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AMELIORATIVE EFFECT OF CURCUMIN ON AFLATOXIN-INDUCED TOXICITY IN DNA, RNA AND PROTEIN IN LIVER AND KIDNEY OF MICE

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Abstract: The present investigation is an attempt to evaluate the ameliorative effect of curcumin on aflatoxin-induced toxicity in liver and kidney of mice. Aflatoxin was obtained by growing *Aspergillus parasiticus* in SMKY liquid medium. 70 male mice were divided into 7 groups (37-40 g body weight) including untreated control, vehicle control (0.2 mL olive oil/animal/day), curcumin control (50 mg/kg body weight/animal), aflatoxin low dose and high dose (750 and 1500 mg/kg body weight). Other two groups were administered curcumin along with low dose aflatoxin and high dose aflatoxin. The treatment was given for 45 days. On 46th day the animals were sacrificed by cervical dislocation. Liver and kidney were removed and weighed. Homogenates were prepared and analyzed for DNA, RNA and protein content. The results revealed dose-dependent significant reduction in DNA, RNA and protein contents in the liver and kidney of mice. Oral administration of aflatoxin along with curcumin significantly ameliorates, as compared to aflatoxin alone treated groups, in all parameters. It is concluded that curcumin ameliorates aflatoxin-induced toxicity in liver and kidney of mice.

Keywords: aflatoxin, curcumin, liver, kidney, toxicity

Since long time ago, and presently also all over the world, we are facing the principal problem of agriculture related to the storage and contamination of the food/feed stuffs at any stage. Major causes of contaminations are fungi. Aflatoxins (AFs) are secondary toxic fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. These contaminated food/feed stuffs also lead to contaminated animal products such as milk, eggs etc. as when they are consumed by animals.

Aflatoxin B_1 is the most potent mutagen among aflatoxins and a strong parallel correlation exists between the ability of the aflatoxins to be mutagenic and carcinogenic. Microsomal activation is an absolute necessity for mutagenicity with aflatoxins. Aflatoxin B_1 cause chromosomal aberrations and DNA breakage in plant and animal cells (1). Bilgrami and Sinha (2) recorded chromosomal abnormalities such as clumping, fragmentation, meiotic polyploidy and euploidy in mice fed with aflatoxin-contaminated diets. These results underscore the mutagenic potential of the toxin and also recorded dominant lethal mutations in mice. A significant decrease in the fertility of females mated to treated males was observed. This was caused by gross genetic damage (mutation) induced by aflatoxin in the germ cells of treated mates. Yakicier et al. (3) revealed a relationship between chromosome 16q homozygous deletions and R249S p53 mutations in tumors where the patient had been exposed to aflatoxin B_1 ($p = 0.002$).

Loarca et al. (4) reported that AFB_1 is a direct acting mutagen and that ellagic acid inhibits AFB_1 direct acting mutagenicity. It was also observed that comutagenicity of coumarin (1,2-benzopyrene) with AFB_1 and human liver S9 fraction increased due to enhanced AFB_1 bioactivation (5).

Curcumin acts as the most active antioxidant of turmeric. It has been investigated that the food additives such as turmeric when added to the medium containing *Aspergillus parasiticus* inhibited the growth of mycelium. The concentration needed for 50% inhibition was approximately 2.5 mg/mL of the medium (6). Statistically non-significant incidence of structural aberrations and absence of numerical aberrations showed *Curcuma longa* to be neither clastogenic nor mitoclastic. Thus turmeric when incorporated into the diet in different amounts had no adverse cytogenetic and mutagenic effects in the rats test system as it is revealed by the long-term observation for a period of four generations (7). Various studies conducted at the NIN (National Institute for Nutrition), Hyderabad, India, on turmeric and its active principle curcumin suggest

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that it can have impact on all the stages of carcinogenesis. It prevents activation of carcinogens and attack of electrophiles on DNA, acts as antioxidant and antipromoter, retards the conversion of preneoplasia in addition to repairing the damage to DNA. The present investigation was an attempt to evaluate ameliorative effects of curcumin on aflatoxin-induced changes in DNA, RNA and protein in liver and kidney of 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 (8).

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 system toluene : isoamyl alcohol : methanol (90:32:2, v/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 B_1 and B_2 showed blue fluorescent spots; aflatoxin G_1 and G_2 showed bluish-green fluorescent spots. Chemical confirmation of aflatoxins was done by spraying with trifluroacetic acid (TFA) and 25% sulfuric acid (10).

Each spot was scraped separately, dissolved in cold methanol and subjected to UV-Vis spectrophotometric analysis according to the method of Nabney and Nesbitt (11). Dried aflatoxin extract containing B_1, B_2, G_1 and G_2 in the ratio of 8:3:2:1, respectively, was used in olive oil carrier for treating the experimental animals.

