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Dietary supplementation of curcumin ameliorate aflatoxn B_1 induced oxidative stress in mice

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Abstract

Studies were carried out to evaluate the effect of dietary supplementation of curcumin on toxicopathology of aflatoxicosis in laboratory mouse following short-term exposure to aflatoxin B_1. Sexually matured Swiss albino mice of either sex weighing between 28-30g were divided into two groups consisting of six animals each. Animals in group I (positive control) were fed with aflatoxin B_1 mixed in standard rodent chow (7.5 ppb) and group II (treatment) received aflatoxin B1 (7.5 ppb) along with curcumin (1g/kg feed) for a period of 14 days. At term, oxidative stress and hepatotoxicity were assessed by determining tissue and serum specific enzymes and by histopathology. Short term exposure to aflatoxin-B_1 significantly ($P \leq 0.05$) decreased hepatic GSH*r* levels on account of oxidative stress as evidenced by elevated TBARS levels (group I). However, concomitant administration of curcumin has significantly ($P \leq 0.05$) reduced the TBARS and protected depletion of hepatic GSH*r* levels. Curcumin supplementation (group II) has markedly reduced the toxicopathological lesions in liver induced by aflatoxin B_1 and consequent reduction ($P \leq 0.05$) in serum levels of both aminotransferase (s) and phosphatase. Thus, the present study demonstrated the potential benefits of dietary supplementation of curcumin in ameliorating aflatoxin induced oxidative stress in mice

Keywords: aflatoxin-B_1, curcumin, lipid peroxidation, mice and toxicopathology

Introduction

Aflatoxin B_1 (AFB_1) is a natural toxin produced as a secondary metabolite by the mould *Aspergillus flavus* and one of the most potent hepatocarcinogens known. Aflatoxicosis in poultry, ducks, laboratory animals and swine is a major concern in areas of the world where temperature and humidity are conducive to growth of the mould, such as India, Africa and Southeast Asian countries. Acute or chronic toxicosis can result from exposure to feed or bedding contaminated with toxins that may be produced during growth of various saprophytic or phytopathogenic fungi or moulds on cereals, hay, straw, pastures, or any other fodder or feed ingredients like maize, sorghum, groundnut, pea nut etc.

Analysis of AFB_1 metabolites in the bile of rats indicated that a major product of metabolism was the glutathione conjugate of the AFB_1-8,9-epoxide (Degen and Neumann, 1978). These conjugates are formed by the action of a glutathione S-transferase, identified as $GSTA_5$ (Hayes *et al.*

1991). Other metabolic pathways were also demonstrated, including the reduction of the cytotoxic AFB_1 dialdehyde to AFB_1-dialcohol catalyzed by $AKR7A_1$ (Ellis *et al*. 1993). As both the glutathione conjugate and the dialcohol products were less toxic than the parental compounds, these were deemed to represent detoxification steps, and the $GSTA_5$ and $AKR7A_1$ enzymes were seen to be pivotal in protecting cells against AFB_1 toxicity.

Curcumin is a yellow colored pigment present in the rhizome of *Curcuma longa* (turmeric), a perennial herb belonging to *Zingiberaceae* family. Curcumin is non-toxic, highly promising natural antioxidant, hepatoprotective, hypocholesteremic, anticarcinogenic and antimutagenic compound having a wide spectrum of biological applications. It is expected that curcumin may become a novel agent in the near future to control various diseases, including inflammatory disorders, carcinogenesis and oxidative stress-induced pathogenesis in man and animals (Chattopadhyay *et al*. 2004).

