Indian Journal of Experimental Biology
Vol. 42, April 2004, pp. 373-377

Modulation of antioxidant potential in liver of mice by kernel oil of cashew nut (*Anacardium occidentale*) and its lack of tumour promoting ability in DMBA induced skin papillomagenesis

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Received 16 October 2003; revised 12 December 2003

Cashew nut shell oil has been reported to possess tumour promoting property. Therefore an attempt has been made to study the modulatory effect of cashew nut (*Anacardium occidentale*) kernel oil on antioxidant potential in liver of Swiss albino mice and also to see whether it has tumour promoting ability like the shell oil. The animals were treated orally with two doses (50 and 100 μl/animal/day) of kernel oil of cashew nut for 10 days. The kernel oil was found to enhance the specific activities of SOD, catalase, GST, methylglyoxalase I and levels of GSH. These results suggested that cashew nut kernel oil had an ability to increase the antioxidant status of animals. The decreased level of lipid peroxidation supported this possibility. The tumour promoting property of the kernel oil was also examined and found that cashew nut kernel oil did not exhibit any solitary carcinogenic activity.

Keywords: Antioxidant, Modulation of antioxidant, Liver, Kernel oil, Cashew nut, *Anacardium occidentalte*, Tumour, DMBA, Skin papillomagenesis
IPC Code: Int Cl.[7] A61K 35/00

Cashew nut (*Anacardium occidentale*) has been reported as a stimulant, a rejuvenator and an appetizer in the sixteenth century Ayurvedic texts. It was popular even with the people of the Indus Valley civilisation[2]. Its aqueous extract has a protective action against diabetes induced by streptozoticin[3]. The kernel of cashew nut is highly nutritious, rich in vitamin A, D and K as well as minerals and fatty acids. The cashew nut oil consists mostly of glycerides of oleic (73.8%), linoleic (7.7%) stearic and palmitic acids[1]. The cashew nut oil finds its application in cosmetic industry because of natural richness in vitamin E which protects the kernel oil itself against oxidation and imparts a free radical scavenging activity to its formulated product[2].

On the contrary, experimental findings have reported the cashew nut shell oil to be a tumour promoting agent, in a DMBA induced skin papillomagenesis in mice[4]. The delicious edible kernels are processed products of cashew nut and the possible contamination of kernel with the shell oil cannot be overlooked. Epidemic eczematous dermatitis after consuming cashew nut contaminated with the shell oil constituents have been reported[5]. It will be of paramount interest to see whether the cashew nut kernel oil has tumour promoting property.

In the present study, an attempt has been made to study the modulatory effect of cashew nut (*Anacardium occidentale*) kernel oil on the antioxidant potential in the liver of Swiss albino mice and also to see whether it has tumour promoting ability like the shell oil of cashew nut.

Materials and Methods

Chemicals — 7,12-Dimethylbenz(a)anthracene (DMBA), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), pyrogallol, 2,6 dichlorophenol indophenol (DCPIP), ethylenediamine tetraacetic acid (EDTA), bovine serum albumin (BSA), thiobarbituric acid (TBA), reduced nicotinamide adenine dinucleotide (NADH), and methylglyoxal were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The rest of the chemicals used were of analytical grade.

Animals—Random bred female Swiss albino mice (6 weeks old), used in the present study, were maintained in the animal facility of the University with a

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12 hr light /dark cycle and provided with standard food pellets and tap water. All the animals were cared for according to the 'Principles of Laboratory Animal Care' (NIH, USA) and under strict adherence to the guidelines issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Preparation of kernel oil—The cashew nut kernel of variety - 210 was purchased locally. The required amount of cashew nut kernel was powdered and was mixed with double the volume of acetone. The mixture was stirred and left for 3-4 days after which the same was filtered. The acetone was allowed to evaporate naturally from the filtrate at room temperature (37°C) for 3 days followed by slight heating in the water bath (45°C) such that a concentrated cashew nut kernel oil was obtained. The animals were administered the kernel oil orally.

