

CURCUMIN AMELIORATES AFLATOXIN-INDUCED LIPID-PEROXIDATION IN LIVER AND KIDNEY OF MICE

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Abstract: The present investigation was an attempt to evaluate the ameliorative effect of curcumin on aflatoxin-induced lipid peroxidation in liver and kidney of mice. Aflatoxin was obtained by growing *Aspergillus parasiticus* in SMKY liquid medium. Pure curcumin (97% purity) was purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. Young adult male albino mice were orally administered with low dose and high dose (750 and 1500 µg/kg body weight) with and without curcumin (2 mg/0.2 mL olive oil/animal/day) for 45 days. On 46th day the animals were sacrificed by cervical dislocation. Liver and kidney were removed and weighed. Homogenates were prepared for measuring lipid-peroxidation along with changes in catalase, superoxide dismutase, glutathione, glutathione-peroxidase and total ascorbic acid. The results revealed concentration dependent increase in lipid peroxidation along with reduction in enzymatic and non-enzymatic antioxidants. Treatment with curcumin along with aflatoxin ameliorates aflatoxin-induced lipid peroxidation in liver and kidney of mice by ameliorating both enzymatic and non-enzymatic antioxidants.

Keywords: aflatoxin, lipid peroxidation, curcumin, liver, kidney

Presently the world is facing a drastic problem regarding diseases. Every now and then we come to know about a new disease and its related causes. But the base of many of the diseases is food/feed stuff contamination which can be caused at any stage of production and storage. These contaminations are basically due to fungi such as aflatoxins.

Aflatoxins are a group of closely related secondary toxic fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*, of relatively low molecular weight organic compounds characterized by their diversity, their frequent specificity and their production during the stationary phase crop in field is undoubtedly influenced by insect damage. *Aspergillus flavus* is also known to infect intact kernels of maize by colonizing and growth down the external silk (1).

Possibly an increase in AFB₁ cause significant increase in hepatic lipid peroxide level. Shen et al. (2) found that AFB₁ induced lipid peroxidation in rat liver and this lipid peroxidation was closely related to liver cell injury. A time- and dose-dependent increase in 8-hydroxy-deoxyguanosine (8-OHdG) was observed in DNA after a single intraperitoneal injection of AFB₁. It indicates that AFB₁ causes oxidative DNA damage in rat liver, which may involve, hydroxyl radicals as the initiating species (3). Therefore, factors interfering with the

generation or action of OH[•] would affect the formation of 8-OHdG.

Verma et al. (4) showed that aflatoxin treatment caused significant rises in intracellular calcium in liver, kidney, testis, adipose tissues, heart and skeletal muscle of rabbits. One of the study (5) suggested that calcium activated catabolic processes are involved in cytotoxicity. Fagian et al. (6) demonstrated that reversible permeabilization induced by calcium plus pro-oxidant is associated with oxidation of membrane protein thiols, forming cross-linked aggregates. Castilho et al. (7) proposed that calcium plus pro-oxidant significantly reduced mitochondrial GSH and NADPH, substrates of the antioxidant enzyme glutathione peroxidase and glutathione reductase respectively favoring accumulation of H₂O₂. Turrens et al. (8) demonstrated that accumulation of calcium in mitochondria mobilized iron which in turn could stimulate the production of OH[•] from H₂O₂.

Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals thereby preventing or delaying damage to the cells and tissues. There are much evidence in highlighting the role of antioxidants which may protect our body against certain conditions such as heart disease, stroke and cancers. It has been proposed that the mechanisms leading to these

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diseases may be promoted by free radicals and that antioxidants may oppose the action of these molecules. Now the concept has come of „eco-friendly antioxidants” which are obtained from nature and its use do not show any side effects and also they are friendly to environment and economically affordable.

One of them is curcumin, an active component of yellow turmeric (*Curcuma longa*). *In vitro* curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H₂O₂ and nitrite radical generation by activated macrophages, which play an important role in inflammation also. Curcumin lowers the production of ROS *in vivo* (9). Curcumin reduces oxidized proteins in amyloid pathology in Alzheimer transgenic mice (10). It also decreases lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates (11). Curcumin shows anti-coagulant activity by inhibiting collagen and adrenaline-induced platelet aggregation *in vitro* as well as *in vivo* in rat thoracic aorta (12).

