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RESEARCH ARTICLE

STUDY OF THE NEUROPROTECTIVE EFFECT OF ALPHA LIPOIC ACID ON ACRYLONITRILE INDUCED CELLULAR & MITOCHONDRIAL DYSFUNCTION BY ENHANCEMENT OF OXIDATIVE STRESS IN RAT HIPPOCAMPUS MAPPING.

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Abstract

Acrylonitrile, one of the nitrile derivatives causes neurotoxicity. It has been suggested that mechanism involved can be generation of free radicals and mitochondria play an important role in this process. It has been reported that ALA (Alpha lipoic acid) by the virtue of its antioxidant nature may prevent free radical induced neurotoxicity. In this work, we investigated the effects of Acrylonitrile (50mg/kg body weight) and Alpha lipoic acid (100mg/kg body weight) on hippocampal cellular and mitochondrial enzyme activities such as SOD (superoxide dismutase), GPx (glutathione peroxidase), CAT (catalase) and GSH (reduced glutathione) and MnSOD (manganese Superoxide dismutase), GPx, GST (glutathione-s-transferase) and GSH respectively. Moreover, we also studied the damage on mitochondrial membrane by measuring MDA (malondialdehyde) level. The activities of TCA cycle enzymes such as ICDH (isocitrate dehydrogenase), SDH (succinate dehydrogenase), α-KDH (Alpha ketoglutarate dehydrogenase) and MDH (malate dehydrogenase) were significantly reduced after Acrylonitrile administration. Subsequent treatment with ALA significantly alleviated the depletion in the level of these enzymes. We measured the activities of ETC enzymes NADH dehydrogenase and Cytochrome C oxidase. Acrylonitrile administration significantly down regulated the activities of these enzymes whereas ALA restored the levels of these enzymes. In conclusion, our data indicate that ALA effectively protects the hippocampal cellular and mitochondrial damage provoked by Acrylonitrile.

Introduction:-

Apart from all tissues in the body brain regions are more susceptible to oxidative stress. Neuronal cells meet huge oxygen demand due to their high-energy expenditure, which particularly constitutes a major site in oxidative damage (Ozdemir*et al*., 2005). Generation of free radicals disrupt the brain redox status that leads to oxidation of membrane lipids, nucleic acids and proteins which lead to degradation of neuronal cells (Rajamani*et al*., 2007).

The mitochondria are involved in diverse processes that modulate cell operation such as cell cycle regulation and apoptosis. Mitochondrial dysfunctions play crucial roles in many neurodegenerative disorders and neuronal damage.

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The main source of ROS in hippocampal mitochondria is the impairment of oxidative phosphorylation in ATP production. The superoxide radical is generated by the transfer of electron between the enzyme complexes of electron transport chain (Sas*et al*., 2007). Mitochondrial antioxidant defense mechanisms counteracts these reactive species, but are exhausted when there is inordinate production of free radicals leading to disruption of mitochondrial membrane (Trushina*et al*., 2007). Free radicals culminating to membrane transition permeability pore (MTPP) results in insult of various mitochondrial enzymes (Michael *et al*., 2007).

Previous documentations finger towards nitriles causing cellular toxicity (El-Sayed *et al*., 2008) and mitochondrial dysfunction that pushes the cell towards apoptosis by the release of cytochrome c and activation of several apoptotic factors (Sas*et al*., 2007). Acrylonitrile is one of those neurotoxicants that play a key role in the pathophysiology of neuronal damage. Previous reports stated that it caused severe profligation to neuronal cells by enormous generation of oxidative free radicals (Tariq *et al*., 2002). Ample literatures suggest that natural compounds with scavenging properties prevent the tissue from the attack of oxidative free radicals (Celik*et al*., 2002) generated by Acrylonitrile.

Antioxidants play a vital role in affecting various neurodegenerative disorders by quenching reactive free radicals (Preston *et al*., 2001). ALA is a potent antioxidant utilized in prevention and cure of various neuronal diseases as proved by previous reports (Garcia-Estrada *et al*., 2003). Mounting evidence shows that ALA acts as a cofactor of enzymes involved in the oxidative phosphorylation in production of ATP in mitochondria. As documented by previous study ALA reprocesses endogenous antioxidants and hence quenches the free radicals due to its dual effect. Therefore records of earlier investigations revealed the exceptional quality of alpha lipoic acid in mitigating healing effects on free radical induced cellular (Poon *et al*., 2005) as well as mitochondrial damage (Savitha*et al*., 2006). The goal of our study is to gain insight into the Acrylonitrile induced oxidative damage in cytosol and mitochondria of hippocampal region that is counteracted by antioxidant effect of ALA.

