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Protective Potential of *Curcuma longa* and *Curcumin* on Aflatoxin B_1 Induced Hepatotoxicity in Swiss Albino Mice

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ABSTRACT

Aflatoxin, a class of mycotoxins produced by fungal spices of genus *Aspergillus* are contaminants of feed ingredients routinely used for poultry rations. This is considered to be one of the most potent hepatotoxin and a well known hepatocarcinogen. Hence the present aim of the study is to elucidate hepatoprotective potential of *Curcuma longa* and *Curcumin* on aflatoxin B_1 induced histobiochemical alterations in Swiss albino mice. Toxicity was developed by oral administration of AFB_1 at a dose of (2 µg/kg body wt) for 45 days in male mice. *Curcuma longa* (100 and 200 mg/kg body weight) and *Curcumin* (50 and 100 mg/kg body wt.) was given simultaneously for 45 days. The enhanced level of tissue lipid peroxide in AFB_1 treated mice was accompanied by decrease in the levels of reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase. Administration of *Curcuma longa* and *Curcumin* lowered the level of lipid peroxidation and enhanced the antioxidant status of animal. Histological examination of the liver revealed pathophysiological changes in aflatoxin group and treatment with *Curcuma longa* and *Curcumin* improved liver histology. Our results suggest that Turmeric rhizome powder (*Curcuma longa*) and *Curcumin* showed protective effects against AFB_1 induced toxicity by modulating lipid peroxidation and augmenting antioxidant defense system.

INTRODUCTION

Aflatoxins, a class of mycotoxins produced by fungal species of genus *Aspergillus* (*A. flavus* and *A. parasiticus*) are contaminants of feed ingredients routinely used for poultry rations [1]. Epidemiological and experimental studies have shown that aflatoxins are hepatotoxic, hepatocarcinogenic, mutagenic and teratogenic [2,3]. Aflatoxins cause oxidative stress by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidants in aflatoxin-treated animals [2-4]. Among the different types of aflatoxin, AFB_1 is the most potent and prevalent and is often found in high concentration in cereal grains and peanut meal [5]. Aflatoxins causes a variety of effects in poultry including poor growth, efficiency of feed consumption [6], liver pathology, immuno suppression [7] and changes in relative organ weight [8], and increased susceptibility to the some environmental and infectious factor [9].

Medicinal plants and their active principles have received great attention as potential anti per oxidative agent [10]. The rhizome of *Curcuma longa* (Turmeric) has been widely used as a spice and coloring agent in many foods. In the tropical region of Asia, turmeric has also been used as a traditional remedy for treatment of inflammation and other diseases. *Curcuma longa*, a perennial herb that is known, as turmeric is a member of family Zingiberaceae. The plant extracts were found to have antifungal[11] immunomodulatory [12] antioxidative [13] and antimutagenic activities. Keeping the above mentioned medicinal properties of *Curcuma longa*, the present study was designed to determine if turmeric rhizome powder and *Curcumin* could modify aflatoxin B_1 induced hepatotoxicity in male Swiss albino mice.

MATERIALS AND METHODS

Chemicals

Aflatoxin B_1 was purchased from HIMEDIA (India). All other chemicals used were of analytical grade and obtained from Sisco Research Laboratories (India), Qualigens (India/Germany), SD Fine Chemicals (India), HIMEDIA (India), and Central Drug House (India).

Animals

Male Swiss albino mice weighing 15–30 g (2–2.5 months) were obtained from Haryana Agricultural University, Hissar, India for experimental purpose. The Animal Ethics Committee of Banasthali University, Banasthali, India has approved the experimental protocols. All experiments were conducted on adult male albino mice (*Mus musculus* L) weighing 25–35 g (3–4 months old). They were housed in polypropylene cages in an air-conditioned room with temperature maintained at 25°C±3°C,

relative humidity of 50% ±5% and 12-h alternating light and dark cycles. The mice were provided with a nutritionally adequate chow diet (Hindustan Lever Limited, India) and drinking water *adlibitum* throughout the study.

