AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Effect of Tamoxifen on Mitochondria – An *In Vitro* Study

Seema Surendran[1], Vijayalakshmi Krishnamurthy[1*]

1. Department of Biochemistry, Bharathi Women's College (Autonomous), Chennai – 600 108, Tamil Nadu, India.

ABSTRACT

Mitochondrion plays an important role in cellular metabolism and in energy production. Free radicals contribute damage to mitochondria during various pathological conditions. Tamoxifen is an anti-estrogen drug given to treat breast cancer. Tamoxifen and its metabolites induce varied cellular effects. It was therefore planned to study the impact of tamoxifen on mitochondria to observe its mechanism of action in promoting cell death. Mitochondrion was isolated from sheep liver and different concentrations of tamoxifen were added to study its impact on oxidative stress. Tamoxifen induced swelling of mitochondria which in turn produced lipid peroxides in a dose-dependent manner. Tamoxifen significantly increased the levels of nitrite and nitrate in mitochondria with depletion of glutathione. It was observed that tamoxifen increased NADH oxidation leading to the release of calcium from mitochondria. These changes observed are correlated with the mechanism of action of tamoxifen in treating breast cancer.

Keywords: Mitochondria, Tamoxifen, Swelling, Oxidative Stress, Calcium.

*Corresponding Author Email: seemasuren@gmail.com

Received 27 February 2012, Accepted 10 March 2012

Please cite this article in press as: Vijayalakshmi K *et al.*, Effect of Tamoxifen on Mitochondria – An *In Vitro* Study. American Journal of PharmTech Research 2012.

INTRODUCTION

Mitochondria are filamentous or granular cytoplasmic organelles of all aerobic cells of higher animals, plants, certain micro-organisms including algae, protozoa and fungi. The mitochondria have lipoprotein framework which contains many enzymes and coenzymes required for energy metabolism [1]. Mitochondria are membrane enclosed organelles distributed throughout the cytosol of most eukaryotic cells. Their main function is the conversion of the potential energy of food molecules into ATP. The cell uses this energy to perform the specific work necessary for cell survival and function[2]. Mitochondria are an active source of the free radicals like superoxide (O_2°) and nitric oxide (NO), whose production accounts for about 2% and 0.5% respectively, of mitochondrial O_2 uptake under physiological conditions[3].

Tamoxifen is a drug commonly used for the treatment of breast cancer. Tamoxifen competes with estrogen for binding sites on estrogen receptors in breast tissues of the body. The competition with estrogen limits cell growth in breast tissues, making it an effective treatment for breast cancer. Tamoxifen is used to treat breast cancer in both pre and post menopausal women with advanced or metastatic breast cancer[4]. Tamoxifen is currently used by more women with breast cancer than any other drug. Tamoxifen is the most widely prescribed cancer medication in the world[5]. The aim of the present investigation is to study the effect of tamoxifen on mitochondria in *in vitro* condition to evaluate its role on mitochondria and to study its mechanism of action in promoting cell death.

MATERIALS AND METHODS

Sheep liver was obtained from healthy animals from slaughter houses which did not suffer from any infections. The tissue was brought in an ice-cold container. It was immediately cleaned to remove adjoining adipose tissue and membranous materials. It was weighed and cut into small pieces and subjected to differential centrifugation for isolation of mitochondria. The mitochondria were isolated[6] and protein was estimated.[7,8] Tamoxifen citrate tablets were purchased from Pharma pharmaceutical company.

Swelling of mitochondria

Mitochondrial swelling was observed by the changes in turbidity at 520nm in a spectrophotometer.[9] Mitochondria was subjected to different concentrations of tamoxifen (0.05mM, 0.1mM, 0.2mM, 0.3mM, 0.4mM and 0.5mM) for different time intervals.

Estimation of lipid peroxide

Lipid peroxide content was determined by thiobarbituric acid reaction[10]. The lipid peroxide formed during swelling was measured and expressed as nanomoles of malonaldehyde/mg protein. A calibration graph was established with malonaldehyde as standard.

Estimation of reduced glutathione

The total reduced glutathione content of mitochondria during swelling process was estimated by the method of Moron *et al.*,[11] to estimate glutathione content was expressed as of nanomoles/mg protein.

Estimation of nitrite

The nitrite content of the mitochondria during the process of swelling was determined.[12,13] The nitrite formed was expressed as nanomoles/mg protein.

