

PROTECTIVE EFFECT OF GINGER EXTRACT AGAINST ROS GENERATION DURING MITOCHONDRIAL STRESS IN SELECTED TISSUES UNDER ETHANOL WITHDRAWAL CONDITION

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ABSTRACT

Alcohol intoxication and sudden withdrawal from chronic alcohol intake certainly induces stress due to the ability of cellular adaptation to environmental and physiological constraints that necessitates a fine tuning of the control of mitochondrial respiration, in response to changes in energy demand and substrate delivery. Accordingly, different tissues present with large differences in the composition of the OXPHOS machinery and the organization of mitochondria, which could reflect their variable physiological activity. In addition, mitochondria also participate in fundamental cell signaling and apoptotic processes, the regulation of which may also lead to changes in organellar content, composition, and functionality. Recently, different studies have evidenced important differences in the mitochondrial proteome between tissues, but their functional implications. Here, we have analyzed the diversity of mitochondrial oxidative phosphorylation between tissues and its consequences for the control of energy production under the condition of ethanol withdrawal induced stress. For this, we determined the compositional and functional features of the respiratory chain on mitochondria isolated from rat muscle, heart, liver, kidney, and brain and compared the activities of respiratory complexes during supplementation with nutraceuticals like silymarin, alpha – lipoic acid and ginger in ethanol withdrawal induced stress.

KEY WORDS

Alcohol Intoxication; Ethanol Withdrawal Induced Stress, Mitochondrial Respiration; Silymarin, Alpha – Lipoic Acid and Ginger

INTRODUCTION

Oxidative stress caused by increased generation of reactive oxygen species (ROS) had been suggested to play the major role in pathology of so many organs. Acute and chronic ethanol treatment has been shown to increase the production of reactive oxygen species, lower cellular antioxidant levels, and enhance oxidative stress in many tissues (Dey and Cederbaum, 2006). Ethanol withdrawal (EW) provokes the intense generation of reactive oxygen species (ROS). The oxidative stress was more severe during EW than

ethanol exposure *per se* and was accompanied with cell death. Taken together, these findings suggest that EW induces destructive oxidative stress both in *in vivo* and *in vitro* conditions (Jung and Metzger, 2010). It has been proposed that oxidative damage due to alcohol withdrawal is more intense than chronic ethanol consumption itself (Koch-Weser et al., 1976). Alcohol withdrawal induce overproduction of reactive oxygen species (ROS) [e.g., hydroxyl radicals ($\text{OH}^{\cdot}$), superoxide anions ($\text{O}_2^{\cdot-}$) and hydrogen peroxide (H_2O_2)] and reactive nitrogen species (RNS) [e.g.,

nitric oxide (NO) and peroxynitrite (OONO^{-1}), called the oxidative and nitrosative stresses. These free radicals may be produced by many of the free radical producing-enzyme systems including mitochondria, cyclooxygenase (COX), xanthine oxidase, NADPH oxidase (NOX), and inducible nitric oxide synthase (iNOS) in response to the activation of proinflammatory mediators (Lo et al., 2003; Madrigal et al., 2004; Shen et al., 2008). ROS and RNS damage tissues by attacking DNA or inducing lipid peroxidation or protein nitrosylation of the cell membrane and organelles (Lo et al., 2003)

Within a cell, mitochondria largely contribute to the production of ROS via the respiratory chain (Lenaz, 1998; 2001). The electron transfer chain or respiratory chain consists of four major multisubunit complexes designated as NADH-coenzyme Q (CoQ) reductase (Complex I), succinate-CoQ reductase (SQR) (Complex II), ubiquinol-cytochrome c reductase (Complex III) and cytochrome c oxidase (Complex IV). Previous studies have emphasized the decrease in the activities of respiratory chain enzymes during aging (Modi et al., 2008). The increase in the lipid peroxidation products MDA and HNE and the decline in the activities of those critical enzymes involved in respiratory chain could be co-related (Navarro et al., 2002). The level of lipid peroxidation would have imposed an effect on the functional capacities of the proteins, thereby affecting their activities in aged animals. Since the electron transport chain complexes are membrane-bound and sensitive to the lipid microenvironment (Keller et al., 1997), oxidative damage to the inner mitochondrial membrane would have an adverse impact on the electron transport chain activities.

This study was undertaken to investigate the effectiveness of dietary supplementation with α -lipoic acid, Silymarin and ginger extract during chronic ethanol consumption and ethanol withdrawal-induced mitochondrial adaptations in rat. These compounds were specifically selected because they are used in the treatment of mitochondrial cytopathies and have been shown to reduce oxidative stress and enhance mitochondrial function in humans (Tarnopolsky, 2008; Rodriguez et al., 2007; Liu and Ames, 2005). Alpha-Lipoic acid has also been shown to improve mitochondrial function (Hagen et al.,

1999). Silymarin and its active component silibinin/silybin both exhibit strong anti-oxidant activity (Koksal et al., 2009; Sangeetha et al., 2009). Antioxidant properties of ginger extract are well established (Chung et al., 2005, 2012). The role of mitochondrial dysfunction is found in many disorders associated with ethanol consumption and this further intensifies the need to understand the potential role of dietary supplements as a therapeutic intervention in treating alcohol related diseases which provide a new hope.

MATERIAL AND METHODS

Plant material and extraction:

Aqueous ginger extract was prepared from locally available ginger roots. Ginger rhizomes were purchased fresh from the local market of Tirupati and were authenticated by staff in the Department of Botany at Sri Venkateswara University, Tirupati in India. Whole rhizome of ginger was thoroughly washed, sliced, grated, and ground to a fine paste. A weighed quantity (30gm) of the paste was subjected to continuous hot extraction in a soxhlet apparatus using double distilled water. The extract was evaporated under reduced pressure using a rotary evaporator and then lyophilized until all of the solvent was removed. This aqueous ginger extract (AGE) was stored at 4°C.

Chemicals: Silymarin and α -Lipoic acid were purchased from Sigma-Aldrich Co. LLC. All other reagents used were of analytical grade.

Animals

The study involved young (2–3 months old; 200 - 220g) Wistar strain male albino rats purchased from Sri Venkateswara Traders Pvt. Limited, in Bangalore and were maintained in the animal house of the department in polypropylene cages. Standard conditions of humidity (50% relative humidity), room temperature (25 - 28°C) and 12 h light/dark cycle (6:00 A.M. to 6:00 P.M.) were maintained. A standard rodent diet (M/s Hindustan Lever Ltd., Mumbai), and water was provided *ad libitum*. All experimental procedures were approved by the CPCSEA on Animal Care, Govt. of India, bearing the CPCSEA No. 438 / 01/a / CPCSEA / IAEC / SVU / KSR-1 (dt: 11.09.2008).

