Semecarpus anacardium nut extract promotes the antioxidant defence system and inhibits anaerobic metabolism during development of lymphoma

Nibha VERMA and Manjula VINAYAK

Biochemistry and Molecular Biology Laboratory, Centre of Advance Study in Zoology, Banaras Hindu University, Varanasi 221005, India

Synopsis

Antioxidants are substances that fight against ROS (reactive oxygen species) and protect the cells from their damaging effects. Production of ROS during cellular metabolism is balanced by their removal by antioxidants. Any condition leading to increased levels of ROS results in oxidative stress, which promotes a large number of human diseases, including cancer. Therefore antioxidants may be regarded as potential anticarcinogens, as they may slow down or prevent development of cancer by reducing oxidative stress. Fruits and vegetables are rich source of antioxidants. Moreover, a number of phytochemicals present in medicinal plants are known to possess antioxidant activity. Therefore the aim of the present study was to investigate antioxidant activity of the aqueous extract of nuts of the medicinal plant Semecarpus anacardium in AKR mouse liver during the development of lymphoma. Antioxidant action was monitored by the activities of antioxidant enzymes catalase, superoxide dismutase and glutathione transferase. The effect of S. anacardium was also studied by observing the activity of LDH (lactate dehydrogenase), an enzyme of anaerobic metabolism. LDH activity serves as a tumour marker. The activities of antioxidant enzymes decreased gradually as lymphoma developed in mouse. However, LDH activity increased progressively. Administration of the aqueous extract of S. anacardium to lymphoma-transplanted mouse led to an increase in the activities of antioxidant enzymes, whereas LDH activity decreased significantly, indicating a decrease in carcinogenesis. The aqueous extract was found to be more effective than doxorubicin, a classical anticarcinogenic drug, with respect to its action on antioxidant enzymes and LDH in the liver of mice with developing lymphomas.

Key words: antioxidant enzyme, cancer, Dalton’s lymphoma, reactive oxygen species (ROS), Semecarpus anacardium

INTRODUCTION

ROS (reactive oxygen species) are produced during aerobic metabolism in an organism and are continuously removed by the antioxidant defence system. Antioxidants neutralize ROS as natural byproducts of normal cell processes and thus protect cells from the damage caused by ROS. A low concentration of ROS is indispensable in many biochemical processes, including cell proliferation, differentiation and apoptosis [1], as well as defence against micro-organisms [2]. Overproduction of ROS by exposure to radiation or due to impaired action of antioxidants damages macromolecules, such as DNA, protein and lip-ids [3,4], which leads to oxidative stress [5]. Oxidative stress has been implicated to be involved in a large number of human diseases, such as atherosclerosis, pulmonary fibrosis, neurodegenerative diseases and cancer [6,7]. It is suggested to be an early event in carcinogenesis. Therefore antioxidants are important in reducing the risk of cancer or in monitoring tumour growth. Impairment of the antioxidant defence system has been reported in various cancers [8]. Organisms have an endogenous antioxidant defence mechanism to maintain redox balance. The cellular antioxidant defence system operates mainly via antioxidant enzymes, such as CAT (catalase), SOD (superoxide dismutase), glutathione peroxidase, GST (glutathione transferase) and glutathione reductase in addition to non-enzymatic
antioxidants, such as glutathione, ascorbic acid and flavanoids. These enzymes neutralize highly reactive free radicals and thus prevent uncontrolled generation of ROS. CAT removes $\text{H}_2\text{O}_2$ by converting it into $\text{H}_2\text{O}$ and $\text{O}_2$. GST promotes ionization of the sulfydryl groups into glutathione by increasing its nucleophilicity, so that reactive electrophilic substances can preferentially bind to glutathione, rather than to other cellular nucleophiles. SOD catalyses spontaneous dismutation of superoxide radicals into $\text{H}_2\text{O}_2$, which is further detoxified by CAT and glutathione peroxidase [3]. The higher metabolic rates that occur in cancerous cells lead to a high level of ROS production. Accumulation of ROS impairs the antioxidant defence system, which is one of the major cause of initiation and promotion of malignancy. Therefore enhancing the antioxidant defence system may favour down-regulation of tumour growth. Malignant cells are known to maintain a high rate of glycolysis, even under aerobic conditions, leading to a high rate of lactate formation. Elevated levels of LDH (lactate dehydrogenase) are associated with various malignancies. Therefore regulation of glycolytic or anaerobic metabolism by down-regulation of the activity of LDH may serve to prevent cancer progression.

