

Original Research Article

Effect of curcumin on aflatoxin B₁-induced toxicity in rats: Biochemical and selected oxidative stress parameters

ABSTRACT

Objective: The aim of the present study was to investigate the protective effect of curcumin against aflatoxin B₁ (AFB₁) induced hepatotoxicity.

Materials and Methods: Twenty-eight healthy adult male rats were divided into four groups. Rats in the first group served as control whereas, rats in the second, third and fourth groups received oral curcumin (15mg/5ml/kg B.wt.), AFB₁ (3mg/kg B.wt.) as single i.p injection and both curcumin and AFB₁, respectively for 5 weeks.

Results: Biochemical analysis of serum samples indicated a significant increase in aspartate transaminase (AST) and alanine transaminase (ALT) activities and total cholesterol and creatinine concentrations along with significant decrease in protein content of AFB₁ intoxicated rats compared to control group. Oral administration of curcumin along with injected AFB₁ restored AST, ALT, total cholesterol, creatinine and total protein near to control values. Biochemical analysis of liver antioxidants revealed a significant ($P < 0.05$) reduction in catalase (CAT) and superoxide dismutase (SOD) activities and hepatic reduced glutathione (GSH) content in rats injected with AFB₁ compared to control. On the contrary, oral administration of curcumin along with injected AFB₁ enhanced hepatic CAT and SOD activities and GSH concentration towards the control values, suggesting that curcumin could improve the antioxidant status in AFB₁ induced oxidative stress. The Biochemical findings were supported by histopathology of liver tissues which indicated vacuolar degeneration and necrotizing

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changes in liver of rats intoxicated with AFB₁ and significant amelioration of these effects in these rats whenever treated with curcumin.

Conclusion: Conclusively, oral administration of curcumin along with AFB₁ caused significant inhibition in AFB₁-induced hepatotoxicity in rats by increasing the bioavailability of GSH and activation of antioxidant enzymes.

Keywords: Medicinal plants, Antioxidants, Oxidative stress, Biomarkers, liver

1. INTRODUCTION

Mycotoxins are toxic metabolites produced by a large number of fungi under a wide range of environmental condition. Many of these fungi invade cereals, nuts and grains that are eventually used in the manufacture of animal feeds. Aflatoxins are secondary metabolites of the moulds *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus tamarii* and *Aspergillus niger* [1]. AFB₁ is by far the most potent teratogen, mutagen and hepatocarcinogen of all aflatoxins [2]. The carcinogenic potential of AFB₁ following oral administration has been shown in several animal species, including rodents, non-human primates and fish [3]. The biological effects of AFB₁ in animals are related to their level in the feed and to the animal's susceptibility. Epidemiologic, clinical, and experimental studies have revealed that aflatoxins are hepatotoxic, hepatocarcinogenic, and mutagenic [4]. AFB₁ can cause lipid peroxidation in the rat liver, which is closely related to liver cell injury [5,6]. Bosch-Morell et al. [7] have

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47 demonstrated the involvement of oxidative stress in retinal detachment by detecting
48 lipid peroxidation products in subretinal fluid of patients undergoing surgery. Medicinal
49 plants and their active principles had received great attention as potentially
50 antiperoxidative agents [8-15]. Turmeric is a perennial herb that grows to a height of
51 three to five feet and is cultivated extensively in Asia (India and China) and other

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54 countries with tropical climate. Curcumin, the active ingredient from the spice turmeric
55 is a potent antioxidant and anti-inflammatory agent with hepatoprotective,
56 anticarcinogenic and antimicrobial properties [16]. The aim of the present study was to
57 investigate the antitoxic effect of curcumin in AFB₁ intoxicated rats by evaluation of
58 selected serum biochemical parameters, hepatic oxidative stress biomarkers and
59 histopathology of affected liver.

60 2. MATERIALS AND METHODS

61 2.1. Chemicals

62 Curcumin, AFB₁ and DMSO were purchased from Sigma Chemical Co. (St. Louis,
63 MO, USA). All other chemicals and buffers were of analytical grade.

64 2.2. Experimental animals

65 Twenty-eight healthy adult male inbred wistar rats weighing between 150–200g were
66 obtained from the laboratory animal house of the Faculty of Veterinary Medicine and
67 Animal Recourses, King Faisal University, Saudi Arabia. They were maintained in
68 accordance with the national guidelines and protocols, approved by the University
69 Animal Ethics Committee, King Faisal University, Saudi Arabia. They were housed in
70 clean and disinfected plastic cages. Commercial basal pelleted diet and water were
71 provided *ad libitum*. Rats were subjected to natural photoperiod of 12hr light:dark cycle
72 throughout the experimental period (5 weeks). The experimental animals were housed
73 in air-conditioned rooms at 21-23°C and 60-65% of relative humidity. All rats received
74 basal diet for two weeks before the start of the experiment for adaptation and to ensure
75 normal growth and behavior.

