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Alterations in antioxidant status of rats following intake of ginger through diet

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Abstract

Ginger is known to possess potent antioxidant and anti-inflammatory properties. Therefore, *in vivo* studies in rats were initiated to investigate if ginger fed through diet can improve their antioxidant status. Inbred male Wistar/NIN rats were divided into four groups containing six animals per group. The 1st group received a stock diet whereas the 2nd, 3rd and 4th groups were fed with a diet incorporating ginger powder at 0.5%, 1% and 5% levels for a period of one month. After one month of feeding, rats were sacrificed and their livers and kidneys collected for the analysis of antioxidant enzymes like superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx), and to estimate lipid peroxidation and protein oxidation. The levels of all the three enzymes, which are the important components of antioxidative defenses, were significantly stimulated in the livers of groups fed with ginger. The significant reduction in lipid peroxidation in livers and kidneys and inhibition of protein oxidative products in livers indicated the antioxidant potential of ginger when consumed naturally through diet. The findings reported suggest that regular intake of ginger through diet can protect against oxidative tissue damage.

Keywords: Ginger; Antioxidant enzymes; Superoxide dismutase (SOD); Catalase; Glutathione peroxidase (GSHPx); Thiobarbituric acid reactive substances (TBARS)

1. Introduction

Reactive oxygen species (ROS) are generated spontaneously in the living cell during metabolism. Antioxidants act as a major defense against ROS mediated toxicity by protecting membrane and cytosolic compounds. These compounds exhibit a wide spectrum of activity against toxicants/carcinogens and have the capacity to intervene in carcinogen metabolism. The antioxidant defenses include natural and synthetic antioxidants and the antioxidant enzymes present in the biological system (Sies, 1991). Plant products exert their antioxidant effect by quenching free radicals. By reducing exposure to free radicals and by increasing intake of antioxidant nutrients it is possible to reduce the risk due to free radical health problems associated with the aging process, cancer and atherosclerosis (Ames, Shigenaga, & Hagen, 1993).

Currently there is a strong interest in the study of natural compounds with free radical scavenging capacity. Dietary antioxidants reduce free radical formation and as a consequence oxidative stress in general, by way of countering LDL oxidation and platelet aggregation and by inhibition of the synthesis of proinflammatory cytokines (Kushi et al., 1996). Lipid peroxidation in tissues results in the production and propagation of free radical reactions primarily involving membrane polyunsaturated fatty acids (PUFAs). This has been implicated in the pathogenesis of numerous diseases including atherosclerosis, diabetes, cancer and rheumatoid arthritis as well as in drug associated toxicity and aging (Halliwell & Gutteridge, 1989).

Oxidative stress causes damage to nucleic acids, membrane lipids and proteins. Proteins are susceptible to oxidation by ROS, where the type of damage is characteristic of the denaturing species. The formation of protein carbonyls

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is considered as a biomarker of primary oxidative stress (Griffiths, 2001). Oxidative modification of proteins may occur via metal-catalyzed oxidation, radiation mediated oxidation and oxidation by oxides of nitrogen. Increase in carbonyl levels has been detected in several diseases such as rheumatoid arthritis, cardiovascular diseases etc. (Reznick, Witt, Matsumoto, & Packer, 1992). Metal catalysed protein oxidation leading to an increase in carbonyl formation has also been detected in aged rat livers (Starke-Reed & Oliver, 1989).

