JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 1283–1290

Received January 9, 2003
Accepted May 30, 2003
DOI 10.1211/0022357021710
ISSN 0022-3573

Therapeutic effect of *Semecarpus anacardium* Linn. nut milk extract on carbohydrate metabolizing and mitochondrial TCA cycle and respiratory chain enzymes in mammary carcinoma rats

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Abstract

Semecarpus anacardium Linn. of the family Anacardiaceae has many applications in the Ayurvedic and Siddha systems of medicine. We have evaluated the effect of *S. anacardium* nut milk extract on carbohydrate metabolizing enzymes and mitochondrial tricarboxylic acid cycle and respiratory enzymes in liver and kidney mitochondria of dimethyl benzanthracene-induced mammary carcinoma in Sprague–Dawley rats. Mammary carcinoma-bearing rats showed a significant rise in glycolytic enzymes (hexokinase, phosphoglucoisomerase and aldolase) and a simultaneous fall in gluconeogenic enzymes (glucose-6-phosphatase and fructose 1,6-diphosphatase). The activities of mitochondrial enzymes isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH-dehydrogenase and cytochrome C oxidase were significantly lowered in mammary carcinoma-bearing rats when compared with control rats. *S. anacardium* nut extract administration to tumour-induced animals significantly lowered the glycolytic enzyme activities (hexokinase, phosphoglucoisomerase and aldolase) and there was a rise in gluconeogenic enzymes (glucose-6-phosphatase and fructose 1,6-diphosphatase), which indicated an antitumour and anticancer effect. Comparison of normal control rats and rats administered *S. anacardium* only as drug control animals showed no significant variations in enzyme activities. *S. anacardium* nut extract administration to dimethyl benzanthracene-tumour-induced animals significantly increased the activities of mitochondrial enzymes, thereby suggesting its role in mitochondrial energy production.

Introduction

Breast cancer is a life threatening disease confronting the female population worldwide. Approximately 80% of the world's population rely on the use of traditional medicine, which is predominantly based on plant materials (Subramaniam & Pushpagadan 1999).

Traditional medicines have been the starting point for the discovery of many important modern drugs. This has led to chemical and pharmacological investigations and general biological screening programmes of medicinal plants all over the world. In recent years there has been considerable emphasis on the identification of plant products as possible anticarcinogens with antioxidant properties. *Semecarpus anacardium* Linn. (Anacardiaceae) commonly known as 'marking nut' has high priority and applicability in the indigenous system of medicine against various ailments (Premalatha & Sachdanandam 1999). It is a deciduous tree, distributed in the sub-Himalayan tract and in tropical parts of India. The nut of *S. anacardium*, known as the marking nut, is reported useful for a variety of ailments such as insanity, fever, asthma, dysentery, neurological disorders, cardiac troubles, enlargement of spleen, alopecia, ulcers, corns, leprosy, leucoderma, piles, rheumatism, and cancer (Sivarajan & Indira 1994). The fruits and oil have been claimed to be highly efficacious in the treatment of neuritis, leprosy, helminthic infection, and venereal disorders.

The chloroform extract of the nut possesses antitumour activity against various experimental tumours such as leukaemia L1210, P388, B16 melanoma and glioma 26

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(Chitinis et al 1980). Also, the acetylated oil of *S. anacardium* potentiated the efficacy of widely used anticancer drugs such as mitomycin, fluorouracil and methotrexate (Indap et al 1983). Many medicinal properties such as acid, hot, digestive, antimicrobial, anti-inflammatory, anti-helminthic and anti-amoebic properties have been reported from nut extract. These properties can be attributed to the presence of various constituents in the nut extract, including biflavonoids (Ishartulla et al 1977), phenolic compounds (Prakasha Rao et al 1973), bhilawanols (Gedam et al 1974), sterols and glycosides (Indap et al 1983). Semecarpoflavonone (Murthy 1983), jeediflavonone (Murthy 1984), galluflavonone (Murthy 1985), nallaflavonone (Murthy 1987), semecarpetin (Murthy 1988), and anacardiflavonone (Murthy 1992) are biflavonoids that have been identified so far from *S. anacardium* nuts. Bhilawanol, a catechol derivative (Prakasha Rao & Ramachandra Rao 1970), and a monohydroxy phenol called semecarpol have been isolated from these nuts also (Govindachari et al 1971). Gulati & Ohiman (1984) carried out phytochemical studies with *S. anacardium* nuts and yielded 2.8% total and 0.65% acid soluble ash. Further analysis revealed the presence of iron, copper, sodium, calcium and aluminum in traces.

