Modulatory effect of *Gynandropsis gynandra* L. on glucose metabolizing enzymes in aflatoxin B\textsubscript{1}-induced hepatocellular carcinoma in rats

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The modulation of glucose-metabolizing enzymes activities play a vital role in the depletion of energy metabolism and leads to inhibition of cancer growth. In the present study, the effect of *Gynandropsis gynandra* L. extract on aflatoxin B\textsubscript{1} (AFB\textsubscript{1})-induced hepatocellular carcinoma (HCC) was studied on glucose-metabolizing enzymes in rats. A significant increase ($p<0.001$) in the activities of the key glycolytic enzymes viz., hexokinase and phosphoglucoisomerase, with a significant decrease ($p<0.001$) in the gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase were observed in HCC-bearing rats, when compared with the control. Administration of *G. gynandra* extract caused a significant decrease in the activities of glycolytic enzymes and an increase in the gluconeogenic enzymes activities to near normal values. Thus, findings suggest the *G. gynandra* extract has a definite modulating role on the key enzymes of glucose metabolism in HCC. The modulatory effect may be due to the phytoactive constituents present in the extract of *G. gynandra*.

Keywords: Aflatoxin B\textsubscript{1}, Glucose-metabolizing enzymes, *Gynandropsis gynandra*, Hepatocellular carcinoma, Modulatory effect

*Gynandropsis gynandra* L. (Capparidaceae) (English: “Caravella” and Tamil: “Velai keerai”) is an important medicinal herb distributed in the tropical and sub-tropical parts of the world, including India. The plant has been used in the traditional medicine for the treatment of rheumatism, headache, epileptic fits, stomach ache, conjunctivitis, stiffneck, scurvy, earaches, and severe infection of threadworms. Many compounds, mainly flavonoids and flavone glycoside, β-carotene, tannins have been identified from the plant. The aqueous extract of the whole plant shows antibacterial and antioxidant activities, whereas alcoholic extract exhibits anticancer activity against human epidermal carcinoma and hepatoma 129 in mouse. Previous studies from our laboratory show anti-inflammatory activity of the crude powder and 50% hydroalcoholic extract of the aerial parts of *G. gynandra*. The 50% hydroalcoholic extract of the plant has also shown anticancer effect in aflatoxin B\textsubscript{1} (AFB\textsubscript{1})-induced hepatocellular carcinoma (HCC) in rats and preventive effect against AFB\textsubscript{1}-induced lipid peroxidation.

The development of tumors is accompanied by characteristic alterations in the activities of enzymes, particularly those involved in carbohydrate metabolism. The growth rate of hepatomas and their glycolytic enzyme activities are significantly correlated. Accelerated rate of glucose transport and alterations in the cellular levels and regulatory properties of key glycolytic enzymes accounts for the abnormal metabolic properties of many tumors. Studies show that alteration in the patterns of glucose metabolism and relevant genes is coordinated with activities of glycolytic and gluconeogenic enzymes during the development of tumor. As a definite correlation exists between tumor progression and the activities of glycolytic and gluconeogenic enzymes, alterations in their activities can be used as a marker of diagnosis and prognosis.

In the present study, the effect of *G. gynandra* extract has been studied on glucose-metabolizing enzymes in aflatoxin B\textsubscript{1} (AFB\textsubscript{1})-induced hepatocellular carcinoma (HCC) in rats.

**Materials and Methods**

**Materials**

Aflatoxin B\textsubscript{1} (AFB\textsubscript{1}) was purchased from Sigma Chemicals Co., St. Louis, Mo, USA. All other chemicals and reagents used were of the highest purity analytical grade and obtained from local firms.

The whole plant of *G. gynandra* was collected during September to November 2005 from Athikkottai, Thanjavur, Tamil Nadu. Aerial parts were rinsed in distilled water to remove impurities, cut into pieces, dried under shade for a week, 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.

**Animals and treatment**

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* and maintained under standard laboratory condition with a 12 h light and dark cycle. All the animal experiments were carried out according to the guidelines of the Institutional Animal Ethics Committee.

