

Dietary sesame (*Sesamum indicum* cultivar Linn) oil inhibits iron-induced oxidative stress in rats

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The high stability of sesame oil against oxidative deterioration is attributed to lignans in its non-glycerol fraction. The present study evaluates the effects of feeding sesame lignans (sesamin and sesamol) on Fe²⁺-induced oxidative stress in rats. Three groups, each of sixteen male weanling WNIN rats, were fed diets containing 200 g casein/kg and 100 g oil/kg (group 1, groundnut oil; group 2, sesame oil; group 3, sesame oil + sesamin (0.4 g/kg). After 45 d of feeding, eight rats from each group were injected with saline (9 g NaCl/l, controls) intraperitoneally while the remaining eight rats were injected with 30 mg Fe²⁺/kg body weight as ferrous sulfate in normal saline. The animals were killed after 90 min to evaluate hepatic function and antioxidant status. Compared with those fed groundnut oil (group 1), sesame oil-fed rats (groups 2 and 3) had lower levels of hepatic thiobarbituric acid-reactive substances, serum glutamate:oxaloacetate transaminase activities and serum glutamate pyruvate transaminase activities, indicating protection against Fe-induced oxidative stress. Despite similar tocopherol levels in the three diets, hepatic α -tocopherol levels were higher in rats fed the sesame-oil diets (groups 2 and 3) compared with controls (group 1). However, activities of hepatic antioxidant enzymes (superoxide dismutase and glutathione peroxidase) were significantly ($P < 0.05$) increased only in rats fed higher levels of lignans (group 3). These observations suggest that sesame lignans may have sparing effects on tocopherols. The increased bioavailability of tocopherols in the presence of dietary lignans might be due to the regeneration of oxidized tocopherols. The synergistic effects of lignans with tocopherols has nutritional and therapeutic implications.

Sesame lignans: Sesamin: Sesamol: Iron-induced oxidative stress *in vivo*: Antioxidant effects

Sesame (*Sesamum indicum*) has long been categorized as a traditional health food in India and Asian countries. Budowski & Markley (1951) and Meydani (1992) reported that consumption of sesame oil can delay the process of ageing. Topical application of sesame oil has been practised for centuries (Press *et al.* 1974). However, the scientific basis underlying the medicinal effects of sesame oil are not well understood. Recently sesamin, the major lipid soluble component of sesame seed unsaponifiables has been shown to exert diverse physiological functions (Hirose *et al.* 1992; Sugano & Akimoto, 1993; Matsumura *et al.* 1995, 1998) including hypocholesterolaemic effects in human subjects (Hirata *et al.* 1996). Sesamin (5 and 10 g/kg) has been shown to have hypocholesterolaemic and antihypertensive effects, to protect the liver from damage induced by alcohol, and to protect against CCl₄- and 1,2 dimethylbenz[a]anthracene-induced rat mammary carcinogenesis (Hirose *et al.* 1992). Sesamol, the second major sesame lignan, has been shown to inhibit proliferation by inducing apoptosis in human lymphoid leukaemia Molt 4B cells (Miyahara *et al.* 2001). Sesamol (10 g/kg) is metabolized to sesamol and sesamolol *in vivo* and inhibits lipid peroxidation (Kang *et al.* 1998). Sesamol and sesamolol, the degradation products of sesamol, have also been shown to inhibit the

formation of lipid peroxides in the oil and contribute to the higher stability of sesame oil (Fukuda *et al.* 1986a,b). Thus, it is evident from literature that the physiological effects of sesame lignans have been demonstrated at much greater supplementary levels than their consumption through dietary sources.

In an attempt to evaluate the antioxidant effects of individual sesame lignans, sesamin and sesamol were isolated and crystallized from an Indian sesame cultivar with a high lignan content (*Sesamum indicum* cultivar Linn; Hemalatha & Ghafoorunissa, 2004). Using *in vitro* lipid peroxidation systems, we showed that sesame lignans potentiate the inhibitory effects of tocopherols and tocotrienols (Ghafoorunissa *et al.* 2004). The present study was conducted to evaluate whether feeding sesame oil (1.2–1.6 g lignans/kg sesame seed or oil) to rats modulates oxidative stress and antioxidant status against Fe-induced oxidative damage.