Experimental design: The rats were randomly grouped into four (n = 4) and treated as follows:

- I. Control received normal saline daily (p.o.),
- II. The ethanol group of rats were treated as following (n=4)
 - i. **EtOH**-Group that received 20% Ethanol for 6 weeks (orally)
 - ii. **Ext+EtOH** -Group that received 20% Ethanol for 6 weeks and received 200 mg/kg body weight of ginger extract (orally) simultaneously daily
 - iii. **Sil+EtOH** -Group that received 20% Ethanol for 6 weeks and received 100 mg/kg body weight of silymarin (orally) simultaneously daily
 - iv. **LA+EtOH** -Group that received 20% Ethanol for 6 weeks and received 100 mg/kg body weight of Lipoc Acid (intra peritoneal (i.p.)) simultaneously daily.
 - v. Also maintained are ethanol(EtOH) groups that are given combined combinations of lipoic acid+ silymarin; lipoic acid+ ginger extract, ginger extract+silymarin at the same doses described above.
- III. The withdrawal group of rats were treated as following (n=4)
 - i. **EW**-Group that received 20% Ethanol for 6 weeks and subjected to ethanol withdrawal for 72hrs
 - ii. **Ext+EW** -Group that received 20% Ethanol for 6 weeks and subjected to ethanol withdrawal for 72 hrs and received 200 mg/kg body weight of ginger extract (orally) simultaneously daily
 - iii. **Sil+EW**-Group that received 20% Ethanol for 6 weeks and subjected to ethanol withdrawal for 72 hrs and received 100 mg/kg body weight of silymarin (orally) simultaneously daily
 - iv. **LA+ EW**-Group that received 20% Ethanol for 6 weeks and subjected to ethanol withdrawal for 72 hrs and received 100 mg/kg body weight of Lipoc Acid (intra peritoneal (i.p.)) simultaneously daily.
 - v. Also maintained are ethanol withdrawal (EW) groups that are given combined combinations

of lipoic acid+ silymarin; lipoic acid+ ginger extract, ginger extract+silymarin at the same doses described above.

Tissue collection

After the period of last dose of ethanol treatment, the animals were sacrificed exactly after 72 hrs y cervical dislocation. The tissues namely brain liver, heart and kidney were dissected, washed with ice-cold saline, blotted, dry freezed in liquid nitrogen and immediately transferred to the ice chamber at -20°C.

Preparation of rat liver and kidney mitochondria.

Liver was collected in isolation medium A (in mM: 250 sucrose, 10 Tris-HCl, pH 7.6, and 1 K⁺ EGTA) and homogenized. The homogenate was centrifuged at 1,000 g for 5 min. The supernatant was strained through gauze and centrifuged at 7,000 g for 10 min. The resulting pellet was resuspended in ice-cold isolation medium B (in mM: 250 sucrose, 10 Tris-HCl, pH 7.6, and 0.1 K⁺ EGTA), and a new series of centrifugations (1,000 and 7,000 g) was performed. The last mitochondrial pellet was resuspended in a minimum volume of isolation medium B to obtain a mitochondrial concentration of between 50 and 70 mg/ml.

Isolation of cardiac mitochondria

Frozen powdered tissue of the whole heart (about 1 g) was thawed in 10 volumes of ice-cold homogenization buffer (30 mM KH₂PO₄, 5 mM EDTA, 0.3 M sucrose, pH 7.0) with 0.3 mM phenylmethylsulfonyl fluoride (PMSF) and homogenized in Potter-Elvehjem homogenizer. Cardiac mitochondrial fraction was isolated from individual tissue homogenates by differential centrifugation.

Preparation of rat brain mitochondria

Brain mitochondria were isolated from whole brain homogenized in isolation buffer (in mM: 250 sucrose, 10 Tris-HCl, pH 7.4, and 0.5 K⁺ EDTA). The homogenate was centrifuged at 1,000 g for 5 min. The supernatant was strained through gauze and recentrifuged at 7,000 g for 10 min. The resulting pellet was resuspended in ice-cold isolation buffer, and a new series of centrifugations (1,000 and 7,000

g) was performed. The crude mitochondrial pellet was resuspended in a final volume of 10 ml in 3% Ficoll medium (3% Ficoll, 250 mM sucrose, 10 mM Tris-HCl, pH 7.4, and 0.5 mM K^+ EDTA). This suspension was carefully layered onto 20 ml of 6% Ficoll medium (6% Ficoll, 250 mM sucrose, 10 mM Tris-HCl, pH 7.4, and 0.5 mM K^+ EDTA) and centrifuged for 30 min at 11,500 *g*. The mitochondrial pellet was resuspended in isolation medium and centrifuged for 10 min at 12,500 *g*. The mitochondria were made up to a concentration of ~50 mg protein/ml in the isolation buffer.

The purity of mitochondria was assessed by the assay of specific marker enzyme; succinate dehydrogenase was assayed by the method of Slater and Bonner, 1952. Mitochondrial protein was estimated by the method of Lowry et al., 1951.

Biochemical Estimation

1. Measurement of NADH-cytochrome C reductase (Complex I) activity

Membrane bound mitochondrial activities were assayed spectrophotometrically in 100 mmol/L phosphate buffer (pH 7.4) at 30°C. For the determination of NADH-cytochrome C reductase activity, 2 mg of mitochondrial fragments were added with 0.2 mM NADH, 0.1 mM cytochrome C, and 1 mM KCN and absorption at 550 nm was followed (Yonetani, 1967). Enzyme activity was expressed in nanomoles cytochrome C reduced per minute per milligram of protein.

2. Measurement of succinate dehydrogenase (SDH) (Complex II) activity

Succinate dehydrogenase activity was determined spectrophotometrically by measuring the decrease in absorbance at 600 nm caused by the reduction of 2,6-dichlorophenol indophenols by succinate by the method of Vrbáček et al., 2007 with slight modifications. To 1 ml phosphate buffer (0.1 mol/L, pH 7.6), 0.1 ml EDTA (1 mmol/L), 0.1 ml of Bovine Serum Albumin (1 mg/ml), 0.1 ml of sodium succinate (5 mM), 2.0 ml water, 0.1 ml potassium cyanide (1 mM) was added. To the above mixture, 0.2 ml of 1 in 5 diluted mitochondrial suspension was added. 0.1 ml of 2,6-dichlorophenol indophenols (0.1 mM) was added, mixed well and the absorbance was measured

spectrophotometrically at 600 nm immediately against a reagent blank without the mitochondrial fraction and chromogen. The extinction co-efficient of 21 mmolL⁻¹ cm⁻¹ was used for calculation. The specific activity of the enzyme was expressed in nanomoles of succinate oxidized/min/mg protein.

3. Measurement of ubiquinol cytochrome c reductase (Complex III)

The oxidation of 6.5 mM decylubiquinol by complex III was determined by using cytochrome c (III) as an electron acceptor (Krahenbuhl et al., 1991). The assay was carried out in basic medium supplemented with 2.5 mg/ml BSA, 15 μM cytochrome c (III), and 5 μg/ml rotenone. The reaction was started with 10 μg of mitochondrial protein, and the enzyme activity was measured at 550 nm. The extinction coefficient used for cytochrome c was 18.5 mM⁻¹·cm⁻¹. The specific activity of the enzyme was expressed in nmoles of cytochrome c reduced/min/mg protein.

4. Measurement of cytochrome oxidase activity (Complex IV)

Cytochrome oxidase activity was determined with phosphate buffer (0.1 mol/L, pH 7.0) containing 0.1 mmol/L reduced cytochrome C, prepared by reduction with excess NaBH₄ and HCl, as the decrease in absorbance over 3 min at 550 nm (Yonetani, 1967). Enzymatic activity was calculated in terms of the pseudo-first order reaction constant for cytochrome C oxidation (k) per milligram of protein and expressed as the initial rate of cytochrome C oxidation in nanomoles cytochrome C oxidised per minute per milligram protein in the presence of 0.1 mmol/L cytochrome C.

