



HEPATOPROTECTIVE ACTIVITY OF A POLYHERBAL FORMULATION-CHATHURMUKA CHOORANAM

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ABSTRACT

Aflatoxins (AFB1) are secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* which they contaminate the animals and humans to produce liver toxicity. The present study is to assess the effect of hydroethanolic extract of the polyherbal formulation Chathurmuka chooranam (CMC) on AFB1 induced Wistar rats. AFB1 is induced at a dosage of 250 µg/kg/*i.p* for 7 days and the hydroethanolic extract at a rate of 250 and 500 mg/kg/*p.o* for the next 7

days. The effect of hematological parameters (WBC, RBC and Hb), serum marker enzymes (AST,ALT and ALP), lipid profile (Total cholesterol, triglycerides, LDL,VLDL and HDL) and other biochemical parameters(urea, uric acid, creatinine and bilirubin) was evaluated. The results revealed there was an increment in the levels of haematological parameters, decrement in the activities of serum marker enzymes and normal level of lipid profile after treatment with the polyherbal extract to the AFB1 induced animals. In conclusion, the present study demonstrate the effect of aflatoxin induced toxicity was reversed after the treatment with polyherbal extract.

KEYWORDS : Aflatoxin B1, CMC, Serum marker enzymes, Haematological parameters.

INTRODUCTION

Aflatoxin B1 are hepatocarcinogenic and hepatoxin, which is difficult to eradicate from the environment because of favorable climatic condition to grow and toxicity produce by the

fungi (Hendrickse, 1991). Aflatoxin B1-8, 9-epoxide, a secondary metabolite from AFB1 exerts as hepatotoxin to cause liver cancer in dose dependent manner. The synergistic interaction between AFB1 and hepatitis B virus results in hepatocellular carcinoma (van Walbeek, 1969). AFB1 epoxide binds with DNA to form AFB1-DNA adduct. The adduct escapes from the detoxification process by Glutathione-S-Transferase and cause lipid peroxidation in the system which may be responsible for the initiation of carcinogenesis (Shen et al., 1996). The mutation in the p53 tumor suppressor gene was identified in liver tumors when exposure to AFB1 induced toxicity (Staib et al., 2003).

Antioxidants play an important role in detoxification pathway and in the elimination of free radical induced damage, which is the cause for many diseases. Plant derived biological products are the natural defender and having therapeutic activity by triggering antioxidants (Mittal et al., 2001). The Chathurmuka chooranam formulation is a compact of five plants namely the roots of *Plumbago zeylanica*, rhizome of *Curculigo orchoides* and *Asparagus racemosus*, nut of *Semecarpus anacardium* and whole plant of *Tinospora cordifolia*. In traditional medicine, the formulation is used as an antitoxin, blood purifier etc., The syngersitic effect of five plants in the formulation may be useful in the elucidation of anticancer potential in Wistar rats.

MATERIALS AND METHODS

Preparation of plant extract

The five medicinal plants were authenticated by Botanical survey of India, Coimbatore and ABS botanical garden, Salem. The plants were shade dried and coarsely powdered. 100 g of dried powder was cold macerated with 50 % hydro ethanol with occasional stirring for 3 days. After 3 days, the suspension was filtered through a fine muslin cloth and the filtrate was evaporated to dryness at low temperature (<40°C) under reduced pressure in a rotatory evaporator. The yield of plant extract was found to be 9.64%. The sample was stored in an airtight desiccator and used for further analysis.

Chemicals

Aflatoxin-B1 was purchased from Sigma-Aldrich chemicals Co.,USA. All other chemicals used were of analytical grade.

Experimental regimen

Male Wistar albino rats were divided into 5 groups (n = 6).The rats are weighed 100±120 gms. During the study, the animals received normal laboratory diet and water *ad libitum*. The

rats were acclimatized to laboratory conditions for 10 days before commencement of the experiment. The clearance of the ethical committee for experimentation on animals was obtained before the start of the experiment (Proposal No: 202/2013/IAEC).

Grouping of animals

Group I: Normal control received 0.5ml DMSO/ rat/ 7 days.

Group II: Hepatoma control received a total of 7 doses of 250 µg/kg/dose for 7 days. The AFB1 was dissolved in DMSO and administered *i.p.*

Group III: Test received a total of 7 doses of 250 µg/kg/ *i.p.* for 7 days and then 250 mg/kg/*p.o.* of hydro-ethanolic extract of CMC formulation from 7th day to 14th day.

Group IV: Test received a total of 7 doses of 250 µg/kg/ *i.p.* for 7 days and then 500 mg/kg/*p.o.* of hydro-ethanolic extract of CMC formulation from 7th day to 14th day.

Group V: Test received Methotrexate 0.5 mg/kg/dose/ *i.m.* for 2 days after AFB1 pretreated.

