Original Article

Azathioprine induced hepatotoxicity due to oxidative stress, protective aspect of Quercetin in rats

Sunil Kumar[a,*], Vijay K. Sharma[b], Amit Kumar[c]

[a]Department of Pharmaceutical Sciences, Kharvel Subharti College of Pharmacy, Subharti University, Meerut, Uttar Pradesh, India.
[b]Department of Pharmaceutical Sciences, Dr. K N Modi Institute of Pharmaceutical Education and Research, Modi Nagar, Ghaziabad, Uttar Pradesh, India.
[c]Department of Biotechnology, Dr. K N Modi Institute of Pharmaceutical Education and Research, Modi Nagar, Ghaziabad, Uttar Pradesh, India.

Corresponding Author. Tel.: +91 9012406375, E-mail address: sunilak2813@gmail.com

ABSTRACT

Mitochondrion plays the most prominent role in the production of energy and cell cycle regulation. Administration of immunosuppressant drug AZA (azathioprine) adversely affects the hepatic mitochondria, which may culminate in hepatotoxicity. The present study is aimed at evaluating the role of QE (quercetin) in AZA provoked hepatic injury. Male Wister rats were used for the experimentation. AZA was administered as a single intraperitoneal injection (50 mg/kg body weight) on the 7th day of the experiment. A prominent depletion in the levels of mitochondrial antioxidants such as MnSOD (manganese Superoxide dismutase), GPx (glutathione peroxidase) and GSH (reduced glutathione) was observed in AZA induced rats. There was a poignant deterioration noticed in mitochondrial membrane, which was observed by measuring levels of MDA (malondialdehyde). Simultaneous decrease in the levels of TCA (tricarboxylic acid) enzymes such as ICDH (isocitrate dehydrogenase) α-KGDH (α-ketoglutarate dehydrogenase) SDH (succinate dehydrogenase) and MDH (malate dehydrogenase) were observed. Decrease in the levels of these enzymes suggests a loss in mitochondrial function and integrity. The supplementation of QE (50 mg/kg body weight) restored the depleted levels of enzymes and above hepatic mitochondrial abnormalities to near normalcy. Thus, our study emphasizes on antioxidant property of QE in improving the mitochondrial functions in AZA induced hepatic degradation.

1. INTRODUCTION

Liver is that organ of body, which performs the function of detoxifying all substances, which are ingested by humans; therefore, hepatic cells are most susceptible to damage by metabolites of various allopathic drugs. These drugs cause significant hepatic damage due to formation of highly toxic metabolites. AZA, one of the prime agents employed in organ transplantations [1] and autoimmune diseases [2] as delineated by previous records has earned quite a name as hepatotoxic agent, which impedes immunosuppressant therapies. Mounting documents conveyed that toxicity profile of AZA is attributed to its cellular biotransformation which involves as eligible pathway for generation of free radicals [3] that concomitantly executes to mitochondrial hepatic mortification [4]. Mitochondria are responsible for various functions such as generating the energy currency of the cell that is ATP, cell cycle regulation, growth and death. Ironically, mitochondria become the ultimate targets of free radicals, which are generated during the transfer of electrons within enzyme complexes via its own electron transport chain [5].

There is enough substantial evidence to suggest that free radical load hampers electron transport chain, gets intercalated between oxidative phosphorylation thereby destroying enzymes doeing in it, subsequently forwarding to mitochondrial swelling and eventually hepatic necrosis [4,6]. Due to excessive production of free radicals, antioxidants in liver mitochondria become inadequate to quench them thus leading to more free radicals, more oxidative stress and more mitochondrial damage.

As previous experimentations already unveiled that AZA stimulated marked exacerbation hepatic cells, investigation to attenuate its toxic effects in mitochondria is unending. Our never

lasting hunger for the near impeccable protective miracle leads us to the clue that our nature made constituents can contend with the unsurpassable in world. Bioflavonoids have been exploited extensively since umpteen years in various researches due to their antibacterial, antiviral, anti-inflammatory and antioxidant effects [7]. One of these is flavonoid QE; copiously acquired in tea and onions, its cardio protective has been already established [8]. Adequate experimental proof has been gathered regarding antioxidant effects of QE in which it scavenges free radicals thereby defending cell against mitochondrial damage [9].