Plant material

Experimental plant *Curcuma longa* was collected from local market, Newai in powdered form and was identified by a plant taxonomist/botanist of our department as a local variety and *Curcumin* was purchased from CDH pvt. Lmt.

Preparation of ethanolic extract of *Curcuma longa*

The coarse powder of *Curcuma longa* (50 g**)** was extracted with ethanol (300ml) using a soxhlet apparatus for 48 h at 60°C. The ethanolic extract thus obtained was dried under reduced pressure at a room temperature not exceeding 40ºC to get the solid extract The yield was 2.5g/kg. Suspension of the extract was prepared in DMSO and was used to assess various biochemical parameters.

Animal treatment and Sample collection

In the present study 60 adult Male Swiss albino mice (*Mus musculus* L) weighing 25–35 g (3–4 months old) were used for biochemical and histological studies. The Groups for each parameter were treated orally, once, daily as follows –

Group 1, received 2ml of DMSO served as Control.

Group 2, received aflatoxin B_1 (2µg/Kg body weight) dissolved in DMSO.

Group 3 and 4, were administered with ethanolic *Curcuma longa* extract at a dose of 100mg/kg body weight and 200mg/kg body weight, respectively.

Group 5 and 6, were administered with *Curcumin* at a dose of 50mg/kg body weight and 100mg/kg body weight, respectively.

Group 7 and 8, were administered with aflatoxin B_1 (2µg/Kg body weight) and *Curcuma longa (*100mg/kg body weight and 200mg/kg body weight*) respectively*.

Group 9 and 10, were administered with aflatoxin B_1 (2µg/Kg body weight) and *Curcumin* (50mg/kg body weight and 100mg/kg body weight*)* respectively

Curcuma longa extract and *Curcumin* were given at an interval of 30 minutes of AFB_1 administration. The dose of aflatoxin B_1 was decided on the basis of previously published report [14] and mentioned doses of *Curcuma longa and Curcumin* were also selected on the basis of earlier published report [15] and on the basis of experiments performed in the laboratory. The duration of treatment for each group was of 45 days. After administration of last dose, the animals were given rest overnight and then on the next day they were sacrificed under light ether anesthesia. The liver lobules were excised, cleaned, washed with ice-cold saline (pH 7.4), blotted and used for various biochemical and histological assays. Liver lobules (3/4 part) were used for biochemical and remaining lobules were used for histological examination.

Biochemical assays

Post Mitochondrial supernatant (PMS) was prepared using method of Mohandas *et al.* [*16*] with some modifications. Liver was excised, cleaned, washed and homogenized in chilled phosphate buffer saline (0.1M, pH7.4) using a Potter elvejem homogenizer. The homogenate was filtered through muslin cloth and centrifuged at 10,000 rpm for 15- 20 min at 4°C to get enzyme fraction. The resulted supernatant was used for various biochemical assays. Lipid peroxidation was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids [17]. Superoxide dismutase (SOD) activity was assessed according to the method of Marklund and Marklund [18]. Catalase (CAT) activity was assayed following the method of Aebi [19]. Reduced glutathione (GSH) content was determined according to the method of Jollow *et al.* [20]. Activity of glutathione-S trasferase (GST) was determined by the method of Habig *et al.*[21] and total protein content was estimated by the method of Lowry *et al.* [22] using bovine serum albumin as standard.

Histological/ Histopathological assay

Liver lobules were dissected from the animals and fixed in buffered 10% formalin at room temperature for 72 h. It was then thoroughly washed under running water and dehydrated in ascending grades of ethyl alcohol, cleared, and embedded in soft paraffin. Tissue sections of about 6 mm were cut, stained with hematoxylin and eosin, and examined with a light microscope.

Statistical analysis

Data are expressed as the mean ±S.E. Statistical analysis was done using analysis of variance (ANOVA) followed by multiple comparison test tukey using the Statistical Package for the Social Sciences (S.P.S.S. 11). The level of significance was set at $p<0.05$, $p<0.02$, $p<0.01$, $p<0.001$.

