Original Article

Curcumin Ameliorates Aflatoxin-Induced Changes in Caput and Cauda Epididymis of Mice

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Abstract

Background: The main aim of the present study was to evaluate the ameliorative effect of curcumin on aflatoxin-induced changes in caput and cauda epididymis of mice.

Materials and Methods: Aflatoxin was obtained by growing *Aspergillus parasiticus* in sucrose - magnesium sulphate - potassium nitrate - yeast extract (SMKY) liquid medium. 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. Aflatoxin was orally administered in 25 [low dose (LD)] and 50 [high dose (HD)] µg/0.2 ml olive oil/animal/day (750 and 1500 µg/kg body weight) respectively with and without curcumin for 45 days. On day 46, animals were sacrificed by cervical dislocation. Caput and cauda epididymis were quickly isolated, blotted free of blood, weighed and used for biochemical studies.

Results: Our results revealed that oral administration of aflatoxin for 45 days caused, as compared with vehicle control, significant reduction in the activities of succinate dehydrogenase and adenosine triphosphatase as well as sialic acid and protein contents in both caput and cauda epididymides. The effect was dose-dependent.

Conclusion: As compared with aflatoxin alone treated animals, curcumin administration along with aflatoxin significantly ameliorates aflatoxin-induced changes in caput and cauda epididymis. Ameliorative effect of curcumin on aflatoxin-induced changes in caput and cauda epididymis of mice might be due to its antioxidative property.

Keywords: Aflatoxin, Curcumin, Amelioration, Cauda, Protein

Introduction

Aflatoxin research has a dramatic beginning. In 1960, 100,000 turkeys, at various poultry farms in England died in opisthotonous following earlier signs of anorexia, lethargy and muscular weakness. At post-mortem, subcutaneous haemorrhage and pale coloured liver with extensive necrosis and biliary proliferation were recorded (1). This mysterious disease was named 'Turkey X disease'. It has been established that aflatoxins (B_1, B_2, G_1, and G_2) are highly substituted coumarin derivatives that contain a fused dihydrofurofuran moiety. The B-toxins have been characterized by the fusion of a cyclopentenone ring to the lactone ring of the coumarin structure, whereas G-toxins contained an additional fused lactone ring. Aflatoxin B_1 (AFB_1), and to a lesser extent aflatoxin G_1 (AFG_1), are responsible for the biological potency of aflatoxin-contaminated meals. These two toxins possess an unsaturated bond at the 2, 3 positions (8, 9 position according to international union of pure and applied chemistry (IUPAC) nomenclature) on the terminal furan ring. Aflatoxin B_2 (AFB_2) and AFG_2 are essentially biologically inactive unless these agents are first metabolically oxidized to AFB_1 and AFG_1 *in vivo*.

Aflatoxins are a group of closely related secondary toxic fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. These are relatively low molecular weight organic compounds characterized by their diversity, their frequent specificity with regard to the taxonomy of the producing organisms and their production during the stationary phase of the batch cultures. Aflatoxins can enter human and animal dietary systems by indirect or direct contamination. Indirect contamination of food/feed can occur when an ingredient during the preparation process has previously been contaminated with toxin producing molds. Although the molds can be killed or

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removed during processing, aflatoxins often remain in the final product. Contamination of cereals and oil seeds represents the main point of entry of many mycotoxins into food chains. In direct contamination the product becomes infected with toxigenic molds with subsequent toxin production. Almost all foods and feeds are susceptible to moldy growth at some stage during their production, processing, transport and storage.

The damage can be to mitochondrial DNA (adducts and mutation), mitochondrial membranes, as well as to the disruption of energy production adenosine triphosphate (ATP production) (2). The mycotoxin alters energy linked functions of Adenosine diphosphate (ADP) phosphorylation, Flavin adenine dinucleotide (FAD) and NAD-linked oxidizing substrates, (3) and α-ketoglutarate-succinate cytochrome reductases (4). It causes ultrastructural changes in mitochondria (5) and also induces mitochondria directed apoptosis (6).