Materials and methods:-

Drugs and chemicals:

Acrylonitrile and ALA were purchased from Sigma Aldrich Chemical Company (Bangalore, India) and Hi-Media Lab (Nasik, India) respectively. The remaining chemicals were of highest purity and analytical grade.

Animals:-

The study was performed on male albino rats of Wistar strain (average weight 150-180 g) obtained from Experimental Animal Care Centre, Kharvel Subharti College of Pharmacy, Subharti University, Meerut, U.P, 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 controlled condition of temperature (25±20 °C) and were acclimatized 12 ± 1 hr. day and night rhythm during the experimental period. They were given 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 hrs. but allowed free access to water throughout. The experiment was conducted according to strict guidelines of the committee.

Experimental Protocol:-

The experimental animals were randomized into four groups of six rats each as follows:
Group 1: Control rats received normal saline (2ml/kg body weight) for 7 days.
Group 2: Rats received Acrylonitrile (50mg/kg body weight) dissolved in saline and administered intraperitoneally for 7 days.
Group 3: Rats received Alpha lipoic acid (100mg/kg body weight) alone orally for 7 days.
Group 4: Rats received Alpha lipoic acid (100mg/kg body weight) dissolved in saline and administered by oral gavage once daily 30 minutes before Acrylonitrile (50mg/kg body weight) for 7 days.

After the 7 days of experimental period (i.e., on the 8^{th} day), all the animals were anaesthetized and decapitated. Brain tissues were immediately excised and rinsed in ice-cold physiological saline. The hippocampus region was isolated and homogenized in 0.01 M Tris – HCL buffer (pH 7.4) and aliquots of this homogenate were used for the assays. Blood was collected and serum was separated for analysis of biochemical parameters.

Biochemical Estimations:-

Estimation of Antioxidant Enzymes in Cytosol:-

Superoxide dismutase was assayed following the method of Misra and Fridovich*et al.* (1972). Absorbance was measured at 480nm in a Shimadzu UV spectrophotometer. The enzyme activity was expressed as units/min/100 mg protein. One unit of SOD activity is the amount of protein required to give 50% inhibition of epinephrine auto oxidation. The method of Beers *et al.* (1952) was used to assess the activity of Catalase. The method of Rotruck*et al.* (1973) was used to estimate the activity of glutathione peroxidase. The enzyme activity was expressed as nmoles of glutathione oxidised/min/mg protein. The level of total reduced glutathione in the brain tissue was measured by the method of Moron *et al.* (1979).

Mitochondrial Studies:-

Isolation of brain mitochondria:-

The mitochondria of brain were isolated by the method of Johnson and Lardy *et al.* (1967). 10% (w/v) homogenate was prepared in 0.05 M Tris-HCl buffer containing 0.25 M sucrose and centrifuged at 600 ×g for 10 minutes. The supernatant fraction was decanted and centrifuged at 15,000 ×g for 5 minutes. The resultant mitochondrial pellet was then washed and resuspended in the same buffer.

Determination of mitochondrial antioxidant enzymes:-

The mitochondrial superoxide dismutase activity was assayed by the method of Misra and Fridovich*et al.* (1972). The mitochondrial glutathione peroxidase activity was assayed by the method of Rotruck*et al.* (1973). The activity of glutathione-s-transferase was assayed by the method of Habig*et al.* (1974). The reduced glutathione in brain mitochondria was determined according to the method of Moron *et al.* (1979).

Determination of mitochondrial lipid peroxides:-

The brain mitochondrial lipid peroxide content was determined by the thiobarbituric acid (TBA) reaction described by Ohkawa*et al.* (1979).

