Effect of Genistein on Modulating Lipid Peroxidation and Membrane-bound Enzymes in *N*-Nitrosodiethylamine-induced and Phenobarbital-promoted Rat Liver Carcinogenesis

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Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors worldwide and a leading cause of cancer-related death in the world. The importance of diet in the control of several major cancers is now widely recognized, and it is generally agreed that a plant-based diet may afford a significant degree of protection. The current study was designed to evaluate the effect of genistein on *N*-nitrosodiethylamine (DEN)-induced (200 mg/kg body weight; by single i.p injection) and Phenobarbital-promoted (0.05% through drinking water for 14 successive weeks) liver cancer in Wistar albino rats. Decreases ($p < 0.001$) in the activities of Na^+/K^+ ATPase and Mg^{2+} ATPase and increases ($p < 0.001$) in Ca^{2+} ATPase activity were observed in erythrocytes membrane and tissue ATPase of liver cancer-bearing animals when compared with control groups. The change in the activities of these enzymes in membrane and tissue were indicative of the persistent deteriorating effect of DEN in cancer-bearing animals. These enzyme activities were reversed to near normal value in animals treated with genistein. From our results we conclude that genistein may play an important role in preserving membrane asymmetry by suppressing free radicals implying a role for genistein against tumorigenesis.

Key words —— genistein, lipid peroxidation, membrane ATPase enzymes, *N*-nitrosodiethylamine Rats

INTRODUCTION

The importance of diet in the control of several major cancers is now widely recognized, and it is generally agreed that a plant-based diet may afford a significant degree of protection.[1)] A number of studies have reported that consumption of soy and soy products is associated with some degree of protection against either induced or spontaneous cancers in animals as well as reduced cancer risks in several human epidemiological studies. Isoflavones are among the potential chemopreventive agents that have been identified in soybeans and the principal isoflavone found in soybeans is genistein. Genistein has received much attention as a potential anticancer agent due to its wide-ranging effects on a number of cellular processes. It has also shown to be an anti-oxidant, to posses weak oestrogenic and antioestrogenic properties and to inhibit the activity of ribosomal S6 kinase, tyrosine kinase and topoisomerase as well as angiogenesis *in vitro*.[2)]

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors worldwide and a leading cause of cancer-related death in the world killing million people < 1.25 annually.[3)] *N*-nitrosodiethylamine (DEN) is the most important environmental carcinogen among the nitrosamines and primarily induces tumors of the liver.[4)] Administration of DEN to rats results in lipid peroxidation (LPO) and enhanced chemiluminescence in liver preneoplastic nodules, indicating formation of activated oxygen species.[5)] DEN also produces 8-hydroxyguanine (8-OH).[6)] an indicator of oxidative damage to DNA and the most abundant of > 20 types of modifications produced under conditions of oxidative stress. This premutagenic DNA damage

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results in specific types of mutation and is likely involved in carcinogenesis. In the present study, we examined the effect of genistein on LPO and activities of membrane ATPases enzymes to assess the protective role of genistein on DEN-induced Phenobarbital (PB)-promoted hepatocellular carcinogenesis in Wistar albino rats.

MATERIALS AND METHODS

Source of Chemicals —— DEN and genistein were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. All other chemicals used for experiments were of analytical grade.

Animals —— Male albino rats of the Wistar strain, at the age of 6 weeks (procured from Tamil Nadu Veterinary College, Chennai, India) were divided into five groups of 6 rats each. All rats were housed in pure polypropylene cages and maintained at a diurnal 12-hr light/dark cycle with constant temperature and humidity. The animals were fed a commercial rat diet. Animals had ad libitum access to food and water. This research work on Wistar albino rats was sanctioned and approved by the Institutional animal ethical committee (vide letter No. PGIBMS/Dr.S/2002/3960/dated 27/12/2002/IAEC/No.02/021/02).

Experimental Design —— Group I served as control animals and was given vehicle alone for 15 days. Group II animals were induced with DEN by a single intraperitoneal injection (200 mg/kg body weight in saline). After 2 weeks recovery, carcinogenic effect was promoted by PB (0.05% PB). PB was administered to the animals through drinking water $\leq$ 14 successive weeks.[7] Group III animals were treated with genistein (15 mg/kg body weight suspension in olive oil) subcutaneously for 15 days before they were treated with DEN (as in group II). Group IV animals were post-treated with genistein (as in Group III) for 15 days after the induction of HCC by DEN (after 16 weeks as in Group II). Group V animals were treated with genistein (as in group III) alone for 15 days to study the cytotoxicity (if any) induced by genistein.