Therefore in concordance with the previous established theory, QE was chosen as a novel remedy for ameliorating hepatic mitochondrial damage caused by generation of free radicals due to AZA intoxication.

2. MATERIALS AND METHODS

Drugs and chemicals

AZA was purchased from Sigma Aldrich Chemical Company, Bangalore, India and QE was obtained from Hi-Media Lab, Nasik, India. All the remaining chemicals used were of analytical grade.

Experimental Protocol

Animals

The study was performed on male albino rats of Wistar strain (average weight of 150-180 g), which were obtained from Experimental Animal Care Centre, Vel's College of Pharmacy, Chennai, India. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, Ministry of welfare, Chennai. The animals were housed under conditions of controlled temp. 25 ($\pm2^0$C) and acclimatized to 12 ± 1 hr day and night rhythm during the experimental period. They were provided with food and water supplied by Hindustan Lever Ltd., Mumbai, India under the trade name Gold Mohur rat feed and water *ad libitum*. Before experimentation the animals were deprived of food for 24 hr but allowed free access to water throughout. Experiment was conducted under the strict guidelines laid by the committee.

The experimental animals were randomized into four groups of six rats each as follows:

Group I: Control rats received normal saline (2ml/kg body weight) for 7 days.

Group II: A single intraperitoneal injection of AZA (50 mg/kg body weight, suspended in saline) was administered to rats on the 7th day.

Group III: Intraperitoneal injection of QE (50 mg/kg body weight, suspended in saline) was given to rats for 7 days.

Group IV: QE (50 mg/kg body weight, suspended in saline) was administered to rats as in Group III, on the 7th day of experimental period 1hr after administration of QE, single dose of AZA (50 mg/kg body weight, suspended in saline) was given intraperitoneal as in Group II.

After the 7 days of experimental period (i.e., on the 8th day), the animals were anesthetized and decapitated. The liver tissues were immediately excised and rinsed in ice-cold physiological saline. The tissues were homogenized in 0.01 M Tris-HCL buffer (pH 7.4) and aliquots of this homogenate were used for the assays. Blood was collected immediately and the sera were separated by centrifugation. The liver homogenate was centrifuged and mitochondria were isolated. Homogenate and sera were used for several biochemical estimations.

Isolation of liver mitochondria

The mitochondria of liver were isolated by the method of Johnson and Lardy [10]. 10% (w/v) homogenate was prepared in 0.05 M Tris-HCl buffer containing 0.25 M sucrose and centrifuged at 600 κg for 10 minutes. The supernatant fraction was decanted and centrifuged at 15,000 κg for 5 minutes. The resultant mitochondrial pellet was then washed and resuspended in the same buffer.

Determination of mitochondrial antioxidant enzymes

The mitochondrial SOD activity was assayed by the method of Misra and Fridovich [11]. The mitochondrial GP_X activity was assayed by the method of Rotruck [12]. The GSH in liver mitochondria was determined according to the method of Moran [13].

Determination of mitochondrial lipid peroxides

The liver mitochondrial lipid peroxide content was determined by the thiobarbituric acid (TBA) reaction described by Ohkawa [14].

Determination of TCA cycle enzymes:

The activity of ICDH was assayed by the method of King [15]. The activity of α-KGDH was assayed by the method of Reed and Mukherjee [16]. The activity of SDH was assayed according to the method of Slater and Bonner [17], in which the rate of reduction of potassium ferricyanide was measured by decreased in optical density at 400 nm, in the presence of adequate amount of potassium cyanide to inhibit cytochrome C oxidase. The activity of MDH was assayed by the method of Mehler [18]. The substrate used was oxaloacetate and determination of enzyme activity was carried out by measuring the rate of oxidation of NADH.

Statistical analysis

All the grouped data were statistically evaluated with Statistical Package for Social Sciences (SPSS), Version 7.5. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. A '*P*' value of less than 0.05 was considered to indicate statistical significance. All the results were expressed as mean + S.D. for six animals in each group.