Antioxidants are abundant in fruits and vegetables, as well as in other foods, including nuts, some meats, poultry and fish. Beta-carotene, lutein, lycopene, vitamin A, vitamin C and vitamin E are important antioxidant nutrients. In addition, phytochemicals present in several medicinal plants are known to possess antioxidant activities. Compounds that can enhance the antioxidant defence system in organisms with cancer may be regarded as potential anticarcinogens.

*Semecarpus anacardium* is used to treat dysentery, tumours, fever, loss of appetite and urinary discharge. The antitumour activity of the nut extract has been reported in lymphocytic leukaemia in mice, as observed by their median survival time [8a]. However, antioxidant activity of *S. anacardium* is not well documented. Therefore, in the present study, the antioxidant ability of the aqueous extract of *S. anacardium* was analysed during development of lymphomas in AKR mice [8b]. The antioxidant activities of the enzymes CAT, SOD and GST were used as parameters to monitor the antioxidant capacity of the medicinal plant. LDH was assayed to analyse the regulatory effect of *S. anacardium* on anaerobic metabolism. Regulation of the antioxidant defence system and LDH activity of the aqueous extract of *S. anacardium* was compared with that of doxorubicin, a traditional anticarcinogenic drug [9]. It was found to be more capable of enhancing the antioxidant defence system and to down-regulate the anaerobic metabolism of DL (Dalton’s lymphoma ascite transplanted) mouse and, therefore, may be regarded to have better anticarcinogenic action.

**MATERIALS AND METHODS**

**Chemicals**

Chemicals of analytical and molecular biology grade were used in the present study. PMSF, EDTA, NAD, NADH, PMS (phenazine methosulfate) and SDS were purchased from Sigma Chemicals; dithiothreitol, bis-acrylamide and APS (ammonium persulfate) from Merck; TEMED ($N,N,N',N'$-tetramethylethlyenediamine), NBT (Nitro Blue Tetracylium), CDNB (1-chloro-2,4-dinitrobenzene), GSH and Coomassie Brilliant Blue G-250 from Sisco Research Laboratories.

**Preparation of aqueous extract of *S. anacardium***

The aqueous extract of nuts of *S. anacardium* was prepared as follows. Nuts were crushed and boiled in distilled water for 30 min. The aqueous extract was filtered and dried for 4–5 days. Various doses of the extract were used for the treatment of DL mice.

**Animals**

Mice of AKR strain were used for the present study because of their short lifespan and high susceptibility to tumour development. The mouse colony was maintained at 25 ± 2°C under a 12 h light/12 h dark cycle with *ad libitum* supply of standard mice feed and drinking water [10]. The average life span of an AKR mouse is approx. 18 months.

**Induction of lymphoma in mouse**

Normal adult (15–20 week old) AKR mice were used for induction of lymphoma by serial transplantation of live Dalton’s lymphoma ascitic cells. Each mouse received approx. 5 × 10⁶ cells in 1 ml of PBS (pH 7.4) via intraperitoneal injection [11].

**Treatment of DL mouse**

The aqueous extract of nuts was given to DL mice orally, using a metallic pipe, as described previously [9]. DL mice were grouped into six batches, and each batch included six mice. Batch 1 were normal adult mice; batch 2 were normal mice treated with aqueous extract of *S. anacardium* (6.0 mg per mouse); batches 3–6 were DL mice, out of which mice of batches 4–6 were given aqueous extract of *S. anacardium* with doses of 1.5, 3.0 and 6.0 mg per mouse (0.5, 1.0 and 2.0 g/kg of body mass) respectively. Doses were given at 24 h intervals from the day following DL implantation.