One study showed curcumin to be eight times more powerful than vitamin E in preventing lipid peroxidation. Taken in group arrangements such as C-complex, curcuminoids are three times as potent in neutralizing free-radical molecules. Several studies have demonstrated curcumin's ability to reduce oxidative stress (13). The ability of curcumin was also examined to protect against lead-induced damage to hippocampal cells of male Wistar rats, as well as lipid peroxidation induced by lead and cadmium in rat brain homogenate (14).

The present investigation was an attempt to evaluate ameliorative activity of curcumin on lipid peroxidation and relatively on superoxide dismutase, catalase, glutathione, glutathione peroxidase and total ascorbic acid in liver and kidney of mice affected by induced-toxicity of aflatoxin in mice.

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 [15]. Culture filtrates were extracted with analytical grade chloroform (1:2, v/v) and passed through a bed of anhydrous sodium sulfate. The chloroform extract was evaporated to dryness and stored. Dried aflatoxin extract was dissolved in fresh chloroform and used for chemical analysis. 100 µL aflatoxin extract was first fractionated on silica gel G coated

activated TLC plates along with aflatoxin standard (a gift from the International Agency for Research on Cancer, Lyon, France). The plates were developed in solvent consisting of toluene : isopentyl alcohol : methanol (90:32:2, v/v) (9). The air-dried plates were observed under long-wave UV light (360 nm) for aflatoxins. Different components of aflatoxins were initially identified usually by comparing the color and intensity of fluorescence as well as polarity of sample spots with standard. 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 (TFA) and 25% sulfuric acid [16].

Each spot was scraped separately, dissolved in chilled methanol and subjected to spectrophotometric analysis according to the method of Nabney and Nesbitt [17] using UV-Vis spectrophotometer. Dried aflatoxin extract containing B₁, B₂, G₁ and G₂ in the ratio of 8:3:2:1, respectively, was used for treating the experimental animals in olive oil carrier.

Curcumin was purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai, India.

Young inbred, Swiss strain male albino mice (*Mus musculus*), weighing approximately 37–40 g, were obtained from Cadila Health Care, Ahmedabad, India. They were provided feed and water *ad libitum* and maintained under laboratory conditions. Seventy such animals were divided into seven groups and caged separately. Group 1 (control) animals were maintained without any treatment. Animals of Group 2 (vehicle control) received olive oil (0.2 mL/animal/day). Animals of Group 3 received curcumin (2 mg/0.2 mL olive oil/animal/day) for 45 days and served as positive controls. Animals of Group 4 and 5 were orally administered aflatoxins at a dose of 25 (low dose) and 50 (high dose) µg/0.2 mL olive oil/animal/day (750 and 1500 µg/kg body weight), respectively, for 45 days. Group 6 and 7 animals were orally administered low dose and high dose of aflatoxin along with curcumin (2 mg/0.2 mL olive oil/animal/day), respectively, for 45 days. All the treatments were given orally using a feeding tube attached to a hypodermic syringe.

On completion of the treatment, the mice were weighed and were sacrificed by cervical dislocation. The liver and kidney were isolated, blotted free of blood, homogenized and used for determination of various parameters. Lipid peroxidation was estimated by the method of Ohkawa et al. (18), superoxide dismutase was estimated by the method of Kakkar et al. (19), Catalase was estimated by the

Table 1. Effect of curcumin on aflatoxin-induced lipid-peroxidation and antioxidant defense mechanisms in liver of mice.

