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ETHANOLIC LEAVES EXTRACT OF *TRIANTHEMA PORTULACASTRUM* L. AMELIORATES AFLATOXIN B_1 INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

Aflatoxins are potent hepatotoxic and hepatocarcinogenic agents. Reactive oxygen species 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 ethanolic leaves extract of Trianthema portulacastrum on aflatoxin B_1 (AFB_1)-induced hepatotoxicity in a rat model. The hepatoprotection of T. portulacastrum is compared with silymarin, a well known standard hepatoprotectant. Lactate dehydrogenase, alkaline phosphatase, alanine and aspartate aminotransferases were found to be significantly increased in the serum and decreased in the liver of AFB_1 administered (1 mg/kg bw, orally) rats, suggesting hepatic damage. Marked increase in the lipid peroxide levels and a concomitant decrease in the enzymic (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glucose-6- phosphate dehydrogenase and glutathione-S-transferase) and nonenzymic (reduced glutathione, vitamin C and vitamin E) antioxidants in the hepatic tissue were observed in AFB_1 administered rats. Pretreatment with T. portulacastrum (100 mg/kg/p.o) and silymarin (100 mg/kg /p.o) for 7 days reverted the condition to near normal. The results of this study indicate that the ethanolic leaves extract of T. portulacastrum is a potent hepatoprotectant as silymarin.

KEY WORDS

Aflatoxin B_1, Trianthema portulacastrum, Hepatotoxic, Hepatocarcinogenic.

INTRODUCTION

Aflatoxins are secondary toxic fungal metabolites produced by *Aspergillus flavus* and *A. parasiticus*. There are four naturally occurring aflatoxins, the most hepatotoxic being aflatoxin B_1 (AFB_1), and three structurally similar compounds namely aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). Aflatoxins not only contaminate our food stuffs but are also found in edible tissues, milk and eggs after consumption of contaminated feed by farm animals (1,2).

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Aflatoxins are well known for their hepatotoxic and hepatocarcinogenic effects (3). The carcinogenic mechanism of AFB_1 has been extensively studied. 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 (4, 5). The formation of AFB_1- DNA adducts is regarded as a critical step in the initiation of AFB_1 induced hepatocarcinogenesis (6).

Although the mechanism underlying the hepatotoxicity of aflatoxins is not fully understood, several reports suggest that toxicity may ensue through the generation of intracellular reactive oxygen species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide (H_2O_2) during the metabolic processing of AFB_1 by cytochrome P_{450} in the liver (7, 8). These species may attack soluble cell compounds as well as

membranes, eventually leading to the impairment of cell functioning and cytolysis (9). Peroxidative damages induced in the cell are encountered by elaborate defense mechanisms, including enzymatic and non-enzymatic antioxidants (10). Biological compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effects of ROS and other free radicals.

Silymarin is a purified extract of *Silybum marianum* Gaertn, composed mainly of flavonolignans like silybin, silibinin and its diastereoisomers isosilybin, silydianin and silychristin (11). Silymarin is frequently used in the treatment of liver diseases where it is capable of protecting liver cells directly by stabilizing the membrane permeability through inhibiting lipid peroxidation (LPO) and preventing liver glutathione depletion (12-14).

Trianthema portulacastrum L. (Aizoaceae) is a prostrate, glabrous, succulent annual herb found almost throughout India as a weed in cultivated and wastelands. The plant is bitter, hot, alexiteric, analgesic, stomachic, laxative, and serves as alterative cures for bronchitis, heart disease, blood anaemia, inflammation, and piles ascites. The root applied to the eye cures corneal ulcers, itching, dimness of sight and night blindness (15). A decoction of the herb is used as a vermifuge and is useful in rheumatitis. It is also an antidote to alcoholic poison (16) has remarkable effect in the reduction of Diethylnitosoamine - induced hepatocarcinogenesis (17) and lowering effect of paracetamol and thioacetamide induced hepatotoxicity in rats (18) as well as antioxidant activity (19). The bitter and nauseous root is given in combination with ginger as a cathartic, (15). These findings prompted us to evaluate the effect of *T. portulacastrum* supplementation in AFB_1 induced hepatotoxicity in rats and compare its antihepatotoxic efficacy with an established drug-silymarin.

