dl) in comparison with AFB$_1$ alone (group 2) these data were shown in table 2 and Fig. 3. Hypoproteinaemia may be due to decreased feed consumption or the known biological activities of aflatoxin as inhibition of protein synthesis (6). Also aflatoxin has been found to inhibit RNA polymerase in vivo and subsequently impair protein
synthesis (25). Similarly, the decline in protein concentration in the testis of aflatoxin treated mice could be due to a decline in the protein biosynthesis by forming adducts with DNA, RNA and protein, an inhibition of RNA synthesis or DNA dependent RNA polymerase activity, as well as degranulation of endoplasmic reticulum (8,17). Also, Reitman et al (37) reported a concentration-dependent inhibition of protein synthesis in the liver of rat. Also, this reduction with reduction in serum levels of albumin and globulin, were noticed in rats (1,14). The decrease in albumin and globulin by AFB1 may be due to inhibition of synthesis of specific immunoglobulins (43). From the data in this table we noticed a clear sign of protective effect of vitamin E on rats exposed to AFB1, as it rise the serum levels of total protein.; Albumin. and globulin of intoxicated rats.

Serum levels of GOT and GPT in rats treated with AFB1 and AFB1 plus vitamin E were tabulated in table 3 and illustrated in Fig. 4 where we noticed that AFB1 treatment increased serum level of GOT (19.35) than that of the control (14.44) while in group treated with AFB1 + vitamin E the level decreased (15.29) but still slightly higher than control. Similar results were recorded in case of serum concentration of GPT where AFB1 increased it (13.79) than that of the control (8.67), while AFB1 plus vitamin E lowered it (9.36) but still somewhat higher than the control. From these data we can notice that vitamin E gave some protection against the hepatotoxic effect of AFB1. The increase in GPT activity may be due to the toxic effect of AFB1 on the liver parenchyma, this was confirmed in our histopathological study where we noticed that liver of rats in group 2 (received 7.5 ug AFB1/200 gm b.wt for three weeks) showed congestion and petechial haemorrhage on their cut surface macroscopically, but microscopically hydropic and vacuolar degeneration with cellular infiltration of the portal area were noticed. Nearly the same results were noticed by El-Shewy (14) in the rats received the same dose. Simillary, Shaaban et al. (39) noticed progressive hepatic degeneration, necrosis and bile duct hyperplasia in rats and chickens fed on stored grains containing AFB1 and AFB2. However, Ibeh et al. (23) recorded degeneration of the membranes in hepatocytes, mitochondria and endoplasmic reticulum in rats received similar dose. Meanwhile, rats in group 3 received 0.9 mg Vit.E/200 g b.wt in combination with AFB1 showed mild histopathological lesions in liver, a result similar to that recorded by Ibeh and Saxena (22). Vitamin E offers protection against liver damage induced by AFB1 (16.28).

The increase in GOT activity could be attributed to the toxic effects upon heart muscle, liver cells and kidney and consequently liberating their intracellular enzyme
into the blood stream as indicated by Harper (18) who described that GOT level increase in disease of heart muscle while GPT is more specific for liver damage. Similarly AFB$_1$ increased serum levels of alkaline phosphatase and transaminase concentrations in rats (1).

Regarding the effects of AFB$_1$ and AFB$_1$ plus Vitamin E on serum level of creatinin in rats, table 3 and Fig. 5 showed that daily treatment of rats with AFB$_1$ for 3 successive weeks resulting in a significant increase in serum creatinin than that of the control (0.97 mg/dl and 0.80 mg/dl, respectively). Pretreatment of rats with vitamin E for one week before the beginning of AFB$_1$ treatment than in the same line of AFB$_1$ resulted in lowering creatinin level (0.85 mg/dl) in comparison to that of AFB$_1$ alone (0.97 mg/dl) but still slightly higher than that of the control (0.80 mg/dl). Similar results were observed in serum urea level after treatment with AFB$_1$, where AFB$_1$ resulted in a significant increase in serum urea concentration (38.02 mg/dl) than that of the control (26.38 mg/dl). AFB$_1$ plus vitamin E showed a moderate concentration between both AFB$_1$ and control (31.91 mg/dl). These results showed additional sign of protective effect of vitamin E against urotoxic effect of AFB$_1$ (represented by serum levels of creatinine) on rats. Similarly addition of hydrated sodium calcium aluminum silicate (HSCAS) of montmorillonite to AF-contaminated diet resulted in a significant improvement in the biochemical parameters including creatinine and urea nitrogen (1).