The antioxidant enzymes in the tissues can effectively scavenge free radicals that are generated during xenobiotic metabolism (Perctval, 1998). Since ancient times spices have been added to different types of food to improve the flavour. Now it is well known that spices possess antioxidant activity and prevent oxidation of lipids in foodstuffs. Kikusaki and Nakatani (1993) reported that chemical constituents like gingerols and shogaols present in ginger exhibited strong antioxidative activity. Sekiwa, Kubota, and Kobayashi (2000) reported that novel glucosides related to gingerdiol from ginger has antioxidative activity using the linoleic acid model system and by their DPPH radical scavenging ability. Gingerol, the pungent factor in ginger oleoresin, inhibited phospholipid peroxidation induced by the $FeCl_3$ ascorbate system (Aeschbach et al., 1994). These *in vitro* observations prompted us to investigate if ginger consumption through diet can improve the *in vivo* antioxidant status. Therefore, the levels of antioxidant enzymes, namely, SOD, catalase and GSHPx (Cytosol and RBC) were measured. Further inhibition of lipid peroxidation and protein oxidation was estimated in tissues of rats (liver and kidney) fed with ginger through their diet for one month.

2. Materials and methods

2.1. Reagents

All chemicals were of analytical grade. Dimethyl triamine penta acetic acid (DTPA), Tris HCl buffer, Pyrogallol, Beta nicotinamide adenine dinucleotide phosphate reduced (β-NADPH), Glutathione (GSH), Glutathione reductase, 2-thiobarbituric acid (TBA), 1,1,3,3-tetra ethoxy propane (TEP), 2,4-dinitrophenyl hydrazine(DNPH), guanidine hydrochloride were purchased from Sigma Chemical Co. (St.Louis, MO, USA). All other general chemicals were of highest purity available in the local market.

2.2. Preparation of test diets

Ginger was purchased from a local market, peeled, washed, coarsely minced, air dried and pulverized with a blender to a fine powder. This was added (0.5 g), 1.0 g and 5 g to the stock diet and mixed so as to yield diets of three compositions containing 0.5%, 1% and 5% ginger, respectively. The amount of ginger powder present in the 15 g diet which the rat consumed daily corresponds to 0.075, 0.15 and 0.75 g which in turn corresponds to 0.25 g, 0.5 g and 2.5 g of fresh ginger/15 g of rat diet. The stock diet contained wheat flour 15%, roasted Bengal gram flour 58%, groundnut flour 10%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt mixture 4% and vitamin mixture 0.2%.

2.3. Study design

The procurement of animals for experiment was undertaken by following the ethical guidelines of the Institutes Animal Ethical Clearance Committee. Inbred male NIN/Wistar rats, aged about 8–10 weeks, were used for the study. Age matched and weight matched rats were divided into four groups containing 6 animals per group. The control group received a stock diet throughout the experimental period whereas the experimental groups were fed with the diet incorporated with ginger powder at 0.5%, 1% and 5% levels for a period of one month. The rats were maintained at 22 ± 1.2 °C with 50–55% relative humidity and 12 h light/dark cycle. Food and water were given ad libitum. The weekly food intake and the body weight of each animal were recorded at the beginning and end of the experiment.

At the end of 1 month feeding, blood samples were collected from the orbital plexus. The animals were sacrificed and the organs, namely the liver and kidney, were collected and frozen under liquid nitrogen immediately and stored at −80 °C. The tissues were then minced and a 20% (W/V) homogenate was prepared in cold buffer using a polytron homogenizer (Kinematica) for a minute. They were processed as per the standard procedures for estimation of antioxidant parameters.

2.4. Measurement of SOD activity

SOD was estimated in liver cytosol according to the method of Marklund and Marklund (1974). The method is indirect and is based on the ability of the enzyme to inhibit O_2 dependent auto-oxidation of pyrogallol. The reaction mixture contained Tris HCl buffer (50 mM, pH 8.2), DTPA (1 mM), cytosol (0.1 ml), the reaction was started by the addition of pyrogallol (1 mM) in a total volume of 1 ml and the increase in absorbance at 420 nm was followed for 30 s. A control was run which had pyrogallol alone without cytosol (enzyme). One unit of SOD activity is the amount of the enzyme that inhibits the rate of auto-oxidation of pyrogallol by 50%. Activity of SOD was expressed as units/mg protein/min.

