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ORIGINAL ARTICLE

Modulatory Role of *Shorea robusta* Bark on Glucose-metabolizing Enzymes in Diethylnitrosamine Induced Hepatocellular Carcinoma in Rats

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ABSTRACT

Introduction: The modulations of glucose-metabolizing enzyme activities play a vital rolein the depletion of energy metabolism and leads to inhibition of cancer growth. **Objective:** To find the effect of *shorearobusta* bark extract on glucose-metbolizing enzymes in diethylnitrosamine (DEN) induced hepatocellular carcinoma rats. **Materials and Methods:** Biochemical evaluation of glucose metabolizing enzyme were done in before and after *shorearobusta* bark extract (500mg/kg) treatment in DEN induced rats. **Results:** A significant increasein the activities of the key glycolytic enzymes viz., hexokinase and phosphoglucoisomerase, with a significant decrease in the gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatasewere observed in HCC bearing rats, when compared with the control. Administration of *shorearobusta* extract caused a significant decrease in theactivities of glycolytic enzymes and an increase in the gluconeogenic enzymes activities to near normal values. **Conclusion:** The current findings suggest that the *S. robusta* extract has a definite modulating role on the key enzymes ofglucose-metabolism in HCC. The modulatory effect may be due to the phytoactive constituents present in the extract of *S. robusta*.

Key words: Diethylnitrosamine, glucose-metabolizing enzymes, hepatocellular carcinoma, *Shorea robusta*

SUMMARY

- Administration of *shorea robusta* bark extract caused a significant decrease in the activities of glycolytic enzymes and an increase in the gluconeogenic enzymes activities to near normal values. The S. *robusta* extract has modulatory activity on the carbohydrate metabolism in DEN-induced HCC bearing rats through a mechanism that which does not provoke any acute biochemical disturbances in the metabolic pathways of glycolysis and gluconeogenesis. The modulatory effect of S. *robusta* extract may be attributed to the presence of active compounds such as polyphenols and flavonoids.

Abbreviations used: HCC: Hepatocellular Carcinoma, SRBE: Shorearobusta bark extract; HEX: Hexokinase; PGI: Phosphoglucoisomerase; DEN: Diethylnitrosamine.

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INTRODUCTION

In developing countries about 35% of prescribed drugs are derived from natural products. Many investigations are being carried out worldwide to discover naturally occurring compounds which can suppress or prevent the progress of carcinogenesis.[1] In recent years, there has been considerable emphasis on the identification of plant products with antioxidant property, as free radicals are considered to play a major role in most of the diseases including cancer. The medicinal value of the chosen plant *Shorea robusta* bark has been extensively worked out. However, its therapeutic efficacy in anticancer activity has not been evaluated. *S. robusta* is a tropical hardwood found and developed in the South-East Asia. It prospers most commonly in Indonesia but can also be seen in Malaysia, the Philippines and certain parts of the Southern India.[3] Bark is a dark brown and thick, with longitudinal fissures deep in poles, becoming shallow in mature trees; provides effective protection against fire. Traditionally the plant is used for dysentery, ulcers, jaundice, wounds, gonorrhea, and leprosy. The bark used as astringent, acrid, cooling, antihelmintic, alexeteric, anodyne, constipating, urinary astringent, union promoter depurative, and tonic.[2] The major chemical constituents of *S. robusta* are reported to contain flavonoids, steroids, terpenoids, phenols, and cardioglycosides.[3,4]

The development of tumors is accompanied by characteristic alterations in the activities of enzymes, particularly those involved in carbohydrate metabolism.[5,6] The growth rate of hepatomas and their glycolytic enzymes activities are significantly correlated.[7,8] Many tumors accelerated the rate of glucose transport, alteration in the cellular levels and regulatory properties of key glycolytic enzymes.[9] Previous studies show that alteration in the patterns of glucose metabolism and relevant genes are coordinated with activities of glycolytic and gluconeogenic enzymes during the development of tumor.[10] As a definite correlation exists between tumor progression and the activities of glycolytic and gluconeogenic enzymes,[11] alterations in their activities can be used as a marker of diagnosis and prognosis. In the present study the effect of *S. robusta* bark extract (SRBE) has been studied on glucose-metabolizing enzymes in diethylnitrosamine (DEN) induced hepatocellular carcinoma (HCC) in rats.

