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Influence of *Tinospora cordifolia* Root Extract Supplementation on Hematological and Serological Parameters of Male Mice Exposed to Aflatoxin B₁

¹Veena Sharma, ¹Rekha Gupta, ¹Nidhi Mishra and ²Shatruhan Sharma

¹Department of Bioscience and Biotechnology, Banasthali University, Rajasthan-304022, India

²MAI-Jaipur, Rajasthan, India

Abstract: This study was undertaken to evaluate the effectiveness of ethanolic extract of *Tinospora cordifolia* root extract in alleviating the toxicity of aflatoxin B₁ (AFB₁) in male swiss albino mice. Forty eight male swiss albino mice (30±5 g) were exposed to aflatoxin B₁ (2 µg 30/g body weight, orally) either individually or in combination with *Tinospora cordifolia* (50, 100, 200 mg kg⁻¹, orally) once daily for 25 days. Evaluation was made for haemato-serological parameters. The results suggested that aflatoxin B₁ exposure led to significant fall (p<0.01) in haemoglobin, erythrocyte count and significant rise (p<0.01) in platelet count, total leukocyte count, segmented neutrophil, lymphocyte count (p<0.05), serum glutamate oxaloacetate transaminase, Serum glutamate pyruvate transaminase and alkaline phosphatase whereas no significant difference was seen in haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin content. Treatment with plant extract alone had no negative effects on most of the parameters. Concomitant administration of all three plant doses along with aflatoxin significantly diminished the adverse effect of aflatoxin and altered most of the haematological and serological variables.

Key words: Aflatoxin, blood profile, clinical biochemistry, preventive, *Tinospora cordifolia*

INTRODUCTION

Aflatoxins are a group of structurally similar difuranocoumarins elaborated as secondary metabolites produced by genus *Aspergillus* and are considered as most potent naturally occurring carcinogens (Kaaya *et al.*, 2006; Prabakaran and Dhanapal, 2009; Mohamed and Metwally, 2009). When livestock eat aflatoxin-contaminated feed, it causes many health problems. Aflatoxin B₁ acts as a hepatotoxicant, hepatocarcinogen and mutagen (Alwakeel, 2009). The acute toxic effects of AFB₁ include hemorrhaging and death. Chronic exposure of aflatoxin affects growth rate, feed efficiency and susceptibility towards the bacterial and viral diseases (Yousef *et al.*, 2003). Lipid peroxidation and oxidative DNA damage are the principal manifestation of AFB₁-induced toxicity which could be mitigated by antioxidants (Patel and Sail, 2006).

Tinospora cordifolia Miers (Menispermaceae) is a glabrous climbing succulent shrub, commonly found in hedges. It is widely used in Ayurvedic medicine in India as tonic, vitalizer and as remedy for diabetes mellitus and metabolic disorders (Puramil *et al.*, 2007). Previous studies showed that the plant possess antidiabetic

(Prince *et al.*, 2004), immunomodulatory (Ranjith *et al.*, 2008) and hepatoprotective (Sharma and Pandey, 2010a) activities. Roots are effective in treatment of gynecological disorders, spleen troubles (Ishnava and Mohan, 2009). The leaves and stem extract of *T. cordifolia* are capable of scavenging lead-induced hematological alterations (Sharma and Pandey, 2010b).

Some authors have shown that *T. cordifolia* roots possess antiulcer (Bafna and Balaraman, 2005) and antistress activity (Singh *et al.*, 2006). To the best of our knowledge and literature survey, no attempt was made to study its regulatory role in aflatoxicosis. Keeping, this view in mind, the present study was planned to determine the effects of ethanolic root extract of *Tinospora cordifolia* on aflatoxin-induced hematological and serological alterations in male swiss albino mice.

MATERIALS AND METHODS

Chemicals: Crystalline AFB₁ (from *Aspergillus flavus*), 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*) weighing (25±5 g) were procured from Haryana Agricultural University, Hissar (Haryana, India). The animals were housed under standard laboratory conditions of light (12 h light-dark cycle), temperature (25±2), 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. Only male mice were used because previous studies have indicated that these were more sensitive to aflatoxin treatment than female.

Preparation of aflatoxin B₁ and ethanolic extract of *Tinospora cordifolia*: Crystalline aflatoxin B₁ was dissolved in dimethylsulfoxide and further diluted with distilled water to the required concentration. The final gavage solution of AFB₁ contained 1% dimethyl sulfoxide.