Estimation of nitrate

The nitrate content was estimated by converting it into nitrite [14] and expressed as nanomoles/mg protein during the process of swelling.

Estimation of NADH oxidation

The impact of tamoxifen on NADH oxidation was studied. The decrease in absorbance at 340nm is directly proportional to the rate of oxidation of NADH. [15] The rate of oxidation of NADH was expressed as nanomoles of NADH oxidized/minute/mg protein.

Estimation of calcium

Calcium released due to the process of swelling was estimated using atomic absorption spectroscopic method.[16] The levels of calcium are expressed as nanomoles of calcium/mg mitochondrial protein.

Statistical analysis

The values are expressed as mean ± SD. Statistical analysis was done by Student's's't' test and 'p' value was arrived at to assess the statistical significance of changes observed. P value less than 0.02 was considered significant.

RESULTS AND DISCUSSION

Swelling is a well known and common response of mitochondria to many unfavorable influences both *in vivo* and *in vitro*. It is possible to divide the variety of factors affecting swelling into four groups according to the swelling kinetics[17]. Table 1 shows the effect of tamoxifen on mitochondrial swelling. The effect of different concentrations of tamoxifen on the swelling of mitochondria was studied during different time intervals (Figure 1).

Table 1: Percentage change in Mitochondrial Swelling induced by Tamoxifen after 1 hr

Concentration of Tamoxifen (mM)	% Swelling
0.05	13.5
0.1	24.3
0.2	37.8
0.3	45.9
0.4	59.4
0.5	64.8