3. RESULTS

The activities of mitochondrial antioxidant enzymes MnSOD and GPx were significantly ($p < 0.05$) lower in the hepatic

mitochondria of AZA induced rats (Group II) as compared to that of control rats (group I). Pretreatment of rats with QE + AZA on the 7th day of the experimental period (group IV) significantly reversed all the AZA induced alterations in the activities of mitochondrial antioxidants. The rats receiving QE alone (group III), did not show any significant change when compared with control rats indicating that it does not produce oxidative stress upto this concentration (Table 1).

Table 1. Effect of Azathioprine and Quercetin on the activities of antioxidant enzymes MnSOD and GPx on liver mitochondria*.

GROUPS	MnSOD	GPx
Group I (Control)	37.15 ± 1.38	29.44 ±1.44
Group II (AZA)	24.22 ± 0.86*a	13.80 ±0.99*a
Group III (QE)	39.68 ± 1.16 NS	28.92 ±1.37 NS
Group IV (AZA+QE)	35.11 ±2.08*b	24.34 ±1.14*b

*Results are expressed as mean ±S.D. for 6 rats. Units: MnSOD, min/100mg Protein; GPx, min/mg Protein. Comparisons are made between: a- Group I and Group II; b- Group II and Group IV. *Statistically significant (p < 0.05); NS-non significant.

The level of hepatic mitochondrial TCA cycle enzymes ICDH, KGDH, SDH and MDH were significantly depleted in mitochondria of liver tissue in AZA induced rats (group II), when compared to control rats (group I). These enzyme levels were reversed in QE pretreatment (group IV), which demonstrate amelioration in levels when compared to the AZA induced rats (group II). The rats receiving QE alone (group III), did not show any significant change when compared to control rats (group I) (Table 2).

Table 2. Effect of Azathioprine and Quercetin on the activities of liver mitochondria TCA cycle enzymes*.

GROUPS	ICDH	KGDH	SDH	MDH
Group I (Control)	788.18 ± 37.23	250.18 ± 11.86	43.57 ± 2.35	257.57 ± 3.88
Group II (AZA)	467.47 ±26.99*a	166.18 ±7.99*a	12.65 ±4.55*,a	199.41 ±6.25*,a
Group III (QE)	767.09 ±23.12NS	249.76 ±12.84 NS	43.94 ±1.47 NS	260.64 ±5.58 NS
Group IV (AZA+QE)	756.34 ±16.77*b	238.89 ±10.11*b	26.25 ±1.35*,b	234.02 ±3.57*,b

*Results are expressed as mean ±S.D. for 6 rats. Units: ICDH, nmole of α-ketoglutarate formed/h/mg protein; KDH, nmole of ferricyanide formed/h/mg protein; SDH, nmole of succinate oxidized/min/mg protein; MDH, nmole of NADH oxidized/min/mg protein. Comparisons are made between: a- Group I and Group II; b- Group II and Group IV. *Statistically significant (p < 0.05); NS-non significant.

The level of MDA was significantly (p < 0.05) ameliorated in liver mitochondria of AZA induced rats (group II) when compared to control rats. The MDA level was found to be lowered by the QE pretreatment (Graph 1) on 7th day on AZA treated rats (group IV), which depicts the alteration in the level of AZA induced rats (group II) compared to QE treated rats (group IV). The rats receiving QE alone (group III) did not show any significant change when compared to control rats indicating that it does not have any adverse effect (Figure 1).
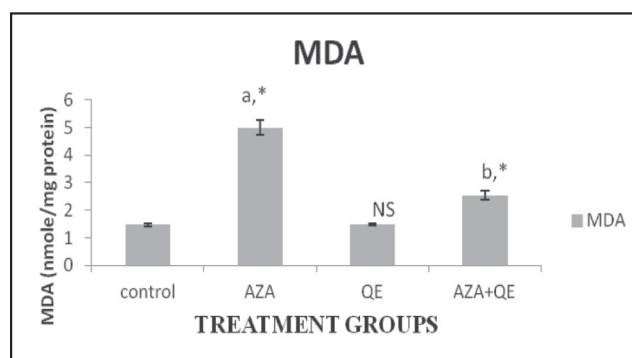

Fig. 1. Levels of MDA in the liver mitochondria of the rats and expressed as μmole mg⁻¹ protein. Comparisons are made between: a – Group I and Group II; b – Group II and Group IV. *Statistically significant (p < 0.05); NS-non significant.

