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Research Article

Hypoglycaemic and Hypolipidemic activity of *Tinospora cordifolia* root extract on aflatoxin B_1-induced toxicity in mice

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ABSTRACT

Aflatoxin, a fungal mycotoxins are potent hepatotoxic and hepatocarcinogenic agent. *Tinospora cordifolia* (Menispermaceae) is an ayurvedic herb and has wide range of traditional use in different diseases. The aim of this study was to evaluate the hypolipidemic and hypoglycaemic effect of ethanolic extract of *Tinospora cordifolia* root on aflatoxin B_1-induced toxicity. Aflatoxin was administered orally (2µg /30g body weight, 0.2 ml^{-1} day^{-1}) to mice of each group except control, group III, group IV and group V. Different doses of plant extract of *Tinospora cordifolia* were given to all groups except control and aflatoxin B_1 administered group. The entire study was carried out for 75 days and animals were scarified after an interval of 25 days till the completion of study. From the current study it was illustrated that the *Tinospora cordifolia* significantly recovered the body weight, liver weight, kidney weight and also showed the hypolipidemic and hypoglycaemic activity by lowering down the level of cholesterol, triglycerides, LDL, VLDL, blood glucose and enhancing the level of HDL cholesterol. The overall data indicated that *Tinospora cordifolia* possess potent hypolipidemic effect against aflatoxin B_1-induced atherosclerosis, and the main mechanism involved in protection could be associated with its strong hypoglycaemic property.

Keywords: Atherosclerosis; Glucose; Lipid profile; Mycotoxins; Protection

INTRODUCTION

Aflatoxin B_1 (AFB_1), a secondary fungal metabolite, has the highest potency as a toxin and classified as group I carcinogen by international agency for research on cancer (Anonymous, 1993).They are widely present in agricultural products such as peanuts, corn, whole wheat and rye breads, oilseeds, fermented beverages made from grains, milk, cheese, meat, fruit juice and numerous other agricultural commodities (Abdel-Wahhab, 2006). Epidemiological studies have established that contamination of food with AFB_1, is one of the important risk factor responsible for human liver cancer (Wogan, 1992).Carcinogenesis in liver is also associated with many other ailments like atherosclerosis and cardiovascular diseases that are the leading cause of mortality and morbidity in worldwide (Yokozawa *et al*., 2003). Hypercholesterolemia and hyperlipidemia contributed in the development of coronary heart diseases. Cholesterol that metabolized in liver, decides the risk of developing cardiovascular diseases, higher the level of cholesterol then greater will be the risk of cardiovascular diseases.

At present atherosclerosis is treated with popular statins and there is an increasing trend in the prescription of physicians to treat hyperlipidemia using statins. The current antihyperlipedemic drugs have lot of adverse effects and there is a need for alternative agents to control atherosclerosis with minimum side effects. Natural products always found to be reliable source for several ailments, their popularity and contribution is undoubtedly worthless.

Tinospora cordifolia, an Indian medicinal plant is well known in the folklore medicine as antidiabetic, antipyretic, antiulcer, antioxidant, hepatoprotective, immunomodulatory and also for its hypolipidemic properties (Maurya *et al*., 1997; Prince and Mennon, 1999, 2000).The plant is reported to have alkaloids like tinosporine, palmatine and glycosides like tinocordiside, tinocordifolioside and also some terpenoids (Maury and Handa, 1998; Chintalwar *et al*., 1999; Sharma and Pandey, 2010).

Earlier reports on *T.cordifolia* reveals that the pharmacological screening has been done for anti-inflammatory, immunomodulatory, anticancer, antidiabetic, antiulcer, antirheumatic activities but antihyperlipedemic activity was not done on root parts. Hence the present study was undertaken to evaluate hypolipidemic and hypoglycaemic activity of ethanolic extract of *Tinospora cordifolia* root on aflatoxin in-

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duced hyperlipidemia and hyperglycaemia in swiss albino mice.