The rats were divided into four groups of six animals each. Group I animals received a single intraperitoneal dose (0.5 ml) of DMSO. Hepatocellular carcinoma (HCC) was induced in group II animals by a single dose of AFB$_1$ in DMSO (2 mg kg$^{-1}$ body wt$^{12,18}$) intraperitoneally, and confirmed by histological examination (not presented here). HCC was also induced in group III animals and, they were treated with the drug *G. gynandra* extract (250 mg kg$^{-1}$ body wt$^{10,11,12}$ orally, twice daily for 3 weeks) by intragastric intubation. Group IV animals served as control and received the same dosage of drug as group III animals by the same route.

At the end of experiments, rats were fasted overnight and sacrificed 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 determination of the activities of hexokinase,$^{20}$ phosphoglucosomerase,$^{21}$ glucose-6-phosphatase,$^{22}$ fructose-1,6-bisphosphatase.$^{23}$ Total protein was estimated by employing the method of Lowry *et al.*$^{24}$

**Statistical analysis**

The values were mean ± SD for six rats in each group and statistically 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 were considered significant. Statistical package for social studies (SPSS) 7.5 version was used for this analysis.

**Results and Discussion**

The activities of glycolytic enzymes viz., hexokinase, phosphoglucosomerase and gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-bisphosphatase in liver homogenates are shown in Table 1. Significant increase (*p*<0.001) in the activities of hexokinase and phosphoglucosomerase, with a significant decrease (*p*<0.001) in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase in liver homogenate were observed in the HCC bearing group II animals, when compared with control. Whereas in group III animals, the enzyme activities were reversed almost to near normal levels. Group IV drug control animals did not show any significant variation in glycolytic and gluconeogenic enzymes, when compared with group I.

The cancer cells possess an abnormal pattern of energy metabolism, when compared with the normal cells. Studies on experimental hepatomas have shown that metabolic alterations in the tumors are often accompanied by changes in the activities of various enzymes, including key enzymes of carbohydrate metabolism.$^{10}$ Many cancer cell lines have shown a marked preferential utilization of glycolytic metabolism to meet their increased energy demands. Rapidly growing, highly malignant tumour cells can obtain up to 60% of their total ATP production from glycolysis.$^{25}$ An elevated rate of glycolysis in tumour

<table>
<thead>
<tr>
<th></th>
<th>Group I (Normal control)</th>
<th>Group II (AFB$_1$ control)</th>
<th>Group III (AFB$_1$+Drug)</th>
<th>Group IV (Drug control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase$^a$</td>
<td>17.51 ± 0.49</td>
<td>35.98 ± 0.50*</td>
<td>19.47 ± 0.68*</td>
<td>17.57 ± 0.34$^\text{NS}$</td>
</tr>
<tr>
<td>Phosphoglucosomerase$^a$</td>
<td>13.31 ± 0.45</td>
<td>33.67 ± 0.38*</td>
<td>21.54 ± 0.41*</td>
<td>13.18 ± 0.28$^\text{NS}$</td>
</tr>
<tr>
<td>Glucose-6-phosphatase$^a$</td>
<td>12.20 ± 0.47</td>
<td>9.58 ± 0.53*</td>
<td>11.42 ± 0.59*</td>
<td>11.95 ± 0.55$^\text{NS}$</td>
</tr>
<tr>
<td>Fructose-1, 6-bisphosphatase$^a$</td>
<td>18.71 ± 0.60</td>
<td>13.31 ± 0.45*</td>
<td>18.40 ± 0.32*</td>
<td>18.02 ± 0.33$^\text{NS}$</td>
</tr>
</tbody>
</table>