2.5. Glutathione peroxidase assay (GSHPx)

(a) GSHPx in the cytosolic fraction of liver was determined by a modified coupled assay procedure of Paglia and Valentine (1967). This method measures the rate of glutathione oxidation by hydrogen peroxide as catalyzed by GSHPx present in the sample by the addition of endogenous glutathione reductase and NADPH that converts oxidized glutathione (GSSG) to the reduced form. The rate

of GSSG formation is measured by following the decrease in absorbance of the reaction mixture at 340 nm as NADPH is converted to NADP. Selenium dependent GSHPx was measured using hydrogen peroxide as substrate. The activity of GSHPx was calculated as the amount of NADPH oxidized per minute using the molar absorption coefficient of 6.22×10^{-6} for NADPH. Enzyme activity was expressed as *n*-moles of NADPH oxidized per mg protein per minute.

(b) GSHPx in the whole blood was estimated using the European Community Food Linked Agro-Industrial Research (EC-FLAIR) common assay (Belsten & Wright, 1995). The following method is based on the original method of Paglia and Valentine (1967). This was modified by the addition of dithiothreitol for enzyme stabilization. *tert*-Butyl hydroperoxide 2.5 μM (0.1 ml) which was used as the initiator was also pre-equilibrated at 37 °C and was mixed quickly with the above and absorbance was measured over 2 min at 340 nm in Hitachi Spectrophotometer at 37 °C after allowing 1 min initial lag phase for the restabilisation of temperature. The mean change in absorbance/min was calculated for the sample and control. The units of the activity were calculated as nmol β-NADPH oxidized/min/mg haemoglobin (Hb) using the Hb concentration of the sample in mg/ml.

2.6. Catalase activity

The activity of catalase in liver homogenate was measured according to the method of Aebi (1983). The decomposition of hydrogen peroxide can be followed directly by the decrease in absorbance at 240 nm per unit time. Hydrogen peroxide was diluted with phosphate buffer to 100 ml. Ten percent of liver homogenate was centrifuged for 10 min at 700×*g*. To an aliquot of the supernatant, ethanol was added to a final concentration of 0.17 M (0.01 ml ethanol/ml) and samples were kept for 30 min in an ice water bath. After 30 min, Triton X 100 was added to a concentration of 1%, followed by the addition of cold buffer to achieve a 100-fold dilution. An aliquot of the diluted homogenate (50–100 μl) equivalent to 20–100 μg protein was taken and made upto 2 ml with phosphate buffer and the reaction was started by the addition of 1 ml of hydrogen peroxide. The decrease in absorbance at 240 nm was followed over 2 min. A control was also run containing either enzyme or substrate. The activity of catalase was calculated from the change in absorbance at 240 nm. One unit of catalase activity was calculated using $K = (2.303/t)(\log A_1/A_2)$, where '$K$' is the rate constant, 't' is the time in minutes, A_1 is absorbance at time 0 and A_2 is absorbance at 2 min. Specific activity was calculated as K/mg protein.

2.7. Estimation of lipid peroxidation and protein oxidation

Thiobarbituric acid reactive substances (TBARS) for the measurement of lipid peroxidation in liver and kidney homogenates of rats was carried out according to the procedure of Wilbur, Bernheim, and Shapiro (1949). Malondialdehyde (MDA), which is the end product of lipid peroxidation, was estimated utilizing its property to react with TBA. To 1 ml of 20% liver and kidney homogenate prepared in 1.15% KCl, 0.5 ml of 20% of trichloro acetic acid (TCA) was added followed by 1 ml of 0.67% TBA. This was heated in a tightly stoppered tube for 10 min in a boiling water bath. After cooling, the tubes were centrifuged to separate the precipitated protein. The absorbance of the pink colour produced was read at 340 nm in a spectrophotometer. The amount of MDA produced was calculated using 1,3,3-tetra ethoxy propane as a standard and the result of peroxidation was expressed as *n*-moles of MDA formed per mg of protein. Protein was estimated by the method of Lowry, Rosenbrongh, Farr, and Rendall (1951).