**Assay of antioxidant enzymes**

The assay of enzyme activities is believed to be the most important parameter in determining the biological impact of antioxidant enzymes. Therefore spectrophotometric and activity gel assays were used to measure the activity of the antioxidant enzymes, such as CAT, SOD and GST. The enzyme activities were determined in the liver of normal, treated normal, DL and treated DL mice. Activities are expressed as units of enzyme per ml of extract, and specific activities as units per mg of protein. Protein concentration was estimated according to the method of Bradford [12].

Liver homogenates were prepared in 50 mM phosphate buffer (pH 7.4) containing 1 mM PMSF, and the samples were
centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was used for assay of antioxidant enzymes, CAT, SOD and GST.

**CAT**
The activity of CAT was measured using a spectrophotometric method as described by Aebi [13], with minor modifications [10], which determines the decomposition of H₂O₂ at 240 nm. The assay mixture (1 ml) contained phosphate buffer (pH 7.0), H₂O₂ and the enzyme source. One unit is the amount of enzyme that decomposes 1 μmol of H₂O₂ per min at 37 °C.

The activity gel assay of CAT was performed by native PAGE (7.5% polyacrylamide), and the gel was stained using the ferric cyanide method [14]. Minor modifications were carried out as described by Pathak et al. [10]. The principle of active staining involves the reaction of H₂O₂ with potassium ferricyanide, thereby reducing it to ferrocyanide. The peroxide is oxidized to molecular oxygen. Ferric chloride reacts with ferrocyanide to form a stable insoluble Prussian Blue pigment. CAT indicates its location by scavenging H₂O₂, causing transparent bands on the blue gel. The intensity of bands was analysed using an Alpha image analyser system.

**SOD**
The activity of SOD was measured according to the method described in [15], with minor modifications [10], by measuring the reduction of NBT at 560 nm. One unit is defined as the amount of enzyme causing half the maximum inhibition of NBT reduction.

Isozymes of SOD were separated by PAGE (10% gel) and were analysed by the photochemical method described by Fridovich [16], with minor modifications [10].

**GST**
The enzyme was assayed by measuring the formation of the conjugate of glutathione and CDNB, according to the method described by Habig et al. [17]. The reaction was performed in a final volume of 1 ml containing cellular protein, 1 mM CDNB.
Figure 2  Changes in activity of SOD in mouse liver during development of lymphoma
(A) Native PAGE (7.5% gel) and activity staining of SOD. (B) Densitometric scanning of SOD. Light grey bars, CuZn-SOD; dark grey bars, Mn-SOD. (C) Specific activity of SOD. N, normal; U, unit. *P < 0.05, significant difference compared with normal control.

Figure 3  Specific activity of GST in mouse liver during development of lymphoma
N, normal; U, unit. *P < 0.05, significant difference compared with normal control.
and 1 mM GSH in 100 mM phosphate buffer (pH 7.0). The activity was calculated by using a molar absorption coefficient of 9.6 mM$^{-1}$·cm$^{-1}$ for CDNB. The activity is expressed as the amount of enzyme forming 1 nmol of CDNB–GSH complex per min.

**Assay of LDH**

The LDH assay was performed in serum by a spectrophotometric method, as well as by native PAGE analysis, according to the method described by Pathak and Vinayak [18]. The enzyme activity was determined by measuring the conversion of NADH into NAD$^+$ in a pyruvate-utilizing reaction at 1 min intervals for 10 min at 340 nm. LDH isozymes were separated by PAGE (7.5% gel) and activity staining was carried out with NBT/PMS in the dark.

**Statistical analysis**

ANOVA followed by Bonferroni $t$ test was performed to evaluate the significant difference between the different groups and comparison was made with DL control mice. Results are
expressed as the means ± S.E.M. from three independent experiments. $P < 0.05$ was considered statistically significant (*). Significant differences were determined by using SigmaStat (version 2.0).

RESULTS

The induction of lymphoma with serial transplantation of live ascites cells into adult mouse leads to the development of lymphoma, with an accumulation of ascites fluid in the peritoneum. Angiogenesis was also observed. The body mass of the mice was increased and liver was found to be increased in size. The lifespan of a DL mouse was found to be $21 \pm 2$ days after induction of lymphoma; however, a normal mouse lives for approx. 18 months.