Biochemical Analysis —— At the end of the experimental period, the animals were killed by cervical decapitation. Blood and liver tissues were collected, tissues were immediately excised, weighed and homogenized in Tris-HCL buffer 0.1 M (pH 7.4). Erythrocyte membranes were isolated according to the method of Dodge *et al.*[8] with slight modification as proposed by Quist.[9]

Na^+/K^+-ATPase was estimated by the method of Bonting.[10] The activity of $Ca2^+$-ATPase was assayed by the method of Hjerten and Pan.[11] The activity of Mg^{2+}-ATPase was assayed by the method of Ohnishi *et al.*[12] Inorganic phosphorous was estimated according to the method of Fiske and Subbarow.[13] Protein content was estimated by the method of Lowry *et al.*[14] The level of lipid peroxides was assayed by the method of Ohkawa *et al.*[15]

Statistical Analysis —— Data are presented as the mean ± standard deviation (S.D.). One-way analysis of variance (ANOVA) was used to detect significant changes between the groups. The Student least significant difference (LSD) method was used to compare the means of different groups and the significance was denoted by its 'p' value. Commercial software program (Sigma STAT, Version 7.5) was used for statistical analysis.

RESULTS

Figure 1 shows the level of LPO in liver of control and experimental animals. It was found that cancer-bearing (Group II) animals showed a significant ($p < 0.001$) increase in the LPO in liver when compared with control animals (Group I). Genistein-treated (Group III and Group IV) animals showed a significant decrease ($p < 0.001$) in the levels of LPO in liver when compared with HCC-bearing (Group II) animals. There was no significant difference in the LPO between the genistein alone-treated group (Group V) and control animals (Group I).

Table 1 presents the tumor incidence of control and experimental animals. Group III and Group IV animals treated with genistein show a significant decrease ($p < 0.001$) in tumor incidence when compared with cancer-bearing Group II animals. The genistein alone-treated Group V animals displayed values equivalent to those of the control group.

Table 2 shows the activities of ATPases in erythrocyte membrane of various experimental groups. Cancer-bearing animals (Group II) showed a significant decrease ($p < 0.001$) in the activities of Na^+/K^+-ATPase and Mg^{2+}-ATPase with a significant ($p < 0.001$) increase in the activities of Ca^{2+}-ATPase when compared with control animals (Group I). This change in the ATPases activities

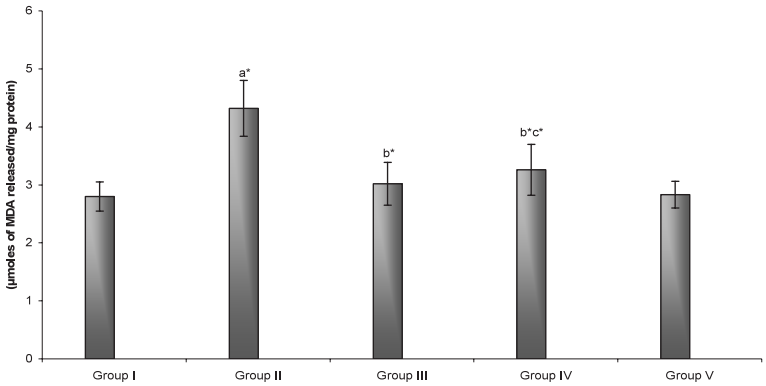

Fig. 1. Level of LPO in Liver of Control and Experimental Animals

Values are expressed as mean ± S.D. for six rats in each group. a: as compared with Group I, b: as compared with Group II, c: as compared with Group III. Statistical significance: $^{*}p < 0.001$.

Table 1. Effect of Genistein on the Number of Tumor-bearing Animals in Control and Experimental Groups

Particulars	Group I	Group II	Group III	Group IV	Group V
No. of animals	6	6	6	6	6
No. of tumor incidence	0	6	3	4	0
No. of tumors/rat	0	$3.75 \pm 0.41^{a*}$	$1.35 \pm 0.15^{b*}$	$1.75 \pm 0.19^{b*c*}$	0

Values are expressed as mean ± S.D. for six rats in each group. a: as compared with Group I, b: as compared with Group II, c: as compared with Group III. Statistical significance: $^{*}p < 0.001$.