Proximate principles, minerals and vitamins

The values given are per 100 g of edible portion: protein 26.4 g, fat 36.4 g, minerals 3.6 g, fibre 104 g, carbohydrates 28.4 g, energy 587 kcal, calcium 295 mg, phosphorous 836 mg, iron 6.1 mg, carotene 0 mg, thiamine 0.35 mg, riboflavin 0.15 mg, niacin 2.7 mg, and vitamin C 0 mg.

Phytochemical study of the Siddha preparation of the *S. anacardium* nut extract (Serankottai nei) revealed the presence of phenols, flavonoids and carbohydrates (Vijayalakshmi et al 1996). Nut extract was tested for cytotoxicity on COLO 320 tumour cells, using the microculture tetrazolium (MTT) assay. The IC50 value (the concentration causing 50% growth inhibition of tumour cells) was used as a parameter for cytotoxicity. The nuts displayed a strong cytotoxic effect with an IC50 value of 1.6 mg mL^{-1}. Recent studies carried out on anacartin forte, an Ayurveda marking nut preparation, have shown promising results in the treatment of human cancers of the oesophagus, urinary bladder and liver, and of leukaemia (Vad 1973).

Carbohydrate metabolism plays a characteristic role in cancerous conditions (Eigenbrodt 1994). One of the most common and profound phenotypes of malignant tissues, particularly those with the highest growth rates, is their capacity to utilize and catabolize glucose at high rates. The high glycolytic rate is important for rapidly proliferating cancers not only as a major energy source but also to provide such cells with precursors for nucleotide and lipid biosynthesis.

Mitochondria are the intracellular organelles responsible for ATP synthesis through the coupling of oxidative phosphorylation to respiration in human and animal cells. The principal mitochondrial substrate is pyruvate formed essentially by glycolysis. Pyruvate carbons enter the tricarboxylic acid cycle (TCA cycle) in the mitochondrial matrix, in which substrates are oxidized with the formation of CO_2 and the reduction of NAD^+ and FADH to NADH and $FADH_2$, respectively. These provide electrons to the respiratory chain upon their re-oxidation. Electron transfer from the TCA cycle to the respiratory chain by NADH and $FADH_2$ promotes the generation of ATP.

Mitochondria are the major intracellular source during oxidative phosphorylation (Sohal & Sohal 1991) and are the primary target of reactive oxygen species (ROS), which are generated under normal conditions as by-products of aerobic metabolism in animal and human cells. It has been established that defects in the respiratory chain lead to enhanced production of ROS and free radicals in mitochondria, resulting in mitochondrial DNA mutations which indirectly impair glucose sensing by reducing intracellular concentrations of ATP (Shoffner & Wallace 1994), an important metabolic fuel. Increased oxidation of glucose derived acetyl residues is attributed to Ca^{2+} dependent activation of NAD-isocitrate dehydrogenase and α-ketoglutarate dehydrogenase (Rasschaert & Malaisse 1992).

Hence, we have aimed to evaluate the effect of *S. anacardium* nut extract on carbohydrate metabolizing and mitochondrial TCA cycle and respiratory chain enzymes in dimethyl benzanthracene (DMBA)-induced mammary carcinoma-bearing rats.

Materials and Methods

Formulation of the drug Serankottai nei

The formulation was prepared according to a recipe from the Formulary of Siddha Medicine (1972) as follows. *S. anacardium* purified nuts (200 g) were boiled with 500 mL milk. The decoction was decanted and 500 mL milk was added to the boiled nuts and boiled again for 30 min. The decoction was recovered and the process was repeated with 500 mL milk. The three portions of milk nut decoction were combined, mixed with ghee (1.5 kg) and boiled until dehydrated. This final product was used for the investigation.

Animals

Female albino Sprague–Dawley rats between 50–55 days old were used (National Institute of Nutrition, Hyderabad, India). The animals were housed in individual, well ventilated cages (12-h light:dark cycle) and were fed a commercial rat pelleted diet (M/s.Hindustan Lever foods, Bombay, India) with water freely available. This project received approval from the Animal Ethics Committee by CPCSEA205/IAEC No.02/019/02.