Pure curcumin (97% purity) 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/0.2mL 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 aflatoxins along with curcumin (2 mg/0.2mL olive oil/animal/day), respectively, for 45 days. All the treatments were given orally using a feeding tube attached to a hypodermic syringe.

Table 1. Effect of curcumin on aflatoxin-induced biochemical changes in the 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
Deoxyribonucleic acid (µmoles/100 mg tissue weight)	281.29 ± 0.21	281.32 ± 0.21	281.37 ± 0.15	148.98 ± 0.28^{abcefg}	81.01 ± 0.11^{abcdfg}	278.16 ± 0.38^{abcdeg}	197.03 ± 0.26^{abcdef}
Ribonucleic acid (µmoles/100 mg tissue weight)	428.11 ± 0.46	428.09 ± 1.12	428.12 ± 0.23	320.17 ± 0.25^{abcefg}	252.81 ± 0.34^{abcdfg}	420.79 ± 0.33^{abcdeg}	391.17 ± 0.26^{abcdef}
Protein (mg/100 mg tissue weight)	24.35 ± 0.04	24.38 ± 0.16	24.42 ± 0.14	20.11 ± 0.06^{abcefg}	14.15 ± 0.07^{abcdfg}	23.34 ± 0.12^{abcdeg}	21.64 ± 0.11^{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 : $p < 0.05$, as compared to group 6 : $p < 0.05$, ^{g} as compared to group 7 : $p < 0.05$

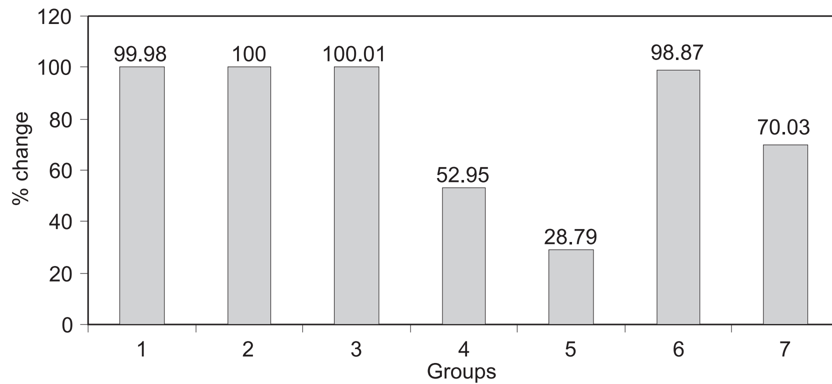

Figure 1. Percent change in DNA content (from vehicle control) in liver

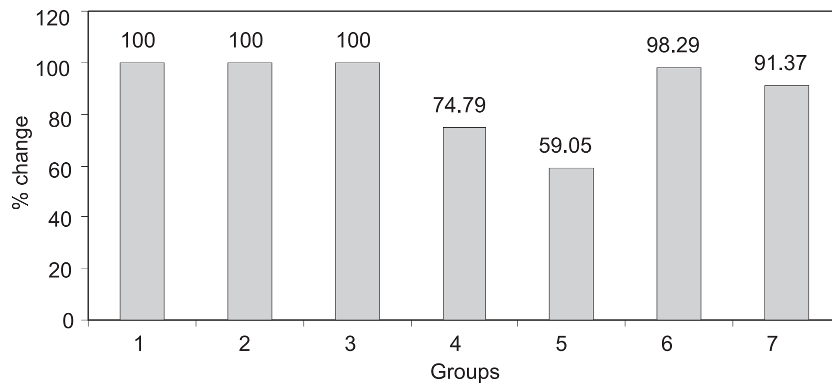

Figure 2. Percent change in RNA content (from vehicle control) in liver

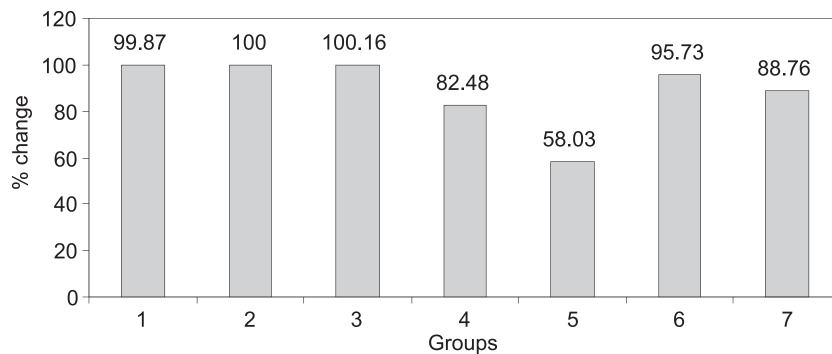

Figure 3. Percent change in protein content (from vehicle control) in liver

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 and were homogenized in the respective medium according to different parameters. DNA was estimated by the method of Giles and Meyer (12), RNA was estimated by the method of Mejboum (13), and protein was estimated by the method of Lowry et al. (14). 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 $p < 0.05$. Comparisons of p-values between different groups were performed.

