International Journal of Pure and Applied Zoology
Volume 2, Issue 4, pp: 321-328, 2014

ISSN (Print) : 2320-9577
ISSN (Online): 2320-9585
http://www.ijpaz.com

Research Article

ACHYRANTHES ASPERA L. *AMELIORATES* AFLATOXIN B1-INDUCED PEROXIDATIVE HEPATIC DAMAGE IN RATS

P. Vanitha Pappa* and C. Padmalatha
Department of Zoology, Rani Anna Government College for Women,
Tirunelveli- 627008, Tamil Nadu, India

Article History: Received 22nd August 2014; Accepted 10th October 2014; Published 16th October 2014

ABSTRACT

Aflatoxins are potent hepatotoxic and hepatocarcinogenic agents. Reactive oxygen species (ROS) formation and consequent peroxidative damage caused by aflatoxin are considered to be the main mechanisms leading to hepatotoxicity. The present investigation aims at assessing the hepatoprotective effect of methanolic root extract of *Acyranthes aspera* L. on aflatoxin B_1 (AFB_1)-induced hepatotoxicity in a rat model. The hepatoprotection of *A. aspera* is compared with silymarin, a well known standard hepatoprotectant. Lactate dehydrogenase (LDH), alkaline phosphatase (ALKP), alanine and aspartate aminotransferases (ALT, AST) were found to be significantly increased in the serum and decreased in the liver of AFB_1 administered (1 mg/kg body mass, orally) rats, suggesting hepatic damage. Marked increase in the lipid peroxide levels (LPO) and a concomitant decrease in the enzymic superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G_6PD) and glutathione-S-transferase (GST)) and nonenzymic reduced glutathione (GSH), vitamin C and vitamin E (antioxidants) in the hepatic tissue were observed in AFB1 administered rats. Pretreatment with *A. aspera* (100 mg/kg body mass, orally) and silymarin (25 mg/kg body mass, orally) for 7 days reverted the condition to near normal. The results of this study indicated that *A. aspera* is a potent hepatoprotectant as silymarin.

Keywords: *Aspergillus flavus, Achyranthes aspera*, Aflatoxins, Hepatotoxicity.

INTRODUCTION

Aflatoxins are secondary toxic fungal metabolites produced by *Aspergillus flavus* and *A. parasiticus*. There are four naturally occurring aflatoxins namely AFB_1, AFB_2, AFG_1 ,AFG_2. Aflatoxins not only contaminate our food stuffs but are also found in edible tissues, milk and eggs through the consumption of contaminated feed by farm animals (Bennett and Klich, 2003; Fink-Gremmels, 1999). Aflatoxins are well known for their hepatotoxic and hepatocarcinogenic effects (Wogan, 1999). The carcinogenic mechanism of AFB_1 has been studied extensively. It has been shown that AFB_1 is activated by hepatic cytochrome P450 enzyme system to produce a highly reactive intermediate, AFB_1-8, 9-epoxide, which subsequently binds to nucleophilic sites in DNA, and the major adduct 8, 9-dihydro-8-(N^7guanyl) - 9-hydroxy-AFB_1 (AFB_1 N^7-Gua) is formed (Sharma and Farmer, 2004; Klein *et al*., 2002). The formation of AFB1- DNA adducts is regarded as a critical step in the initiation of AFB_1-induced hepato carcinogenesis (Preston and Williams, 2005).

Achyranthes aspera L. (Amaranthaceae) is a herbaceous, 1 m to 2 m high stems erect, pubescent swollen at the nodes, leaves opposite, short petioled, margins undulate. Flowers are numerous, stiffly deflected against the pubescent rachis in elongate terminal spike, 20-30 cm. Long, urticle oblong cylindrical, enclosed in the hardened perianth, brown. Seeds oblong-ovoid

*Corresponding author e-mail: biovanitha@rediffmail.com

wildly growing plant found **t**hroughout India, propagating through seeds. Betaine and Achyranthine are the principal alkaloids, identified from the whole plant of *A. aspera*. Seeds contain *Achyranthes* saponin A and its ester, named as *Achyranthes* saponin B3. The presence of ecdysterone is also reported. Shoots contain an essential oil, tannins and glycosides. The dried dehusked seeds contain aminoacids. Alcoholic and aqueous extracts of the roots cause fall in blood pressure but the chloroform extract raised the blood pressure in dogs. Achyranthine produced hypertension and depression of the heart, dilation of blood vessels in dogs, spasmogenic effect in frog rectal muscle and diuretic and purgative effect in albino rats (Neogi *et al.*, 1970). Alcoholic extract has hypoglycemic activity (Dhar *et al*., 1968). Seed saponins increased the contraction of the isolated heart of frog, guinea pig and rabbit. The effect was quicker in onset and shorter in duration than that exerted by digoxin. Saponins have phosphorylase activity in heart. (Ram *et al.*, 2004). Saponins have diuretic activity in albino rats and in dogs.