Histopathological details of the liver show that hepatocytes were closely packed and well arranged in normal mouse. However, in DL mouse liver, dividing cells were present; hepatocytes were impaired in shape and arrangement. The nucleus was enlarged, sinusoids were increased and there was a large number of blood cells (lymphocytes). The number of lymphocytes was found to be increased in DL mouse compared with normal [10].

Changes in enzyme activities during growth of lymphoma

The activities of the antioxidant enzymes CAT, SOD and GST, as well as that of LDH, were monitored during development of lymphoma from the day after lymphoma transplantation until D18 (day 18). The activities of the enzymes were found to vary gradually with the development of lymphoma.
**Activities of the enzymes of the antioxidant defence system**

The activities of CAT, SOD and GST were observed to decrease progressively in a similar pattern as the lymphoma advanced. CAT activity is only approx. 33% at D18 compared with normal mouse liver (Figure 1A). The activity gel assay showed a similar pattern of decreasing activity with development of lymphoma (Figures 1B and 1C). However, activity of SOD is decreased to a lesser extent as compared with that of CAT. SOD activity is decreased to 63% of the activity in normal mouse liver at D18 (Figure 2A). Both isozymes, Cu/Zn-SOD and Mn-SOD, show a gradual decrease in activity with advancement of lymphoma, as observed by native PAGE (Figures 2B and 2C). The band for Cu/Zn-SOD was visible up to D9, indicating that cytosolic isoforms are inactivated quickly with the development of lymphoma. Although CuZn-SOD band is not clearly visible after D9 in Figure 2(A), the corresponding densitometric scanning of SOD in Figure 2(B) indicates that Cu/Zn-SOD activity is present up to D18, however, to a lesser extent. On the other hand, the intensity of the mitochondrial isozyme, Mn-SOD also decreased after DL transplantation up to D18. Spectrometric assay confirmed the decrease in total activity (Figure 2C). The decrease in SOD activity may be due to oxidative stress.

Similarly, the activity of GST was found to decrease gradually with progression of lymphoma and it reached up to 55% activity on D18, as compared with that in normal mouse liver (Figure 3).

**Activity of LDH**

The activity of LDH was monitored in serum of normal mice, as well as in DL mice. The variation in LDH activity was analysed with progression of lymphoma from D1 to D18 after lymphoma transplantation. The activity increases gradually from D1 to D18. It increased up to 12-fold on D18 (Figure 4C). The level of...
isozyme A4 was found to increase with the progress of lymphoma (Figures 4A and 4B), as observed by the activity gel assay.

**Effect of *S. anacardium* on enzyme activity of antioxidant enzymes in DL mice**

The aqueous extract, prepared from nuts of *S. anacardium*, was tested for its effects on the enzymes comprising the antioxidant defence system, i.e., CAT, SOD and GST, in DL mouse liver on D18. Three different doses were selected. The modulation in enzyme activities was found to be dose-dependent.

The lower activity of CAT in DL mouse liver was found to increase gradually with increasing doses of *S. anacardium*. The most effective dose is 6.0 mg per mouse per day. It leads to an approx. 3.7-fold increase in enzyme activity, as compared with DL control mouse liver, which is equivalent to 68% of the activity in normal mouse liver (Figure 5). Although a significant difference in the activity is observed between normal and treated normal mice in the activity gel assay (Figures 5A and 5B), the specific activity of CAT, measured by spectrometric assay does not show significant difference (Figure 5C). Similarly, lower activities of SOD and GST in DL mouse are also enhanced by different doses of *S. anacardium*. Maximum elevation in SOD activity is brought about by 6.0 mg of nut extract per mouse per day. The increase in enzyme activity was 1.2-fold, as compared with DL control mouse liver, which corresponds to 73% of normal mouse liver (Figure 6). In the case of GST activity, a 3.0-mg dose of *S. anacardium* led to a higher activation, compared with a 6.0-mg dose. It resulted in the activity increasing 3.3-fold, as compared with DL control mouse liver, which corresponds to 76% of normal mouse liver (Figure 7).