Parameters	Experimental Groups						
	1	2	3	4	5	6	7
	Untreated control	Vehicle control	Curcumin control	Low dose aflatoxin	High dose aflatoxin	Low dose aflatoxin + curcumin	High dose aflatoxin + curcumin
Lipid peroxidation (nmoles MDA/mg protein/60 min)	3.25 ± 0.11	3.26 ± 0.01	3.24 ± 0.04	4.96 ± 0.01 ^{abcde}	6.35 ± 0.11 ^{abcde}	3.74 ± 0.01 ^{abcde}	4.22 ± 0.08 ^{abcde}
Catalase activity (µmoles H ₂ O ₂ consumed/mg protein/min)	40.379 ± 0.116	40.380 ± 0.137	40.382 ± 0.032	29.401 ± 0.095 ^{abcde}	20.462 ± 0.119 ^{abcde}	39.374 ± 0.029 ^{abcde}	32.733 ± 0.022 ^{abcde}
Superoxide dismutase activity (units/mg protein)	5.216 ± 0.001	5.214 ± 0.002	5.218 ± 0.007	2.214 ± 0.001 ^{abcde}	0.845 ± 0.002 ^{abcde}	5.048 ± 0.012 ^{abcde}	4.160 ± 0.007 ^{abcde}
Glutathione peroxidase activity (nmoles NADPH consumed/mg protein/min)	10.215 ± 0.002	10.644 ± 0.002	10.842 ± 0.002	7.216 ± 0.002 ^{abcde}	4.244 ± 0.003 ^{abcde}	9.928 ± 0.003 ^{de}	8.789 ± 0.001 ^e
Glutathione (µg/100 mg tissue weight)	95.72 ± 0.27	95.74 ± 0.23	96.77 ± 0.28	70.33 ± 0.26 ^{abcde}	55.23 ± 0.23 ^{abcde}	92.38 ± 0.31 ^{abcde}	79.73 ± 0.34 ^{abcde}
Total ascorbic acid (mg/g tissue weight)	16.94 ± 0.03	16.97 ± 0.02	16.99 ± 0.01	9.51 ± 0.02 ^{abcde}	6.32 ± 0.02 ^{abcde}	15.41 ± 0.04 ^{abcde}	12.64 ± 0.02 ^{abcde}

Values are the mean ± S.E.M.; n = 10; ^a as compared to group 1, p < 0.05; ^b as compared to group 2, p < 0.05; ^c as compared to group 3, p < 0.05; ^d as compared to group 4, p < 0.05; ^e as compared to group 5, p < 0.05; ^f as compared to group 6, p < 0.05; ^g as compared to group 7, p < 0.05.

method of Luck (20), Glutathione was estimated by the method of Grunert and Philips (21), Glutathione peroxidase was estimated by the method of Pagila and Valentine (22) and total ascorbic acid was estimated by the method of Roe and Kuether (23). For all the parameters a minimum of 10 replicates were used and the data were statistically analyzed using one way analysis of variance (ANOVA) followed by Tukey test. The levels of significance was accepted with p < 0.05.

RESULTS AND DISCUSSION

Table 1 shows the effects of aflatoxin and aflatoxin plus curcumin on lipid peroxidation and activities of catalase, superoxide dismutase and glutathione peroxidase as well as glutathione and total ascorbic acid contents in the liver of mice. Also percent changes from vehicle control (Group 2) are shown in Figures 1.1 – 1.6, respectively. The results revealed no significant alterations between different groups of controls (Groups 1 – 3).

The level of lipid peroxidation was significantly higher, as compared with vehicle control (Group 2), in aflatoxin-treated mice. The effect was dose-dependent (LD: 52.14%; HD: 94.78%; Figure 1.1). As compared to the vehicle control (Groups 2), aflatoxin treatment for 45 days caused significant reduction in the activities of catalase (LD: 27.19%; HD: 49.33% Figure 1.2), superoxide dismutase (LD: 57.54%; HD: 83.80%; Figure 1.3) and glutathione peroxidase (LD: 32.21%, HD: 60.13%; Figure 1.4), as well as glutathione (LD: 26.55%; HD: 42.32%; Figure 1.5) and total ascorbic acid (LD: 43.96%; HD: 62.76%; Figure 1.6) contents in the liver of mice. The effect was almost dose-dependent. Thus, aflatoxin treatment caused dose-dependent increase in lipid peroxidation by decreasing the antioxidative defense mechanism of the cell.

