VITAMIN E AMELIORATES AFLATOXIN-INDUCED ALTERATIONS IN THE EPIDIDYMIS OF MICE

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Abstract: The present investigation was an attempt to evaluate the effect of aflatoxin on biochemical and histopathological changes in the epididymis of mice and its possible amelioration on pre-treatment with vitamin E. Adult male albino mice were orally administered with 25 and 50 mg of aflatoxin/animal/day (750 and 1500 mg/kg body weight) for 45 days. Epididymis was isolated and processed for biochemical analysis. As compared with the control, absolute and relative epididymal weights were significantly reduced in aflatoxin-treated mice. Aflatoxin treatment caused significant, dose-dependent reduction in protein and sialic acid contents in caput and cauda epididymis than that of vehicle control. While activities of succinic dehydrogenase and adenosine triphosphatase were significantly reduced, acid phosphatase activity was significantly higher in caput and cauda epididymis of aflatoxin-treated mice than that of vehicle control. Pyknosis of epithelial cell nuclei, disorganization of epithelium, clumping of stereocilia and lumen devoid of sperms in caput and cauda epididymis were observed. Thus, pre-treatment with vitamin E (2 mg/0.2 mL olive oil/animal/day) significantly ameliorated aflatoxin-induced changes, measured by biochemical and histopathological parameters.

Keywords: aflatoxin, vitamin E, epididymis, biochemical changes

Aflatoxins are food-borne toxicants produced by Aspergillus flavus and A. parasiticus. This mycotoxin represents a major public health problem especially in Southeast Asia and sub-Saharan Africa where it contaminates human food supplies. Aflatoxins are also found in edible tissues, milk and eggs after ingestion of aflatoxin-contaminated food by farm animals (1). Aflatoxins are well known hepatotoxic and hepatocarcinogenic agents (2, 3).

In previous studies we observed reduced weight of testis, scattered and disorganized cell population in the seminiferous tubules (4) as well as biochemical changes in the testis (5) in mice orally administered with 25 and 50 mg of aflatoxin/animal/day for 45 days. All these changes were significantly ameliorated on pre-treatment with vitamin E.

The mammalian epididymis is an elongated coiled duct suspended within the mesorchium and is firmly or loosely bound to the tunica albuginea. The epididymal tubular lumen is continuous with the lumina of vas efferentes in the testis and ends in the vas deferens. The gross division of epididymis comprises of the caput, corpus and cauda epididymis.

Vitamin E deficient female rats suffer death and resorption of the fetuses. In male animals deficiency results in testicular atrophy, with degeneration of the germinal epithelium of the seminiferous tubules. This lesion responds to vitamin E. The nervous system is also affected in deficiency, with the development of central nervous system necrosis. Vitamin E can prevent these changes. Vitamin E deficient animals show anemia and exudative diathesis, i.e. leakage of blood plasma from capillaries in subcutaneous tissues. There is also increased erythrocyte hemolysis in deficiency of vitamin E.

The aim of the present investigation was to study the effect of aflatoxin on histopathological and biochemical changes in the epididymis of mice. In addition, possible ameliorative effect of vitamin E pre-treatment was also evaluated.

EXPERIMENTAL

Aspergillus parasiticus (NRRL 3240) obtained from the Indian Agricultural Research Institute, New Delhi, India was grown on sucrose-magnesium sulfate-potassium nitrate-yeast extract (SMKY) liquid medium at 28 ± 2°C for 10 days (6). Culture filtrates were extracted with chloroform (1 : 2, v/v) and passed through anhydrous sodium sulfate. The chloroform extract was evaporated to dryness.