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MATERIALS AND METHODS

Animals

Male albino rats of wistar strain approximately weighing 180–220 g were used in this study. They were healthy animals procured from Sri Venkateswara Enterprises, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (temperature 27°C ± 2°C and 12 h light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. They were acclimatization to the environment for 1 week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India.

Chemicals

Nitro blue tetrazolium, ethylene diamine tetraacetic acid (EDTA), trichloro acetic acid, thiobarbituric acid, 1-chloro-2,4-dintiro benzene, 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (reduced), glutathione (oxidized), DEN and L-ascorbic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Plant materials and preparation of plant extract

The barks of the *S. robusta* were collected from the Koli hills, Tamil Nadu, India. The collected plant materials were washed, sliced and completely dried in a hot-air oven at 37°C. The dried materials were ground into make a fine powder and used for the extraction. Three hundred grams of the powered plants were extracted with ethanol (70%) using "Soxhlet Apparatus" for 48 h. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in the refrigerator until used. The extract contains both polar and nonpolar phytocomponents. For experiments 500 mg/kg body weight of SRBE was used. This effective dose was selected based on dose dependent studies of SRBE carried out in our laboratory.

Dosage fixation

Different doses of SRBE (50 mg, 100 mg, 250 mg, 500 mg, and 750 mg/kg body weight) were treated for 4 weeks in rats. The effective dose of SRBE was assessed based on the contents of liver lipid peroxidation (oxidative damage marker). Supplementations of SRBE at doses of 250 mg, 500 mg, and 750 mg/kg body weight for 4 weeks were found to be effective in aged rats. Among these doses, the minimal effective dose 500 mg was fixed as therapeutic dosage for the subsequent studies.

Experimental design

Body weights of the animals were recorded, and they were divided into four groups of six animals each as follows:

Group I: Normal control rats fed with standard diet and served as a control, which received saline

Group II: Rats treated with *S. robusta* bark alone by oral gavage daily at a dose of 500 mg/kg body weight (based on effective dosage fixation studies) for 16 weeks

Group III: Rats induced with HCC by providing 0.01% DEN through drinking water for 16 weeks

Group IV: Rats pretreated with *S. robusta* bark intragastrically at the dose of (500 mg/kg body weight) for 1 week before the administration of DEN, *S. robusta* bark, and DEN administration continued till the end of the experiment (i.e. 16 weeks).

Collection of samples

On completion of the experimental period, animals were anesthetized with thiopentone sodium (50 mg/kg). The blood was collected with or without EDTA as an anticoagulant. Blood, plasma, and serum were separated for the estimation of various biochemical parameters. The Liver was dissected out, washed in ice-cold saline, and weighed. A known weight of them was used for homogenate preparation and used for various biochemical analyzes.

Evaluation of biochemical parameters

The total protein in the liver was estimated by the method of Lowry *et al*. The activities of carbohydrate enzymes hexokinase (HEX) was assayed by the method of Brandstrup *et al*.,[12] phosphoglucoisomerase (PGI) was assayed by the method of Horrocks *et al*.,[13] glucose-6-phosphatease was assayed according to the method of Koide and Oda,[14] fructose-1,6-diphosphatase was assayed by the method of Gancedo and Gancedo.[15] The phosphorus content of the supernatant was estimated according to the method described by Fiske and Subbarow.[16]

Statistical analysis

Values were expressed as mean ± standard deviation for six rats in the each group and statistically significant differences between mean values were determined by one-way analysis of variance followed by the Tukey's test for multiple comparisons.[17] Statistical analysis carried out by Graph Pad Instat software (Graph Pad Software, San Diego, CA, USA) 3 version was used. A value of $P < 0.001$ was considered statistically significant.

RESULTS

The activities of glycolytic enzymes viz., HEX, phosphoglucoisomerase, and gluconeogenic enzymes, glucose-6-phosphatase, and fructose 1,6-bisphasphatase in liver homogenates are shown in Figures 1-3. A significant increase in the activities of HEX and PGI, with a significant decrease in the activities of glucose-6-phosphatase and fructose1,6-bisphosphatase in liver homogenate, were observed in the HCC bearing Group III animals when compared with control. Whereas in Group IV *S. robusta* and DEN-treated animals the enzyme activities were reversed almost to near normal levels. Group II *S. robusta* alone treated animals did not show any significant variation in glycolytic and gluconeogenic enzymes when compared with Group I rats.