The level of mitochondrial GSH was significantly reduced in AZA treated rats (group II). The mitochondrial GSH level was significantly normalized by pretreatment of QE on the 7th day with subsequent administration of AZA (group IV). The rats receiving QE alone did not show any significant change (group III) when compared to control rats (group I), indicating that it does not produce any harmful effects (Figure 2).
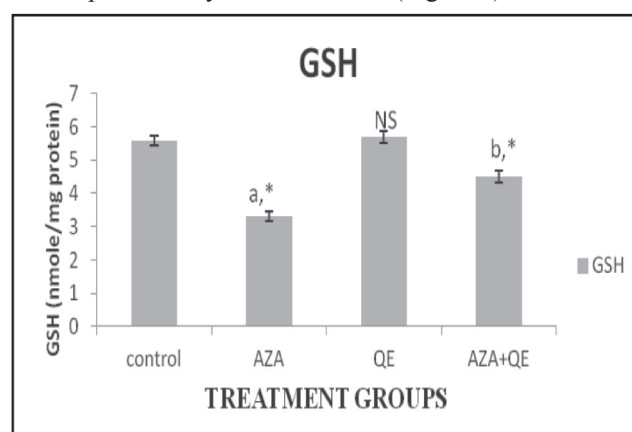

Fig. 2. Levels of mitochondrial GSH in the liver of the rats and expressed as μmole mg-1 protein. Comparisons are made between: a – Group I and Group II; b – Group II and Group IV. *Statistically significant (p < 0.05); NS-non significant.

Therefore through the above enzyme estimations, it was proved that quercetin is efficient in protecting degradation of hepatic mitochondria induced by administration of AZA.

4. DISCUSSION

One of the most frequently employed drug as immunosuppressant therapy in organ transplantation is azathioprine [6-(1-methyl-4-nitro-5-imidazolyl) thioprine] [1]. Its complicacy in the transplantation therapy is due to its adverse reactions which execute to profligation of liver. As rationalized earlier, AZA toxicity is related to its biotransformation and its oxidized mechanism is the principal pathway for generating the free radicals [3]. QE, as assured by previous documents, is extremely virile to fight against mitochondrial degradation caused by free radicals [9]. There is a substantial evidence to prove that QE protects azathioprine induced hepatic cellular damage [19]. Therefore, we examined the protective effect of quercetin against azathioprine induced deterioration at the subcellular level.

An alteration in the equilibration between production of free radicals and the quenching action of antioxidant system is denoted as "oxidative stress", which is associated with chronic diseases. Evidence of data revealed that free radicals generated during the electron transfer in the respiratory complexes might lead to deposition of these radicals in mitochondria that might impinge on its physiology [20]. The homeostasis of reactive oxygen species in the mitochondria is regulated by GSH [21]. GSH is a tripeptide [22] which counteracts against oxidative stress in the mitochondria by quenching free radicals [23].

The attenuation in the antioxidant system in mitochondrial matrix as a consequence of hampered GSH levels has been shown to enhance oxidative damage to the ETC. This severe effect on ETC indirectly inhibits GSH synthesis there by exacerbating the effect of mitochondrial oxidative free radicals ultimately leading to hepatic damage [6, 24]. Culminating evidences proved that during AZA disposition, GSH is consumed as a co substrate, so that it leads to depleted levels in mitochondria [25]. It was observed in our study that on treatment with AZA, mitochondrial GSH was reduced, also our results depicted an increment in the GSH levels after quercetin administration that may be due to fact that it affects GSH/GSSG ratio in hepatic mitochondria.