Induction of mammary carcinoma

Mammary carcinoma was induced in eight-week-old rats using 7,12 dimethyl benz(a)anthrancene (DMBA; 25 mg) dissolved in olive oil, and administered through gastric intubation. Two months post-DMBA-period the rats

were palpated regularly to determine the appearance of mammary tumour. The tumour was allowed to grow for one month more to attain considerable mass and size. After three months, mammary carcinoma was confirmed by histological examination.

Experimental design

The animals were divided into four groups of six animals each. Group 1 animals were normal healthy animals that served as the control. Group 2 animals were administered DMBA 25 mg mL^{-1} dissolved in olive oil by gastric intubation to induce mammary carcinomas. Group 3 were mammary carcinoma-induced animals (as in group 2) but in addition, after three months, treatment began with *S. anacardium* nut milk extract (200 mg kg^{-1}) dissolved in olive oil administered by gastric intubation daily for fourteen days. Group 4 animals were administered *S. anacardium* only on the same dosage as group 3 and by a similar route.

Biochemical analysis

At the end of the experimental period, animals were fasted overnight and then killed by cervical decapitation. The liver and kidneys from all the animals were removed, washed in ice-cold isotonic saline and blotted individually on ash-free filter paper. The tissues were homogenized in 0.1 M Tris HCl (pH 7.4) and sucrose–EDTA buffer (pH 7.2) for estimation of carbohydrate metabolizing and mitochondrial enzymes, respectively. Mitochondria were isolated from fresh liver and kidney tissues (Johnson & Lardy 1967).

Carbohydrate metabolizing enzyme assays

Hexokinase activity was measured with respect to the amount of glucose utilized after the addition of ATP (Branstrup et al 1957). Aldolase activity was assayed according to the method of King (1965a) with fructose 1,6-diphosphate as substrate and dinitrophenyl hydrazine as colouring reagent. Glucose-6-phosphate dehydrogenase activity was measured using 2,6 dichlorophenol indophenol dye according to the method of Ells & Kirkman (1961). The activity of glucose-6-phosphatase and of fructose 1,6-diphosphatase (Gancedo & Gancedo 1971) was assayed with respect to the amount of inorganic phosphorus liberated after the addition of their respective substrate, glucose-6-phosphate or fructose 1,6-diphosphate.

Mitochondrial TCA cycle and respiratory chain enzyme assays

The purity of the mitochondria was assessed by estimating succinate dehydrogenase activity. The activity of isocitrate dehydrogenase was assayed by the method of King (1965b). α-Ketoglutarate activity was assayed according to the colorimetric determination of ferrocyanide produced by the decarboxylation of α-ketoglutarate with ferricyanide as electron acceptor (Reed & Mukherjee 1969). Succinate dehydrogenase activity was assayed by the method of Slater & Bonner (1952) in which the rate of reduction of potassium ferricyanide was measured in the presence of potassium cyanide. The activity of malate dehydrogenase was estimated by the method of Mehler et al (1948). The method of Minakami et al (1962) was followed for the determination of reduced nicotinamide dinucleotide (NADH) dehydrogenase activity. The activity of cytochrome C oxidase was assayed by the method of Wharton & Tzagoloff (1967).

Statistical analysis

The effects of the various groups on the various biological outcomes were statistically evaluated using an analysis of variance. Individual differences between treatments were examined using Tukey's HSD test. In all cases $P < 0.05$ denoted significance.

Results

The activities of glycolytic enzymes (hexokinase, aldolase, phosphoglucoisomerase) and gluconeogenic enzymes (glucose-6-phosphatase and fructose 1,6-diphosphatase) in the liver and kidney of control and experimental animals are shown in Tables 1 and 2, respectively.

The activity of hexokinase, aldolase, and phosphoglucoisomerase were significantly increased, whereas the activity of the gluconeogenic enzymes (glucose-6-phosphatase and fructose 1,6 diphosphatase) (liver and kidney) was significantly decreased in cancerous group 2 when compared with control (group 1) animals. Administration of drug for 14 days in mammary carcinoma-bearing animals (group 3) showed significantly reduced activity of hexokinase, aldolase and phosphoglucoisomerase ($P < 0.05$) and significantly increased activity of glucose-6-phosphatase and fructose 1,6 diphosphatase when compared with cancerous group 2 ($P < 0.05$). However, drug-alone-treated animals (group 4) did not show any significant changes when compared with control animals (group 1).

