Aflatoxins are among the most common myco-
toxins to which humans are exposed. They are high-
ly substituted coumarin derivatives containing a
fused dihydrofurofuran moiety. Epidemiological
and experimental studies have shown that aflatoxins
are hepatotoxic, hepatocarcinogenic, mutagenic and
teratogenic (1). Aflatoxins cause oxidative stress by
increasing lipid peroxidation and decreasing enzy-
matic and non-enzymatic antioxidants in aflatoxin-
treated animals (2, 3).

Tea is one of the most widely consumed bev-
erages, second only to water. Tea flavonoids
demonstrate antioxidant activity (4) and while not a
replacement for fruit and vegetables, the antioxidant
activity of tea has been compared to that of fruit and
vegetables in a number of studies. Several studies
have found that black tea and green tea offered pro-
tection against oxidative damage to red blood cells
induced by a variety of inducers, e.g. H$_2$O$_2$.

The aim of the present investigation was to
evaluate the ameliorative effect of black tea extract
on aflatoxin-induced lipid peroxidation in the kid-
ney of mice.

EXPERIMENTAL

A toxigenic strain of Aspergillus parasiticus
var. globosus (MTCC 411) obtained from Institute
of Microbial Technology, Chandigarh, India, was
grown on sucrose-magnesium sulfate-potassium
nitrate-yeast extract (SMKY) liquid medium at 28 ±
2°C for 10 days (5). Fifty mL of SMKY liquid medi-
um was taken in a 500 mL Erlenmeyer flask and
sterilized at 15 lb pressure for 20 min. The sterilized
medium was inoculated with 0.5 mL spore suspen-
sion of Aspergillus parasiticus having 10$^8$
conidia/mL aseptically (under laminar flow) and
incubated at 28 ± 2°C for 10 days. The contents of
the autoclaved flasks were filtered through
Whatman filter paper No. 1. Pooled culture filtrates
were extracted twice with chloroform (1:2, v/v) in a
separating funnel and the lowermost chloroform
layer was passed through the bed of anhydrous sodi-
um sulfate (Na$_2$SO$_4$). The chloroform extract was
subsequently evaporated to dryness and the residue
was dissolved in 1 mL of chloroform. Samples were
stored in vials.

Silica gel G coated activated TLC plates were
spotted with 100 mL of aflatoxins standard (a gift
from the International Agency for Research on
Cancer, Lyon, France) and developed in a solvent
consisting of toluene:iso-amyl alcohol:methanol
(90:32:2, v/v/v) (6). The air-dried plates were
observed under long-wave UV light (360 nm) for
aflatoxins. Aflatoxins were chemically confirmed
by spraying with trifluoroacetate and 25% sulfuric
acid. Each spot was scraped separately, dissolved in
chilled methanol and subjected to spectrophotomet-
ic analysis at 360 nm (7).

Young adult inbred Swiss strain male Albino
mice (Mus musculus) weighing approximately 30-
35 g were obtained from Alembic Ltd., Baroda,
India. Animals were provided with animal feed and water ad-libitum and maintained under 12 h light/dark cycles at 26 ± 2°C. Animal feed was prepared as per the formulation given by the National Institute of Occupational Health, Ahmedabad, India and was confirmed to be free of mycotoxins. Guidelines for care and use of animals in Scientific Research 1991 published by Indian National Science Academy, New Delhi, India, was followed.

Seventy animals were divided into seven groups and caged separately. Group 1 (untreated control) animals were maintained without any treatment. Animals of group 2 and 3 received olive oil (0.2 mL/animal/day) and black tea extract (2% in drinking water), respectively, for 30 days and served as pretreatment controls. Animals of group 4 and 5 were orally administered with aflatoxin in 25 and 50 µg/0.2 mL olive oil/animal/day (750 and 1,500 µg/kg body weight) respectively, for 30 days. Group 6 and 7 animals were orally treated with aflatoxin as mentioned for group 4 and 5 animals and given 2% black tea extract instead of drinking water for 30 days.

Olive oil was obtained from Figaro, Madrid, Spain. 80 g of black tea solids (Lipton Yellow Label of Hindustan Lever Ltd., Mumbai, India) and 4 litres of deionized water were used to produce a 2% tea infusion (8). Aflatoxin was dissolved in olive oil; hence it was administered as a vehicle alone in group 2. As different isomers of aflatoxin exist together in the food-stuffs, we preferred to carry out the experiment with mixed aflatoxins. The dose of aflatoxin was based on LD50 value of aflatoxin, i.e. 9 mg/kg body weight for male mice (9). The effective dose of black tea was based on earlier work in group 2. As different isomers of aflatoxin exist together in the food-stuffs, we preferred to carry out the experiment with mixed aflatoxins. The dose of aflatoxin was based on LD50 value of aflatoxin, i.e. 9 mg/kg body weight for male mice (9). The effective dose of black tea was based on earlier work in group 2. As different isomers of aflatoxin exist together in the food-stuffs, we preferred to carry out the experiment with mixed aflatoxins.