MATERIALS & 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, Hissar (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. A prior approval was obtained from the institutional animal ethics committee on 14 November 2007 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 Krishi Vigyan Kendra, 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 40ºC 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 entire period of study, i.e., till 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)

Tinospora cordifolia root (RTc) extract were given at an interval of 30 min of Aflatoxin B_1 administration.

Mice body weight and organ weight (Liver and Kidney) were taken after scheduled interval of 25th, 50th and 75th day and the blood samples were collected from the retro-orbital venous plexus of all animals. Blood glucose concentration was determined by using one-touch ultra glucometer (Johnson & Johnson Co., USA) and compatible blood glucose strips (Henry, 1994). Serum was prepared by centrifugation of blood samples at 860×g for 20 min, and was stored at -20ºC until used for analysis. Serum total cholesterolerol ((TC), Triglycerides (TG) and high density lipoprotein (HDL)-cholesterol were determined by using respective diagnostic kits (Erba Mannheim Cholesterol kit, Transasia bio-Medicals Ltd; Daman).Very low –density lipoprotein (VLDL) and low density lipoprotein (LDL)-Cholesterol were calculated as per Friedvald's equation.

VLDL-cholesterol = Serum triglyceride/5

LDL-cholesterol=Serum total cholesterol–VLDL cholesterol–HDL cholesterol

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

The effect of ethanolic RTc, AFB_1 and their combination on body weight, liver and kidney weight was presented in Table 1. Aflatoxin B_1 exposure led to significant fall ($P<0.05$) in body weight and liver weight on 25th day of study whereas, significant fall is also noticed on 50th and 75th day but at $P<0.01$ significance level. Kidney weight was decreased significantly ($P<0.01$) on 50th and 75th day of study whereas, it was comparable to control on 25th. Groups of mice which received different doses of plant extracts along with aflatoxin showed significantly enhanced ($P<0.01$) values of body weight during entire period of study however liver and kidney weight showed no significant difference on 25th day as compared to their respective values of group II mice. On 50th and 75th day of study liver and kidney weight were enhanced significantly ($P<0.01$) as compared to their respective values of aflatoxin B_1 administered group.

Table 1: *In vivo* effect of ethanolic extract of *Tinospora cordifolia* root on body weight, liver and kidney weight of mice treated with aflatoxin B1

Treatments (mean ± S.E.M.)									
Parameters (g)	**DAT**	**(Gp I)**	**(Gp II)**	**(Gp III)**	**(Gp IV)**	**(Gp V)**	**(Gp VI)**	**(Gp VII)**	**(Gp VIII)**
Body Weight	25	32.16± 1.95 ^{c}	27.83± 1.21 ^{b}	33.34± 1.21 ^{NS,c}	35.16± 1.34 ^{NS,c}	31.83± 1.57 ^{NS,c}	32.12± 1.09 ^{NS,c}	33.00± 2.51^{NS,c}	31.6± 2.21^{NS,c}
	50	32.33± 2.05 ^{c}	27.00± 1.29 ^{a}	33.01± 0.87^{NS,c}	35.00± 0.81 ^{b,c}	32.83± 0.89 ^{NS,c}	33.02± 0.81 ^{NS,c}	34.00± 0.81^{NS,c}	32.33± 0.94^{NS,c}
	75	34.00± 0.81 ^{c}	26.33± 1.49 ^{a}	34.21± 0.93^{NS,c}	35.83± 1.34 ^{NS,c}	33.33± 1.10 ^{NS,c}	33.92± 0.65^{NS,c}	34.16± 1.06^{NS,c}	33.16± 1.34^{NS,c}
Liver Weight	25	1.97± 0.26 ^{d}	1.57± 0.13 ^{b}	1.83± 0.13^{NS}	1.89± 0.12 ^{NS}	1.80± 0.14 ^{NS}	1.92± 0.13^{NS}	1.95± 0.13^{NS}	1.95± 0.30^{NS}
	50	2.05± 0.16 ^{c}	1.55± 0.12 ^{a}	1.94± 0.12^{NS}	1.95± 0.20 ^{NS,c}	1.95± 0.14 ^{NS,c}	1.95± 0.09^{NS,c}	2.05± 0.11^{NS,c}	1.96± 0.11^{NS,c}
	75	2.13± 0.12 ^{c}	1.53± 0.10 ^{a}	2.18± 0.08^{NS}	2.15± 0.06 ^{NS,c}	2.06± 0.07 ^{NS,c}	2.08± 0.06^{NS,c}	2.07± 0.08^{NS,c}	2.09± 0.07^{NS,c}
Kidney Weight	25	0.57± 0.04 ^{NS}	0.49± 0.02 ^{NS}	0.58± 0.01^{NS}	0.59± 0.02 ^{NS}	0.57± 0.10 ^{NS}	0.56± 0.02^{NS}	0.58± 0.04^{NS}	0.55± 0.05^{NS}
	50	0.55± 0.03 ^{c}	0.45± 0.03 ^{a}	0.59± 0.02^{NS,c}	0.57± 0.03 ^{NS,c}	0.58± 0.03 ^{NS,c}	0.58± 0.03^{NS,c}	0.59± 0.01^{NS,c}	0.56± 0.03^{NS,c}
	75	0.55± 0.09 ^{c}	0.43± 0.02 ^{a}	0.56± 0.02^{NS,c}	0.55± 0.03 ^{NS,c}	0.60± 0.01 ^{b,c}	0.60± 0.01^{a,c}	0.61± 0.02^{a,c}	0.57± 0.02^{NS,c}