The experimental plant material was collected from Krishi Vigyan Kendra, Banasthali University, District Tonk, India during the month of October 2009. It was identified as *Tinospora 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, shade-dried then powdered with a mechanical grinder, passing through sieve No. 40 and stored in tight container. Ethanolic extract of the dried roots of *Tinospora cordifolia* was prepared by soxhlet method (Soxhlet Apparatus, Tarson; India) 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.

Animal treatment and sample collection: Male Swiss albino mice (30±5 g) were randomized into eight groups comprising of six animals in each groups for haematological and serological studies. All these animals were treated orally by gavage, once daily as below, for 25 days:

Group 1: Control (Normal saline, 0.9%)

Group 2: Aflatoxin B₁ (AFB₁) (2 µg 30/g body weight)

Group 3: *Tinospora cordifolia* (50 mg kg⁻¹ body weight)

Group 4: *Tinospora cordifolia* (100 mg kg⁻¹ body weight)

Group 5: *Tinospora cordifolia* (200 mg kg⁻¹ body weight)

Group 6: AFB₁ + *Tinospora cordifolia* (50 mg kg⁻¹ body weight)

Group 7: AFB₁ + *Tinospora cordifolia* (100 mg kg⁻¹ body weight)

Group 8: AFB₁ + *Tinospora cordifolia* (200 mg kg⁻¹ body weight)

Tinospora cordifolia root (RTc) extract were given at an interval of 30 min of Aflatoxin B₁ administration.

At the end of 25th day the blood samples were collected from the retro-orbital venous plexus of all animals.

Noncoagulated blood was used for hematological parameters viz., hemoglobin estimation was done using Sahli's hemoglobin meter (Haden, 1939), total erythrocyte count and total leukocyte count by haemocytometer (Berkson *et al.*, 1940), PCV by Wintrob method (Wintrob, 1932) and differential leukocyte count was done by Leishman's staining method (Sharma, 2007). Mean corpuscular hemoglobin, Mean corpuscular hemoglobin content and Mean corpuscular volume were also calculated (Mitruka and Rownsly, 1981; Jandl, 1987). Serum was prepared by centrifugation of blood samples at 860×g for 20 min and stored at -20°C until used for analysis. Activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957), activity of Alkaline Phosphatase (ALP) was determined according to the protocol described in a laboratory practical manual (Hawk *et al.*, 1954).

Statistical analysis: The results are expressed as Mean±standard error Mean±SEM. 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

Results of the hematological analysis in Table 1 show that AFB₁ alone caused a significant decrease (p<0.01) in hemoglobin content, erythrocyte count and lymphocyte count (p<0.05) as compared to respective control values. Significant increase (p<0.05) was seen in platelet count, total leukocyte count and segmented neutrophil whereas no significant difference was seen in MCH, MCV, MCHC and hematocrit values in aflatoxin treated group as

Table 1: Effect of RTc extract either alone or in combination with AFB₁ on haematological variables in Swiss albino mice