Biochemical parameters

The whole blood obtained from the animals after scarification was used for Haematological parameters (RBC,WBC, Hb) (Mukherjee, 1988) and lipid profile (cholesterol (Parekh and Jung,1970), triglycerides (Foster and Dunn,1973), LDL,VLDL (Ahmadi and Boroumand,2008) and HDL (Gordon *et al.*,1977). The serum separated from blood was used for AST (King,1965a), ALT and ACP (King,1965), urea (Natelson *et al.*,1951), uric acid (Caraway and Seligson,1963), bilirubin (Malloy and Evelyn,1937) and creatinine (Jaffe *et al.*,1886).

Statistical analysis

The results were articulated as mean ± standard deviation. Statistical analysis was carried between the experimental groups using one way analysis of variance (ANOVA) employing statistical package for social science (SPSS Version 1.6). Post hoc testing was performed for inter-group comparisons using Duncan's multiple range test. The level of significance was set as ($P < 0.05$).

RESULTS

The alterations in the haematological parameters, namely RBC, WBC and Hb in the control and experimental groups of animals were presented in Table-1. It was noted that RBC count,

WBC count and Hb content in the AFB1 and methotrexate group was markedly ($P<0.05$) declined as compared to normal animals, but after the treatment with the hydro-ethanolic extract maximum recovery was observed in the haematopoietic system.

Table 1: Effect of hydroethanolic extract of CMC formulation on the level of RBC,WBC and Hb in control and experimental animals

Groups	RBC(millions/cu.mm)	WBC(10^3 cells/cu.mm)	Hb(g/dl)
Group I	8.5± 0.89	1.98±0.67	14.03± 0.75
Group II	4.6 ±0.74 ^a	3.26±0.57 ^a	10.73± 0.95 ^a
Group III	7.8 ±0.77 ^b	8.21± 0.49 ^{ab}	14±1.89 ^b
Group IV	8.3± 0.79 ^b	8.75± 0.39 ^{ab}	14.65± 1.97 ^b
Group V	5.7± 0.73 ^{ab}	4.26± 0.75 ^{ab}	11.1± 1.13 ^a

Values are mean ± SD of six samples in each group

a – Group I vs Group II, III, IV, V ; b – Group II vs Group III, IV, V

a, b are significant at 5% ($P<0.05$).

The AST is more elevated than ALT in the condition of HCC induced animals and AST is very sensitive to tumor growth than ALT (Moss and Neal, 1985). AST, ALT and ALP activities in the experimental and control animals were depicted in Table-2. A 2-fold increase in the activities of transaminases and ALP were found in toxin induced rats. In the CMC formulation treated groups there was a significant ($P<0.05$) decrease in the activity of transaminase enzymes.

Table 2 : Effect of hydroethanolic extract of CMC formulation on the activities of AST, ALT and ALP in control and experimental animals

Groups	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
Group I	165.1±1.26	84.4±1.51	158.4±1.64
Group II	256.9±1.51 ^a	126.1±1.06 ^a	282.1±1.76 ^a
Group III	174±1.87 ^{ab}	109.3±1.54 ^{ab}	213.05±1.53 ^{ab}
Group IV	155.6±1.43 ^{ab}	95.7±2.17 ^{ab}	195.8±1.15 ^{ab}
Group V	198.2±1.05 ^{ab}	121.4±1.54 ^{ab}	241±1.25 ^{ab}

Values are mean ± SD of six samples in each group

a – Group I vs Group II, III, IV, V; b – Group II vs Group III, IV, V

a, b are significant at 5% ($P<0.05$)

Table-3 shows the level of cholesterol, triglycerides, LDL, VLDL and HDL in the serum of control and experimental group of rats. The results showed that the level of cholesterol and triglycerides, manifested a significant increase ($P<0.05$) in the aflatoxin and methotrexate

drug treated group as compared to control. But, in the CMC formulation treated group, the levels return to near normal. An increased level of low density lipoprotein (LDL-c), very low density lipoprotein (VLDL-c) and a decrease in high density lipoprotein (HDL-c) level of AFB1 induced animals was observed which indicates hepatic injury and biliary obstruction.

Table 3: Effect of hydroethanolic extract of CMC formulation on the levels of lipid profile in control and experimental animals

Groups	Total cholesterol(mg/dl)	Total triglycerides(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)	HDL(mg/dl)
Group I	102.1± 2.31	108.8±1.75	30.1± 1.47	24±2.09	47.6±1.96
Group II	120.1 ±1.47 ^a	166±1.78 ^a	127.3± 1.63 ^a	41.6±2.16 ^a	39.3±1.21 ^a
Group III	118.1 ±1.72 ^a	145.1± 2.31 ^{ab}	46.1±2.42 ^{ab}	38±1.41 ^{ab}	43.8±1.47 ^{ab}
Group IV	99± 2.19 ^{ab}	121.1± 1.94 ^{ab}	37.3± 2.97 ^{ab}	33.1±1.47 ^{ab}	47±1.41 ^b
Group V	121.3± 2.25 ^a	150.8± 1.47 ^{ab}	99.1± 1.72 ^{ab}	39±2.28 ^{ab}	41.6±1.63 ^{ab}

