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Hepatoprotective Activity of Ethanol Extracts of *Amaranthus viridis* Linn on Aflatoxcin B_1 Induced Rats.

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Abstract

The pharmacological evaluation of ethanol extract of *Amaranthus viridis* Linn *(EEAV)* in Aflatoxin B1 (AFB1) induced hepatocellular carcinoma, AFB1 causes oxidative stress and cellular damage its lead to enhanced formation of reactive oxygen spices (ROS) which associated with increases in biochemical parameters like serum glutamate pyruvate transaminase (SGPT), serum glutamate oxalacetic acid (SGOT), serum alkaline phosphatase (SALP), ã-glutamate trans peptidase(GGT), bilirubin, lipid levels as well as decrease in the levels of total protein and uric acid. EEAV was administered orally (100 & 200 mg/kg) for 14 days to hepatocarcinoma bearing rats. The levels of lipid peroxides and activity of enzymatic antioxidants level were determined by liver homogenates. Marked increase in lipid peroxide levels and concomitant decrease in enzymatic antioxidants levels were observed in carcinoma induced rats, while EEAV treatment reversed the conditions to near normal levels. Liver histopathology showed that EEAV reduced the Incidence of liver lesions, lymphocytic infiltrations and hepatic necrosis induced by AFB1 in rats. These results suggest that EEAV could protect liver against the AFB1-induced oxidative damage in rats, which may be due to its capability to induce the *in vivo* antioxidant system.

Key words: Hepatocellular Carcinoma, *Amaranthus viridis* Linn, *Reactive* oxygen spices

INTRODUCTION:

Primary hepatocellular carcinoma (HCC) is a tumor of considerable epidemiologic, clinical and pathological interest. It is the sixth most common cancer worldwide and the third most common cause of death from cancer[1] Global incidence of HCC accounts for 5-6% of all cancers in humans with more in males than females. Exposure to contaminated food with mycotoxins such as Aflatoxin B1 (AFB1), Fuminosin B1(FB1), T-2 toxins and some chemical carcinogens like diethyl nitrosamine, NNitrosobis (2-oxopropyl) amine etc are found to be risk for developing HCC. AFB1 is a mycotoxin metabolite obtained from the Aspergillus flavus and A. parasiticus found in food stuffs, oil seeds, corn, rice and peanuts which are stored improperly[2&3]. Aflatoxin B1 is considered as a potent carcinogen classified by the International Agency for Research on cancer (IARC) and is a genotoxic hepatocarcinogen that cause HCC. AFB1 is metabolized to AFB1 8, 9-epoxide by hepatic microsomal enzymes (CYP 450) that forms AFB1-N7 guanine adducts leading to genetic changes that cause DNA strand breakage and oxidative damage[4], *Amaranthus viridis* (Amaranthaceae**)** is an annual or perennial herbaceous bush and has several forms with varying colors of flowers. It is native to China and grown widely as an ornamental plant throughout India [5]. The flowers are considered emollient, and an infusion of the petals is used as a demulcent. Its decotion is given in bronchial catarrhin India. Previous studies show that the plant possesses anti-complimentary, antidiarrhetic and antiphologisticactivities. The leaves and flowers have been found to be effective in the treatment of heart disorders [6]. No reports are available on the antidiabetic activity of *Amaranthus viridis* Linn. Hence, the present study focuses on the scientific investigation of antidiabetic activity of Amaranthus viridis leaves.

MATERIALS AND METHODS

Chemicals

AFB1 (Sigma, St. Louis, Mo) was dissolved in dimethylsulphoxide (DMSO) immediately before administration.Reduced Glutathione (GSH), 1-Chloro-2,4-dinitrobenzene (CDNB), Glutathione Oxidised (GSSG),Epinephrine, Nicotinamide Adenine DinucleotidePhosphate (NADPH) was obtained from Sigma, Aldrich Ltd..

Animals

Adult Wistar albino male rats weighing 150-200gWere used in the pharmacological studies, The animals were maintained in well-ventilated room temperature with natural 12h ± 1h day Night cycle in the propylene cages. Tap water ad *libitum* throughout the experimental period.

Plant collection and Authentication

The plant material of *Amaranthus viridisLinn* used for the investigation was collected from ABS botanical garden yercaud hills. The plant was identified and authenticated by Dr. P.Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai and a voucher specimen is kept in the Herbarium of the same center.

Extraction

The leaves were dried under shade and powdered with help of mechanical grinder and made in to coarse powder. The powdered material was defatted with Petroleum ether (40-60ºC) and extract with ethanol by continuous hot percolation method extraction6. It was fractionated by n.hexane, Chloroform and ethanol The ethanol extract was concentrated using rotary evaporator and dried under vacuum[7.] The ethanol extract contains different type of phytochemicals. The ethanol fraction was found to be positive for the Presence of flavonoids. So ethanol fraction was used for further investigation.

Experimental groups

The rats were divided into five groups with six animals in each group and the given dose schedule has given below:

Group I: Animals were given a single administration (p.o) of 0.5 ml vehicle (2% w/v aqueous Tween 80) and after 6 weeks, 0.5 ml of vehicle/day was administered (p.o) for next 14 days. This group served as normal control.