Materials and methods

Chemicals

Reduced NADPH, GSSG, GSH, glutathione reductase, cumene hydroperoxide, butylated hydroxytoluene,

Abbreviations: SGOT, serum glutamate:oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminase; TBARS, thiobarbituric acid-reactive substances.

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thiobarbituric acid, tetraethoxypropane, pyrogallol, tocopherols (α - and γ -) and sesamol were from Sigma Chemical Co. (St Louis, MO, USA). H_2O_2 was obtained from E-Merck (Mumbai, India) and diethylene triamine penta acetic acid was from Loba Chemie (Mumbai, India). Assay kits for serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and HPLC solvents were obtained from Qualigens India Ltd (Mumbai, India). All other chemicals used were of analytical grade procured from local sources.

Sesamin was isolated and crystallized from a high-lignan sesame variety (Hemalatha & Ghafoorunissa, 2004) according to Budowski (1950) and purified by recrystallization in absolute ethyl alcohol. Groundnut oil (Dhara-National Dairy Development Board, Anand, Gujarat, India) and sesame oil (Agmark label; Mansion brand; Samalkot, Andhra Pradesh, India) were purchased from the local market.

Animals, diets and study design

The Institute's Animal Ethics committee approved the animal experimental protocols. The institutional guidelines for the care and use of laboratory animals were followed strictly. Male weanling WNIN rats with a mean body weight of 35 g (n 48) were obtained from the National Centre for Laboratory Animal Sciences at the National Institute of Nutrition, Hyderabad, India. Rats were randomly divided into three groups (sixteen per group; a control group and two experimental groups) and housed individually in polypropylene cages at a temperature of $24 \pm 2^\circ\text{C}$ and with a 12 h light–dark cycle. Rats were fed *ad libitum* (Table 1; 200 g casein/kg, 100 g oil/kg (group 1, groundnut oil; group 2, sesame oil; group 3, sesame oil + sesamin (0.4 g/kg)). The total tocopherol content was similar in all three diets and the diets differed only in their lignan content (Table 1). Although the total PUFA composition and SFA content were similar in the groundnut- and sesame-oil groups, the two oils differed in their individual SFA contents (Table 2).

Dietary intakes were recorded daily and body weights were monitored once per week. After 45 d of feeding, animals were fasted overnight and oxidative stress was

Table 1. Composition of diets (g/kg)

Ingredients	Group 1*	Group 2*	Group 3*
Casein	200	200	200
Cellulose	50	50	50
Starch	600	600	600
Choline chloride	2	2	2
Methionine	3	3	3
Vitamin mix†	10	10	10
Mineral mix†	35	35	35
Oil‡	100	100	100
α -Tocopherol	0.03	–	–
γ -Tocopherol	0.04	0.07	0.07
Sesamin	0.0	0.8	1.2
Sesamol	0.0	0.4	0.4

* Group 1, groundnut oil; group 2, sesame oil; group 3, sesame oil + sesamin (0.4 g/kg diet).

† Prepared as per AIN formulations (Reeves *et al.* 1993).

‡ Groundnut oil or sesame oil or sesame oil + sesamin (0.4/kg diet).

Table 2. Fatty acid composition of oils (g/100 g total fatty acids)

Serial no.	Fatty acids	Groundnut oil	Sesame oil
1	16:0	13.0	8.0
2	18:0	3.3	10.0
3	20:0	2.1	–
4	24:0	4.9	0.2
5	18:1	44.0	43.0
6	18:2 n -6	33.0	38.0

induced in eight rats per group by a single intraperitoneal injection of 30 mg Fe^{2+} as ferrous sulfate in normal saline (9 g NaCl/l)/kg body weight (Hu *et al.* 1990). The remaining eight rats in each group were injected with normal saline and served as controls.