Values are mean ± SD of six samples in each group

a – Group I vs Group II, III, IV, V ; b – Group II vs Group III, IV, V

a, b are significant at 5% (P<0.05)

Table-4 shows the level of urea, uric acid, creatinine and bilirubin in the experimental and control animals of AFB1 induced and CMC treated. The levels of urea, creatinine and bilirubin was found to be significantly (P<0.05) elevated, but the uric acid level was found to be 2-fold decreased in AFB1 group of animals. In methotrexate group, the amount of urea gets elevated than AFB1 group. On management with polyherbal extract of lower (250 mg/kg body weight) and higher (500mg/kg body weight) doses, a prominent recovery of the uric acid was noticed as compared with the normal group.

Table 4: Effect of hydroethanolic extract of CMC formulation on the levels of urea, uric acid and creatinine in control and experimental animals

Groups	Urea(mg/dl)	Uric acid(mg/dl)	Creatinine (mg/dl)	Bilirubin(mg/dl)
Group I	41.7± 1.76	1.29±0.11	0.81± 0.05	0.43±0.02
Group II	46.5 ±1.95 ^a	0.67±0.04	0.91± 0.02	0.91±0.08
Group III	44.8 ±1.20 ^{ab}	1.25± 0.09	0.90± 0.08	0.54±0.10
Group IV	34.7± 1.59 ^{ab}	1.36± 0.15 ^b	0.85± 0.06	0.67±0.09
Group V	48.1± 1.80 ^a	1.11± 0.07	0.88± 0.09	0.50±0.11

Values are mean ± SD of six samples in each group

a – Group I vs Group II, III, IV, V ; b – Group II vs Group III, IV, V

a, b are significant at 5% (P<0.05)

DISCUSSION

Aflatoxin in rats cause aflatoxicosis which selectively binds and forms radicals, which are overcome by the defense mechanism of antioxidants in the metabolic reactions. The effect of AFB1 on antioxidants in Wistar rats were previously demonstrated (Saraswathi *et al.*, 2013). The effect of CMC formulation on AFB1 induced lipid profile, biochemical parameters had been documented in this article.

The decreased level of hematological parameters was mainly due to hemolytic anemia in the cancer induced animals. The animals developed tumour shows decreased level of haemoglobin and RBC, this condition is due to the chronic level of the disease and inhibitory level of erythroid precursors. The inability of the damaged hepatic parenchyma in the cancer induced animals to produce erythropoietinogen will reveal the decreased level of erythrocytes in the circulation (Bhaumik and Sharma, 2002). The polyherbal formulation treated animals shows reduced anaemia and has satisfactory improvement on the blood cells system.

The serum cholesterol and triglyceride levels was found to be down regulated in this study. Several studies reported that that high blood cholesterol level and hyper-lipidemia may be the consequence which frequently associated with AFB1 induced cancer. The increased cholesterol and triglyceride levels in blood serum is mainly due to disrupted cholesterol metabolism by liver cells, Kupffer cells and hepatic cells. Brinda *et al.*, (2013), Edrington *et al.* (1995). Any physiological or pharmacological changes in the liver will affect the metabolism of the lipid content either to increase or decrease (West *et al.*, 1992). Hepatic lesions and damage are reported with the elevated level of LDL and VLDL cholesterol with AFB1 infection (Abdel-Wahhab *et al.*, 2010). However, there is a significant change in LDL, VLDL and HDL cholesterol levels were recorded in the groups treated with CMC formulation, indicating normal functioning of the liver.

The increased level of ALT and AST activity observed in the present study was due to the hepatotoxic effect of aflatoxin characterized by the impairment of carbohydrate and lipid metabolism and inhibition of protein synthesis (Arawind *et al.*, 2003; Basmacioglu *et al.*, 2005). The extent of cellular damage and change in the activities of the transaminases is based on the kind of toxin and its capability to produce toxic liver injuries (Devi and Devaki, 1998).

The increased level of bilirubin is mainly due to the hyperbilirubinemia condition and liver damage. The unconjugated bilirubin was increased in the blood stream and was released from the damaged hepatocytes to the biliary tract (Wolf *et al.*, 1997) in the AFB1 induced groups. A significant reduction in the level of urea and creatinine shows that prophylaxis of the CMC formulation in improving kidney function. The elevated level of bilirubin, urea and creatinine in AFB1 group was brought back to near normal by the administration of CMC formulation. Additionally, significant increase in the level of uric acid was observed, which may be due to the poly herbal extract that might work as a promising agent to prevent mesangial cell proliferation (Makino *et al.*, 2000).

CONCLUSION

It can be concluded that AFB1 is a hepato carcinogen and its toxic effects can be reverted by CMC formulation which demonstrates the hepatoprotective effect of CMC formulation.

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