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Research Article

PREVENTIVE EFFECTS OF *TINOSPORA CORDIFOLIA* EXTRACT AGAINST AFLATOXIN-B_1 INDUCED OXIDATIVE STRESS IN SWISS ALBINO MICE

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ABSTRACT

Aflatoxins are potent hepatotoxic and hepatocarcinogenic agents. Reactive oxygen species and consequent peroxidative damage caused by aflatoxin are considered to be the main mechanisms leading to carcinoma. The present investigation aimed at assessing the protective role of ethanolic root extract of *Tinospora cordifolia* on aflatoxin B_1 induced toxicity in mice model. The level of alkaline phosphatase, alanine and aspartate aminotransferase were found to be increased significantly in serum of AFB_1 administered (2µg/30g b.wt., orally) mice, suggesting hepatic and renal damage. Marked increase in lipid peroxide and a concomitant decrease in enzymatic (superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase) and non-enzymatic (reduced glutathione, vitamin c and protein) antioxidant in hepatic and renal tissues were observed in aflatoxin administered animals. AFB_1 supplementation also affects the hematological indices by decreasing the level of hemoglobin, erythrocytes, hematocrit, lymphocyte and elevating the level of leukocyte, platelet and neutrophil counts. Histopathological examination of liver and kidney also corroborated the biochemical findings. Concomitant treatment with different doses of plant extract along with aflatoxin for 75 days reverted the condition near to normal. The results of present study suggested that *Tinospora cordifolia* extract protect against aflatoxin induced carcinoma and other hematological abnormalities.

Keywords: Aflatoxin, Biochemical Variables, Blood, Hepatotoxic, Mice, *Tinospora cordifolia*

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequent cancers among humans, with 0.25-1 million newly diagnosed cases every year.[1] The consumption of AFB_1-contaminated diet is an important factor in prognosis of HCC.[2] The carcinogenic mechanism of AFB_1 has been extensively studied. It has been shown that AFB_1 is activated by cytochrome p450 enzyme system and produce a highly reactive intermediate, AFB_1-8-9-epoxide which subsequently bind to nucleophilic site of DNA, and the major adducts 8,9-dihydro-8-(N^7 guanyl)-9-hydroxy-AFB_1 (AFB_1N^7-Gua) is formed.[3,4] Several reports also suggest that intracellular reactive oxygen species (ROS) like superoxide anions, hydroxyl and hydrogen peroxide radicals were generated during metabolic processing of AFB_1 by cytochrome p450 in liver.[5,6] The formation of AFB_1-DNA adduct and generation of free radicals is a critical step is the initiation of AFB_1 induced HCC as these radicals initiate lipid peroxidation, a damaging process in biological system that leads to diminished antioxidant status.[7]

Chemoprevention by synthetic and dietary compounds is an effective means of controlling the incidence of hepatocellular carcinoma (HCC) either by preventing or regulating the process of carcinogenesis.[8] Chemopreventive agents are reported to exert anticarcinogenic effects by modulating free radical induced by lipid peroxidation.[9, 10] In the recent year there has been considerable interest in natural products with antioxidant property in human diet. *Tinospora cordifolia*, commonly called as Guduchi, belongs to the family of Menispermaceae, is widely distributed throughout the planes of India. The whole plant is used for therapeutic purpose. It is reported that the plant is bitter but nontoxic and also has ability to scavenge free radicals. Phytochemical investigation of plant revealed that it contains a variety of constituents belongs to different classes such as alkaloids, diterpenoids lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides.[11] The plant also possess remarkable and notable medicinal properties such as antidiabetic[12], antipyretic[13], antioxidant[14,15], antiallergic[16], hepatoprotective[17,18], Immunomodulatory[19], blood purification[20] and antineoplastic.[21]

Keeping the medicinal properties of this plant in mind the present study was undertaken to investigate the preventive role of *T.cordifolia* extract against AFB_1-mediated changes in peroxidation level, enzymatic, non enzymatic antioxidant, blood profile, serum profile and histopathological changes in male albino mice.

MATERIALS AND METHODS

Chemicals

Crystalline AFB_1 (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*) were procured from Haryana Agricultural University; Hissar (Haryana, India).Only male mice were used because previous studies have indicated that these were more sensitive to aflatoxin treatment than female. 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. 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 aflatoxin B_1 was dissolved in dimethylsulfoxide and further diluted with distilled water to the required concentration. The final gavage solution of AFB_1 contained 1% dimethyl sulfoxide.