RESULTS

Effect on body weight and organ weight

The change in body weight and organ weight of animals were detected at the end of experiment in different groups and are presented in Table No.1. At the termination of experiment, the AFB_1 treated animals showed significant decrease in percent body weight (6.18%, $p<0.001$) as compared to control group (1.13%), indicating a condition of weight lose. But treatment with low dose and high dose of *Curcuma longa* and *Curcumin* showed significant increase in body weight (1.73%, 2.20%, 6.20%9.01%, in group III –group VI respectively, $P<0.001$) when compared to control animals. When animals were treated with low dose and high dose of *Curcuma longa* and *Curcumin* along with AFB_1 (group VII-group X) exhibited 3.97%, 0.53%, 1.07% and 2.22% respectively, $P<0.001$ increase in body weight compared to relevant AFB_1 treated groups.

The relative weight of liver (6.56%) in AFB_1 treated group insignificantly increased ($p>0.05$) when compared with control group (6.47%). Mice on treatment with low dose and high dose of *Curcuma longa* showed increased values of 6.75% and 6.63% but treatment with low and high dose of *Curcumin* showed decreased values of 6.38% and 6.12% respectively when compared to control group .On the other hand mice administered with low and high dose of *Curcuma longa* and *Curcumin* along with AFB_1 showed decrease liver weight ($p>0.05$ in all the groups except group VIII $p<0.05$) values of 5.96%, 5.64%, 5.87%, and 5.87% respectively as compared to AFB_1 exposed group.

Biochemical parameters

Effect of AFB_1, *Curcuma longa* and *Curcumin* either alone or in different combination with AFB_1 on lipid peroxidation, total

Table No.1: Preventive effects of Curcuma longa and Curcumin extracts on body weight and organ weight of mice exposed to aflatoxin B_1

Parameters / Groups	Body Weight (in gms)		Difference (%)	Liver (in gms)	
	Initial	Final		Absolute	Relative (%)
Control	29 ± 1.46	29.33 ± 0.80	1.14	1.90 ± 0.20	6.47
Aflatoxin B_1 (2ug/kg body weight)	32.33 ± 0.76	30.33 ± 0.08	6.19[k]	1.99 ± 0.04	6.56
Curcuma longa (low dose)-100mg/kg body weight	28.83 ± 1.16	29.33 ± 1.02	1.73[k]	1.98±0.61	6.75
Curcuma longa (high dose)-200mg/kg body weight	30.33 ± 1.20	31± 0.81	2.201[k]	2.05 ± 0.18	6.63
Curcumin (low dose)-50mg/kg body weight	29.5 ± 1.23	31.33 ± 0.98	6.20[k]	2±0.05	6.38
Curcumin (high dose)-100mg/kg body weight	29.5 ± 1.25	32.16 ± 1.42	9.02[k]	1.97 ± 0.05	6.12
Curcuma longa (low dose)+AFB_1	30.83 ± 0.47	29.66 ± 0.49	3.98[a]	1.77 ± 0.04	5.96
Curcuma longa (high dose)+AFB_1	31.5 ± 0.76	31.33 ± 0.49	0.54[a]	1.77 ± 0.03	5.64[d]
Curcumin (low dose)+AFB_1	31.66 ± 1.14	32 ± 0.63	1.07[a]	1.88 ± 0.07	5.87
Curcumin (high dose)+AFB_1	30.16 ± 0.87	30.83 ± 0.94	2.22[a]	1.81 ± 0.04	5.87

Values are the mean ±S.E.M.; n =6 (The details are given in materials and methods)
As compare to group 1 (control) k = p<0.001; l = p<0.01; m = p<0.02; n= p<0.05
As compare to group 2(aflatoxin) a = p<0.001; b = p<0.01; c = p<0.02; d = p<0.05

protein and antioxidant related parameters in liver of mice (Table No.2). Aflatoxin B_1 at a dose of 2µg/kg body weight caused significant (p<0.01) increase in the level of TBA reactive product and significant decrease in SOD (p<0.01), GST (p<0.001), and GSH (p<0.001) content in hepatic tissue of mice, in comparison to control group1 animals.CAT and total protein content decreased insignificantly in group 2 when compared with control animals.