2.8. Protein carbonyls in liver

Carbonyl groups were assayed by the method described by Uchida et al. (1998). Protein samples (50 μl liver cytosol diluted with water to 500 μl) were added to an equal volume of 0.1% 2, 4-dinitro phenyl hydrazine in 2 M HCl and left for 1 h at room temperature. Twenty percent TCA was added to this mixture and the precipitate extracted three times with 0.5 ml ethanol/ethyl acetate (1:1). The precipitates were dissolved in 8 M-guanidine hydrochloride, 13 mM ethylene diamene tetra acetic acid (EDTA), 133 mM Tris, pH 7.4 and the carbonyl content was determined at 370 nm with the use of molar absorbance coefficient of 22,000 mol/l per mole of protein.

2.9. Statistical analysis

The data was analyzed by the analysis of variance (ANOVA). Testing of mean values in different groups was done by Duncan's multiple range test. SPSS 10 Window version was used for the statistical analysis (Middle Brooks, 1977).

3. Results

3.1. Effect of ginger feeding to rats on in vivo antioxidant enzymes

Significant increase of liver SOD activity was observed in the ginger fed groups compared to the control group that was not fed with ginger ($p < 0.05$). At 0.5%, 1.0% and 5.0% ginger feeding a stimulation of 76%, 107% and 141% of hepatic SOD against the control was observed. There was an increase in the activity of catalase compared to control ($p < 0.05$). At 0.5%, 1.0% and 5.0% of ginger feeding a stimulation of 37%, 65% and 94% against control was observed. The activity of GSHPx in liver cytosol was more in ginger fed groups compared to the control ($p < 0.05$), and the extent of stimulation observed at 0.5%, 1.0% and 5.0% levels was 11%, 21% and 30%, respectively. The activity of GSHPx in whole blood was also

Table 1
Antioxidant status in ginger fed rats in liver

Groups	SOD (U/mg protein)	Catalase (U/mg protein)	GSHPx	
			Cytosol (oxidized/mg prot/min)	RBC (U/gm Hb)
Control	2.9 ± 0.5^{a}	40.4 ± 3.5^{a}	332 ± 38.6^{a}	267 ± 35.1^{a}
0.5% Ginger	5.1 ± 1.2^{b}	55.4 ± 10.1^{b}	370 ± 29.9^{a}	312 ± 29.2^{b}
1.0% Ginger	6.1 ± 0.9^{b}	66.7 ± 7.9^{c}	401 ± 27.6^{b}	328 ± 6.8^{b}
5.0% Ginger	7.0 ± 0.9^{bc}	78.4 ± 2.7^{d}	433 ± 23.8^{c}	379 ± 25.8^{c}

Values are Mean ± SD of 6 rats per group. Vertical comparisons between groups: ab, bc, bd, cd, ac, ad are significantly different at $p < 0.05$ by Duncan's multiple range test.

Table 2
Inhibition of lipid peroxidation in rat liver and kidney

Groups	Liver		Kidney	
	MDA (nmol/mg protein)	Percent inhibition	MDA (nmol/mg protein)	Percent inhibition
Control	3.4 ± 0.5^{a}	–	2.9 ± 0.5^{a}	–
0.5% Ginger	2.2 ± 0.8^{b}	35	2.1 ± 0.5^{b}	27
1.0% Ginger	1.5 ± 0.3^{c}	56	1.6 ± 0.6^{c}	45
5.0% Ginger	1.4 ± 0.2^{c}	59	1.2 ± 0.6^{c}	59

Values are mean ± SD of 6 rats per group. Vertical comparisons: ab, ac, bc are significantly different at $p < 0.0.5$ by Duncan's multiple range test.

found to be significant ($p < 0.05$) and the percentage stimulation was 17%, 23% and 42%, respectively (Table 1).