The experimental plant material was collected from Krishi Vigyan Kendra, Banasthali University; India. 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 and shade-dried. Ethanolic extract of the dried roots of *Tinospora cordifolia* was prepared by soxhlet method using 300ml ethanol for 50g (dry weight) of dried root powder .The ethanolic extract thus obtained was dried under reduced pressure at a room temperature not exceeding 40º 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 group and were administered orally by gavage, once daily as below, for 75 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)

The doses of Aflatoxin & plant extract were decided on the basis of previously published reports.[22, 23] The mice from each groups were sacrificed by cervical dislocation on 75th day of the study. Liver and kidney sample from the sacrificed mice were quickly removed and cleaned to make them free from extraneous material and perfused with ice-cold saline for biochemical and histopathological evaluation. Post-mitochondrial supernatant (PMS) was prepared using method of Mohandas *et al.*[24] with some modifications. The various histo-biochemical variables viz., lipid peroxidation[25], Superoxide dismutase[26], Catalase[27], glutathione-S-transferase[28], reduced glutathione[29], glutathione peroxidase [24], glutathione reductase[30], Ascorbic acid[31] and Protein[32] were performed.

Noncoagulated blood was used for hematological parameters viz., hemoglobin estimation was done using Sahli's hemoglobin meter[33], total erythrocyte count and total leukocyte count by hemocytometer[34], PCV by Wintrob method[35], and Differential leukocyte count by Leishman's staining method. Mean corpuscular hemoglobin, Mean corpuscular hemoglobin content & Mean corpuscular volume were also calculated.[36, 37]

Serum was obtained by centrifugation of blood samples at 860×g for 20 min, and was stored at -20ºC until used for analysis. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel.[38] Activity of alkaline phosphatase (ALP) was determined according to the protocol described in a laboratory practical manual.[39]

Histopathological evaluation of liver and kidney tissues were done according to the method of Luna.[40]

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 package16. 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

Biochemical parameters

Table 1 depicts the levels of lipid peroxide, ascorbic acid, protein and reduced glutathione in normal and experimental mice liver and kidney. The lipid peroxide activity was significantly raised ($P<0.01$) in aflatoxin toxicated mice but the activities of ascorbic acid, protein and reduced glutathione were found to fall significantly ($P<0.01$) as compared with normal mice. Administration of all doses of RTc extract (low, medium and high), decreased the level of LPO and increased the level of ascorbic acid, protein and reduced glutathione significantly ($P<0.01$) when compared with aflatoxin alone receiving group of mice (group II).

Antioxidant Parameters

Table 2 shows levels of superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase and glutathione reductase in liver & kidney of normal and experimental mice. The level of SOD, CAT, GST, GPx and GR were decreased significantly ($P<0.01$) in liver and kidney of aflatoxin toxicated mice group when compared with normal mice. The RTc extract treatment brought the activities of all these antioxidant enzymes back to normal level to some extent when compared with group II mice.

Hematological variables

Data presented in Table 3 shows the effect of RTc extract either alone or in combination with AFB_1 on hematological variables.

AFB_1 alone administration caused a significant decrease ($P<0.01$) in hemoglobin, hematocrit, total erythrocyte count, lymphocyte count and significant increase ($P<0.01$) in WBC, platelet and neutrophil count when compared with control values. The levels of Hb, PCV, RBC were increased significantly ($P<0.01$) and it were comparable to control in the groups of mice that receive combination of different doses plant extract along with AFB_1. On the other hand, the level of WBC, platelet and neutrophil count were reduced significantly ($P<0.01$) in the group of mice that received RTc extract and AFB_1 simultaneously in comparison to group II mice.

Serological parameters

Table 4 shows SGOT, SGPT and ALP activities in the serum of normal and treated mice. The values of SGOT, SGPT and ALP were elevated significantly ($P<0.01$) when compared with respective values of normal mice. RTc extract supplemented mice showed decrease ($P<0.01$) in SGOT, SGPT and ALP values; RTc extract reversed the toxic effect of aflatoxin to some extent.

Histological/histopathological examination

Histopathological examination of liver (Fig 1) revealed no tumor and other pathological abnormalities in mice administered with all doses of plant extract and normal saline but mice administered with aflatoxin alone showed hepatocellular carcinoma. Mice supplemented with aflatoxin along with RTc (50) and RTc (100) showed normal hepatic parenchyma whereas mice administered with RTc (200) along with aflatoxin showed scattered vacuolated hepatocytes in the mid and periportal zones.

Histopatholical examination of kidney (Fig 2) showed normal kidney parenchyma in mice administered with different doses of plant and normal saline whereas group of mice that receive aflatoxin alone showed vacuolation, necrosis and shedding of tubular epithelial cells. Mice supplemented with different doses of plant along with aflatoxin prevent the toxic effect of aflatoxin and showed normal kidney architecture.