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: *In vivo* effect of ethanolic extract of *Tinospora cordifolia* root on total cholesterol, triglycerides and HDL level of mice treated with aflatoxin B1

Treatments (mean ± S.E.M.)									
Parameters (mg/dl)	**DAT**	**(Gp I)**	**(Gp II)**	**(Gp III)**	**(Gp IV)**	**(Gp V)**	**(Gp VI)**	**(Gp VII)**	**(Gp VIII)**
TC	25	77.09± 2.20^{c}	159.79± 2.8 ^{a}	65.34± 1.13^{a,c}	63.79± 3.84 ^{a,c}	69.91± 3.31^{b,c}	125.43± 3.24^{a,c}	115.30± 4.01 ^{a,c}	133.02± 5.49 ^{a,c}
	50	75.13± 1.22^{c}	161.38± 4.25^{a}	63.34± 2.19^{a,c}	61.34±2.87 ^{a,c}	66.57± 2.25^{a,c}	117.89± 3.67^{a,c}	109.16± 1.94 ^{a,c}	125.86± 2.80 ^{a,c}
	75	75.47± 2.50^{c}	167.61± 4.13^{a}	61.23± 1.17^{a,c}	58.96± 2.04 ^{a,c}	65.24± 2.43^{b,c}	109.87± 2.67^{a,c}	98.64± 4.25 ^{a,c}	114.84± 2.88 ^{a,c}
TG	25	84.13± 3.56^{c}	139.3± 2.16 ^{a}	65.23± 1.13^{a,c}	64.56± 3.68 ^{a,c}	67.98± 5.31^{a,c}	115.34± 3.22^{a,c}	105.64± 4.01 ^{a,c}	121.72± 5.49 ^{a,c}
	50	83.85± 1.56^{c}	145.49± 2.18^{a}	63.92± 1.26^{a,c}	63.35± 1.83 ^{a,c}	64.27± 1.41^{a,c}	106.34± 1.78^{a,c}	99.59± 4.77 ^{a,c}	120.04± 1.94 ^{a,c}
	75	68.15± 2.51^{c}	184.34± 1.96^{a}	62.95± 1.45^{a,c}	62.19± 1.83 ^{a,c}	65.08± 2.60^{NS,c}	98.56± 2.16^{a,c}	91.39± 2.90 ^{a,c}	112.89± 3.07 ^{a,c}
HDL	25	27.10± 1.56^{c}	18.39± 0.98 ^{a}	23.34± 1.67^{a,c}	24.97± 2.93^{NS,c}	22.72± 2.13 ^{a,c}	21.56± 0.87^{a,c}	22.48± 0.42 ^{a,c}	20.88± 0.54 ^{a,c}
	50	29.94± 3.16^{c}	18.65± 1.83 ^{a}	23.55± 1.13^{a,d}	23.68± 1.58 ^{a,d}	23.17± 2.11 ^{a,d}	22.92± 1.21^{a,d}	23.62± 2.62 ^{a,d}	22.62± 1.26 ^{a,d}
	75	26.62± 1.51 ^{c}	16.02± 1.08 ^{a}	23.56± 1.76 ^{a,c}	25.13± 0.79^{NS,c}	23.38± 0.67 ^{a,c}	22.72± 0.88 ^{a,c}	23.69± 0.77 ^{a,c}	22.24± 1.24 ^{a,c}

