BIOCHEMICAL AND ANTIOXIDANT PROFILES IN AFLATOXIN B1 INDUCED LIVER DAMAGE AND ITS AMELIORATION BY USING AGED GARLIC EXTRACT IN WISTAR ALBINO RATS

K. Sarvesha[1], M.L. Satyanarayana[2], S. Rao[3], S. Yathiraj[4] and N.B. Shridhar[5]

Veterinary College, Hebbal, KVAFSU, Bengaluru- 560 024 .

ABSTRACT

The present study was conducted to evaluate hepatoprotective effect of aged garlic extract in aflatoxin B1 induced liver damage in Wistar albino rats. In this study the liver damage was induced by inoculating aflatoxin B1 intraperitoneally at the dose rate of 4 mg/kg body weight on day 12 of the experimental study. Aged garlic extract was used at the dose rate of 200 mg/kg body weight orally through orogastric tube for a period of 28 days against aflatoxin B1 induced liver injury. In aflatoxin B1 treated rats there was significant increase in liver enzymes and significant decrease in the enzymic antioxidant profile was observed, but these liver enzymes and antioxidant levels were with in the normal range in aged garlic extract and aflatoxin B1 treatment rats, indicating hepatoprotective effect of aged garlic extract against aflatoxin B1 induced liver damage.

Key words: Aged garlic extract (AGE), Aflatoxin B1 (AFB1), Antioxidants

INTRODUCTION

Aflatoxin B1 (AFB1) is a metabolite produced by the fungi, Aspergillus flavus and Aspergillus parasiticus, which are contaminants of human and animal food grains. Corn is probably the most important source of AFB1 for both human and animal consumption (Wood, 1989). AFB1 has been reported to cause acute hepatotoxicity and liver carcinomas in people and laboratory animals (Roebuck and Maxuitenko, 1994). They are metabolized to a highly reactive 8,9-epoxide that binds to cellular macromolecules, primarily in the periportal region of the liver. AFB1-induced liver injury manifests itself as periportal parenchymal cell necrosis, hemorrhage, and injury to intrahepatic bile ducts. The liver lesions along with loss of appetite and lethargy in exposed animals are collectively referred to as aflatoxicosis.

Garlic is an important medicinal herb and is known to possess scientifically proven medicinal properties. Garlic ranks highly among herbs that help to prevent diseases largely due to its high content of organosulphur compounds and its antioxidant activity. Aged garlic extract (garlic stored at room temperature (37°C) for 20 months) is a richer source of antioxidants than fresh garlic (Biren et al., 2006).

1 Ph.D scholar, Department of Pathology, Veterinary College, Hebbal, KVAFSU, Bengaluru- 560 024 E. mail: sarveshkgowda@gmail.com Telephone: 09972201719; 2 Professor, Department of Pathology, Veterinary College, Hebbal, KVAFSU, Bengaluru.; 3 Professor, Department of Pathology, Veterinary College, Hebbal, KVAFSU, Bengaluru.; 4 Dean, Veterinary College, Hebbal, KVAFSU, Bengaluru 560 024.; 5 Associate Professor, Department of Pharmacology and Toxicology, Veterinary College, Hebbal, KVAFSU, Bengaluru-560 024.

Tadi et al. (1991) reported that the AGE protected liver against toxicity induced by benzopyrene and Aflatoxin B1, two potent free radical producing environmental carcinogens. Thus, the present study was undertaken to determine the hepatoprotective effect of AGE in AFB1 induced liver damage in Wistar albino rats.

MATERIALS AND METHODS

The present study was conducted at the Department of Veterinary Pathology, Veterinary College, Bangalore-560024.

Animals: Wistar albino rats weighing between 150 to 300 g were used for the present study. The animals were obtained from the Central Animal Facility, Indian Institute of Sciences, Bangalore 560 012. The study was approved by the Institutional Animal Ethics Committee (IAEC). The animals were fed with standard commercial rat feed obtained from M/S Godrej Agrovet Ltd Mumbai – 400079.

Aflatoxin B1: To induce hepatotoxicity in rats, single dose of commercially available AFB1 preparation (Sigma-Aldrich Chemicals Ltd) was used in the present study, which was given intraperitoneally at the dose rate of 4 mg/kg body weight on day 12 of experimental period.

Aged garlic extract: was obtained from M/S Vet Care Pvt. Ltd, Yelahanka, Bangalore and was administered daily at a dose rate of 200 mg/kg body weight, orally through orogastric tube for 28 days.

Experimental design: In the present study the experimental animals were divided into 4 groups with 6 animals in each group. Group I served as normal control used for studying baseline values of the parameters. Group II (vehicle control) received DMSO (0.3 ml/animal) on day 12 of experimental period. Rats in Groups III and IV were injected with a single dose of AFB1 intraperitoneally after fasting overnight at the dose of 4mg/kg body weight, in 3 per cent DMSO on day 12 of the experimental period. The rats belonging to Group IV were administered AGE suspended in distilled water, orally through orogastric tube at the dose rate of 200 mg/kg body weight from day one to twenty eight of the experimental period. The volume of administered AGE was 1ml for each animal.