Table 2. Effect of Genistein on the Activities of Erythrocyte Membrane ATPases in Control and Experimental Animals

Particulars	Group I	Group II	Group III	Group IV	Group V
Na^+/K^+	2.72 ± 0.24	$1.84 \pm 0.20^{a*}$	$2.58 \pm 0.32^{b*}$	$2.37 \pm 0.29^{b*c*}$	2.73 ± 0.27
Ca^{2+}	3.52 ± 0.32	$5.11 \pm 0.63^{a*}$	$3.40 \pm 0.42^{b*}$	$2.85 \pm 0.35^{b*c*}$	3.56 ± 0.29
Mg^{2+}	1.63 ± 0.14	$0.53 \pm 0.05^{a*}$	$1.46 \pm 0.18^{b*}$	$1.07 \pm 0.13^{b*c*}$	1.57 ± 0.13

Values are expressed as mean ± S.D. for six rats in each group. Units: µmoles of inorganic phosphorus formed per min/mg protein. a: as compared with Group I, b: as compared with Group II, c: as compared with Group III. Statistical significance: $^{*}p < 0.001$.

Table 3. Effect of Genistein on the Activities of Membrane Bound ATPases in Liver of Control and Experimental Animals

Particulars	Group I	Group II	Group III	Group IV	Group V
Na^+/K^+	1.87 ± 0.18	$1.03 \pm 0.11^{a*}$	$1.64 \pm 0.20^{b*}$	$1.39 \pm 0.17^{b*c*}$	1.85 ± 0.16
Ca^{2+}	1.62 ± 0.14	$2.27 \pm 0.25^{a*}$	$1.65 \pm 0.20^{b*}$	$1.81 \pm 0.22^{b*c*}$	1.63 ± 0.13
Mg^{2+}	2.65 ± 0.24	$1.78 \pm 0.19^{a*}$	$2.57 \pm 0.32^{b*}$	$2.32 \pm 0.29^{b*c*}$	2.63 ± 0.21

Values are expressed as mean ± S.D. for six rats in each group. Units: µmoles of inorganic phosphorus formed per min/mg protein. a: as compared with Group I, b: as compared with Group II, c: as compared with Group III. Statistical significance: $^{*}p < 0.001$.

was significantly ($p < 0.001$) reverted in Group III and Group IV ($p < 0.001$) genistein-treated animals to near normal values. However, genistein alone-treated animals (Group V) did not show any significant change when compared with control animals (Group I).

Table 3 shows the effect of genistein on the activities of ATPases in liver tissues of control and experimental animals. Decrease ($p < 0.001$) in the activities of Na^+/K^+ ATPase and Mg^{2+} ATPase and increase ($p < 0.001$) in Ca^{2+} ATPase activity was seen in cancer-bearing Group II animals when compared with control animals. There was a significant increase in the activities of Na^+/K^+ ATPase ($p < 0.001$) and Mg^{2+} ATPase ($p < 0.001$) and significant decrease in the activity of Ca^{2+} ATPase ($p < 0.001$) in genistein-treated (Group III and Group IV) animals when compared with Group II animals. There was no significant difference in the activities of ATPases between Group V animals and control.

DISCUSSION

The search for new chemopreventive and antitumor agents that are more effective and less toxic than existing agents has kindled great interest in phytochemicals.[16] A number of studies in animal models demonstrate that dietary soybean and genistein inhibit the growth of transplantable tumors in rats[17,18] and dietary soybean isoflavones were shown to reduce the induction of tumors in rats by chemical carcinogens.[19]

In the present study, we observed that treatment with genistein significantly reduced tumor incidence. Since genistein is believed to have a potential role in reducing tumor incidence[20] and the size of the tumor nodules the effects we observed might possibly have been caused by this agent. Although it is evident that not all the hepatocytes nodules became cancerous during the lifespan of the animals, numerous observations support the concept that the nodules are precursors of HCC.[21] Moreover, there is a large body of evidence to show a correlation between the number and size of hyperplastic nodules and hepatocarcinoma in both experimental and human disease.[22] In view of this, inhibition of nodule growth and enhancement of their regression by genistein, as observed in our study, may be important for anticarcinogenic effect.

