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RESEARCH ARTICLE

Chemopreventive Action of *Gynandropsis Gynandra* L., against Aflatoxin-B_1 in Rats

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Abstract: One of the most impressive finding in the field of chemoprevention is to prevent the occurrence of cancer by modulating the biotransformation of carcinogens. Our study was designed to elucidate the possible mechanism of chemoprevention by *Gynandropsis gynandra* through the biotransformation of unmetabolized aflatoxin B_1 (AFB_1) excretion in urine, distribution in liver by the phase I and phase II detoxification enzymes and the prevention of DNA damage caused by AFB_1. The animals were pre-treated with the extract of *Gynandropsis gynandra* for seven days followed by a single injection of AFB_1 dissolved in dimethyl sulphoxide (DMSO). The urine samples were collected on days one and three after AFB_1 injection and analyzed for the unmetabolized AFB_1 concentration. On day three after the injection, unmetabolized AFB_1 and GSH content in the liver was measured followed by the activities of Cyt-P_{450}, GST and QR. Pretreatment with the drug showed an enhanced rate of unmetabolized AFB_1 excretion in the urine and a diminished distribution in the liver with maintained activities of the phase I and II enzymes. These results indicate that *G.gynandra* extract affords a potent chemoprevention against AFB_1 through modulating the rate of biotransformation and detoxification and further prevented the DNA damage that was observed in AFB_1 induced male albino rats.

Keywords: Chemoprevention, Biotransformation, Aflatoxin B_1, *G. gynandra*.

Introduction

Cancer chemoprevention is defined as the prophylactic use of drugs or dietary components to block, inhibit or reverse the development of cancer in normal or pre-neoplastic tissue. The World Health Organization has called the attention of many countries to the ever increasing interest of the public in the use of herbal medicines and encourages to identify and exploit these aspects of traditional medicine that provide safe and effective remedies[1]. It is thought that increased capacity to detoxify chemical carcinogens and reactive oxygen species represents an important mechanism of chemoprotection[2]. The concept of chemoprevention was based in part of epidemiological observations suggested that high intake of vegetables could be associated with reduced risk of cancer[3]. Notably cruciferous vegetables including broccoli, cabbage, cauliflower and brussels sprout are particularly beneficial in preventing carcinogensis[4].

Gynandropsis gynandra (L.) Briq (Capparidaceae) distributed throughout the tropical and sub-tropical areas have been mentioned in the Indian system of medicine for its usefulness in various ailments[5]. Previous report from our laboratory has demonstrated the preventive effect of *G.gynandra*[6] and *Aegle marmelose* leaf extract[7] against AFB_1 induced lipid peroxidation and maintenance of the antioxidant defenses and potentially regulated the altered glucose metabolizing enzymes during AFB_1-induced carcinogenesis in rats[8]. Chemical investigation of the plant has afforded several compounds such as cleome, hexacosanol, free β-sitosterol, and kaempferol[9]. In the current study, an attempt has been made to study the potential role of the hydroalcoholic extract of *G.gynandra* on biotransformation and detoxification of AFB_1 in male albino rats.

Experimental

Drug

Whole plant of *G.gynandra* was collected during the months of September to November. The aerial part of the plant was rinsed in distilled water to remove the impurities. Then the plant material was cut into pieces and dried under shade for a week time. The shade-dried material was coarsely powdered and extracted in 50% alcohol (v/v) using a soxhlet apparatus. The extract was filtered and evaporated to separate the solvent and the residue. The semi-solid residue thus obtained was stored in desiccator until further use.

Chemicals & Reagents

Aflatoxin B_1, Glutathione, NADPH and CDNB were purchased form Sigma Chemicals Co., St. Louis, Mo. All other chemicals and reagents used were of highest purity analytical grade obtained from local firms.

Animals

Albino male rats of wistar strain weighing 80-120 g were used for the study. The rats were fed with commercial pelleted rat chow and water *ad libitum.* They were maintained under standard laboratory condition with 12 h light and dark cycle. All the animal experiments were carried out according to the guidelines of Institutional Animal ethics committee.

Experimental plan

The previously acclimatized rats (2 weeks) were divided into four groups of six animals each. Group I rats were maintained as normal control. Group II rats were considered as AFB_1 positive control. Group III and IV rats were orally treated with the plant extract at a dose of 250 mg/kg body weight[6] twice a day for 7 days. After the last dose of extract treatment, rats in the Groups II and III were intraperitoneally injected with AFB_1 (1 mg/kg b.wt.) dissolved in DMSO[10].

