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Research Article

ANTIAFLATOXICOGENIC ACTIVITIES OF *TINOSPORA CORDIFOLIA* ROOT EXTRACT AGAINST AFLATOXIN B_1- INDUCED RENAL DAMAGE

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ABSTRACT

The present study is an effort to identify a potent chemopreventive agent against various diseases (including aflatoxicosis) in which oxidative stress plays an important causative role. Here, we investigated the antiaflatoxicogenic activity of ethanolic extract of *Tinospora cordifolia* against aflatoxin induced renal damage. The results revealed that administration of AFB_1 induces oxidative stress in kidney of AFB_1-treated mice through elevating the level of malondialdehyde (MDA) and depleting the level of tissue antioxidants viz., superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione–S-transferase (GST), ascorbic acid and protein. Pathological changes in kidney tissue also supported the biochemical findings. Supplementation of ethanolic extract of *Tinospora cordifolia* root during post exposure therapy effectively ameliorates the deviation induced in kidney of animal in response to AFB_1 administration may be due to presence of antioxidant alkaloids viz., tinosporine, tinosporic acid, tinosporol, choline (V), isocolumbine, palmatine and magnoflorine. The further studies warrant the investigation of exact active principle (s) present in the extract responsible for the observed effects employing various carcinogenic models.

Keywords: Aflatoxin B_1, Antioxidants, Kidney, Mice, *Tinospora cordifolia*

INTRODUCTION

Aflatoxins, a group of secondary metabolites produced by certain fungal species are known to contaminate items of alimentary importance. Aflatoxin is not only contaminating our food stuffs, but also found in edible tissues, milk and eggs after consumption of contaminated feed by farm animals[1, 2].

AFB_1 is well known to be potent mutagenic, carcinogenic, immunosuppressive, teratogenic and also inhibited several metabolic activities, causing liver, kidney and heart damage[3-5]. These toxins have been incriminated as cause of high mortality in livestock and some cause death in human being[6].

The toxicity and carcinogenicity of AFB_1 has been extensively studied and thought to be directly linked to its bioactivation by hepatic cytochrome P_{450} enzyme system, that results the formation of highly reactive intermediate $AFB_1$8,9-epoxide.This intermediate subsequently bind to nucleophilic sites of DNA and major adduct 8,9 dihydro-8-(N^7guanyl)-9 hydroxy-AFB_1 (AFB_1N^7-Gua) is formed[7]. The formation of AFB_1-DNA adducts is a critical step in the initiation of AFB_1-induced carcinogenesis[8]. Although the mechanism underlying the toxicity of aflatoxin is not fully understood, several reports suggests that toxicity may ensue through the generation of intracellular reactive oxygen species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide (H_2O_2) during processing of AFB_1 by cytochrome P_{450} in the liver[9].These species may attack soluble cell compounds as well as membrane, eventually leading to the impairment of cell functioning and cytolysis[10]. Damage of hepatocytes and the nephrocytes is believed to be the results of this process.

The use of synthetic chemicals has greatly contributed to management of such damage, but indiscriminate application of chemicals has led to a number of ecological and medicinal problems due to residual toxicity, teratogenicity, carcinogenicity, hormonal imbalance, spermatotoxicity etc[11]. Interest in medicinal plant burgeoned by the increased efficiency of new plant-derived drugs and growing interest in natural products. The use of plants as medicines dates to the down of history[12, 13]. Medicinal plants serves as therapeutic alternative, super choice, or in some cases, as only effective treatments.

Among the promising medicinal plants *T.cordifolia* (Menispermaceae) is an amazing herb with rich historical background. It is widely used in Indian Ayurvedic medicine as a tonic, vitalizer and as a remedy for metabolic disorders[14]. Previous studies showed that the plant posses antidiabetic[15], immunomodulatory[16], hepatoprotective[17] and antipyretic[18] activities. Some authors have reported that *T.cordifolia* roots possess antiulcer[19] and antistress[20] activities.

There are no available reports on the antioxidant action of *T.cordifolia* roots on aflatoxicosis. Therefore we made an attempt to evaluate the antioxidant property of an ethanolic *T.cordifolia* root extract in aflatoxin induced toxicity toxicated mice kidney.