Blood and tissue collections

Blood was collected from the orbital sinus 90 min after the intraperitoneal injection of ferrous sulfate and before killing; serum was separated and stored at -70°C until further analysis. Rats were killed by CO_2 inhalation, the abdomen was cut open quickly and the liver perfused thoroughly with isotonic saline (9 g NaCl/l), excised, blotted dry and divided into multiple samples. One of the samples was used to assess the thiobarbituric acid-reactive substances (TBARS) in the liver homogenate on the same day, while the other samples were quickly frozen in liquid N_2 and stored at -70°C .

Tissue processing

Livers collected from each rat were homogenized in 10 mM-Tris-HCl buffer, pH 7.4 containing 0.2 mol KCl/l, using a Polytron homogenizer (Kinematica, Lucerne, Switzerland) for 2 min at 4°C . The homogenate was subjected to differential centrifugation (Sorval OTD-65B ultracentrifuge using 50.1 type rotor; Newtown, CT, USA) at 800 g for 10 min, 14 000 g for 10 min, 105 000 g for 80 min at 4°C ; the supernatant fractions were collected and stored frozen at -70°C until further analysis. The protein contents of these supernatant fractions were determined according to a modified Lowry's method (Schacterle & Pollack, 1973).

Tocopherols in liver homogenate

Total lipids were extracted (Bligh & Dyer, 1959) from liver homogenate (corresponding to about 10 mg protein), reconstituted in 50 μ l *n*-hexane and mixed well; a 20 μ l sample was analysed for tocol profile by reverse-phase HPLC (Shimadzu LC-10A HPLC system; Chiyoda-ku, Tokyo, Japan), using a Shodex C_{18} column (4.6 mm internal diameter \times 250 mm; Shodex, Minat-ku, Tokyo, Japan) connected to a manual valve injector and a Shimadzu UV-Vis detector SPD-10A (Shimadzu). The mobile phase comprised acetonitrile–methanol–dichloromethane (60:35:5, by vol.) at a flow rate of 2 ml/min (Barrie & Linda, 1989). The chromatograms were recorded in a Shimadzu C-R6A Chromatopac and the peaks quantified by comparison with those of authentic tocols (α -, γ - and δ -tocopherol, and α -tocotrienol) used as external standards.

Lipid peroxidation

The lipid peroxide content in liver homogenate was measured as TBARS (Slater & Sawyer, 1971) using 1,1,3,3-tetraethoxypropane as an authentic external standard.

Antioxidant enzymes

Catalase. The activity of catalase was determined in the supernatant fraction of liver homogenate (800 g, 50–100 µg protein) by following the decomposition of H₂O₂ at 240 nm for 1 min (Aebi, 1983).

Superoxide dismutase. Superoxide dismutase was assayed in the supernatant fraction (105 000 g, 200–300 µg protein) by following the inhibition of auto-oxidation of pyrogallol at 420 nm for 1 min (Marklund & Marklund, 1974).

Glutathione peroxidase. Glutathione peroxidase was assayed in the supernatant fraction (105 000 g, 100–200 µg protein) using cumene hydroperoxide as the substrate. The activity was expressed as the amount of NADPH oxidized in 1 min at 340 nm (Paglia & Valentine, 1967).

Statistical analysis

All the results are expressed as mean values with their standard errors for six to eight independent observations, with all measurements being performed in duplicate. Data were analysed for statistical significance by two-way ANOVA using the SPSS statistical package (version 10.0; SPSS Inc., Chicago, IL, USA). *Post hoc* multiple comparison tests for significant differences among groups using least significant difference were performed only for oils. Similar *post hoc* tests were not performed for stress, because there were only two stress conditions, i.e. with and without stress. The values were considered significantly different if *P* values were <0.05.

Results

Diet intake, weight gain and liver weights

There were no significant differences in food intake, body weight gain or liver weights among the three groups of rats (results not shown).

Serum enzymes

Under basal conditions, feeding sesame-oil diets (groups 2 and 3) significantly decreased the SGPT (Fig. 1(B)) activity compared with groundnut-oil diets (group 1). The decrease in SGOT activity (Fig. 1(A)) was, however, significant (*P*<0.05) only in rats fed the sesame-oil + sesamin diet (group 3) compared with rats fed the groundnut-oil (group 1) and sesame-oil (group 2) diets. Regardless of the type of oil fed, induction of oxidative stress with Fe caused a significant increase in the activities of SGOT and SGPT. Even under the conditions of Fe-induced oxidative stress, the activities of SGOT and SGPT (Fig. 1) were least in group 3, whereas the activities in groups 2 and 3 were not different from one another.