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Dried aflatoxin extract was dissolved in fresh chloroform and used for chemical analysis. 100 mL of aflatoxin extract was fractionated on silica gel G coated activated TLC plates (Merck) along with standard (a gift from International Agency for Research on Cancer, Lyon, France). The plates were developed in a solvent consisting of toluene : isopentyl alcohol : methanol (90 : 32 : 2, v/v/v) (7). The air dried plates were observed under long-wave UV light (360 nm) for aflatoxins. Different components of aflatoxins were initially identified visually by comparing the color and intensity of fluorescence as well as polarity of sample spots with standards. Aflatoxin B₁ and B₂ showed blue fluorescent spots, aflatoxin G₁ and G₂ showed bluish green fluorescent spots. Chemical confirmation of aflatoxin was done by spraying with trifluoroacetic acid and 25% sulfuric acid (8).

Each spot was scraped separately, dissolved in chilled methanol and subjected to spectrophotometric analysis at 360 nm (9). The concentrations of aflatoxins were calculated using molar extinction coefficients. Other isomers were non-detectable. The dried aflatoxin extract containing B₁, B₂, G₁ and G₂ in the ratio of 8 : 3 : 2 : 1, respectively, was used in olive oil carrier for treating the experimental animals. As different isomers of aflatoxins exist together in the form of mixture under natural conditions, we preferred to carry out the experiments with mixed aflatoxins.

Young inbred Swiss strain male albino mice (Mus musculus) weighing approximately 32-34 g were obtained from Cadila Health Care, Ahmedabad, India. Animals were provided with animal feed (prepared as per the formulation given by the National Institute of Occupational Health, Ahmedabad, India) and water ad-libitum and maintained under laboratory conditions.

Seventy male mice were randomly divided into seven groups and caged separately. Group 1 (untreated control) animals were maintained without any treatment. Animals of Group 2 and 3 received olive oil (0.2 mL/animal/day) and vitamin E (2 mg/0.2 mL olive oil/animal/day), respectively, for 45 days. Animals of Group 4 and 5 were orally administered with 25 and 50 mg aflatoxin/0.2 mL of olive oil/animal/day (750 and 1500 mg/kg body weight), respectively, for 45 days. In addition to aflatoxin treatment as mentioned for Group 4 and 5 animals, animals of Group 6 and 7 also received vitamin E (2 mg/0.2 mL of olive oil/animal/day) orally 1 h prior to the administration of aflatoxin for 45 days.

For administration, aflatoxin and vitamin E were dissolved in olive oil. Hence, it was used as a vehicle in Group 2 animals. Vitamin E (2 mg/animal/day) was administered in Group 3 animals to see its effect alone, if any. The dose of aflatoxin was based on LD₅₀ value (9 mg/kg body weight) (10). The effective dose of vitamin E was based on earlier work (11). Duration of the treatment (45 days) was based on cumulative toxicity of aflatoxin and duration of spermatogenesis and spermiation (12).

On completion of the treatment, mice were sacrificed by cervical dislocation. The caput and cauda epididymis were quickly isolated, blotted free of blood, weighed to the nearest mg and the relative weight was calculated.

Histological studies were carried out using the standard technique of hematoxylin and eosin staining. The caput and cauda epididymis were fixed for 18 h in alcoholic Bouin’s fixative. The tissue were dehydrated by passing through ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. 5 µm thick sections were cut on a rotary microtome and stained with Ehrlich’s hematoxylin-eosin (alcohol soluble), dehydrated in alcohol, cleared in xylene, mounted in DPX and examined microscopically.

The concentration of protein was measured by the method of Lowry et al. (13). Sialic acid concentration was estimated in the testis by the method of Jourdain et al. (14). The activity of succinic dehydrogenase was measured by the method of Beatty et al. (15). Adenosine triphosphatase was measured by the method of Quinn and White (16). The inorganic phosphate content released at the end of the reaction was measured by the method of Fiske and Subbarow (17). The acid phosphatase activity was assayed by the method of Bessey et al. (18).

The Student t-test was used for statistical analysis of the data and p < 0.05 was considered significant. Comparisons of p-values were performed between different groups.