LPO is regarded as one of the basic mechanisms of cellular damage caused by free radicals. DEN is the most important environmental carcinogen among nitrosamine in interacting with membrane lipids and consequently inducing free radical formation.[5] Free radicals react with lipids causing peroxidation, resulting in the release of products such as malondialdehyde, hydrogen peroxide, and hydroxyl radicals. An increase in lipid peroxides indicates serious damage to cell membranes, inhibition of several important enzymes, reduced cellular function, and cell death.[23] Administration of genistein significantly reduced membrane lipid peroxides in cancer-bearing animals. LPO can be suppressed by enzymatic inactivation of free radicals.[24] It has been reported that the hydrogenation of double bond between carbon two and three (C2–C3) of the C-ring decreased the antiperoxidative effects.[25,26] This represents the antioxidant potency of genistein and its ability to protect the membrane from free radical-mediated injuries, suggesting its anticarcinogenic effect.

In malignancy, the cell membrane plays a crucial role in stimulation and control of cell adhesiveness, mortality, and proliferation in a much-damaged condition.[27] The protection of membranes is of potential importance in the treatment of disease processes. ATPases are membrane-bound enzymatic proteins that maintain ionic gradients between aqueous intra-and extracellular phases.[28] Membrane-bound enzymes such as Na^+/K^+-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase are responsible for the transport of sodium/potassium, magnesium and calcium ions across the cell membranes at the expense of ATP by hydrolysis.[29] The activities of ATPases in erythrocyte membrane and liver tissues have been shown inhibited in cancer-bearing animals. These finding are similar to those reported in various cancers.[30]

In the present study the decrease in the activities of Na^+/K^+-ATPase and Mg^{2+}-ATPase in erythrocyte membrane and liver cancer-bearing animals may be due to increased production of free radicals leadind to cell injury.[5] Free radicals are clearly involved in the pathogenesis of various diseases such as atherosclerosis, inflammatory diseases, and cancer.[31] Free radicals have been suggested to exert their cytotoxic effects by causing peroxidation of membrane phospholipids.[32] Damage of plasma membrane occurs directly through interaction with the membrane components such as the ion-dependent ATPases and ion channels and indirectly as a consequence of overt cytosolic damage. Inhibiting function of ion-dependent ATPases leads to disturbances in ion homeostasis resulting in impaired signal transduction, altered cellular metabolism, changes in cell membrane permeability and integrity, an elevation in membrane fluidity, and disturbances of vital function.

Ca^{2+} ATPase is also located in the plasma membrane pumping Ca^{2+} out of the cell and thereby helping to maintain the concentration gradient of Ca^{2+} between the cytosol and the extracellular fluid (ECF). Many ATPases, including Ca^{2+}-ATPases, contain essential free sulfhydryl groups. Impairment of this enzyme may be due to peroxidative stress, which may act on the sulphydryl groups present in the active site of Ca^{2+} ATPase.[33] Thiol modification (*i.e.* loss of protein sulfhydryl groups) has been recognized as a critical event in cytotoxicity.[34] Damage to these thiol moieties may result in inhibition of Ca^{2+}-ATPases, function and increase in the intracellular Ca^{2+} concentration may result.[35] Elevated Ca^{2+} is thought related to tumor cell invasion. Imamura *et al.*[36] found that the *in vitro*

invasion of highly invasive cancer cells is initiated by addition of serum, which induces an increase in intracellular pH as well as a transient elevation of Ca^{2+}, and that addition of serum to poorly invasive cancer cells does not increase intracellular pH or Ca^{2+}. There was also an increasing awareness that plasma membrane calcium (PMC) ATPase alterations are associated with tumorigenesis.[37] There are four isoforms of PMC ATPase (PMCA1–4) with additional isoform diversity generated from the primary PMCA transcripts by alternative splicing.[38, 39] Indeed, relative greater expression of PMCA1 mRNA expression was observed in tumorigenic breast cancer MCF-7 and MDA-MB-231 cells compared with non-tumorigenic MCF-10A cells.[40] Thus, perturbed PMCA regulation or function may be important in diseases such as cancer.

In the present study, a decrease in the activities of Na^+/K^+ ATPase and Mg^{2+}-ATPase and an increase in the activities of Ca^{2+} ATPase were found in cancer-bearing animals. Genistein treatment significantly restored the tissue ATPase activities to near normal values. The results suggest that genistein protects ion pump ATPases, presumably by limiting the degree of oxidation and levels of oxidation by products due to an increase in the reduced glutathione (GSH) content in rats treated with genistein.

In conclusion, the results of our studies demonstrate that genistein can modulate ATPase enzymes and reduce free radical formation in DEN-induced PB-promoted liver cancer in albino rats. Thus inhibitory effect of genistein may play an important role in preserving membrane asymmetry by suppressing free radicals, implying efficacy of this agents against tumorigenesis.