The activity of TCA cycle enzymes (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase) and respiratory chain enzymes (NADH dehydrogenase and cytochrome C oxidase) in the liver and kidney mitochondria of experimental animals are shown in Tables 3 and 4, respectively. The activity of the mitochondrial enzymes (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase) and respiratory chain enzymes (NADH dehydrogenase and cytochrome C oxidase) were significantly decreased in mammary carcinoma-bearing animals (group 2) ($P < 0.05$) when compared with control animals. Mammary carcinoma-bearing animals treated with *S. anacardium* nut extract showed a significant increase in mitochondrial enzyme activity ($P < 0.05$). Normal rats treated with nut extract showed no significant variation.

Table 1 Effect of *Semecarpus anacardium* on glycolytic and gluconeogenic enzyme activity in liver of control and experimental animals.

Enzyme (Units (mg protein)$^{-1}$)	Group 1 (control)	Group 2 (tumour induced)	Group 3 (tumour induced + drug treated)	Group 4 (drug control)
Hexokinase	15.62 ± 1.34	29.45 ± 2.33*	18.36 ± 1.48*	14.76 ± 1.22^{NS}
Aldolase	22.84 ± 2.15	32.43 ± 2.87*	26.60 ± 2.25*	21.50 ± 2.07^{NS}
Phosphoglucoisomerase	25.73 ± 2.38	35.56 ± 3.12*	27.33 ± 2.47*	25.23 ± 2.19^{NS}
Glucose-6-phosphatase	21.35 ± 1.81	10.84 ± 1.06*	17.94 ± 1.65*	21.70 ± 1.86^{NS}
Fructose 1,6-diphosphatase	31.17 ± 2.63	20.83 ± 1.69*	28.77 ± 2.35*	32.42 ± 2.74^{NS}

Values are expressed as mean ± s.d. for six animals. One unit of enzyme activity was expressed as: hexokinase – nmol glucose-6-phosphate; phosphoglucoisomerse – nmol fructose; aldolase – nmol glyceraldehyde formed min^{-1} (mg protein)$^{-1}$ at 37 °C. Glucose-6-phosphatase and fructose 1, 6-diphosphatase – nmol inorganic phosphorus released min^{-1} (mg protein)$^{-1}$ at 37 °C. *$P < 0.05$, group 1 compared with group 2; group 2 compared with group 3. ^{NS}Not significant, group 1 compared with group 4.

Table 2 Effect of *Semecarpus anacardium* on glycolytic and gluconeogenic enzyme activities in kidney of control and experimental animals.

Enzyme (Units (mg protein)$^{-1}$)	Group 1 (control)	Group 2 (tumour induced)	Group 3 (tumour induced + drug treated)	Group 4 (drug control)
Hexokinase	10.76 ± 1.02	17.81 ± 1.40*	12.92 ± 1.19*	10.21 ± 0.96^{NS}
Aldolase	24.42 ± 2.16	30.26 ± 2.72*	26.34 ± 2.23*	23.84 ± 2.04^{NS}
Phosphoglucoisomerase	19.52 ± 1.74	28.47 ± 2.62*	22.16 ± 2.13*	20.19 ± 1.86^{NS}
Glucose-6-phosphatase	29.72 ± 2.73	17.64 ± 1.67*	25.62 ± 2.23*	30.34 ± 2.75^{NS}
Fructose 1,6-diphosphatase	35.27 ± 3.14	24.58 ± 2.24*	31.05 ± 2.92*	35.68 ± 3.11^{NS}

Values are expressed as mean ± s.d. for six animals. One unit of enzyme activity was expressed as: hexokinase – nmol glucose-6-phosphate; phosphoglucoisomerse – nmol fructose; aldolase – nmol glyceraldehyde formed min^{-1} (mg protein)$^{-1}$ at 37 °C. Glucose-6-phosphatase and fructose 1, 6-diphosphatase – nmol inorganic phosphorus released min^{-1} (mg protein)$^{-1}$ at 37 °C. *$P < 0.05$, group 1 compared with group 2; group 2 compared with group 3. ^{NS}Not significant, group 1 compared with group 4.