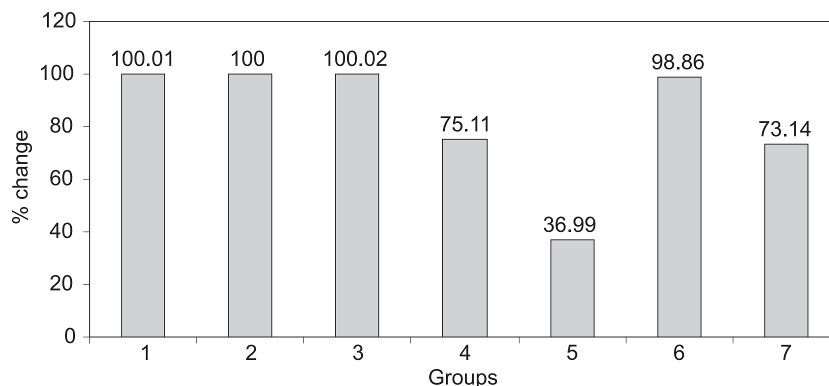

Figure 4. Percent change in DNA content (from vehicle control) in kidney

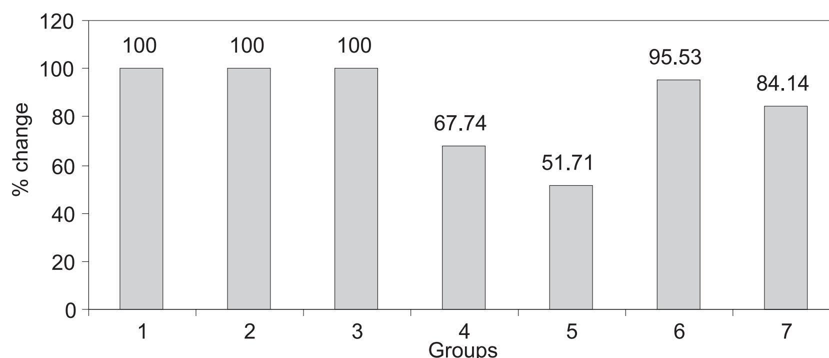

Figure 5. Percent change in RNA content (from vehicle control) in kidney

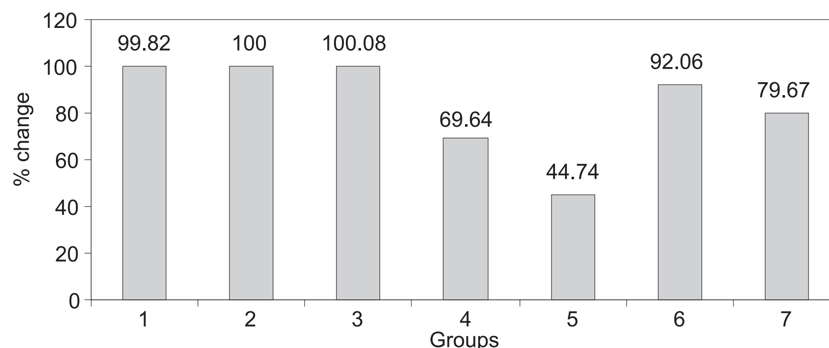

Figure 6. Percent change in protein content (from vehicle control) in kidney

RESULTS AND DISCUSSION

Liver

The effects of aflatoxin and aflatoxin plus curcumin treatment on biochemical changes in liver are shown in Table 1. Also percent changes from vehicle control (Group 2) are shown in Figures 1-3. No significant difference in DNA, RNA and protein contents were observed between different groups of controls (Groups 1 – 3).

Oral administration of aflatoxin for 45 days caused, as compared to vehicle control (Group 2), significant, dose-dependent reduction in DNA (LD: 47.05%; HD: 71.21%; Figure 1), RNA (LD: 25.21%; HD: 40.95%; Figure 2) and protein (LD: 17.52%; HD: 41.97%; Figure 3) contents.