These free radical species initiate the oxidation of mitochondrial polyunsaturated fatty acids (PUFA), protein, and sterol as revealed by mounting literatures. The formation of conjugated dienes by the PUFA through oxidative radicals may propagate synthesis of lipid peroxides that cause impairment in the integrity and function of hepatic mitochondrial membrane [26]. We observed an alteration in mitochondrial MDA levels in AZA intoxicated rats, which is in sync with the previous literatures indicating increased lipid peroxidation that could be attributed to deterioration of antioxidant defense mechanism [27]. The QE treatment in our study exhibited near normalcy in MDA levels by quenching peroxide radicals thereby proving its efficacy in maintaining membrane integrity.

Mitochondrial endogenous radical load is diminished by enzymatic scavengers like Mn-SOD and GPx [28]. Superoxide radical is formed directly by accepting electron from the molecular oxygen, its accumulation leads to inhibition of ETC [29]. This superoxide is dismutated into H_2O_2 by the mitochondrial MnSOD [30]. GPx converts H_2O_2 in to H_2O [28] in mitochondria. Activities of Mn-SOD depict hampered levels after induction with azathioprine as documented by previous reports. The hampered levels of GPx in AZA intoxicated rats may be due to the unavailability of the thiol substrate [27]. Pre-treatment with the quercetin significantly restored the mitochondrial levels of Mn-SOD and GPx to near normal levels [31, 9].

The most organized enzyme of TCA cycle is α-KGDH. It catalyzes the conversion of α-ketoglutarate, Co-A and NAD^+ to succinyl Co-A, CO_2 and NADH [32]. Due to the degradation of mitochondrial membrane via free radicals, aldehydic products are formed which inhibit the activity of this enzyme as corroborated earlier [26]. Hydrogen peroxide attacks the enzyme; leading to its diminished activity thereby, stopping the formation of NADH, which consequently does not, participates in ETC, resulting in depleted ATP levels [32]. ICDH controls the redox balance in mitochondria. It restores NADPH, which is adept in regenerating GSH. Due to the free radical attack, electron transfer gets hampered leading to its loss in activity [33, 34]. SDH, a component of ETC and MDH, both are susceptible to inactivation by oxidative stress. Their activity gets lost due to thiolation of cysteine residues [35, 36]. We observed a significant decrease in the levels of mitochondrial enzymes such as ICDH, SDH, MDH and α-KGDH in AZA intoxicated rats, which were in concordance with previous studies [27]. In our experiment quercetin, protected rats depicted an increase in the activities of ICDH, SDH, MDH and α-KGDH.

AZA provoked mitochondrial membrane damage and subsequent degradation of respiratory enzymes thus leading to imbalanced transaction of substrates and ions in mitochondria, thereby pushing mitochondrial membrane to expand leading to mitochondrial swelling [4].

5. CONCLUSION

Concluding with the present observation quercetin may reduce oxidative mitochondrial damage by normalizing enzymes, which get disproportionate by AZA intoxication; moreover, QE maintains endogenous mitochondrial antioxidants status and exerts membrane stabilization action by declining lipid peroxidation. This confirms that quercetin due to its antioxidant property protects mitochondrial damage induced by AZA and regulates energy production which is required for the cell normal homeostasis. Thus, the present work highlights the cytoprotective role of QE in AZA induced hepatotoxicity at the subcellular level.

REFERENCES

[1] Raza, M.; Ahmad, M.; Gado, A.; Al-Shabanah, O.A. A comparison of hepatoprotective activities of aminoguanidine and N-acetylcysteine in rat against the toxic damage induced by azathioprine. *Comp Biochem Physiol C Toxicol Pharmacol.* 2003, *134*, 451-6.

[2] Nash, C.L.; Sutherland, L.R. Medical management of inflammatory bowel disease: old and new perspectives. *Curr Opin Gastroenterol.* 2001, *17*, 336–341.

[3] Lee, A.U.; Farrell, G.C. Mechanism of azathioprine-induced injury to hepatocytes: roles of glutathione depletion and mitochondrial injury. *J Hepatol.* 2001, *35*, 756-64.

[4] Menor, C.; Fernandez–Moreno, M.D.; Fueyo, J.A.; Escribano O.; Olleros, T.; Arriaza, E.; Cara, C.; Lorusso, M.; Di Paola, M.; Roman, I.D.; Guijarro, L.G. Azathioprine acts upon rat hepatocyte mitochondria and stress activated protein kinases leading to necrosis: Protective role of N-acetyl –L-cysteine. *The Journal of Pharmacology and Experimental Therapeutics.* 2004, *311*, 668-676.