Table 3 Effect of *Semecarpus anacardium* on the TCA cycle and respiratory chain enzyme activity in liver of control and experimental animals.

Parameters	Group 1 (control)	Group 2 (tumour induced)	Group 3 (tumour induced + drug treated)	Group 4 (drug control)
Isocitrate dehydrogenase	830.00 ± 44.00	535.00 ± 27.00*	790.00 ± 40.00*	840.00 ± 43.00^{NS}
α-Ketoglutarate dehydrogenase	95.10 ± 8.15	63.25 ± 5.90*	89.30 ± 8.00*	97.70 ± 8.60^{NS}
Succinate dehydrogenase	25.40 ± 2.14	14.65 ± 1.18*	23.60 ± 1.80*	28.34 ± 2.36^{NS}
Malate dehydrogenase	360.80 ± 26.24	240.95 ± 18.36*	345.30 ± 23.42*	365.38 ± 25.49^{NS}
NADH dehydrogenase	29.46 ± 2.23	15.59 ± 1.33*	26.17 ± 1.94*	28.86 ± 2.15^{NS}
Cytochrome C oxidase	6.17 ± 0.44	3.45 ± 0.18*	5.88 ± 0.37*	6.38 ± 0.48^{NS}

Values are expressed as mean ± s.d. for six animals. Isocitrate dehydrogenase was expressed as nmol α-ketoglutarate h^{-1} (mg protein)$^{-1}$. α-Ketoglutarate dehydrogenase was expressed as nmol ferricyanide h^{-1} (mg protein)$^{-1}$. Succinate dehydrogenase, malate dehydrogenase, NADH dehydrogenase were expressed as μmol NADH oxidized min^{-1} (mg protein)$^{-1}$. Cytochrome C oxidase was expressed as optical density $\times 10^{-2}$ min (mg protein)$^{-1}$. *$P < 0.05$, group 1 compared with group 2; group 2 compared with group 3. ^{NS}Not significant, group 1 compared with group 4.

Discussion

The control of mitochondrial biogenesis in mammalian cells is relatively little known despite the vital roles of these organelles in virtually all eukaryotic cells. Malignant cells have a diminished respiratory rate, coupled with an excessive rate of aerobic glycolysis. In this study we have observed an increase in activity of the glycolytic enzymes hexokinase, phosphoglucoisomerase and aldolase in mammary carcinoma rats. This allowed us to infer the elevated rate of glycolysis in tumour conditions since tumour cell proliferation is dependent on

Table 4 Effect of *Semecarpus anacardium* on the TCA cycle and respiratory chain enzyme activities in kidney of control and experimental animals.

Parameters	Group 1 (control)	Group 2 (tumour induced)	Group 3 (tumour induced + drug treated)	Group 4 (drug control)
Isocitrate dehydrogenase	870.00 ± 52.00	630.00 ± 35.20*	845.00 ± 49.00*	890.00 ± 60.00^{NS}
α-Ketoglutarate dehydrogenase	50.20 ± 4.30	36.35 ± 3.25*	46.40 ± 3.56*	53.70 ± 4.73^{NS}
Succinate dehydrogenase	10.64 ± 0.86	4.95 ± 0.47*	8.92 ± 0.89*	11.08 ± 0.93^{NS}
Malate dehydrogenase	226.24 ± 18.42	140.16 ± 12.72*	215.00 ± 17.50*	228.43 ± 20.15^{NS}
NADH dehydrogenase	19.21 ± 1.33	10.48 ± 0.73*	16.66 ± 1.14*	20.70 ± 1.65^{NS}
Cytochrome C oxidase	4.25 ± 0.31	2.01 ± 0.13*	3.90 ± 0.28*	4.30 ± 0.34^{NS}

