Efficacy of *Terminalia arjuna* (Roxb.) on N-nitrosodiethylamine induced hepatocellular carcinoma in rats

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The effect of ethanolic extract of *Terminalia arjuna* bark on carbohydrate metabolizing enzymes of N-nitrosodiethylamine induced hepatocellular carcinoma in Wistar albino rats were studied. The plasma and liver glycolytic enzymes such as hexokinase, phosphoglucoisomerase, aldolase were significantly increased in cancer induced animals while glyconeogenic enzyme, glucose-6-phosphatase was decreased. These enzymes were reverted significantly to near normal range in treated animals after oral administration of *T. arjuna* for 28 days. The modulation of the enzymes constitute the depletion of energy metabolism leads to inhibition of cancer growth. This inhibitory activity may be due to the anticancer activity of constituents present in the ethanolic extract of *T. arjuna*.

**Keywords**: *Terminalia arjuna*, Hepatocellular carcinoma, Carbohydrate metabolizing enzymes

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Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver and among the most common cancers worldwide. The attributable risk factors for HCC are viral infection, aflatoxin exposure in diets, cigarette smoking, alcohol consumption and oral contraceptives. For many years cancer chemotherapy has been dominated by potent drugs that either interrupt the synthesis of DNA or destroy its structure once it has formed. Unfortunately, their toxicity is not limited to cancer cells and normal cells are also harmed. Efforts to develop less toxic drugs that affect only malignant cells and mechanism based approach are necessary in cancer chemotherapy. According to WHO estimates, more than 80% people in developing countries depend on traditional medicine for their primary health needs. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as traditional system of medicine in India. A wide variety of chemical agents have been shown to possess chemopreventive properties against a broad spectrum of cancer. A recent survey that more than 60% of cancer patients use vitamins or herbs at some point in their therapy.

*Terminalia arjuna* Roxburgh a tropical woody tree growing throughout India and locally known as kumbuk; Arjuna in English, is a member of the Combretaceaee family, which includes 250 species of *Terminalia*. A few randomized clinical trials of unstandardised concoction of the tree bark have been performed in coronary heart disease (CHD) patients in India. Besides, the plant exhibits variety of medicinal properties such as fungicidal, antibacterial, antifertility and antihuman immunodeficiency virus induced disease. The plant was found to be useful in the treatment of cancer also. Kapoor reported that the powdered bark is traditionally used as diuretic and general tonic in cases of cirrhosis of the liver. In this context Pettit is of the opinion than that a compounds of *T. arjuna* possess cancer cell growth inhibitory activity against various cell lines such as P388, OVCAR-3, SF-295, A498, NCI-4460, KM20L2 and SK-MEL-5. Thus there is a paucity of information regarding the anticancer activity of *T. arjuna* against liver cancer. Hence, the present study has been undertaken to evaluate the anticancer activity of ethanolic extract of *T. arjuna* on carbohydrate metabolizing enzymes such as hexokinase (HEX), phosphoglucoisomerase (PGI), aldolase (ALD) and glucose-6-phosphatase (G6P) in N-nitrosodiethylamine induced liver cancer in rats.
Materials and Methods

Plant material—The fresh bark of Terminalia arjuna was collected during September 2002 in Chennai, Tamil Nadu, India. The plant was authenticated by botanist of Captain Srinivasa Murti Drug Research Centre for Ayurveda, Chennai, Tamil Nadu, India. A voucher specimen (No. 064) has been deposited in the herbarium of the same department.

Preparation of plant extract—The shade dried T.arjuna bark was coarsely powdered (1kg) and soaked in 1000 ml of ethanol for 10 days at room temperature. The extract was filtered and concentrated to obtain the solid residue and the final weight was noted. The yield of the total ethanolic extract was 8.5%. Primary phytochemical screening of the ethanolic extract of T. arjuna bark revealed the presence of triterpenoids, phenol, flavonoids, tannin and saponins.

Animals—Healthy male Wistar albino rats (60-80 g) used in the present study were obtained from Tamil Nadu Veterinary College and University, Chennai, Tamil Nadu, India. The animals were maintained at 25° ± 2° C and were provided with standard commercially available pellet diet (M/s Hindustan Lever Limited, Mumbai, India) and water ad libitum.

Experimental design—The rats were divided into four groups of 6 animals each. Group I animals were given drinking water, Groups II and III animals were administered with single i.p injection of N-nitrosodimethylamine (DEN, Sigma Chemical Company, USA) at a dose of 200 mg/kg body weight for 28 days. Group IV animals served as plant extract control.

Collection of plasma and liver homogenate—After the experimental period all the animals were sacrificed by decapitation. Blood was collected in tubes containing EDTA and centrifuged at 2000 × g for 15 min and plasma was collected. Liver was perfused in situ with cold 0.15 M NaCl at 37°C and homogenized in ice-cold 0.1M Tris-HCl buffer (pH 7.4).

Biochemical assays—Hexokinase, phosphoglucomerase, aldolase and glucose-6-phosphatase were assayed according to the method of Branstrup10, Horrocks11, King12 and Gancedo13, respectively.

Statistical analysis—Statistical differences were calculated by independent sample-‘t’ test by using SPSS 7.5 student version. The mean ± SE was compared in each group. Comparisons were made between group I and II, group I and III, group I and IV and group II and III.