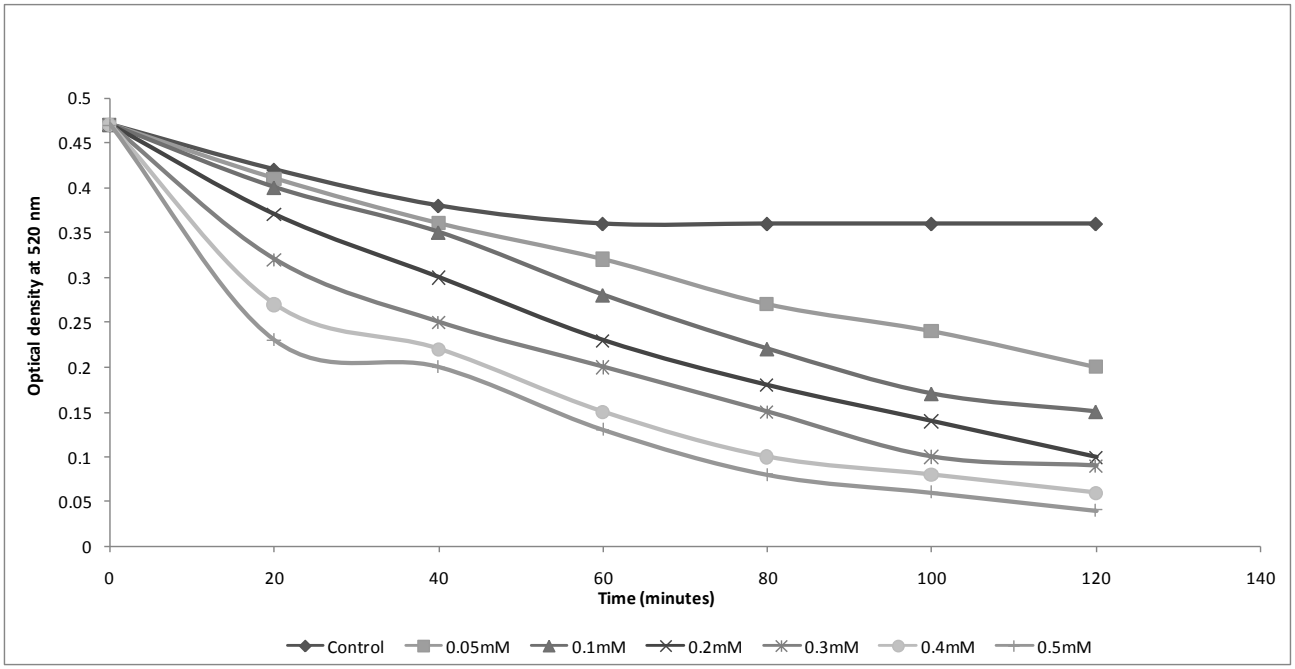

Figure 1: Effect of Tamoxifen on Mitochondrial Swelling

0.05mM concentration of tamoxifen produced 13.5 % swelling after 1 hr whereas 0.4mM concentration (*in vivo* concentration) of tamoxifen induced nearly 60% swelling of mitochondria. After 2 hrs of tamoxifen treatment, 0.05mM tamoxifen induced 46% swelling as compared to 84% swelling induced by 0.4mM concentration of tamoxifen. Thus it is observed that swelling was maximum during first 1 hr, for 0.4mM and 0.5mM concentrations of tamoxifen. 0.05mM concentration of tamoxifen shows nearly 3 times the % of swelling (2 hours) than that was observed during the first 1 hour. The % of swelling increased with increase in tamoxifen concentration. After 1 hr it was noticed that 0.5 mM concentration of tamoxifen could produce 64.8 % swelling.

At the end of 2 hours as seen in Table 2, 0.5mM concentration of tamoxifen could nearly produce 89 % swelling. Therefore, for further *in vitro* studies we adopted 120 minutes (2 hrs) as our maximum time interval. Mitochondrial swelling can be induced by calcium with cytochrome

C release[18]. The swelling of mitochondria was promoted by long chain fatty acids such as myristate indicating a protonophoric mechanism.[19] Tamoxifen-induced swelling might lead to uncoupling of oxidative phosphorylation and changes in membrane potential.

Table 2: Percentage change in Mitochondrial swelling induced by Tamoxifen after 2 hr

Concentration of Tamoxifen (mM)	% Swelling
0.05	45.9
0.1	59.4
0.2	72.9
0.3	75.9
0.4	83.7
0.5	89.1

Table 3: Production of Lipid Peroxides during the process of Mitochondrial Swelling induced by Tamoxifen after 2 hrs

Control (No Drug)	0.05 mM	0.1 mM	0.2 mM	0.3 mM	0.4 mM	0.5 mM
3.5±0.1	4±0.4^{NS}	4.2±0.5*	4.5±0.6*	5±0.8**	10±0.8***	11.1±0.6****

Values are mean ± s.d. for 6 different mitochondrial preparations

Values are expressed as nanomoles/mg mitochondrial protein

Comparison between control and different concentrations of tamoxifen were carried and the levels of significance was arrived at ^{NS} – Non-Significant P<0.02* P<0.01** P<0.002*** P<0.001****

It is observed from Table 3 that tamoxifen at a concentration of 0.3mM, 0.4mM and 0.5mM induced the production of lipid peroxides very significantly than at 0.05mM, 0.1mM and 0.2mM concentrations. This shows that tamoxifen at higher concentrations could induce the production of lipid peroxides in mitochondria more significantly. Lipid peroxidation in mitochondria brings about swelling of mitochondria, uncoupling of oxidative phosphorylation and inhibition of respiration[20]. Lipid peroxidation leads to the degradation of cytochromes[21]. Lipid peroxides decrease the respiratory control ratio (RCR) of mitochondria and results in the lysis of mitochondria[9]. The increased level of lipid peroxides in mitochondria brings about impairment in mitochondrial function such as respiration[22]. Tamoxifen induces oxidative stress within the carcinoma cells in *in vitro* conditions[23]. Lipid peroxidation impairs the flow of electrons along the respiratory chain which may cause an increase in the reactive oxygen species in mitochondria [24]. The increase in lipid peroxidation may also be due to insufficiency of the protective antioxidant system mainly glutathione which may be depleted by peroxy nitrite[25]. Thus it is observed from our study that tamoxifen at different concentrations leads to the production of lipid peroxides.

Table 4 shows the levels of glutathione during mitochondrial swelling induced by tamoxifen in 2 hrs. Glutathione levels are depleted significantly from 0.1mM concentration of tamoxifen. Glutathione is required for detoxification of hydroperoxides generated by respiratory chain. Lipid peroxidation modulates the level of glutathione in mitochondria[26]. Oxidized glutathione leads to the opening of mitochondrial transition pore[27]. It is observed from Table 3 that tamoxifen leads to the production of lipid peroxides. Hence this depletion in glutathione level could be due to the production of lipid peroxides by tamoxifen.

