Aflatoxins are secondary toxic fungal metabolites produced by Aspergillus flavus and Aspergillus parasiticus. They not only contaminate our foodstuffs but are 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 (1, 2).

Aflatoxins are well known for its hepatotoxic and hepatocarcinogenic effects (3). Aflatoxin B₁ (AFB₁) is activated to AFB₁-8,9-oxide and forms adduct primarily at N7 position of guanine and is responsible for its mutagenic and carcinogenic effects (4, 5). In addition, lipid-peroxidation and oxidative DNA damage are also the manifestations of aflatoxin B₁-induced toxicity. Souza et al. (6) have reported significant rise in lipid peroxidation in the liver of rats 72 h after a single intraperitoneal dose of AFB₁. Shen et al. (7) have also reported significantly raised aflatoxin B₁-induced lipid peroxidation in rat liver. Seminiferous tubules derangement in the testis of aflatoxin-treated mice was reported by Nair and Verma (8). A significant reduction in epididymal sperm motility, count and fertility rate was also observed. This may be due to oxidative stress, which is generally correlated with cellular damage (6, 9). The increased lipid peroxidation in liver and kidney of aflatoxin-treated animals was also reported (10).

Curcumin, the most active compound of turmeric has antioxidative properties. It is one of the most potent free radical scavengers (11). Curcumin exerts its protective effect against nicotine-induced lung toxicity by modulation of the biochemical marker enzymes, lipid peroxidation and augmenting antioxidant defense mechanism (12). In our earlier studies we reported ameliorative effect of curcumin on aflatoxin-induced hemolysis in vitro (13).

The present investigation was an attempt to evaluate the possible ameliorative effect of curcumin on aflatoxin-induced lipid peroxidation in liver, kidney and testis of mice *in vitro*. Tissues were collected from healthy Swiss strain male albino mice *Mus musculus* weighing 30-35 g. The homogenates were treated with aflatoxin (2-10 mg/mL) with and without curcumin (25-200 mg/mL). The results revealed that addition of aflatoxin (2-10 mg/mL) to homogenates caused significant increase in lipid-peroxidation which was maximal at 6 mg/mL aflatoxin concentration. However, concurrent addition of aflatoxin (6 mg/mL) and curcumin (25-200 mg/mL) caused concentration-dependent amelioration in aflatoxin-induced lipid peroxidation.

**Keywords:** aflatoxin, curcumin, amelioration, lipid peroxidation.

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**EXPERIMENTAL**

Aflatoxin was produced by growing *Aspergillus parasiticus* (NRRL 3240) on SMKY liquid medium for 10 days at 28 ± 2°C as described by Diener and Davis (14). The obtained culture filtrates were extracted with chloroform and concentration of aflatoxin was quantified spectrophotometrically (15).

Curcumin was purchased from Hi-Media Laboratories Pvt. Ltd. Mumbai, India. Liver, kidney and testis were collected from healthy Swiss strain male albino mice *Mus musculus* weighing 30-35 g.

The effect of curcumin on aflatoxin-induced lipid-peroxidation *in vitro* was measured in liver, kidney and testis homogenates by quantification of thiobarbituric acid (TBA) reactive substances in accordance with the method described by Ohkawa et al. (16).
Liver, kidney and testis homogenates (10%) were prepared in 0.1 M phosphate buffered saline (pH 7.4).

(A) Reaction mixture contained 0.2 mL of homogenate, 10 mM H₂O₂ and varying concentrations of aflatoxin solution (2–10 mg/mL) in saline.

(B) Other tubes containing curcumin control (200 mg/mL).

(C) Set of tubes containing curcumin of different concentrations (25-200 mg/mL) along with aflatoxin (6 mg/mL).

Reaction was initiated by addition of H₂O₂ and the mixture was incubated at 37°C for 30 min with occasional shaking. Lipid peroxides (TBARS) were estimated using 8.1% sodium dodecyl sulfate, 20% acetic acid and 1% thiobarbatic acid solution. Care was taken to adjust the pH of 20% acetic acid to 3.5 using 1 M NaOH. The blank for each sample was prepared by substituting the TBA solution with distilled water. The final volume was adjusted with 0.1 M phosphate buffered saline. The solution was mixed and heated in a water bath at 95°C for 60 min. The tubes were immediately cooled and aliquots were centrifuged at 1000 g for 15 min. The absorbance of the resulting supernatant fraction was read at 532 nm against blank on a Systronics 118 UV-Vis spectrophotometer at 532 nm. The results were expressed as TBARS formed/mg protein/60 min.

All chemicals used were of analytical grade. Data were expressed as the means ± S.E.M. Statistical analysis was performed using Student’s t test.

RESULTS AND DISCUSSION

Table 1 shows the effect of aflatoxin on production of thiobarbatic acid reactive substances (TBARS) in liver, kidney and testis homogenates in vitro. Percent changes in lipid peroxidation are shown in Figure 1. The results revealed that the H₂O₂-induced lipid peroxidation increased as the concentration of aflatoxin was increased (2-10 mg/mL) in liver, kidney and testis homogenates and it remained always higher than in the control. An increase was significantly different from the control. The maximal increase was at 6 mg/mL.

Table 2 shows the effect of curcumin on aflatoxin-induced lipid-peroxidation in homogenates of
Curcumin ameliorates aflatoxin-induced lipid peroxidation in liver, kidney and testis. The results revealed that concurrent addition of curcumin (25-200 µg/mL) and aflatoxin (6 mg/mL) caused significant amelioration in aflatoxin-induced lipid peroxidation. The effect was concentration-dependent with maximum amelioration at 200 mg/mL of curcumin (Fig. 2).

The present study (in vitro) revealed that the addition of aflatoxin to liver, kidney and testis homogenates caused significant, persistent and concentration-dependent increase in H₂O₂ induced TBARS (Table 1) suggesting that it causes lipid peroxidation. It is known that H₂O₂ is an oxidant, which can form free radicals with ferrous ion and with oxygen (Haber Weiss reaction).

The concurrent addition of curcumin and aflatoxin to liver, kidney and testis homogenates significantly reduced aflatoxin-induced lipid peroxidation (Table 2). This could be due to antioxidative property of curcumin. The results revealed that the addition of curcumin to homogenates results in a decrease in lipid peroxidation. The effect was concentration-dependent. One of the studies showed curcumin to be eight times more powerful antioxidative agent than that of vitamin E in preventing lipid peroxida-
Several studies have demonstrated curcumin ability to reduce oxidative stress (17-19).

CONCLUSION

It can be concluded that curcumin acts as effective compound playing an important role in ameliorating aflatoxin-induced lipid-peroxidation in liver, kidney and testis homogenates due to its antioxidative property.

REFERENCES


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