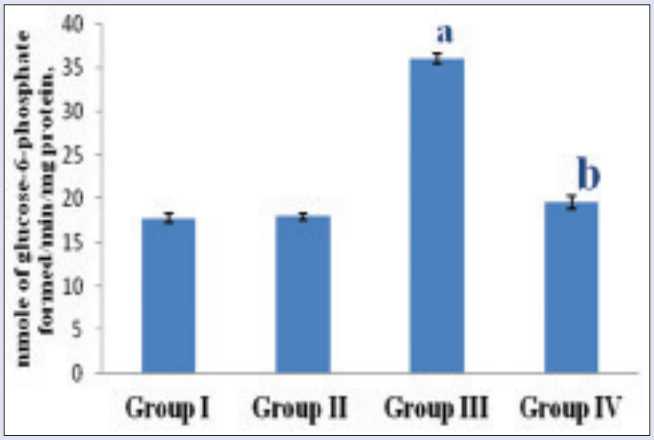

Figure 1: Effect of *Shorea robusta* bark on liver hexokinase in control and experimental rats. [a]$P < 0.001$ significantly different compared with Groups I and II control animals. [b]$P < 0.001$ significantly different compared with Group III animals

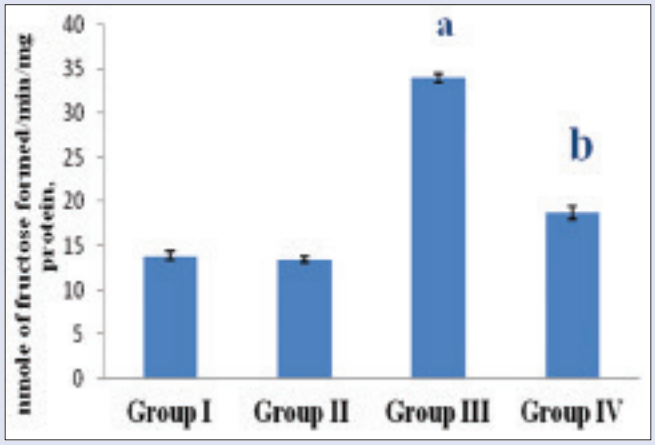

Figure 2: Effect of *Shorea robusta* bark on liver phosphoglucoisomerase in control and experimental rats. [a]$P < 0.001$ significantly different compared with Groups I and II control animals. [b]$P < 0.001$ significantly different compared with Group III animals

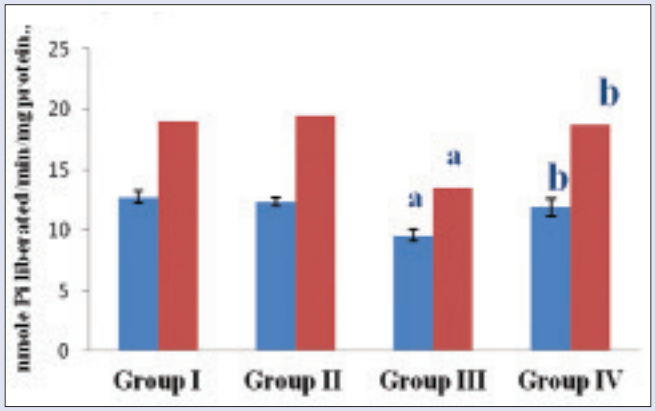

Figure 3: Effect of *Shorea robusta* bark on liver glucose-6-phosphatase and fructose-1,6-diphosphatase in control and experimental rats. [a]$P < 0.001$ significantly different compared with Groups I and II control animals. [b]$P < 0.001$ significantly different compared with Group III animals

DISCUSSION

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.[7,18] Many cancer cell lines have shown a marked preferential utilization of glycolytic metabolism to meet their increased energy demands. Rapidly growing, highly malignant tumor cells can obtain up to 60% of their total ATP production from glycolysis.[5] An elevated rate of glycolysis in tumor cells results an increase in the intracellular concentration of glucose-6-phosphate, a key precursor in the *de nove* synthesis of nucleic acids, phospholipids, and other macromolecules. An enhanced rate of synthesis of the abovementioned compounds is essential to keep pace with rapid cell division and membrane biosynthesis during tumor growth.[18,19]

