RESEARCH ARTICLE

THE AMELIORATIVE EFFECT OF TURMERIC AGAINST AFLATOXIN TOXICITY IN MALE ALBINO RATS FED ON MOULDY BREAD

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ABSTRACT
Moulds are found in many foodstuffs and produce toxic substances known as mycotoxins which are dangerous to the human health and livestock. Aflatoxins (AFs) are the most important mycotoxins affecting the human health and trade in the world. There is an inevitable exposure to AFs in developing countries. The current study aimed to investigate the ameliorative role of turmeric (tur) against AFs toxicity in adult male albino rats (Rattus norvegicus) fed on mouldy bread. Twenty-four rats were equally allotted into 4 groups: GpI (control group: fed on dry normal bread), GpII (mouldy bread group: fed on mouldy bread), GpIII (fed on normal bread + 60 mg tur/kg body weight/day), and GpIV (fed on mouldy bread + 60 mg tur/kg body weight/day). Total AFs concentration was measured in mouldy bread, and accordingly the daily dose of AFs was 0.272 mg/kg body weight of rat. After 30 days, the effect of tur on AFs toxicity was evaluated by investigating the activity of aminotransferases, kidney function, lipid profile, and glucose level in serum, as well as genotoxicity and histopathological alterations. Feeding rats with mouldy bread containing AFs led to a significant increase in the activities of serum aspartate and alanine aminotransferases, the concentrations of serum creatinine, urea, total cholesterol, triacylglycerol, low-density lipoprotein cholesterol, and glucose, as well as genotoxicity in bone marrow cells and histopathological lesions in the liver and kidneys tissues. However, tur supplementation alleviated significantly almost all the above-mentioned harmful effects of AFs.

INTRODUCTION
Bread is the main food in developing countries, but it is susceptible to fungal contamination during different stages of production and storing. Developing countries in Africa have high temperatures and high humidity, which are proper conditions for mould growth and mycotoxins production[1]. Many people in the developing countries remove the visible mouldy portions from the bread and consume the remaining of it. However, invisible fungal growth can spread inside the bread loaf starting to produce mycotoxins[2]. Others consume mouldy bread after heating, thinking that heat eliminate any hazard. Unfortunately, mycotoxins produced by these fungi...
either before baking (contaminated wheat for instance) or after baking (due to mishandling) are heat-resistant\[3\].

Mycotoxins are natural toxic compounds produced by fungal genera such as *Aspergillus* and *Penicillium* as secondary metabolites\[4\]. Among these, Aflatoxins (AFs) are the most toxic\[5\]. AFs are very toxic to humans and their animals\[6\]. They have hepatotoxic, nephrotoxic, neurotoxic, carcinogenic, mutagenic, teratogenic, and immunosuppressive effects\[7\]. They include B1, B2, G1 and G2, among which Aflatoxin B1 (AFB1, a common contaminant in food) has the most toxicity including mutagenicity and carcinogenicity\[8\].

The liver is the principal target organ for AFs\[9\]. Several reports have shown that other organs are affected by the toxicity of AFs like kidney\[10\], and lung\[11\]. AFB1 toxicity is attributed to the production of free radicals resulting in lipid peroxidation, DNA damage, and mutations\[10\]. It is also converted to the more toxic epoxide, AFB1-8,9-epoxide (AFBO), by the microsomal CYP450 which binds to macromolecules such as DNA and proteins\[6\].

The dried rhizomes of turmeric plant (*Curcuma longa* linn) plant rhizome was purchased from a local market and powdered after being identified in the herbarium of the Faculty of Science, Fayoum University, Egypt.

**MATERIAL AND METHODS**

**Turmeric**

*Curcuma longa* linn plant rhizome was purchased from a local market and powdered after being identified in the herbarium of the Faculty of Science, Fayoum University, Egypt.