Treatments (Mean±SEM)								
Parameters	Control (Group 1)	AFB ₁ (Group 2)	RTc (50) (Group 3)	RTc (100) (Group 4)	RTc (200) (Group 5)	AFB ₁ +RTc (50) (Group 6)	AFB ₁ +RTc (100) (Group 7)	AFB ₁ +RTc (200) (Group 8)
Hb (g dL ⁻¹)	12.81±0.93 ^c	10.15±0.31 ^a	16.12±0.52 ^{bc}	17.11±0.60 ^{bc}	14.18±0.48 ^c	13.12±0.45 ^c	13.86±0.94 ^c	12.91±1.17 ^c
PCV (%)	43.76±3.53	35.5±0.35	48.12±1.23 ^{bc}	58.3±2.27 ^{bc}	43.5±3.07	39.23±3.12	38.75±6.52	40.93±4.02
RBC (10 ⁶ mm ⁻³)	8.76±0.56 ^c	7.39±0.37 ^a	11.24±0.22 ^{bc}	11.85±0.33 ^{bc}	9.26±0.39 ^c	8.48±0.35 ^d	8.50±0.71 ^d	8.39±0.82
MCV (fL)	50.9±2.29	52.12±2.74	47.12±1.34	47.9±0.71	46.98±2.65 ^d	46.23±2.56 ^c	45.86±3.74 ^c	48.03±1.93
MCH (Pg)	14.6±0.84	13.36±1.68	14.92±0.92 ^d	14.43±0.41	15.3±0.69 ^d	15.92±0.67	16.56±0.93 ^{bc}	15.18±0.84
MCHC (g dL ⁻¹)	29.23±0.64	26.38±0.91	30.92±0.78 ^{bd}	30.15±1.1	32.63±1.55 ^c	33.84±2.31	36.48±4.40 ^c	31.61±1.27 ^c
WBC (10 ³ mm ⁻³)	17.27±1.21 ^c	22.90±2.29 ^a	18.09±0.56 ^c	17.63±0.79 ^c	18.26±2.28 ^c	19.96±0.92 ^{bc}	18.42±1.10 ^c	20.09±1.69 ^{bc}
Platelet (10 ³ mm ⁻³)	729.16±5.95 ^c	17.27±8.63 ^a	672.5±4.53 ^c	642.6±8.52 ^c	720.6±9.93 ^c	1124.3±4.67 ^{bc}	1088.6±13.06 ^{bc}	1199.58.22 ^{bc}
Neutrophil (%)	17.66±2.42 ^c	22.83±2.33 ^a	15.75±1.34 ^c	15.33±2.42 ^c	16.83±1.77 ^c	19.97±1.34	20.16±3.48	19.83±2.11
Lymphocyte (%)	88.8±7.60 ^d	73.88±7.37 ^b	82.34±2.12	84.1±11.30	79.85±6.32	84.34±2.56	87.36±2.92	78.5±8.44

AFB₁: Aflatoxin B₁; Hb: Haemoglobin; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin content, MCV: Mean corpuscular volume; RTc: Root of *Tinospora cordifolia*. Values are Mean±SE of six mice. Significant differences in data are shown as a p<0.01 and b p<0.05 when compared with control (Group 1) and c p<0.01 and d p<0.05 when compared with aflatoxin treated group (Group 2)

Table 2: Effect of RTc extract either alone or in combination with AFB₁ on some serological variables in Swiss albino mice

Treatments (Mean±SEM)								
Parameters	Control (Group 1)	AFB ₁ (Group 2)	RTc(50) (Group 3)	RTc(100) (Group 4)	RTc(200) (Group 5)	AFB ₁ +RTc(50) (Group 6)	AFB ₁ +RTc(100) (Group 7)	AFB ₁ +RTc (200) (Group 8)
SGOT (Units mL ⁻¹)	10.80±0.82 ^c	43.39±2.43 ^a	10.24±0.92 ^c	10.27±1.21 ^c	10.19±1.23 ^c	29.78±2.34 ^{bc}	24.83±4.24 ^{bc}	34.94±2.27 ^{bc}
SGPT (Units mL ⁻¹)	11.96±1.63 ^c	41.32±5.33 ^a	11.19±0.45 ^c	12.32±2.56 ^c	9.99±0.82 ^c	30.23±2.21 ^{bc}	25.34±3.24 ^{bc}	36.66±2.05 ^a
ALP (KA Units mL ⁻¹)	5.26±0.70 ^c	11.13±0.90 ^a	5.79±0.56 ^c	6.00±0.42 ^c	5.42±0.86 ^c	9.24±0.89 ^{bc}	8.64±0.71 ^{bc}	9.92±0.19 ^a

ALP: Alkaline phosphatase; SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase. Values are mean±SE of six mice. Significant differences in data are shown as a p<0.01 and b p<0.05 when compared with control (Group 1) and c p<0.01 and d p<0.05 when compared with aflatoxin treated group (Group 2)

compared to control group. Group of mice treated with Rtc (50) (group 3) and Rtc (100) (Group 4) showed no significant difference in total leukocyte count, platelet count, lymphocyte count, segmented neutrophil count as compared to control group whereas significant increase (p<0.01) was seen in erythrocyte count, haemoglobin concentration and haematocrit value as compared to control group. Group 5 which received Rtc (200) showed no significant difference in all mentioned hematological parameters as compared to control group which was treated with normal saline.

Simultaneous administration of doses of plant extract separately but along with aflatoxin showed no significant difference in hematocrit value, lymphocyte count and segmented neutrophil as compared to aflatoxin treated group. Groups of mice which received RTc (50) and RTc (100) along with AFB₁ showed significant increase (p<0.05) in erythrocyte count, in group 6 and 7, respectively whereas group 8 showed no significant difference when compared to group of mice which received aflatoxin.