Oral administration of curcumin along with aflatoxin significantly ameliorates aflatoxin-induced lipid peroxidation. This could be due to the significantly higher activities of superoxide dismutase,

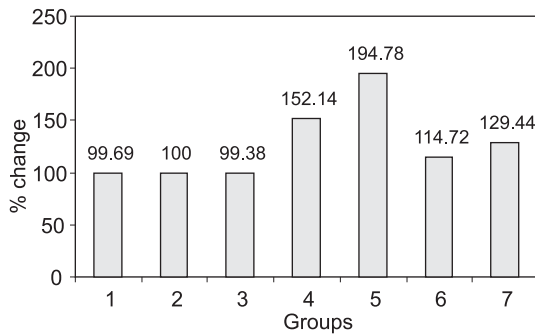


Figure 1.1. Percent change in lipid peroxidation (from vehicle control) in liver.

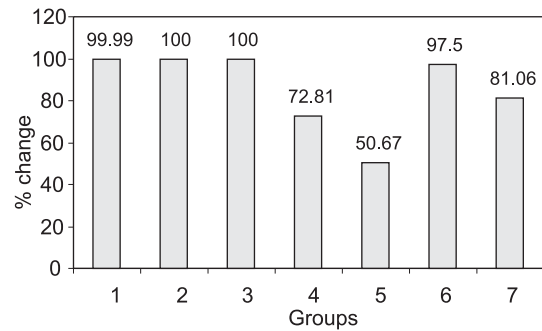


Figure 1.2. Percent change in catalase activity (from vehicle control) in liver.

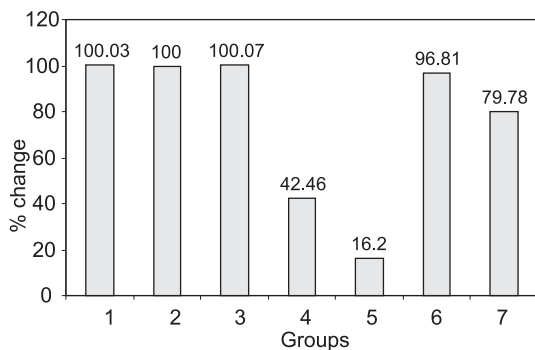


Figure 1.3. Percent change in superoxide dismutase activity (from vehicle control) in liver.

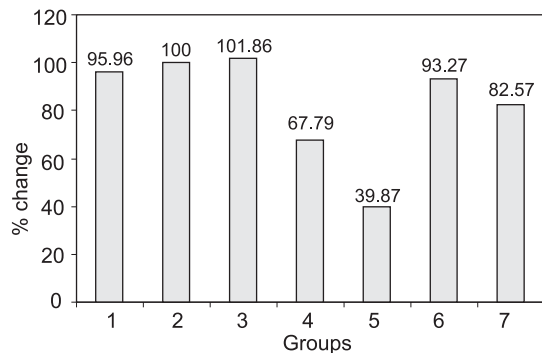


Figure 1.4. Percent change in glutathione peroxidase activity (from vehicle control) in liver.

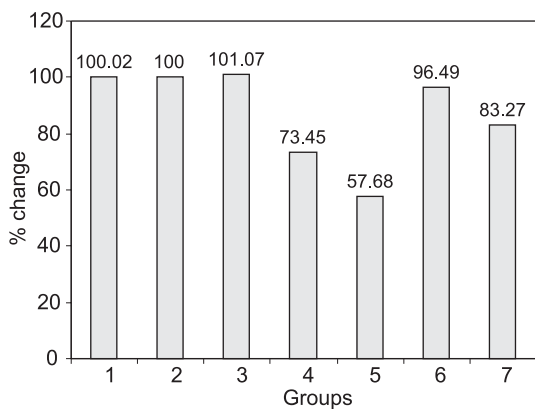


Figure 1.5. Percent change in glutathione content (from vehicle control) in liver.