Group .II: Animals were given a single dose (i.p) of 0.5ml dimethyl sulphoxide (DMSO) and after 6 weeks, 0.5ml of vehicle /day was administered (p.o) for 14 days. This group served as DMSO control.

Group .III: Hepatocellular carcinoma was induced in these animals by a single dose of AFB1 in DMSO (2mg/kg body weight i.p). After 6 weeks, the animals were sacrificed by cervical decapitation and the hepatocellularcarcinomathis group served as AFB1 intoxicated hepatocarcinoma control.

Group .IV: Hepatocellular carcinoma was induced in these animals by a single dose of AFB1 in DMSO (2mg/kg body weight i.p). Six weeks after the induction of AFB1, the animals were treated with 100 mg/kg bodyweight (p.o) of ethanol fraction of *Amaranthus viridis* Linn, For14 days. On the 15th day, the animals were sacrificed by cervical decapitation and various biochemical parameters were analyzed.

Group .V: Hepatocellular carcinoma was induced in these animals by a single dose of AFB1 in DMSO (2mg/kg body weight i.p). Six weeks after the induction ofAFB1, the animals were treated with 200 mg/kg bodyweight (p.o) of ethanol fraction of *Amaranthus viridis* Linn for 14 days. On the 15th day, the animals were sacrificed by cervical decapitation and various biochemical parameters were analyzed.

Biochemical analysis

At the end of the experimental period, animals were sacrificed by cervical decapitation under light ether anesthesia and blood was collected, serum was separated by centrifuging at 3,000rpm for 10 min.(7) The above collected serum was used for the assay of marker enzymes, such as glutamate oxaloacetate transaminase(GOT), glutamate pyruvate transaminase (GPT),alkaline phosphatase (ALP), serum total bilirubin ,serum uric acid , serum total cholesterol, HDL cholesterol and total protein[8&9]. Serum ã-glutamyl transpeptidase (ã -GT) was determined by the methods of Szasz 1969. the liver a 10% homogenate was prepared in Tris-HCl buffer (0.1 M, pH.7.4). The homogenate was centrifuged and the supernatant was used for the assay of total protein and cytoprotective enzymes, namely glutathione peroxidase (GPx) glutathione-Transferees(GST), glutathione reductase (GR) ,superoxide dismutase (SOD) catalase (CAT) and lipid peroxidation (LPO).[50] All the enzymatic assayswere taken at particular nm using Shimadzu spectrophotometer, UV-1601 model[10].

Statistical analysis

Values are expressed as Mean ± SD. The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet.s.t. Test. Comparisons were made between Group I and Group II, Group I and Group III, Group III and Group IV, Group III and Group V. p values <0.05 were considered as significant

RESULTS AND DISCUSSION

Effect of *EEAV* on serum enzymes Level, bilirubin, uric acid, Ã-GT and protein.

The effect of ethanol fraction of *Amaranthus viridis* Linn on serum transaminases, alkaline phosphatase, bilirubin, uric acid, *ã*-GT and total protein levels in AFB1 intoxicated rats are summarized in Table 1. Hepatic damage induced byAFB1 caused significant ($p< 0.001$) rise in marker enzymes SGPT, SGOT, ALP, bilirubin, and *ã*-GT in Group III hepatocarcinoma bearing animals when compared with that of normal control group animals. Whereas treatment with EEAV at the doses of 100 and 200 mg/kg, p.o, significantly ($p< 0.001$) decreased the elevated serum marker enzymes, bilirubin and *ã*-GT levels as compared with that ofrespective hepatocarcinoma control group. The uric acid and total protein levels were significantly decreased inAFB1 intoxicated rats. Administration of EEAV at tested dose levels reversed the altered uric acid and total protein to near normal levels as compared with that of hepatocarcinoma bearing control group. Comparison between Group I and Group II shows no significant Variation in marker enzyme levels, bilirubin, uric acid, *ã*-GT and protein levels indicating no appreciable adverse effect due to the administration of DMSO in GroupII animals

Effect of EEAV on serum cholesterol and HDL cholesterol.

From the Fig.1, it is clear that the total cholesterol level were increased significantly ($p<0.001$) and HDL Cholesterol level were decreased significantly ($p<0.001$)in Group III hepatocarcinoma bearing animals, when compared with that of Group I normal control animals. Treatment with the ethanol fraction of *Amaranthus viridis* Linn at 100 and 200 mg/kg, p.o dose level significantly($p<0.001$) decreased the total cholesterol level, when compared with that of Group III hepatocarcinoma bearing animals. EEAV at 100 and 200 mg/kg dose level showed significant ($p<0.001$) increase in serum HDL-cholesterol levels in AFB1 challenged rats. The DMSO treated groupII did not show any significant difference in the level of total cholesterol and HDL- cholesterol, when compared tonormal control group I.