Determination of TCA cycle enzymes:-

The activity of Isocitrate dehydrogenase was assayed by the method of King *et al.* (1965). The activity of α-ketoglutarate dehydrogenase was assayed by the method of Reed and Mukherjee *et al.* (1969). The activity of succinate dehydrogenase was assayed according to the method of Slater and Bonner *et al.* (1952). The activity of malate dehydrogenase was assayed by the method of Mehler*et al.* (1948). The substrate used was oxaloacetate and determination of enzyme activity was carried out by measuring the rate of oxidation of NADH.

Determination of enzyme complexes of electron transport chain:-

The activity of NADH dehydrogenase was assayed according to the method of Minakami*et al.* (1962). Cytochrome c oxidase activity was assayed by the method of Pearl *et al.* (1963).

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.

Results:-

We observed the effects of Acrylonitrile and ALA on cellular antioxidants SOD, CAT and GPx (Table 1). The results depicted that the activities of enzymes were significantly ($p < 0.05$) decreased in hippocampus of Acrylonitrile induced rats (group 2) when compared to control rats (group 1). When ALA was co-treated with Acrylonitrile in (group 4) rats, significant amelioration in the levels of enzymes was observed. Rats receiving ALA alone (group 3) did not show any significant difference when compared to control rats (group 1).

The activities of mitochondrial antioxidant enzymes MnSOD, GPx and GST were significantly ($p < 0.05$) lower in the mitochondria of hippocampus of Acrylonitrile induced rats (group 2) as compared to that of control rats (group 1). Co-treatment of rats with ALA + Acrylonitrile (group 4) significantly reverses all these Acrylonitrile induced alterations in the activities of mitochondrial antioxidants (Table 2). The rats receiving ALA alone (group 3) did not show any significant change when compared with control rats indicating that it does not produce oxidative stress.

Table 3 depicts the level of mitochondrial TCA cycle enzymes ICDH, KDH, SDH and MDH that were significantly reduced in mitochondria of hippocampus in Acrylonitrile induced rats (group 2) when compared to control rats (group1). These enzyme levels were reversed in ALA+ Acrylonitrile co treated rats (group 4) which shows alteration in levels when compared to the Acrylonitrile induced rats (group2). The rats receiving ALA alone (group 3) did not show any significant change when compared to control rats (group1) indicating that it does not have any adverse effects.

Graph 1 exhibits the level of cellular GSH in rats. Level of Acrylonitrile induced (group 2) rats show depleted level of enzyme as compared to control rats (group 1). Whereas ALA+ Acrylonitrile co-treated (group 4) rats reversed the levels bringing it to normal status when compared to Acrylonitrile induced (group 2) rats. The rats receiving ALA alone did not show any significant change (group 3) when compared to control rats (group 1).

Graph 2 shows the levels of MDA were significantly ($p < 0.05$) increased mitochondrial hippocampus of Acrylonitrile induced rats (group 2) when compared to control rats (group 1). The MDA level is reversed by the ALA + Acrylonitrile treated rats (group 4) which shows the alteration in the level of when compared to Acrylonitrile induced rats (group 2). The rats receiving ALA alone (group 3) did not show any significant change when compared with control rats indicating that it does not have any adverse effect (Graph 1).

Graph 3 exhibits the level of mitochondrial GSH that was significantly reduced in Acrylonitrile induced rats (group 2). The mitochondrial GSH level is reversed by ALA+ Acrylonitrile co treated rats (group 4). The rats receiving ALA alone did not show any significant change (group 3) when compared to control rats (group 1) indicating that it does not have any toxic effect upto this concentration (Graph 2).

Graph 4 and Graph 5 state the levels of respiratory marker enzymes NADH dehydrogenase and cytochrome c oxidase respectively, the levels were significantly increased in mitochondria of hippocampus tissue in Acrylonitrile induced rats (group 2) when compared to control rats (group 1). The levels of these enzymes are reversed by ALA+ Acrylonitrile co treated rats (group 4). The rats receiving ALA alone (group 3) did not show any significant change when compared to control rats (group 1).