Table 4: Levels of Glutathione during the process of Mitochondrial Swelling induced by Tamoxifen after 2 hours

Control (No Drug)	0.05 mM	0.1 mM	0.2 mM	0.3 mM	0.4 mM	0.5 mM
22±2.0	16.5±1.5**	14.6±1.3****	9.5±0.9****	9.2±0.8****	4.8±0.4****	2.2±0.1****

Values are mean ± s.d. for 6 different mitochondrial preparations

Values are expressed as nanomoles/mg mitochondrial protein

Comparison between control and different concentrations of tamoxifen were carried and the levels of significance was arrived at $P<0.01^{**}$ $P<0.001^{****}$

Table 5: Levels of Nitrite during the process of Mitochondrial Swelling induced by Tamoxifen after 2 hours

Control (No Drug)	0.05 mM	0.1 mM	0.2 mM	0.3 mM	0.4 mM	0.5 mM
1.3±0.1	1.6±0.1**	1.8±0.17***	2.2±0.2****	2.5±0.22****	3.4±0.32****	3.8±0.3****

Values are mean ± s.d. for 6 different mitochondrial preparations

Values are expressed as nanomoles/mg mitochondrial protein

Comparison between control and different concentrations of tamoxifen were carried and the levels of significance was arrived at $P<0.01^{**}$ $P<0.002^{***}$ $P<0.001^{****}$

Table 5 shows the levels of nitrite produced during the process of mitochondrial swelling induced by tamoxifen in 2 hrs. Tamoxifen at 0.05mM concentration significantly caused the production of nitrite. Table 6 shows the levels of nitrate formed during the process of mitochondrial swelling induced by tamoxifen. 0.2mM concentration of tamoxifen produced significantly large amounts of nitrate. It is observed from Table 5 and 6 that 0.05mM concentration of tamoxifen significantly lead to the formation of nitric oxide, whereas at the same concentration there was not much formation of lipid peroxides (Table 3). This shows that tamoxifen first leads to the production of nitrate and nitrite more significantly than lipid peroxides. NO inhibits mitochondrial respiration in dose-dependent manner.[28,29,30] NO inhibits cytochrome oxidase activity[31]. NO produces a decrease in membrane potential of mitochondria.[32] NO production in mitochondria leads to the formation of peroxy nitrite which nitrates or oxidizes

the amino acids of mitochondrial proteins[33].Peroxy nitrite formed in mitochondria is scavenged by glutathione and glutathione peroxidase[34]. The decrease in glutathione level noted in our study (Table 4) may be due to the increased production of NO (Table 5).

Tamoxifen induces significant apoptosis (programmed cell death) in human breast cancer cells. Tamoxifen induces apoptosis by activating NO synthase[35]. The increase in nitrate and nitrite levels (Table 5 and 6) noted in our study could be due to the activation of nitric oxide synthase induced by tamoxifen. Tamoxifen may induce apoptosis through the formation of NO.

Table 6: Levels of Nitrate during the process of Mitochondrial Swelling induced by Tamoxifen after 2 hours

Control (No Drug)	0.05 mM	0.1 mM	0.2 mM	0.3 mM	0.4 mM	0.5 mM
0.74±0.06	0.85±0.05*	0.92±0.08**	1.3±0.12****	1.6±0.14****	2.11±0.2****	2.8±0.1****

Values are mean ± s.d. for 6 different mitochondrial preparations

Values are expressed as nanomoles/mg mitochondrial protein

Comparison between control and different concentrations of tamoxifen were carried and the levels of significance was arrived at P<0.02* P<0.01** P<0.001****