3.2. Inhibition of lipid peroxidation in rat liver and kidney

The levels of MDA formed in liver homogenates of rats fed with ginger at different concentrations, namely 0.5%, 1% and 5%, were reduced significantly ($p < 0.05$) as compared to the control group that was not fed with ginger. The percentages of inhibition were 35%, 56% and 59%, respectively. As was observed in liver, significant reduction ($p < 0.05$) in levels of lipid peroxides in kidney was observed in all the ginger fed groups compared to the control group. At 0.5%, 1.0% and 5.0% of ginger feeding, an inhibition of 27%, 45% and 59% was observed (Table 2).

3.3. Inhibition of protein oxidation in rat liver

Reduction in the carbonyl levels was observed at all levels of ginger feeding compared to the control group. However, the only significant decrease ($p < 0.05$) was observed with the 5.0% level of feeding.

Percentages of inhibition at 0.5%, 1% and 5.0% ginger feeding was 23%, 14% and 36%, respectively compared to the control group (Table 3).

Table 3
Inhibition of protein oxidation in rat liver

Groups	Protein carbonyls (nmol/mg protein)	% Inhibition
Control	2.2 ± 0.9^{a}	–
0.5% Ginger	1.7 ± 0.5^{a}	23
1.0% Ginger	1.9 ± 0.4^{a}	14
5.0% Ginger	1.4 ± 0.4^{b}	36

Values are mean ± SD of 6 rats per group. Control vs. 5% ginger $p < 0.05$ by Duncan's multiple range test.

4. Discussion

Oxidative damage in tissues can be limited by the defense system of the host. These defenses appear to be inducible by nutrients/non-nutrients in the diet. In addition to the protective effects of antioxidant enzymes, consumption of dietary antioxidants through the diet plays an important modulatory role against endogenous oxidative damage. Among the defenses are enzymes such as SOD, catalase and GSHPx. The levels of all the three enzymes, which are important components of enzymatic antioxidative defenses, were significantly stimulated in liver tissue of rats fed with ginger at all levels. Levels of these enzymes modulate the oxidative damage within the tissues which can be quantitated in terms of lipid peroxidation and protein oxidation. The *in vitro* antioxidant activity of gingerol and other constituents of ginger have been previously reported by Kikusaki and Nakatani (1993). Inhibition of xanthine oxidase activity responsible for the generation of reactive oxygen species, such as superoxide anion has been documented with gingerol (Chang, Chang, Lu, & Chiang, 1994).

Low levels of antioxidant enzymes were observed in human lung cancer tissue as compared to normal lung tissue suggesting that elevated levels of reactive oxygen metabolites could have resulted in mutation in lung carcinoma cell DNA and other vital cellular components (Guner, Islekel, Oxteken, Hazan, & Acikel, 1996). Increased flux of oxy-radicals and loss of cellular redox haemostasis is known to cause "oxidative stress", and DNA damage leading to tumorigenesis (Cerutti, 1994 & Halliwell, 1994).

Our earlier findings in an *in vitro* study has shown that DNA damage was inhibited when induced by benzo(*a*)pyrene in human peripheral blood lymphocytes of male smokers, male non-smokers and females (Nirmala, Prasanna

Krishna, & Polasa, 2007a,b). *In vivo* antimutagenic effect of ginger was also observed in rats that were fed with ginger through the diet (Nirmala et al., 2007a,b). These findings suggest that the antimutagenic and chemopreventive potential of ginger could be due to its antioxidant activity.