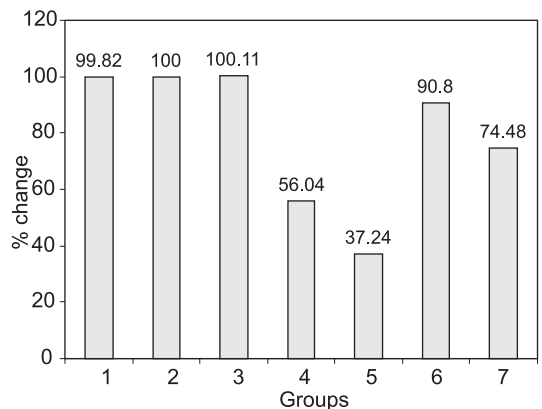


Figure 1.6. Percent change in total ascorbic acid content (from vehicle control) in liver.

catalase and glutathione peroxidase, as well as glutathione and total ascorbic acid contents in the liver of mice receiving aflatoxin plus curcumin (Groups 6 and 7) as compared with those groups treated with aflatoxin alone (Groups 4 and 5).

Table 2 shows the effect of aflatoxin and aflatoxin plus curcumin on lipid peroxidation and activities of catalase, superoxide dismutase and glutathione peroxidase, as well as glutathione and total ascorbic acid contents in the kidney of

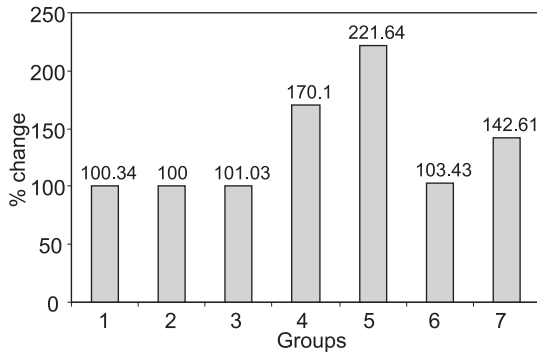


Figure 2.1. Percent change in lipid peroxidation (from vehicle control) in kidney.

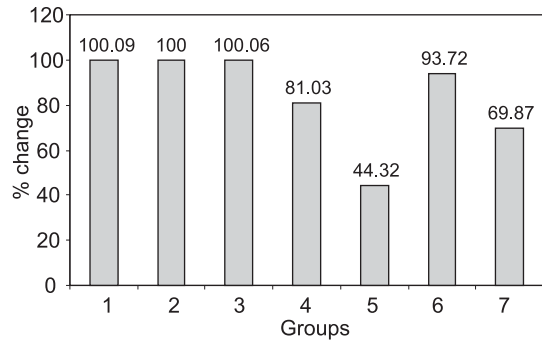


Figure 2.2. Percent change in catalase activity (from vehicle control) in kidney.

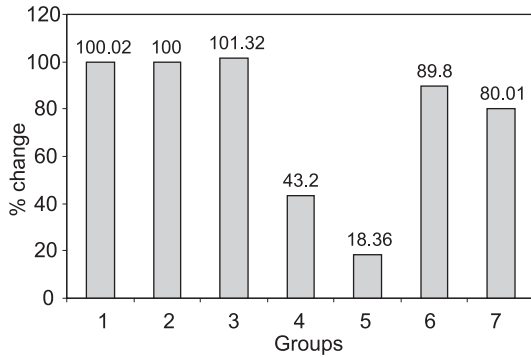


Figure 2.3. Percent change in superoxide dismutase activity (from vehicle control) in kidney.

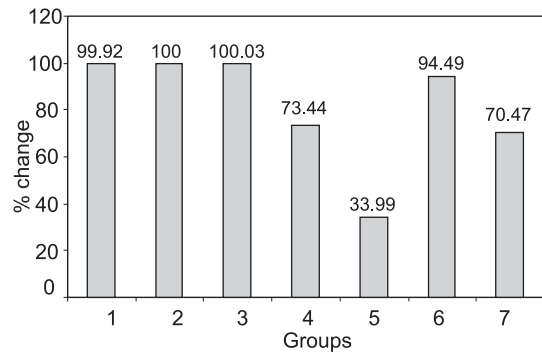


Figure 2.4. Percent change in glutathione peroxidase activity (from vehicle control) in kidney.