MATERIALS AND METHODS

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

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 (60-80%) - temperature (25 ± 2°C) and light (12 h light/12 h dark) and keep over standard rat pellet diet (M/s Pranav Agro Industries Ltd, Sangli, India) and water *ad libitum*. The animal experiments were conducted according to National Institute of Health guidelines for the care and use of laboratory animals (20).

The leaves of *T. portulacastrum* L. were collected during Jan-Feb.2002 from Tiruchirapalli, Tamilnadu, India. The plant was authenticated by comparison with reference specimens preserved at the Rapinat Herbarium of St.Joseph's College, Tiruchirapalli. Voucher Herbarium Specimens (Collection number 299, 300, 6711, 12114, 26644) are kept in the Herbarium for future references.

Coarse powder from the shadow dried leaves of *T. portulacastrum* L. (500 g) was extracted with ethanol using a soxhlet apparatus. The ethanolic extract thus obtained was dried under reduced pressure and temperature not exceeding 40°C to get 100 g of the crude extract.

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

The final gavage solution of AFB_1 contained 1% dimethyl sulfoxide. At the end of the 10-day experimental period (72 h after AFB_1 administration), blood was withdrawn, animals were sacrificed and the livers were 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.

Serum was also separated and used for assay the following hepatospecific enzymes. The activities of Lactate dehydrogenase (LDH), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in liver and serum were estimated by the method of King (21-23). Protein content was determined by the method of Lowry et al (24) using bovine serum albumin as a standard. LPO was determined by the procedure of Hogberg et al (25). 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 (26).

The unit of enzyme activity is defined as the enzyme required to give 50% inhibition of pyrogallol autooxidation. Catalase (CAT) was assayed by the method of Sinha (27). In this method, dichromate in acetic acid was reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchloric acid as an unstable intermediate. Chromic acetate thus produced was measured colorimetrically at 610 nm.

Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al (28) based on the reaction between glutathione remaining after the action of GPx and 5,5'- dithiobis-(2-nitrobenzoic acid) to form a complex that absorbs maximally at 412 nm. Glutathione reductase (GR) that utilizes NADPH to convert oxidized glutathione (GSSG) to the reduced form was assayed by the method of Staal et al (29). Glucose-6-phosphate dehydrogenase (G6PD) was assayed by the method of Beutler (30) wherein the increase in absorbance was measured when the reaction was started by the addition of glucose-6-phosphate. Glutathione-S-transferase (G-S-T) was assayed by the method of Habig et al (31). Total reduced glutathione (GSH) was determined by the method of Moron et al (32). Vitamin C was estimated by the method of Omaye et al (33). Vitamin E was estimated according to the procedure of Desai (34).

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. 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 ethanolic leaves extract of *T. portulacastrum* (group 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 ethanolic leaves extract of *T. portulacastrum* 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 *T. portulacastrum* 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). Ethanolic leaves extract of *T. portulacastrum* and silymarin pre-treated 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). Ethanolic leaves extract of *T. portulacastrum* and silymarin pre-treatment restored the levels of these non-enzymatic

Table 1: Alterations in serum and tissue enzyme activities in AFB_1 induced animals and the effect of ethanolic leaves extract of *T. portulacastrum* and silymarin pre-treatment (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	12.29±1.09^{a***}	4.09±0.42	4.21±0.34	4.52±0.49^{b***}	4.57±0.41^{b***}
AST	0.52±0.03	1.21±0.11^{a***}	0.50±0.06	0.49±0.05	0.57±0.05^{b***}	0.56±0.06^{b***}
ALT	0.63±0.06	1.39±0.14^{a***}	0.65±0.07	0.62±0.04	0.69±0.05^{b***}	0.68±0.07^{b***}
ALP	1.98±0.20	4.51±0.35^{a***}	1.92±0.16	1.93±0.18	2.18±0.19^{b***}	2.16±0.23^{b***}
Liver LDH	10.15±1.12	6.74±0.49^{a***}	10.08±0.87	10.17±0.98	9.10±0.71^{b***}	9.30±0.82^{b***}
AST	0.16±0.02	0.09±0.01^{a***}	0.15±0.02	0.17±0.02	0.14±0.01^{b***}	0.15±0.01^{b***}
ALT	0.12±0.01	0.06±0.01^{a***}	0.13±0.02	0.12±0.01	0.11±0.02^{b***}	0.11±0.01^{b***}
ALP	1.75±0.19	0.68±0.06^{a***}	1.71±0.14	1.77±0.16	1.61±0.14^{b***}	1.63±0.13^{b***}