The kidneys showed focal area of cloudy swelling of convoluted tubules, leucocytic infiltration of interstitial tissue in the rats of group II. El-Shewy (14) detected nearly the same lesions. On the other hand, the kidneys of group 3 showed slight congestion of the blood vessels. Similar observation was indicated by Ibeh and Saxena (22).

Data of table 4 and Fig. 7 showed the effect of AFB$_1$ alone or with vitamin E on testicular weight (gm) and relative testicular weight, where we noticed that AFB$_1$ lowered the testicular weight and relative testicular weight (5.37 mg and 3.12% respectively) in comparison to the control (7.1 gm and 3.39%, respectively). Addition of vitamin E produced a significant rise in both items (6.13 gm and 3.62%, respectively) in comparison to that of AFB$_1$, but still lower than that of the control. Similarly Ibeh and Saxena (22) noticed that AFB$_1$ treated rats showed a lower testicular weight and relative testicular weight, but addition of alphatocopherol improved both. The mechanism(s) of cellular interaction with aflatoxin is not yet fully understood. It appears that the toxicity of aflatoxin on the cell membrane is mediated through the generation of free radicles. The presence of vitamin E in the third group of rats provided a protective influence over the toxicity of administered aflatoxin on
the testicular cells and tissue. Another possibility is that vitamin E may have dampened the unsaturation of the aflatoxin molecules by accepting electrons thereby slowing down the rate of its activation and metabolism (22).

In group 2 received AFB$_1$ alone, the present study revealed that, the testis showed congestion of testicular blood vessels, intertubular oedema, degeneration of the lining epithelium of semineferous tubules and necrosis of some semineferous tubules. Egbunike (15) detected sever testicular degeneration and impaired spermatogenesis in rats chemically treated with AFB$_1$. Piskac et al. (35) found regressive changes in the germinal epithelium of the tubules and interstitial tissue in rats administered 1 mg/kg AFB$_1$. Ortatati and Verma (33) observed degeneration and desquamation in the epithelium of the seminiferous tubules in roosters received 20 ppm AFB$_1$ orally for 8 weeks. While the testis of rats in Group 3 showed nearly normal histological structure of seminiferous tubules, and in agreement with Ibeh and Saxena (22) who stated that vitamin E reduced pathological changes of the testis.

Concerning the testosterone concentrations in serum of rats dosed AFB$_1$ alone or plus vitamin E, data of table 4 and Fig. 7 showed that AFB$_1$ highly significantly lowered serum testosterone concentration (6.02 ng/ml ) than that of the control rats (8.013 ng/ml). An intermediate concentrations were recorded after dosing AFB$_1$ + Vitamin E ( 7.047 ng/ml). Similarly in quail feeding AFB1 for four weeks lowered plasma testosterone concentrations (24). Moreover, similar results were recorded in roosters and rabbits (33 and 36 respectively) and in rats (3). The mechanism by which aflatoxin produces an impact on the reproductive system could be related to its toxicity on the liver (23), which may be manifested by the desquamation of the membranes by hepatocytes, the mitochondria, the cytosol, and the endoplasmic reticulum. The net effect of this cellular damage can induce inhibition of enzyme synthesis and/ or enzyme activities (48); or inhibition of lipid metabolism or fatty acid synthesis, which may derail the capacity of the hepatocytes to handle the conversion of intermediate biomolecules, such as precursor molecules for hormones, e.g., testosterone and progesterone. The depression of the normal level of these hormones, or their total absence, could participate in a wide range of degenerative changes in sexual organs of aflatoxicotic animals (23).