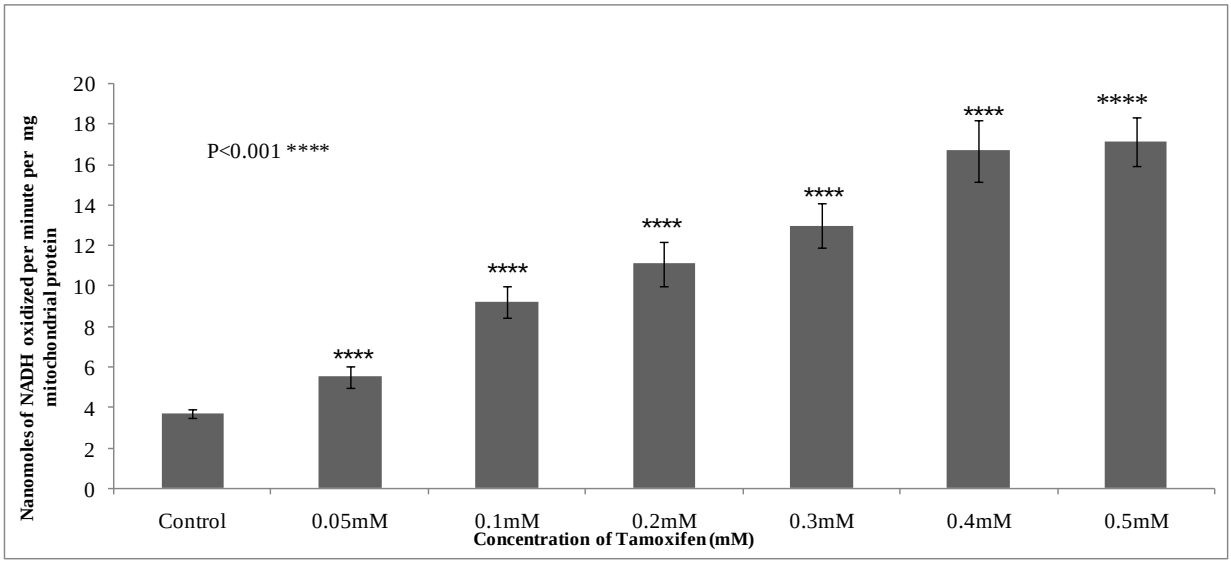

Figure 2: NADH Oxidation induced by Tamoxifen after 2 hours

Figure.2 shows NADH oxidation of mitochondria induced by tamoxifen. The intact mitochondria are impermeable to exogenous NADH[15]. This property of NADH makes it very suitable to test mitochondrial integrity. NADH which absorbs maximally at 340nm was used as a substrate for β-hydroxybutyric acid, malic acid and glutamate dehydrogenase[16]. The decrease in absorbance at 340nm is proportional to the rate of oxidation of NADH. It is observed from the Figure .2 that tamoxifen leads to increased permeability of mitochondrial membrane to NADH. The increase in permeability of mitochondrial membrane to NADH is significantly high even at

0.05mM concentration of tamoxifen. This suggests that mitochondria become more permeable to NADH after adding tamoxifen. The rate of exogenous NADH oxidation is proportional to swelling of mitochondria. An increase in the rate of NADH oxidation is due to the result of outer membrane disruption caused by swelling of mitochondria[36]. It is observed from Table 1 and 2 that tamoxifen causes swelling of mitochondria and this could be the reason for the increased rate of NADH oxidation observed.

Table 7 shows calcium released during the process of mitochondrial swelling induced by tamoxifen after 2 hrs. The percentage release of calcium increased significantly with increased concentration of tamoxifen. Calcium is a largest divalent cation that crosses links with macromolecules and induces conformational changes in them. Calcium may also compete with magnesium for binding and may inhibit the activity of the other. Many calcium bound proteins are found outside the cell[37]. Mitochondrial calcium influx occurs primarily via, a calcium uniporter that utilizes the negative membrane potential as a driving force[38]. Tamoxifen causes an increase in cytoplasmic free calcium levels[39]. Calcium induces the release of cytochrome C from mitochondria[40]. Mitochondrial swelling is associated with cytochrome C release[41]. It is observed from our study that tamoxifen may induce mitochondrial swelling which may lead to the release of cytochrome C and calcium from mitochondria. Further studies are to be carried out to study the exact mechanism of action of tamoxifen.

Table 7: Percentage release of Calcium during the process of Mitochondrial Swelling induced by Tamoxifen after 2 hours

Concentration of Tamoxifen (mM)	% of Calcium release
0.05	18.1
0.1	27.2
0.2	36.36
0.3	45.45
0.4	54.54
0.5	63.6

CONCLUSIONS

Tamoxifen causes the production of nitric oxide and lipid peroxide in mitochondria. This production of nitric oxide leads to the formation of lipid peroxides. The formation of nitric and lipid peroxide may lead to depletion of glutathione. The production of free radicals (RNS and ROS) may in turn damage the mitochondrial membrane. Due to the damage of mitochondrial membrane, calcium may be released, leading to increased permeability of membrane for NADH.As a result of these changes, mitochondria may undergo swelling. The swelling of mitochondria may lead to the dysfunctioning of mitochondria by lowering ATP synthesis and

energy production. As a result of the decreased energy production in mitochondria they may be induced to apoptosis. Tamoxifen may probably induce cell death through the above said processes. This could be one of the mechanisms of tamoxifen to kill cancer cells. Oxidative stress, nitrosative stress, changes in mitochondrial permeability transition and changes in membrane fluidity may be the path through which tamoxifen induces apoptosis.