**Effect of *S. anacardium* on LDH activity**

The activity of LDH, an enzyme of anaerobic metabolism, was decreased with the treatment of DL mice with an aqueous extract of *S. anacardium*, as compared with control DL mice. However, the LDH activity remained 6–8-fold higher than that in normal mouse liver (Figure 8). The higher activity of LDH is an indicator of growth of the lymphoma, even after treatment with aqueous extract of *S. anacardium*. A similar pattern of variation in enzyme activities was found when the enzymes were assayed by spectrophotometric methods, as well as by activity gel staining.

On the basis of the above results, 6.0 mg of nut extract of *S. anacardium* per mouse per day was selected to study its effect on activities of antioxidant enzymes and LDH during the growth of lymphoma.

**Effect of *S. anacardium* on antioxidant enzymes during development of lymphoma**

Treatment of mice with *S. anacardium* during development of lymphoma led to modulation in the activities of antioxidant enzymes, as well as LDH, the enzyme of anaerobic metabolism.

The antioxidant enzyme CAT was activated in mouse liver when the mice were treated with *S. anacardium*. The increase in activity was gradual, as the DL mice received the drug treatment, with a maximum activity at D18. The activity reached up to approx. 77% of normal mouse liver at D18, which was decreased up to 63% at D18 in untreated DL mice (Figure 9). The result shows that aqueous extract of *S. anacardium* contributed to the antioxidant defence system of the organism by elevating CAT activity during development of lymphoma. Similar results were obtained in the case of other enzymes of the antioxidant defence system. SOD activity was increased gradually on treatment of DL mice with an aqueous extract of *S. anacardium*. The highest enhancement in SOD activity was found at D18, when the activity was elevated up to 84% of normal mouse liver as compared with 63% activity in DL mouse without drug treatment (Figure 10). The result demonstrated that an aqueous extract of *S. anacardium* promotes the activity of the antioxidant enzyme SOD. In the case of GST, a rise in the activity was observed from D1 to D18 in DL mice on receiving treatment with *S. anacardium* (Figure 11). The activity is enhanced to 76% of normal mouse liver from 55% activity found in untreated control DL mouse.

**Effect of *S. anacardium* on LDH during development of lymphoma**

The increased activity of LDH with development of lymphoma was decreased by treatment of DL mice with an aqueous extract of *S. anacardium* during development of lymphoma (Figure 12). However, the activity of LDH remained high, as compared with normal mouse liver. It decreased up to 9.4-fold more than normal mouse liver, and was 12-fold in untreated control DL mouse liver.

The increase in the activity of the antioxidant enzymes, as well as the decrease in the activity of LDH in lymphoma-bearing mice, was found to be better on treatment with the aqueous extract of *S. anacardium* as compared with treatment with doxorubicin, a traditional anticancer drug, which has been investigated previously [9].
DISCUSSION

The balance between the generation of ROS and its removal by the antioxidant defence system is under tight homoeostatic regulation in normal organisms. Imbalance between the two processes leads to various pathological conditions, including cardiovascular diseases, neurodegenerative diseases, metabolic dysfunction, cancer and premature aging [19]. Accumulation of ROS causes a decrease in the enzyme activities of the antioxidant defence system. Lower activities of these antioxidant enzymes have been reported in many animal and human cancers. A down-regulation in the expression of CAT in carcinoma was reported by Kwei et al. [20]. Antioxidants are the major protective agents of an organism against oxidative damage. Therefore any agent leading to an increase in the impaired activities of antioxidant enzymes would contribute to anticarcinogenic action. The present study demonstrated that the aqueous extract of S. anacardium possesses
antioxidant agents, which are capable of promoting the cellular antioxidant defence system by elevating activities of antioxidant enzymes, CAT, SOD and GST, in DL mouse liver. The lower activities of antioxidant enzymes in DL mouse are increased towards normal level. Overexpression of CAT has been suggested to serve as a powerful inhibitor of tumorigenesis and malignancy [21]. Therefore enhancement of CAT activity in DL mice when treated with *S. anacardium* may serve as inhibitor of tumorigenesis.