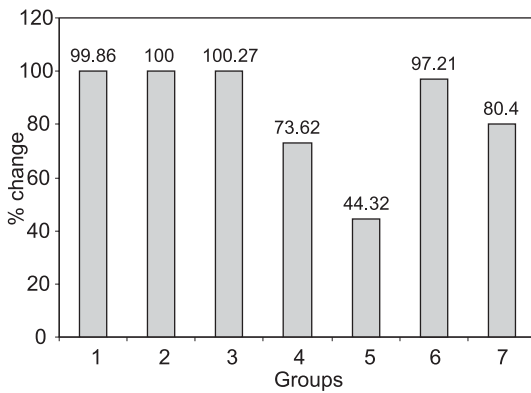


Figure 2.5. Percent change in glutathione content (from vehicle control) in kidney.

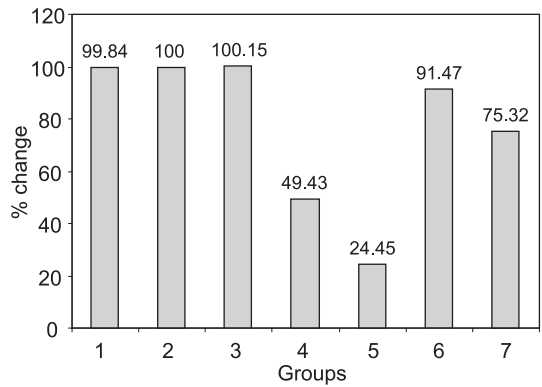


Figure 2.6. Percent change in total ascorbic acid content (from vehicle control) in kidney.

mice. Also percent changes from vehicle control (Group 2) are shown in Figures. 2.1 – 2.6, respectively. The results revealed no significant alterations between different groups of controls (Groups 1 – 3).

The level of lipid peroxidation was significantly higher, as compared with vehicle control (Group 2), in aflatoxin-treated mice. The effect was dose-dependent (LD: 70.10%; HD: 121.64%; Figure 2.1). As compared to vehicle control (Group 2),

Table 2. Effect of curcumin on aflatoxin-induced lipid-peroxidation and antioxidative defense mechanisms in kidney of mice.

Parameters	Experimental Groups						
	1	2	3	4	5	6	7
	Untreated control	Vehicle control	Curcumin control	Low dose aflatoxin	High dose aflatoxin	Low dose aflatoxin + curcumin	High dose aflatoxin + curcumin
Lipid peroxidation (nmoles MDA/mg protein/60 min)	2.92 ± 0.08	2.91 ± 0.08	2.94 ± 0.04	4.95 ± 0.01 ^{abcdfg}	6.45 ± 0.09 ^{abcdfg}	3.01 ± 0.01 ^{cdteg}	4.15 ± 0.03 ^{abcdef}
Catalase activity (m moles H ₂ O ₂ consumed/mg protein/min)	32.56 ± 0.19	32.53 ± 0.12	32.55 ± 0.13	26.36 ± 0.29 ^{abcdfg}	14.42 ± 0.21 ^{abcdfg}	30.49 ± 0.10 ^{abcdeg}	22.73 ± 0.07 ^{abcdef}
Superoxide dismutase activity (units/mg protein)	7.774 ± 0.134	7.772 ± 0.058	7.875 ± 0.041	3.358 ± 0.077 ^{abcdfg}	1.427 ± 0.091 ^{abcdfg}	6.980 ± 0.227 ^{bcdeg}	6.219 ± 0.122 ^{abcdef}
Glutathione peroxidase activity (nmoles NADPH consumed/mg protein/min)	6.314 ± 0.001	6.319 ± 0.002	6.321 ± 0.003	4.641 ± 0.002 ^{abcdfg}	2.148 ± 0.003 ^{abcdfg}	5.971 ± 0.008 ^{abcdeg}	4.453 ± 0.011 ^{abcdef}
Glutathione (mg/100 mg tissue weight)	75.38 ± 0.31	75.48 ± 0.32	75.69 ± 0.38	55.57 ± 0.25 ^{abcdfg}	33.46 ± 0.15 ^{abcdfg}	73.38 ± 0.37 ^{abcdeg}	60.69 ± 0.26 ^{abcdef}
Total ascorbic acid (mg/g tissue weight)	13.23 ± 0.01	13.25 ± 0.02	13.27 ± 0.02	6.55 ± 0.03 ^{abcdfg}	3.24 ± 0.03 ^{abcdfg}	12.12 ± 0.02 ^{abcdeg}	9.98 ± 0.02 ^{abcdef}