MATERIALS AND METHODS

Plant material

Roots of *A. aspera* were collected during January-February 2005 from Rasipuram, Tamil Nadu, India. The plant was authenticated by comparison with reference specimen preserved at St. Xavier's College, Palayamkottai.

Preparation of plant extracts

Coarse powder from the shadow dried roots of *A. aspera* (500g) was extracted to exhaustion with methanol (13%) using soxhlet apparatus. Silymarin is a purified extract of *Silybum marianum* Gaertn, composed mainly of flavonolignans like silybin, silibinin and its diastereoisomers isosilybin, silydianin and silychristin (Franschini *et al*., 2002). Silymarin is frequently used in the treatment of liver diseases. To evaluate anti hepatotoxic efficacy of *A. aspera* in AFB_1-induced hepatotoxicity in rats, silymarin is used as comparative drug.

Drugs and chemicals

AFB_1 and 1, 1, 3, 3-tetraethoxypropane were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Animal model

Male albino rats of Wistar strain (*Rattus norvegicus*) weighing 140 ± 20 g (12–14 weeks) were obtained from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The rats were maintained under standard conditions of humidity, temperature (25 ± 2° C) and light (12 h light/12 h dark) and fed a standard rat pellet diet (M/s Pranav Agro Industries Ltd, Sangli, India); drinking water was given *ad libitum*. The animal experiments were conducted according to National Institute of Health guidelines for the care and use of laboratory animals (1985) and ethical clearance was obtained (SPK/CPCSEA/1641/ZOOL/2007).

Experimental design

Animals were divided into six groups of six rats each as follows. Group I served as control. Group II were given AFB_1 (1 mg/kg) as a single oral administration on the 8^{th} day of the experimental period. Group III received single dose of aflatoxin and silymarin (25 mg/ kg) orally for seven days. Group IV received *A. aspera* root extract (100 mg/kg) orally for first seven days and followed by AFB_1 administration (1 mg/kg) on day 8. Group V received *A. aspera* (dosage and duration were as group IV) after aflatoxin administration. Group VI received silymarin (dosage and duration as group III) followed by AFB_1 administration (1 mg/kg) on day 8. *A. aspera* and silymarin were dissolved in olive oil. AFB_1 was dissolved in dimethyl sulfoxide and further diluted with distilled water to the required concentration.

Assessment of liver functions

At the end of the 10-day experimental period (72 h after AFB_1 administration), the animals were killed by decapitation. Blood samples were collected and the serum was separated for

enzyme assays. The liver was excised immediately, rinsed in ice-cold physiological saline and homogenized in Tris–HCl buffer (0.1 M, pH 7.4) to give a 10% homogenate. Aliquots of the tissue homogenate were suitably processed for the assessment of following biochemical parameters.

Biochemical parameters

The activities of Lactate dehydrogenase (LDH), alkaline phosphatase (ALKP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in liver and serum were estimated by the method of King (1965 a, b, c). LPO was determined by the procedure of Hogberg *et al*., (1974). Malondialdehyde (MDA), formed as an end product of the peroxidation of lipids, served as an index of oxidative stress. Superoxide dismutase (SOD) was assayed according to the method of Marklund and Marklund (1974). Catalase (CAT) was assayed by the method of Sinha (1972). In this, dichromate in acetic acid was reduced to chromic acetate when heated in the presence of H_2O_2. Chromic acetate thus produced was measured colorimetrically at 610 nm.