After three weeks of dosing rats with AFB$_1$, a highly significant reduction in the percentage of morphologically normal sperm and epididymal sperm count (91.20 and 0.80 x 10$^6$/ml, respectively) in relation to control rats (97.40 and 1.40 x 10$^6$/ml, respectively). Dosing aflatoxicated rats vitamin E raised the percentage of
normal sperm and the epididymal sperm count (94.40 and 1.00 x10^6, respectively) in comparison with that of aflatoxicated rats, but still lower than that of normal rats. These results were shown in table 4 and illustrated in Figs. 8 and 9, respectively. Similar results were noticed in rats and infertile men in Benin City, Nigeria (23), also AFB_1 and vitamin E produce a similar results in rats (22). Moreover, AFB_1 decreased sperm concentration in the epididymis and sperm motility whereas sperm abnormalities increased in mice (2) indicating disruption of the spermatogenic as well as androgenic compartments of the testis by AFB_1 and an alteration of epididymal function towards the post-testicular sperm maturation process by AFB_1. Ibeh et al (23) mentioned that other routes by which the impact of aflatoxin could be left on the male reproductive system include: frank lysis of sperm cells as a result of constant reversible reaction with the mycotoxin; binding of the toxin to free and / or bound amino acids in the seminal fluid may depress the motility of spermatozoa by way of inhibiting their chemotactic response and the formation of aflatoxin adducts with nucleic acids, which may give rise to mutations of the spermatogonia. Verma and Nair (45) reported that reduced succinic dehydrogenase and ATPase activity could explain the reduced sperm count and motility and increased number of non-viable spermatozoa observed in aflatoxin-treated mice.

Concerning sperm abnormalities, sperm with double head; double middle piece and double tail with deformed head after dosing rats with 7.5 ug AFB_1/ 200 g b.wt. for three successive weeks were shown in Fig. 13 while Fig. 14 showed sperm with double tail and deformed head after dosing AFB_1 with Vitamin E. During spermatogenesis and spermiogenesis many macromolecules are synthesized. Sertoli cells secrete both serum proteins and testis-specific proteins, including androgen binding protein, inhibin, sertoli-derived growth factors and cyclic protein 2. Therefore, the reduction in these macromolecules could be responsible for the reduction in spermatogenesis and spermiogenesis (44). Sialic acid is a sialomucoprotein essential for the maintenance of the structural integrity of the sperm membrane and sperm maturation. Therefore, a reduction in the sialic acid concentration in the testis could be responsible for morphological abnormalities observed in spermatozoa (45). The protective effect of vitamin E could be due to a significant recovery in the antioxidant capacity of the cell. The antioxidative function of Vitamin E is mainly due to its reaction with membrane phospholipid bilayers to break the chain reaction initiated by hydroxyl radical (40). Also, a reduction in toxicity of free radical by vitamin E in association with a reduction in aflatoxin metabolism seems to be responsible for the protective influence (44).
A congestion of superficial testicular blood vessels was noticed after dosing rats 7.5 ug AFB1/200 g b.wt. for three successive weeks, this was previously recorded in rats given AFB1 for 14 days by Ibeh and Saxena (22).

Effect of AFB1 alone or plus vitamin E on some haematological parameters were shown in table 5 and illustrated in Figs from 10 to 11, where we noticed that AFB1 significantly reduced RBCs, WBCs count and Hb concentration (gm%) (6.11 x 10^6 /mm^3; 6.03 x 10^6 /mm^3 and 13.85 , respectively) than that of the control (10.11 x10^6/ mm^3;11.44 x 10^6 /mm^3 and 18.35, respectively). Dosing vitamin E in combination with AFB1 improved these figures in comparison to AFB1 alone (8.196 x 10^6/mm^3; 9.95 x 10^6/mm^3  and 15.08, respectively). Also AFB1 significantly lowered PCV; and MCHC (28.66; 48.33 ,respectively) than that of the control (33.33 and 55.06, respectively), but significantly increased MCV cm^3 and MCH pg (46.91 and 18.39, respectively). Addition of vitamin E lowered these figures when we compare with AFB1 (36.60 and 18.39, respectively). Weekly and Uewell (47) noticed that PCV was decreased in chronic AFB1 treated rats, while in mice no changes were observed in RBCs but leukocyte count were decreased in a dose-related manner (51). Also, AFB1 (2.5 mg/kg diet) treated rats significantly reduced haemoglobin ;erythrocytes and leukocytes and addition of HSCAS or montmorillonite (5g/kg) to AF-contaminated diet resulted in a significant improvement in these haematological parameters (1). The type of anaemia here is macrocytic hypochromic anaemia (increase in MCV and decrease in MCHC), i.e. haemorrhagic or haemolytic anaemia, this was previously reported in chicken (13).