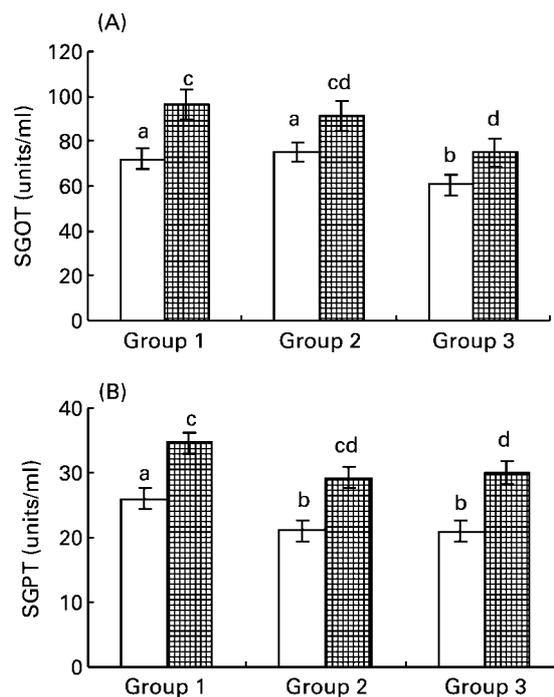


Fig. 1. Liver function tests in rats fed groundnut oil (group 1), sesame oil (group 2) and sesame oil + sesamin (group 3) diets, without (□) and with (▨) Fe-induced oxidative stress. (A), serum glutamate oxalate transaminase (SGOT); (B) serum glutamate pyruvate transaminase (SGPT). Values are means with their standard errors for six to eight samples analysed. For details of diets and procedures, see p. 582. Data were analysed by two-way ANOVA followed by *post hoc* analysis using LSD. (A), SGOT: *F* ratio for oils 3.324 (*P*<0.05), stress 9.628 (*P*<0.05); (B), SGPT: *F* ratio for oils 5.281 (*P*<0.05), stress 13.574 (*P*<0.05). ^{a,b,c,d} Mean values with unlike superscript letters were significantly different among the three groups under basal and Fe-induced stress conditions (*P*<0.05).

Lipid peroxidation in hepatic tissue

Basal levels of TBARS (Fig. 2(A)) in liver homogenates were comparable among the three groups, whereas induction of oxidative stress resulted in a twofold increase in TBARS in rats fed the groundnut-oil (group 1) diet (*P*<0.05), no such increase was observed in rats fed the sesame-oil diets (groups 2 and 3).

Tocopherol content in liver

Basal levels of tocopherol in liver (Fig. 2(B)) were significantly higher (*P*<0.05) in rats fed the sesame-oil diets (groups 2 and 3) than those receiving the groundnut-oil (group 1) diet. However, there was no difference between the hepatic tocopherol concentrations of rats fed diets containing sesame oil (groups 2 and 3). Induction of oxidative stress with Fe²⁺ did not cause significant changes in the hepatic tocopherol concentrations in any of the groups.

Antioxidant enzymes

There were no significant differences among the three groups of rats in the basal activities of catalase, superoxide dismutase and glutathione peroxidase in liver (Fig. 3). In addition,