Effect of EEAV on liver enzymic antioxidants

The effect of EEAV on the activities of enzymatic antioxidants viz., superoxide dismutase, catalase, Glutathione peroxidase Glutathione-S-transferase and Glutathione reductase in liver homogenates is shown in Table 2. The levels of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase were significantly decreased ($p<0.001$) in Group III hepatocarcinoma bearing animals, when compared with that of group I normal control rats. Treatment with EEAV at doses 100 and 200mg/kgrecovered these decreased enzyme activities

produced byAFB1 towards normalization in a dose dependent manner. Comparison between Group I and Group II shows no significant variation in enzyme antioxidant levels indicating no appreciable adverse side effect due to the administration of DMSO in Group II animals.

Effect of EEAV on *invivo* lipid peroxidation

The localization of radical formation resulting in lipid peroxidation, measured as MDA in rat liver homogenate, content in the liver homogenate was significantly (p <0.001) increased in AFB1 intoxicated group compared to normal control group. Treatment with EEAV resulted in significant decrease in the level of lipid peroxidation products (MDA) in AFB1challenged rats. This observation leads to the inference that the EEAV treatment counteracts the abnormal increase in lipid peroxidation induced by AFB1.DMSO treated group II did not show any significant difference in the level of lipid peroxides, when compared to normal control group I.

CONCLUSION

In conclusion, biochemical alterations observed in EEAV status seem to be mainly due to an oxy radical mediated mechanism, involving LPO, under conditions of reducedantioxidant defenses. Recently more research has beenfocused on the role of flavonoids in cancer prevention because epidemiological investigations suggest that increased intake of fruits and vegetables are associated with the reduced risks of certain cancers. In preliminary phytochemical studies the ethanol fraction of *Amaranthus viridis* Linn used in this study showed positive results for the presence of flavonoid.. In this study, the hepatoprotective effects of EEAV may be due to its ability to scavenge free radicals and thus can prevent the AFB1-induced hepatotoxicity in rats by its antioxidant function which may be in part due to the presence of these flavonoids. Further studies on isolation of active constituent(s) present in the ethanol fraction of *Amaranthus viridis* Linn is under progress.

Table 1. Effect of ethanol fraction of *Amaranthus viridis* Linn on serum transaminases, alkaline phosphatase, bilirubin, uric acid, *ã*-GT and total protein levels in AFB1 intoxicated rats.

Parameters	Group I (Normal Control	Group II (DMSO Control)	Group III (AFB1 intoxicated	Group IV (AFB1 + 100mg/kg EEAV)	Group V (AFB1 + 200mg/kg EEAV)
SGOT (U/ml)	42.71±4.81	42.33±4.12NS a	176.81±7.83b*	120.59±4.28c*	87.46±5.21d*
SGPT (U/ml)	48.16±3.24	51.24±4.14a	135.92±7.48b*	115.8±4.52c*	76.32±3.42d*
ALP (KA units)	67.43±5.34	66.44±3.24a	164.44±7.22b*	122.45±5.60c*	89.45±5.12d*
Bilirubin(mg/dl	0.63±0.18	0.66±0.16a	2.50±0.35b*	5.46±0.33c*	0.98±0.22d*
Total protein(mg/dl)	7.14±0.12	6.99±0.28a	4.89±0.32b*	5.74±0.43c*	6.51±0.54d*
Uri acid(mg/dl)	2.98±0.32	2.72±0.28a	1.33±0.18b*	2.11±0.23c*	2.46±0.22d*
ã-GT (mU/ml)	28.62±3.26	29.48±4.12	192.51±7.21b*	121.35±6.24c*	85.32±5.14d*
HDL	23.22±2.31	17.70 ± 5.10a	24.10±4.11b	24.30 ± 3.20c*	25.21±34.12d*

Values are given as Mean ± SD of six animals in each group.
Comparisons were made between (a) group I and group II, (b) group I and group III, (c) group III and group IV, (d) groupIII andgroupV.
Statistical differences are expressed as * p < 0.001, NS-non significant.

Table 2 Effect of ethanol fraction of *Amaranthus viridis* Linn on activities of on antioxidant enzyme levels in liver.

Parameters	GROUP I Normal	GROUP II DMSO Control	GROUP III AFB 1 Intoxification	GROUP IV AFB 1+100mg EEAV	GROUP V AFB 1+200mg EEAV
SOD	7.21±0.49	6.97±0.11a NS	2.16±0.12b*	4.12±0.22c*	5.42±0.33d*
CAT	98.32±0.49	98.83±5.62aNS	49.27±4.16b*	64.15±4.48c*	89.33±5.14d*
GPx	5.17±0.38	4.96±029aNS	1.33±0.12b*	3.63±0.14c*	4.38±0.21d*
GST	19.84±1.63	20.22±1.46aNS	12.13±1.12b*	15.23±1.21c*	17.16±1.13d*
GR	0.812±0.06	0.83±0.04aNS	0.23±0.01b*	0.38±0.02c*	0.61±0.03d*
LPO	4.47±0.45	33.47±3.25aNS	40.31±3.32b*	8.19±0.22c*	6.21±0.22d*

Values are given as Mean ± SD of six animals in each group.
Comparisons were made between (a) group I and group II, (b) group I and group III, (c) group III and group IV, (d) groupIII andgroupV.
Statistical differences are expressed as * p < 0.001, NS-non significant.

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