[5] Sas, K.; Robotka, H.; Toldi, J.; Vecsei, L. Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *Journal of the Neurological Sciences.* 2007, *257*, 221-239.

[6] Ehrhart, J.; Gluck, M.; Mieyal, J.; Zeevalk, G.D. Functional glutaredoxin (thioltransferase) activity in rat brain and liver mitochondria. *Parkinsonism and related disorders.* **2002**, *8*, 395-400.

[7] Molina, M.F.; Sanchez-Reus, I.; Iglesias, I.; Benedi, J. Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse liver. *Biol Pharm Bull.* 2003, *26*, 1398-402.

[8] Boots, A.W.; Li, H.; Schins, R.P.; Duffin, R.; Heemskerk, J.W.; Bast, A.; Haenen, G.R. The quercetin paradox. *Toxicology and applied pharmacology* 2007, *222*, 89-96.

[9] Guzy, J.; Kusnir, J.; Marekova, M.; Chavkova, Z.; Dubayova, K.; Mojzisova, G.; Mirossay, L.; Mojzis, J. Effect of Quercetin on Daunorubicin-Induced Heart Mitochondria Changes in Rats. *Physiol. Res.* 2003, *52*, 773-780.

[10] Johnson, D.; Lardy, H. Isolation of liver or kidney mitochondria. *Methods Enzymol.* 1967, *10*, 94-96.

[11] Misra, H.P.; Fridovich, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem.* 1972, *247*, 3170-3175.

[12] Rotruck, J.T.; Pope, A.L.; Ganther, H.; Swanson, A.B.; Hafeman, D.G.; Hoektsra, W.G. Selenium: biochemical role as a component of glutathione peroxidase. *Science.* 1973, *179*, 588-590.

[13] Moran, M.S.; Depierre, J.W.; Mannervik, B. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. *Biochim Biophys Acta.* 1979, *582*, 67-68.

[14] Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979, *95*, 351-358.

[15] King, J. The hydrolases-acid and alkaline phosphatases. In: Practical clinical enzymology (King JC, ed.), *Van D Nostrand Company, London* 1965a, 199-208.

[16] Reed, L.J.; Mukherjee, B.B. α-Ketoglutarate dehydrogenase complex from Escheriachia coli. *Method Enzymol.* 1969, *13*, 55–61.

[17] Slater, E.C.; Bonner, W.D. The effect of fluoride on succinic oxidase system. *Biochem J.* 1952, *52*, 185-196.

[18] Mehler, A.H.; Kornberg, A.; Grisolia, S.; Ochoa, S. The enzymatic mechanism of oxidation-reductions between malate or isocitrate and pyruvate. *J Biol Chem.* 1948, *174*, 961-977.

[19] Shanmugarajan, T.S.; Prithwish, N.; Somasundaram, I.; Arunsundar, M.; Niladri, M.; Lavande, J.P.; Ravichandiran, V. Mitigation of Azathioprine-Induced Oxidative Hepatic Injury by the Flavonoid Quercetin in Wistar Rats. *Toxicology Mechanisms and Methods.* 2008, *18*, 653 – 660.

[20] Fariss, M.W.; Chan, C.B.; Patel, M.; Van Houten, B.; Orrenius, S. Role of mitochondria in toxic oxidative stress. *Molecular interventions.* 2005, *5*, 94-111.

[21] Garcia-Ruiz, C.; Morales, A.; Colell, A.; Rodes, J.; Yi, J.R.; Kaplowitz, N.; Fernandez-Checa, J.C. Evidence that the rat hepatic mitochondrial carrier is distinct from the sinusoidal and canalicular transporters for reduced glutathione. Expression studies in Xenopus laevis oocytes. *The journal of biological chemistry.* 1995, *270*, 15946-15949.

[22] Vergauwen, B.; Pauwels, F.; Vaneechoutte, M.; Van Beeumen, J.J. Exogenous Glutathione Completes the Defense against Oxidative Stress in *Haemophilus influenza. Journal of bacteriology.* 2003, *185*, 1572–1581.