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REFERENCES

1) Messina, M. J., Parsky, V., Stetchell, K. D. and Barnes, S. (1994) Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr. Cancer,* **21**, 113–131.
2) Peterson, G. (1995) Evaluation of the biochemical targets of genistein tumor cells. *J. Nutr.*, **125**, 784S–789S.
3) Qaglia, A., Bhattacharya, S. and Dhillon, A. P. (2001) Limitation of the histopathological diagnosis and prognostic assessment of hepatocellular carcinoma. *Histopathology*, **38**, 167–174.
4) Thirunavukkarasu, S. and Sakthisekaran, D. (2001) Effect of selenium on *N*-nitrosodiethylamine induced multistage heatocarcinogenesis with reference to lipid peroxidation and enzyme antioxidants. *Cell Biochem. Funct.*, **19**, 27–35.
5) Nakae, D., Kobayashi, Y., Akai, H. and Nabuaki, A. (1997) Involvement of 8-hydroxyguanine formation in the inhibition of rat liver carcinogenesis by low dose levels of N-nitrosodiethylamine. *Cancer Res.*, **57**, 1281–1287.
6) Ampy, F. R. and Williams, A. O. (1986) Diethylnitrosamine metabolism: I. In viro activation of Diethylnitrosamine to mutagenic substance(s) by hepatic and renal tissues from three inbred strains of mice. *Life Sci.*, **39**, 923–930.
7) Yoshiji, H., Nakae, D., Kinugasa, T., Matsuzaki, M., Denda, A. and Suiji, T. (1991) Inhibitory effect of the iron deficiency on the induction of putative preneoplastic foci rat liver initiated with diethylnitrosamine and promoted by Phenobarbital. *Br. J. Cancer*, **64**, 839–842.
8) Dodge, J. D., Mitchell, G. and Honatian, D. J. (1963) The preparation and chemical characteristics of hemoglobin free ghosts of human red blood cells. *Arch. Biochem. Biophys.*, **180**, 119–130.
9) Quist, E. H. (1980) Regulation of erythrocyte membrane shape by calcium ion. *Biochim. Biophys. Res. Commun.*, **92**, 631–637.
10) Bonting, S. L. (1970) *Membranes and Ion Transport* (Bittar, E. E., Ed.), Wiley Interscience, London, pp. 257–263.
11) Hjerten, S. and Pan, H. (1983) Purification and characterization of two forms of a low affinity calcium ion ATPase from erythrocyte membrane. *Biochim. Biophys. Acta*, **728**, 281–288.
12) Ohnishi, T., Suzuki, T., Suzuki, Y. and Ozawa, K. (1982) A comparative study of plasma membrane magnesium ion ATPase activities in normal, regenerating and malignant cells. *Biochim. Biophys. Acta*, **684**, 64–74.
13) Fiske, C. H. and Subbarrow, Y. (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.*, **66**, 375–400.
14) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
15) Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.*, **95**, 351–358.