Discussion:-

Nitrile derivatives are extensively acquired in day-to-day life, among which Acrylonitrile exhibits behavioral syndrome in rodents, which is depicted by hyperactivity and repetitive head movements. Several preceding explorations revealed that Acrylonitrile and its metabolite (i.e. N-hydroxyl Acrylonitrile) exposure augmented synthesis of reactive species thereby culminating in oxidative stress (Kamenduli*set al.*, 1999). Massive free radical generation evoked cellular and sub-cellular damage as culminated by previous literatures (Sagara*et al*., 2002; Sas*et al*., 2007). ALA is a potent free radical scavenger, which is having the potential to cross blood brain barrier, due to this property, it recovers the neuronal cells from free radical damage (Arivazhagan*et al*., 2002). Our present investigation is rationalized on the effect of ALA on Acrylonitrile induced hippocampal oxidative damage on cellular and subcellular level, owing to its antioxidant effect and its profound ability to regenerate cellular and mitochondrial antioxidants.

To combat the oxidative free radicals cells have their own antioxidant defense system comprising of enzymic antioxidants, SOD (superoxide dismutase) which carries out the process of conversion of highly reactive superoxide to hydrogen peroxide, hydrogen peroxide in turn is converted to water and oxygen by the action of CAT (catalase). GPx (glutathione peroxidase) is an enzymic antioxidant, which quenches hydrogen peroxide in the presence of GSH (reduced glutathione). GSH is a cellular non-enzymic antioxidant, which apart from quenching free radicals maintains the redox status of the cell (Nita *et al*., 2001; Zhang *et al*., 2006).

Previous studies depicted that oxidative stress due to nitrile intoxication caused marked reduction in the activity of antioxidants SOD, CAT, GPx and GSH (Unnisa*et al*., 2005). In our study, Acrylonitrile being nitrile caused neurotoxicity at the cellular level as was demonstrated by the depleted activity of SOD, CAT, GPx and GSH. Mounting reports have already stated that ALA significantly restored the level of enzymes (Shanmugarajan*et al*., 2008) which is in harmony with our present study. For the further confirmation of Acrylonitrile provoked oxidative hippocampal damage, we laid emphasis on its damaging effects on cellular and its mitochondrial level.

Earlier reports revealed that nitriles initiate lipid peroxidation (LPO) by generating free radicals, which cause deleterious effect on mitochondrial membrane (both inner and outer) thereby leading to distortion and loss of membrane integrity (Kristal *et al*., 1997). Mitochondrial membrane repercussions provoked by generation of lipid peroxyl radicals ($LOO^{\cdot}$) give rise to degradation product MDA (malondialdehyde) (El-Sayed *et al*., 2008), which can be applied to assess lipid peroxidation in hippocampal mitochondria. Acrylonitrile induced rats show increased levels of MDA due to disruption of hippocampal mitochondrial membrane. As assured by indigenous studies, ALA prevents mitochondrial lipid bilayer from oxidative free radical attack (Savitha*et al*., 2006). In our study ALA treatment demonstrated decreased levels of MDA due to its free radical quenching effect and membrane stabilizing effect.

To combat the mitochondrial membrane alteration due to oxidative stress, cells possess inherent antioxidant defense system (McMurray *et al*., 2007). GSH is an important constituent of antioxidant defense system, ubiquitous in all brain cells hence it was intriguing to suss-out that intracellular reduced glutathione (GSH) pools in brain cells were exhausted by reactive oxygen species (Vali*et al*., 2007). Much evidence has been conglomerated to state that mitochondrial GSH undergoes oxidation of its thiol (-SH) group due to nitrile intoxication which eventually causes down regulation of mitochondria (Prabhakaran*et al*., 2006) therefore Acrylonitrile being a nitrile had potential to deplete the level and activity of GSH. As scrutinized earlier ALA engrosses in the uptake of cysteine therefore involves in alterations in levels of GSH (Arivazhagan*et al*., 2002). Therefore, our study revealed that ALA co-treatment regained the activity of mitochondrial GSH. Mitochondrial antioxidant manganese Superoxide dismutase (MnSOD) is a metalloenzyme, which quenches superoxide radicals by converting to molecular oxygen and hydrogen peroxide. Glutathione peroxidase (GPx) eliminates the hydrogen peroxide by converting to H_2O as reported by previous documents (Moreira *et al*., 2007). Nitriles show attenuation in the levels of mitochondrial antioxidants (MnSOD, GPx and GST) by generating enormous free radicals as reported by literatures (El-Sayed *et al*., 2008). Acrylonitrile induction depicted lower levels of hippocampal mitochondrial antioxidant enzymes such as MnSOD, GPx and GST. In our experiment the activity of hippocampal mitochondrial antioxidant enzymes (MnSOD, GPx and GST) were found to be increased upon treatment with ALA.