Oral administration of ethanolic *Curcuma longa* extract at a dose of 100 and 200 mg/kg body weight insignificantly decreased the TBA reactive product as compared to control animals (group 1). Moreover low and high dose of ethanolic *Curcuma longa* extract treatment also lead to insignificant increase in the CAT activity and GSH and total protein content. In comparison to control animals (group 1), SOD activity increased significantly in group 4 (p<0.001) and insignificantly increased in group 3 treated animals, whereas on the other hand GST activity increased significantly in group 3 (p<0.02) and group 4 (p<0.001) respectively, in comparison to control animals (group 1).

Intake of *Curcumin* at a dose of 50 and 100 mg/kg body weight showed insignificant decrease in TBA reactive product, while administration of same dose significantly (p<0.001) elevated SOD, GST activity and total protein content (p<0.01 and p<0.001) in group 5 and 6 respectively when compared with control mice (group 1). Significant rise of GSH in group 6 (p<0.001) and insignificant rise in group 5 were also recorded with both dose of *Curcumin*. In comparison to control animals, activity of CAT increased insignificantly in group 5 and group 6 animals. Administration of low and high dose of *Curcuma longa* and *Curcumin* along with AFB_1 to mice in group 7,8 and 9 insignificantly decreased the TBA reactive product and significantly (p<0.001) decreased the TBA reactive products in group 10, animals when compared with AFB_1 treated animals(group 2).

In animals supplemented with *Curcuma longa* and *Curcumin* along with AFB_1 significantly (p<0.001) increased the SOD, GST and GSH content in group 8, 9 and 10 except group 7, where insignificant increase was noticed, when compared to AFB_1 treated animals (group 2). Moreover, low and high dose of *Curcuma longa* and *Curcumin* when given along with AFB_1 led to insignificant elevation in the CAT activity and significant elevation in total protein content in group 9 and group 10 but insignificant increase in total protein content was observed in group 7 and group 8, when compared to AFB_1 treated animals (group 2).

Histological/Histopathological Analysis

The liver of control mice (group 1) showed normal hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective

Table No.2: Protective effect of Curcuma longa and Curcumin on hepatic biochemical parameters in male mice treated with Aflatoxin B_1.

Parameters / Groups	Protein (mg/100ml)	GST (nmols/min/ mg protein using CDNB as substrate)	GSH (ug/100 mg tissue weight)	LPO (n moles MDA/ mgprotein /60 min)	SOD (units/mg protein)	CAT (H_2O_2 consumed/ mg protein/min)
Control	93.83 ± 3.87	175.44 ± 0.58	62 ± 1.36	6.00 ± 0.11	8.47 ± 0.36	36.02±2.64
Aflatoxin B_1 (2ug/kg body weight)	89.00 ± 2.46	170.44 ± 0.58^{k}	45.16 ± 1.70^{k}	6.90 ± 0.17^{l}	7.06 ± 0.39^{l}	32.78 ± 2.05
C. L. (low dose) 100mg/kg body weight	95.05 ± 0.38	178.48 ± 0.53^{m}	63.5 ± 0.42	5.92 ± 0.18	9.18 ± 0.22	36.84 ± 2.09
C. L. (high dose) 200mg/kg body weight	99.89 ± 0.44	181.42 ± 0.49^{k}	65.83 ± 0.60	5.83 ± 0.14	10.15 ± 0.15^{k}	38.18 ± 1.33
***Curcumin* (low dose) 50mg/kg body weight**	105.26 ± 2.07^{l}	180.43 ± 0.64^{k}	67 ± 1.69	5.79 ± 0.15	11.04 ± 0.23^{k}	39.69 ± 1.63
***Curcumin* (high dose) 100mg/kg body weight**	110.03 ± 0.93^{k}	185.67 ± 0.57^{k}	73 ± 2.09^{k}	5.54 ± 0.08	12.47 ± 0.22^{k}	41.26 ± 1.94
C. L. (low dose) +AFB_1	90.84 ± 0.55	172.26 ± 0.64	50.5 ± 2.48	6.86 ± 0.14	8.05 ± 0.13	35.93 ± 1.58
C.L. (high dose) +AFB_1	92.76 ± 0.57	176.47 ± 0.55^{a}	57 ± 1.46^{a}	6.35 ± 0.15	9.48 ± 0.23^{a}	37.25 ± 2.30
***Curcumin* (low dose) +AFB_1**	96.49 ± 2.64^{a}	174.27 ± 0.61^{a}	60.66 ± 0.49^{a}	6.26 ± 0.13	10.38 ± 0.21^{a}	37.32 ± 1.97
***Curcumin* (high dose) +AFB_1**	106.22 ± 1.40^{a}	178.51 ± 0.58^{a}	67.66 ± 0.49^{a}	6.13 ± 0.17^{a}	12.15 ± 0.24^{a}	39.22 ± 1.67

