Antioxidant Potential of *Aegle marmelos* Leaves Against Aflatoxin B₁ Induced Liver Toxicity in Rats

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**Abstract**: *Aegle marmelos* leaves have been used for treatment of various liver ailments from time immemorial. Pre-treatment of *A. marmelos* leaves crude powder at its therapeutic dose (1000 mg/kg body weight) was carried out to determine the efficacy on aflatoxin B₁ (AFB₁) (1 mg/kg body weight) induced liver toxicity in rats. The crude powder of *A. marmelos* was found to be a potent inducer of the phase II detoxifying enzyme GST and promotes the antioxidant activity by controlling lipid peroxidation in the liver of AFB₁ intoxicated male albino (Wistar) rats.

**Keywords**: Aflatoxin B₁, *Aegle marmelos*, Detoxification, Antioxidants

**Introduction**

Mycotoxicosis due to aflatoxins is a major health hazard to man. Acute and sub-acute poisoning affects the liver of the population consuming aflatoxin contaminated foods. Aflatoxins are produced by the common fungal molds *Aspergillus flavus* and *Aspergillus parasiticus*. They are implicated in the high incidence of hepatocellular and lung carcinoma in humans and animals. The degree that AFB₁ contributes to risk of HCC may be influenced by both genetic and environmental factors. An earlier report shows that AFB₁ at a dose of 1mg/kg body weight enhances the rate of lipid peroxidation thereby causing hepatotoxicity. 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, as these radicals initiate lipid peroxidation, a damaging process in biological systems that leads to diminished antioxidant status.

Medicinal plants and their active principles have attracted the focus of attention as potential chemopreventive agents. *Aegle marmelos* Linn. (Rutaceae), has high priority and applicability in the indigenous system of traditional medicines from time immemorial. The plant have various important medicinal properties, especially the fresh juice of the leaves is used in the treatment of jaundice. It has been reported that a number of chemical constituents are present in *A. marmelos* such as, alkaloids, coumarins, terpenoids, fatty acids
and amino acids. In leaves rutin, flavon-3-ols, anthocyanins, leucanthoyannis, flavone-glycosides and tannis. Further these active principles have been reported to exert their anti-carcinogenic effects by modulating the free radical-induced lipid peroxidation and antioxidant potentials.

The current focus of chemoprevention is on the control of intermediate biomarkers which are capable of detected during earlier stages can be correlated with inhibition of carcinogenic process. The naturally occurring sulfydryl compound Glutathione (GSH) and the GSH-dependent enzymes, Glutathione peroxidase (GPx) Glutathione reductase (GR) and Glutathione-S-Transferase (GST) have been considered as significant biomarkers of chemoprevention owing to their antioxidant and detoxification properties. The structural and functional damage caused by lipid peroxidation may lead to an increase in the production of thiobarbituric acid reactive substances (TBARS), which was evidenced in the AFB1 induced rat hepatocytes.

The present study was undertaken to investigate the protective role of A.marmelos (leaves) crude powder pre-treatment on AFB1-induced alterations in the levels of TBARS, GSH in liver and kidney and the activities of the GSH-dependent enzymes in liver of male albino (Wistar) rats.

**Experimental**

Fresh leaves of A.marmelos were collected in the month of September and air-dried. The dried materials were powdered, sieved through a mesh cloth and was used as drug in the crude form in physiological saline.

**Chemicals**

Aflatoxin B1, reduced and oxidized glutathione, FAD, NADH, NADPH and Thiobarbituric acid were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals used were of highest available purity obtained from local firms.

**Animals and Diet**

Male albino rats (Wistar strain) weighing about 110-130 g were used for the experiments. They were maintained under standard experimental conditions (temperature 27±1 °C; relative humidity 60±5% and 12 h light/dark cycle) and fed with standard pelleted diet and water ad libitum. All the animal experiments were carried out according to the guidelines of Institutional Animal Ethics Committee.

**Experimental design**

The animals were divided into 4 groups of six each. Group I animals received a single dose of 3% DMSO (0.5 mL i.p.,) and served as normal control. Group II and III animals were injected with AFB1 (1mg/kg body weight), intraperitoneally in 3% DMSO. Group III and IV animals received oral administration of drug (100 g/kg body weight) in physiological saline twice daily for 7 days followed by a single injection of AFB1 (1 kg/kg body weight), after the last dose of drug treatment to the group III animals alone. The animals were killed by cervical dislocation 72 h after the AFB1 injection. Liver and kidney tissues were immediately excised, weighed and then homogenized to get a 10% tissue homogenate using 0.1 M Tris-HCl buffer (pH 7.4) and used for the estimations.
Biochemical analysis

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was assayed in tissue samples as described by Ohkawa et al.,12. GSH was estimated by the method of Ellman13. The activity of GST was assayed by the method of Habig et al.,14. Activities of GPx and GR were assayed by the method of Rotruck et al.,15 and Horn et al.,16 respectively. Tissue protein was estimated by the method of Lowry et al.,17 using bovine serum albumin as standard.