Statistical analysis

All of the data presented in this study correspond to the mean value of *n* experiments ± SD, with *n* ≥ 3. Comparison of the data obtained from isolated liver, heart, brain and renal mitochondria were performed with Student's *t*-test, using Excel software (Microsoft). Two sets of data were considered as statistically different when *p* < 0.001 or *p* < 0.01 or *P* < 0.05.

RESULTS

Changes in activities of electron transport chain complexes

To evaluate the effect of alcohol and withdrawal on mitochondrial function, enzyme activities of complexes I-IV in hepatic, cardiac, cerebral and renal mitochondria isolated from young (3-month old) wistar rats are measured (Fig 1,4,7,10). There were no significant difference in the complex I activities between alcohol and withdrawal rats, the activity of withdrawal rats decreased to 76 ± 1 % of alcoholic ($p < 0.01$) (Fig 2, 5, 8, 11). Also complex II activity was significantly changed only in withdrawal rats, however, the decrease was less pronounced (to 86 ± 3 % of alcoholic, $p < 0.01$) than that of complex I. On the other hand, antimycin A-sensitive complex III activity

decreased significantly in both alcoholic (to 90 ± 4 % of adult, $p < 0.05$) and withdrawal rats (to 83 ± 2 % of adult, $p < 0.01$) (Fig 3, 6, 9, 12). The most pronounced changes were observed in KCN-sensitive complex IV (COX) activity, comparing to adult the activity decreased to 79 ± 3 % ($p < 0.01$) in alcoholic and 63 ± 4 % ($p < 0.001$) in withdrawal rats. The alcohol withdrawal-related changes tend to get normalized activities on treatment with ginger extract and lipoic acid in all the tissues tested. The activities of the complexes under various treatments in different organs are given (Fig 1-12). In contrast, the activities of mitochondrial complexes in rats treated with silymarin alone as well in rats given lipoic acid only were significantly lower than those of normal rats.

Fig 1. Mitochondrial complexes in liver in groups treated with ginger extract, silymarin and lipoic acid

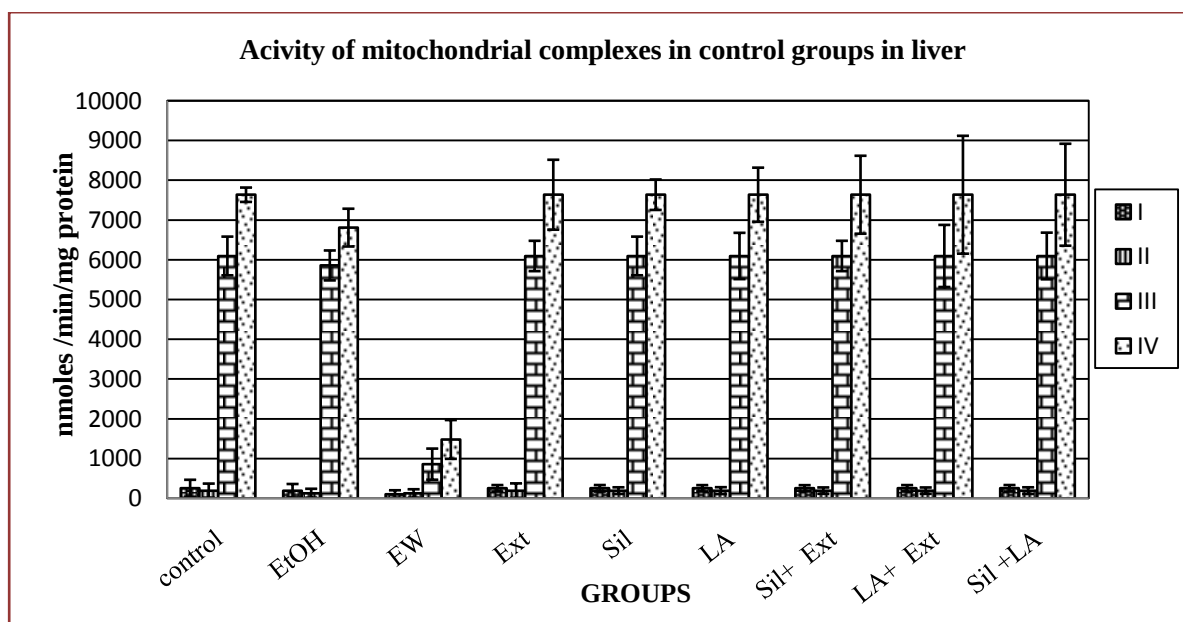


Fig 2. Mitochondrial complexes in liver during ethanol intoxication in groups treated with silymarin, lipoic acid and ginger extract

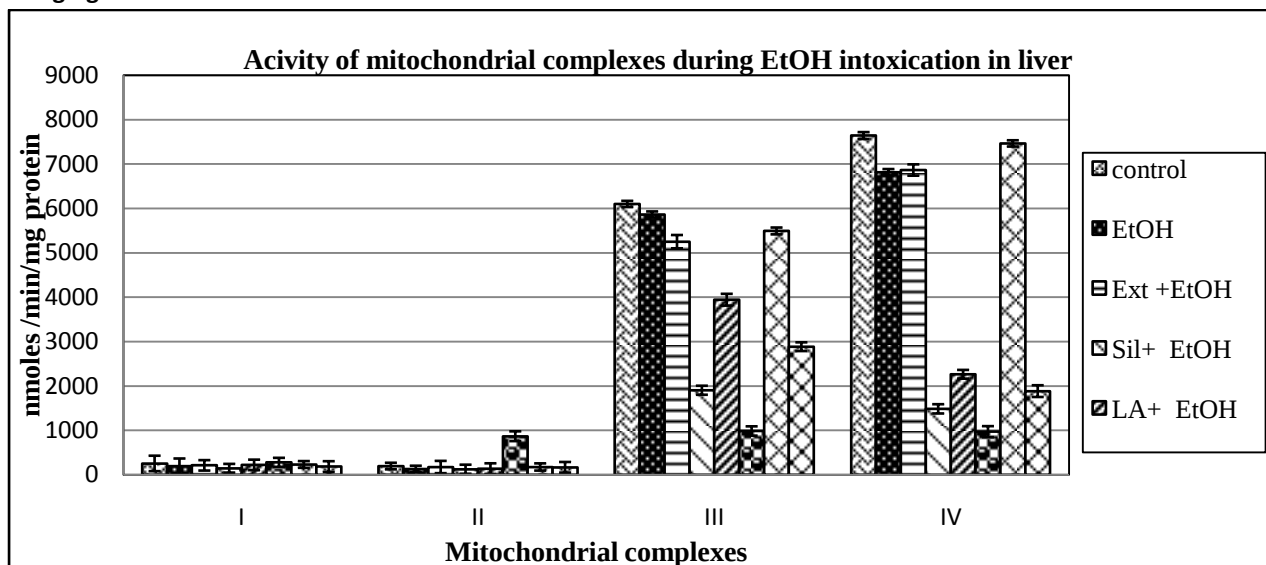


Fig 3. Mitochondrial complexes in liver during ethanol withdrawal in groups treated with silymarin, lipoic acid and ginger extract