Same groups of mice (Group 6, 7 and 8) also showed significant increase (p<0.01) in haemoglobin content as compared to respective value of aflatoxin treated group of mice whereas significant decrease (p<0.01) were seen in platelet count and leukocyte count, respectively as compared to aflatoxin treated group of mice.

Groups of mice which were treated with aflatoxin alone showed a significant increase (p<0.01) in SGOT, SGPT and ALP as compared to control group values. Groups of mice which received plant extract alone (Group 3, 4) showed no significant difference when compared to control group. Co administration of RTc (50) and RTc (100) along with aflatoxin showed significant fall (p<0.01) in SGOT, SGPT and ALP level on 25 day of study. Group of mice which receive RTc (200) along with aflatoxin showed significant decline (p<0.01) in SGOT level on 25 day of study but SGPT and ALP level was comparable to aflatoxin administered group (Table 2).

DISCUSSION

In the present study, toxicity of AFB₁ was expressed as significant alteration in hematological and serological variables. Significant reduction in Hb and TEC in AFB₁ treated group might be due to lower oxygen supply to different tissues, resulting in low energy production. These observations are in agreement with previous finding of Basmacioglu *et al.* (2005). Decrease in RBC has been attributed to reduction/disturbance in the erythropoiesis in bone marrow (Panda *et al.*, 1975) and a faster rate of destruction of peripheral RBC in spleen (Gupta *et al.*, 1967). Reduction in Hb can be related to the decreased size of RBC, impaired biosynthesis of haeme in

bone marrow or due to an increased rate of destruction/reduction in the rate of formation of TEC. Simultaneous administration of plant extract along with AFB₁ showed improvement or significant effect on almost all blood parameters. The observations corroborated with the report of Sharma and Pandey (2010b). In Ayurvedic literature, *Tinospora cordifolia* has been reported to be a blood purifier (Kirtikar and Basu, 1993) that possibly acts by stimulating liver and spleen which remove defective and damaged RBCs from peripheral blood circulation. RTc stimulate hemopoiesis in the bone marrow and therefore higher Hb level was observed, the increase in TLC in RTc treated mice in comparison to control indicated the immunostimulatory role of RTc and could therefore be attributed to the already known immunomodulatory constituents presents in *T. cordifolia*. In the present study increased TEC and other hematological parameters could be the results of immunomodulatory and other properties of *T. cordifolia* (Weiss *et al.*, 1990; Rege *et al.*, 1993).

Several soluble enzymes of blood serum have been considered as indicators of hepatic dysfunction and damage (Shakoori *et al.*, 1994). Transaminases (AST and ALT) is important and critical enzyme in the biological processes (Harper *et al.*, 1979). Alkaline Phosphatase (ALP) enzyme is a sensitive biomarker to metallic salts since it is a membrane bound enzymes related to the transport of various metabolites (Lakshmi *et al.*, 1991; Coleman, 1992). The various isoenzymes of ALP are ubiquitous through out the body, although they are mainly present in liver, bone, intestine, kidney, placenta and white blood cells (Tietz, 1976). The increment of the activities of AST, ALT and ALP is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al.*, 1993) which gives an indication on the hepatotoxic effect of aflatoxin B₁. Also Ossuna and Edds (1982) reported that the increased values of AST and ALP could be related to the liver necrosis and hepatotoxic effects of AFB₁ which corroborates with our study. In addition, the increase of ALP in serum could be a result of damage of liver cells and bile duct obstruction due to proliferation of its cells/or related to the progressive liver necrosis (Newberne and Butler, 1969). Thus, the observed increase in serum AST, ALT and ALP could be attributed in part to the concomitant hepatic necrosis as induced by AFB₁. RTc extract treatment during AFB₁ intoxication tried to down-regulate the above-mentioned molecular mechanism of enzyme action. These results showed similar trend with that of Bishayi *et al.* (2002).

In the conclusion, the hematological and serum biochemical values were significantly affected by AFB₁

treatment; the addition of different doses of RTc significantly recovered the adverse effects of AFB₁ on hematological and serum biochemical values of mice. The protective effects of RTc (100) is higher than RTc (50) and RTc (200) against the toxic effects of AFB₁. These improvements contribute to a solution of AFB₁ toxicity and may become a base of biological detoxification.

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