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 is 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 diseases may be promoted by free radicals and that antioxidants may oppose the action of these molecules. The concept eco-friendly antioxidants are obtained from nature obtained from nature that do not show side-effects, are environmentally friendly and economically affordable.

Curcumin reduces oxidized proteins in amyloid pathology in Alzheimer's transgenic mice (7). Turmeric (*Curcuma longa*) and its active ingredient, curcumin, have been shown to scavenge free radicals and thereby act as good antioxidants (8). It has been investigated that the addition of food additives such as turmeric to medium containing Aspergillus parasiticus inhibited the growth of mycelium. The concentration needed for 50% inhibition was approximately 2.5 mg/ml of the medium (9). Both turmeric and curcumin have been shown to inhibit the aflatoxin-induced toxicity in experimental ducklings. The weight change induced by the toxin is partially reversed by the simultaneous administration of turmeric and curcumin. The most remarkable feature is the histopathological findings in which the aflatoxin-induced injury is remarkably reduced in animals treated with curcumin and turmeric (9). Curcumin reduces tumours that have been induced in mice by the injection of 10^6 Datton's lymphoma tumour cells into the intraperitoneal cavity. This property of curcumin would be an ideal drug which could be of value as a chemopreventive agent against carcinogen activation (10).

One study has shown curcumin to be eight times more powerful than that of 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 (11).

The aim of the present study was to investigate the biochemical alterations in the caput and cauda epididymides of mice induced by aflatoxin-treatment and its possible amelioration by curcumin.

Materials and Methods

Aspergillus parasiticus (NRRL 3240) was procured from the Indian Agricultural Research Institute, New Delhi, India. It was grown on sucrose-magnesium sulphate-potassium nitrate-yeast extract (SMKY) liquid medium at 28 ± 2ºC for ten days (12). Culture filtrates of aflatoxin were extracted with chloroform (1:2, v/v) and passed through a bed of anhydrous sodium sulphate. The chloroform extract was evaporated to dryness and stored. Next, dried aflatoxin extract was dissolved in fresh chloroform and used for the experiment. 100 µl aflatoxin extract was first fractionated on silica gel G coated activated Thin layer chromatography (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 : iso-amyl alcohol : methanol (90: 32: 2, v/v) (13). The air-dried plates were observed under long-wave UV light (360 nm) for aflatoxins. Aflatoxin B_1 and B_2 showed blue fluorescent spots; aflatoxin G_1 and G_2 showed bluish-green fluorescent spots. Chemical confirmation of aflatoxin was done by spraying trifluro acetic acid (TFA) and 25% sulfuric acid (14).

Each spot was scraped separately, dissolved in chilled methanol and subjected to spectrophotometric analysis according to the method of Nabney and Nesbitt (15) using UV-Vis spectrophotometer. Dried aflatoxin extract which contained B_1, B_2, G_1 and G_2 in the ratio of 8: 3: 2: 1 respectively was used for treating the experimental animals in an olive oil carrier.

Young inbred, Swiss strain male albino mice (*Mus musculus*), weighing approximately 37-40 g, of ages 10-11 weeks were obtained from Cadila Health Care, Ahmedabad, India. They were provided feed and water ad libitum and

maintained under laboratory conditions. There were 70 animals divided into seven groups and caged separately. Group 1 (untreated 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 an antidote control. Animals of Groups 4 and 5 were orally administered with aflatoxins at a dose of 25 [low dose (LD)] and 50 [high dose (HD)] µg/0.2ml olive oil/animal/day (750 and 1500 µg/kg body weight) respectively for 45 days. Groups 6 and 7 animals were orally administered both LD HD aflatoxin 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.

On completion of the treatment, the mice were weighed and sacrificed by cervical dislocation. The caput and cauda epididymis were isolated, blotted free of blood, weighed, and homogenates were prepared for respective parameters. Succinate dehydrogenase activity was assayed by the method of Beatty et al. (16). Adenosine triphosphatase activity was measured by the method of Quinn and White (17). Sialic acid and protein contents were estimated by the method of Jourdian et al. (18) and Lowery et al. (19), respectively.