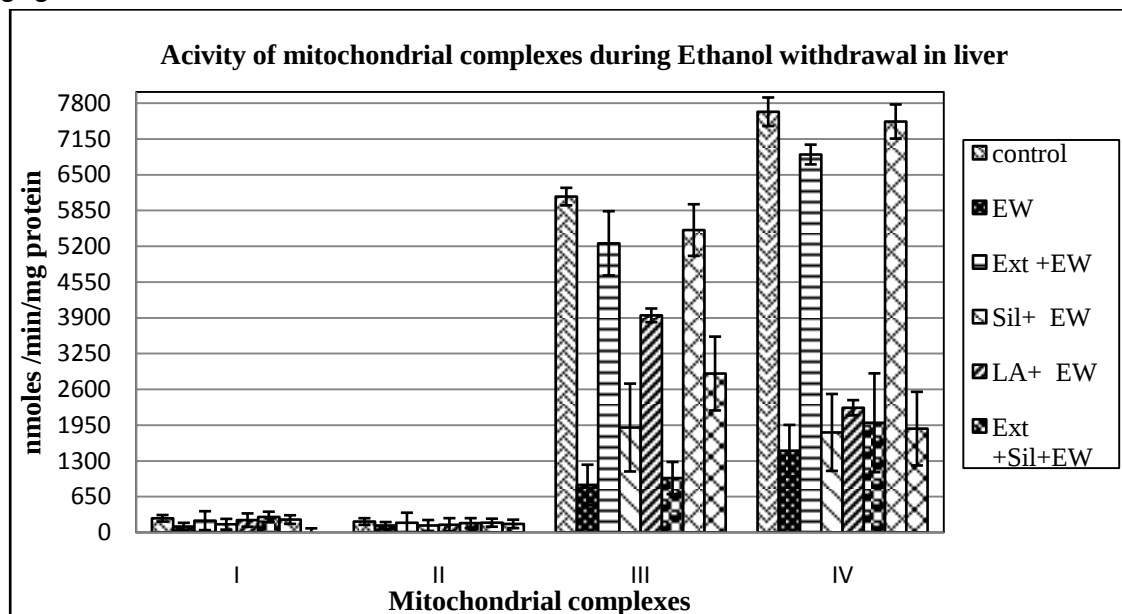


Fig 4. Mitochondrial complexes in brain in groups treated with ginger extract, silymarin and lipoic acid

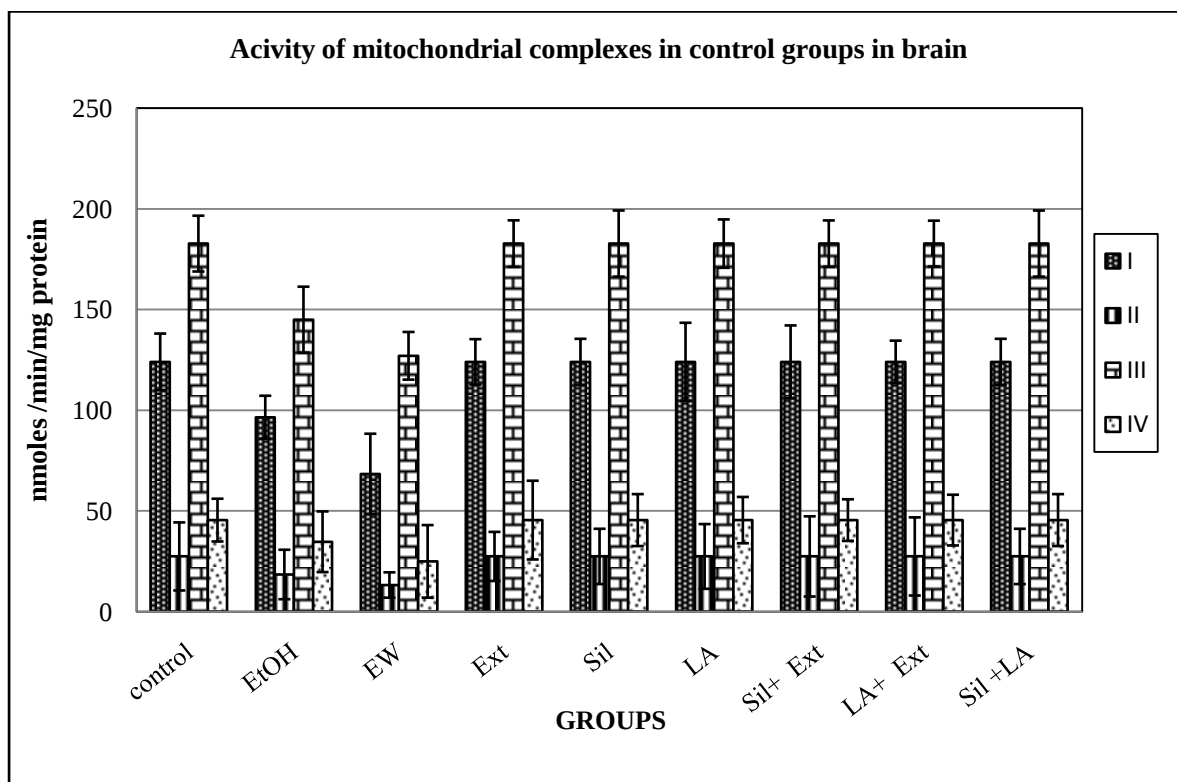


Fig 5. Mitochondrial complexes in brain during ethanol intoxication in groups treated with silymarin, lipoic acid and ginger extract