Abbreviation-GST: glutathione S-transferase; GSH: reduced glutathione; LPO: lipid peroxidase; SOD: superoxide dismutase; CAT: catalase; C.L.: Curcuma longa

Values are the mean ±S.E.M; n =6

k = p<0.001 as compared to normal animals
l = p<0.01 as compared to normal animals
m = p<0.02 as compared to normal animals
n = p<0.05 as compared to normal animals

a = p<0.001 as compared to AFB_1 exposed animals
b = p<0.01 as compared to AFB_1 exposed animals
c = p<0.02 as compared to AFB_1 exposed animals
d = p<0.05 as compared to AFB_1 exposed animals

tissue. Hepatocytes are arranged in trabecules running radially from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus (Fig 1). The livers of mice exposed to AFB_1 (group 2) for 45 days revealed disruption of the normal structural organization of the hepatic lobules and loss of the characteristic cord-like arrangement of the normal liver cells. The central and portal veins were congested. Many hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization. The nuclei of these cells were pyknotic. The central vein and sinusoids between hepatocytes were dilated (Fig 2). Animals treated with low and high doses of *Curcuma longa* (groups 3 and 4) and *Curcumin* (groups 5 and 6) showed normal structure of hepatic cells without any damage. Animals treated with low doses of *Curcuma longa* and *Curcumin* (Groups 7 and 9) showed that the majority of these histopathological changes were diminished but some hepatocytes appeared with vacuolized cytoplasm and the central vein appeared congested (Fig 3and 5). In the high dose groups (Groups 8 and 10), the liver restored most of its normal structure and was able to diminish the fibrosis, congestion, incidence of inflammatory cells infiltration, centrilobular hepatocytes swelling, hepatocytes vacuolization, fatty changes, and hemorrhagic clots (Fig 4 and 6).

DISCUSSION

Aflatoxin especially AFB_1 are the most common mycoyoxins to which humans and animals are exposed. Aflatoxins are hepatotoxic, hepatocarcinogenic, mutagenic, teratogenic [2,3] and causes oxidative stress by increasing lipid peroxidation and decreasing antioxidant in treated animals [2-4].

The inhibition of body weight appears to be solely the result of the profound effect of AFB_1 [23]. These adverse effects of AFB_1 are due to anorexia, inhibition of protein and DNA synthesis and lipogenesis. Lower body weight gain, and heavier liver observed in mice treated with AFB_1 alone are consistent with previous report [8]. The co-current administration improved both bodyweight gain and liver weight in comparison to AFB_1 treated mice, suggesting antioxidant protection by *Curcuma longa* and