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).The animals were housed under standard laboratory conditions of light (12 h light-dark cycle), temperature (25±2ºC), 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 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

Sixty adult male Swiss albino mice (Mus musculus) weighing 30±5 g were placed in two groups of 12 and 48 mice each and were administered orally by gavage, once daily as below, for 25 consecutive days. The groups were as follows:

Group I - Control (Normal saline, 0.9%)

Group II - Aflatoxin B_1 (AFB_1) 2µg/30 g b. wt

After 25 days, aflatoxin–exposed mice were divided into four groups of 12 mice each and were given following treatments orally by gavage, once daily for 20 consecutive days.

Group II A - No treatment

Group II B - *T.cordifolia* (Root) 50 mg/kg b.wt

Group II C - *T.cordifolia* (Root) 100 mg/kg b.wt

Group II D - *T.cordifolia* (Root) 200 mg/kg b.wt

The doses of Aflatoxin and plant extract were decided on the basis of previously published reports[21, 22].The mice from each group were sacrificed by cervical dislocation after post exposure therapy. 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.*[23] with some modifications. The various biochemical variables viz., lipid peroxidation[24], superoxide dismutase[25], catalase[26], glutathione-S-transferase[27], reduced glutathione[28], glutathione peroxidase[23], glutathione reductase[29], ascorbic acid[30] and protein[31] were performed. Histopathological evaluation of kidney tissue was done according to the method of Luna[32].

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

Table 1 shows the biochemical status of animals, i.e., level of LPO, SOD, CAT, GSH, GST, GPx, GR, ascorbic acid and protein content. The renal LPO level was significantly increased (P<0.01) whereas SOD, CAT, GSH, GST, GR, GPx, ascorbic acid and protein level were significantly decreased (P<0.01) in aflatoxin treated group than that of control group (group I).When post exposure therapy was done for 20 days after AFB_1 exposure, it was found that all plant doses (low, medium and high) showed significant increased (P<0.01) in the activity of CAT, SOD, GST, and GSH when compared with aflatoxin administered group (group IIA).Group IIB and group IID showed GPx and GR activity comparable to aflatoxin alone receiving group but group IIC showed significant improvement (P<0.01) in the activity of these enzymes as compared to that of aflatoxin toxicated mice. The level of LPO was significantly decreased (P<0.01) in group IIB and IIC mice whereas group IID mice also showed significant increased in this enzyme but at P<0.05 significant level than that of group IIA mice. The ascorbic acid concentration was significantly elevated (P<0.05) in only group IIC mice whereas as it was comparable to aflatoxin receiving group in group IIB and group IID. The mean values of protein was enhanced significantly (P<0.05) in group IIB and group IID mice however it also showed significant improvement in group IIC mice but at P<0.01 level.