Urine collection

The 24 h urine samples were collected on days 1 and 3 after the AFB_1 injection from the control and experimental animals with no food during the collection period. The joints between the collecting vessel and the outlet of the metabolic cage were sealed to prevent evaporation. Photolysis of aflatoxin was prevented by reducing the lighting level of the room. After measuring the volume, the urine samples were preserved in a freezed condition with a drop of HCl until analysis.

Biochemical analysis

After 3 days of the AFB_1 injection, the animals were killed by cervical decapitation under mild ether anesthesia. The liver was removed after perfusion with physiological saline, blotted

dry, weighed and homogenized in tris-HCl buffer 0.1 M (pH 7.4). The 10% homogenate was used for the estimation of Glutathione[11], determination of the activities of Cyt-P_{450}[12], Glutathione-S-transferase[13] and Quinone reductase[14]. The total protein was estimated by employing the method of Lowry *et al*.,[15].

Analysis of AFB_1

Urine sample was extracted with acetone and liver homogenate was extracted with an acetone: water mixture (23:1 v/v) and the filtrates were collected. The unmetabolized AFB_1 was estimated in urine and liver filtrates by the method of Romer[16]. High performance thin layer chromatography (HPTLC) was employed for the analysis of AFB_1 concentration.

DNA fragment analysis

Liver DNA was extracted by employing the method of Stanley *et al*.,[17]. The precipitated DNA was resuspended in TEN buffer and electrophoresed in 1.5% agarose gel containing ethidium bromide and fragments of DNA was observed under UV densitometer.

Statistical analysis

Values are mean ± SD for six rats in the 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 this analysis.

Results and Discussion

The molecular basis of resistance and susceptibility to the adverse effects of chemical carcinogens is the subject of several scientific papers. Only limited information is available on the efficiency of the drug metabolizing system during the fate of the AFB_1 in the target tissues. Cyt-P_{450} plays a vital role in the oxidation pathway of AFB_1 that leads to the formation of the aflatoxin M_1 (AFM_1), aflatoxin Q_1 (AFQ_1) and the exo-8, 9–epoxide which is responsible for its mutagenic and hepatocarcinogenic potential[18]. The reactive intermediate AFB_1-8,9-epoxide produced by microsomal Cyt-P_{450} subsequently reacts with the cellular macromolecules such as protein and DNA specifically with the 7th position of guanine[19]. Enhancement in the activity of Cyt-P_{450} in the liver of the group II AFB_1 control animals (Table 1) is well correlated with the increased activation of the AFB_1 to its metabolites which is in good correlation with the decreased elimination of unmetabolized AFB_1 (Figure 1) observed in the urine samples of these animals.

Table 1. Levels of Cyt-P_{450}, Glutathione (GSH), Glutathione S-transferase (GST) and uinine reductase (QR) in the liver of control and experimental animals

GROUPS	Cyt-P_{450}, nmoles/mg protein	GSH, mg/g tissue	GST $^{\$}$	QR$^{@}$
GROUP I	0.184±0.04	0.267±0.04	0.413±0.034	0.739±0.037
GROUP II	0.431±0.02*	0.100±0.013*	0.173±0.025*	0.335±0.030*
GROUP III	0.240±0.04*	0.203±0.025*	0.296±0.029*	0.629±0.025*
GROUP IV	0.194±0.01^{NS}	0.362±0.008*	0.532±0.051*	0.932±0.039^{NS}

*Values are expressed as mean ±S.D. for six animals. Statistical significance: Group I vs. II & IV Group II vs. III. P values *<0.05; NS, not significant. $^{\$}$nmoles of CDNB-GSH conjugate formed m^{-1} mg^{-1} protein. $^{@}$nmoles of DCPIP reduced per minute*

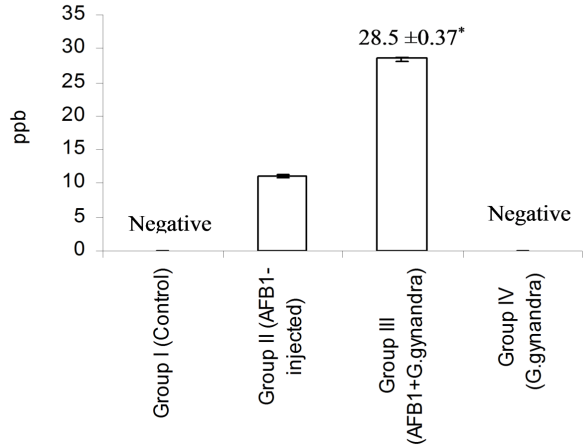

Figure 1. AFB1 in urine samples of control and experimental rats

*(Values are expressed as mean ± SD for six animals in each group. Statistical significance: Group II vs. III. P values *<0.05; NS, not significant)*

Animals pretreated with the hydro alcoholic extract of *G.gynandra* showed a decreased activity of Cyt-P_{450} enzyme, which could be seen through the increased excretion of unmetabolized AFB_1 in the urine sample. Further it can be related to the decreased rate of metabolism by the liver and increased excretion of the kidney as reported in earlier studies[20]. These observations are also in agreement with the variation in the activities of both phases I and II enzymes along with the GSH content (Table 1).