Glutathione peroxidase (GPx) was assayed by the method of Rotruck *et al.,* (1973) based on the reaction between glutathione remaining after the action of GPx and 5,5′- dithiobis-(2-nitrobenzoic acid). Glutathione reductase (GR) that utilizes NADPH to convert oxidized glutathione (GSSG) to the reduced form was assayed by the method of Staal *et al*., (1969). Glucose-6-phosphate dehydrogenase (G6PD) was assayed by the method of Beutler (1983); wherein, the absorbance was measured when the reaction was started by the addition of glucose-6-phosphate. Glutathione-S-transferase (GST) was assayed by the method of Habig *et al*., (1974). Total reduced glutathione (GSH) was determined by the method of Moron *et al*., (1979). Vitamin C was estimated by the method of Omaye *et al*., (1979). Vitamin E was estimated according to the procedure of Desai (1984).

Statistical analysis

The results are expressed as mean ± standard deviation (SD). Differences between groups were assessed by one-way analysis of variance using the SPSS software package for Windows (1988). Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test; significance at P-values <0.001, <0.01, <0.05 have been given respective symbols in the Tables.

RESULTS

Table 1 reveals the abnormal activities of serum and liver enzymes in rats that indicate the cellular damage caused by AFB_1 treatment. The activities of serum LDH, AST, ALT and ALP were increased by 2.98-, 2.33-, 2.01- and 2.78-fold, respectively, in group II animals when compared with control. Activities of these marker enzymes were significantly (P<0.001) decreased in the liver of AFB_1 administered animals. The *Achyranthes aspera* (group IV, V) and silymarin (group VI) pre-treated animals restored these enzyme levels to nearly that of control values (P<0.001) indicating the hepatoprotective role of the drug.

Table 2 shows the effects of *Achyranthes aspera* and silymarin on AFB_1-induced LPO and antioxidant status. The 3.46-fold rise in LPO seen in the AFB_1 group was maintained at near normal levels by *Achyranthes aspera* and silymarin pre-treatment. A significant decrease (P<0.001) in the activities of enzymatic antioxidants (SOD, CAT, GPx, GR, G6PD and GST) was seen in the AFB_1 treated animals (group II). *A. aspera* and silymarin pretreated rats did not show any decrease in the activities of antioxidants. GSH, vitamin C and vitamin E levels were decreased by 57.74%, 42.95% and 25.69% respectively in the AFB_1 administered rats (Table 3). *A. aspera* and silymarin pre-treatment restored the levels of these non-enzymatic antioxidants towards the control level (P<0.001, P<0.01; as against group II), thereby indicating that prophylaxis taken against aflatoxicosis using *A. aspera* and silymarin protects the liver against oxidative stress-induced depletion of antioxidants.

Table 1. Alterations in serum and tissue enzyme activities in AFB_1-induced animals and the prophylactic measures of *A. aspera* and silymarin (values are expressed as mean ± SD for six animals in each group).

Enzyme assays (U/mg protein)	Group I	Group II	Group III	Group IV	Group V	Group VI
Serum LDH	4.13±0.36[a]	12.29±1.09[b]	4.09±0.42 [a]	4.21±0.34 [a***]	4.52±0.49[a]	4.57±0.41[a***]
AST	0.52±0.03[a]	1.21±0.11 [b]	0.50±0.06 [a]	0.49±0.05 [a***]	0.57±0.05[a]	0.56±0.06[a***]
ALT	0.63±0.06[a]	1.39±0.14 [b]	0.65±0.07 [a]	0.62±0.04 [a***]	0.69±0.05[a]	0.68±0.07[a***]
ALKP	1.98±0.20[a]	4.51±0.35 [b]	1.92±0.16 [a]	1.93±0.18 [a***]	2.18±0.19[a]	2.16±0.23[a***]
Liver LDH	10.15±1.12[a]	6.74±0.49 [b]	10.08±.87 [a]	10.17±.98 [a***]	9.10±0.71[c]	9.30±0.82[c***]
AST	0.16±0.02 [a]	0.09±0.01 [b]	0.15±0.02 [a]	0.17±0.02 [a***]	0.14±0.01[a]	0.15±0.01[a***]
ALT	0.12±0.01 [a]	0.06±0.01 [b]	0.13±0.02 [a]	0.12±0.01 [a***]	0.11±0.02[a]	0.11±0.01[a***]
ALP	1.75±0.19 [a]	0.68±0.06 [b]	1.71±0.14 [a]	1.77±0.16 [a***]	1.61±0.14[a]	1.63±0.13[a***]

Note: LDH: µmol×10−1 of pyruvate liberated/min; AST, ALT: µmol×10−2 of pyruvate liberated/min; ALP: µmol×10−2 of phenol liberated/min.
The values in each row superscribed with different alphabets are statistically significant (LSD test:*P<0.05, **P<0.01, ***P<0.001)

Table 2. Alterations in lipid peroxidation and antioxidant enzymes in the liver of AFB1-induced animals and the prophylactic measures of *A. aspera* and silymarin (values are expressed as mean ± SD for six animals in each group).