**Animals and experimental design**

The current experimental study was performed by following the National Health Institute (NIH) guidelines for Ethical Conduct in the Care and Use of Laboratory Animals. Twenty-four adult male albino Wistar rats (*Rattus norvegicus*), 165 ± 5 g, were purchased from the National Research Center (Giza, Egypt) and kept under firmly hygienic conditions for two weeks to acclimatize before starting the experiment. During this period, rats were provided with standard food pellets and tap water *ad libitum*.

Rats were then divided into four groups (6 rats each) as follows: Group I, control group, fed on dry normal bread; Group II, mouldy bread group, fed on dry mouldy bread; Group III, tur group, fed on dry normal bread and received 60 mg tur/kg body weight/day\[15\]. Group IV, mouldy bread + tur group, fed on dry mouldy bread and received 60 mg tur/kg body weight/day. The experiment extended for 30 consecutive days.

**Calculation of AFs dose**

Total AFs concentration was measured in bread samples to determine the average amount of AFs consumed by each rat in the mouldy bread fed groups (30 g bread/rat/day). Determination of total AFs concentration was performed by using enzyme-linked immunosorbent assay (Cat. No. 981AFL01LM-96, Helica Biosystems Inc, Santa Ana, USA) as per manufacturer’s protocol. Optical density was then measured using a plate reader (BioTek Instruments, Inc., Winoaosi, VT, USA). Calculation of the mean dose of the consumed AFs/kg body weight of rat/day was performed according to the following equation:
Mean dose of the consumed AFs/kg body weight of rat/day = [Mean concentration of AFs in the bread samples (µg/kg) × Mean of the consumed bread weight/rat/day (g)] / Mean of the body weight of rats (g)

Dissection and sampling
Rats were anesthetized with xylazine and ketamine and killed by cervical dislocation, as per the procedure given by the Institutional Animal Ethics Committee. Blood was obtained, left for clotting at room temperature, and centrifuged at 1200 x g for 10 minutes. Serum was then collected to be used in the biochemical analyses. The liver and kidney were excised, cut into small pieces, and preserved in fixative for histological examination.

Biochemical analyses
Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activities were kinetically investigated using commercial kits (Spinreact, Barcelona, Spain) according to the manufacturer’s instructions. Blood glucose as well as kidney function represented by urea and creatinine levels were assessed using colorimetric kits (Biodiagnostic, Giza, Egypt), according to the manufacturer’s instructions using a Jenway spectrophotometer (Staffordshire, UK). Serum total cholesterol (TC), total triacylglycerol (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using standard colorimetric kits purchased from Biosystems (Barcelona, Spain) according to the methods of Allain et al. Fossati and Prencipe, Grove, respectively. Low-density lipoprotein cholesterol LDL-C was determined according to the Friedewald equation: LDL-C (mg/dL) = TC – HDL-C – TG

Statistical analyses
The results were statistically analyzed using analysis of variance (F-test) followed by Duncan’s multiple range test to determine differences in means using Statistical Analysis Systems (SAS, 2000, version 6.2, SAS Institute, Cary, North Carolina, USA).

RESULTS
Effect of tur supplementation on AFs-induced biochemical disturbance in serum
Total AFs concentration in mouldy bread was 1496 ± 7 µg/kg; accordingly, the daily dose of AFs was 0.272 mg/kg body weight of rat. Rats fed on the mouldy bread only (GpII) showed a significant elevation in the serum AST and ALT activities (P<0.01). Addition of tur to AFs-contaminated diet (GpIV) had a protective effect evidenced by the significantly decrease in the serum activities of aminotransferases (P<0.01), as compared with GpII (Table 1). Mouldy bread intake led to the dysfunction of the kidney as indicated by the significant increase in serum creatinine and urea levels (P<0.01) of GpII compared by Krishna and Hayashi. Bone marrow cells were extracted from femurs using 1.0 mL bovine calf serum. Smears of bone marrow cells were then made on glass slides, left to dry, fixed in methanol for 5 minutes and then stained with May-Grünwald and Giemsa for 5 minutes. For each rat, 2000 polychromatic erythrocytes (PCEs) were examined blindly for the presence of micronuclei under light microscope.