Earlier documents have stated that excessive free radical load hampers various complexes of ETC (electron transport chain). NADH-dehydrogenase (flavin-linked) is complex I in ETC, it is responsible for removing electrons from NADH and transferring them to ubiquinone (Q) for ATP production (Preston *et al*., 2001). Cytochrome-c-oxidase is complex IV in ETC; it carries out the transfer of electron to molecular oxygen thus producing water (Sas*et al*., 2007). Free radicals deplete activity of GSH which decrease production of reducing equivalents NADH and NADPH that causes marked attenuation in activities of NADH-dehydrogenase and cytochrome-c-oxidase (Mythili*et al*., 2005; Vali*et al*., 2007). Cytochrome c oxidase was previously documented to be oppressed by nitrile causing generation of more free radicals therefore Acrylonitrile being a nitrile had sufficient capacity to generate enormous free radicals (Kamenduli*set al*., 1999) leading to depletion in activity of cytochrome c oxidase. We observed the functional loss in the activity of NADH-dehydrogenase in Acrylonitrile treatment due to enormous free radical generation. Previous documented evidence stated that ALA effectively increased the level of NADH dehydrogenase and cytochrome-c-oxidase (Arivazhagan*et al*., 2001). In our study, administration of ALA ameliorated the levels of these complexes.

H_2O_2 as reported previously (Tretter et al., 2005) directly inhibits αlpha ketoglutarate dehydrogenase (α-KDH) and succinate dehydrogenase (SDH). Superoxide reacts with nitric oxide radical to form peroxynitrite (Sas*et al*., 2007) which inactivates α-KDH. SDH is also complex II in ETC of mitochondria and its activity gets hampered when its thiol groups are oxidized due to excessive free radicals. Free radicals damage the enzyme isocitrate dehydrogenase (ICDH) which is unable to produce NADPH, thereby leading to decreased production of GSH (Mythili*et al*., 2005). Malate dehydrogenase (MDH) gets inactivated by being susceptible to modification and degradation by oxidative free radicals (Sharma *et al*., 2007). In our present experimentation, we noticed that Acrylonitrile via free radical production inactivates and depletes TCA cycle enzymes like α-KDH, SDH, ICDH and MDH. Substantial amount of evidence proves that ALA preserves and protects the activity and levels of these enzymes. Culminating evidences reported earlier that ALA is involved in decarboxylation reactions by acting as coenzyme for various dehydrogenase complexes in mitochondria (Sharma *et al*., 2007; Arivazhagan*et al*., 2001). In our study, we noticed that alpha lipoic acid on contrary increased the abated levels of alpha ketoglutarate dehydrogenase (α-KDH), succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH) and malate dehydrogenase (MDH).

Concluding, together with the previous reports, the data bespeak that ALA exerts a potent antioxidant action on Acrylonitrile induced cellular and mitochondrial hippocampal damage, so it may have potential consumption in neurotoxic disorders caused by Acrylonitrile by having a casual access to all brain cells.

Table 1:- Effect of Acrylonitrile and Alpha lipoic acid on the activities of cellular enzymic antioxidants in hippocampus of control and experimental rats.

Groups	Control (Group 1)	Acrylonitrile Induced (Group 2)	ALA Alone (Group 3)	ALA+ Acrylonitrile (Group 4)
SOD	7.81 ±0.10	4.52 ±0.02[a],*	7.75 ±0.03 [NS]	6.72 ±0.04 [b],*
CAT	3.37 ±0.03	1.95 ±0.02 [a],*	3.28 ±0.05 [NS]	3.13 ±0.02 [b],*
GPx	4.49 ±0.04	2.63 ±0.02 [a],*	4.46 ±0.04[NS]	4.22 ±0.03 [b],*

SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; NS, non-significant.
Results are expressed as mean ±S.D. for six rats. Units: SOD, min/100mg/protein; CAT, µmoles H_2O_2 consumed min/mg/protein; GPx, nmoles of glutathione oxidized/min/mg/protein.
Comparisons are made between the following:
[a]Group I and Group II; [b]Group II and Group IV;
[NS]Group I and Group III, *Statistically significant ($p < 0.05$);

Table 2:- Effect of Acrylonitrile and Alpha lipoic acid on the activities of mitochondrial enzymic antioxidants in hippocampus of control and experimental rats.