Para meters	Group I	Group II	Group III	Group IV	Group V	Group VI
LPO	1.08±0.13[a]	3.74±0.41[b]	1.12±0.11[a]	1.05±0.12 [a***]	1.21±0.17 [a]	1.19±0.20 [a***]
CAT	340.82±31.71[a]	210.11±17.25[b]	349.36±29.42 [c]	338.54±25.62 [a***]	324.73±26[d]	317.76±33.56[e***]
SOD	7.55±0.87[a]	4.21±0.45[b]	7.62±0.81 [a]	7.46±0.75 [a***]	7.15±0.51[c]	7.09±0.57[c***]
GPx	5.49±0.61[a]	3.09±0.28[b]	5.36±0.56 [a]	5.52±0.58 [a***]	5.27±0.32[a]	5.19±0.45[a***]
GR	0.28±0.03[a]	0.16±0.02[b]	0.27±0.03 [a]	0.28±0.04 [a***]	0.30±0.03[a]	0.27±0.02[a***]
G6PD	2.12±0.20[a]	1.21±0.13[b]	2.05±0.19 [a]	2.10±0.18[a***]	1.94±0.21[a]	1.85±0.15[a***]
GST	1.11±0.14[a]	0.61±0.07[b]	1.21±0.13[a]	1.17±0.12[a***]	1.00±0.12[a]	1.02±0.06[a***]

Note: LPO: nmol of MDA formed/mg protein; CAT: µmol of H_2O_2 consumed/min/mg protein; SOD: units/mg protein; GPx: µg of GSH utilized/min/mg protein; GR: nmol of NADPH oxidized/min/mg protein; G6PD: nmol of NADPH formed/min/ mg protein; GST: nmol of 1-chloro-2, 4-dinitrobenzene–GSH conjugate formed/ min/mg protein.
The values in each row superscribed with different alphabets are statistically significant
The symbols represent statistical significance: *P<0.05, **P<0.01, ***P<0.001.

Table 3. Alterations in non-enzymatic antioxidant status in the liver of AFB_1-induced animals and the prophylactic measures of *A. aspera* and silymarin (values are expressed as mean ± SD for six animals in each group)

Tissue antioxidants	Group I	Group II	Group III	Group IV	Group V	Group VI
GSH	5.17±0.57[a]	0.34±0.31[b]	5.07±0.51 [a]	5.18±0.52 [a***]	4.94±0.39 [a**]	4.89±0.52[a***]
Vitamin C	3.12±0.38[a]	1.78±0.13 [b]	3.16±0.31 [a]	3.19±0.33 [a***]	3.08±0.25 [a**]	3.11±0.46[a***]
Vitamin E	1.44±0.09[a]	1.07±0.09 [b]	1.41±0.14 [a]	1.48±0.14 [a***]	1.43±0.12 [a**]	1.40±0.17[a***]

Note: GSH, Vitamin C and Vitamin E—µg/mg protein.
The values in each row superscribed with different alphabets are statistically significant
The symbols represent statistical significance: *P<0.05, **P<0.01, ***P<0.001.

DISCUSSION

The hepatotoxic effect of AFB_1 has been well documented in a variety of animal species (Mishra and Das, 2003; Cheng *et al*., 2001; Wogan, 1999). Increased activities of serum ALT, AST, LDH and ALP are well known diagnostic indicators of hepatic injury (Wang *et al*., 1991; Liu *et al*., 2001). In cases such as liver damage with hepatocellular lesions, these enzymes are released from the liver into the blood stream (Plaa and Hewitt, 1986).

The activities of amino transferases (ALT and AST), LDH and ALKP were decreased in AFB_1 administed rats indicates impaired liver function (Kalengayi and Desmet, 1975; Yin *et al.,* 1980). Amino transferases being an important class of enzymes linking carbohydrate and amino acid metabolism, have connection with intermediates of citric acid cycle. These enzymes are markers of liver injury since liver is the major site of metabolism. ALKP is a membrane bound enzyme and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites (Plaa and Hewitt, 1986).