16) Perin, N. and Notani, N. (2001) Global variation in cancer incidence and mortality. *Curr. Oncol.*, **85**, 465–474.
17) Schleicher, R., Lamartiniere, C. A., Zheng, M. and Zhang, M. (1999) The inhibitory effect of genistein on the growth and metastasis of a transplantable rat accessory gland carcinoma. *Cancer Lett.*, **136**, 195–201.
18) Zhou, J. R., Gugger, E. T., Tanaka, T., Guo, Y., Blackburn, G. L. and Clinton, S. K. (1999) Soybean phytochemicals inhibit the growth of transplantable prostate carcinoma and tumour angiogenesis in mice. *J. Nutr.*, **129**, 1628–1635.
19) Onozawa, M., Kawamori, T., Baba, M., Fukuda, K., Toda, T., Sato, H., Ohtani, M., Akaza, H., Sugimura, T. and Wakabayashi, K. (1999) Effects of a soybean isoflavone mixture on carcinogenesis in prostate and seminal vesicles of F344 rats. *Jpn. J. Cancer Res.*, **90**, 393–398.
20) Ringstad, J., Jacobsen, B. K., Tretli, S. and Thomassen, Y. (1998) Serum selenium concentrations associated with risk of cancer. *J. Clin. Pathol.*, **41**, 454–457.
21) Hacker, H. J., Steinberg, P. and Bannasch, P. (1998) Pyruvate kinaseisoenzyme shift from L-type to M2-type is a late event in hepatocarcinogenesis induced in rats by a choline-deficient/DL-ethionine-supplemented diet. *Carcinogenesis*, **19**, 99–107.
22) Farber, E. (1990) Clonal adaptation during carcinogenesis. *Biochim. Pharmacol.*, **39**, 1837–1846.
23) Pompella, A., Romani, A. and Benditti, M. (1991) Comporti Lose of membrane protein thiols and lipid peroxidation of allyl alcohol hepatotoxicity. *Biochem. Pharmacol.*, **41**, 1225–1259.
24) Mora, A., Paya, M., Ribos, J. L. and Alcaraz, M. J. (1990) Structural activity relationships of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation. *Biochem. Pharmacol.*, **140**, 793–797.
25) Cholbi, M. R., Paya, M. and Alcaraz, M. J. (1991) Inhibitory effects of phenolic compounds on Cl-induced microsomal lipid peroxidation. *Experentia*, **41**, 251–268.
26) Van Acker, S. A. B. E., Best, A. and Van Der Jijgh, W. J. E. (1998) Structural aspects of antioxidant activity of flavonoids, In *Flavonoids in Health and Disease* (Rice-Evans, C.A. and Packer, L., Eds.), Marcel Dekker, Inc., New York, pp. 221–251.
27) Optatka, A., Friedman, J. E. and Kosenheck, K. (1985) *Cell membranes and cancer* (Galeotti, T., Cittadini, A., Novi, G., Papa, S. and Smets, L.A., Eds.), Elsevier Science Publisher, Amsterdam, pp. 117–124.
28) Ademo Lu, E., GökkuÕu, C., Palandüz, O. and Vitamin, E. (2000) ATPases: Protection of ATPase activities by vitamin E suplementation in various Tissues of hypercholesterolemic rats. *Int. J. Vitam. Nutr. Res.*, **70**, 3–7.
29) Skekhoren, M. A. and Bonting, S. L. (1981) Transport ATPases: Properties and functions. *Physiol. Rev.*, **61**, 1–76.
30) Randak, L., Auerswald, E. A., Machleidt, A. and Mechleidt, W. (1999) Inhibition of ATPase and adenylate kinase activities of the second nucleotide binding of the cystic fibrosis conditions by genistein. *Biochem. J.*, **340**, 227–235.
31) Lux, O. and Naidoo, D. (1995) Biological variability of superoxide dismutase and glutathione peroxidase in blood. *Redox Rep.*, **1**, 331–335.
32) GökkuÕu, C., Ademo Lu, E., Türko Lu, U. M., Öz, H. and Öz, F. (1996) Thymosin protects liver and aorta from oxidative damage in atherosclerotic rabbits. *Life Sci.*, **59**, 1059–1067.
33) Jain, K. S. and Sohet, S. B. (1981) Calcium potentiates the peroxidation of erythrocyte membrane. *Lipids*, **642**, 46–54.
34) Pascoe, G. A., Olafsdottir, K. and Reed, D. J. (1987) Vitamin E protection against chemical induced injury. I. Maintenance of cellular protein thiols as a cytoprotective mechanism. *Arch. Biochem. Biophys.*, **256**, 150–158.
35) Pruijn, F. P., Haenen, G. R. M. M. and Bast, A. (1991) Interplay between vitamin E, glutathione and dihydrolipoic acid in protection against lipid peroxidation. *Fat Sci. Technol.*, **93**, 216–221.
36) Imamura, F., Horai, T., Mukai, K. and Akedo, H. (1991) Serum requirement for in vitro invasion by tumor cells. *Jpn. J. Cancer Res.*, **82**, 493–496.
37) Carafoli, E. (1994) Biogenesis: plasma membrane calcium ATPase: 15 years of work on the purified enzyme. *FASEB J.*, **8**, 993–1002.
38) Strehler, E. E. and Zacharias, D. A. (2001) Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. *Physiol. Rev.*, **81**, 21–50.
39) Kamrava, M. R., Michener, C. M. and Kohn, E. C. (2002) Antisense-mediated Inhibition of the Plasma Membrane Calcium-ATPase Suppresses Proliferation of MCF-7 Cells. *Pharmaceutical News*, **9**, 435–442.
40) Lee, W. J., Roberts-Thomson, S. J., Holman, N. A., May, F. J., Lehrbach, G. M. and Monteith, G. R. (2002) Expression of plasma membrane calcium pump isoform mRNAs in breast cancer cell lines. *Cell. Signal.*, **14**, 1015–1022.