DISSCUSSION

Carcinogenesis is a multistep process begins with cellular transformation and process by hyper proliferation leading to metastatic lesions.[41] This progress can be activated by carcinogenotoxic substance such as aflatoxin that develops cancers in specific organ of experimental animals. However, relatively little is known about the mechanism of its toxicity, it was discovered that AFB_1 was able to induce chromosomal damage through the release of free radicals.[42] This finding suggested that oxidative damage might contributed to the carcinogenic effects of AFB_1. Aflatoxin cause oxidative stress by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidant in aflatoxin treated mice.[43,44] Oxidative damage usually refers to impairment of the function of cellular component by reactive oxygen species such as superoxide anion (O_2^-), hydroxyl ion (OH^-), and hydrogen peroxide (H_2O_2).These agents initiate cell injury by extracting hydrogen atom from polyunsaturated fatty acid and cause a degenerative process known as lipid peroxidation.[45] To protect them against oxidative stress, cell have developed antioxidant defense and repair system which prevent the accumulation of oxidatively damaged molecules. The antioxidant defense system include enzyme like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase, as well as small molecules ascorbic acid.[46]A significant increase in production of MDA and decrease in the level of glutathione was observed in AFB_1 administered animal. Malondialdehyde, a major end product of lipid peroxidation, cross-links with DNA, protein and nucleotides on the

same or opposite stand there by promoting carcinogenesis. Elevation in the level of lipid peroxide in the present study is in accordance with the previous reports given by Shen *et al*., 1994.[47] Simultaneous administration of RTc root extract in aflatoxin treated animal has effectively controlled the rate of lipid peroxidation. The presence of epoxy clerodane diterpene in *T.cordifolia* may attribute to this effect because they are proved to be potential inhibitor of lipid peroxidation.[48]The increased level of lipid peroxide is further evidenced by the decrease in the activity of SOD, CAT and GPx in the liver and kidney tissues of AFB_1 injected mice.

Table 1: Effect of RTc extract either alone or in combination with AFB_1 on LPO, Ascorbic acid , Protein and GSH level in mice liver and Kidney

Treatments (mean ± S.E.M.)									
Parameters	**Tissues**	**(Group I)**	**(Group II)**	**(Group III)**	**(Group IV)**	**(Group V)**	**(Group VI)**	**(Group VII)**	**(Group VIII)**
LPO(nmols TBARS h-^{1}g-^{1}tissue)	Liver	8.08±0.40^{c}	30.12±1.17^{a}	7.99±0.59^{NS,c}	7.96±0.25^{NS,c}	8.06±0.14^{NS,c}	10.14±0.84^{a,c}	9.59±0.41^{a,c}	10.85±0.42^{a,c}
	Kidney	8.47±0.23^{c}	27.10±1.22^{a}	8.29±0.26^{NS,c}	8.18±0.14^{NS,c}	8.47±0.35^{NS,c}	9.75±0.52^{NS,c}	9.23±0.43^{NS,c}	10.26±0.33^{a,c}
Asc(mg ascorbic acid/g tissue)	Liver	6.44±0.26^{c}	1.60±0.05^{a}	8.14±0.76^{a,c}	8.87±0.23^{a,c}	7.73±0.17^{a,c}	6.24±0.12^{NS,c}	6.29±0.14^{NS,c}	6.02±0.11^{b,c}
	Kidney	5.95±0.05^{c}	1.24±0.08^{a}	6.22±0.26^{a,c}	6.37±0.24^{a,c}	5.89±0.03^{NS,c}	5.90±0.14^{NS,c}	6.02±0.10^{NS,c}	5.45±0.05^{a,c}
Protein (mg g-^{1}fresh wt)	Liver	92.71±3.15^{c}	42.47±2.14^{a}	90.92±1.49^{NS,c}	92.97±1.90^{NS,c}	90.05±2.49^{NS,c}	86.67±2.24^{a,c}	89.54±1.74^{NS,c}	82.28±1.38^{a,c}
	Kidney	63.08±2.31^{c}	19.74±1.42^{a}	70.63±2.23^{a,c}	72.38±1.96^{a,c}	64.97±0.84^{NS,c}	52.19±1.23^{a,c}	56.27±1.76^{a,c}	50.73±2.19^{a,c}
GSH(nmols GSH g-^{1} tissue)	Liver	262.14±2.31^{c}	121.90±2.65^{a}	284.18±1.49^{a,c}	288.67±2.10^{a,c}	280.05±1.74^{a,c}	264.17±1.48^{b,c}	263.20±1.98^{NS,c}	266.64±1.99^{b,c}
	Kidney	272.21±2.88^{c}	97.72±3.18^{a}	312.13±2.62^{a,c}	325.29±4.42^{a,c}	305.93±2.49^{a,c}	267.17±4.21^{NS,c}	269.34±2.73^{NS,c}	268.71±2.76^{NS,c}