Histopathology
Small portions from the liver and kidney tissues were excised and fixed in neutral buffered formalin. After washing, they were dehydrated through ascending series of alcohol, cleared in xylol, and embedded in paraffin wax. Tissue sections were cut at a thickness of 5 µm using a microtome and stained with hematoxylin and eosin (H&E) according to Bancroft and Gamble, 2008.

Micronucleus test
Micronucleus assay was performed as a measure of genotoxicity. After rats were killed by cervical dislocation, bone marrow cells were prepared and stained as described

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with GpI. However, addition of tur to AFs-contaminated diet (GpIV) was effective in restoring the creatinine and urea levels to the normal value of the control group ($P > 0.05$), as shown in Table “1”.

As depicted in Table “1”, mouldy bread intake led to a significant elevation in serum TC, TG, and LDL-C concentrations ($P < 0.01$). A significant reduction in the levels of these parameters, as well as a significant increase in HDL-C, was detected after treatment with tur ($P < 0.01$) in GpIV as compared with GpII.

AFs-contaminated diet led to a significant elevation ($P < 0.01$) in the serum glucose level when compared to rats fed on normal bread (GpI). Tur treatment decreased significantly the glucose levels ($P < 0.01$) in mouldy bread-treated group, as shown in Table “1”.

Table 1: Serum biochemical parameters of male albino rats fed on mouldy bread and/or turmeric for 30 consecutive days.

<table>
<thead>
<tr>
<th></th>
<th>GpI</th>
<th>GpII</th>
<th>GpIII</th>
<th>GpIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>48.0±3.0c</td>
<td>91.7±1.5a</td>
<td>55.3±4.2b,c</td>
<td>59.1±0.7b</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>29.3±2.4b</td>
<td>88.7±5.9a</td>
<td>30.7±3.7b</td>
<td>38.3±1.1b</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.63±0.02b</td>
<td>1.30±0.09a</td>
<td>0.61±0.03b</td>
<td>0.73±0.05b</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>22.7±0.6c</td>
<td>38.0±1.1a</td>
<td>24.7±0.2b,c</td>
<td>25.0±0.7b</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>89.3±1.5c</td>
<td>177.6±3.4a</td>
<td>96.3±5.6c</td>
<td>111.7±5.9b</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dL)</td>
<td>79.0±5.2b</td>
<td>129.3±10.3a</td>
<td>77.1±4.9b</td>
<td>89.7±9.2b</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>36.7±1.3b,c</td>
<td>31.7±2.0c</td>
<td>40.0±2.0a,b</td>
<td>43.7±1.4a</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>61.7±2.4b</td>
<td>119.3±3.0a</td>
<td>62.3±2.1b</td>
<td>55.3±2.6b</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>79.0±3.3b</td>
<td>100.0±1.7a</td>
<td>78.7±3.7b</td>
<td>82.0±1.3b</td>
</tr>
</tbody>
</table>

Data are represented as means of six samples ± standard error. Means with different letters for each parameter are significantly different ($P < 0.01$). GpI: control group, GpII: mouldy bread group, GpIII: turmeric group, and GpIV: mouldy bread + turmeric group.

Effect of tur supplementation on AFs-induced genotoxicity
Genotoxicity was assessed in different groups using micronucleus assay. There was a highly significant increase ($P < 0.0001$) in the number of micronucleated polychromatic erythrocytes (MnPCE) after AFs exposure in GpII compared with GpI. However, this effect was markedly attenuated by using tur during AFs exposure in GpIV compared with GpII, as shown in Figure “1”.

Effect of tur supplementation on AFs-induced histological alterations in liver and kidney
Hepatic toxicity and nephric toxicity were obvious by histopathological investigation as shown in Figures “2 and 3”. Rats fed on mouldy bread (GpII) showed many histopathological lesions in the liver such as lymphocyte infiltration, karyolysis, pyknosis, and necrosis. Additionally, histological alterations were also manifested in the kidney in GpII such as glomeruli shrinkage, hemorrhage, and necrosis. However, combined treatment with tur + AFs-contaminated diet (GpIV) showed marked restoration of normal hepatic and renal architectures.

