Ameliorative Effects of *Tinospora Cordifolia* Root Extract on Histopathological and Biochemical Changes Induced by Aflatoxin-B₁ in Mice Kidney

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**ABSTRACT**

The present study was planned to investigate the ability of the *Tinospora cordifolia* to scavenge free radicals generated during aflatoxicosis. A total no. of 48 male Swiss albino mice (30 ± 5 g) were exposed to Aflatoxin B₁ (AFB₁) (2 μg/30 g b.wt, orally) either individually or in combination with *T. cordifolia* (50, 100, 200 mg/kg, orally) once daily for 25 days. AFB₁ exposure led to significant rise in thiobarbituric acid reactive substances (TBARS) and fall in superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), ascorbic acid, and protein content. *T. cordifolia* was found to show protective effect by lowering down the content of TBARS and enhancing the GSH, ascorbic acid, protein, and the activities of antioxidant enzymes viz., SOD, CAT, glutathione peroxidase, GST, and GR in kidney. Histopathological analysis of kidney samples also confirmed the protective values and antioxidant activity of ethanolic extract of herb. *T. cordifolia* showed protection against aflatoxin-induced nephrotoxicity due to the presence of alkaloids such as a choline, tinosporin, isocolumbin, palmatine, tetrahydropalmatine, and magnoflorine.

**Key words**: Aflatoxin, antioxidant, mice, oxidative stress, *Tinospora cordifolia*

**INTRODUCTION**

Aflatoxin (AF) is polysubstituted bifuranocoumarins that are secondary fungal metabolites produced by the *flavus/parasiticus* group of the genus *Aspergillus*.¹ The toxic effects of AF in livestock have been well documented. When livestock eat AF-contaminated feed, it may cause many health problems.² Epidemiological and experimental studies have shown that AF is hepatotoxic, hepatocarcinogenic, mutagenic, and teratogenic.³ AF cause oxidative stress by increasing lipid peroxidation and decreasing enzymatic and nonenzymatic antioxidants in treated animals.⁴ Oxidative damage in the cell or tissue occurs when the concentration of reactive oxygen species (superoxide radical, hydroxyl radical, and hydrogen peroxide) generated exceeds the antioxidant capacity of the cell⁵ or when the antioxidant capacity of the cell decreases. Levels of nonenzymatic antioxidants (glutathione, ascorbic acid) and enzymatic antioxidants glutathione peroxidase, catalase [CAT], and superoxide dismutase [SOD]) are the major determinants of the antioxidant defense mechanism of the cell.

Management of AF toxicity without any side effect is still a challenge to the medicinal field, as presently available drugs for aflatoxicosis have one or other adverse effects.
In recent years, the herbal remedy for the unsolved medicinal problems is gaining importance in research field. *Tinospora cordifolia* (Menispermacae) is widely used in Ayurvedic medicine as a tonic, vitalizer, and as a remedy for metabolic disorder. The plant stem has been considered as an indigenous source of medicines to have antidiabetic, immunomodulatory, antihepatotoxic, and antipyretic actions. The roots of *T. cordifolia* possess antifaulcer and antistress action. In the present study, the attempts were made to evaluate the anticarcinogenic activity of *T. cordifolia*, its effect on the kidney morphology and biochemical variables in AF-toxicated kidney of mice, since AF nephropathy is one of the major complication in long-term aflatoxicosis.

**MATERIAL AND METHODS**

**Chemicals**

Crystalline aflatoxin B$_1$ (AFB$_1$) (from *Aspergillus flavus*) was purchased from HIMEDIA (India). All other chemicals used were of analytical grade and obtained from SD fine chemicals (Mumbai, India), SRL (India), CDH (India), and Qualigens (India/Germany).

**Animals**

Healthy male Swiss albino mice (*Mus musculus*) were procured from Haryana Agricultural University, Hisar (Haryana, India). Only male mice were used because previous studies have indicated that these were more sensitive to AF treatment than female. The animals were housed under standard laboratory conditions of light (12 hours light-dark cycle); temperature, 25 ± 2°C; humidity, 55 ± 5%, and fed with standard mice pellet diet (Hindustan Liver Limited, India) and tap water *ad libitum* in animal house of Banasthali University according to internationally accepted principle. A prior approval was obtained from the institutional animal ethics committee for the study protocol. After 1 week of acclimatization, mice were used for experimental purpose.

**Preparation of aflatoxin B$_1$ and ethanolic extract of *Tinospora cordifolia***

Crystalline AFB$_1$ was dissolved in dimethylsulfoxide and further diluted with distilled water to the required concentration. The final gavage solution of AFB$_1$ contained 1% dimethylsulfoxide.

