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CURCUMIN AMELIORATES AFLATOXIN-INDUCED CHANGES IN SDH
AND ATPase ACTIVITIES 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 changes in activities of succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase) 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) aflatoxin 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 and organs were removed to prepare homogenates for measuring changes in enzyme activities such as succinate dehydrogenase and adenosine triphosphatase. The results showed that in liver and kidney of mice activities of both the enzymes succinate dehydrogenase and adenosine triphosphatase were found to be reduced in the groups treated with low dose and high dose of aflatoxin, which were ameliorated by the treatment of curcumin along with aflatoxin in other groups. Thus, curcumin along with aflatoxin ameliorates aflatoxin-induced changes in succinate dehydrogenase and adenosine triphosphatase activities in liver and kidney of mice.

Keywords: aflatoxin, succinate dehydrogenase, adenosine triphosphatase, curcumin, liver, kidney

Aflatoxin research had a dramatic beginning. In 1960, one hundred thousand turkeys, in various poultry farms of England died in opisthotonus following earlier signs of anorexia, lethargy and muscular weakness. At post-mortem, subcutaneous hemorrhage and pale colored liver with extensive necrosis and biliary proliferation were recorded (1). This mysterious disease was named as „Turkey X” disease. Concurrent with loss of turkey poults, large scale losses of ducklings, partridges and pheasant poults were also reported (2). Investigations revealed that the common ingredients of feed in various poultry farms were incorporated in imported Brazilian peanut meal. Occurrence of these incidences attracted many groups of scientists to work together for isolation and characterization of the compound present in the feed which was imported from Brazil.

Aflatoxins are a group of closely related secondary toxic fungal metabolites produced by *Aspergillus flavus* and *A. parasiticus*, of 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. During early period of recognition it was considered as a part of storage flora of inadequate post-harvest storage and poor storage conditions during distribution of commodities such as maize, groundnuts, peanuts, barley etc. However, it has now become increasingly recognized that the contamination of some commodities especially peanuts and maize with aflatoxin is a far more complex phenomenon which may involve infection and aflatoxin production in the field. Although contamination of crop in field is undoubtedly influenced by insect damage, *A. flavus* is also known to infect intact kernels of maize by colonizing and growth down the external silk (3).

The effects of aflatoxin in ruminants are similar to those of non-ruminants. Young animals are comparatively more susceptible to aflatoxin than the adults (4). Lynch *et al.* (5) recorded slight hyperplasia in hepatic cells from the end of the first month of aflatoxin feeding in cattle; this phenomenon increased during the second and third months until centrilobular degeneration of the hepatic cells became apparent. However, during the fourth month

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central necrosis of the hepatic cells was marked by proliferation of the bile ducts and occlusion of the centrilobular veins. Calves develop a disease that features blindness, circling and falling down, twitching of ears and grinding of teeth. Spasm of the rectum is seen in most cases. Death usually follows within two days of onset of severe clinical signs. Postmortem findings revealed pale, firm and fibrosed liver. Histologically, the main changes in liver were centrilobular necrosis bile duct proliferation and veno-occlusive disease. The kidneys are yellow and surrounded by wet fat. Ascites and edema of the mesentery (enteritis) and rectal eversion are common findings (6).

The damage can be to mitochondrial DNA (adducts and mutation), mitochondrial membranes, as well as to disruption of energy production (production of ATP) (7).

Curcumin is the basic ingredient of turmeric and turmeric is widely used in the Unani and Ayurvedic methods of treatment in many diseases related to vital organs also. Chemical structure of curcumin was determined by Roughley and Whiting (8). It has a melting point at 176-177°C, forms a reddish-brown salt with alkali and is soluble in ethanol, alkali, acetone, acetic acid and chloroform. Curcumin is found to be the biologically active compound in turmeric.

The increase in fatty acid content after ethanol-induced liver damage is significantly decreased by curcumin treatment and arachidonic acid level is increased (9). Alcohol and water extracts of *Curcuma longa* shows anti-inflammatory effects (10).