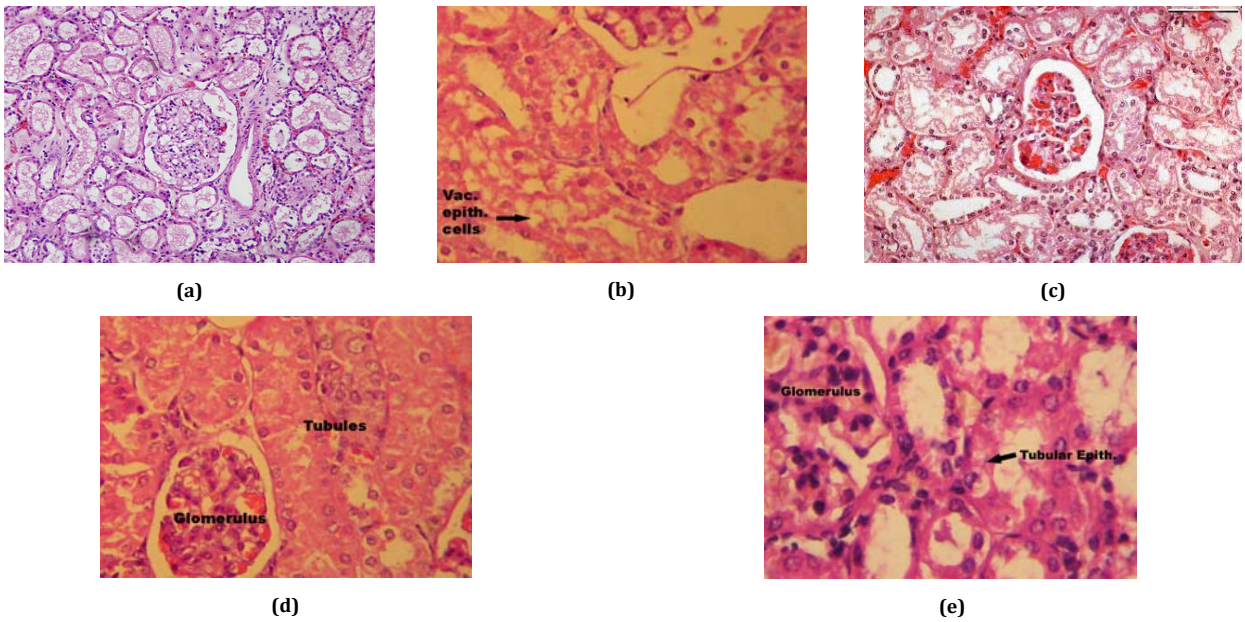

Fig. 1: Effect of ethanolic RTc extract on kidney histopathology in mice treated with Aflatoxin B_1 during post exposure therapy

Cross section of kidney of mice treated with AFB_1 and RTc (40X). Kidney From Control mice (a) to which normal saline was given for 45 days shows normal glomerulus and tubules in the cortical area whereas kidney of mice to which no treatment was given for 20 days after 25 days of aflatoxin administration (b) shows vacuolation and necrosis of tubular epithelial cells. Kidney section of mice to which RTc (50) was given for 20 days after 25 days of aflatoxin administration (c) shows normal glomerulus and tubules in the cortical zone whereas kidney section of mice to which RTc (100) was given for 20 days after 25 days of aflatoxin administration (d) the section shows normal glomerulus and tubules. Section of kidney of mice to which RTc (200) was given for 20 days after 25 days of aflatoxin administration (e) shows a congested glomerulus and tubules.

Table 1: Effect of ethanolic RTc extract on non-enzymatic, enzymatic parameters and level of TBARS in the kidney of mice treated with aflatoxin B_1 during post exposure therapy

Parameters	Control (Group I)	AFB_1 (Group IIA)	AFB_1+RTc(50) (Group IIB)	AFB_1+RTc(100) (Group IIC)	AFB_1+RTc(200) (Group IID)
LPO(nmols TBARS $h^{-1}g^{-1}$tissue)	8. 37±0. 22	18.61±0.71 [a]	15.45±0.58 [c]	14.48±1.10 [c]	16.40±0.95 [d]
SOD(Unit mg^{-1}Protein h^{-1}	12.42±0.12	5.53±0.08 [a]	7.31±0.04 [c]	7.55±0.05 [c]	7.26±0.02 [c]
CAT(µ mol H_2O_2 consumed min^{-1} mg^{-1} protein)	83.15±1.71	47.55±1.83 [a]	56.84±1.71 [c]	59.50±1.97 [c]	53.33±2.33 [c]
GSH(nmols GSH g^{-1} tissue)	267.57±3.84	164.80±3.56 [a]	251.72±3.66 [c]	258.66±2.4 [c]	248.91±3.61 [c]
GST(nmols CDNB conjugates formed $min^{-1}mg^{-1}$ protein)	166.69±1.60	106.34±1.60 [a]	123.09±2.24 [c]	126.08±2.43 [c]	120.62±1.51 [c]
GPx(µg of glutathione utilized $min^{-1}mg^{-1}$protein)	24.04±1.33	15.77±1.00 [a]	17.23±0.78 [NS]	18.58±1.03 [c]	17.17±0.69 [NS]
GR(nmoles NADPH oxidized $min^{-1}mg^{-1}$protein)	140.96±2.48	123.31±1.31 [a]	126.14±2.22 [NS]	128.92±1.55 [c]	124.44±1.39 [NS]
Asc(mg ascorbic acid/g tissue)	5.74±0.04	3.96±0.08 [a]	4.33±0.04 [NS]	4.83±0.06 [d]	4.11±0.06 [NS]
Protein (mg g^{-1}fresh wt)	51.17±3.70	34.65±1.63 [a]	39.29±1.76 [d]	40.30±1.15 [c]	38.65±1.17 [d]