The experimental plant material was collected from medicinal plant garden, Banasthali University, India. It was identified as *T. cordifolia* by a plant taxonomist of our department and its sample has been preserved and documented in the herbarium of our University. The hanging aerial roots were washed thoroughly with distilled water and shade-dried. Ethanolic extract of the dried roots of *T. cordifolia* was prepared by Soxhlet method using 300 ml ethanol for 50 g (dry weight) of dried root powder. The ethanolic extract thus obtained was dried under reduced pressure at a room temperature not exceeding 40°C to get a yield of 7% from the crude extract. The extract, devoid of alcohol, was used for required concentration.

**Experimental design**

Male Swiss albino mice (30 ± 5 g) were randomized into eight groups comprising of six animals in each groups and were administered orally by gavage, once daily as below, for 25 days:

- **Group I**- Control (Normal saline, 0.9%)
- **Group II**- AFB$_1$ (2 μg/30 g body weight)
- **Group III**- *T. cordifolia* (50 mg/kg body weight)
- **Group IV**- *T. cordifolia* (100 mg/kg body weight)
- **Group V**- *T. cordifolia* (200 mg/kg body weight)
- **Group VI**- AFB$_1$ + *T. cordifolia* (50 mg/kg body weight)
- **Group VII**- AFB$_1$ + *T. cordifolia* (100 mg/kg body weight)
- **Group VIII**- AFB$_1$ + *T. cordifolia* (200 mg/kg body weight)

On 25$^{th}$ day of the study, the animals were scarified by cervical dislocation. Kidney sample from the sacrificed mice were quickly removed and cleaned to make them free of extraneous material and perfused with ice-cold saline for biochemical and histopathological evaluation. Post-mitochondrial supernatant was prepared using method of Mohandas et al.,$^8$ with some modifications. The various biochemical variables viz., lipid peroxidation,$^9$ SOD,$^10$ CAT,$^{11}$ glutathione-S-transferase (GST),$^{12}$ reduced glutathione (GSH),$^{13}$ glutathione peroxidase,$^{14}$ glutathione reductase (GR),$^{15}$ ascorbic acid,$^{16}$ and protein$^{17}$ were analyzed. Histopathological evaluation of kidney tissue was done according to the method of Luna.$^{18}$

**Statistical analysis**

The results are expressed as mean ± standard error (S.E.M.). Statistical significance between the different groups was determined by one way analysis of variance (ANOVA) using the SPSS software package.$^{16}$ Post hoc testing was performed for inter-group comparisons using the Tukey multiple comparison test. The level of significance was set at $P<0.05$.

**RESULTS AND DISCUSSION**

The biochemical status of animals, i.e., levels of LPO, SOD, CAT, GSH, GST, GPx, GR, ascorbic acid, and protein content are given in Table 1. AFB$_1$ exposure led to significant rise ($P<0.01$) in thiobarbituric acid reactive substances.
(TBARS) (20.71 ± 0.92 nmoles TBARS h⁻¹·g⁻¹) and fall in SOD (5.12 ± 0.61 Unit mg⁻¹·protein h⁻¹), CAT (43.86 ± 2.08μmol H₂O₂ consumed min⁻¹·mg⁻¹·protein), GSH (154.88 ± 7.36 nmolsGHg⁻¹·tissue), GST (97.65 ± 2.20 nmoles CDNB conjugates formed min⁻¹·mg⁻¹·protein), GPx (14.20 ± 0.26 μg of glutathione utilized min⁻¹·mg⁻¹·protein), GR (115.09 ± 1.06 nmoles NADPH oxidized min⁻¹·mg⁻¹·protein), ascorbic acid (3.75 ± 0.17 mg ascorbic acid/g tissue), and protein content (32.71 ± 0.94 mg g⁻¹·fresh wt) as compared with respective control values (P<0.01). Significant increase (P<0.05) in CAT activity was shown in group III and group IV mice, whereas group of mice which received plant extract alone (group III, group IV, and group V) showed significant enhancement (P<0.01) in protein content. Glutathione peroxidase activity was significantly increased (P<0.01) in the group III and group V mice, whereas GSH activity was also significantly improved (P<0.01) in group of mice which received RTc (50) and RTc (100).