Values are the mean ± S.E.M.; n = 10; ^a as compared to group 1, p < 0.05; ^b as compared to group 2, p < 0.05; ^c as compared to group 3, p < 0.05; ^d as compared to group 4, p < 0.05; ^e as compared to group 5, p < 0.05; ^f as compared to group 6, p < 0.05; ^g as compared to group 7, p < 0.05.

aflatoxin treatment for 45 days caused significant reduction in the activities of catalase (LD: 18.97%; HD: 55.68%; Figure 2.2), superoxide dismutase (LD: 56.80%; HD: 81.64%; Figure 2.3) and glutathione peroxidase (LD: 26.56%; HD: 66.01%; Figure 2.4), as well as glutathione (LD: 26.38%; HD: 55.68%; Figure 2.5) and total ascorbic acid (LD: 50.57%; HD: 75.55%; Figure 2.6) contents in the kidney of mice. The effect was almost dose-dependent. Thus, aflatoxin treatment caused dose-dependent increase in lipid peroxidation by decreasing the antioxidative defense mechanism of the cell.

Oral administration of curcumin along with aflatoxin significantly ameliorates aflatoxin-induced lipid peroxidation. This could be due to significantly higher activities of superoxide dismutase, catalase, glutathione peroxidase, as well as glutathione and total ascorbic acid contents (Groups 6 and 7), as compared with those given aflatoxin alone (Groups 4 and 5) (Table 2).

Oral administration of aflatoxin for 45 days caused significant increase in lipid peroxidation in liver and kidney of aflatoxin-treated mice, as compared to controls. Lipid peroxidation is regarded as one of the primary key events in cellular damage (24) and the relationship between GSH levels, lipid peroxidation and cell lysis has been reported (25). Carcinogens like aflatoxin B₁, which generate epoxides, have been found to conjugate readily with GSH (26). Liver cells, which are lethally injured by several toxins, exhibit marked alterations in intracellular Ca²⁺ homeostasis after excessive accumulation of Ca²⁺ (5). During hepatocellular necrosis, excessive intracellular Ca²⁺ is known to thrust the metabolism in an unmanageable disorder, which leads to mitochondrial dysfunction, inhibition of enzymes and denaturation of structural proteins (6). Verma et al. (4) showed that aflatoxin treatment caused significant increase in intracellular calcium in the liver and kidney of rabbits. Toskulkaio and Glinsukon (27) reported that excessive accumulation of hepatic

intracellular Ca^{2+} might be responsible for potentiation of hepatotoxicity in rats treated with both ethanol and AFB_1 . It is secondary effect of the dysfunction of mitochondria caused by lipid peroxidation and reduction of hepatic adenosine triphosphatase activity, which participate in Ca^{2+} extrusion and uptake mechanisms (28). The increased lipid peroxidation in aflatoxin-treated animals is in agreement with findings reported previously for rat liver (29, 30) as well as liver, kidney (31) and testis (32) in mice.