RESULTS AND DISCUSSION

Oral administration of aflatoxin for 45 days caused, as compared with control, significant, dose-dependent reductions in absolute and relative weight, protein and sialic acid contents in the caput epididymis of aflatoxin-treated mice. Vitamin E pretreatment significantly ameliorated aflatoxin-induced changes only in low dose aflatoxin-treated mice (Table 1).

Activities of adenosine triphosphatase and succinic dehydrogenase were significantly lower in the caput epididymis of aflatoxin-treated mice as com-
Table 1. Effect of pretreatment with vitamin E on aflatoxin-induced changes in caput epididymis of mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups*</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute weight (mg)</td>
<td></td>
<td>19.59 ± 0.48</td>
<td>19.77 ± 0.43</td>
<td>19.48 ± 0.41</td>
<td>17.90 ± 0.48</td>
<td>16.47 ± 0.47</td>
<td>20.70 ± 0.47</td>
<td>17.62 ± 0.46NS</td>
</tr>
<tr>
<td>Relative weight (mg/100 g body weight)</td>
<td></td>
<td>56.62 ± 0.29</td>
<td>58.89 ± 0.28</td>
<td>57.68 ± 0.32</td>
<td>54.32 ± 0.39***</td>
<td>52.16 ± 0.35***</td>
<td>55.60 ± 0.31**</td>
<td>53.11 ± 0.33NS</td>
</tr>
<tr>
<td>Protein (mg/100 mg tissue weight)</td>
<td></td>
<td>11.42 ± 0.38</td>
<td>11.60 ± 0.41</td>
<td>11.90 ± 0.48</td>
<td>8.93 ± 0.37***</td>
<td>7.59 ± 0.37***</td>
<td>10.87 ± 0.38**</td>
<td>8.70 ± 0.39NS</td>
</tr>
<tr>
<td>Sialic acid (µg/mg tissue weight)</td>
<td></td>
<td>6.94 ± 0.36</td>
<td>7.28 ± 0.31</td>
<td>7.73 ± 0.35</td>
<td>5.27 ± 0.44**</td>
<td>4.67 ± 0.49**</td>
<td>7.28 ± 0.26**</td>
<td>5.67 ± 0.38NS</td>
</tr>
<tr>
<td>Adenosine triphosphatase (µmol i.p. released/mg protein/15 min)</td>
<td></td>
<td>1.54 ± 0.04</td>
<td>1.58 ± 0.03</td>
<td>1.62 ± 0.03**</td>
<td>1.37 ± 0.03**</td>
<td>1.19 ± 0.02***</td>
<td>1.62 ± 0.05***</td>
<td>1.30 ± 0.02 NS</td>
</tr>
<tr>
<td>Succinic dehydrogenase activity (µg formazan formed/mg protein/15 min)</td>
<td></td>
<td>15.76 ± 0.41</td>
<td>15.44 ± 0.80</td>
<td>15.99 ± 0.53</td>
<td>11.74 ± 0.42***</td>
<td>10.46 ± 0.47***</td>
<td>13.92 ± 0.33***</td>
<td>12.46 ± 0.60***</td>
</tr>
<tr>
<td>Acid phosphatase activity (µmoles p-nitrophenol released/mg protein/30 min)</td>
<td></td>
<td>0.295 ± 0.008</td>
<td>0.305 ± 0.010</td>
<td>0.304 ± 0.010</td>
<td>0.375 ± 0.008***</td>
<td>0.342 ± 0.010***</td>
<td>0.320 ± 0.007***</td>
<td>0.404 ± 0.008***</td>
</tr>
</tbody>
</table>

*Group 1: untreated control; Group 2: olive oil control; Group 3: Vitamin E control; Group 4: AF-25 µg treated; Group 5: AF-50 µg treated; Group 6: AF-25 µg treated + vitamin E; Group 7: AF-50 µg treated + vitamin E. Values are the mean ± SEM, n = 10