Abbreviations: - AFB1, Aflatoxin B1; Asc, Ascorbic acid; GSH, Reduced Glutathione; LPO, Lipid peroxidase; 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 I) and c $p<0.01$ and d $p<0.05$ when compared with aflatoxin treated group (group II).NS (Statically not significant)

Table 2: Effect of RTc extract either alone or in combination with AFB_1 on antioxidant parameters in mice liver and kidney

Treatments (mean ± S.E.M.)									
Parameters	**Tissues**	**(Group I)**	**(Group II)**	**(Group III)**	**(Group IV)**	**(Group V)**	**(Group VI)**	**(Group VII)**	**(Group VIII)**
SOD(Unit mg-^{1}Protein h-^{1}	Liver	14.86±0.11^{c}	2.47±0.09^{a}	14.59±0.16^{NS,c}	14.98±0.11^{NS,c}	13.08±0.20^{a,c}	12.23±0.23^{a,c}	13.30±0.19^{a,c}	11.27±0.28^{a,c}
	kidney	12.26±0.21^{c}	1.52±0.02^{a}	13.26±0.46^{a,c}	12.58±0.34^{NS,c}	13.53±0.50^{a,c}	12.16±0.23^{NS,c}	12.45±0.38^{NS,c}	10.84±0.30^{a,c}
CAT(µ mol H_2O_2 consumed min-^{1} mg-^{1} protein)	Liver	225.39±4.03^{c}	98.09±3.13^{a}	231.12±1.56^{NS,c}	233.42±3.86^{a,c}	230.62±1.99^{NS,c}	210.14±1.65^{a,c}	219.32±2.43^{b,c}	204.78±2.04^{a,c}
	Kidney	82.93±1.88^{c}	18.22±0.92^{a}	86.13±1.32^{NS,c}	85.66±2.64^{NS,c}	89.24±1.25^{a,c}	75.87±2.29^{a,c}	78.63±1.96^{a,c}	69.55±0.77^{a,c}
GST(nmols CDNB conjugates formed min-^{1}mg-^{1} protein)	Liver	205.28±2.27^{c}	52.08±2.26^{a}	195±1.49^{a,c}	196.18±1.92^{a,c}	193.20±1.89^{a,c}	192.16±3.19^{a,c}	201.07±2.90^{NS,c}	185.16±2.72^{a,c}
	Kidney	165.53±2.09^{c}	67.77±1.79^{a}	171.23±1.71$^{NS, c}$	175.49±2.43$^{NS, c}$	168.20±1.47$^{NS, c}$	163.45±2.19$^{a, c}$	171.32±2.30$^{NS, c}$	158.43±2.23$^{NS, c}$
GPx(µg of glutathione utilized min-^{1}mg-^{1}protein)	Liver	57.8±1.99^{c}	16.87±1.49^{a}	59.12±1.42^{NS,c}	60.69±1.41^{NS,c}	57.49±1.92^{NS,c}	55.13±1.56^{NS,c}	57.40±3.97^{NS,c}	50.51±1.76^{a,c}
	Kidney	28.73±1.53^{c}	6.41±0.34^{a}	32.92±0.36^{a,c}	30.25±0.46$^{NS, c}$	33.49±1.02$^{a, c}$	30.12±0.32$^{a, c}$	31.89±1.42$^{a, c}$	26.99±1.46$^{NS, c}$
GR(n moles NADPH oxidized min-^{1}mg-^{1}protein)	Liver	146.23±2.19^{c}	98.82±2.46^{a}	149.19±1.92^{NS,c}	154.12±2.57^{a,c}	146.48±1.93^{NS,c}	145.24±1.29^{NS,c}	149.03±1.84$^{NS, c}$	140.50±2.33^{a,c}
	Kidney	141.26±1.86^{c}	93.69±2.17^{a}	147.24±1.43$^{a, c}$	151.29±2.60$^{a, c}$	143.78±2.25$^{NS, c}$	145.16±1.65$^{a, c}$	147.01±1.44$^{a, c}$	138.05±2.37$^{NS, c}$

Abbreviations:- AFB1, Aflatoxin B1; CAT, Catalase; GPx, Glutathione peroxidase; GR, Glutathione reductase; GST, Glutathione-S-transferase; RTc, Root of *Tinospora cordifolia*; SOD, Superoxide dismutase

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 I) and c $p<0.01$ and d $p<0.05$ when compared with aflatoxin treated group (group II).NS (Statically not significant)

Table 3: Effect of RTc extract either alone or in combination with AFB_1 on haematological variables in Swiss albino mice