When all the doses of *T. cordifolia* root extract were administered along with AF, it was found that all doses significantly decreased (P<0.01) the level of Lipid peroxidation (13.26 ± 0.42; 12.54 ± 1.28; 15.19 ± 0.94 μg of lipid peroxidation g⁻¹·fresh wt) as compared with AF-treated animal. Co-administration of plant with all doses along with AF significantly increased (P<0.01) the CAT (62.15 ± 1.92; 65.78 ± 3.28; 123.87 ± 1.43 Unit mg⁻¹·protein), SOD (8.45 ± 0.34; 8.37 ± 0.34; 8.45 ± 0.02 Unit mg⁻¹·protein), GR (123.87 ± 1.43; 121.01 ± 1.26; 125.57 ± 3.29 Unit mg⁻¹·protein), GST (267.46 ± 3.29; 265.65 ± 6.19; 212.13 ± 3.29 nmoles CDNB conjugates formed min⁻¹·mg⁻¹·protein), GPx (18.93 ± 0.29; 20.95 ± 1.64; 17.43 ± 0.58 μg of glutathione utilized min⁻¹·mg⁻¹·protein), ascorbic acid (3.75 ± 0.17; 4.92 ± 0.05; 4.18 ± 0.10 mg ascorbic acid/g tissue), and protein content (41.21 ± 0.92; 42.56 ± 0.81; 40.88 ± 1.06 mg g⁻¹·fresh wt) as compared with respective values of group II mice. Group VI and group VIII mice also showed significant improvement (P<0.01) in SOD (8.21 ± 0.43; 8.59 ± 0.86 Unit mg⁻¹·protein h⁻¹), whereas group VII mice showed significant improvement (P<0.05) in SOD (7.92 ± 1.07 Unit mg⁻¹·protein h⁻¹) as compared with the values of AF-administered group (group II).

Histopathological examination of the mice kidney revealed normal architecture in control (a), RTc (50) (c), RTc (100) (d), and RTc (200) (e) treated group. However, kidney from AFB₃-treated mice (b) revealed the vacuolar degeneration of tubular epithelial cells. The kidney of mice treated with AFB₃ + RTc (50) (f) shows normal tubules with congested glomerulus and AFB₃ + RTc (100) (g) showed congested glomerulus and tubules with vacuolated epithelial cells, whereas AFB₃ + RTc (200) (h) showed vacuolation of tubular epithelium but no shedding of tubular cells into the lumen [Figure 1].

### Table 1: Effect of ethanolic extract of Tinospora cordifolia root on non-enzymatic, enzymatic parameters and level of TBARS in the kidney of mice treated with aflatoxin B₁

| Parameters                  | Control (Group I) | AFB₁ (Group II) | AFB₁+RTc (50) (Group III) | AFB₁+RTc (100) (Group IV) | AFB₁+RTc (200) (Group V) | AFB₁+RTc (100) + PO₄ (Group VI) | AFB₁+RTc (200) + PO₄ (Group VII) | AFB₁+RTc (100) + PO₄ + AFB₂ (Group VIII) |
|-----------------------------|-------------------|----------------|---------------------------|---------------------------|--------------------------|-----------------------------|-----------------------------|----------------------------------|
| Lipid peroxidation (μg)     | 13.26 ± 0.42      | 20.71 ± 0.92   | 8.45 ± 0.34               | 12.54 ± 0.28              | 15.19 ± 0.94             | 18.93 ± 0.29                | 17.43 ± 0.58                 | 15.19 ± 0.94                     |
| SOD (Unit mg⁻¹·protein)     | 8.37 ± 0.34       | 8.45 ± 0.34    | 8.37 ± 0.34               | 8.45 ± 0.02               | 8.45 ± 0.02              | 8.45 ± 0.02                | 8.45 ± 0.02                  | 8.45 ± 0.02                      |
| GR (Unit mg⁻¹·protein)      | 123.87 ± 1.43     | 121.01 ± 1.26  | 125.57 ± 3.29             | 127.00 ± 3.29             | 125.89 ± 2.61            | 125.89 ± 2.61               | 125.89 ± 2.61                | 125.89 ± 2.61                     |
| GST (nmols CDNB conjugates formed min⁻¹·mg⁻¹·protein) | 267.46 ± 3.29 | 265.65 ± 6.19 | 212.13 ± 3.29             | 212.13 ± 3.29             | 212.13 ± 3.29            | 212.13 ± 3.29               | 212.13 ± 3.29                | 212.13 ± 3.29                     |
| GPx (μg of glutathione utilized min⁻¹·mg⁻¹·protein) | 18.93 ± 0.29 | 20.95 ± 1.64  | 17.43 ± 0.58              | 15.19 ± 0.94              | 17.43 ± 0.58             | 17.43 ± 0.58                | 17.43 ± 0.58                  | 17.43 ± 0.58                     |
| Ascorbic acid (mg)          | 3.75 ± 0.17       | 4.92 ± 0.05    | 4.18 ± 0.10               | 4.18 ± 0.10               | 4.92 ± 0.05              | 4.92 ± 0.05                  | 4.92 ± 0.05                  | 4.92 ± 0.05                      |
| Protein (mg g⁻¹·fresh wt)   | 41.03 ± 1.71      | 41.03 ± 1.71   | 41.03 ± 1.71              | 41.03 ± 1.71              | 41.03 ± 1.71             | 41.03 ± 1.71                | 41.03 ± 1.71                 | 41.03 ± 1.71                     |
We observed significant fall in the activities of kidney SOD and CAT in the mice fed with AF alone. SOD and CAT are the main antioxidant enzyme in the body, which scavenge unwanted $O_2^-$, $H_2O_2$, and ROOH produced by free radical. SOD catalyzes superoxide radical dismutation and CAT decomposes hydrogen peroxide.$^{[19]}$ The decreased enzyme activities and increased TBARS levels produced by AFB$_1$ can be attributed to lower ability of the tissue, which cannot scavenge free radicals and prevent the action of lipid peroxidation. In present study, reduction in GSH, GST, and ascorbic acid content in the kidney was shown after AF treatment. GSH and GST play a critical role in the protection of tissues from deleterious effects of activated AFB$_1$. GSH is a tripeptide containing cysteine that has a reactive –SH group with reductive potency. It can act as a nonenzymatic antioxidant by direct interaction of –SH group with ROS, as a cofactor or coenzyme.$^{[20]}$ During the free radical scavenging action, ascorbic acid is transformed into L-dehydroascorbate.$^{[21]}$ GSH is required for conversion of L-dehydroascorbate back to ascorbate. The fall in the level of GSH decreases the conversion of L-dehydroascorbate to ascorbic acid in AF-treated animals. GST catalyzes the conjugation of AFB$_1$-8, 9-epoxide with GSH to form AFB$_1$-epoxide-GSH conjugate, thereby decreasing the intracellular glutathione content.$^{[22]}$ These observations support our findings where we observed a significant decline in the level of GSH and GST in AFB$_1$-induced animal.

