Preventive role of *Gynandropsis gynandra* L., against aflatoxin B₁ induced lipid peroxidation and antioxidant defense mechanism in rat

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*G. gynandra* extract was found to potentially diminish the rate of lipid peroxidation, with a significant increase in the levels of enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic (reduced glutathione, vitamins E and C, and uric acid) antioxidants, which were found, altered during aflatoxin B₁ (AFB₁) injection. The result confirmed that *G. gynandra* extract exerts its chemopreventive efficacy by preventing the rate of lipid peroxidation and influenced the enzymatic and non-enzymatic antioxidants in AFB₁ induced male albino rats.

Keywords: AFB₁, Antioxidants, *Gynandropsis gynandra*, Lipid peroxidation

Chemoprevention by synthetic and dietary compounds is an effective mean of controlling the incidence of hepatocellular carcinoma (HCC) either by preventing or controlling the process of carcinogenesis. In recent years there has been considerable interest in natural products with antioxidant property in human diet. Antioxidant supplement have attracted the focus of attention as potentials in the prevention of diseases caused by oxidative damage. Chemopreventive agents are reported to exert anticarcinogenic effects by modulating free radical induced lipid peroxidation.

*Gynandropsis gynandra* (L.,) Brig (Syn., *G. pentaphylla*), commonly called as velai keerai in Tamil, belongs to the family of *Capparidaceae* which is widely distributed in the tropical and sub-tropical parts of the world including India. The leaves and seeds are used in indigenous medicine in the treatment of several diseases including neuralgia, rheumatism and other local pains. The leaves are applied to boils to prevent the formation of pus. The plant is reported for its use against snakebite and the juice of the leaf as a remedy for nostalgia, antitick property and antibacterial activity. Preliminary report of Uniyal and Tewari showed that the alcoholic extract of the entire plant is found to possess anticancer activity against human epidermal carcinoma of the nasopharynx and hepatoma 129 in mice. The crude powder and alcoholic extract of aerial part of the plant exerts strong anti-inflammatory activity.

Phytochemical analysis of the plant revealed that the seeds contain cleomin, hexacosanol, free β-sitosterol and kaempferol. Flavonoids such as quercetin and kaempferol are recognized as antioxidants and inhibitors of carcinogenesis.

Glucosinolates and isothiocyanates are also found to be strong chemopreventing agents, which are essentially involved in the detoxification process. There is now agreement among oncologists that the incidence of cancer is determined by the factors in the environment and it is surprising that fungal contaminated diet is suggested to be responsible for about 30-70% of the cause of cancer. The consumption of aflatoxin-contaminated diet is an important factor in the prognosis of hepatoma. Aflatoxin B₁ (AFB₁), the most common mycotoxin is a potent mutagen and naturally occurring hepatocarcinogen. AFB₁ induced lipid peroxidation is one of the main manifestations of oxidative cellular damage. Oxidation of AFB₁ results in the formation of 8,9-epoxide intermediate, dihydrodiol metabolite and eventually dialcohol product via the action of AFB₁ aldehyde reductase. In particular, DNA damage and formation of DNA adducts may be the critical step in carcinogenesis as these radicals initiate lipid peroxidation, a damaging process in biological systems that leads to diminished antioxidant status.

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The present study is undertaken to investigate the preventive role of *G. gynandra* extract on AFB₁–mediated changes in the peroxidation level, enzymatic and non-enzymatic antioxidants in male albino rats.

**Materials and Methods**

**Animals**—Adult male albino rats (24) of Wistar strain weighing 80–120g were used. The rats were fed with commercial pelleted rat chow and water *ad libitum*. They were maintained under standard laboratory conditions with 12:12 hr light and dark cycle. All the experiments were carried out according to the guidelines of Institutional animal ethics committee.

**Chemicals**—Aflatoxin B₁ (AFB₁), glutathione, 5,5′-dithio-bis(2-nitrobenzoic acid), were purchased from Sigma Chemicals Co., St. Louis, Mo. All other chemicals and reagents used were of analytical grade with high purity and purchased locally.

**Plant material**—The whole plant of *G. gynandra* was collected during September to November from Athikkottai, Thanjavur (District). The aerial part of the plant was cut into pieces and dried under shade for a week. The shade dried plant material was coarsely powdered and extracted in 50% alcohol using a soxhlet apparatus. The extract was filtered and evaporated to obtain a semisolid residue for further use.

**Experimental design**—Animals were randomized into control and experimental groups and divided into 4 groups of 6 each. Animals in group I without any treatment served as normal control. Group II animals were injected with a single dose of AFB₁ (1mg/kg body weight, ip). Animals in groups III and IV were treated with 250mg/kg body weight, of *G. gynandra* extract through intragastric intubation, twice daily for 7 days. After the last dose of treatment the animals were fasted overnight and the animals in the group III were injected intraperitoneally (ip) with a single dose of the AFB₁ (1mg/kg body weight) in 3% DMSO.

Three days after the injection of AFB₁, the animals were sacrificed by cervical dislocation. The blood sample was collected in heparin rinsed tubes to separate the plasma. The liver and kidney were removed after perfusion with physiological saline, blotted dry, weighted and homogenized in Tris-HCl buffer of 0.1 M with pH 7.4. The 10% homogenate was used for the biochemical analysis.

Malondialdehyde (MDA) released from the endogenous lipid peroxidation was assayed in the homogenates as described by Ohkawa *et al.* Activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) were determined. The plasma vitamin E was estimated by Quaife and Diu with slight modifications as per Baker and Frank. Vitamin C was estimated as per Omaye *et al.* Uric acid was estimated by the method of Caraway. The protein content in the tissue samples was estimated following the method of Lowry *et al.* using BSA as standard.