Treatments (mean ± S.E.M.)								
Parameters	**(Group I)**	**(Group II)**	**(Group III)**	**(Group IV)**	**(Group V)**	**(Group VI)**	**(Group VII)**	**(Group VIII)**
Hb (g/dl)	13.39±0.23[c]	7.17±0.45[a]	17.09±0.32 a,c	17.99±0.36[a,c]	14.10±0.45[NS ,c]	13.01±0.12[NS , c]	13.41±0.39[N S, c]	12.75±0.48[NS c]
PCV (%)	43.80±0.56 [c]	32.52±1.52 [a]	48.34±2.1 [a,c]	50.36±1.45 a,c	45.92±0.73[NS , c]	40.13±2.31[NS , c]	41.57±1.05[N S,c]	46.60±1.27[NS , c]
RBC(10^6/mm^3) Abbreviations: - AFB_1	8.94±0.41 [c]	5.74±0.38 [a]	12.49±0.67 a,c	13.23±0.44 a,c	11.16±0.33 [a, c]	8.51±0.22 [NS, c]	8.53±0.44 NS,c	8.36±0.27 [NS,c]
MCV(fL)	48.75±2.63 [c]	56.83±4.11 [a]	39.98±1.65 a,c	38.09±1.50 a,c	41.36±1.35 a,c	47.92±1.45[NS ,c]	48.76±1.66[N S, c]	48.92±2.99[NS ,c]
MCH(pg)	14.99±0.49 [c]	12.53±1.16 [a]	13.13±0.92 NS	13.60±0.29 NS	12.63±0.47 [a, NS]	15.70±0.34[NS ,c]	15.77±1.21[N S,c]	15.25±0.58[NS ,c]
MCHC(g/dl)	30.56±0.71 [c]	22.05±1.10 [a]	34.23±0.45 a,c	35.74±0.75 a,c	30.69±0.65 NS,c	31.93±0.93 NS, c	32.30±1.70 NS, c	30.69±1.63[NS ,c]
WBC (10^3/mm^3)	17.21±0.78 [c]	23.92±0.57 [a]	17.92±0.67 NS,c	17.25±0.79[NS ,c]	17.92±2.28 NS,c	19.42±0.92 a,c	18.02±1.10[N S,c]	19.92±1.69 a,c
Platelet(10^3/mm^3)	717.33±7.86[c]	1734.16±6.91[a]	624.78±3.24 a,c	607.66±5.96 a,c	697.33±6.04 a,c	913.12±4.78 a,c	8.31.33±5.6[a, c]	931.83±5.98 a,c
Neutrophil (%)	16±1.41[c]	24.16±1.06 [a]	13.45±0.92 b,c	12.83±0.68 a,c	14.66±1.10[NS ,c]	16.93±1.67[NS ,c]	16.83±0.68[N S,c]	17.00±0.57[NS ,c]
LyLLymphocyte (%)	92.04±2.81 [c]	62.97±1.84[a]	86.65±2.67 a,c	89.55±3.04 NS,c	83.95±3.46 a,c	87.67±2.45 b,c	90.47±2.38 NS,c	85.99±2.65 b,c

Abbreviations:- AFB1, Aflatoxin B1; Hb, Haemoglobin; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin content, MCV, Mean corpuscular volume; PCV, Packed cell volume; RTc, Root of *Tinospora cordifolia* ; WBC, White blood corpuscles