[23] Beer, S.M.; Taylor, E.R.; Brown S.E.; Dahm, C.C.; Costa, N.J.; Runswick, M.J.; Murphy, M.P. Glutaredoxin 2 Catalyzes the Reversible Oxidation and Glutathionylation of Mitochondrial Membrane Thiol Proteins. *The journal of biological chemistry.* 2004, *279*, 47939–47951.

[24] Fernandez-checa, J. C.; Kaplowitz, N. Hepatic mitochondrial glutathione: transport and role in disease and toxicity. *Toxicology and applied pharmacology.* 2005, *204*, 263-273.

[25] Tapner, M.J.; Jones, B.E.; Wu, W.M.; Farrell, G.C. Toxicity of low dose azathioprine and 6-mercaptopurine in rat hepatocytes. Roles of xanthine oxidase and mitochondrial injury. *J Hepatol.* 2004, *40*, 454-63.

[26] Martinez–Cruz, F.; Osuna, C.; Guerrero, J.M. Mitochondrial damage induced by fetal hyperphenylalaninemia in the rat brain and liver: Its prevention by melatonin, Vitamin E and Vitamin C. *Neuroscience Letters.* 2006, *392*, 1-4.

[27] Shanmugarajan, T.S.; Devaki, T. Hepatic Perturbations Provoked by Azathioprine: A Paradigm to rationalize the cytoprotective Potential of *Ficus Hispida Linn. Toxicology Mechanisms and methods.* 2009, *19*, 129-34.

[28] Higuchi, H.; Adachi, M.; Miura, S.; Gores, G.J.; Ishii, H. The Mitochondrial Permeability Transition Contributes to Acute Ethanol-Induced Apoptosis Induced Apoptosis in Rat Hepatocytes. *Hepatology.* 2001, *34*, 320-328.

[29] Vali, S.; Mythri, R.B.; Jagatha, B.; Padiadpu, J.; Ramanujan, K.S.; Andersen, J.K.; Gorin, F.; Bharath, M.M. Integrating glutathione metabolism and mitochondrial dysfunction with implications for Parkinson's disease: a dynamic model. *Neuroscience.* 2007, *149*, 917-30.

[30] Preston T.J.; Abadi, A.; Wilson, L.; Singh, G. Mitochondrial contributions to cancer cell physiology: potential for drug development. *Advanced Drug Delivery Reviews.* 2001, *49*, 45-61

[31] Kebieche, M.; Lakroun, Z.; Lahouel, M.; Bouayed, J.; Meraihi, Z.; Soulimani, R. Evaluation of epirubicin-induced acute oxidative stress toxicity in rat liver cells and mitochondria, and the prevention of toxicity through quercetin administration. *Exp Toxicol Pathol.* 2008, *61*, 161-7.

[32] Tretter, L.; Adam-Vizi, V. Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress *Phil Trans R Soc Lond B Biol Sci.* 2005, *360*, 2335-2345.

[33] Lee, S.M.; Koh, H.J.; Park, D.C.; Song, B.J.; Huh, T.L.; Park, J.W. Cytosolic NADP(+)-dependent isocitrate dehydrogenase status modulates oxidative damage to cells. *Free Radic Biol Med.* 2002, *32*, 1185-96.

[34] Plaut, G.W.; Cook, M.; Aogaichi, T. The subcellular location of isozymes of NADP-isocitrate dehydrogenase in tissues from pig, ox and rat. *Biochim Biophys Acta.* 1983, *760*, 300-8.

[35] Le-Quoc, K.; Le-Quoc, D.; Gaudemer, Y. Evidence for the existence of classes of sulphydryl groups essential for membrane-bound succinate dehydrogenase activity. *Biochemistry.* 1981, *20*, 1705-1710.

[36] Sharma, A.B.; Sun, J.; Howard, L.L.; Williams, A.G.Jr.; Mallet, R.T. Oxidative stress reversibly inactivates myocardial enzymes during cardiac arrest. *Am J Physiol Heart Circ Physiol.* 2007, *292*, H198–H206.