DISCUSSION
AFs contaminated bread induced genotoxicity and histopathological lesions that were observed in the liver and kidney,
as shown in the current and previous studies\cite{22-26}. The liver is the most affected organ by AFB1\cite{9}. Histological anomalies resulting from AFB1 toxicity in the liver leads to malfunctioning represented by disturbed lipid metabolism, lipid accumulation in the liver, and elevated hepatocyte apoptosis. In addition, AFB1 toxicity has been mainly attributed to the conversion into AFBO by cytochrome P450 isoenzymes\cite{6,27}. AFBO can then readily react with the cellular components such as protein\cite{28} and nucleic acids leading to metabolic disturbances and genotoxicity\cite{29}.

The current study showed that rats fed on mouldy bread manifested a significant elevation in the AST and ALT serum activities indicating the toxic effect of AFs present in mouldy bread on the liver and may be on other tissues like muscles. This was supported by the lipid profile analysis in the current study, as dyslipidemia and hypercholesterolemia may lead to an enhance in the oxidative stress and the lipid peroxidation causing tissue leakage of aminotransferases\cite{30}. In the current study, dyslipidemia which occurred as a result of feeding rats on mouldy bread was represented by disturbances in the levels of TC, TG, and LDL-C.

The kidney is the organ where the blood is cleared from metabolic waste products and is also involved in biochemical homeostasis maintenance. Nephrotoxicity was represented in the current study by both histopathological lesions in the kidney and the subsequent elevation of creatinine and urea. This result was consistent with previously published studies reported that AFs intake causes nephrotoxicity in chicken, fish, and mice\cite{31-33}. Elevated creatinine and urea levels can be attributed to the inability of the kidney tubules to eliminate them from the blood\cite{34}. It can also be attributed to the increase in the transformation of phosphocreatine to creatinine in the muscle because of the excessive usage of phosphocreatine in muscular contraction\cite{34}.

**Figure 1:** (a) Photomicrograph showing normochromatic erythrocytes (NCE), polychromatic erythrocytes (PCE) and micronucleated polychromatic erythrocytes (MnPCE). (b) Number of MnPCE after different treatments. GpI: control group, GpII: mouldy bread group, GpIII: turmeric group, and GpIV: mouldy bread + turmeric group. Data are represented as mean number of MnPCE in 20000 PCEs ± standard error. ****: Highly significantly \((P<0.0001)\) compared to the control group.
Figure 2: Photomicrographs of hepatic tissues from different groups after staining with hematoxylin and eosin. (a) GpI (control group) showing normal hepatic architecture represented by regular hepatic strands (H), blood sinusoids (S), and central veins (CV). (b) GpII (mouldy bread group) showing many histopathological lesions such as lymphocyte infiltration (*), karyolysis (arrow), pyknosis (arrowhead), necrosis (double arrowheads). (c) GpIII (turmeric group) showing normal hepatic architecture. (d) GpIV (mouldy bread + turmeric group) showing almost the same hepatic architecture like that of the control group. Scale bar = 20 µm.

In addition, consuming mouldy bread contaminated with AFs led to an elevation in the serum glucose level. Alsuhaihani\textsuperscript{[35]} reported that the rates of glycogenolysis and glycolysis are accelerated in AFs-treated rats. Moreover, it has been reported that the increase in glucose utilization and the decrease in glucose 6-phosphate dehydrogenase (a glycolytic enzyme) are the primary reasons for the increase in blood glucose following AFs exposure\textsuperscript{[29]}. The elevation of blood glucose in rats treated with AFs may also be attributed to the disturbance in the endocrine system responsible for regulating plasma glucose, which requires being assessed in future studies.
AFs induced hepatic and nephric toxicities, represented by biochemical and histological parameters, were alleviated by tur administration. Cur prevents AFB1 toxicity by inhibiting cytochrome P450 isoenzymes, and therefore reducing the formation of AFs metabolites including AFBO\cite{36}. Co-administration of mouldy bread with tur mitigated liver damage as indicated by the significant reduction in AST and ALT activities. Therefore, it can be used as a hepatoprotective supplement. The results of the present study confirmed the previously reported hepatoprotective role of cur against the hepatotoxicity induced by ochratoxin A mycotoxin\cite{37}.