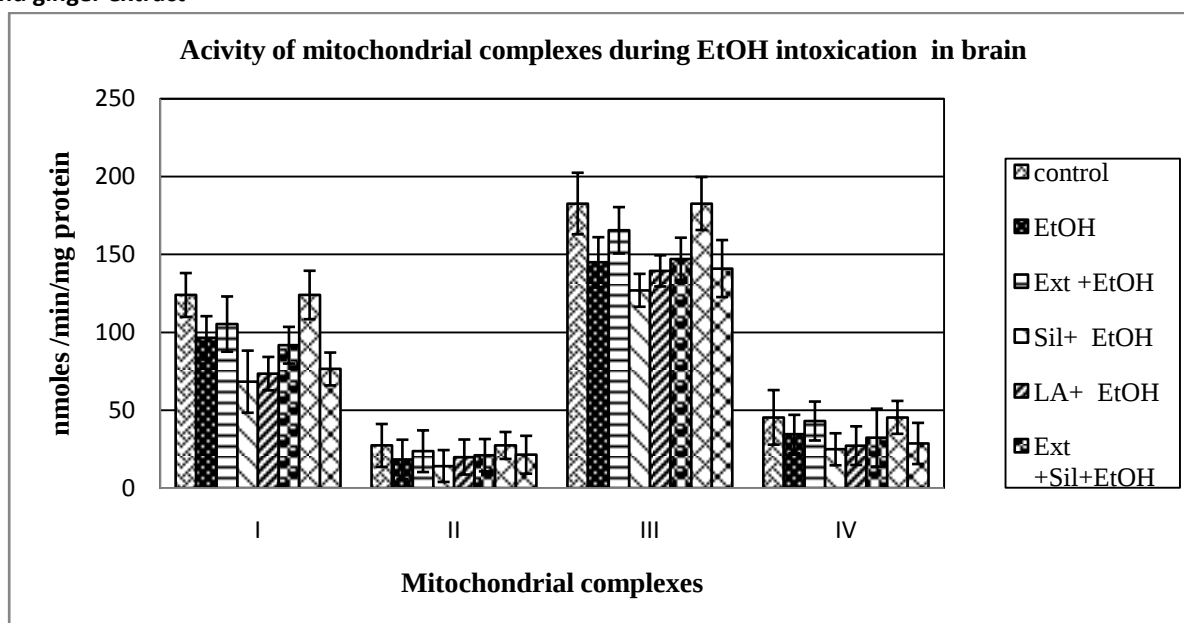


Fig 6. Mitochondrial complexes in brain during ethanol withdrawal in groups treated with silymarin, lipoic acid and ginger extract

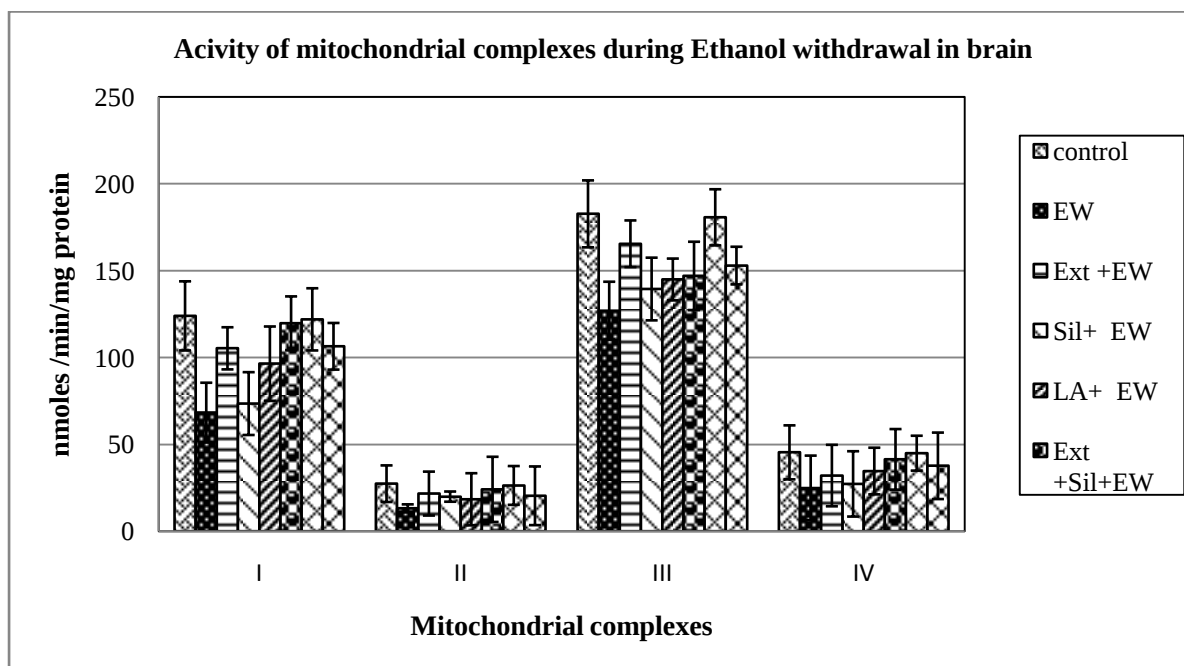


Fig 7. Mitochondrial complexes in Heart in groups treated with ginger extract , silymarin and lipoic acid

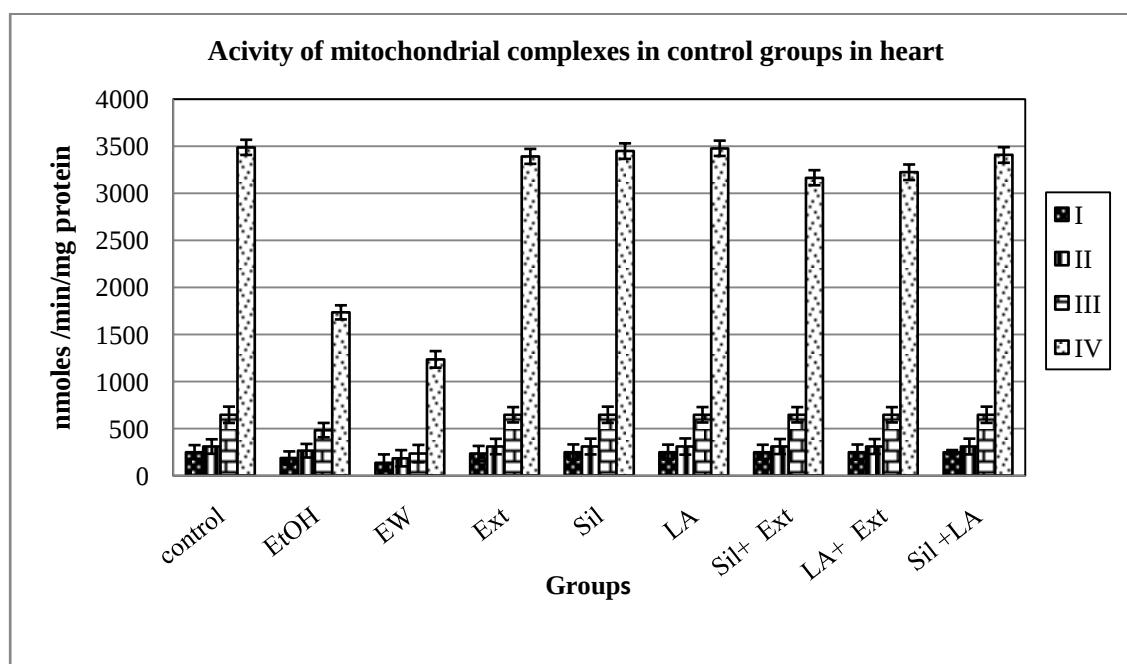


Fig 8. Mitochondrial complexes in Heart during ethanol intoxication in groups treated with silymarin, lipoic acid and ginger extract