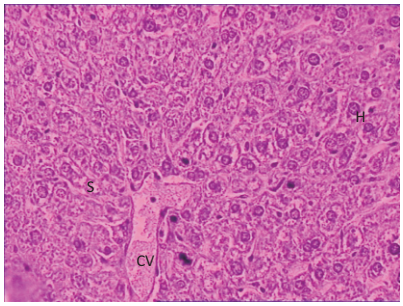


Fig 1

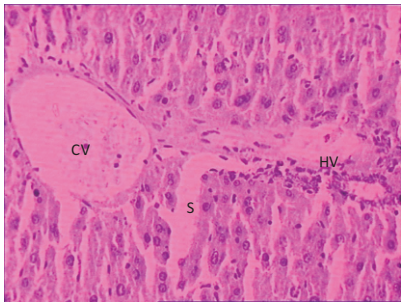
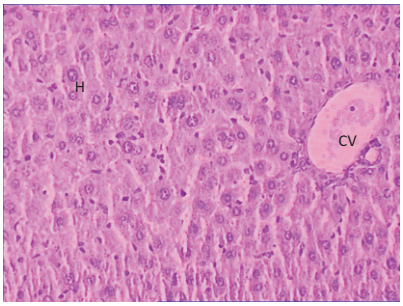


Fig 2



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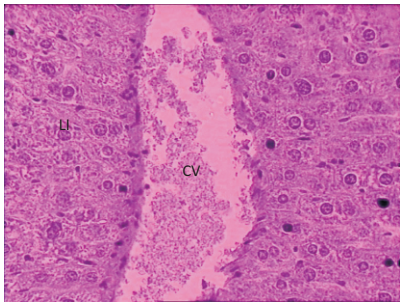

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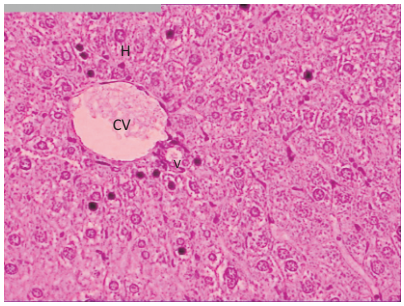

Fig 5

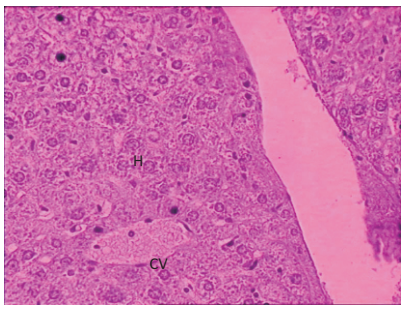

Fig 6

Fig. 1: Transverse section of the liver of a control mouse (X= 40x). **Fig. 2:** Transverse section of the liver of a mouse treated with AFB1 (X= 40x). **Fig. 3 and 4:** T.S. of liver section obtained from a mouse treated low dose of curcuma longa and Curcumin with AFB1 (X= 40x). **Fig. 5 and 6:** T.S. liver section obtained from a mouse after treatment with AFB1 and high dose of curcuma longa and Curcumin showing improvement of hepatic tirssue (X= 40x).
Here- CV-central vein; H- hepatocytes; K- kuffer cells; S- sinusoids; Hv-hepatocellular infileration; LI-lymphocyte infilteration

Curcumin. Curcumin, the major antioxidant ingredient of turmeric, is known to inhibit the biotransformation of AFB_1 to aflatoxicol in liver [24] and is also responsible for its anti-mutagenic and anticarcinogenic action [25].

In the present study results revealed that reduced level of total protein is indicative of the toxic effect of AFB_1 in liver. Aflatoxin is known to impair protein biosynthesis by forming adducts with DNA, RNA and proteins, inhibits RNA synthesis, DNA-dependent RNA polymerase activity and causes degranulation of endoplasmic reticulum [26]. 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, heart, liver and kidney [27] of aflatoxin-fed animals. Our results showed that *Curcuma longa* & *Curcumin* treatment along with aflatoxin ameliorates aflatoxin-induced changes in protein contents in the liver of mice. The amelioration in protein contents might be due to increased DNA synthesis and reduction in harmful adduct formation [28].