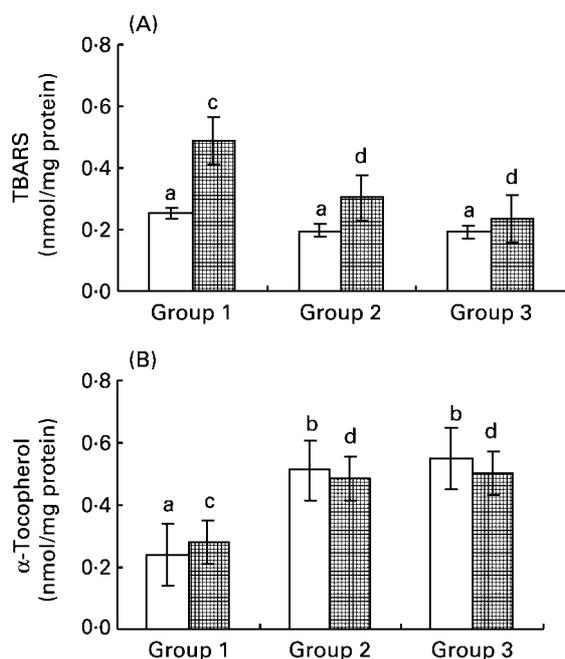


Fig. 2. Oxidative stress and antioxidant status of rats fed groundnut oil (group 1), sesame oil (group 2) and sesame oil + sesamin (group 3) diets, without (\square) and with (\blacksquare) Fe-induced oxidative stress. (A), thiobarbituric acid-reactive substances (TBARS) in liver homogenate; (B), α -tocopherol in liver homogenate. Values are means with their standard errors for six to eight samples analysed. For details of diets and procedures, see p. 582. Data were analysed by two-way ANOVA followed by *post hoc* analysis using LSD. (A), TBARS: *F* ratio for oils 4.71 ($P < 0.05$), stress 8.56 ($P < 0.05$); (B), α -tocopherol: *F* ratio for oils 12.48 ($P < 0.05$), stress 0.05 (NS). ^{a,b,c,d}Mean values with unlike superscript letters were significantly different among the three groups under basal and Fe-induced stress conditions ($P < 0.05$).

induction of oxidative stress with Fe^{2+} did not cause any significant increase in the activities of these enzymes in any group of rats. A significant increase ($P < 0.05$) was observed in the superoxide dismutase and glutathione peroxidase activities in rats fed the diet containing sesame oil + sesamin (group 3) compared with those fed the groundnut- or sesame-oil diets (groups 1 and 3).

Discussion

Fe overload leads to chronic hepatotoxicity, resulting in increased oxidative stress (Niederau *et al.* 1985). The present study demonstrates the effects of sesame lignans included in the diets of rats subjected to oxidative stress with Fe^{2+} . A dose of 30 mg Fe^{2+} /kg body weight is the maximum concentration reported to be tolerated by rodents (Hu *et al.* 1990) and has been shown to induce hepatotoxicity (Bhattacharya *et al.* 2000). However, in the present study 30 mg Fe^{2+} induced moderate hepatotoxicity, as assessed by SGOT and SGPT activities.

Feeding sesamin to rats at 2–5 g/kg has been shown to increase liver weight (Sugano *et al.* 1990; Fujiwara *et al.* 1995; Gu *et al.* 1995, 1998; Nakabayashi *et al.* 1995; Yamashita *et al.* 1995). However, in the present study liver weights remained unaltered in rats fed the sesame-oil diets (1.2–1.6 g sesamin + sesamol/kg), as has been