Sample collection: Two animals from each group were sacrificed humanely under anaesthesia on day 14, 21 and 28 of experimental period. Blood samples were collected from the rats in each group on day 0, 14, 21 and 28 of trial. Blood samples were allowed to clot and centrifuged at 1500 rpm for 5 min to separate the sera and stored at -200C for biochemical estimation. The tissue samples were collected on day 14, 21 and 28 of experimental study for liver antioxidants estimation.

ALT, AST and creatinine estimation: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Creatinine kits were purchased from M/S Span Diagnostics Ltd, Sachin 394230 (Surat). All other chemicals and reagents used for the present study were of analytical grade. ALT, AST and creatinine were estimated by the method of International Federation of Clinical Chemistry (IFCC) using semi-automatic analyser (ARTOSE, Bangalore).

Estimation of antioxidant enzymes: Immediately after sacrificing the animals, the liver tissues were processed for estimation of antioxidant enzymes as per the method of Bruce and Baudry (1995). The liver samples were washed in ice cold normal saline to remove blood and was blotted dry and stored at -20^{0}C for further analysis. Liver was crushed in tissue homogenizer with 0.005 M phosphate buffer (pH 7.4) to make it 10 per cent liver homogenate w/v (1g of liver tissue crushed in 10 ml of 0.005 m phosphate buffer (pH 7.4). This homogenate solution was centrifuged at 15,000 g for 1 h at 4^{0}C and the supernatant obtained was

used for the estimation of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) levels. Superoxide dismutase (EC 1.15.1.1) was determined by the method described by Marklund and Marklund (1974). Catalase (EC 1.11.1.6) was estimated by the method of Claiborne (1985). Glutathione peroxidase (EC 1.11.1.6) was determined by the method described by Rotruck et al. (1973).

Statistical analysis: The data generated from the experimental study were subjected to two way analysis of variance (ANOVA) test using Graph Pad Prism version 5 for windows.

RESULTS AND DISCUSSION

The liver damage was induced in rats by administering single dose of AFB1 4mg/kg body weight by intraperitoneal route on day 12 of the experiment. The serum samples collected from rats of different groups on day 0, 14, 21 and 28 of the experimental study was used for the estimation of ALT, AST, and Creatinine. Results of the same are presented in Table 1.

Table - 1

Comparison of serum ALT, AST and CRT of rats administered with DMSO, AFB1 and AGE

Parameter	Day	Group I (Control)	Group II (Vehicle Control)	Group III (AFB_1)	Group IV (AFB_1+AGE)
ALT (U/L)	0	38.67±0.92	38.99±1.20	41.62±2.29	40.06±1.34
	14	39.17±0.84^b	38.67±1.03^b	110.92±4.68^a	39.76±0.80^b
	21	39.28±0.80^b	39.04±0.97^b	118.26±3.77^a	38.34±0.80^b
	28	37.16±0.99^b	37.16±1.00^b	116.81±3.00^a	38.17±1.04^b
AST (U/L)	0	85.24±4.50	91.71±6.40	95.44±5.08	99.24±4.37
	14	86.82±4.37^b	93.80±6.47^b	200.21±5.92^a	98.65±3.23^b
	21	87.73±6.42^b	90.74±6.39^b	194.57±3.50^a	94.08±3.35^b
	28	84.30±5.10^b	81.75±4.35^b	195.51±0.70^a	101.07±3.14^b
CRT (mg/dl)	0	0.54±0.02	0.52±0.02	0.55±0.02	0.52±0.03
	14	0.50±0.02	0.50±0.03	0.57±0.03	0.50±0.03
	21	0.49±0.02	0.52±0.02	0.55±0.03	0.49±0.04
	28	0.46±0.03	0.49±0.02	0.55±0.01	0.53±0.04

Mean ± SE values of ALT, AST and CRT at 0 day (n=6), 14th day (n=6), 21st day (n=4) and 28th day (n=2) of the study. a: Group I is compared with other groups, b: Group III is compared with other groups, n: Number of rats. Means bearing common superscript do not differ significantly at ($P \leq 0.05$).

In the present study a significant elevation was observed in the levels of ALT and AST in the serum of AFB1 administered rats. This observation was in accordance with that of Hong and Yan (2002); Farombi et al. (2005) and Adel et al. (2008) who also recorded significant increase in the ALT and AST upon AFB1 administration to rats. This might be due to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular organellar damage. Serum AST, ALT and ALP are biomarkers in the diagnosis of hepatic damage because they are released into the circulation after cellular damage.

ALT and AST levels were decreased significantly in AGE treated rats administered with AFB1. Our results are in line with the various research findings of Yamasaki and Lau (1997) who also found decreased levels of aminotransferases in garlic treated animals to protect vascular endothelial cells from oxidant injury. It could be attributed to the protective action of AGE on the hepatocytes through its antioxidant and detoxifying enzyme systems property.

No significant difference was observed in the levels of creatinine between treated and control Groups. It may be pertinent to mention here that aflatoxins are generally regarded as hepatotoxins. Mild to moderate damage to the kidney in the present study was an anticipated response.