6-Paradol and 6-dehydroparadol, minor chemical constituents of ginger, inhibited TPA induced ear oedema and ornithine decarboxylase (ODC) activity and tumor promotion in mouse skin. Antitumor promotion effects of 6-paradol and 6-dehydroparadol were as efficient as that of curcumin, one of the well-investigated chemopreventive agents (Chung, Jung, Surh, Lee, & Park, 2001). The anti-inflammatory effect is due to the inhibition of the arachidonic acid cascade. Gingerol, shogaol and other chemicals in ginger inhibit prostaglandin and leukotriene biosynthesis through suppression of 5-lipooxygenase synthetase (Srivastava & Mustafa, 1992). Since tumor promotion is associated with oxidative stress and inflammation, agents that possess antioxidant properties capable of mitigating these effects can act as antitumor promoters.

The significant reduction in MDA in liver and kidney tissues of ginger fed rats at 0.5%, 1% and 5% suggests that the antioxidant status of host tissue can be enhanced by consuming ginger through the diet. Inhibition of protein oxidative products, which was measured in the liver, reinforces our hypothesis that daily ginger ingestion can result in improved antioxidant status. Dehydrogingerone, a synthetic analogue of zingerone, showed mild inhibition of lipid peroxidation by acting as free radical scavenger (Rajkumar & Rao, 1993). Concomitant dietary feeding of ginger 1% w/w during administration of malathion (20 ppm) for 4 weeks significantly attenuated malathion induced lipid peroxidation and oxidative stress in rats (Ahmed, Seth, Pasha, & Banerjee, 2000). Strengthening the body defenses by improving the antioxidant status will undoubtedly protect against the oxidative stress involved in the etiology of many chronic diseases.

Low levels of tissue antioxidant enzymes are likely to result in high levels of tissue damage that are reflected as lipid peroxides, protein carbonyls, etc. Conversely elevated levels of antioxidant enzymes would reduce this oxidative damage to tissues, as has been observed in this study in terms of decreased lipid peroxides at 1% and 5% levels of ginger and inhibition of protein carbonyls at 5% level. Although the literature is replete with reports on *in vitro* antioxidant activity of dietary substances, limited information is available to demonstrate this protective effect when consumed naturally through the diet. In a diet survey study conducted by Thimmayamma et al. (1983) it has been shown that human intake of ginger is 2.6 g/consumption unit/day. The level of 5% ginger used in this study corresponds to the levels that are consumed in India. Since these quantities can be easily incorporated and consumed by humans, a regular intake of ginger may be suggested to boost antioxidant status, which in turn will reduce the oxidative damage to tissues involved in etiology of complications associated with degenerative diseases.

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References

Aebi, H. E. (1983). In H. O. Bergmeyer (Ed.). *Catalase, methods enzymology* (Vol. 3, p. 2). New York: Academic Press.

Aeschbach, R., Loliger, J., Scott, B. C., Murcia, A., Buetle, J., Halliwell, B., et al. (1994). Antioxidant actions of thymol carbacrol 6-gingerol, zingerone and hydroxytyrosol. *Food Chemical Toxicology, 32*, 31–36.

Ahmed, R. S., Seth, V., Pasha, S. T., & Banerjee, B. D. (2000). Influence of dietary ginger (*Zingiber officinale Roscoe*) on oxidative stress induced by malathion in rats. *Food Chemical Toxicology, 38*, 443–450.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of National Academy of Sciences, 90*, 7915–7922.

Cerutti, P. A. (1994). Oxy-radicals and cancer. *Lancet, 344*, 862–863.

Chang, W. S., Chang, Y.-H., Lu, F.-J., & Chiang, H. C. (1994). Inhibitory effects of phenolics on xanthine oxidase. *Anticancer Research, 14*, 501–506.

Chung, W. Y., Jung, Y. J., Surh, Y. J., Lee, S. S., & Park, K. K. (2001). Antioxidative and antitumor promoting effects of 6-paradol and its homologs. *Mutation Research, 496*, 199–206.

Belsten, J. L., & Wright, A. J. (1995). European Community—FLAIR common assay for whole-blood glutathione peroxidase (GSH-Px); results of an inter-laboratory trial. *European Journal of Clinical Nutrition, 49*, 921–927.