As compared to control a*: p < 0.05; a**: p < 0.01; a***: p < 0.001 (Student t-test)
As compared to group 4 b*: p < 0.02; b**: p < 0.01; b***: p < 0.001
As compared to control 5 c*: p < 0.05; c**: p < 0.02; c***: p < 0.001
NS = Non-significant
Table 2. Effect of pretreatment with vitamin E on aflatoxin-induced changes in cauda epididymis of mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Absolute weight (mg)</td>
<td>18.49 ± 0.54</td>
</tr>
<tr>
<td>Relative weight (mg/100 gm body weight)</td>
<td>53.71 ± 0.49</td>
</tr>
<tr>
<td>Protein (mg/100 mg tissue weight)</td>
<td>14.60 ± 0.33</td>
</tr>
<tr>
<td>Sialic acid (mg/mg tissue weight)</td>
<td>7.71 ± 0.35</td>
</tr>
<tr>
<td>Adenosine triphosphatase</td>
<td>1.61 ± 0.01</td>
</tr>
<tr>
<td>Succinic dehydrogenase activity (µg of formazan formed/mg protein(15 min))</td>
<td>17.18 ± 0.57</td>
</tr>
<tr>
<td>Acid phosphatase activity</td>
<td>0.258 ± 0.04</td>
</tr>
</tbody>
</table>

*Group designation – see Table 1. Values are the mean ± SEM, n = 10
As compared to control a*: p < 0.05; a**: p < 0.02; a***: p < 0.01; a****: p < 0.001 (Student’s t-test)
As compared to group 4 b*: p < 0.05; b**: p < 0.02; b***: p < 0.01; b****: p < 0.001
As compared to group 5 c*: p < 0.05; c**: p < 0.01
NS= Non-significant
Vitamin E ameliorates aflatoxin-induced alterations in the epididymis of mice compared to the control. However, the activity of acid phosphatase was significantly higher, as compared to the control, in aflatoxin-treated mice. Vitamin E pretreatment significantly ameliorated aflatoxin-induced changes in the activities of adenosine triphosphatase, succinic dehydrogenase and acid phosphatase (Table 2).

The caput epididymis of control animals consisted of several tubules with pseudostratified epithelium having stereocilia. The intertubular interstitium was observed between the tubules. The aflatoxin treatment for 45 days brought about significant structural alterations in the caput epididymis of mice. The pyknosis of epithelial cell nuclei was observed. The stereocilia showed clumping. The lumen was devoid of sperm bundles. Pretreatment with vitamin E for 45 days caused significant amelioration in aflatoxin-induced changes in caput epididymis. The epithelium was well organized with distinct nuclei. The epithelium showed the presence of stereocilia and sperm bundles were seen in the tubular lumen (Figure 1).

Oral administration of aflatoxin for 45 days caused significant, dose-dependent reduction in absolute and relative weights of cauda epididymis in mice as compared with that of vehicle control. Vitamin E pretreatment significantly ameliorated aflatoxin-induced changes in absolute weight of cauda epididymis only in low dose aflatoxin-treated group as compared to that of low dose aflatoxin alone-treated group (Table 2).

As shown in Table 2, aflatoxin treatment for 45 days caused dose-dependent significant reduction in protein concentration as compared to the controls. However, the concentration of sialic acid was significantly reduced only in high dose aflatoxin-treated group of mice as compared to the controls. Activities of adenosine triphosphatase and succinic dehydrogenase were significantly lower in cauda epididymis of aflatoxin-treated mice as compared to the controls. However, there was dose-dependent significant increase in the activity of acid phosphatase in aflatoxin-treated mice as compared to the controls. Vitamin E pretreatment significantly ameliorated aflatoxin-induced changes in activities of adenosine triphosphatase, succinic dehydrogenase and acid phosphatase (only in low dose group) as compared to the animals given aflatoxin alone.

The cauda epididymis of control animals consisted of many tubules with a larger diameter in comparison to the caput epididymis, with a pseudostratified epithelium having stereocilia. The intertubular interstitium was present between tubules. The lumen of the tubules contained sperm bundles.