Enzyme units: LDH: $\mu mol \times 10^{-1}$ of pyruvate liberated/min; AST, ALT: $\mu mol \times 10^{-2}$ of pyruvate liberated/min; ALP: $\mu mol \times 10^{-2}$ of phenol liberated/min. Comparisons are made between: a-group I and group II, III, IV, V, VI; b-group II and group V, VI; c-group Vand group VI; *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 effect of ethanolic leaves extract of *T. portulacastrum* and silymarin pre-treatment (values are expressed as mean ± SD for six animals in each group)

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
LPO	1.08±0.13	3.74±0.41^{a***}	1.12±0.11	1.05±0.12	1.21±0.17^{b***}	1.19±0.20^{b***}
CAT	340.82±31.71	210.11±17.25^{a***}	349.36±29.42	338.54±25.62	324.73±26.32^{b***}	317.76±33.56^{b***}
SOD	7.55±0.87	4.21±0.45^{a***}	7.62±0.81	7.46±0.75	7.15±0.51^{b***}	7.09±0.57^{b***}
GPx	5.49±0.61	3.09±0.28^{a***}	5.36±0.56	5.52±0.58	5.27±0.32^{b***}	5.19±0.45^{b***}
GR	0.28±0.03	0.16±0.02^{a***}	0.27±0.03	0.28±0.04	0.30±0.03^{b***}	0.27±0.02^{b***}
G6PD	2.12±0.20	1.21±0.13^{a***}	2.05±0.19	2.10±0.18	1.94±0.21^{b***}	1.85±0.$^{15a*\ b***}$
GST	1.11±0.14	0.61±0.07^{a***}	1.21±0.13	1.17±0.12	1.00±0.12^{b***}	1.02±0.06^{b***}

Units: 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; Comparisons are made between: a-group I and group II, III, IV, V, VI; b -group II and group V, VI; c-group V and group VI; *P<0.05, **P<0.01, ***P<0.001.

antioxidants towards the control level (P<0.001, P<0.01; as against group II), thereby indicating that ethanolic leaves extract of *T. portulacastrum* and silymarin pre-treatment protects the liver against oxidative stress-induced depletion of antioxidants.

The recovery of biochemical parameters near the normal value is not appropriate (65% protection afforded by drugs). Also compare the hepatoprotective action of *T portulacastrum* with those of silymarin

DISCUSSION

The hepatotoxic effect of AFB_1 has been well documented in a variety of animal species (3, 35, 36). Increased activities of serum ALT, AST, LDH and ALP are well known diagnostic indicators of hepatic injury. In cases such as liver damage with hepatocellular lesions, these enzymes are released from the liver into the blood stream (37). The results obtained by us indicate a significantly recovered in the activities of these marker enzymes in serum, which is in accordance with the previous reports (38, 39). Pre-treatment with *T. portulacastrum* significantly lowered the levels of these enzymes, and the values were comparable with that of the control animals and silymarin pre-treated group. This suggests the hepatoprotective role of ethanolic leaves extract of *T. portulacastrum.*

In the present study, a decrease in the activities of amino transferases, LDH and ALP was observed in the liver following AFB_1 administration. Amino transferases (ALT and AST) being an important class of enzymes linking carbohydrate and amino acid metabolism, have established a relationship between the intermediates of citric acid cycle. These enzymes are markers of liver injury since liver is the major site of metabolism. The marked decrease in the activity of hepatic LDH with AFB_1 treatment indicates impaired liver function. ALP is a membrane bound enzyme and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites (37).