Lower activities of SOD and GST after transplantation of lymphoma into the mice may be due to oxidative stress, leading to a further increase in the level of ROS and thus contributing to development of lymphoma. The increase in the activity of SOD on treatment with the medicinal plant strengthened the antioxidant defence system in lymphoma-bearing mice. Although the treatment decreased SOD activity in normal mice, the activity increased sharply in DL-transplanted mice treated with the aqueous extract of *S. anacardium*. Therefore the treatment of DL mice with the aqueous extract of *S. anacardium* indicates its promising therapeutical value.

Induction in the activity of SOD has been proposed to be of value for longevity in elderly individuals and has been predicted to provide resistance to malignancy or fatal cardiovascular events [22]. Purified SOD (Cu/Zn-SOD) from garlic decreases superoxide radical in tumour cell lines and therefore SOD is suggested for use in pharmacological applications [23]. Overexpression of Mn-SOD in certain tumours has been reported to enhance migration and invasion of fibrosarcoma cells. The migration and invasion of Mn-SOD-expressing cells was inhibited following overexpression of CAT, indicating that the invasive phenotype of SOD-expressing cells is H$_2$O$_2$-dependent [24]. The report highlights the significance of both antioxidant enzymes SOD and CAT in providing defence against oxidative stress.

Similarly, higher GST activity when mice were treated with aqueous extract of *S. anacardium* during lymphoma growth may contribute to the organism’s defence against toxic compounds, such as carcinogens. GST is known to neutralize potentially toxic electrophilic xenobiotics by promoting their conjugation with nucleophilic glutathione. The GST-T1 null genotype was correlated with an increase in the risk of Hodgkin’s lymphoma.

---

**Figure 9** Changes in activity of CAT in liver of mice treated with *S. anacardium* during development of lymphoma

(A) Native PAGE (7.5%) and activity staining of CAT. (B) Densitometric scanning of CAT. (C) Specific activity of CAT. DL, DL control; D1–D18, D1 to D18 mice treated with *S. anacardium*; N, normal control; NT, normal mice treated with 6.0 mg of *S. anacardium*; U, unit. *P < 0.05, significant difference compared with DL mice.
**Figure 10** Changes in activity of SOD in liver of mice treated with *S. anacardium* during development of lymphoma

(A) Native PAGE (7.5%) and activity staining of SOD. Light grey bars, CuZn-SOD; dark grey bars, Mn-SOD.

(B) Densitometric scanning of SOD. Light grey bars, CuZn-SOD; dark grey bars, Mn-SOD.

(C) Specific activity of SOD. DL, DL control; D1–D18, D1 to D18 mice treated with *S. anacardium*; N, normal control; NT, normal mice treated with 6.0 mg of *S. anacardium*; U, unit. *P < 0.05, significant difference compared with DL mice.

**Figure 11** Specific activity of GST in liver of mice treated with *S. anacardium* during development of lymphoma

DL, DL control; D1–D18, D1 to D18 mice treated with *S. anacardium*; N, normal control; NT, normal mice treated with 6.0 mg of *S. anacardium*; U, unit. *P < 0.05, significant difference compared with DL mice.
Figure 12 Changes in activity of LDH in serum of mice treated with *S. anacardium* during development of lymphoma

(A) Native PAGE (7.5%) and activity staining of LDH. (B) Densitometric scanning of *A*₄ isozymes of LDH. (C) Specific activity of LDH. DL, DL control; D1–D18, D1 to D18 mice treated with *S. anacardium*; N, normal control; NT, normal mice treated with 6.0 mg of *S. anacardium*; U, unit. *P* < 0.05, significant difference compared with DL mice.

Lower activity of antioxidant enzymes may be due to the accumulation of ROS in lymphoma-bearing mice, causing oxidative stress. ROS damages protein and therefore adversely affects the activities of the enzymes. ROS accumulation is a common factor in several cancers. Growth factors, such as FGF-2 (fibroblast growth factor 2), have been reported to generate *H*₂*O*₂ and *O*₂⁻, which mediates mitogenic signalling by receptor tyrosine kinases [26]. There are several mechanisms proposed for antioxidant action. Chemical antioxidants are known to donate an electron to a free radical, converting it into a less reactive form, i.e. a non-radical. Similarly, stable radical formers, such as melanin, scavenge an odd electron to form a stable radical species. According to the third mechanism of antioxidant action, a macromolecule binds to a radical-generating compound, deexcites an excited state species or quenches a free radical, i.e. it mimics SOD action. The aqueous extract of *S. anacardium* may follow one or more of these mechanisms and thus contribute to enhancement of the activity of antioxidant enzymes by removing oxidative stress.