*Statistical significance: Group I vs Groups II & IV Groups II vs Group III

*P* values *<*0.001; NS, not significant

$^a$nmoles of glucose-6-phosphate formed min$^{-1}$ mg protein$^{-1}$; $^b$nmoles of fructose formed min$^{-1}$ mg protein$^{-1}$; $^c$nmoles of inorganic phosphorus liberated min$^{-1}$ mg protein$^{-1}$
cells results in an increase in the intracellular concentration of glucose-6-phosphate, a key precursor in the de novo synthesis of nucleic acids, phospholipids and other macromolecules. An enhanced rate of synthesis of the above mentioned compounds are essential to keep pace with rapid cell division and membrane biosynthesis during tumour growth.16

A direct correlation has been observed between glycolytic activity and hexokinase in a variety of tumour cell lines. Hexokinase levels are important in determining the glycolytic capacity of cancer cells.26 Increased activities of hexokinase and phosphoglucoisomerase during development of tumour cells observed in the present study are in agreement with the findings of earlier study,27 wherein the increased activities of glycolytic enzymes have been found to correlate with the degree of malignancy in different tumor types. High levels of hexokinase reported in Novikoff and Zajdela hepatomas16 signify the functional importance of hexokinase in tumor cells to utilize excess glucose for the production of ATP. Elevated level of phospho-glucoisomerase reported in sarcoma, and in cancers of lung, rectum and breast28 is an indicator of metastatic growth and increases specifically after metastasis.29 Its increased activity in liver of AFB1-induced HCC rats may be due to its level in malignant tissues.28

Glucogenes is a biochemical process almost completely restricted to the liver.30 Gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-bisphosphatase have shown a preferential localization in different zones of hepatic lobules, thus diseases affecting this organ can be diagnosed by the measurement of activity of certain enzymes of this pathway.31,32 The progressive failure of glucogenes, manifested most extensively in rapidly growing tumours such as hepatomas is explained partly by a marked decrease or complete absence of glucose-6-phosphatase and fructose-1,6-bisphosphatase activities.33

The inhibition of activities of gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase in group II AFB1-induced rats was in accordance with the earlier report.33 Glucose-6-phosphatase is also reduced in residual liver tissue of Novikoff hepatoma.34 Decreased rate of glucose-6-phosphatase mediated dephosphorylation is also reported in malignant cells.35 Decreased activity of fructose-1,6-bisphosphatase, the key regulatory enzyme for the synthesis of glucose-6-phosphatase from pyruvic acid observed in liver of group II rats is supported by an earlier report,36 which reported that in Novikoff hepatoma, there appears to be an absence of fructose-1,6-bisphosphatase in the tumour and consequently a block in the pathway, leading to the synthesis of glucose-6-phosphate from pyruvate.

A sharp drop in the activities of hexokinase and phosphoglucoisomerase and a significant increase (P<0.001) in the activities of liver glucose-6-phosphatase and fructose-1,6-bisphosphatase observed on oral administration of the extract of G. gynandra to AFB1-induced group III rats correspond to the return of the tumor towards its normal states and are consistent with earlier reports on the herbal extracts, which have shown effect on glucose-metabolizing enzymes.

Comparison of groups I and IV animals shows no significant variation in the key regulatory enzyme activities of both glycolytic and gluconeogenic pathways. It could be presumed that the G. gynandra extract has modulatory activity on the carbohydrate metabolism in AFB1-induced HCC bearing rats through a mechanism that does not provoke any acute biochemical disturbances in the metabolic pathways of glycolysis and gluconeogenesis. The modulatory effect of G. gynandra extract may be attributed to the presence of active compounds such as polyphenols and flavonoids. Earlier studies have also shown that Semecarpus anacardium and Terminalia arjuna, which are rich in flavonoids and polyphenols modulate the glucose-metabolizing enzymes in HCC rats.37,38 The extract treatment might lead to depletion of energy metabolism in cancer tissue by inhibiting the glycolytic enzymes and regulating the gluconeogenic enzymes.

In conclusion, the findings suggest the G. gynandra extract has a definite modulating role on the key enzymes of glucose metabolism in hepatocellular carcinoma and this may be through its potency in the normalization of abnormal cell’s behavior.

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