Groups	Control (Group 1)	Acrylonitrile Induced (Group 2)	ALA Alone (Group 3)	ALA+ Acrylonitrile (Group 4)
MnSOD	21.91 ± 0.54	12.42 ± 0.65 [a],*	22.28 ± 0.64 [NS]	18.77 ± 0.46 [b],*
GPx	59.35 ±1.08	30.12 ± 1.58 [a],*	60.56 ± 0.97 [NS]	44.95 ± 1.56 [b],*
GST	36.61 ± 0.45	15.17 ± 0.63 [a],*	37.61 ± 1.2 [NS]	27 ± 0.46 [b],*

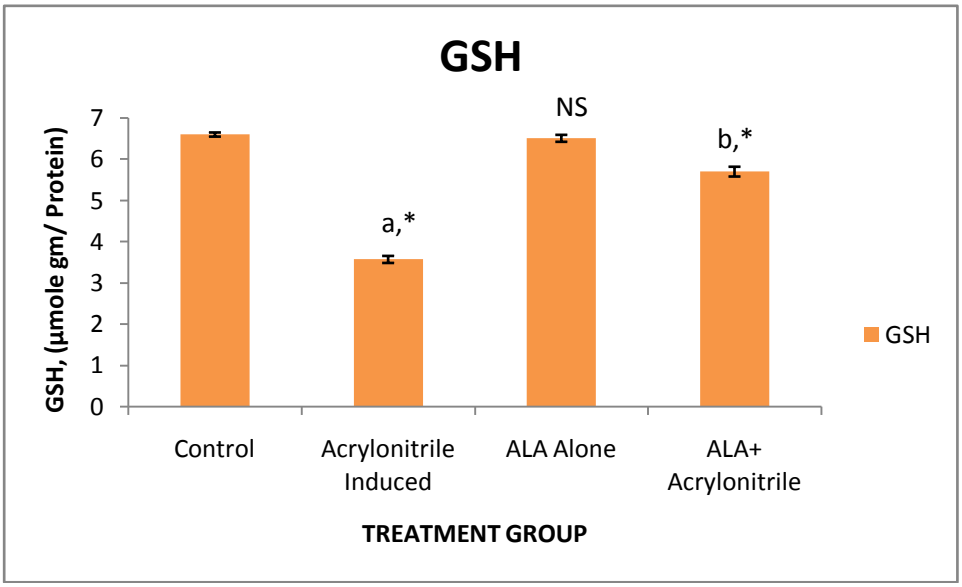
MnSOD, manganese Superoxide dismutase; GPx, glutathione peroxidase; GST, glutathione-s-transferase; NS-non significant.
Results are expressed as mean ±S.D. for six rats. Units: MnSOD, min/100mg Protein; GPx, min/mg Protein; GST, n moles of CDNB conjugated/min/mg protein. Comparisons are made between the following:
[a]Group I and Group II; [b]Group II and Group IV;
[NS]Group I and Group III, *Statistically significant ($p < 0.05$);

Table 3:- Effect of Acrylonitrile and Alpha lipoic acid on the activities of mitochondrial TCA cycle enzymes in hippocampus of control and experimental rats.

Groups	Control (Group 1)	Acrylonitrile Induced (Group 2)	ALA Alone (Group 3)	ALA+ Acrylonitrile (Group 4)
SDH	43.57 ±2.35	12.66 ±4.55 [a],*	43.95 ±1.47 [NS]	26.25 ±1.35 [b],*
MDH	875.85 ±32.65	553.43 ±26.38 [a],*	870.05 ±31.42 [NS]	711.59 ±26.26 [b],*
KGDH	54.28 ±3.97	23.43 ±1.83 [a],*	52.84 ±2.73 [NS]	38.66 ±1.53 [b],*
ICDH	155.57 ±3.88	99.41 ±6.25 [a],*	160.64 ±5.58 [NS]	134.02 ±3.57 [b],*