Results and Discussion

The results of the efficacy of T. arjuna on plasma carbohydrate metabolizing enzymes of DEN induced hepatocellular carcinoma was presented in Table I. The levels of HEX, PGI and ALD in plasma of group II animals were elevated significantly where as G6P were decreased. In group III T. arjuna treated animals these enzymes were returned significantly to near normal range. Correspondingly the activities of HEX, PGI, ALD were increased significantly in liver tissue of group II animals when compare with group I animals (Fig.1). On the other hand, the levels of G6P in liver tissues of group II animals were decreased. These enzymes levels were normalized in T. arjuna treated animals. However, there were no significant changes observed in group III and group IV animals when compared with group I control animals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tbody>
<tr>
<td>HEX</td>
<td>11.11 ± 0.43</td>
<td>19.15 ± 0.41 a**</td>
<td>12.16 ± 0.40 aNS b***</td>
<td>9.95 ± 0.34 aNS</td>
</tr>
<tr>
<td>PGI</td>
<td>12.66 ± 0.31</td>
<td>18.12 ± 0.38 a**</td>
<td>13.07 ± 0.46 aNS b***</td>
<td>12.00 ± 0.51 aNS</td>
</tr>
<tr>
<td>ALD</td>
<td>12.52 ± 0.44</td>
<td>15.15 ± 0.36 a**</td>
<td>13.08 ± 0.35 aNS b***</td>
<td>13.16 ± 0.44 aNS</td>
</tr>
<tr>
<td>G6P</td>
<td>31.05 ± 0.49</td>
<td>21.13 ± 0.48 a**</td>
<td>29.02 ± 0.44 a NS b***</td>
<td>32.08 ± 0.51 aNS</td>
</tr>
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Unit: HEX: n moles of glucose-6-phosphate liberated/mg protein/min; PGI: n moles of fructose liberated/mg protein/min; ALD: nmol of glycerol released/mg protein/min and G6P: n moles of inorganic phosphorus liberated/mg protein/min.

a: as compared with group I; b: as compared with group II.

P values: *<0.001; **<0.01; *<0.05 and NS=Not significant.
The proportion of glucose-6-phosphate metabolized via phosphoglucoisomerase may be expected to influence cancer tissues is due to the large amount of glucose elevated level of hexokinase exceeding that of the glycolytic pathway. In hepatoma cells, type II metabolism studies the group II cancer bearing animals also show outer mitochondrial hexokinase activity is over expressed and binds to the hexokinase which catalyses the first step in the metabolism is dependent on elevated levels of hexokinase, phosphoglucoisomerase and aldolase in cancer cells.

The most common biochemical phenotype of highly malignant, rapidly growing tumors is their ability to utilize glucose at high rates. Numerous studies have demonstrated that highly malignant tumors, i.e. those that are poorly differentiated and grow rapidly, exhibit the capacity to metabolize glucose to lactate at much higher rates than normal cells. The growth rate of hepatomas and their glycolytic enzymes activities are significantly correlated. In the present study increased activities of hexokinase, phosphoglucoisomerase and aldolase in cancer bearing animals may be due to the elevated rate of glycolysis in determining the glycolytic capacity of cancer cells.

It is well known that the high rate of glucose metabolism is dependent on elevated levels of hexokinase which catalyses the first step in the glycolytic pathway. In hepatoma cells, type II hexokinase activity is over expressed and binds to the outer mitochondrial membrane. In the present studies the group II cancer bearing animals also show elevated level of hexokinase exceeding that of the normal rats. This increased level of hexokinase in cancer tissues is due to the large amount of glucose metabolism. Alteration in the activity of phosphoglucoisomerasede may be expected to influence the proportion of glucose-6-phosphate metabolized via the glycolytic pathway. This enzyme is an indicator of metabolic growth and increases in cancer condition. The group II cancer bearing animals show increased level of phosphoglucoisomerase in liver and plasma, may be due to the higher rate of glycolysis in liver and subsequent leakage of this enzyme into the blood. Aldolase, is a key enzyme in glycolytic pathway and elevated levels of this enzyme were reported in metastatic conditions of breast tissues. In group II cancer bearing animals the activities of aldolase was increased. Aldolase was less frequently raised than phosphoglucoisomerase.

The reduction in gluconeogenesis is manifested rapidly in growing tumors in which the levels of glucose-6-phosphatase were decreased. Schamhart reported decreased activities of glucose-6-phosphatase in hepatoma cells, revealing the progressive failure of gluconeogenesis in liver tumor. In the present investigation, glucose-6-phosphatase was inhibited in group II cancer bearing animals. This is in accordance with the findings of Graham. On the contrary, a significant decrease of glycolytic enzymes and the increase of gluconeogenic enzymes level in drug treated group III animals indicate the regression of cancer. This can be attributed to the anticancer potency of T. arjuna bark extract.

Generally large number of recognized inhibitors of mutagens/carcinogens are basically of plant origin and of highly diverse chemical nature, using Ames assay reported that tannin fraction of T. arjuna possesses antimutagenic activity against 4-nitrophenylenediamine (NPD) in TA 98, tester strain of Salmonella typhimurium. According to them anticarcinogenic and antimutagenic activity of plant phenols is due to an interaction of the compound with target tissue DNA. Teel also suggested that ellagic acid of T. arjuna masks the sites of DNA. Therefore, the anticancer activity of T. arjuna on DEN induced liver cancer may be attributed to the possible binding of one of the active compound with DNA and interruption of the macromolecule synthesis. This may leads to depletion of energy metabolism in cancer tissues and normalize the abnormal cells behaviour. This anticancer activity of T. arjuna on regulation of carbohydrate metabolism can provide new important information and potentially new approach to cancer chemotherapy.

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