Values are expressed as mean ± s.d. for six animals. Isocitrate dehydrogenase was expressed as nmol α-ketoglutarate h^{-1} (mg protein)$^{-1}$. α-Ketoglutarate dehydrogenase was expressed as nmol ferricyanide h^{-1} (mg protein)$^{-1}$. Succinate dehydrogenase, malate dehydrogenase, NADH dehydrogenase were expressed as μmol NADH oxidized min^{-1} (mg protein)$^{-1}$. Cytochrome C oxidase was expressed as optical density × 10^{-2} min (mg protein)$^{-1}$. *$P < 0.05$, group 1 compared with group 2; group 2 compared with group 3. ^{NS}Not significant, group 1 compared with group 4.

glucose availability; these cells acquire the major part of their energy from the glycolytic pathway (Taper & Roberfroid 1999). The degree of elevation of these enzymes is directly related to the extent of morphological differentiation and growth rate of hepatomas (Gerbracht et al 1993). Hexokinase plays a critical role in initiating and maintaining the high glucose catabolic rates of rapidly growing tumours (Mathupale et al 1997) and accomplishes the entry of glucose into the glycolytic pathway by phosphorylation to glucose-6-phosphate. The proliferating cells undergo a shift from oxidative to glycolytic metabolism, where the energy requirements of the rapidly dividing cells are provided by ATP from glycolysis (Golshani Hebroni & Bessman 1997). The mitochondrial porin-bound hexokinase II is increased under the control of mutated p53. This would direct mitochondrial ATP preferentially to glucose-6-phosphate synthesis and hence is expected to increase the biosynthetic pentose phosphate pathway. Hence, the glycolytic capacity of cancer cells depends totally on hexokinase activity for its metabolic fuel (Arora & Pederson 1988). In this study, the observed increase in the activity of hexokinase in mammary carcinoma animals might have been due to the increased metabolic need of energy fuel for proliferating tumour cells and simultaneously increased activity of hexokinase. Administration of *S. anacardium* for 14 days to mammary carcinoma-bearing rats significantly reduced the enzyme activity and this may have been due to its antitumour effects.

Phosphoglucoisomerase serves as a good index of tumour growth and is significantly elevated in cancerous animals. In agreement with our study, Campbell & King (1962) reported that phosphoglucoisomerase was an indicator of metastatic growth and was elevated in patients with neoplasms, especially after metastasis. Alterations in the activity of phosphoglucoisomerase might be expected to influence the proportion of glucose-6-phosphate metabolized via the glycolytic pathway (Ebrahim et al 1996). Aldolase, another key enzyme in the glycolytic pathway, was increased in diethylnitrosamine-induced tumour conditions. Aldolase was found to be elevated in tumour-bearing animals and in breast cancer (Mazurek et al 2001). The elevated activity of phosphoglucoisomerase and aldolase may be due to cell impairment and necrosis. In experimental carcinogenesis the cells are subjected to carcinogen-induced damage, and very often exhibit glycolysis after a period of increased oxygen uptake. The high glycolytic rate of most tumours can be adopted as a major source of energy in the deranged cell. Increase in the activity of glycolysis results from the rise of the tumour growth rate and is accompanied by a decrease in the activity of the pentose phosphate pathway and respiratory chain (Racker 1980). The activity of gluconeogenic enzymes like glucose-6-phosphatase and fructose 1,6-diphosphatase are inhibited significantly in tumour-bearing animals. Lactate production from glucose rises and concomitantly glucose production from pyruvate decreases during the progression of tumour growth. The observed reduction in activity of these enzymes in tumour-bearing animals may be due to the higher lactate production of neoplastic tissues, and it has been proved that tumour utilizes a large proportion of lactate for glycolysis and protein synthesis. A crucial point in regulation of aerobic glycolysis and of energy metabolism in general is represented by the transport of metabolites across the mitochondrial membrane from the cytosol to the matrix space of mitochondria.

Drug (*S. anacardium*)-treated animals, showed a significant drop in the activity of glycolytic enzyme and concomitant elevation in the activity of gluconeogenic enzymes. This modulation may be due to the anticancer activity of the drug either by inhibiting the glycolytic enzyme activity or by suppression of tumour progression (Sujatha et al 1999). The activity may be attributed to the presence of flavonoid in the drug extract, which has an effective role over aerobic glycolysis (Suolinna et al 1975). The inhibition of hexokinase activity may be due to the flavonoids which have structural resemblance between adenosine and the hydroxylated glycosylated benzopyrene nucleus (Havsteen 1983). Flavonoids play a role

in inhibiting migration of cancer cells and malignant cell proliferation (Stavric & Matula 1992). Suolinna et al (1975) reported that inhibition of glycolysis is by the action of flavonoids, which interfere with the generation of ADP, Pi which are required for glycolysis. Comparison of groups 1 and 4 showed no significant variations in enzyme activity (Sujatha & Sachdanandam 2000).