However, cosupplementation of RTc extract with AFB$_1$ increased SOD and CAT activities and reduced TBARS levels. Any compound, natural or synthetic, with antioxidant property may inhibit free radical generation by direct scavenging of the free radicals and subsequent transformation of the antioxidant species into less toxic product. Therefore, removing $O_2^-$ and $OH^-$ is probably one of the most effective defense mechanism of living body against disease. Photochemical studies of T. cordifolia have revealed the presence of alkaloids such as a choline (V), tinosporin, isocolumbin, palmatine, tetrahydropalmatine (VI), and magnoflorine in the roots and these alkaloids are known to exhibit antioxidant property.$^{[23]}$ These constituent accelerates dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. Hydrogen

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Figure 1: Cross section of kidney in mice treated with AFB$_1$ and RTc (40x). Kidney from control (a), RTc (50) (c), RTc (100) (d), and RTc (200) (e) treated mice show normal architecture. However, kidney from AFB$_1$-treated mice (b) revealed the vacuolar degeneration of tubular epithelial cells. The kidney of mice treated with AFB$_1$ + RTc (50) (f) shows normal tubules with congested glomerulus and AFB$_1$ + RTc (100) (g) showed congested glomerulus and tubules with vacuolated epithelial cells, whereas AFB$_1$ + RTc (200) (h) showed vacuolation of tubular epithelium but no shedding of tubular cells into the lumen.
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peroxide produced is further removed by CAT.\textsuperscript{[24]} Therefore, 

\textit{Tinospora} induced SOD activity, in conjugation with CAT 

antagonizes free-radical-induced injury. Extract treatment 

significantly reduced lipid peroxidation, as measured by 

Malondialdehyde production, and eliminates the 

possibility of oxidative stress due to the administration of 

\textit{AFB\textsubscript{1}} to mice. It is also reported that \textit{T. cordifolia} exhibits an appreciable amount of Vit C (41.36 mg/g of extract) and glutathione (6.86 mg/g of extract) and both of these 

are known to be effective in direct scavenging of a wide 

variety of free radicals.\textsuperscript{[25]} Also, Vit (C) and GSH present 

in \textit{T. cordifolia} are potent lipid peroxidation chain-breaking 

agent and therefore further add to the protective role 

of the herbs against lipid peroxidation. Thus, \textit{Tinospora} 

induced unregulated antioxidant enzyme status, owing to 

an increase in the specific activities of SOD, CAT points 

to an extended functional balance between pro-oxidant 

and antioxidant pathways. The results of present study 

demonstrate that \textit{T. cordifolia} showed protection against 

AF-induced nephrotoxicity due to the presence of alkaloids 

such as a choline (V), tinosporin, isocolumbin, palmatine, 

tetrahydropalmatine (VI), and magnoflorine. The 

treatment with \textit{Tinospora} resulted in striking induction in 

the specific activities of detoxifying enzymes in the kidney, 

which strongly suggests a possible role of \textit{T. cordifolia} 

in cancer chemoprevention.

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