Preparation of homogenate, cytosol and microsome fractions—For assay of the antioxidant profile, mice were administered 50 or 100 µl of cashew nut kernel oil/ animal/ day orally for 10 days and then sacrificed by cervical dislocation and the entire liver was then perfused immediately with cold NaCl (0.9%) and thereafter carefully removed, trimmed free of extraneous tissue and rinsed in chilled 0.15 *M* of Tris-KCl buffer (0.15 *M* KCl + 10 m*M* Tris-HCl, *p*H 7.4). The liver was then blotted dry, weighed quickly and homogenized in ice cold 0.15 *M* of Tris-KCl buffer (*p*H 7.4) to yield 10% (w/v) homogenate. An aliquot of this homogenate (0.5 ml) was used for estimation of reduced glutathione levels while the remainder was centrifuged at 10,500 *g* for 20 min at 4°C using RC 5C Sorvall centrifuge (SM 24 rotor). The resultant supernatant was further centrifuged at 1,05,000 *g* for 60 min at 4°C in a Beckman ultracentrifuge (Model-L8 70M). The supernatant (cytosol fraction), after discarding any floating lipid layer and appropriate dilution, was used for the assay of antioxidant enzymes. The pellet representing the microsome was re-suspended in the homogenizing buffer and was used for determining lipid peroxidation.

Assay of antioxidant enzymes, glutathione content and peroxidative damage—Specific activity of catalase was estimated by the method of Aebi[6]. Superoxide dismutase was assayed utilizing the technique of Marklund and Marklund[7]. The cytosolic glutathione S-transferase activity was determined spectrophotometrically according to the method of Habig et al[8]. DT-diaphorase activity was measured as described by Ernster *et al*[9]. Glutathione content was estimated as total non-protein sulphydryl group by the method described by Moron *et al*[10]. Glyoxalase I was assayed according to the method of Racker as described by Thornalley[11]. Lipid peroxidation in the microsomes was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method, as described by Varshney and Kale[12].

Protein determination—Protein was determined by the method of Lowry *et al*[13], using bovine serum albumin (BSA) as standard at 660 nm.

Mouse skin papillomagenesis and its chemomodulation—DMBA induced skin papillomagenesis was studied in Swiss albino mice as described by Banerjee and Rao[4]. The hairs on the dorsal scapular region (2 cm diam.) of the mice were clipped off three days before application of chemical or modulator. Animals in the resting phase of hair growth cycle were selected for the experiment. The animals were assorted into the following groups.

Group I (n=10)-The animals were topically administered with 0.1 ml of acetone which was used as a vehicle for dilution of the cashew nut kernel oil. This group served as the negative control.

Group II (n=15)-A single dose of DMBA (0.05 mg/0.05 ml acetone) was topically applied to these animals in the shaven area to achieve initiation. After a gap of one week, croton oil (2%) was topically applied thrice a week until termination of the experiment. This group of mice served as the positive control group.

Group III (n=15)-A single dose of DMBA (0.05 mg/0.05 ml acetone) was topically applied to the animals on the shaven area to achieve initiation of skin papillomagenesis. Cashew nut kernel oil (2.5%) was topically applied three times a week until termination of the experiment.

Group IV (n=15)-A single dose of DMBA (0.05 mg/0.05 ml acetone) was topically applied to the animals on the shaven area to achieve initiation of skin papillomagenesis. After a gap of one week, cashew nut kernel oil (5%) was topically applied three times a week until termination of the experiment.

Group V (n=15)-Cashew nut kernel oil (5%) was topically applied to the animals on the shaven area until termination of the experiment.

The body weight of animals were recorded at regular intervals. Skin papillomas of size more than 1 mm in diam. appearing in the shaven area were re-

corded at weekly intervals and was included in the data analysis only if they persisted for more than two weeks. Duration of the experimentation was 120 days.

Statistical analysis—After calculating the mean and the standard deviation, Mann Whitney-Rank sum test was performed to obtain a significant difference between the treated groups and the control group. A value at $P < 0.05$ was considered to indicate a significant difference between the control and the experimental groups.

Results and Discussion

Animals were treated with two doses of cashew nut kernel oil (50 and 100 μl/animal/day) for ten days. Both the doses did not exhibit any adverse effects on the body weight profile and liver somatic index of animals (Table 1). It was important that the specific activity of hepatic catalase exhibited an increase by 1.53 and 2.03 fold as compared to the controls in the group of animals orally treated with low dose (50 μl) and high dose (100 μl) of cashew nut kernel oil respectively (Table 2). Further, the specific activity of SOD also increased by 1.11 fold in the group of animals treated with higher dose of the kernel oil (100 μl). Intracellular SOD and catalase are natural antioxidants and known to act as selective scavengers of superoxide radical and hydrogen peroxide. Both the enzymes are important members of the defense system against oxidative stress.