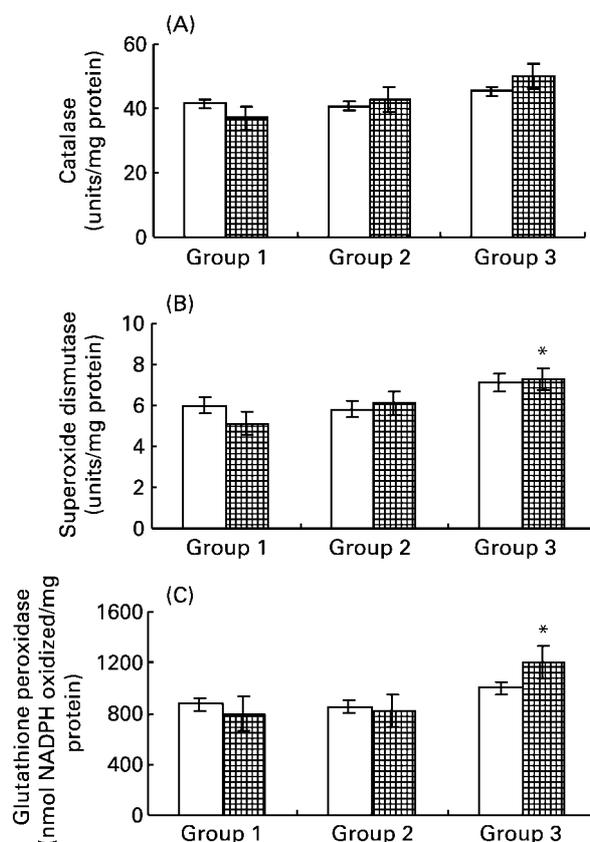


Fig. 3. Antioxidant enzyme activities in rats fed groundnut oil (group 1), sesame oil (group 2) and sesame oil + sesamin (group 3) diets, without (\square) and with (\blacksquare) Fe-induced oxidative stress. (A), catalase activity; (B), superoxide dismutase (SOD) activity; (C), glutathione peroxidase (Gpx) activity. Values are means with their standard errors for six to eight samples analysed. For details of diets and procedures, see p. 582. Data were analysed by two-way ANOVA followed by *post hoc* analysis using LSD. (A), catalase: *F* ratio for oils 1.796 (NS), stress 0.041 (NS); (B), SOD: *F* ratio for oils 4.364 ($P < 0.05$), stress 0.112 (NS); (C), Gpx: *F* ratio for oils 3.24 ($P < 0.05$), stress 0.197 (NS). Mean values were significantly different from those of groups 1 and 2: * $P < 0.05$.

reported by Kamal-Eldin *et al.* (1995) and Satchithanandam *et al.* (1996). Under basal and Fe^{2+} -induced oxidative stress conditions, SGOT and SGPT activities were lower in rats fed the sesame-oil + sesamin diets (group 3). These observations suggest that dietary lignans provided through consumption of sesame seeds or oil may protect the liver against Fe-induced oxidative damage. Akimoto *et al.* (1993) showed that sesamin lowered the ethanol inhalation-induced increase in SGPT activity. It has been shown that lignans (schisantherin compounds) containing the methylenedioxy group protect the liver from injury against viral hepatitis (as evident from lower SGPT levels) in mice and human subjects (Macrae & Towers, 1984; Gao & Rao, 1993). Therefore, the hepatoprotective effects of sesame oil observed in the present study could be due to the methylenedioxy group present in sesame lignans (sesamin and sesamol). However, the mechanism needs to be studied.

Sesamin at 2 g/kg has been shown to maintain blood and hepatic γ -tocopherol concentrations, resulting in enhanced suppression of lipid peroxidation (Yamashita *et al.* 1992,

1995). Sesamin (2 g/kg) and α -tocopherol have also been shown to synergistically suppress lipid peroxidation in rats fed a high-docosahexaenoic acid diet (Yamashita *et al.* 2000). Kang *et al.* (1998) have shown that the concentration of TBARS in the liver and kidney of rats fed 10 g sesamol/kg diet was low. In the present study, rats fed the sesame-oil diet (group 3; 0.8 g sesamin + 0.4 g sesamol/kg, a dose of sesamin that is about 50% of that reported by Yamashita *et al.* (1992, 1995)) had maximum protection against Fe^{2+} -induced lipid peroxidation; this appears to be due to the increase in hepatic α -tocopherol (Fig. 2(B)). Therefore, the protection afforded by rats fed the sesame-oil diets (groups 2 and 3) may be due to the combined effects of sesamin and sesamol. However, in response to Fe-induced stress, levels of TBARS in group 1 rats (control) were two- to threefold lower than those reported by Pulla Reddy & Lokesh (1994). Further, regardless of the source of dietary oil, α -tocopherol was the major isomer in liver (Fig. 2) of rats, while γ -tocopherol was present only in negligible amounts (results not shown). Yamashita *et al.* (1995) reported that α -tocopherol was the predominant isomer in serum and tissues of rats fed adequate α -tocopherol and sesame diets, while γ -tocopherol was detected in rats fed deficient or low levels of α -tocopherol.