A direct correlation has been observed between glycolytic activity and HEX in a variety of tumor cell lines. HEX levels are important in determining the glycolytic capacity of cancer cells.[20,21] Increased activities of HEX and PGI during development of tumor cells observed in the present study are in agreement with the finding of earlier study,[22] wherein the increased activities of glycolytic enzymes have been found to correlate with the degree of malignance in tumor tissues. High levels of HEX reported in Novikoff and Zajdela hepatomas and aflatoxin-B1 induced liver carcinoma [22-24] signify the functional importance of HEX in tumor cells to utilize excess glucose for the production of ATP. Elevated level of PGI reported in sarcoma, and in cancers of the lung, rectum and breast is an indicator of metastatic growth and increases specifically after metastasis.[25,26] Its increased activity in the liver of DEN-induced HCC rate may be due to its level in malignant tissues.[29]

Gluconeogenesis is a biochemical process almost completely restricted to the liver.[27] 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 the activity of certain enzymes of this pathway.[10,28] The progressive failure of gluconeogenesis, manifested most extensively in rapidly growing tumors such as hepatomas is explained partly by marked decrease or complete absence of glucose-6-phosphatase and fructose-1,6-bisphosphatase activities.[6]

The inhibition of activities of gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphasphatase in Group III DEN-induced rats was in accordance with the earlier report.[29,30] Glucose-6-phosphatse is reduced in the residual liver tissue of Group III DEN-induced rats was in accordance with the earlier report.[29,30] Glucose-6-phosphatse is also reduced in liver tissue of aflatoxin-B1 induced liver carcinoma.[21] Decreased rate of glucose-6-phosphatase mediated dephosphorylation is also reported in malignant cells.[31] Decreased activity of fructose-1,6-bisphosphatase, the key regulatory enzyme for the synthesis of glucose-6-phosphate from pyruvic acid observed in liver of Group III rats is supported by the earlier report,[22] which reported that in aflatoxin-B1 induced liver carcinoma, there appears to be a decreased fructose-1,6-bisphosphatase in the tumor and consequently a block in the pathway, leading to the synthesis of glucose-6-phosphate from pyruvate.

A sharp drop in the activities of HEX and phosphoglucoisomerase and a significant increase in the activities of liver glucose-6-phospatase and fructose-1,6-bisphosphatase observed on oral administration of the extract of *S. robusta* to DEN-induced Group III rats correspond to the return of the tumor toward its normal states and are consistent with earlier reports[32] on the herbal extracts, which have shown effect on glucose-metabolizing enzymes.

Comparison of Groups I and II animals are shown that no significant variation in the key regulatory enzyme activities of both glycolytic and gluconeogenic pathways. It could be presumed that the *S. robusta* extract has modulatory activity on the carbohydrate metabolism in DEN-induced HCC bearing rats through a mechanism that which does not provoke any acute biochemical disturbances in the metabolic pathways of glycolysis and gluconeogenesis. The modulatory effect of *S. robusta* extract may be attributed to the presence of active compounds such as polyphenols and flavonoids. Earlier studies have also shown that *Semecarpus anacardium*, *Hygrophila auriculata,* and *Terminalia arjuna*, which are rich in flavonoids and polyphenols modulate the glucose-metabolizing enzymes in HCC rats.[29,30,33] The extract treatment might lead to the depletion of energy metabolism in cancer tissues by inhibiting the glycolytic enzymes and regulating the gluconeogenic enzymes.

In this study, an alteration in the levels of carbohydrate metabolizing key enzymes was observed on DEN-treated rats. It can be concluded from the present data that the altered levels of HEX, PGI, fructose-1,6-bisphasphatase, glucose-6-phosphatase in HCC bearing rats were reverted significantly to near normal with the ethanolic extract of *S. robusta* bark treated rats. The plant extract might interrupt the energy requirement of tumor tissue and lead to the suppression of tumor growth due to the presence of phenols and flavonoids.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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