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).

The effect of ethanolic RTc, AFB_1 and their combination on total cholesterol, triglyceride and high density lipoprotein level were depicted in Table 2.Groups of mice which were administered with aflatoxin alone showed a significant increase (P<0.01) in cholesterol, triglycerides and significant decrease in HDL as compared to their respective control values during entire period of study. Co-supplementation of AFB_1 along with all doses

Table 3: *In vivo* effect of ethanolic extract of *Tinospora cordifolia* root on LDL, VLDL and blood glucose level of mice treated with aflatoxin B1

Treatments (mean ± S.E.M.)									
Parameters (mg/dl)	**DAT**	**(Gp I)**	**(Gp II)**	**(Gp III)**	**(Gp IV)**	**(Gp V)**	**(Gp VI)**	**(Gp VII)**	**(Gp VIII)**
LDL	25	33.16± 2.35^{c}	113.4± 1.82 ^{a}	28.17± 2.19 ^{b,c}	22.88± 4.59 ^{b,c}	32.59± 2.42 ^{NS,c}	82.16± 2.12^{a,c}	71.69± 4.74 ^{a,c}	87.79± 3.83 ^{a,c}
	50	28.42± 3.34 ^{c}	113.63± 5.28 ^{a}	26.78± 1.23 ^{NS,c}	24.99± 3.92 ^{NS,c}	30.54± 3.13 ^{NS,c}	71.67± 2.31^{a,c}	65.53± 2.53 ^{a,c}	78.57± 3.20 ^{a,c}
	75	32.47± 2.03 ^{c}	113.72± 2.99 ^{a}	23.14± 1.76 ^{a,c}	21.72± 1.76 ^{a,c}	29.2± 2.83 ^{NS,c}	67.34± 2.67^{a,c}	56.63± 4.51 ^{a,c}	70.04± 2.51 ^{a,c}
VLDL	25	16.80± 0.72 ^{c}	27.90± 0.44 ^{a}	13.12± 0.77^{a,c}	12.93± 0.71 ^{a,c}	13.59± 1.06 ^{a,c}	23.12± 0.89^{a,c}	21.26± 0.77 ^{a,c}	24.34± 1.09 ^{a,c}
	50	16.78± 0.33 ^{c}	29.09± 0.43 ^{a}	12.92± 0.45^{a,c}	12.66± 0.36 ^{a,c}	12.85± 0.28 ^{a,c}	22.19± 0.56^{a,c}	20.01± 0.97 ^{a,c}	24.67± 1.05 ^{a,c}
	75	13.82± 0.71 ^{c}	37.86± 2.38 ^{a}	12.88± 0.66^{a,c}	12.81± 2.09 ^{NS,c}	13.01± 0.52 ^{NS,c}	20.56± 0.55^{a,c}	18.40± 0.67 ^{a,c}	22.62± 0.59 ^{a,c}
Glucose	25	156.00± 13.31 ^{c}	228± 6.42 ^{a}	124.13± 1.51 ^{a,c}	120.16± 14.21^{a,c}	136.83± 15.31^{NS,c}	155.23± 3.32^{NS,c}	148.33± 22.42^{NS,c}	164.5± 14.58^{NS,c}
	50	145.83± 3.43 ^{c}	234± 5.25 ^{a}	122.12± 2.34 ^{a,c}	115± 2.00 ^{a,c}	126.33± 5.55 ^{a,c}	149.43± 4.12^{NS,c}	147.5± 6.84 ^{NS,c}	159.11± 5.58 ^{NS,c}
	75	139.16± 2.26 ^{c}	244± 2.76 ^{a}	115.67± 2.18 ^{a,c}	113.66± 1.49 ^{a,c}	118.00± 2.38 ^{a,c}	142.43± 2.39^{NS,c}	139.66± 3.39 ^{NS,c}	145.16± 5.92 ^{NS,c}