Several studies have also demonstrated *Curcumin's* ability to reduce oxidative stress [29]. 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.

Carcinogens like aflatoxin B_1, which generate epoxides, have been found to conjugate readily with GSH. Liver cells, which are lethally injured by several toxins, exhibit marked alterations in intracellular Ca^{2+} homeostasis after excessive accumulation of Ca^{2+}. During hepatocellular necrosis, excessive intracellular Ca^{2+} is known to thrust the metabolism in an unmanageable disorder, which leads to mitochondrial dysfunction, inhibition of enzymes and denaturation of structural proteins. Toskulkao and Glinsukon [30] 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. The increased lipid peroxidation in aflatoxin-treated animals is in agreement with findings reported previously for rat liver [3] as well as liver, kidney [31] in mice.

Oxidative stress was originally defined as the disequilibrium between prooxidants and antioxidants in biological systems. Lipid peroxidation is regarded as one of the primary key events in cellular damage and the relationship between GSH levels, lipid peroxidation and cell lysis has been reported. An increase in lipid peroxidation could also 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 in the liver 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 to H_2O_2 and O_2. The H_2O_2 produced can then be decomposed enzymatically by catalase. Thus, significant reduction in these enzyme activities could be responsible for increased lipid peroxidation observed during aflatoxicosis. Significant reductions in glutathione peroxidase [32], superoxide dismutase [3] and catalase [33] have been reported in aflatoxin-fed rat liver. The decline in enzymatic antioxidants could also be due to the reduction in protein biosynthesis [26].

Glutathione content decreased significantly in liver of aflatoxin-treated mice, 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 peroxidation. Several investigators [3, 34] have reported significant reduction in glutathione content in aflatoxin-fed rat liver. *Curcumin*, the major pigment in Curcuminoids of turmeric, is known to protect against AFB_1 by inhibiting the biotransformation of AFB_1 to aflatoxicol in liver [24]. The other beneficial compounds of turmeric are tetrahydro curcumin, niacin, tumerone, culone and cinnamic acid, but these are present in very low concentration and contribute very little to over all antioxidant activity.

Administration of *Curcuma longa* and *Curcumin* reversed the changes induced by aflatoxin B_1 supporting the hypotheses that plant products are effective antioxidative agents. Possible mechanism behind this is that the *Curcumin* scavenges or neutralizes the free radicals, interacts with oxidative cascade, quenches oxygen, inhibits oxidative enzymes like cytochrome P450, and chelates metal ions like Fe^{+2}, inhibits peroxidation of membrane lipids and maintains cell membrane integrity and their function in the liver, lung and kidney.

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 [35] 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 having polyphenolic structure and diketone functional groups. *Curcumin* is a stronger antioxidant inhibitor of lipid peroxidation than other flavonoids, which have a single phenolic hydroxyl group. Effective antioxidant property of *Curcumin* decreases the utilization of vitamins C and E in the liver and thus maintains their levels.

Histopathological examination of liver section of normal mice showed normal hepatic cells with cytoplasam and nucleus whereas, aflatoxin B1 treated animals showed pronounced hepatic histopathological evidences by histological alternations, including hepatocyte vacuolization of the cells, swelling, pyknotic nuclei, dilation of central vein and sinusoids. These findings are in support with Surh *et al.* [36]. The co-current administration of *Curcuma longa* and *Curcumin* (at both doses) and intoxicated with aflatoxin B_1 the normal cellular architecture was retained. Hence, confirming the significant hepatoprotective effect of *Curcuma longa* and *Curcumin* which is confirmed by the results of biochemical studies.

CONCLUSION

Curcuma longa and *Curcumin* exert its protective effect against aflatoxin B_1-induced toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system. The results of the present study suggest that *Curcuma longa* and *Curcumin* can be used as a dietary supplement in order to prevent aflatoxin B_1 induced oxidative stress. This could be due to their antioxidant nature, which combines free radical scavenging with metal chelating properties. The healing effects of Curcuma longa and Curcumin were also confirmed by histological observations.

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