Values are mean ± SD for 6 rats in each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparison values of P<0.05 was considered to be significant. Statistical Package for Social Studies (SPSS) 7.5 version was used for the statistical analysis.

**Results and Discussion**

Oxidative damage usually refers to the impairment of the function of cellular components by reactive oxygen species such as superoxide anions (O₂⁻), hydroxyl ions OH-, and hydrogen peroxide (H₂O₂). These agents initiate or extend cell injury by extracting hydrogen atom from polyunsaturated fatty acid and cause a degenerative process known as lipid peroxidation.

Table 1 shows the levels of malondialdehyde (MDA) and GSH in tissues of liver and kidney in control and experimental groups of animals. A significant (P<0.01) enhancement in the production of MDA and decrease in the level of GSH was observed in AFB₁ injected animals when compared with normal controls. Pretreatment with the extract of *G. gynandra* showed a low level of MDA and maintained GSH level to near normal control when compared with the AFB₁ treated group of animals.

Malondialdehyde, a major end product and index of lipid peroxidation, cross – links DNA and proteins and nucleotides on the same and opposite strand thereby promoting carcinogenesis. Elevation in the level of lipid peroxide observed in the present study is in accordance with the earlier report by Shen *et al.*, who showed an increased level of lipid peroxide in AFB₁ injected animals. Further, they have postulated that the elevated levels of reactive oxygen species following AFB₁ exposure resulted from Cyt-P₄₅₀ metabolism of AFB₁. Pre-treatment with *G. gynandra* extract had effectively controlled the rate of lipid peroxidation, which suggests the beneficial effect of
the extract against AFB1 mediated free radical formation. The presence of flavonoids (kaempferol) may contribute to this effect, because they are proved to be a potential inhibitor of lipid peroxidation34.

The enhanced formation of lipid peroxides is further evidenced by the decrease in the activities of SOD, CAT and GPx in liver and kidney tissues of AFB1 injected rats as compared with the normal control animals (Table 2). Pretreatment of rats with G. gynandra showed slight variations in the activities of enzymatic antioxidants SOD, CAT and GPx than that of control rats.

Due to increase in lipid peroxidation the level of free radicals overcome the saturation level. The decreased SOD activity in the present study may be because of highly reactive oxygen metabolites (ROMs) production. Over production of O2⋅−, itself or OH during oxidative stress due to AFB1 causes membrane damage. Results of the present study are in accordance with the report of Isabel et al35 which states that membrane damage due to over production of free radicals possibly causes conformational changes and, hence, inactivate enzymes such as SOD.

The decreased CAT activity observed in the present study may be because of higher ROMs production, especially O2⋅− which itself affects directly the CAT activity36. Under high rate of free radicals input, the enzyme inactivation prevails and the enzymatic activities are reduced leading to autocatalysis of oxidative damage process37. GPx catalyses the reduction of H2O2 at the expense of reduced GSH, thereby protecting mammalian cells against oxidative damage28,39. The decreased activity of GPx in AFB1 injected animals observed in the present study may be due to the low availability of the substrate GSH.

Pretreatment with the extract of G. gynandra 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 plant extract due to the presence of the flavonoids and β-carotene.

Table 3 shows the significant reduction in the levels of the non enzymatic antioxidants, vitamins E, C and uric acid in plasma of the AFB1 induced animals when compared with the normal control animals. The decreased vit E, C and uric acid observed in AFB1 induced animals observed may be due to excessive utilization of these antioxidants for quenching enormous free radicals produced40. The extract of G. gynandra on prior administration showed no significant reduction in the levels of vitamins E
and C and uric acid in plasma after administration of AFB1. Minimal decrease in the levels of these antioxidants may be due to the presence of the flavonoids and β-carotene in G.gynandra. Flavonoids are reported to have the ability to regenerate the reducing agents such as ascorbic acid,41 which can protect cell membranes and lipoprotein particles from oxidative damage by regenerating the antioxidant form of vitamin E,42 and thus could scavenge a wide variety of reactive oxygen species.

Vitamin E is the major lipid soluble peroxyl radical scavenger that can limit the lipid peroxidation by terminating the chain reactions initiated in the membrane lipids.43 Vitamin C and reduced GSH, the water-soluble antioxidants are able to remove the free radical from cytosol by reacting directly with them.44 The relative magnitude of the contribution of each antioxidant does not necessarily represent the relative importance of the particular antioxidant to the biological system.45

The antioxidants will react cooperatively in vivo, so as to provide greater protection to the organs against radical damage that could not be provided by any single antioxidant compounds. The decreased levels of GSH, vitamins E and C in the AFB1 injected animals in the present study ameliorates the increased rate of lipid peroxidation with a concomitant decrease in the activities of SOD, CAT, and GPx. Pretreatment of the G.gynandra extract had effectively controlled the loss of GSH, vitamins E and C and uric acid and thereby maintained the loss of the activities of SOD, CAT, and GPx.

The extract did not produce any deleterious effect on the antioxidant defense system of the normal animals which is evidenced from the non-significant alteration of the non-enzymatic and enzymatic antioxidants along with the maintained rate of lipid peroxidation in the group IV extract control animals when compared with the normal control group I animals.

From the present observation, free radical scavenging role of the extract against AFB1 induced oxidative stress could be confirmed. Further, the maintained antioxidant defense and MDA production may prove the anticarcinogenic action of the drug. Thus, G.gynandra may be a beneficial chemopreventive agent.

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