REFERENCES

1. Gunning BES, Steer MW. Ultrastructure and Biology of plant cells. Edward Arnold, London, U.K., 1975: 109-11, 130-1,249-59.
2. Peter C Hinkle, Arun Kumar M, Andrea Resetar, David L Harris. Mechanistic stoichiometry of mitochondrial oxidative phosphorylation. Biochemistry 1991; 30(14): 3576-3582.
3. Laura B Valdez, Silvia Lores Arnaiz, Juanita Bustamante, Silvia Alvarez, Lidia E Costa, Alberto Boveris. Free radical chemistry in biological systems. Biol Res 2000; 33(2): 65-70.
4. Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N. Tamoxifen for prevention of Breast cancer: Report of the National Surgical adjuvant breast and bowel project P-1 study. J. Natl. Cancer Inst. 1998; 90: 1371-88.
5. Clorfene-Casten, Liane. Breast Cancer: Poisons, Profits and Prevention. Common Courage Press. Maine, U.S.A. 1996: 93.
6. Johnson D and Lardy HA. Isolation of liver or kidney mitochondria. Methods in Enzymol. 1967; 10: 94-96.
7. Lowry OH, Rosebrough JN, Farr AL and Randall RJ. Protein measurement with the Folin Phenol reagent. J Biol Chem 1951; 193(1): 265-75.
8. Spach P, Wallace P, Cunningham C. Effect of chronic ethanol administration on energy metabolism and phospholipase A2 activity in rat liver. Biochem J 1979; 178: 23-33.
9. Hunter Jr FE, Scott A, Hoffsten PE, Guerra F, Weinstein J, Schneider A, Schutz B, Fink J, Ford L, Smith E. Studies on the mechanism of ascorbate-induced swelling and lysis of isolated liver mitochondria. J Biol Chem 1964; 239: 604-613.
10. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue with thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-354.

11. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase, glutathione-S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979; 582(1): 67-78.
12. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannehaum SR. Analysis of nitrate, nitrite and [^{15}N] nitrate in biological fluids. Anal Biochem 1982; 126: 131-138.
13. Isao Yokoi, Hitoshi Habu, Hideaki Kabuto, Akitane Mori. Analysis of Nitrite, Nitrate and Nitric oxide synthase activity in brain tissue by automated flow injection technique. Methods Enzymol. 1996; 268: 152-159.
14. John S Wishnok, Joseph A Glogowski, Steven R Tannebaum. Quantitation of nitrate, nitrite and nitrosating agents. Methods in Enzymology. 1996; 268: 130-141.
15. Hosein EA, Hofmann I, Linder E. The influence of chronic ethanol feeding to rats on the integrity of liver mitochondrial membrane as assessed with the Mg^{2+}-stimulated ATPase enzyme. Arch Biochem Biophys 1977; 183(1): 64–72.
16. Trudeau DL, Freier EF. Determination of calcium in urine and serum by atomic absorption spectroscopy (AAS). Clin Chem 1967; 13(2): 101-14.
17. Bartley W, Enser MB. The swelling and contraction of isolated rat-liver mitochondria. Biochem J 1964; 93(2): 322–330.
18. Kobayashi T, Kuroda S, Tada M, Houkin K, Iwasaki Y, Abe H. Calcium-induced mitochondrial swelling and cytochrome C release in the brain: its biochemical characteristics and implication in ischemic neuronal injury. Brain Res 2003; 960(1-2): 62-70.
19. Schonfeld P, Weickowski MR, Wojtczak L. Long-chain fatty-acid promoted swelling of mitochondria: further evidence for the protonophoric effect of fatty acids in the inner mitochondrial membrane. FEBS Lett 2000; 471(1): 108-12.
20. Vladimirov YA, Olenev VI, Suslova TB, Cheremisina ZP. Lipid peroxidation in mitochondrial membrane. Adv. Lipid Res. 1980; 17: 173-249.
21. Nakano M, Tsutsumi Y, Ushijima Y. Degradation of thyroxine by the microsomal particles from rat liver. I. Correlation between thyroxine degradation and lipid peroxides. Biochem Biophys Acta 1971; 252(2): 335-47.
22. Neubert D, Wojtczak AB, Lehninger AL. Purification and enzymatic identity of mitochondrial contraction-factors I and II. Proc. Natl. Acad. Sci. U.S.A. 1962; 48: 1651-1658.