The aflatoxin treatment for 45 days caused significant structural alterations in the tubules showed degeneration with pyknosis of epithelial cell nuclei, disorganization of epithelium and clumping of stereocilia in the tubules. The lumen was devoid of sperm bundles. Pre-treatment with vitamin E for 45 days...
The lumen is filled with fluid and spermatozoa and under normal conditions in many mammals makes up approximately 50% of the weight of tissue (12), decreases in epididymal weight are usually associated with decreases in epididymal sperm reserve. Thus reduction in sperm bundles in both caput and cauda epididymis might be responsible for reduction in weight of epididymis.

The aflatoxin treatment caused significant, dose-dependent reductions in protein and sialic acid content in both caput and cauda epididymis of mice (Tables 1, 2). The decline in protein concentration in epididymis of aflatoxin-treated mice could be due to a decline in protein biosynthesis by forming adducts with DNA, RNA and proteins, inhibition of RNA synthesis, DNA-dependent RNA polymerase activity as well as degranulation of endoplasmic reticulum (2). In addition, oxidative DNA damage and lipid peroxidation are also manifestations of aflatoxin-induced toxicity (19, 20). Degenerative changes observed in histopathological studies could also be due to oxidative damage.

Sialic acid is a sialomucoprotein essential for the maintenance of the structural integrity of the sperm membrane and for sperm maturation (21). Epididymal plasma contains high concentration of proteins and sialic acid which helps in sperm maturation and sperm fertilizing capacity (22). Therefore, reductions in total proteins and sialic acid in the epididymis could be responsible for morphological abnormalities observed in cauda epididymal spermatozoa.

The reduction in succinic dehydrogenase and ATPase activity in the epididymis could explain reduced sperm motility and increased number of non-viable spermatozoa observed in aflatoxin-treated mice. A decrease in succinic dehydrogenase, a key mitochondrial enzyme, in caput and cauda epididymis indicates a reduction in aerobic oxidation (23). Reduced aerobic oxidation and ATP generation in the epididymis could be responsible for reduction of ATPase activity. The reduced SDH and ATPase activity could explain the reduced sperm count, sperm motility and increased number of non-viable spermatozoa observed in aflatoxin-treated mice.

Acid phosphatase is very important for the tissues reorganization and tissue repair. Intracellularly acid phosphatase activity is restricted to lysosomes. Increases of acid phosphatase activity in the epididymis of aflatoxin-treated mice could be due to the increased leakage of enzymes.

Epididymis is an androgen-dependent organ. The significant reduction in serum testosterone dur-
Vitamin E ameliorates aflatoxin-induced alterations in the epididymis of mice

Aflatoxicosis has been reported by Bashandy et al. (24) and Verma and Nair (25). Therefore, alterations in the epididymis of aflatoxin-treated mice could be due to direct effect of aflatoxin on the epididymis and/or indirect effect through reduction in serum testosterone concentration.

Vitamin E pretreatment significantly ameliorated aflatoxin-induced alterations in the epididymis of mice. Vitamin E is a potent biological antioxidant. The antioxidative function of vitamin E is mainly due to its reaction with membrane phospholipid bilayers to break the chain reaction initiated by hydroxyl radicals. In addition, vitamin E has higher affinity for aflatoxin and acts by reducing bioavailability of aflatoxin by forming stable associates with it (26).

In parts of India, 100% of maize samples have been found contaminated with aflatoxin in the range of 6,250-15,600 µg/kg (27). Also, farmers and farm workers may be exposed to potentially hazardous concentration of AFB1 particularly during bin cleaning and animal feeding in enclosed chambers (28).

CONCLUSION

It can be concluded that vitamin E pretreatment significantly ameliorates aflatoxin-induced histopathological and biochemical changes in the caput and cauda epididymides of mice.

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REFERENCES


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