GST in another important enzyme known to catalyse antioxidant processes of thiol compounds and in turn protect the cells from electrophiles, free radical induced damage and oxidative stress[14]. The specific activity of hepatic GST also exhibited an increase by 2.04 and 2.19 fold as compared to the control in the group of animals orally treated with low and high dose of cashew nut kernel oil respectively (Table 2). Since GST belongs to the class of phase II enzymes which divert ultimate carcinogens from reaction with critical cellular macromolecules[15], it could be speculated from the increased levels of GST that cashew nut kernel oil probably had the ability to reduce the electrophilic damage to the cell which could lead to initiation of cancer.

DTD is known to play a protective role against oxidative stress[16,17]. Surprisingly, there was no significant change in the specific activity of DTD in the liver of mice on oral administration of the kernel oil. It was possible that the enhanced activities of SOD, catalase and GST might also be compensatory to non-responsiveness of DTD. In case of the antioxidant system such compensatory responses have been reported elsewhere[18,19]. It was significant that the levels of GSH enhanced by 4.38 and 2.32 fold in the group of animals treated with the low and high dose of

Table 1—Modulatory influence of cashew nut (*Anacardium occidentale*) kernel oil on weight gain profiles
[Values are expressed as mean ± SD of at least 6 animals].

Treatment	Body wt (g)		Liver wt × 100/Final body wt
	Initial	Final	
Control	22.33 ± 1.50	24.33 ± 1.50	6.17 ± 0.64
A	24.66 ± 1.63	26.0 ± 1.26	6.25 ± 0.73
B	24.66 ± 1.03	25.33 ± 1.63	6.82 ± 1.26

(A)–50 μl of cashew nut kernel oil/animal/day; (B) – 100 μl of cashew nut kernel oil/animal/day.

Table 2—Modulatory effect of cashew nut (*Anacardium occidentale*) kernel oil on mice hepatic antioxidant profile and lipid peroxidation
[Values are expressed as mean ± SD of at least 6 animals]

Groups	GSH (1)	GST (2)	DTD (3)	SOD (4)	CAT (5)	Gly I (6)	LP (7)
Control	1.03 ± 0.29 (100)	1.10 ± 0.43 (100)	0.03 ± 0.01 (100)	11.49 ± 0.64 (100)	32.29 ± 7.66 (100)	1.80 ± 0.25 (100)	1.87 ± 0.24 (100)
A	4.52± 0.53^{d} (438.40)	2.26 ± 0.40^{c} (204.98)	0.03 ± 0.01 (99.13)	11.60 ± 1.25 (100.94)	49.54 ± 7.97^{c} (153.3)	3.68 ± 0.47^{d} (204.09)	1.60 ± 0.41 (85.6)
B	2.39 ± 0.24^{d} (232.39)	2.42 ± 0.33^{a} (219.76)	0.04 ± 0.01 (106.05)	12.85 ± 0.80^{b} (111.81)	65.79 ± 3.87^{d} (203.73)	4.39 ± 0.91^{d} (243.3)	1.29 ± 0.31^{a} (68.9)

(A) - 50 μl of cashew nut kernel oil/animal/day; (B) – 100 μl of cashew nut kernel oil/animal/day
Values in parentheses represent relative changes in parameters assessed (i.e. levels of activity in liver of mice receiving test substance to activity in liver of control mice). $^{a}P < 0.05$, $^{b} < 0.01$, $^{c} < 0.005$, $^{d} < 0.001$ represents significant changes against the control group. (1) mmole GSH/g tissue. (2) μmole CDNB-GSH conjugate formed/min/mg protein. (3) μmole of DCPIP reduced/min/mg protein. (4) μmole/mg protein. (5) μmole of H_2O_2. consumed/min/mg protein. (6) μmole s – lactoylglutathione formed/min. (7) nmole malondialdehyde formed/mg protein. Abbreviations: GSH – reduced glutathione; GST – glutathione – s – transferase; DTD – DT- diaphorase; SOD - superoxide dismutase; CAT – catalase; LP – lipid peroxidation.