Several researchers have reported decreased activities of ALT, AST, LDH and ALP in liver during AFB_1 treatment, which corroborate with our study (40, 41). The AFB_1-induced decreases in the liver enzymes were significantly inhibited by *T. portulacastrum* pre-treatment in a manner similar to that observed with silymarin, a known hepatoprotectant. The protection rendered by silymarin may be due to their antioxidant

Table 3: Alterations in non-enzymatic antioxidant status in the liver of AFB_1 induced animals and the effect of ethanolic leaves extract of *T. portulacastrum* and silymarin pre-treatment (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	0.34±0.31^{a***}	5.07±0.51	5.18±0.52	4.94±0.39^{b***}	4.89±0.52^{b***}
Vitamin C	3.12±0.38	1.78±0.13^{a***}	3.16±0.31	3.19±0.33	3.08±0.25^{b***}	3.11±0.46^{b***}
Vitamin E	1.44±0.09	1.07±0.09^{a***}	1.41±0.14	1.48±0.14	1.43±0.12^{b***}	1.40±0.17^{b**}

Units: GSH, Vitamin C and Vitamin E-μg/mg protein; Comparisons are made between: a-group I and group II, III, IV, V, VI; b-group II and group V, VI; c-group V and group VI; *P<0.05, **P<0.01, ***P<0.001.

effect and their ability to act as a radical scavenger, thereby protecting membrane permeability (11, 13, 42, 43).

AFB_1 induced free radicals production has been referred to as a possible contributor to hepatotoxicity (7). LPO is one of the main manifestations of oxidative damage initiated by ROS and it has been linked with altered membrane structure and enzyme inactivation. It is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane (44). The present data reveals that AFB_1 administration produced a marked oxidative impact as evidenced from the significant increase in LPO. The increase in lipid peroxides might result from increased production of free radicals and a decrease in antioxidant status. The oxidative stress observed in our study is in accordance with other reports (45, 46) where it has been implicated in AFB_1 induced hepatotoxicity. In this study, *T. portulacastrum* and silymarin pre-treatment 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 (47). 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 (10). 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 (48). This observation supports our finding where we observed a significant decline in the levels of GSH and GST in AFB_1 induced animals.

Silymarin has already been reported to improve the GSH level (14). The restoration of intracellular GSH content and GST activity to normal levels by ethanolic leaves extract of *T. portulacastrum* and silymarin pre-treatment indicates that they play a vital role in mitigating AFB_1 induced oxidative stress and subsequent damage to the liver. 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 (46, 49). The above finding corroborates with our results where we have observed a decline in SOD, CAT and GPx activities.

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 (50). 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.

Ethanolic leaves extract of *T. portulacastrum* increases the GSH status resulting in the increase in SOD activity thereby preventing the deleterious effect of super oxide radicals. Thus *T. portulacastrum* 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 reduction in the activity of GPx on AFB_1 administration may be due to decrease in the availability of substrate (GSH) and also because of alterations in their protein structure by ROS (10).

The increased intracellular GSH content following ethanolic leaves extract of *T. portulacastrum* and silymarin pre-treatment may activate GPx thereby preventing the accumulation of H_2O_2. 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. The present study also showed a similar finding in the levels of these enzymes, indicating increased onslaught of oxidative radicals. Treatment with ethanolic leaves extract of *T. portulacastrum* 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 (51). 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. Ethanolic leaves extract of *T. portulacastrum* and silymarin pre-treatment prevented the significant decline in the level of vitamin C. The increased ascorbate and GSH content in the *T. portulacastrum* and silymarin pre-treated animals regenerates vitamin E and establishes a synergistic effect among them thereby enhancing the antioxidant protection (52).

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 ethanolic leaves extract of *T. portulacastrum* ameliorates AFB_1-induced toxicity due to its combined antioxidant potential as well as hepatoprotective action.