Vitamin C and/or GSH in blood and tissues regenerates oxidized tocopherol to its native form (Leung *et al.* 1982; Scholz *et al.* 1997). The enhancement of vitamin E activity *in vivo* by food components has also been reported (Leung *et al.* 1982; Zhu *et al.* 2000). Sesame lignans have been shown to increase tissue tocopherol levels (Parker *et al.* 2000) by inhibition of the cytochrome P450 3A-dependent *n*-hydroxylase pathway of tocopherol catabolism. Recently, Yamashita *et al.* (2002) have shown that a sesaminol-induced increase in α -tocopherol and α -tocotrienol in plasma and tissues was not due to their enhanced absorption. In the present study, despite similar tocopherol levels in all three diets, the increased bioavailability of α -tocopherol (Fig. 2(B)) in the presence of dietary lignans (groups 2 and 3) appears to be due to inhibition of tocopherol catabolism and/or regeneration of oxidized tocopherols.

Antioxidant enzymes have an important role in the secondary defence mechanism during oxidative stress. In the present study, although the activity of catalase was not affected by the dietary oils or by the induction of oxidative stress with Fe^{2+} , superoxide dismutase activity was greater in rats fed sesame oil + sesamin (group 3). These results suggest that sesame lignans may enhance the ability to 'mop up' superoxide radicals formed during Fe^{2+} -induced oxidative stress. Induction of oxidative stress with Fe^{2+} caused an increasing trend in glutathione peroxidase activity only in group 3. The observation that the hepatic antioxidant enzyme (superoxide dismutase and glutathione peroxidase) activities were significantly higher ($P < 0.05$) in rats fed diets containing sesame oil + sesamin (group 3) than in those fed sesame-oil diets (group 2) supports the dose-dependent effects of dietary sesame lignans on the antioxidant enzyme activities. Sesame antioxidants (sesamin and sesamol) spared superoxide dismutase and catalase in hypoxia-stressed

PC12 cells in a dose-dependent manner, an effect that may be related to their radical scavenging effect (Hou *et al.* 2003). Earlier studies have shown the effects of sesame lignans (sesamin and sesamol) on antioxidant enzyme activities in *in vitro* systems using cell lines (Hou *et al.* 2003), whereas in the present study these effects were demonstrated *in vivo*.

The degradation products of sesamin in liver (Asami *et al.* 1993) and the metabolites of sesamol (sesamol and sesamolol) have been shown to contribute to their antioxidant effects *in vivo* (Kang *et al.* 1998). Using various lipid peroxidation systems, Uchida *et al.* (1996) showed that sesamol and related compounds containing methylene dioxy groups have antioxidant effects. Sesamin and sesamol inhibited lipid peroxidation *in vitro* in the enzymic and non-enzymic lipid peroxidation systems only when incubated with microsomes, and not with mitochondria or boiled microsomes (Ghafoorunissa *et al.* 2004); this suggests that the metabolites of these lignans may have greater antioxidant activities. Earlier, the synergistic effects of sesamin (2 g/kg) and tocopherols in increasing the bioavailability of γ -tocopherol (Kamal-Eldin *et al.* 1995), in inducing hypocholesterolaemia (Nakabayashi *et al.* 1995) and in modulating immune functions (Gu *et al.* 1997) have been documented. Our earlier studies have shown that sesame lignans (sesamol, sesamin and sesamolol) potentiate the antioxidant activities of tocopherols (α or γ -tocopherol or α -tocotrienol) in lipid peroxidation systems *in vitro* (Ghafoorunissa *et al.* 2004). The findings of our *in vitro* study and the present observations suggest that sesame oil (1.0–1.5 g sesamin + sesamol/kg) consumed through dietary sources, along with tocopherols, may counteract the oxidative damage caused by reactive oxygen species during Fe^{2+} -induced oxidative stress. This could be due to the metabolites of sesame lignans and/or tocopherol enhancing effects of sesame lignans.

Since reactive oxygen species are implicated in chronic diseases, current emphasis is to identify newer natural components of food exhibiting antioxidant activity. Considering the widespread production and usage of sesame oil and consumer preference for natural antioxidants, sesame seeds and oil appear to be promising agents with therapeutic potential. However, the mechanism(s) of the antioxidant and hepatoprotective effects of sesame lignans *in vivo*, vis-à-vis the increased levels of tocopherols and increased antioxidant enzyme activities needs to be deciphered.

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