Malignant cells maintain a high glycolytic rate, even under aerobic conditions, leading to high rate of lactate formation from glycolysis [27,28]. Therefore, LDH activity is much higher in tumour tissues than in corresponding normal tissues [29,30]. Similarly, LDH activity is found to be elevated in DL cancerous mice compared with normal mice. Although the treatment of normal mouse with the aqueous extract of *S. anacardium* caused
an increase in the activity of LDH, the activity was decreased significantly after treatment of DL mice. The result indicated that anaerobic metabolism of DL mouse is significantly impaired by treatment with the aqueous extract of *S. anacardium*, contributing to its anticarcinogenic action on DL mouse. Several reports highlight the increase in the A4 isozyme of LDH in tumours of all origins [31,32]. LDH is a tetramer protein. There are five isozymes of LDH, comprising two types of polypeptides (A and B) forming a tetramer. Expression of different LDH isozymes has been associated with clinical diseases, such as cancer, myocardial infarction and liver disorders. The LDH A4 isozyme is increased during promotion of malignancy. Therefore LDH isozymes serve as a useful tumour marker. In the present study, this isozyme was elevated in the serum of DL mice compared with normal mice. Herbal treatment of DL mice significantly decreased LDH activity and that of the A4 isozyme, suggesting that *S. anacardium* down-regulates anaerobic metabolism.

Aqueous extract of *S. anacardium* is found to be more effective than the conventional anticancer drug doxorubicin, as the herbal drug leads to a higher elevation in the activities of antioxidant enzymes that contribute to the strengthening of the antioxidant defence system of the organism. The herbal extract lowers the activity of LDH to a greater extent during the development of the lymphoma, as compared with doxorubicin. The effect of doxorubicin on the activities of antioxidant enzymes and LDH in DL mice has been reported previously [9]. These results encourage the use of medicinal plants, such as *S. anacardium*, as antioxidant, as well as anticarcinogenic, drugs. The nut milk extract of *S. anacardium* has been reported to restore energy metabolism in leukaemic mice [33]. This herbal extract modulates the activities of glutathione-associated enzymes [34]. It also regulates cell proliferation, apoptosis and carcinogenesis [35,36]. *S. anacardium* possesses a series of substituted phenolic compounds and a variety of flavonoids. The traditional use of different preparations of *S. anacardium* with reference to its anti-inflammatory property and antitumour property may be attributed to these phytochemicals. The potent free radical quenching activity of the extract, as shown by an increase in the activities of antioxidant enzymes, may be due to the presence of flavanoids. The water-soluble fraction of *S. anacardium* has been shown to inhibit the activities of COX2 (cyclo-oxygenase 2) and LOX15 (lipoxygenase 15) in rabbit. An anabolic effect of the aqueous extract of *S. anacardium* has been reported in MC 3T3-E1 osteoblast-like cells under inflammatory conditions induced by LPS (lipopolysaccharide) [37]. Similarly in the present study, the aqueous extract of *S. anacardium* may contain phytochemicals which possess antioxidant activities. It will be interesting to analyse and identify the phytochemicals in due course. Phytochemicals, such as anacardic acid or bhilawanol, may be present in the aqueous extract. However, further studies are needed to isolate and locate the phytochemical that is mainly responsible for elevation of the antioxidant action in DL mice.

**FUNDING**

This work was supported by University Grants Commission, India, under CAS programme [grant number F.5-3/2005 (SAP-II)]; the Indian Council of Medical Research for a Senior Research Fellowship [grant number 45/6/2003/BMS/TRM] to N.V.

**REFERENCES**


Received 9 May 2008/7 August 2008; accepted 2 September 2008
Published as Immediate Publication 2 September 2008, doi 10.1042/BSR20080035