The mammary carcinoma-bearing animals showed decreased activity of mitochondrial TCA cycle and respiratory chain enzymes when compared with normal control rats. Decreased activity of these enzymes might be due to the alteration in the morphology and ultrastructure of cancer cells and the ability of mitochondria to undergo metabolic changes when compared with normal cells, and also the number of mitochondria was drastically reduced in tumour cells (Pederson 1978). The decrease in mitochondrial content might be due to the marked deficiency in one or more electron transport chain compounds. In contrast, Pederson (1978) reported that a decrease in mitochondrial content does not seem to be related to tumour growth.

We may speculate also that cancer cells may be able to produce compounds capable of being transported to normal host cells and act as uncoupling agents, thus lowering ATP production in the normal tissues and contributing to tumour-induced cachexia (Argiles & Bieto 1988). Enhanced mitochondrial lipid peroxidation has been reported to inactivate succinate oxidase, succinate dehydrogenase, isocitrate dehydrogenase and the components of the respiratory chain (Tretter et al 1987). Similarly Remus & Firman (1995) reported that the decrease in TCA cycle action may be related to decline in the activity of a ketoglutarate dehydrogenase and pyruvate dehydrogenase complex.

Further supporting our observation, the decreased activity of isocitrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase in kidney has been reported in gentamicin-induced nephrotoxicity (Sandhya et al 1995). Enhanced free radical generation and the loss of mitochondrial respiration and ketoglutarate dehydrogenase activity have been observed in Parkinson's disease, Alzheimer's disease, and cardiac ischaemia/reperfusion injury.

Mitochondria carry out a variety of processes of which oxidative phosphorylation is the most important. Detoxification of oxygen via its reduction into H_2O by the cytochrome oxidase system takes place in the mitochondria. Cytochrome C oxidase and NADH dehydrogenase are the enzymes involved in the electron transport chain and are located in the inner mitochondrial membrane. The role is ultimately linked to the production of useful energy rich compounds such as ATP. In this study, we have observed a decrease in the activity of mitochondrial respiratory chain enzymes in liver and kidney of mammary carcinoma rats that were significantly enhanced upon nut extract therapy. This effect was due to the presence of flavonoids and other constituents present in *S. anacardium* nut extract which could act synergistically or independently in enhancing the activity of TCA cycle enzymes. They act on the already initiated neoplastic cell mitochondria by modulating tumour promotion or by penetrating the malignant cell to modify neoplasia. Lipid peroxidation has been reported to cause a decrease in the rate of oxygen consumption as well as the activity of cytochrome C oxidase in mitochondria (Ishankhodzhaev et al 1987). In an ischaemia model, the measurement of oxidative phosphorylation revealed marked depression in all variables (the ratio of oxygen consumed to ADP phosphorylated to ATP) i.e. ADP/O ratio, respiratory control index and the rate of succinate oxidation. The decrease in respiratory activity during state 3 respiration indicated severe impairment of electron transport activity.

The respiratory process involves the transport of electrons via cytochromes to molecular oxygen. Variations in cytochrome concentrations may affect the transport of electrons via the transport chain and thereby alter the energy production of mitochondria. It has been reported that reduction in functioning of mitochondrial enzymes may be related to a defect in the mitochondrial energy production that would impair protein synthesis and energy production. A decrease in mitochondrial cytochrome content could result in the concomitant loss of oxidative phosphorylation capacity (Schultz & Chan 2001). The close correlation reaction preceding the phase of mitochondrial MDA production between the decline of mitochondrial respiration and the activity of complex III suggests an impairment of respiratory chain in the b–c_1 region, representing one of the functional events in the casual peroxidation reaction (Trumper et al 1988).

Conclusion

Deranged energy metabolism in dimethyl benz(a)anthracene-induced mammary carcinoma in rats was rectified and favourable restoration of glycolysis, TCA cycle and oxidative phosphorylation was achieved by administration of *Semecarpus anacardium* nut milk extract.

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