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)

of RTc leads to significant fall (P<0.01) in cholesterol, triglycerides and significant rise (P<0.01) in HDL level on 25th and 75th day of study whereas on 50th day HDL was also increased significantly but at P<0.05 significance level as compared to group II mice.

The effect of ethanolic RTc, AFB_1 and their combination on low density lipoprotein, very low density lipoprotein and blood glucose level were shown in Table 3.Aflaoxin alone exposure led to a significant rise (P<0.01) in level of LDL, VLDL and blood glucose when compared with control mice. Simultaneous administration of RTc extract along with aflatoxin significantly brought back the level of these variables near to normal at some extent as compared with respective values of aflatoxin supplemented mice.

DISCUSSION

Aflatoxins are a group of fungal toxins that have been associated with severe toxic effects in man and animals (Romos and Hernandez, 1997).The main effects of AF are associated with liver damage. The negative effect of aflatoxin on body, liver and kidney weight (Denli *et al*., 2005) and change of serum variables such as, cholesterol, triglyceride and blood glucose concentration during aflatoxicosis has been reported .The decrease in the body weight of mice treated with aflatoxin alone may effect the balance between orexigenic and anorexigenic circuits that regulate the homeostatic loop of body weight regulation, leading to cachexia (Rastogi *et al*., 2000). In this supports, Abdel Wahhab *et al* (2006) reported that rat administered with AFB_1 showed a significant fall in leptin. Low leptin concentration is usually associated with the high levels of cortisol and IL-6 which together influence the feeding efficiency of rat, causing weight loss (Abdel-Fattah, 2010).This correlation may explain the recorded decrease in body weight of mice ingested with AFB_1. Administration of AFB_1 significantly enhanced the levels of serum triglycerides, HDL and cholesterol of mice that indicate the degenerative changes and hypofunction of liver (Kaplan, 1987).These results agree with the finding of Santurio *et al* (1999). In contrast, Edringto *et al* (1996) reported that aflatoxicosis in chicken's induced strong reductions in serum triglycerides and cholesterol concentration.

Co-supplementation of RTc along with AF leads to significant increase in body weight, liver and kidney weight. The increase in liver weight associated with an increase in the level of microsomal protein is indicative of induced protein synthesis and possibly associated with endoplasmic reticulum, which could be responsible for the increase in mice liver weight and ultimate increase in body weight.

In present study, the serum total cholesterol and triglycerides showed significant reduction. The hypolipidemic effect of RTc is not exclusively depending on dosage of drug, but on the effectiveness of RTc in controlling the blood glucose. High density lipoprotein (HDL) cholesterol is produced in liver and also derived from chylomicron and VLDL catabolism (Feingold *et al*., 1982). HDL serves as an acceptor of lipids from different extra

hepatic cells to liver for ultimate excretion in bile (Schaefer and Robert, 1985).Low density lipoprotein (LDL) cholesterol carrying the lipoprotein in plasma are derived from catabolism of VLDL, but some are synthesized directly in liver and is regulated by dietand hormones.

CONCLUSION

The observation of present study indicated that RTc is much beneficial in enhancing HDL cholesterol levels and lowering the LDL and VLDL cholesterol due to its hypoglycaemic activity.

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