For all parameters, a minimum of ten replicates were used and the data was statistically analyzed using one way Analysis of Variance (ANOVA) followed by the Tukey Test. The levels of significance were accepted with $p<0.05$. Comparisons of P-values between different groups were performed.

Results

Oral administration of aflatoxin for 45 days caused significant reduction in the weights of the caput and cauda epididymides (Tables 1, 2). The effect was dose dependent. Administration of curcumin along with aflatoxin significantly ameliorated aflatoxin-induced changes in the weight of the epididymides.

The ameliorative effect of curcumin on aflatoxin-induced toxicity on the caput is shown in Table 1. No significant difference in activities of succinate dehydrogenase and adenosine triphosphatase as well as sialic acid 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), a significant, dose-dependent reduction in activities of succinate dehydrogenase (LD: 43.09%; HD: 59.99%) and adenosine triphosphatase (LD: 41.52%; HD: 62.90%) as well as sialic acid (LD: 37.53%; HD: 56.88%) and protein (LD: 42.00%; HD: 58.87%) contents (Table 1).

Table 1: Effect of curcumin on aflatoxin-induced changes in the caput epididymis of mice

Parameters	Experimental Groups						
	1	2	3	4	5	6	7
	Untreated control	Vehicle control	Curcumin control	LD aflatoxin	HD aflatoxin	LD aflatoxin + curcumin	HD aflatoxin + curcumin
Succinate dehydrogenase (µg formazon formed/ mg protein/15 min)	17.85 ± 0.29	17.87 ± 0.27	17.89 ± 0.22	10.17 ± 0.21 ^{abcefg}	7.15 ± 0.13 ^{abcdfg}	17.02 ± 0.16 ^{abcdeg}	13.21 ± 0.11 ^{abcdef}
Adenosine triphosphatase activity (µ moles i.p. released/ mg protein/30 min)	1.938 ± 0.011	1.973 ± 0.017	1.986 ± 0.022	1.154 ± 0.038 ^{abcefg}	0.732 ± 0.016 ^{abcdfg}	1.821 ± 0.011 ^{abcdeg}	1.668 ± 0.022 ^{abcdef}
Sialic acid (µg/mg tissue weight)	8.324 ± 0.021	8.327 ± 0.018	8.329 ± 0.021	5.202 ± 0.036 ^{abcefg}	3.591 ± 0.101 ^{abcdfg}	7.947 ± 0.033 ^{abcdeg}	6.562 ± 0.024 ^{abcdef}
Protein (mg/100 mg tissue weight)	10.52 ± 0.09	10.55 ± 0.12	10.59 ± 0.11	6.12 ± 0.06 ^{abcefg}	4.34 ± 0.08 ^{abcdfg}	10.31 ± 0.18 ^{bcdeg}	7.54 ± 0.07 ^{abcdef}

LD: Low dose, HD: High dose
Values are Mean ± SEM; 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

Table 2: Effect of curcumin on aflatoxin-induced changes in the cauda epididymis of mice

Parameters	Experimental Groups						
	1	2	3	4	5	6	7
	Untreated control	Vehicle control	Curcumin control	LD aflatoxin	HD aflatoxin	LD aflatoxin + curcumin	HD aflatoxin + curcumin
Succinate dehydrogenase (µg formazon formed/ mg protein/15 min)	20.94 ± 0.17	20.97 ± 0.17	20.99 ± 0.21	14.11 ± 0.13 [abcefg]	9.89 ± 0.12 [abcdfg]	19.84 ± 0.14 [abcdeg]	16.22 ± 0.12 [abcdef]
Adenosine triphosphatase activity (µ moles i.p. released/mg protein/30 min)	2.86 ± 0.15	2.71 ± 0.04	3.02 ± 0.11	1.56 ± 0.09 [abcefg]	1.04 ± 0.04 [abcdfg]	2.37 ± 0.11 [abcde]	2.11 ± 0.12 [abcde]
Sialic acid (µg/mg tissue weight)	10.212 ± 0.033	10.215 ± 0.041	10.219 ± 0.032	6.215 ± 0.021 [abcefg]	4.261 ± 0.086 [abcdfg]	9.567 ± 0.011 [abcdeg]	8.145 ± 0.021 [abcdef]
Protein (mg/100 mg tissue weight)	13.92 ± 0.09	13.86 ± 0.02	14.02 ± 0.1	8.14 ± 0.04 [abcefg]	4.25 ± 0.05 [abcdfg]	12.38 ± 0.11 [abcdeg]	10.22 ± 0.09 [abcdef]