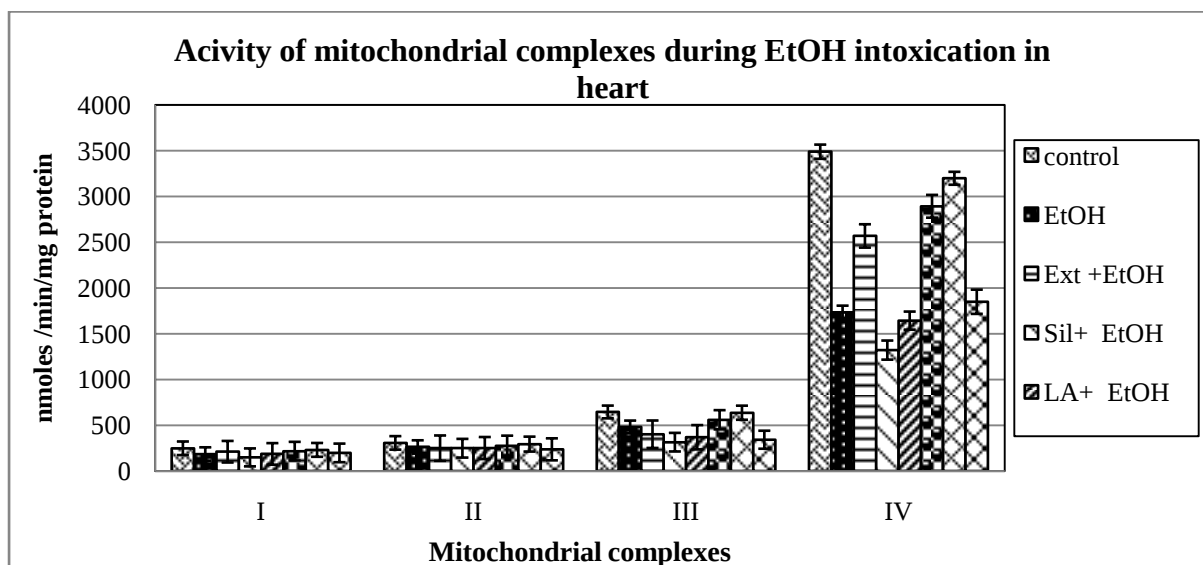


Fig 9. Mitochondrial complexes in Heart during ethanol withdrawal in groups treated with silymarin, lipoic acid and ginger extract

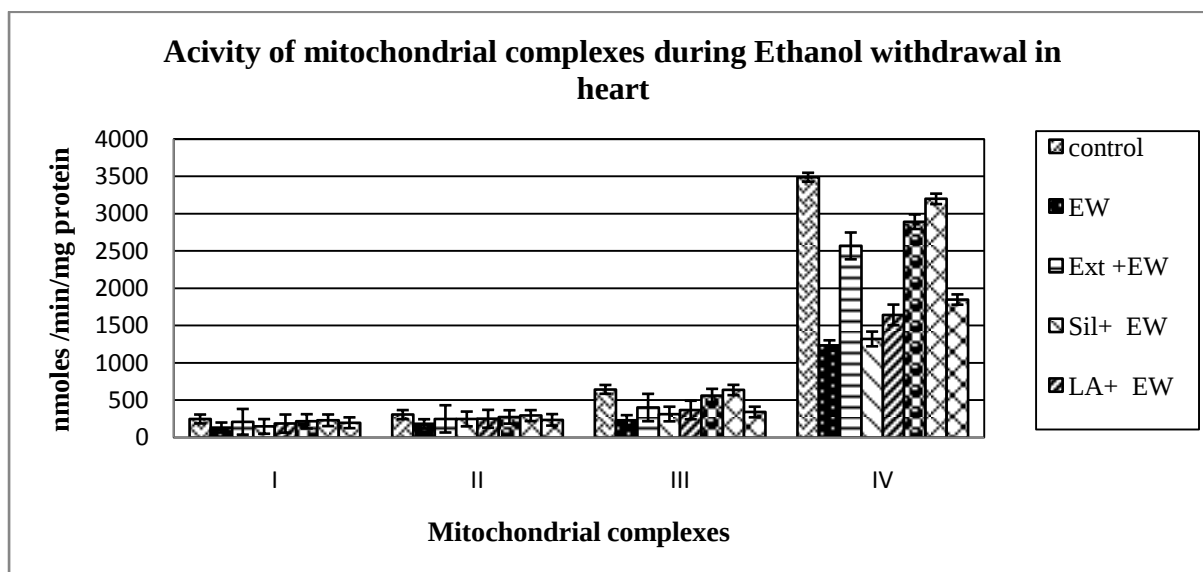


Fig 10. Mitochondrial complexes in kidney in groups treated with ginger extract, silymarin and lipolic acid

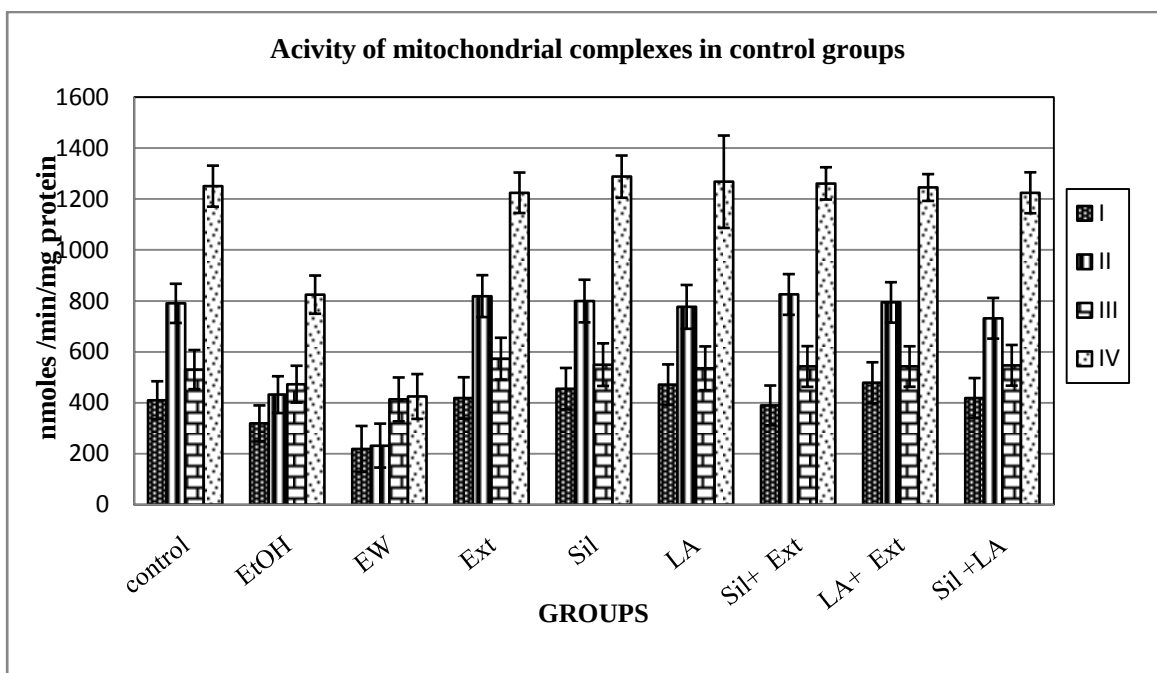


Fig 11. Mitochondrial complexes in kidney during ethanol intoxication in groups treated with silymarin, lipolic acid and ginger extract