Table 3—Effect of cashew nut (*Anacardium occidentale*) kernel oil on DMBA induced skin papillomagenesis in Swiss albino mice [Values are expressed as mean ± SD of 10-15 animals]

Groups/Treatment	Body wt (g)		Tumour incidence (%)	Tumour burden (tumour/ mouse)
	Initial	Final		
I. Only vehicle (acetone)	22.80 ± 1.39	30.40 ± 0.84[d]	Nil	Nil
II. DMBA + 2 % croton oil	26.0 ± 2.13	32.57 ± 3.08[d]	86	4.92 ± 1.08
III. DMBA + 2.5 % cashew nut kernel oil	24.80 ± 2.11	32.26 ± 1.83[d]	Nil	Nil
IV. DMBA + 5 % cashew nut kernel oil	24.13 ± 1.40	32.26 ± 1.66[d]	Nil	Nil
V. 5 % cashew nut kernel oil	25.33 ± 1.79	32.26 ± 2.12[d]	Nil	Nil

[d]$P < 0.001$ represent significant changes against the control. Duration of the experimentation was 120 days.

cashew nut kernel oil respectively (Table 2). Since, GSH is the principle non-protein sulphydryl (NPSH) which has been endowed with an important function in maintaining the reduced milieu of the cells, its enhancement in the present study was a significant observation. Due to conjugating ability it is involved in detoxification of xenobiotics including carcinogens[20,21]. It has a redox potential of about (-) 230 mV which makes it behave as an antioxidant and protect against electrophiles, free radicals and oxidative stress[18,19]. Some of GSH dependent antioxidant reactions are catalyzed by the enzymes GST and methyl glyoxalases.

Methyl glyoxalase system is considered to be vital for biological function. Apart from its involvement in the regulation of cell divison and differentiation, the glyoxalase system is suggested to have antioxidant function as the electrophiles and cytotoxic 2-oxoaldehydes are converted to less reactive chemical species[18,19,22]. There was a significant increase in the specific activity of hepatic glyoxalase I by 2.04 and 2.43 fold in the group of mice orally treated with the low and high dose levels of cashew nut kernel oil respectively (Table 2). The concomitant increase in SOD, GST, catalase, glyoxalase I and GSH perhaps reflected the enhanced antioxidant status of animals which were treated with cashew nut kernel oil for 10 days. Since, cashew nut kernel oil exhibited an enhancement of GST, one of the phase II enzymes, it will be interesting to examine whether this kernel oil could be classified as a type A inhibitor under blocking agents, according to the classification of chemopreventive agents[23]. The enhanced antioxidant status in animals is expected to provide protection against oxidative damage. To confirm this possibility the peroxidative damage was examined in the liver of mice. As expected there was a significant decrease in the level of lipid peroxidation by 6.89 fold in the liver of mice particularly, orally treated with the high dose of cashew nut kernel oil compared to untreated group of animals (Table 2). The kernel oil is known to contain vitamins particularly vitamin E which might have scavenged the free radicals involved in initiation and propagation of lipid peroxidation resulting in its inhibition.

Antioxidant potential of plants is known to be closely linked with their cancer chemopreventive properties. This possible property of cashew nut kernel oil was also tested in the present work. Table 3 depicts the results of DMBA initiated skin papillomagenesis in mice. DMBA induced tumour incidence was 86% and the tumour burden (the number of tumours per mouse) was 4.92 ± 1.08. There was no development of skin papillomas in the group of animals treated with topical application of 2.5 and 5% cashew nut kernel oil. These observations were quite interesting and could perhaps be attributed to the ability of cashew nut kernel oil to enhance the antioxidant status in the animals. As mentioned earlier, the cashew nut shell oil has been found to exhibit a tumour promoting property in DMBA induced mice skin papillomagenesis[4]. The edible cashew nut kernel being the processed product, there could be possibility about its contamination with the shell oil and in turn increased risk of cancer. In view of this, it is important that the cashew nut kernel oil does not have a promoting effect on DMBA induced skin papillomagenesis in mice. Moreover, the results indicated that the kernel oil did not exhibit any solitary carcinogenic activity.

Since, SOD, catalase, GST, methyl glyoxalase I and GSH play an important role in protecting the animals against oxidative damage, their enhanced levels in the present study suggested that the cashew nut kernel oil had an ability to increase the antioxidant potential of animals. It was evident from the decreased level of peroxidative damage in the liver of mice due to treatment with the kernel oil. Unlike, the shell oil, it did not show promoting effect on DMBA induced skin papillomagenesis in mice. The results

also showed that the kernel oil did not exhibit any solitary carcinogenic activity. Further work needs to be done in different model systems to see whether cashew nut kernel oil can be classified as a type A inhibitor under blocking agent.

Acknowledgement

Financial assistance to author (BS) from CSIR, New Delhi is gratefully acknowledged.

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