LD: Low dose, HD: High dose
Values are Mean ± SEM; 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

Oral administration of curcumin along with aflatoxin caused, as compared with the aflatoxin alone treated group, significant amelioration in all parameters of the caput epididymis (Groups 6 and 7). Amelioration was almost complete in all parameters in the LD aflatoxin plus curcumin-treated group (Group 6); however, amelioration was partial in the HD aflatoxin plus curcumin-treated group (Group 7).

Extent of amelioration of adenosine triphosphatase activity and sialic acid content in the caput epididymis of the aflatoxin plus curcumin-treated mice was comparatively more in the HD group (adenosine triphosphatase: 47.44% and sialic acid: 35.68%) than that of the LD group (adenosine triphosphatase: 33.81% and sialic acid: 32.96%); while the extent of amelioration of succinate dehydrogenase activity and protein content was more in the LD group (succinate dehydrogenase: 38.33%; protein: 39.72%; Group 6) than that of the HD group (succinate dehydrogenase: 33.91%; protein: 30.33%; Group 7; Table 1).

The effect of aflatoxin and aflatoxin plus curcumin treatment on biochemical and enzymatic changes in the cauda epididymis are shown in Table 2. No significant differences in succinate dehydrogenase and adenosine triphosphatase activities as well as sialic acid and protein contents were observed between the different groups of controls (Groups 1 - 3).

Oral administration of aflatoxin for 45 days caused, as compared to the vehicle control (Group 2), a significant, dose-dependent reduction in activities of succinate dehydrogenase (LD: 32.72%; HD: 52.84%) and adenosine triphosphatase (LD: 42.44%; HD: 61.63%) as well as sialic acid (LD: 39.16%; HD: 58.29%) and protein (LD: 41.27%; HD: 69.34%) contents (Table 2).

Oral administration of curcumin along with aflatoxin caused significant amelioration in aflatoxin-induced changes in the cauda epididymis of mice (Groups 6 and 7). Amelioration was almost complete in all parameters in the LD aflatoxin-treated group along with curcumin (Group 6); however it was partial in the HD aflatoxin plus curcumin-treated group (Group 7). The extent of amelioration in activities of succinate dehydrogenase and adenosine triphosphatase as well as sialic acid and protein contents in the cauda epididymis of aflatoxin plus curcumin-treated mice were comparatively more in the HD group (succinate dehydrogenase: 30.18%, adenosine triphosphatase: 39.48%, sialic acid: 38.02%, protein: 43.07%; Group 7) than that of the LD group (succinate dehydrogenase: 27.33%, adenosine triphosphatase: 29.89%, sialic acid: 32.81%, protein: 30.59%; Group 6; Table 2).

Discussion

Oral administration of aflatoxin (750 and 1500 µg/kg body weight/day) for 45 days caused significant reduction in the weights of reproductive organs such as the caput and cauda epididymides. The reduction in weights of caput and cauda epidi-

dymides could be due to degenerative changes in the epithelial lining and tubular lumen devoid of sperm bundles. The protein content was significantly reduced in the caput and cauda epididymides of aflatoxin-treated mice which was corroborated with earlier findings reported in the skeletal muscle (20), heart (21), liver (22) and kidneys (23) of aflatoxin-fed rabbits. Aflatoxin impairs protein biosynthesis by forming adducts with DNA, RNA and protein, inhibits RNA synthesis and DNA-dependent RNA polymerase activity and causes degranulation of the endoplasmic reticulum (24).