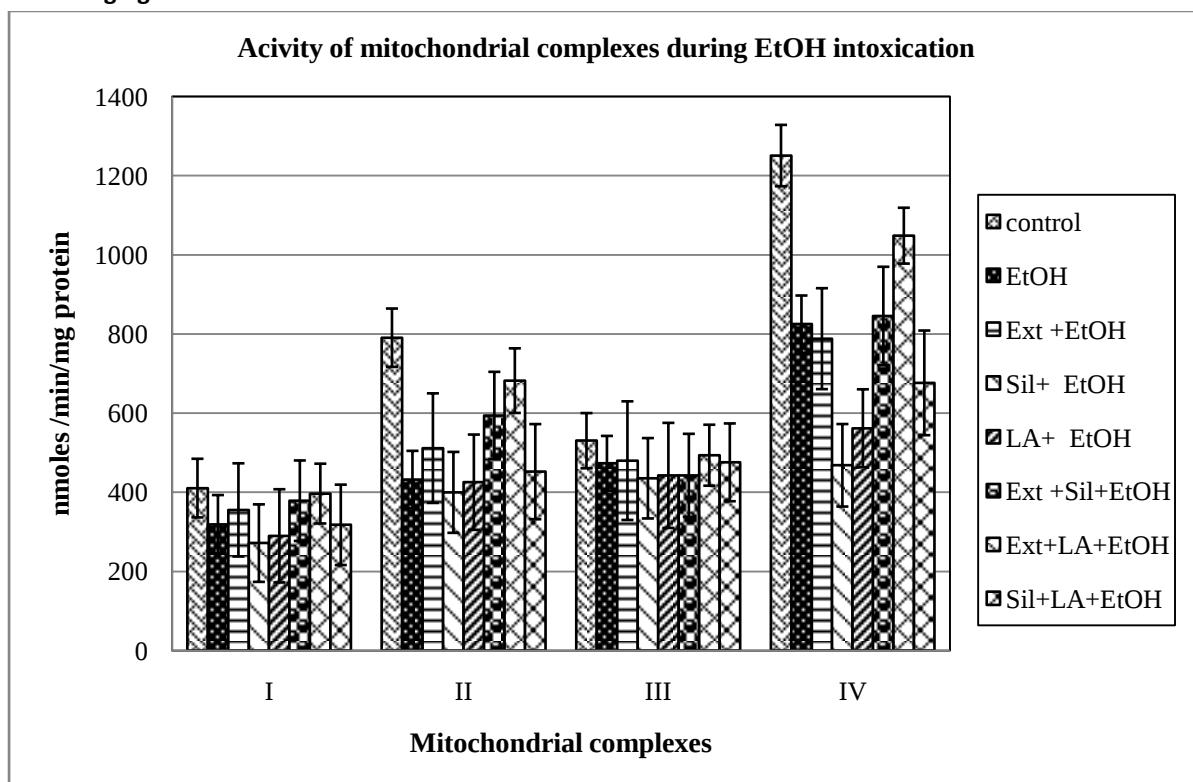
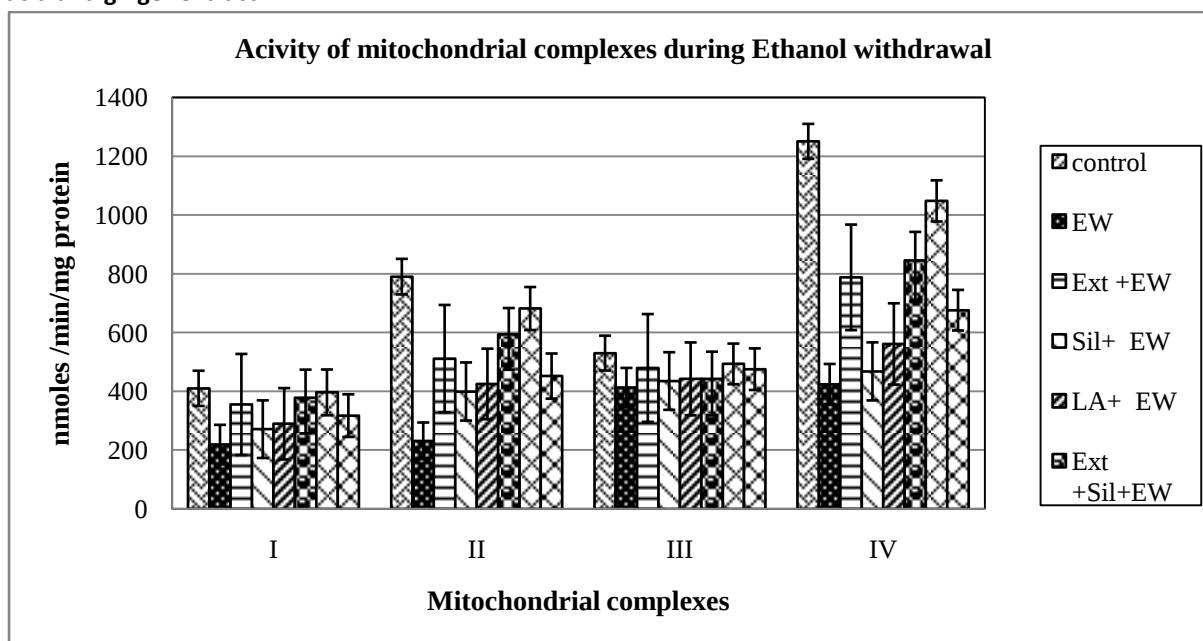


Fig 12. Mitochondrial complexes in Kidney during ethanol withdrawal in groups treated with silymarin, lipoic acid and ginger extract



DISCUSSION

Mitochondria are one of the most important cellular sources of ROS production and are particularly susceptible to oxidative stress (Cadenas and Davies, 2000; Lenaz et al., 2002). Specifically, the mitochondrial respiratory chain represents a major source of ROS production. It has been estimated that during normal cellular metabolism, 1–2% of the electrons which flow into the electron transport chain catalyze the incomplete reduction of O_2 to superoxide radical (Boveris and Chance, 1973). It is well known that the generation of ROS will significantly increase when the function of the electron transport chain is compromised (Cadenas and Davies, 2000; Lenaz et al., 2002). Metabolism of ethanol through alcohol dehydrogenase (ADH) generates cytosolic NADH, which is oxidized indirectly by mitochondrial electron transport depending on hydrogen shuttling mechanisms that involve metabolite carriers in the inner membrane (Sugano et al., 1990). Further oxidation of the resulting acetaldehyde is mediated predominantly by the mitochondrial low K_m aldehyde dehydrogenase, which also generates NADH for oxidation in the mitochondria. Thus, both steps depend on mitochondrial electron transport.