Materials and methods

Swiss albino mice of either sex (n=12; 28-30g) were maintained in the laboratory animal house of veterinary college, Bidar were procured for the experiment. They were acclimatized to laboratory conditions (temperature: 24±1.0°C and humidity 60±5 %). Animals were fed with standard rodent chow (Amruth Feeds Ltd., Pune, India) and given drinking water *ad libitum*. The experimental protocol met the national guidelines on the proper care and use of animals in laboratory research. Six mice (group I) were fed with aflatoxin B_1 (7.5 ppb) containing diet delivered through standard rodent chow and the remaining six animals (group II) received aflatoxin B1 (7.5 ppb) and curcumin (Hi-Media®, Mumbai, India) at 1g/kg feed given concomitantly. Body weights of the animals were recorded at weekly interval. On Day 14 of the experiment, all the animals in both the groups were fasted overnight and sacrificed. Blood samples were collected to harvest serum samples which were stored at -20°C until analysis. Liver tissues from individual animals were dissected out, weighed and frozen immediately in liquid nitrogen until analysis for thiobarbituric acid reacting substance (TBARS) and reduced glutathione (GSH_r) levels. Representative liver samples were also stored in formal-Bouin's fluid for histological examination.

Serum enzymes:

Serum levels of aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) were estimated according to Reitman & Flankel *et al.* (1957). Membrane bound alkaline phosphates levels was determined by IFCC(1983) method.

TBARS and GSHr in hepatic tissue:

Frozen hepatic tissues were trimmed from extraneous material using chilled saline solution and homogenized in 0.25M ice-cold sucrose solution (10%w/v) using pestle and mortar. The homogenates were centrifuged at 700xg for 10 min. to remove cell debris and the supernatant of the hepatic tissue was used for the estimation of TBARS and GSH_r levels.

Histopathology:

Formal-Bouin's fixed tissues were dehydrated in graded alcohols, processed to prepare paraffin embedded blocks and sections of 5-8 µm thickness were obtained. Haematoxylin–eosin (H&E) stained tissues were examined under light microscopy (Sheenan, 1973).

Statistical analysis

All the values were expressed as Mean ±SE. Students't' test was applied to compare the difference between two groups with respect to various parameters studied. A difference of $P \leq 0.05$ was considered as statistically significant.

Results

There was no mortality or significant change in the body weight between the two groups of experimental animals. Serum levels of aminotransferases (AST and ALT) and alkaline phosphatase (ALP) were significantly ($P \leq 0.05$) reduced in curcumin treated (group II) group (Fig.1).

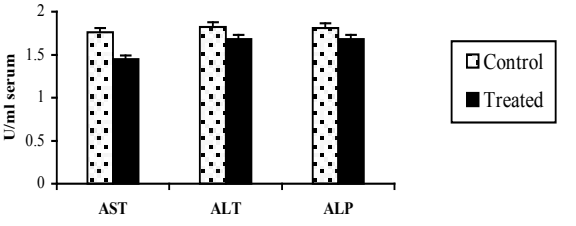

Fig . 1. Serum levels of transaminases (AST & ALT) and alkaline phosphatase (ALP) in control and curcumin treated group (U/ml) at term.

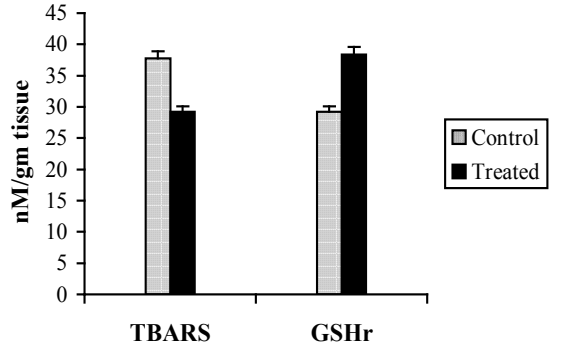

Fig . 2 TBARS (nmoles/g) and reduced GSHr (nmoles/g) levels in hepatic tissue at term.