The tissue samples collected from rats of different groups on day 14, 21 and 28 of the experimental study was used for the estimation of SOD, CAT, and GPx. Results of the same are presented in Table 2.

Table - 2

Comparison of SOD, CAT and GPx activity in liver of rats administered with DMSO, AFB1 and AGE

Parameter	Day	Group I (Control)	Group II (Vehicle Control)	Group III (AFB_1)	Group IV (AFB_1+AGE)
SOD (units/mg protein)	14	5.59 ± 0.66^{b}	5.07 ± 0.24^{b}	2.89 ± 0.05^{a}	5.10 ± 0.12^{b}
	21	6.04 ± 0.78^{b}	6.10 ± 0.12^{b}	2.96 ± 0.33^{a}	5.09 ± 0.11^{b}
	28	5.89 ± 1.12^{b}	5.96 ± 0.33^{b}	2.65 ± 0.36^{a}	5.60 ± 0.79^{b}
CAT (µmol of H_2O_2utilized/min/ mg protein)	14	48.62 ± 3.31^{b}	51.31 ± 2.91^{b}	24.85 ± 0.96^{a}	52.91 ± 3.28^{b}
	21	49.51 ± 0.31^{b}	49.45 ± 2.25^{b}	21.98 ± 0.35^{a}	53.41 ± 4.70^{b}
	28	46.71 ± 2.10^{b}	48.16 ± 3.55^{b}	19.91 ± 0.70^{a}	50.01 ± 3.19^{b}
H_2O_2utilized/min/ mg protein)	14	151.91 ± 4.30^{b}	150.51 ± 1.31^{b}	112.96 ± 6.15^{a}	153.02 ± 6.19^{b}
	21	148.91 ± 0.71^{b}	152.29 ± 5.57^{b}	110.90 ± 1.29^{a}	157.01 ± 3.79^{b}
	28	149.59 ± 2.38^{b}	145.26 ± 6.56^{b}	112.18 ± 4.04^{a}	153.70 ± 4.50^{b}
GPx (units/mg protein)	14	0.50±0.02	0.50±0.03	0.57±0.03	0.50±0.03
	21	0.49±0.02	0.52±0.02	0.55±0.03	0.49±0.04
	28	0.46±0.03	0.49±0.02	0.55±0.01	0.53±0.04

Mean ±SE values of SOD, CAT, GPx activity in Liver of various treatment groups at 14th day (n=2), 21st day (n=2) and 28th day (n=2) of the study. a: Group I is compared with other groups, b: Group III is compared with other groups, n: Number of rats. Means bearing common superscript do not differ significantly at ($P\leq0.05$).

In the present study a significant decrease was observed in the activity of SOD, CAT and GPx in AFB1 treated rats. This could be attributed to production of highly reactive oxygen metabolites (ROMs) and low availability of the substrate glutathione (GSH). These finding are in accordance with the research findings of Sivanesan and Begum (2007) and Sharmila and Murugesan (2009) who also observed a significant decrease in the liver antioxidants upon AFB1 administration to rats. Over production of superoxide anions which itself affects directly the CAT activity. Under high rate of free radicals input, the enzyme inactivation prevails and the enzymatic activities are reduced leading to autocatalysis of oxidative damage process. GPx catalyses the reduction of H_2O_2 at the expense of reduced GSH, thereby protecting mammalian cells against oxidative damage.

AGE+AFB1 treatment afforded maximum protection to the antioxidant enzymes such as SOD, catalase and GPx by influencing the GSH. GSH could directly scavenge and eliminate the AFB1 and thereby minimizing the toxic effects, which definitely indicates the antioxidant potency of the AGE. These finding are in accordance with the reports of Elsaid (2006) who also observed a significant increase in the liver antioxidants upon aqueous garlic extract administration to cyanide intoxicated albino rats. AGE exerted its antioxidant action by scavenging reactive oxygen species, enhancing the cellular antioxidant enzymes and lowering the lipid peroxidation in reducing the AFB1 induced liver damage to hepatocytes.

Oxidative damage usually refers to the impairment of the function of cellular components by reactive oxygen species such as superoxide anions, hydroxyl ions and hydrogen peroxide. These agents initiate or extend cell injury by extracting hydrogen atom from polyunsaturated fatty acid and cause a degenerative process known as lipid peroxidation. The enhanced formation of lipid peroxides is further evidenced by the decrease in the activities of SOD, CAT and GPx in liver tissues of AFB1 injected rats as compared with the normal control animals. Due to increase in lipid peroxidation the level of free radicals overcome the saturation level.

In conclusion hepatic damage can be experimentally induced in rats by administration of AFB1 at the dose rate of 4 mg/kg B.W by intraperitoneal route. Hepatic damage observed in AFB1 toxicity could be attributed to the conversion of AFB1 to its highly reactive 8,9-epoxide by the action of the mixed function mono-oxygenase enzyme systems (cytochrome P450-dependent) in liver. The study revealed that administration of AGE orally through orogastric tube, for 28 days had a significant hepatoprotective effect in AFB1 induced liver damage in rats. The hepatoprotective effect could be attributed to antioxidant, detoxifying enzyme systems and anti-lipid peroxidative effects of AGE.

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