Detoxification of the epoxide metabolites is a result of the enzyme-mediated hydrolysis or conjugation with nucleophile, GSH. The role of GST in catalyzing the reaction of a wide variety of electrophiles with GSH has been well established and the formation of GSH-conjugates of xenobiotics has been associated with the cellular detoxification system[21]. Decrease in the concentration of reduced GSH and the activity of GST and QR are reported to play a significant role in the AFB_1 induced hepatocarcinogenesis[22]. Results of the present study are in consistent with the earlier report in which the GSH content and the activity of GST were found decreased in the AFB_1 induced rats[23]. On pre-treatment, the drug had provided protection to the tissue from the damages mediated by the electrophilic metabolites produced by the action of Cyt-P_{450} through the enhancement in the level of GSH and the activities of GST and QR. The induction of QR is reported to facilitate the bio-reductive reduction of hydroxy quinone, could be conjugated by glucuronide or sulphate and excreted[24]. Thus, the maintained level of GSH, activities of GST and QR suggests further metabolic disposition of chemical carcinogen AFB_1. Further, pretreatment of rats with the *G.gynandra* extract in our present study clearly demonstrates its preventive role on DNA strand breaks in hepatic cells of rats injected with a single dose of AFB_1 (Figure 2). Thus suggesting the formation of less reactive metabolites from AFB_1 due to lower expression of phase I enzyme Cyt-P_{450} which involved in the bioactivation[25] of AFB_1. However simultaneous induction of GSH, GST and QR by the *G. gynandra* may contribute to the detoxification of AFB_1. Pretreatment with the extract of *G.gynandra*, resulted in the inhibition of AFB_1 mutagenicity/carcinogenicity in rats. The present observation is similar to the earlier evidences with dietary allyl sulfides, which have been proved to modulate the drug-metabolizing enzymes and decreased DNA damage in rats injected with chemical

carcinogens such as AFB_1 and *N*-nitrodimethylamine respectively[26]. A recent report by Sheen *et al.,*[27] also indicates that the active principles of garlic might protect hepatocytes from AFB_1-induced DNA damage via increasing the activities of detoxification enzymes GST and GPx.

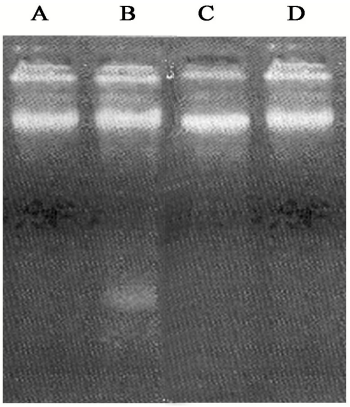

Figure 2. AFB1 induced DNA fragmentation in liver of control and experimental rats

Lane A: Group I – control, Lane B: Group II – AFB_I – Induced, Lane C: Group III – AFB_I + GGE Treated, Lane D : Group IV – GGB alone Treated

The protection against carcinogenicity is based on the alteration of activation/ detoxification balance[28]. Increased activities of GST and QR along with the GSH contents observed in group III animals might reduce the hepatic AFB_1-DNA binding and AFB_1 hepatocarcinogenesis by inactivation of the reactive AFB_1-epoxide produced by the action of Cyt-P_{450}. Vitamin A has been found to inhibit the formation of DNA adduct[29] by AFB_1. *β*-carotene present in *G.gynandra* might be responsible to cause significant inhibition on the formation of AFB_1-DNA adduct in the present study. Plant flavonoids[30] and *β*-carotene[31] are reported to modulate the activities of phase I and II detoxification enzymes also to regulate the hepatic GSH content.

Conclusion

Results from our study proves the chemopreventive ability of *G.gynandra* extract against high risk of aflatoxin exposure and development of HCC by obviating the ill effects of the AFB_1 and its metabolites via., modulating the metabolism with enhanced biotransformation and detoxification. The protection to the cells afforded by the *G.gynandra* is due to the presence of flavonoids and *β*-carotene.

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