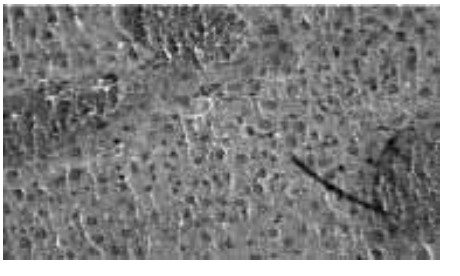

Fig.3. Aflatoxin induced hepatic lesions characterized by extensive haemorrhages, infiltration of inflammatory cells and swelling of nucleus (H & E, 40X)

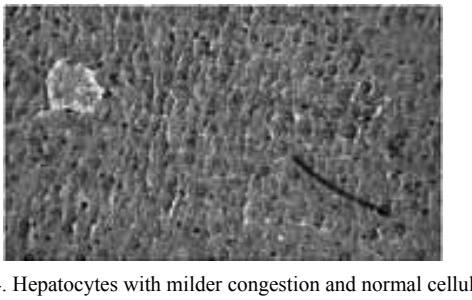

Fig.4. Hepatocytes with milder congestion and normal cellular architecture in curcumin treated group (H & E, 40X)

At termination of the experiment (Day 14), TBARS levels was significantly ($P \leq 0.05$) elevated in the group I (positive control) due to aflatoxin induced oxidative stress when compared with group II which received curcumin concomitantly with dietary aflatoxin exposure. Further, co-administration of curcumin with aflatoxin B_1 (group II) has significantly ($P \leq 0.05$) protected the depletion of hepatic GSH*r* levels in mice (Fig.2)

Histological examination of liver revealed extensive hemorrhages, infiltration of inflammatory cells and swelling of the nucleus in control group (Fig.3), while milder degenerative changes without loss of hepatic architecture was observed in curcumin treated group (Fig.4).

Discussion

Chemical induced cellular alteration varies from simple increase in the metabolism to death of the cell (Giray *et al.* 2001). Alterations in the transaminases and or phosphatase activity are related to the intensity of the cellular damage. The increase in serum transaminase activity (AST and ALT) along with the increase in ALP activity in group I (positive control) is due to the aflatoxin B_1 induced pathological changes in the hepatic tissue. A significant reduction in serum enzyme levels (AST, ALT and ALP) in the group II animals was due to the ameliorating and hepatoprotective role exerted by curcumin. The antioxidant activity of curcumin was reported as early as in 1970s (Sharma, 1976). It acts as a scavenger of oxygen free radicals (Joe and Lokesh, 1994). It can protect haemoglobin from oxidation (Pulla Reddy and Lokesh, 1992).

Curcumin also lowers the reduction of reactive oxygen species (ROS) *in vivo* (Joe and Lokesh, 1994) and also decreases lipid peroxidation in liver microsomes, erythrocyte membranes and brain homogenates which is brought about by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase (Pulla Reddy and Lokesh,1994). In the present study, the TBARS levels were comparatively lesser in the treatment group compared to the control group and the free radical scavenger enzyme reduced glutathione (GSH*r*) levels were also significantly higher in curcumin treated animals as compared to positive control group. It is important to note that oxidative status in animals may vary according to husbandry practices; however we did not monitor stress level under our laboratory conditions.

Acute toxicity was initially attributed to mainly genotoxic effects of the epoxide, dependent on the formation of DNA adducts which at high levels lead to cell death. However, a dialdehyde metabolite of AFB_1that rapidly forms from the epoxide, can form adducts with proteins, and these were proposed to contribute to the acute toxicity (Neal *et al.* 1981). In addition, such cellular necrotic damage caused by AFB_1 dialdehyde may lead to compensatory liver hyperplasia and may promote the incorporation of mutations into the DNA of dividing cells and hence contribute towards carcinogenicity initiated by the AFB_1-exo-epoxide (Roebuck, 2004). Histological studies of hepatic tissue in the control group revealed extensive hemorrhages, infiltration of inflammatory cells and swelling of nucleus suggestive of extensive damage by the epoxides of aflatoxin B_1 where as the changes were milder in the treatment group, with normal hepatic cellular architecture. Thus, dietary supplementation of curcumin had ameliorating role in aflatoxin induced oxidative stress and toxicopathology in mice.

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