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77 4 78 2.3. Experimental design

79 Rats were divided into four groups of 7 rats each (4 animals/cage). Rats of the first
80 group received basal diet and served as control whereas, rats of the second and third
81 groups received curcumin orally (15mg/5ml/kg BW) [15] and single i.p injection of
82 AFB₁ (3mg/kg BW) [17], respectively. Rats in the fourth group received a combination
83 of second and third groups for five weeks.

84 2.4. Sampling and analysis

85 At the end of the experiment, rats were sacrificed, anaesthetized by diethyl ether
86 inhalation and blood and liver samples were collected. Serum was separated by
87 centrifugation
88 for 10 min at 1200g and was immediately frozen at –20°C until the time of
89 analysis. Commercial diagnostic kits (United Diagnostic Industry, UDI, Dammam,
90 Saudi Arabia) were used for determination of glucose (EP37L-660), total proteins
91 (EP56-660), albumin (EP03-570), alanine aminotransferase, ALT (EP07-500), aspartate

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Comment [GS7]: Normal diet

Comment [GS8]: Normal control (NC)

Comment [GS9]: Did they get the injection?

Comment [GS10]: This part is not clear, you have to specify each group separately and what they were fed on and how? Again you need a group of AFB₁ fed animals as another set of control to compare with the animals fed with both. Are all the experimental groups received the same doses? This needs to be addressed and change the experimental design accordingly.

92 amino transferase, AST (EP15-500), blood urea nitrogen, BUN (EP20-420), uric acid
93 (EP61-620), creatinine (EP33K-660), triacylglycerol, TAG (EP59-660), cholesterol
94 (EP24-660), calcium (EP22-660), phosphorus (EP46-660), magnesium (EP50-660),
95 chloride (EP27-500) on ELIPSE full automated chemistry analyzer (Rome, Italy).
96 Concentration of the biochemical constituents was calculated according to the
97 manufacturer's instructions. In addition, portions of the liver were dissected out and
98 trimmed off the attached tissue and stored at -80°C until used for biochemical analysis
99 of GSH concentration and antioxidant enzyme activities. The activities of CAT
100 (nmol/min/gram tissue; Cayman Chemical Company, USA, Catalog No. 707002), SOD
101 (U/ gram tissue; Cayman Chemical Company, USA, Catalog No. 706002) and
102 concentrations of GSH (μM / gram tissue; Cayman Chemical Company, USA, Catalog

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105 No.703002) were determined by ELISA reader (Absorbance Microplate Reader ELx
106 800TM BioTek®, USA). The buffer for preparation of liver tissues homogenate were
107 obtained from Cayman Chemical Company, USA. Results were calculated according to
108 the manufacturer's instructions. Another portion of liver tissue was collected also and
109 cut in small pieces and immersed in neutral buffered formalin for 24 h for
110 histopathological examination. The fixed liver tissue was processed routinely,
111 embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard
112 techniques [18]. The effect of high cholesterol diet induced fatty degeneration was
113 evaluated by assessing the morphological changes in the liver sections stained with
114 hematoxylin and eosin (H and E), using standard techniques.

Comment [GS12]: Which group received such a diet?

115 2.5. Statistical analysis

116 All the grouped data were statistically evaluated and the significance of changes caused
117 by various treatments was determined using one ways ANOVA. Post hoc tests in the
118 Analysis of Variance (ANOVA), containing one factor (Group) and serum biochemical
119 dependent measurements, was used applying GLM- Unianova Procedure. Bartlett's,
120 Brown and Forsythe's Tests for Homogeneity Of Variance assumptions were reasonably
121 met for the one way ANOVA. The tables (1-5) shows the significance difference of
122 means. Inspection of the source tables (6-10) shows that the main effects are significant
123 for some parameters. The pair-wise comparisons of means was determined using the
124 Duncan's Multiple Range Test [19].

125 3. RESULTS

126 3.1. Selected Biochemical Parameters

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129 The present findings indicated that, AFB₁ treatment (group III) induced significant
130 increase ($P < 0.05$) in total cholesterol, ALT and AST activities (Table 1) and creatinine
131 concentration (Table 3) however protein contents (Table 1) was significantly decreased
132 ($P \leq 0.05$) in serum of rats when compared with the control group. Oral administration
133 of curcumin along with injected AFB₁ restored total cholesterol, ALT, AST, creatinine
134 and total protein near to control values.

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135 3.2. Oxidative stress Biomarkers

136 The effects of AFB₁ and curcumin either alone or in combination on oxidative stress

biomarkers of liver tissues of rats are summarized in Table 5. A significant ($P < 0.05$) reduction of CAT and SOD activities and GSH content were evident in rats injected with AFB₁ (group III) compared to normal untreated animals (group I). On the contrary, Oral administration of curcumin along with AFB₁ (group IV) caused significant amelioration in AFB₁-induced effects observed by decreased CAT and SOD enzymes activities ($P \leq 0.05$) and increased reduced glutathione contents ($P \leq 0.001$) compared to the AFB₁ treated rats (group III).