Meningitis and the histopathological lesions in the heart observed in most cases of group II were not recorded in the available literature. On the other hand, these lesions were not seen in rats of group 3.

In agreement with El-Shewy (14), the lung in the group 2 showed sever congestion of pulmonary blood vessels with desquamation of the bronchial epithelium, while in group 3 slight congestion was detected.

CONCLUSIONS AND RECOMMENDATION

Several reports have appeared and suggest that aflatoxins are extremely potent mycotoxins which produce serious health hazards in a variety of animal species. It has also been reported that aflatoxins have a deleterious effect on the reproductive systems of a wide spectrum of domestic animals. Based on the findings in this study, we share the opinion that the presence of aflatoxin in feed and food constitutes a
serious health hazard, but pretreatment of protective doses of vitamin E (alphatocopherol) significantly ameliorates the AFB1-induced changes as decreased body weight; growth rate; total protein; albumin and globulin; increased GOT, GPT, urea and creatinin; lowering testis weight; serum testosterone concentration; lowering percentage of morphologically normal sperms with increasing sperm abnormalities; affect some haematological parameters with degenerative changes in several organs in rats. Therefore, the following suggestive measures should be conducted:

1-Addition of vitamin E (alphatocopherol) to animal feeds as a routine work to give some protection against the presence of aflatoxin according to the dose recommended in this study.
2- However, whatever the outcome of such studies, the incidence of aflatoxin in feed and food is unacceptable. Therefore, efforts should be made to check the source of food contamination by aflatoxin and to lower the permissible limits for AFB1 in Egypt.
3- Feeds must be stored under conditions which prevent mold growth.

REFERENCES


التأثير المُلطف لفيتامين هـ ضد سمية الأفلاتوكسين B1 على الفئران

مع الأخذ في الاعتبار تأثيره على الخصائصين

* إلهام عبد المنعم الشيوى ، ** منى فاروق إبراهيم

قسم الطب الشرعي و السموم بكلية الطب البيطرى جامعة الزقازيق فرع بنها

** زميل قسم الباثولوجى بكلية الطب البيطرى جامعة الزقازيق فرع بنها

الأفلاتوكسينات هي نواتج ثانوية سامة للفطريات تُظهر تأثيرات سامة على الأنسجة والخلايا، ولذلك توجد في الأنظمة الحيوانية وتؤثر على الخصائصين الأولية والثانية سمية الأفلاتوكسينات على الفئران، وتقييم التأثيرات المولدة لفيتامين هـ على سمية الأفلاتوكسينات على الفئران تم تقسيم 20 الفأرة إلى 4 مجموعات كل منها 5 فئران:

- المجموعة الأولى: تركت كمجموعة ضابطة.
- المجموعة الثانية: أعطيت أفلاكتوكسين ب، بجرعة 7.5 ملغ/جم/200 جرام من وزن الجسم عن طريق الفم لمدة أسابيع متتالية.
- المجموعة الثالثة: أعطيت فيتامين هـ 0.5 ملغ/جم/200 جرام من وزن الجسم عن طريق الفم لمدة أسبوع قبل إعطاء أفلاكتوكسين ب، ثم استمر معه لمدة أسابيع متتالية.
- المجموعة الرابعة: أعطيت فيتامين هـ 0.5 ملغ/جم/200 جرام من وزن الجسم عن طريق الفم لمدة أسبوع قبل إعطاء أفلاكتوكسين ب، ثم استمر معه لمدة أسابيع متتالية.

وقد لوحظ أن 

AFB1 ، 

الجلوبيولين ، وزن الخصائص وكذلك وزنها النسبي للجسم ، نسبة الحيوانات المنوية الظهارية السليمة ، تركيز هرمون التستوستيرون في السيرم ، خلايا الدم الحمراء والبيضاء ، الهيموجلوبينين ، MCH ، PCV ، MCHC ، GOT ، GPT ، الكرياتينين ، البروتينات الكلية ، الألبومينات ، MCV ، MCV ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT ، GOT ، GPT 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