On completion of the treatment, the animals were sacrificed by cervical dislocation. Kidneys of all controls and treated groups of animals were quickly isolated, blotted free of blood and utilized for biochemical analysis. The lipid peroxidation in the kidneys of controls and all treated groups of animals was measured by quantification of thiobarbituric acid reactive substances (TBARS) determined by the method of Ohkawa et al. (10) using malondialdehyde (MDA) as a substrate. The activity of superoxide dismutase in the kidney of controls and treated groups of animals was assayed by the modified spectrophotometric method of Kakkar et al. (11). The glutathione peroxidase and catalase activity in the kidneys of controls and all treated groups of mice were assayed by the modified method of Pagila and Valentine (12) and Luck (13), respectively. The concentration of glutathione was estimated in kidneys of controls and all treated groups of mice by the method of Grunert and Philips (14).

The results shown in Table 1 reveal the effect of aflatoxin and aflatoxin plus black tea extract treatment on the lipid peroxidation and antioxidative defense mechanisms in the kidney of mice. Except significant reduction in glutathione content in the kidneys of black tea extract-treated mice, no significant difference was noted between control groups (group 1, 2 and 3).

The aflatoxin treatment for 30 days caused significant, dose-dependent increase in the levels of lipid peroxidation (LD : 41.15%; HD : 60.10%) in the kidney of mice compared to the control (group 1, 2 and 3). The levels of enzymatic antioxidants like catalase (LD : -4.73%; HD : -17.17%), superoxide dismutase (LD : -31.26%; HD : -49.19%) and glutathione peroxidase (LD : -25.33%; HD : -42.67%) and non-enzymatic antioxidants such as glutathione content (LD : -32.64%; HD : -46.52%) were significantly lower in the kidney of aflatoxin-treated mice as compared to the controls.

Aflatoxin plus black tea extract treatment significantly ameliorates aflatoxin-induced lipid peroxidation which could be due to higher enzymatic and non-enzymatic antioxidant levels in the kidney of mice (group 6, 7) as compared to those given aflatoxin alone (group 4, 5). However, there was partial amelioration in the levels of superoxide dismutase and glutathione in the high dose group (group 7) as the values were still significantly lower than the those of the controls.
DISCUSSION

Oral administration of aflatoxin for 30 days caused significant increase in lipid peroxidation in kidney of aflatoxin-treated mice due to reduced enzymatic (catalase, superoxide dismutase, glutathione peroxidase) and non-enzymatic (glutathione) antioxidants as compared to controls (Table 2). Previous studies (2, 3) have also reported similar changes.

Administration of black tea extract in group 3 animals caused, as compared with other controls, significant decrease in non-enzymatic antioxidant such as glutathione. However, treatment did not cause any significant change in lipid peroxidation and in enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase. Black tea extract contains many polyphenols with antioxidative property which is continuously administered to these groups of animals. Thus, significant reduction in endogenous non-enzymatic antioxidants might be due to higher exogenous antioxidants due to black tea administration. Oral administration of black tea extract for 30 days along with aflatoxin caused significant amelioration in aflatoxin-induced lipid peroxidation by increasing the antioxidative activity of the cells. Activities of enzymatic antioxidants (catalase, superoxide dismutase and glutathione peroxidase) as well as non-enzymatic antioxidant, glutathione were significantly increased in kidney of aflatoxin-treated mice (Table 1).

Tea shows many health promoting activities including chemopreventive action during carcinogenesis due to antioxidative polyphenolic constituents. Sengupta et al. (16) have shown significant reduction in the number of aberrant crypt foci and levels of lipid peroxidation among the tea-treated groups. This may be due to an outcome of antioxidative influence of tea components on azoxymethane-induced carcinogenesis (16). 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 et al. (17) 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. Maity et al. (18) had suggested that thearubigin, the most predominant polyphenol of

<table>
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<th>Parameters</th>
<th>Experimental Groups</th>
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<tr>
<td>Lipid peroxidation (nmoles MDA/mg protein/60 min) ± 0.107 ± 0.104 ± 0.240 ± 0.188 abcdfg ± 0.101abcdfg ± 0.106adef ± 0.084cdef</td>
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<td>Catalase activity (µmoles H2O2 consumed/mg protein/min) ± 0.95 ± 0.96 ± 0.87 ± 0.54 ce ± 0.85abcdfg ± 0.61e ± 0.87e</td>
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<td>Superoxide dismutase activity (units/mg protein) ± 0.223 ± 0.283 ± 0.409 ± 0.247 abcef ± 0.167abcdfg ± 0.315de ± 0.173abce</td>
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<tr>
<td>Glutathione peroxidase activity (nmoles NADPH consumed/mg protein/min) ± 0.002 ± 0.006 ± 0.005 ± 0.003 abcdfg ± 0.002deg ± 0.006e</td>
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<tr>
<td>Glutathione (µg/100 mg tissue weight) ± 35.77 ± 36.18 ± 27.75 ± 24.94 ± 21.35 ± 19.57 ± 15.84 ± 15.91 ± 8.14 abcdef</td>
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Values are 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
black tea, ameliorated the appearance of diarrhoea and the disruption of colonic architecture by reducing lipid peroxidation in the inflamed colon. Tea catechins have also been shown to have protective effects on the injury of cerebral ischemia in rats. Tea catechin could decrease the concentration of MDA in brain tissue and serum and it could improve the injury of blood brain barrier (19).

It is thus concluded that oral administration of black tea extract along with aflatoxin significantly ameliorates aflatoxin-induced lipid peroxidation by increasing the antioxidative defense mechanism of the cells.

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REFERENCES


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