Statistical analysis

Values are mean ± SD for six rats in each group and statistical differences between mean values were determined by one way analysis of variance by Tuke’s test for multiple comparisons as post test.

Results and Discussion

Table 1 indicates the level of TBARS and GSH content in liver and kidney of control and experimental animals. The level of TBARS was significantly (p<0.001) increased in group II when compared with group I animals. In group III the level was significantly less when compared to group II. In group IV the level was found to be reduced than group I. GSH content was significantly (p<0.001) decreased in group II when compared to group I. In group III the level was normal when compared with group II. The level of GSH in group IV was maintained as in group I.

Table 1. Levels of TBARS and GSH content in Liver and Kidney of control and experimental rats (mean ± SD; n=6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBARS ‡</td>
<td>0.86±0.009</td>
<td>1.78±0.013 *</td>
<td>0.89±0.006 *</td>
<td>0.80±0.001 *</td>
</tr>
<tr>
<td>GSH §</td>
<td>2.0±0.22</td>
<td>0.6±0.38 *</td>
<td>1.72±0.48 *</td>
<td>2.79±0.45 *</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBARS ‡</td>
<td>0.68±0.007</td>
<td>1.58±0.02 *</td>
<td>1.05±0.002 *</td>
<td>0.65±0.005 *</td>
</tr>
<tr>
<td>GSH §</td>
<td>0.9±0.10</td>
<td>0.5±0.01 **</td>
<td>0.86±0.02 **</td>
<td>1.0±0.14 *</td>
</tr>
</tbody>
</table>

Statistical significance: Group I vs. Group II, Group II vs. Groups III and IV. *P<0.001, **P<0.01

‡nMoles gm⁻¹ tissue. §μMoles gm⁻¹ tissue

Activities of glutathione dependent enzymes in liver of the control and experimental group of rats were tabulated in Table 2. The activities of GST, GPX and GR were found to be significantly (p<0.001) decreased in group II when compared with group I animals. In group III, significant (p<0.001) variation in the activities of the enzymes was observed when compared with group II animals.

Table 2. Activities of glutathione dependent enzymes in liver of control and experimental rats (mean ± SD; n=6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST †</td>
<td>105.15±1.5</td>
<td>87.47±1.2 **</td>
<td>93.18±2.6 **</td>
<td>127.26±2.5 **</td>
</tr>
<tr>
<td>GPx §</td>
<td>2.70±0.13</td>
<td>1.87±0.046 **</td>
<td>2.50±0.041 **</td>
<td>3.26±0.042 **</td>
</tr>
<tr>
<td>GR ‡</td>
<td>2.26±0.18</td>
<td>1.51±0.24 *</td>
<td>2.18±0.26 **</td>
<td>3.27±0.36 *</td>
</tr>
</tbody>
</table>

Statistical significance: Group I vs. Group II, Group II vs. Groups III and IV. *P<0.001, **P<0.01.

†nmoles of CDNB conjugate formed min⁻¹ mg protein⁻¹. §nmoles of GSH oxidized min⁻¹ mg protein⁻¹. ‡U mg protein⁻¹
Wide ranges of studies have shown that several naturally occurring compounds possess significant anti-tumor promoting activity due to their antioxidant nature. The primary requisite for the process of carcinogenesis is the macromolecular damage by free radicals and it is evidenced from elevated level of TBARS due to AFB₁. Thus the enhanced TBARS observed in the present study can be attributed by excessive generation of free radicals by AFB₁. In addition, a decrease in the total GSH content observed in our study might be a consequence of the diminished activities of GPx, GR and GST in AFB₁ induced rats.

Pre-treatment with the drug had minimized the production of lipid peroxides and influenced the concentrations of antioxidants and the detoxifying enzyme profile in liver and kidney of the animals in the present study. It is reported that plant anti-carcinogens have the potential to modulate the biotransformation of carcinogens. Glutathione, a physiologically important nucleophile, in conjunction with GPx, GR and GST is involved in the detoxification of carcinogens and reactive oxygen species. Chemopreventive agents are known to induce GSH and GSH-dependent enzymes at various sites, in addition to the target organs. In particular, inducers of GSH-dependent enzymes GPx, GR and phase II detoxification enzyme GST are considered to be potential chemopreventive agents. GST families are important candidates for involvement in susceptibility to aflatoxin related liver cancer, because they may regulate an individual’s ability to metabolize the ultimate carcinogen of aflatoxin, the exo-epoxide that plays a pivotal role in protection against carcinogenesis. Phase II enzyme induction is a common feature of many chemoprotectants and the evidence is strong that phase II induction before or during exposure to carcinogens can decrease or inhibit carcinogenesis.

The observed protective capacity of the crude powder of Aegle marmelos (leaves) can be attributed to their phytochemical constituents, most of which are recognized as antioxidants and inhibitors of carcinogenesis.

References
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