REFERENCES

1. Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev 2003; 16: 497–516.
2. Fink-Gremmels J. Mycotoxins: their implications for human and animal health. Vet Q 1999; 21: 115–20.
3. Wogan GN. Aflatoxin as a human carcinogen. Hepatol 1999; 30: 573–5.
4. Sharma RA, Farmer PB. Biological relevance of adduct detection to the chemoprevention of cancer. Clin Cancer Res 2004; 10: 4901–12.
5. Klein PJ, Van Vleet TR, Hall JO, Coulombe Jr RA. Biochemical factors underlying the age-related sensitivity of turkeys to aflatoxin B(1). Comp Biochem Physiol C 2002; 132: 193–201.
6. Preston RJ, Williams GM. DNA-reactive carcinogens: mode of action and human cancer hazard. Criterion Rev Toxicol 2005; 35: 673–83.
7. Towner RA, Qian SY, Kadiiska MB, Mason RP. *In vivo* identification of aflatoxin-induced free radicals in rat bile. Free Rad Biol Med 2003; 35: 1330–40.
8. Sohn DH, Kim YC, Oh SH, Park EJ, Li X, Lee BH. Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*. Phytomed 2003; 10: 165–9.
9. Berg D, Youdim MB, Riederer P. Redox imbalance. Cell Tiss Res 2004; 318: 201–13.
10. Janssen YM, Van Houten B, Borm PJ, Mossman BT. Cell and tissue responses to oxidative damage. Lab Invest 1993; 69: 261–74.
11. Franschini F, Demartini G, Esposti D. Pharmacology of silymarin, Linn. Drug Invest 2002; 22: 51–65.
12. Gazak R, Svobodova A, Psotova J, Sedmera P, Prikrylova V, Walterova D, Kren V. Oxidized derivatives of silybin and their antiradical and antioxidant activity. Bioorg Med Chem 2004; 12: 5677–87.
13. Soto C, Recoba R, Barron H, Alvarez C, Favari L. Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Comp Biochem Physiol C 2003; 136: 205–12.
14. Valenzuela A, Monica A, Soledad V, Ricardo G. Selectivity of silymarin on the increase of the glutathione content in different tissues of the rats. Planta Med 1989; 55: 420–22.
15. Kirtikar KR, Basu BD. Indian Medicinal Plants. In: Basu, L.M. (Ed.), 2nd ed., Vol. 2. Allahabad, India, 1933; 1180–81.
16. Shastri BN. Wealth of India-Raw Materials, Vol. 10. CSIR Publication, New Delhi, 1952; 19–23.
17. Bhattacharya S, Chatterjee M. Protective role of *Trianthema portulacastrum* against diethylnitrosamine induced experimental hepatocarcinogenesis. Cancer Lett 1998; 129: 7–13.
18. Kumar G, Sharmila Banu G, Vanitha Pappa P, Sundararajan M, Rajasekara Pandian M. Hepatoprotective activity of *Trianthema portulacastrum* L. against paracetamol and thioacetamide intoxication in albino rats. J Ethnopharmacol 2004; 92: 37–40
19. Kumar G, Sharmila Banu G, Rajasekara Pandian M. Evaluation of the antioxidant activity of *Trianthema portulacastrum.* Indian J Pharmacol 2005; 37: 331-34.
20. National Institute of Health Guide for the Care and Use of laboratory Animals. DHEW Publication (NIH), revised, Office of Science and Health Reports, DRR/NIH, Bethesda, USA, 1985.
21. King J. The dehydrogenases or oxidoreductases - Lactate dehydrogenase. In: King, J. (Ed.), Practical Clinical Enzymology. Van Nostrand Company Ltd, London, 1965a: 83–93.
22. King J. The hydrolases-acid and alkaline phosphatases. In: King J (Ed.), Practical Clinical Enzymology. Van Nostrand Company Ltd, London, 1965b: 191–208.
23. King J. The transferases - alanine and aspartate transaminases. In: King J (Ed.), Practical Clinical Enzymology. Van Nostrand Company Ltd, London, 1965c: 121–38.
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265–75.
25. Hogberg J, Larson RE, Kristoferson A, Orrenius S. NADPH dependent reductase solubilized from microsomes by peroxidation and its activity. Biochem Biophy Res Comm 1974; 56: 836–42.
26. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47: 469–74.
27. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972; 47: 389–94.
28. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588–90.
29. Staal GE, Visser J, Veeger C. Purification and properties of glutathione reductase of human erythrocytes. Biochim Biophys Acta 1969; 185: 39–48.