23. Ferlini C, Scambia G, Marone M, Distefano M, Gaggini C, Ferrandina G, Fattorossi A, Isola G, Benedetti Panici P, Mancuso S. Tamoxifen induces oxidative stress and apoptosis in oestrogen receptor-negative human cancer cell lines. Br J Cancer 1999; 79(2): 257-263.
24. Dominique Pessayre, Alain Berson, Bernard Fromenty, Abdellah Mansouri. Mitochondria in Steatohepatitis. Semin Liver Dis 2001; 21(1): 057-070.
25. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite oxidation of sulfydryls. The cytotoxic potential of superoxide and nitric oxide. J Biol Chem 1991; 266(7): 4244-4250.
26. Maddaiah VT. Glutathione correlated with lipid peroxidation in liver mitochondria of tri iodothyronine-injected hypophysectomized rats. The FASEB Journal 1990; Vol.4: 1513-1518.
27. Cherynyak BV, Bernardi P. The mitochondrial permeability transition pore is modulated by oxidative agents through both pyridine nucleotides and glutathione at two separate sites. Eur J Biochem 1996; 238: 623-630.
28. Cleeter MWJ, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AHV. Reversible inhibition of cytochrome C oxidase, the terminal enzyme of the mitochondrial respiratory chain by NO. FEBS Lett 1994; 345: 50-54.
29. Brown GC, Cooper CE. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett 1994; 356: 295-98.
30. Okada S, Takehara Y, Yabuki M, Yoshioka T, Yasuda T, Inoue M, Utsumi K. Nitric oxide, a physiological modulator of mitochondrial function. Physiol Chem Phys Med 1996; 28(2): 69-82.
31. Clementi E, Brown GC, Feelisch M, Moncada S. Persistent inhibition of cell respiration by nitric oxide: Crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. Proc Natl Acad Sci USA. 1998; 95: 7631-7636.
32. Takehara Y, Kanno T, Yoshioka T, Inoue M, Utsumi K. Oxygen-dependent regulation of mitochondrial energy metabolism by nitric oxide. Arch Biochem Biophys 1995; 323(1): 27-32.
33. Cassina A, Radi R. Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. Arch. Biochem Biophys 1996; 328: 309-316.

34. Sies H, Sharov VS, Klotz LO, Briviba K. Glutathione peroxidase protects against peroxynitrite-mediated oxidations. A new function for selenoproteins as peroxynitrite reductase. J Biol Chem 1997; 272: 27812-27817.
35. Maccarronea M, Cristina Fantinib, Marco Ranallib, Gerry Melinobc, Alessandro Finazzi Agroa, (1998). Activation of nitric oxide synthase is involved in tamoxifen-induced apoptosis in human erythroleukemia K 562 cells. FEBS Lett 1998; 434(3): 421-424.
36. Lemeshko VV, Shekh VE, Aleksenko TV. Intermembrane electron transport in the dynamics of high-amplitude swelling of rat liver mitochondria. Ukr Biokhim Zh. 1995; 67(2): 28-34.
37. Kretsinger RH. Calcium binding proteins. Ann. Rev. Biochem. 1976; 45: 239-66.
38. Gunter TE, Pfeiffer DR. Mechanisms by which mitochondrial transport calcium occurs. American Journal of Physiology. 1990; 258: c755-786.
39. Chang HT, Huang JK, Wang JL, Cheng JS, Lee KC, Lo YK, Liu CP, Chou KJ, Chen WC, Su W, Law YP, Jan CR. Tamoxifen-induced increase in cytoplasmic free Ca2+ levels in human breast cancer cells. Breast Cancer Res Treat. 2002; 71(2): 125-31.
40. Morkuniene R, Jekabsone A, Borutaite V. Estrogens prevents calcium-induced release of cytochrome C from heart mitochondria. FEBS Lett. 2002; 521(1-3): 53-6.
41. Abe T, Takagi N, Nakano M, Tanonaka K, Takeo S. The effects of monobromobimane on calcium and phenylarsineoxide-induced mitochondrial swelling and cytochrome C release in isolated brain mitochondria. Biol. Pharm Bull. 2004; 27(4): 524-7.