Turmeric and curcumin has been found to have hepatoprotective effects in protecting animal livers from a variety of hepatotoxic insults induced by chemicals and drugs. Turmeric and curcumin were also found to reverse biliary hyperplasia, fatty liver and liver necrosis induced by aflatoxin (11). Dietary administration of turmeric (0.05%) and curcumin (0.05% each) to rats significantly reduced the number of gamma-glutamyl transpeptidase positive foci induced by aflatoxin B₁ which is considered as the precursor of hepatocellular neoplasm. These studies indicate the usefulness of antioxidant food additives in ameliorating aflatoxin-induced mutagenicity and carcinogenicity (12).

As, all round the world researchers are making their best to make "Curcumin – a boon drug" against cancer and other diseases in human as well as other animals, and its effectiveness has also been known against aflatoxin-induced cancer, which is world-wide problem.

The present investigation was an attempt to evaluate ameliorative activity of curcumin on activities of succinate dehydrogenase and adenosine triphosphatase 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 (13). 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 of 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/v) (14). 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 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 (TFA) and 25% sulfuric acid (15).

Each spot was scraped separately, dissolved in chilled methanol and subjected to spectrophotometric analysis according to the method of Nabney and Nesbitt (16) 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) $\mu\text{g}/0.2\text{mL}$ olive oil/animal/day (750 and 1500 $\mu\text{g}/\text{kg}$ 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 and were homogenized in the respective medium according to different parameters. Succinate dehydrogenase was estimated by the method of Beatty et al. (17) and adenosine triphosphatase was estimated by the method of Quinn and White (18).

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.

RESULTS AND DISCUSSION

Table 1 shows the effects of aflatoxin and aflatoxin plus curcumin treatment on activities of succinate dehydrogenase and adenosine triphosphatase in the liver of mice. Also percent changes from vehicle control (Group 2) are shown in Figures 1 and 2, respectively. There were no significant changes between untreated control, vehicle control and curcumin-treated groups (Groups 1 – 3).

Oral administration of aflatoxin (Group 4 Low Dose; LD and group 5 High Dose; HD) caused, as compared with vehicle control (Group 2), dose-dependent, significant reductions in the activities of succinate dehydrogenase (LD: 41.30%; HD: 50.73%) and adenosine triphosphatase (LD: 36.63%; HD: 57.00%).

As compared to aflatoxin alone treated groups, oral administration of curcumin along with aflatoxin (Groups 6 and 7) caused significant amelioration in activities of succinate dehydrogenase and adenosine triphosphatase.

Table 2 shows the effect of aflatoxin and aflatoxin plus curcumin treatment on the activities of succinate dehydrogenase and adenosine triphosphatase in the kidney of mice. Also percent changes

Table 1. Effect of curcumin on aflatoxin-induced enzymatic 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
Succinate dehydrogenase (μg formazan formed/mg protein/15 min)	68.21 \pm 0.21	68.43 \pm 0.25	68.73 \pm 0.14	40.17 \pm 0.54 ^{abcd}	33.72 \pm 0.25 ^{abcde}	59.78 \pm 0.21 ^{abcde}	50.59 \pm 0.19 ^{abcdef}
Adenosine triphosphatase activity ($\mu\text{moles } i.p. \text{ released/mg protein/30 min}$)	4.82 \pm 0.02	4.86 \pm 0.02	4.94 \pm 0.01	3.08 \pm 0.03 ^{abcde}	2.09 \pm 0.01 ^{abcde}	4.31 \pm 0.08 ^{abcde}	3.77 \pm 0.03 ^{abcde}

Values are the mean \pm 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, $p < 0.05$.

Table 2. Effect of curcumin on aflatoxin-induced enzymatic changes in the kidney of mice.