3.3. Histopathological examination

Liver of the control and curcumin treated rats showed central veins surrounded by polygonal cells arranged in regular cords separated from each other by sinusoids (Fig. 1). The liver of rats intoxicated with AFB₁ showed distorted lobular architecture and necrobiotic changes ranged from vacuolar degeneration to necrotizing changes. This was associated with mononuclear cell infiltration everywhere (Fig. 2). However, livers treated with AFB₁ along with curcumin appeared more or less recovered and have an almost normal architecture (Fig. 3).

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4. DISCUSSION

4.1. Selected Biochemical parameters

It is well known that, aflatoxin has a harmful and stressful effect on liver tissue. AST and ALT are cytosolic enzymes and are famous biomarkers of liver damage. In the present study, AFB₁ injection was found to cause an increase in serum ALT and AST activities. These results indicated liver injury and necrosis [20,21]. Whenever liver was injured, levels of hepatic transaminases were significantly increased [22-24]. However, administration of curcumin along with injected AFB₁ showed marked recovery.

Comment [GS18]: Is this recovery or prevention? Specify

Regarding the ameliorative effect of curcumin against AFB₁ toxicity, previous reports [25,26] showed a significant hepatoprotective activity of curcumin by lowering the level of serum biomarker enzymes in AFB₁ intoxicated rats. Low total protein level acts as an indicator of the toxic effect of AFB₁ in serum [27]. 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 [27]. Reduction in protein content observed in the current study (group III) may be attributed to increase in the rate of degeneration of liver tissues as underlined by increasing activities of ALT and AST. The injured liver logically is unable to maintain vital biochemical processes particularly protein biosynthesis. These results are in accordance with previous work [28] which reported a decrease in protein content in skeletal muscle, heart, liver and kidney of aflatoxin-fed animals. The present results showed that, curcumin treatment along with injected AFB₁ ameliorates AFB₁-induced changes in protein contents in the serum of rats. The amelioration in protein contents might be due to increased DNA synthesis and reduction in harmful adduct formation [29]. Authors investigated the inhibitory effects of curcumin, garlic squeeze,

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grape seed extract, tea polyphenols, vitamin C and vitamin E on nicotine-DNA adduction *in vivo*. They suggested that these dietary constituents are beneficial in

preventing the harmful adduct formation and thus block the potential carcinogenesis induced by nicotine. Similar results described the ameliorative effect of curcumin against AFB₁ induced low protein contents in Broiler chicks [30] and in mice [31]. After biosynthesis of creatine in the liver, it is taken up from the blood by skeletal muscles and converted into creatine phosphate. Creatine and its phosphate are converted spontaneously into creatinine [32]. The significant appearance ($P < 0.05$) of creatinine in the serum of aflatoxin-fed rats indicated the increased transformation of phosphocreatine to creatinine in muscle which might be due to lesser utilization of phosphocreatine in muscular contraction. The elevated creatinine level in AFB₁ treated rats as observed in the current study, suggests the myotoxic and nephrotoxic effect of AFB₁ in rats [33] which improved upon administration of curcumin.

4.2. Oxidative stress Biomarkers

Oxidative stress was originally defined as the imbalance between prooxidants and antioxidants in biological systems. The significant reduction in the activities of enzymatic antioxidants such as CAT and SOD as well as non- enzymatic antioxidants such as glutathione in the liver of AFB₁-treated rats when compared to the controls indicated that AFB₁ induced oxidative stress and subsequent liver damage. SOD protects cells from oxidative damage by converting free radical superoxide to H₂O₂ and O₂. The H₂O₂ produced can then be decomposed enzymatically by CAT. Significant reductions in SOD [27] and CAT [24] have been reported in aflatoxin-fed rat liver. The significant increase of hepatic antioxidant enzymes CAT and SOD activities observed in this study in rats intoxicated with AFB₁ and treated with curcumin are in accordance

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with previous studies which reported that curcumin is a potent inducer of detoxifying enzymes and thereby prevents the toxicity induced by a chemical carcinogen [15,34]. Glutathione has a beneficial effect by virtue of possessing –SH groups. It helps to protect biological membranes, which are readily susceptible to peroxidation. Carcinogens like AFB₁, which generate epoxides, have been found to conjugate readily with GSH. Lower GSH level would further aggravate the toxic effects of aflatoxin. Many investigators [27, 35] have reported significant reduction in glutathione content in aflatoxin-fed rat liver. The impact of curcumin on GSH has been documented [15].

4.3. Histopathological findings

The histopathological findings supported the biochemical findings and give evidence of liver damage in rats intoxicated with AFB₁. Similar AFB₁-induced hepatic damage has been reported [36]. The relief of hepatic tissues in AFB₁ intoxicated rats treated with curcumin, is consistent with earlier report [37] which suggested that curcumin but not resveratrol has a hepatoprotective effect against aflatoxin B(1)-induced liver injury.

5. CONCLUSION

The results obtained in this present study indicate that, oral administration of curcumin along with AFB₁ caused significant amelioration in AFB₁-induced hepatotoxicity in rats by increasing the bioavailability of GSH and activation of antioxidant enzymes. This suggests that curcumin could improve the antioxidant status in AFB₁-induced oxidative stress.

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