30. Beutler E. Active transport of glutathione disulfide from erythrocytes In: Larson A, Orrenius S, Holmgren A, Mannervik B (Eds.), Functions of Glutathione - Biochemical, Physiological, Toxicological and Clinical Aspects. Raven Press, New York, 1983; 65.

31. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130–39.

32. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979; 582: 67–78.

33. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol 1979; 62: 3–11.

34. Desai ID. Vitamin E analysis methods for animal tissues. Met Enzymol 1984;105: 138–47.

35. Mishra HN, Das C. A review on biological control and metabolism of aflatoxin. Critic Rev Food Sci Nut 2003; 43: 245–64.

36. Cheng YH, Shen TF, Pang VF, Chen BJ. Effects of aflatoxin and carotenoids on growth performance and immune response in mule ducklings. Comp Biochem Physiol C 2001; 128: 19–26.

37. Plaa GL, Hewitt WR. Detection and evolution of chemically induced liver injury. In: Hayes, A.W. (Ed.), Principles and Methods of Toxicology. Raven press, New York 1986; 401–41.

38. Wang CJ, Shiow SJ, Lin JK. Effects of crocetin on the hepatotoxicity and hepatic DNA binding of aflatoxin B_1 in rats. Carcinogenesis 1991; 12: 459–62.

39. Liu J, Yang CF, Wasser S, Shen HM, Tan CE, Ong CN. Protection of salvia miltiorrhiza against aflatoxin B_1 induced hepatocarcinogenesis in Fischer 344 rats dual mechanisms involved. Life Sci 2001; 69: 309–26.

40. Kalengayi MM, Desmet VJ. Sequential histological and histochemical study of the rat liver during aflatoxin B_1 induced carcinogenesis. Cancer Res 1975; 35: 2845–52.

41. Yin SJ, Kao MC, Lee SC. Sequential biochemical and histological changes in rats treated with aflatoxin B_1. Brit J Cancer 1980; 42: 319–25.

42. Sudharsan PT, Mythili Y, Selvakumar E, Varalakshmi P. Cardioprotective effect of pentacyclic triterpene, lupeol and its ester on cyclophosphamide induced oxidative stress. Hum Exp Toxicol 2005; 24: 313–18.

43. Nagaraj M, Sunitha S, Varalakshmi P. Effect of lupeol, a pentacyclic triterpene, on the lipid peroxidation and antioxidant status in rat kidney after chronic cadmium exposure. J Appl Toxicol 2000; 20: 413–17.

44. Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: mechanisms, inhibition, and biological effects. Biochem Biophy Res Comm 2005; 338: 668–76

45. Shen HM, Shi CY, Lee HP, Ong CN. Aflatoxin B_1 induced lipid peroxidation in rat liver. Toxicol Appl Pharmacol 1994; 127: 145–50.

46. Rastogi R, Srivastava AK, Rastogi AK. Long term effect of aflatoxin B(1) on lipid peroxidation in rat liver and kidney: effect of picroliv and silymarin. Phytother Res 2001; 15: 307–10.

47. Larsson P, Busk L, Tjalve H. Hepatic and extrahepatic bioactivation and GSH conjugation of aflatoxin B_1 in sheep. Carcinogenesis 1994; 15: 947–55.

48. Raney KD, Meyer DJ, Ketterer B, Harris TM, Guengerich FP. Glutathione conjugation of aflatoxin B_1 exo- and endo-epoxides by rat and human glutathione S-transferases. Chem Res Toxicol 1992; 5: 470–78.

49. Verma RJ, Nair A. Ameliorative effect of vitamin E on aflatoxin induced lipid peroxidation in the testis of mice. Asian J Androl 2001; 3: 217–21.

50. Johnson F, Giulivi C. Superoxide dismutases and their impact upon human health. Mol Asp Med 2005; 26: 340–52.

51. Singh U, Devaraj S, Jialal I. Vitamin E, oxidative stress and inflammation. Annl Rev Nut 2005; 25: 151–74.

52. Lee IM. Antioxidant vitamins in the prevention of cancer. Proc Assoc Am Phys 1999; 111: 10–15.