Parameters	Experimental groups						
	1	2	3	4	5	6	7
Untreated control	90.93 ± 0.18	90.95 ± 0.22	90.98 ± 0.28	65.21 ± 0.21 ^{abdefg}	53.76 ± 0.25 ^{abdefg}	81.69 ± 0.21 ^{abdefg}	71.15 ± 0.15 ^{abdef}
Succinate dehydrogenase (µg formazan formed/mg protein/15 min)	90.93 ± 0.18	90.95 ± 0.22	90.98 ± 0.28	65.21 ± 0.21 ^{abdefg}	53.76 ± 0.25 ^{abdefg}	81.69 ± 0.21 ^{abdefg}	71.15 ± 0.15 ^{abdef}
Adenosine triphosphatase activity (µmoles <i>i.p.</i> released /mg protein/30 min)	3.71 ± 0.06	3.79 ± 0.02	3.85 ± 0.11	2.39 ± 0.11 ^{abdefg}	1.01 ± 0.01 ^{abdefg}	3.46 ± 0.01 ^{abdefg}	3.23 ± 0.01 ^{abdef}

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.

from vehicle control (Group 2) are shown in Figures 3 and 4, respectively. There was no significant change between untreated control, vehicle control and curcumin-treated groups (Groups 1 – 3).

Oral administration of aflatoxin (Groups 4 and 5) caused, as compared to vehicle control (Group 2), dose-dependent, significant reductions in the activities of succinate dehydrogenase (LD: 28.31%; HD: 40.90%) and adenosine triphosphatase (LD: 36.94%; HD: 73.36%).

As compared to aflatoxin alone treated groups, oral administration of curcumin along with aflatoxin (Groups 6 and 7) caused significant amelioration in these enzymes activities.

Oral administration of aflatoxin for 45 days caused significant reduction in the activities of succinate dehydrogenase and ATPase in liver and kidney of mice. The effect was comparatively more pronounced in high dose aflatoxin-treated group than that of low dose (Tables 1 and 2). Succinate dehydrogenase (SDH) is a key enzyme in the mitochondrial Krebs cycle, which is mainly concerned with the aerobic oxidation of acetyl CoA and the generation of ATP. Putilina and Eschanko (19) explained that among the Krebs cycle dehydrogenases, SDH is more active than any other enzyme. Therefore, reduction in SDH activity clearly indicates reduction in aerobic metabolism, which might be the result of reduced oxygen transport to tissues.

Aflatoxin caused ultrastructural changes in mitochondria. Roy (20) have reported mitochondrial swelling during aflatoxicosis. This could be due to accumulation of calcium which is known to cause mitochondrial dysfunction and reduced ATP generation (21). Aflatoxin alters energy-linked functions of ADP phosphorylation, FAD⁺ and NAD⁺ linked oxidizing substrates (8) and α-ketoglutarate-succinate cytochrome reductases (9). Reduction in hepatic succinate dehydrogenase activity was also reported in broilers (22), rats (23) and mice (24). Inhibition of liver mitochondria and electron transport flow by aflatoxin in rat has been reported by Doherty and Campbell (25).

The enzymatic hydrolysis of ATP by ATPase is an ubiquitous property of cells which is important for intracellular transfer of energy. Reduction in ATPase activity in liver and kidney suggests reduced utilization of ATP produced in the cell. The reduced aerobic oxidation and ATP generation could be responsible for the reduction in ATPase activity. Verma and Nair (24) also reported reduction in ATPase activity in the testis of aflatoxin-treated mice.

Treatment with curcumin along with aflatoxin significantly ameliorates aflatoxin-induced changes

in SDH and ATPase activities in liver and kidney of mice. The ameliorative effect of curcumin might be due to its polyphenolic nature having antioxidative property (25). Antioxidants have the property to protect all membrane lipids and unsaturated fatty acids against oxidative degeneration (26).

CONCLUSION

It is concluded that curcumin efficiently ameliorates aflatoxin-induced toxicity in activities of succinate dehydrogenase and adenosine triphosphatase in liver and kidney of mice. Thus, curcumin proves to be the active component of turmeric.

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