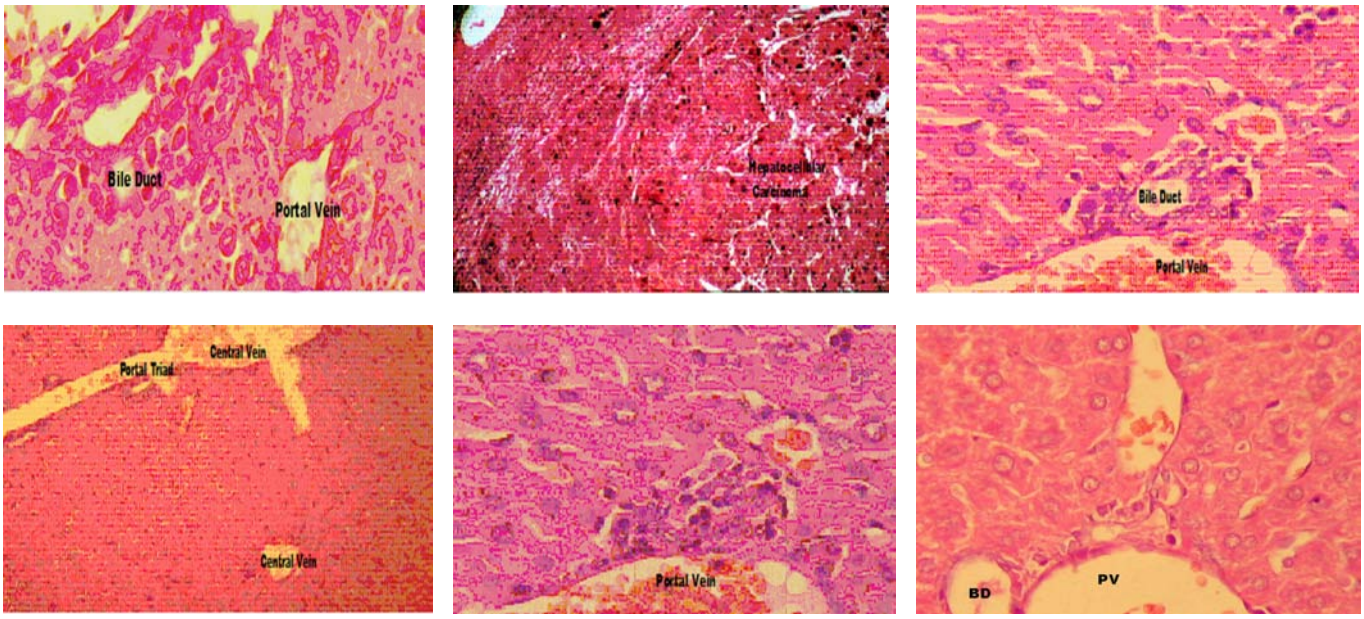
Values are mean± SE of six mice. Significant differences in data are shown as a $p<0.01$ and b $p<0.05$ when compared with control (group I) and c $p<0.01$ and d $p<0.05$ when compared with aflatoxin treated group (group II). NS (Statically not significant)

Table 4: Effect of RTc extract either alone or in combination with AFB1 on some serological variables in Swiss albino mice

Treatments (mean ± S.E.M.)								
Parameters	**(Group I)**	**(Group II)**	**(Group III)**	**(Group IV)**	**(Group V)**	**(Group VI)**	**(Group VII)**	**(Group VIII)**
SGOT(Units /ml)	10.07±0.12 c	51.58±2.37 a	9.48±0.65 NS, c	9.25±0.20 NS, c	9.85±0.14 NS, c	18.34±2.12 a,c	15.01±0.95 a,c	22.11±1.41 a,c
SGPT(Units /ml)	11.25±0.51 c	52.17±1.10 a	9.58±1.12NS, c	9.19±0.22 b, c	9.68±0.33 NS, c	17.67±2.34 a,c	14.80±0.48 a,c	19.71±1.42 a, c
ALP (KA Units /ml)	4.96±0.27c	14.22±0.39a	5.06±0.23 NS, c	4.9±0.14 NS, c	5.11±0.11 NS, c	6.16±0.78 a,c	5.65±0.32 a,c	7.43±0.30 a, c

Abbreviations:- AFB1, Aflatoxin B1; SGOT, Serum glutamate oxaloacetate transaminase; SGPT, Serum glutamate pyruvate transaminase; ALP, Alkaline phosphatase; 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 I) and c $p<0.01$ and d $p<0.05$ when compared with aflatoxin treated group (group II).NS (Statically not significant)



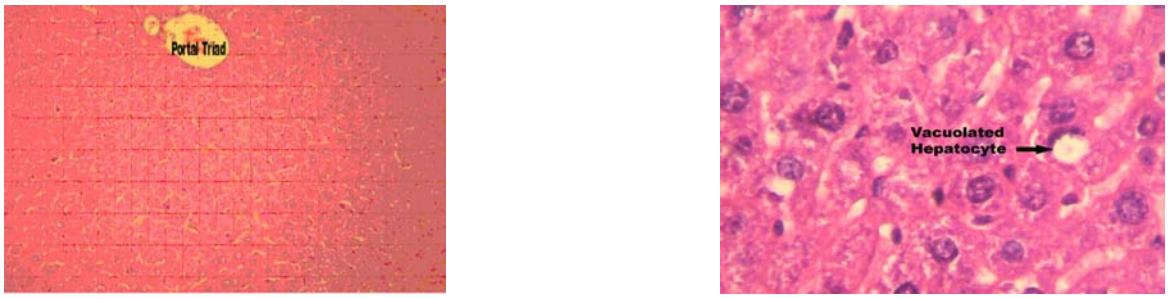


Fig. 1: Effect of RTc extracts either alone or in combination with AFB_1 on liver tissue of mice during concomitant therapy for 75 days