Treatment with curcumin along with aflatoxin caused significant amelioration in liver, as compared to aflatoxin alone treated mice (Groups 6 and 7). Amelioration was almost complete in all param-

Table 2. Effect of curcumin on aflatoxin-induced biochemical changes in the 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
Deoxyribonucleic acid (μmoles/100 mg tissue weight)	265.46 ± 0.73	265.43 ± 0.87	265.49 ± 0.74	199.39 ± 0.72[abcefg]	98.19 ± 0.85[abcdfg]	262.41 ± 0.82[abcdeg]	194.15 ± 0.85[abcdef]
Ribonucleic acid (μmoles/100 mg tissue weight)	312.35 ± 0.38	312.38 ± 0.42	312.41 ± 0.42	211.61 ± 0.25[abcefg]	161.54 ± 0.31[abcdfg]	298.44 ± 0.21[abcdeg]	262.84 ± 0.34[abcdef]
Protein (mg/100 mg tissue weight)	22.89 ± 0.11	22.93 ± 0.09	22.95 ± 0.04	15.97 ± 0.12[abcefg]	10.26 ± 0.12[abcdfg]	21.11 ± 0.09[abcdeg]	18.27 ± 0.13[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 : $p < 0.05$, as compared to group 6 : $p < 0.05$, [g] as compared to group 7 : $p < 0.05$

eters in low dose aflatoxin-treated group along with curcumin (Group 6). DNA, RNA and protein contents in high dose aflatoxin-treated group along with curcumin (Group 7) showed partial amelioration. Extent of amelioration in RNA and protein contents in liver of aflatoxin plus curcumin-treated mice was comparatively higher in high dose group (RNA: 32.32%; protein: 30.73%) (Group 7) than that of low dose group (RNA: 23.50%; protein: 13.25%) (Group 6) (Figures 2 and 3). However, an extent of amelioration in DNA in liver of aflatoxin plus curcumin-treated mice was comparatively higher in low dose group (45.92%) than that of high dose (41.24%) (Figure 1).

Kidney

The effects of aflatoxin and aflatoxin plus curcumin treatment on biochemical changes in kidney are shown in Table 2. Also percent changes from vehicle control (Group 2) are shown in Figures 4-6. No significant difference in DNA, RNA and protein contents were observed between different groups of controls (Groups 1 – 3).

Oral administration of aflatoxin for 45 days caused, as compared to vehicle control (Group 2), significant, dose-dependent reduction in DNA (LD: 24.89%; HD: 63.01%; Figure 4), RNA (LD: 32.26%; HD: 48.29%; Figure 5) and protein (LD: 30.36%; HD: 55.26%; Figure 6) contents.

Oral administration of curcumin along with aflatoxin caused significant amelioration in aflatoxin-induced changes in kidney of mice (Groups 6 and 7). An extent of amelioration in DNA, RNA and protein contents in kidney of aflatoxin plus curcumin-treated mice is comparatively higher in high dose (DNA: 36.15%; RNA: 32.43%; protein: 34.93%) (Group 7) than that of low dose (DNA: 23.75%; RNA: 27.79%; protein: 22.42%) (Group 6) (Figures. 4-6). Amelioration was almost complete in all parameters in low dose aflatoxin along with curcumin-treated groups (Group 6). However, it was partial in high dose aflatoxin plus curcumin-treated group (Group 7).

Aflatoxin treatment caused significant, dose-dependent reduction in concentration of DNA, RNA and protein in the liver and kidney of mice (Tables 1 and 2). Aflatoxin is known to impair protein biosynthesis by forming adducts with DNA, RNA and proteins (15), inhibits RNA synthesis, DNA-dependent RNA polymerase activity and causes degranulation of endoplasmic reticulum (16). Reduction in protein content could also be due to increased necrosis in the liver. Thus reduction in protein biosynthesis as well as increased necrosis could be responsible for a decrease in protein content. Many other investigators have also reported a decrease in protein content in skeletal muscle (17), heart (18) liver and kidney (19) of aflatoxin-fed animals.

Our results showed that curcumin treatment along with aflatoxin significantly ameliorates aflatoxin-induced changes in DNA, RNA and protein contents in the liver and kidney of mice. The amelioration in these contents might be due to increased DNA synthesis and reduction in harmful adduct formation. Cheng et al. (20) investigated the inhibitory effects of curcumin, garlic squeeze, grape seed extract, tea polyphenols, vitamin C and vitamin E on nicotine-DNA adduction *in vivo*. They suggested that these dietary constituents are beneficial to pre-

vent the harmful adduct formation and thus to block the potential carcinogenesis induced by nicotine.

Curcumin have been shown to scavenge the free radicals and thereby act as good antioxidant (21). Several studies have demonstrated curcumin's ability to reduce oxidative stress (22). It appears that curcumin's role as an antioxidant may be due in part to its ability to down-regulate nitric oxide formation, which is a key element in inflammation and may contribute to carcinogenesis. Curcumin lowers cholesterol and triglyceride levels, decreases susceptibility of low density lipoprotein (LDL) to lipid peroxidation and inhibits platelet aggregation. These effects have been noted even with low doses of turmeric (23).

CONCLUSION

It can be concluded that curcumin acts as an effective compound playing major role in ameliorating the adversity caused to DNA, RNA and protein in the liver and kidney of mice induced by aflatoxins.

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