Oxidative stress was originally defined as the disequilibrium between prooxidants and antioxidants in biological systems (33). Therefore, an increase in lipid peroxidation could be due to significant reduction in the activities of enzymatic antioxidants such as catalase, superoxide dismutase and glutathione peroxidase, as well as non-enzymatic antioxidants such as glutathione and total ascorbic acid contents in the liver and kidney of aflatoxin-treated mice, as compared to the controls. Superoxide dismutase protects cells from oxidative damage by breaking down a potentially hazardous free radical superoxide ($\text{O}_2^{\cdot-}$) to H_2O_2 and O_2 . The H_2O_2 produced can then be decomposed enzymatically by catalase and glutathione peroxidase (GSH-Px). Glutathione peroxidase not only decomposes H_2O_2 but can also interact with lipid peroxidation (34). Thus significant reduction in these enzyme activities (Tables 1 and 2) could be responsible for increased lipid peroxidation observed during aflatoxicosis. Significant reductions in glutathione peroxidase (29), superoxide dismutase (30), and catalase (35) have been reported in aflatoxin-fed rat liver.

The decline in enzymatic antioxidants could be due to the reduction in protein biosynthesis. Aflatoxin impairs protein biosynthesis by forming adducts with DNA, RNA and proteins, inhibits RNA synthesis and DNA-dependent RNA polymerase activity as well as causing degranulation of endoplasmic reticulum (36) as mentioned earlier. In addition, oxidative stress may result in damage to critical cellular macromolecules including DNA, lipids and proteins (2). Cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals which can subsequently propagate into MDA, may result in interaction with cellular DNA-MDA adducts (2). Proteins are also easily attacked by ROS directly or indirectly through lipid peroxidation modifying their enzyme activity (37).

Glutathione content decreased significantly in liver and kidney of aflatoxin-treated mice (Table 1

and 2), suggesting its rapid oxidation. Glutathione has a beneficial effect by virtue of possessing -SH groups. It helps to protect biological membranes, which are readily susceptible to injury by peroxidation (34). The aflatoxin 2,3-epoxide may be inactivated by hydrolysis to the dihydrodiol, either spontaneously or via epoxide hydrolase or by conjugation with glutathione by glutathione-S-transferase. Thus, significantly lower GSH level would further aggravate the toxic effects of aflatoxin. Many investigators (30, 38) have reported significant reduction in glutathione content in aflatoxin-fed rat liver.

Present study shows significant reduction in ascorbic acid content in the liver and kidney of aflatoxin-treated mice (Tables 1 and 2). During free radical scavenging action, ascorbic acid is transformed into L-dehydroascorbate (39). Reduced glutathione is required for the conversion of L-dehydroascorbate back to ascorbate (39). The fall in the level of reduced glutathione decreases the conversion of L-dehydroascorbate to ascorbate and this probably explains the lowered level of ascorbic acid in aflatoxin-treated animals.

Oral administration of curcumin along with aflatoxin for 45 days caused significant amelioration in aflatoxin-induced lipid peroxidation by increasing the antioxidative activity of the cells. Activities of enzymatic antioxidants (catalase, superoxide dismutase and glutathione peroxidase) as well as contents of non-enzymatic antioxidants (glutathione and ascorbic acid) were significantly increased in liver and kidney of aflatoxin plus curcumin-treated mice than in those of aflatoxin alone treated mice (Table 1 and 2).

Curcumin shows many health promoting activities including chemopreventive action during carcinogenesis due to antioxidative polyphenolic constituents (40). Smoke shield, which is a formulation containing extracts of turmeric together with extracts of green tea and other spices, was found to elevate antioxidant enzymes such as catalase and superoxide dismutase in blood as well as in liver and kidney of mice. Glutathione levels were also significantly elevated in blood. Administration of smoke shield decreased the lipid peroxidation in serum, liver and kidney. Sreekanth et al. (41) thus explained that smoke shield had potent antioxidant activity, could inhibit phase I enzymes and increase detoxifying enzymes which makes it an effective chemopreventive herbal formulation.

CONCLUSION

It can be concluded that curcumin being an active component of turmeric plays efficient role in ameliorating the toxicity induced by aflatoxin in liver and kidney of mice by reducing lipid-peroxidation to normal along with normalizing the enzymatic and non-enzymatic activities of superoxide dismutase, catalase, glutathione, glutathione peroxidase and total ascorbic acid.

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