Abbreviations: - AFB^1, Aflatoxin B^1; Asc, Ascorbic acid; CAT, Catalase; GPx, Glutathione peroxidase; GR, Glutathione reductase; GSH, Reduced Glutathione; GST, Glutathione-S-transferase; LPO, Lipid peroxidase; 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 IIA).NS as statistically not significant

Histopathological examination of mice kidney revealed in Fig 1. shows normal architecture in control (a) to which normal saline was given for 45 days whereas kidney of mice to which no treatment was given for 20 days after 25 days of aflatoxin administration (b) shows vacuolation and necrosis of tubular epithelial cells. Kidney section of mice to which RTc (50) was given for 20 days after 25 days of aflatoxin administration (c) shows normal glomerulus and tubules in the cortical zone whereas kidney section of mice to which RTc (100) was given for 20 days after 25 days of aflatoxin administration (d) the section shows normal glomerulus and tubules. Section of kidney of mice to which RTc (200) was given for 20 days after 25 days of aflatoxin administration (e) shows a congested glomerulus and tubules.

DISCUSSION

In the present study increase in lipid peroxidation level in the kidney of aflatoxin treated mice was observed. The enzymatic antioxidant defense system is natural protector against lipid peroxidation. SOD, CAT and GPx enzymes are important scavengers of superoxide ion and hydrogen peroxides. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage[33]. GPx is a cytoplasmic and mitochondrial enzyme that detoxifies H_2O_2 in most cells. Glutathione –S-Transferase (GST) is a family of the enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates, which have electrophilic functional groups. They play an important role in detoxification and metabolism of many xenobiotic and endobiotic compounds[34]. Superoxide dismutase is a very important enzyme that functions as a cellular antioxidant. It is present in cell cytoplasm and mitochondria in order to maintain a low concentration of superoxide anions[34].The significant reduction in the activities of antioxidant enzymes (GPx, GST, GR, SOD and CAT) and non enzymatic antioxidant system (GSH) in aflatoxin treated mice kidney (group IIA) as compared to the control group (group I) could be responsible for increased lipid peroxidation levels observed during aflatoxin induced oxidative stress. Similar results have also been reported previously for liver of mice[35]. Protein and ascorbic acid concentration also significantly decreased in aflatoxin treated mice kidney. These observations have been supported by previous findings, which demonstrated that aflatoxin lower the protein contents[36]. During free radical scavenging action, ascorbic acid is transformed into L-dehydroascorbate[37] and reduced glutathione is required for the conversion of L-dehydroascorbate back to ascorbate[37].The fall in the level of reduced glutathione reduced the conversion of L-dehydroascorbate to ascorbate and this probably explains the lowered level of ascorbic acid in the aflatoxin treated animals[35].

However, administration of ethanolic RTc extract decreased the renal TBARS in aflatoxicosis bearing mice due to elevated GSH content and its antiperoxidative activity. Any compound having antioxidant property may alleviate the toxicity of aflatoxin. Therefore removing free radical is probable one of the most effective defense mechanism of body against diseases. Phytochemical studies of *Tinospora* revealed the presence of alkaloids viz., tinosporine, tinosporic acid, tinosporol, choline (V), isocolumbine, palmatine and magnoflorine in the roots of *T.cordifolia*. These alkaloids posses antioxidant property[38] due to its strong free radical scavenging activity. *Tinospora* acts as a bifunctional enzyme which induces both phase I as well as phase II enzyme system of body and significantly attenuate the oxidative stress by modulating cellular enzymatic and non-enzymatic antioxidant defense system. Thus, *Tinospora* upregulated antioxidant system by increasing the activities of SOD, CAT and GPx and maintains the balance between pro-oxidant and antioxidant pathways.

CONCLUSION

Results of the current study demonstrate that *Tinospora cordifolia* increases the activities of phase I and phase II enzymes due the presence of alkaloids having antioxidant property and these enzymes involved in alleviating oxidative stress induced by aflatoxin B_1 in mice kidney.

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