Succinate dehydrogenase (SDH) is a key enzyme of the mitochondrial Kreb's cycle which is mainly concerned with aerobic oxidation of acetyl CoA and generation of ATP. A decrease in SDH activity observed in the caput and cauda epididymides (Tables 1, 2) indicates a reduction in aerobic oxidation, which could be in response to reduced oxygen transport to tissues.

Another possible explanation could be due to the accumulation of calcium which is known to cause mitochondrial dysfunction and reduced ATP generation (25). Roy (26) has reported mitochondrial swelling during aflatoxicosis. In addition, the altered calcium/magnesium ratio, reduced oxidative phosphorylation and ATP generation in heart and skeletal muscle might be a key factor causing the development of lethargy.

The enzymatic hydrolysis of ATP by ATPase is a ubiquitous property of cells which is important for intracellular transfer of energy. The ATPase activity showed a decline in caput and cauda epididymides suggesting a reduced utilization of ATP produced in the cell. Toskulkao and Glinsukon (25) have reported increased accumulation of Ca^{+2} inside mitochondria causing mitochondrial dysfunction and reduction in hepatic ATP content.

Sialic acid is a sialomuco-protein, essential for the maintenance of the structural integrity of the sperm membrane and sperm maturation (27, 28). The altered sialic acid content in caput and cauda epididymides (Tables 1, 2) indicate that the structural integrity of acrosomal membranes of the sperm might be affected. Reduction in sialic acid content during induced aflatoxicosis in mice has been reported by Verma and Nair (29).

The degenerative changes observed in caput and cauda epididymides could be attributed to oxidative stress, which is generally correlated with cellular damage (30). Also, the caput and cauda epididymides are androgen-dependent organs, thus biochemical changes observed in these organs could be due to reduced testosterone levels in aflatoxin-treated mice. Significantly decreased 3β- and 17β-hydroxysteroid dehydrogenase activities and serum testosterone levels were significantly reduced in aflatoxin-treated mice as compared to controls (31).

Administration of curcumin along with aflatoxin caused recovery in all biochemical and enzymatic changes in these reproductive organs. The recovery was more pronounced in animals receiving LD aflatoxin as compared to those receiving HD. Amelioration was due to the antioxidative property of curcumin.

Mechanism of action of curcumin

It has been suggested that the apparent chemopreventive action of curcumin is related to its ability to competitively inhibit cytochrome P450 isoenzymes responsible for the metabolic activation of carcinogens such as benzo [a] pyrene and aflatoxin B_1.

Curcumin selectively inhibits the CYP 1A1/1A2 and 2B1/2B2 isoenzymes in the high-nanomolar to low-micromolar range (32), levels that are certainly achievable, even given the poor absorption kinetics for this agent.

The antioxidant mechanism of curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols and an enol form of diketone the structure of curcumin shows typical radical trapping ability as a chain-breaking antioxidant (33). Generally the nonenzymatic antioxidant process of the phenolic material is thought to be mediated through the following two stages:

$S\text{-}OO^{o} + AH$ --- $SOOH + A^{o}$

$A^{o} + X^{o}$ ---- Nonradical materials

Where S is the substance oxidized, AH is the phenolic antioxidant, A^{o} is the antioxidant radical and X^{o} is another radical species or the same species (34) as A^{o}. A^{o} and X^{o} dimerize to form the nonradical product. Masuda et al. (33) further studied the antioxidant mechanism of curcumin using linoleate as an oxidizable polyunsaturated lipid and proposed that the mechanism involves oxidative coupling reaction at the 3' position of the curcumin with the lipid and a subsequent intramolecular Diels-Alder reaction.

Conclusion

Oral administration of curcumin along with aflatoxin significantly reduced aflatoxin induced toxicity in the caput and cauda epididymides of mice which could be due to its antioxidative property.

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