**Figure 3:** Photomicrographs of renal tissues from different groups after staining with hematoxylin and eosin. (a) GpI (control group) showing normal renal architecture represented by regular glomeruli (G) and bowman’s spaces (B), and normal lining epithelium of both proximal convoluted tubules (P) and distal convoluted tubules (D). (b) GpII (mouldy bread group) showing glomeruli shrinkage (arrow), hemorrhage (arrowhead), and necrosis (double arrowheads). (c) GpIII (turmeric group) showing normal renal architecture. (d) GpIV (mouldy bread + turmeric group) showing high restoration of renal architecture with few necrotic areas (double arrowheads). Scale bar = 20 µm.
Turmeric ameliorates aflatoxin toxicity in rats

Treatment with tur was also sufficient to alleviate the dyslipidemia and kidney dysfunction induced by AFs in the current study. Cur stimulates the change of TC to bile acids (cholesterol catabolism) by raising the activity of cholesterol 7-a-hydroxylase enzyme[38]. Tur supplementation also modulated the hyperglycemia induced in rats fed on mouldy bread. Similarly, tur showed antihyperglycemic activity in experimental diabetic animals[39]. This hypoglycemic effect can be attributed to the regulatory role of cur on the amount of insulin secreted from the pancreas and also its modulatory effect on hepatic enzymes of glycolysis and gluconeogenesis[39].

Mouldy bread induced genotoxicity in the present study in consistence with previous reports[22,40] stating that AFB1 significantly increased chromosomal aberrations and DNA fragmentation in rats. AFBO formation leads to the formation of covalent N guanine-adducts[41]. The genotoxicity of mouldy bread was alleviated by tur administration. The protective effect of tur against AFB1 mutagenicity can be attributed to the role of cur in decreasing reactive oxygen species[42]. The current study addressed the mitigating effect of tur against toxicity induced by mouldy bread containing AFs. The ameliorative effect of tur was revealed by biochemical, histopathological, and cytogenetic analyses. Therefore, tur can be used as a food supplementation to counteract the consequences of AFs exposure.

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This research didn’t receive a grant from any funding agency in the public, commercial, or not-for-profit domains.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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AUTHORS’ CONTRIBUTIONS

All authors contributed equally to the suggestion of the research point, designing the experiment, analyzing the results of the study, and writing and revising the manuscript.

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Turmeric ameliorates aflatoxin toxicity in rats


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تأثير الكركم ضد سمية الأفلاتوكسين في الجرذان المُخفَّض على الخبز المتعفن

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يوجد العفن في العديد من المواد الغذائية وتنتج عنه مواد سامة تعرف بالسموم الفطرية والتي تشكل خطراً على صحة الإنسان والحيوان. يعتبر الأفلاتوكسين من أهم السموم الفطرية التي تتسبب على صحة الإنسان والتجارة في العالم، وذلك نظرًا لPresence of aflatoxins in the world. This study aimed to study the effect of curcumin on the aflatoxicosis in the rat (Rattus norvegicus). The study was conducted on four groups: the control group (fed with a diet containing aflatoxins), the second group (fed with a diet containing aflatoxins + 60 mg curcumin/kg), the third group (fed with a diet containing aflatoxins + 0.272 mg curcumin/kg), and the fourth group (fed with a diet containing aflatoxins + 0.272 mg curcumin/kg + cholesterol). The results showed a significant increase in the level of cholesterol in the blood of the control group compared to the other groups. The results also showed a significant decrease in the level of the study enzymes in the blood of the curcumin groups compared to the control group. Thus, curcumin can be used as a natural remedy to prevent aflatoxicosis and its effects.