The major sites of ROS formation in the electron transport chain are the NADH dehydrogenase complex and the cytochrome b-c1 complex (Sugano et al., 1993; Bailey et al., 1999). The rate of ROS formation increases with higher Δp and, therefore, it depends on the respiratory conditions, notably on the substrate and O_2 supply and the electron transport rate (Skulachev, 1998). Conditions that increase the supply of mitochondrial NAD(P)H and enhance the reducing pressure on the electron transport chain without increasing the rate of respiration promote the formation of O_2 through the electron transport chain (Boveris and Chance, 1973; Skulachev, 1998). It is not surprising, therefore, that both acute and chronic ethanol treatment enhance mitochondrial ROS formation in liver cells, where most ethanol oxidation takes place (Kurose et al., 1997; Higuchi et al., 2001; Bailey et al., 2001). Also not unexpectedly, the liver responds to a prolonged ethanol exposure by enhancing the rate of O_2 uptake (Israel et al., 1975; Rivera et al., 1998). Several mechanisms may be involved. There is some evidence of an increased respiratory activity, either by enhanced ATP utilization (Israel et al., 1975), or as a result of the induction of an uncoupling protein, such as UCP-2,

which enables electron transport without a corresponding increase in ATP utilization (Rashid et al., 1999; Diehl et al., 1999). However, this is likely to represent only a minor contribution to the adaptation of the tissue to ethanol, and the predominant proportion of increased O_2 uptake in the liver represents the activation of ethanol metabolism through alternative pathways. These include ethanol oxidation by the cytochrome P450 isoform CYP2E1, which is up-regulated by chronic ethanol intake (Ingelman-Sundberg et al., 1994), and by catalase, which can provide a significant ethanol oxidation pathway under conditions in which peroxisomal oxidation of fatty acids provides the necessary H_2O_2 for that reaction (Bradford et al., 1999). Importantly, the increased utilization of O_2 through these pathways competes with mitochondrial electron transport, and this may lead to conditions in which a localized and transient hypoxia develops in the tissue. Chronic ethanol treatment also affects mitochondrial oxidative phosphorylation in the liver by suppressing the synthesis of protein subunits that are encoded on mitochondrial DNA (mtDNA) (Cunningham et al., 1990; Cahill and Cunningham, 2000). These include subunits of the main respiratory complexes, NADH dehydrogenase (Complex I), cytochrome b-c1 (Complex III), and cytochrome oxidase (Complex IV), as well as the ATP synthase complex (Complex V). The lower capacity for oxidative phosphorylation further stresses the balance between the reducing pressure imposed by ethanol and the capacity for oxidative phosphorylation. These are precisely the conditions that would promote mitochondrial O_2 formation.

Acute administration of ethanol (5 g/kg, IP) to mice depleted mitochondrial DNA in heart, liver, and brain (Mansouri et al., 2001). ROS produced during ethanol metabolism altered mitochondrial function (Mansouri et al., 2001; Minana et al., 2002). There is, however, overwhelming evidence that ROS production detected in different cells under pathological conditions has a mitochondrial origin. Alcohol-induced oxidative stress end-organ injury (Haorah et al., 2005, 2007; Cullen and Halliday, 1995) is believed to be the results of mitochondrial damage (Kessova and Cederbaum, 2007; Dey and Cederbaum, 2006; Pastorino et al., 1999; Cahill et al., 2002) and decrease of ATP production (Bailey, Pietsch and Cunningham,

1999). Mitochondrial damage and subsequent oxidative production are known to initiate the development of neurological diseases (Lin and Beal, 2006; Maracchioni et al., 2007). Key factors involved in alcohol-elicited mitochondrial dysfunction may arise from altering the outer and inner mitochondrial membranes that regulate the transport of energy substrates via β -oxidation.

The major sites of ROS formation in the respiratory chain are within respiratory Complexes I and III. Further sites, however, may have importance and physiological relevance. It is worth noting that mitochondria from different tissues may vary conspicuously in their capacity to produce ROS using different substrates (Kwong and Sohal, 1998), and this capacity is also related to animal species and age. Studies confirmed that Complex I is a major source of superoxide production in several types of mitochondria. The superoxide production by Complex I is higher during the reverse electron transport from succinate to NAD^+ (Korshunov et al., 1997; Turrens, 2003; Jezek and Hlavata, 2005; Takeshige and Minakami, 1979). There is ample evidence that often ROS production is higher when electrons are channelled through Complex II than through Complex I, while in both cases reaching Complex III, would be in line with the idea that Complex II may be a source of ROS (McLennan and Degli Esposti, 2000; Ishii et al., 1998; Zhang et al., 1998). Complex III (ubiquinone-cytochrome c reductase) represents a confluence point for reducing equivalents from various dehydrogenases: it catalyses the transfer of electrons from ubiquinol ($CoQH_2$) to cytochrome c. The formation of superoxide in Complex III depends on the mechanism of electron transfer, called the Q-cycle (Crofts, 2004).

The present findings revealed the impairment of mitochondrial membrane and the disruption of complex I and V in alcohol abuse and during ethanol withdrawal. Alteration of respiratory complex was indicated by the reduction in mitochondrial complex I and V. There is increasing evidence that pathological states in which Complex I activity is impaired also lead to ROS overproduction (Fato et al., 2008); in cell lines from patients with Complex I deficiency, an inverse

relationship was found between superoxide production and residual enzyme activity (Verkaart et al., 2007). The mechanism of the decrease in oxidative phosphorylation can be related to the reduction in the enzyme activities of the different respiratory chain complexes of the mitochondrial inner membrane (Balaban, 1990; Brown, 1992). In the present study, we have observed a collective decrease in all enzyme complexes. Normalization of enzyme activities of the respiratory chain complexes by ginger extract administration yields no changes during ethanol withdrawal.

Silymarin is most well known for its antioxidant and chemoprotectant effects on the liver (Post-White et al., 2007). Beside liver cells, many other cells have proven to be sensitive to the protective action against toxic agents, including kidney cells, cardiac myositis, immune system cells, neurons, endothelial cells, (Comelli et al., 2007). A single dose of alpha-lipoic acid (100 mg/kg i.p.) resulted in improvement in mitochondrial function, determined by mitochondrial oxygen consumption and complex I, II, and IV activities, in endotoxemic rats (Vanasco et al., 2008). Mitochondrial membrane potential is another indicator of mitochondrial health as it reflects the metabolic activity and integrity of mitochondrial membrane (Distelmaier et al., 2007). Alpha-lipoic acid supplementation (0.5% w/w) improved the average mitochondrial membrane potential in old rats hepatocytes to that of young rats (Hagen et al., 1999).

Even though alpha-lipoic acid has been shown to be effective in improving mitochondrial function and decreasing mitochondrial superoxide in rats (Hagen et al., 1999; El Midaoui et al., 2003), its effect is not entirely specific to mitochondria. The antioxidant effect of alpha-lipoic acid, which is mediated by both the original alpha-lipoic acid compound and its metabolite dihydrolipoic acid, a more potent antioxidant, acts via a number of mechanisms such as metal chelation and direct ROS scavenging (Biewenga et al., 1997). Dihydrolipoic acid can regenerate antioxidants such as glutathione and vitamin C (Biewenga et al., 1997). Dietary supplementation also increases unbound lipoic acid, which can act as a potent antioxidant and ameliorate oxidative stress

both *in vitro* and *in vivo* (Suzuki, et al., 1991; Scott, et al., 1994; Han, et al., 1997; Xu et al., 1996; Lykkesfeldt et al., 1998; Kagen et al., 1992).

In this context, the administration of nutraceuticals is found capable of modulating mitochondrial oxidative alterations and improved most of the investigated stress parameters in the mitochondria of liver, heart, brain and kidney.

CONCLUSION

The results of this work indicated that the combination of ginger and Lipoic acid had protective effects against mitochondrial toxicity in rats better than each of these compounds alone in all the tissue investigated. Silymarin is also found to exhibit mitochondrial protection but as not as efficient as alpha- lipoic acid and ginger combination. These finding might provide a basis for the development of novel therapeutic strategies for protection against mitochondrial damage caused by ethanol administration and the subsequent organ damage.

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Conflict of interest

There is no conflict of interest

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