Cross section of liver treated with AFB_1 and RTc for 75 days (40X). Liver from control mice (a), RTc (50) (c), RTc (100) (d), RTc (200) (e), AFB_1 + RTc (50) (f) and AFB_1+RTc (100) (g) show normal architecture. However, liver from AFB_1 supplemented mice (b) revealed hepatocellular carcinoma but mice treated with AFB_1+RTc (200) shows scattered vacuolated hepatocytes in mid and periportal zone

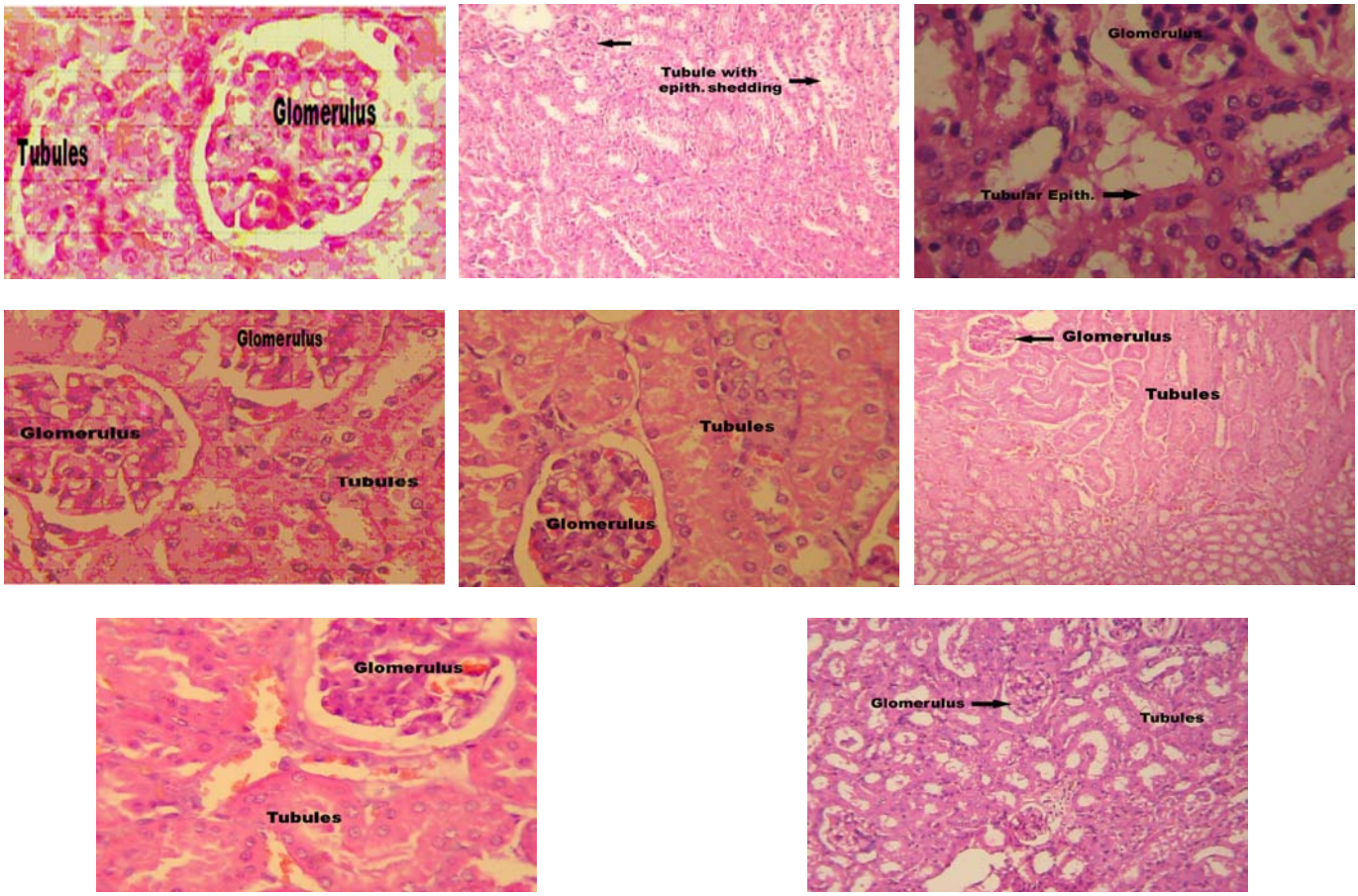


Fig. 2: Effect of RTc extract either alone or in combination with aflatoxin on kidney tissues of mice during concomitant treatment for 75 days