Griffiths, H. R. (2001). Antioxidants and protein oxidation. *Free Radical Research, 33*, 547–558.

Guner, G., Islekel, H., Oxteken, O., Hazan, E., & Acikel, U. (1996). Evaluation of some antioxidant enzymes in lung carcinoma tissue. *Cancer Letters, 103*, 233–239.

Halliwell, B. (1994). Free radicals, antioxidants and human disease:curiosity, cause or consequence? *Lancet, 344*, 721–724.

Halliwell, B., & Gutteridge, J. M. C. (1989). *Free radicals in biology and medicine* (2nd ed.). Tokyo: Japan Scientific Societies Press.

Kikusaki, H., & Nakatani, N. (1993). Antioxidant effect of some ginger constituents. *Journal of Food Science, 58*, 1407–1410.

Kushi, L. H., Folson, A. R., Prineas, R. J., Mink, P. J., Wu, Y., & Bostick, R. M. (1996). Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *New England Journal of Medicine, 334*, 1156–1162.

Lowry, O. H., Rosenbrongh, N. J., Farr, A. L., & Rendall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Marklund, S., & Marklund, G. (1974). Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry, 47*, 469–474.

Middle Brooks, J. (1977). Statistical calculation. How to solve statistical problems. *Annals Arboreum Science, 62*, 21–27.

Nirmala, K., Prasanna Krishna, T., & Polasa, K. (2007a). Protective effect of ginger against benzo(*a*)pyrene induced DNA damage. *International Journal of Cancer Research, 3*(1), 13–24.

Nirmala, K., Prasanna Krishna, T., & Polasa, K. (2007b). In vivo antimutagenic potential of ginger on formation and excretion of urinary mutagens. *International Journal of Cancer Research, 3*, 134–142.

Sies, H. (1991). Oxidative stress: from basic research to clinical application. *American Journal Medical Association, 91*(3c), 31s–38s.

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine, 70*, 158–169.

Perctval, M. (1998). Antioxidants. In *Clinical nutrition insights* (pp. 1–4). Advanced Nutrition Publications Inc.

Rajkumar, D. V., & Rao, M. N. A. (1993). Dihydrogingerone and iso-eugenol as inhibitors of lipid peroxidation and as free radical scavengers. *Biochemical Pharmacology, 46*, 2067–2072.

Reznick, A. Z., Witt, E., Matsumoto, M., & Packer, L. (1992). Vitamin E inhibits protein oxidation in skeletal muscle of resting and exercised rats. *Biochemical Biophysical Research Communication, 189*, 801–806.

Sekiwa, Y., Kubota, K., & Kobayashi, A. (2000). Isolation of novel glucosides related to gingerdiol from ginger and their antioxidative activities. *Agricultural Food Chemistry, 48*(2), 373–377.

Srivastava, K. C., & Mustafa, T. (1992). Ginger (*Zingiber officinale*) in rheumatism and musculoskeletal disorders. *Medical Hypotheses, 39*, 342–348.

Starke-Reed, P. B., & Oliver, C. N. (1989). Protein oxidation and proteolysis during aging and oxidative stress. *Archives of Biochemistry and Biophysics, 275*, 559–567.

Thimmayamma, B. V. S., Parvati, R., & Radhaiah, G. (1983). Use of spices and condiments in the dietaries of urban and rural families. *The Indian Journal of Nutritional and Dietetics, 20*, 153–162.

Uchida, K., Kanematsu, M., Sakai, K., Matsuda, T., Hattori, N., Mizuno, Y., et al. (1998). Protein – bound acrolein: Potential markers for oxidative strength. *Proceedings of National Academy of Sciences (USA), 95*, 4882–4887.

Wilbur, K. M., Bernheim, F., & Shapiro, O. W. (1949). The thiobarbituric acid reagent as a test for the oxidation of unsaturated fatty acids by various agents. *Archives Biochemistry, 24*(2), 305–313.