Hepatic damage was significantly reduced by prophylactic measure of *A. aspera* in a manner similar to that observed with silymarin, a known hepatoprotectant. The protection rendered by silymarin may be due to their antioxidant effect and their ability to act as a radical scavenger, thereby protecting membrane permeability (Sudharsan *et al*., 2005; Nagaraj *et al.,* 2000; Soto *et al*., 2003; Franschini *et al*., 2002).

AFB_1-induced free radicals production has been referred to as a possible contributor to hepatotoxicity (Towner *et al.,* 2003). LPO is one of the main manifestations of oxidative damage initiated by ROS and it has been linked with altered membrane structure, enzyme inactivation and initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane (Niki *et al*., 2005). The increase in lipid peroxidase might result from increased production of free radicals and a decrease in antioxidant status (Shen *et al*., 1994; Rastogi *et al*., 2001). Administration of *A. aspera* and silymarin significantly reduced the AFB_1-induced LPO by their ability to scavenge the free radicals.

GSH and GST play a critical role in the protection of tissues from the deleterious effects of activated AFB_1 (Larsson *et al.,* 1994). GSH is a tripeptide containing cysteine that has a reactive –SH group with reductive potency. It can act as a non-enzymatic antioxidant by direct interaction of –SH group with ROS or it can be involved in the enzymatic detoxification of ROS, as a cofactor or a coenzyme (Janssen *et al*., 1993). GST catalyzes the conjugation of AFB_1-8, 9-epoxides with GSH to form AFB_1- epoxide–GSH conjugates thereby decreasing the intracellular glutathione content (Raney *et al*., 1992). Silymarin improves the GSH level (Valenzuela *et al*., 1989).

Antioxidant enzymes like SOD, CAT and GPx form the first line of defense against ROS and a decrease in their activities was observed with AFB_1 administration (Verma and Nair, 2001; Rastogi *et al*., 2001). SOD is a family of metallo-enzymes that is known to accelerate the dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 which are deleterious to polyunsaturated fatty acids and structural proteins of plasma membrane (Johnson and Giulivi, 2005). The hydrogen peroxide produced by SOD is further removed by CAT. Decline in the activities of these enzymes after AFB_1 administration might be due to the inactivation of these enzymes by ROS.

A. aspera increases the GSH status resulting in the increase in SOD activity thereby preventing the deleterious effect of super oxide radicals. Thus *A.aspera* indirectly influences the activities of SOD and CAT. Selenium dependent GPx removes both H_2O_2 and lipid peroxides by catalyzing the conversion of lipid hydroperoxide to hydroxy acids in the presence of GSH. The activity of GPx, which is a constituent of GSH redox cycle decreased during AFB_1 administration. The decrease in the levels of glutathione metabolizing enzymes (G6PD and GR) in AFB_1 administered rats occurs as a result of impaired flux of glucose-6-phosphate through hexose monophosphate shunt and decreased supply of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for the conversion of GSSG to GSH in the presence of GR. Under conditions of oxidative assault, the NADP+/NADPH ratio will switch in favour of $NADP^+$, indicating decreased G6PD activity. Prophylaxsis with *A. aspera* and silymarin

significantly improved the activities of GR and G6PD.

Vitamin E, a fat-soluble molecule present in the interior of membranes protects against LPO while ascorbate, a water- soluble antioxidant reduces oxidized α-tocopherol and lipid peroxides (Singh *et al*., 2005). GSH depletion can additionally explain the decreased concentration of vitamin C observed in the present study. This vitamin enters the cell mainly in the oxidized form where it is reduced by GSH. *A. aspera* and silymarin administration prevented the significant decline in the level of vitamin C. The increased ascorbate and GSH content in the *A. aspera* and silymarin administered animals regenerates vitamin E and establishes a synergistic effect among them thereby enhancing the antioxidant protection (Lee, 1999).

It showed a trend similar to that of silymarin, a known hepatoprotective agent in protecting liver from AFB_1-induced toxicity. Thus, it may be concluded that *A. aspera* ameliorates AFB_1-induced toxicity due to its combined antioxidant potential as well as hepatoprotective action.

CONCLUSION

The study on the extracts of *A.aspera* further exposes its anti oxidant potential, a thrust area in the modern search for anti oxidant plant drugs. It opens a new avenue for the development of plant drugs against aflatoxicosis.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENT

The authors wish to acknowledge the Principal and HOD of Zoology, Rani Anna Government College for Women, Tirunelveli for the laboratory facilities provided.

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