SOD is a family of metallo-enzyme that accelerate the dismutation of endogenous cytotoxic superoxide radical to H_2O_2 which are deleterious to polyunsaturated fatty acid and structural protein of plasma membrane.[49] The H_2O_2 produced by SOD is further removed by CAT. Decline in the activities of these enzymes after AFB_1 administration might be due to the inactivation of these enzymes by ROS. Ethanolic root extract of *T.cordifolia* increases the GSH status resulting in the increase in SOD activity thereby preventing the deleterious effect of superoxide radicals. Thus, *T.cordifolia* indirectly influences the activity of SOD and CAT. GPx, selenium dependent enzymes removes both H_2O_2 and lipid peroxides by catalyzing the conversion of lipid hydroperoxide to hydroxy acid in the presence of GSH. The activity of GPx decreased during AFB_1 administration may be due to decrease availability of substrate (GSH) and also because of alteration in their protein structure by ROS.[50] The increased level of GSH in *T.cordifolia* treated group may activate GPx thereby preventing accumulation of H_2O_2.GR is enzyme responsible for the conversion of glutathione disulphide (GSSG) back to reduced GSH.GSSG is formed during the detoxification of H_2O_2 by GPx. Reduced GR activity in AFB_1 treated group confirmed the higher production of GSSG due to the higher rate of detoxification of H_2O_2 by GPx. Similar results were also obtained in rats hepatotoxicity by Cascales *et al.*, 1991.[51]GST eliminates the reactive epoxide of aflatoxin by conjugation with GSH. The values showed significant reduction in AFB_1 treated group and non significant changes in RTc supplemented groups indicate that RTc plays important role in

maintenance of GST activity. Vitamin C was also found to be low in AFB_1 intoxicated mice. This may be due to the utilization of vitamin C for scavenging free radical produced by cancerous cell and AFB_1 metabolism or its synthesis in body may be affected by the damaged cell in response of AFB_1 treatment.[52]

Cross Section kidney mice treated with AFB_1 and RTc for 75 days (40X). Groups of mice supplemented with normal saline and different doses of plant extract (low, medium and high) (a, c, d, e) shows normal kidney parenchyma whereas groups of mice that received AFB_1 (b) shows vacuolation, necrosis and shedding of tubular epithelial cells. Mice supplemented with different doses of plant along with AFB_1 (f, g, h) revert the toxic effect of aflatoxin and show normal kidney architecture.

We observed a significant decrease in haemoglobin concentration, PCV and total RBCs resulting in a normocytic normochromic anemia. This decrease in haemopoitic parameters may be due inhibition of protein synthesis as indicated by lower amount of total protein.[53] Previous reports indicated that AFB_1 decreases the total iron binding capacity[54] and affects the metabolism of minerals such as Cu and Zn.[53]These results support our findings that aflatoxin causes normocytic normochromic anaemia. There was a significant enhancement in the WBC counts, which mainly consisted of neutrophils. This increase in WBCs and neutrophils suggested that the toxin is elicited an inflammatory response, and causes alteration in bone marrow system.[55]

However administration of all doses of RTc extract (low, medium and high) produced moderate to significant effect in almost all blood variables. Several reports suggested that *T.cordifolia* is used for treatment of multiple disorders; it also enriches the blood .[56, 57, 58] Simultaneous administration of *T.cordifolia* along with AFB_1 decrease the toxic effect of AFB_1 possibly by stimulating liver and spleen for removing defective and damaged RBCs from blood circulation.[59] The feedback mechanism, however stimulated hemopoisis in the bone marrow, therefore higher haemoglobin was observed. The immunostimulatory role of RTc could be attributed to the already known immunomodulatory constituent present in *T.cordifolia*. RTc has already reported, to contain a large number of bio-active molecules, which elicit protection against several stress and pathological condition by acting through different mechanism such as antioxidant[60], stimulation of cell proliferation [61, 62], immunomodulation[63,64] and anti-inflammatory activity.[65]

ALT, AST and ALP are well known diagnostic indicator for changes in the hepatic tissue and biliary system.[53] In cases such as liver and kidney carcinoma these enzymes are released from the liver and kidney tissue into the blood stream.[66] The results obtained in present study indicated a significant increase in the activities of these marker enzymes in serum, which is in accordance with the previous reports.[67,68] Treatment with RTc significantly lowered the levels of these enzymes, suggests the protective role of RTc extract.

CONCLUSION

In conclusion, we determined that aflatoxin could increase the liver and kidney enzyme levels and affect some hematological and serological parameters. Increase in these parameters may occur due to peroxidation reactions, arising in aflatoxin biotransformation, and these reactions may inflict oxidative injury to cellular components. Administration of RTc extract to mice received aflatoxin B_1, resulted in a significant improvement in all biochemical, hematological, serological indices as well as a significant improvement in histopathological picture of the liver and kidney in different experimental groups. In the light of these results, RTc extract was found to induce the potent protective action in mice and plays a role in prevention of hepatic cellular injury produced by aflatoxin.

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