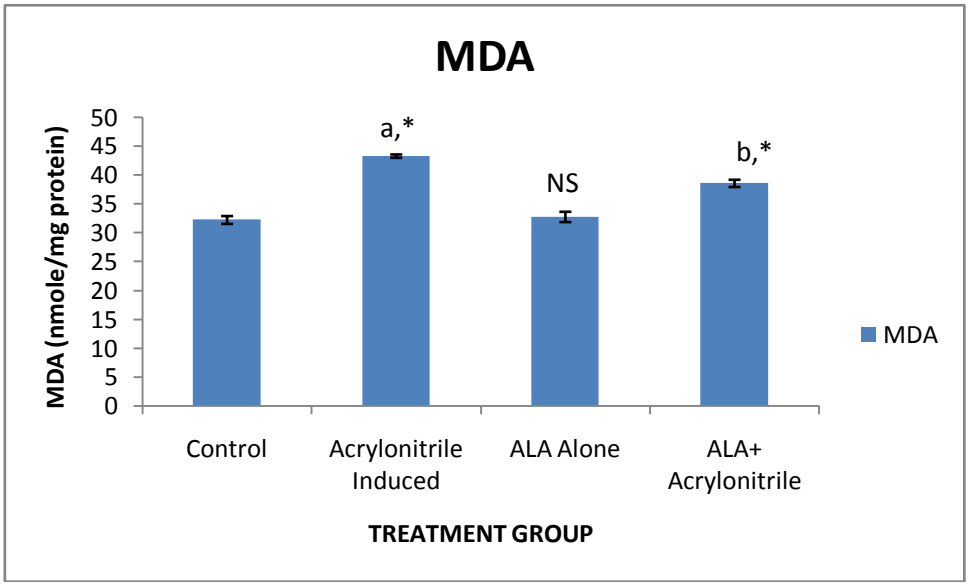
ICDH, isocitrate dehydrogenase; SDH, succinate dehydrogenase; α-KDH, Alpha ketoglutarate dehydrogenase; MDH, malate dehydrogenase; NS, non-significant

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



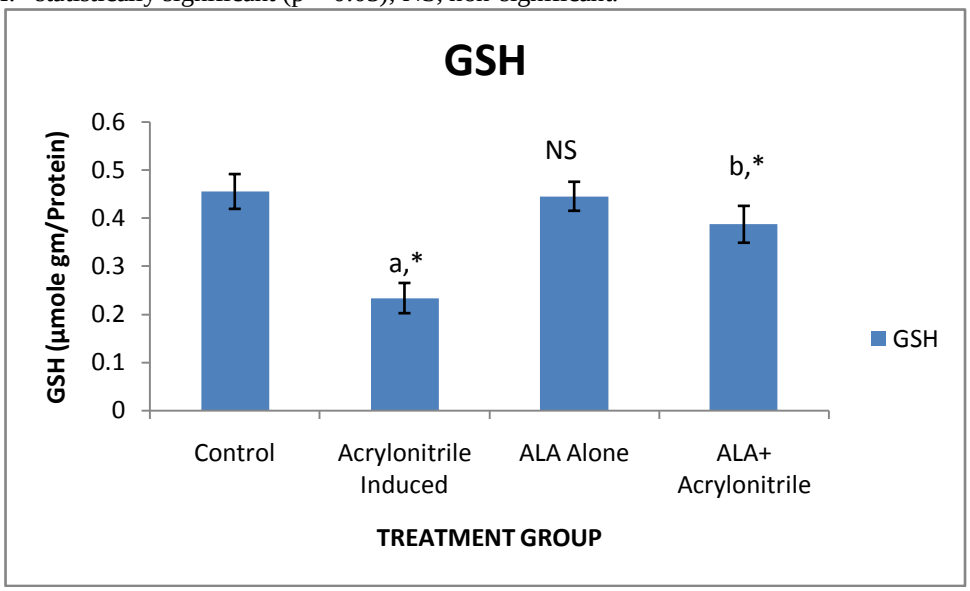
Graph 1:- Levels of cellular GSH (Glutathione) in hippocampus of the experimental rats. Results are given as mean ±S.D. for six rats.

Comparisons are made between the following: a. Group I and Group II; b. Group II and Group IV; NS. Group I and Group III. *statistically significant ($p < 0.05$); NS, non-significant.



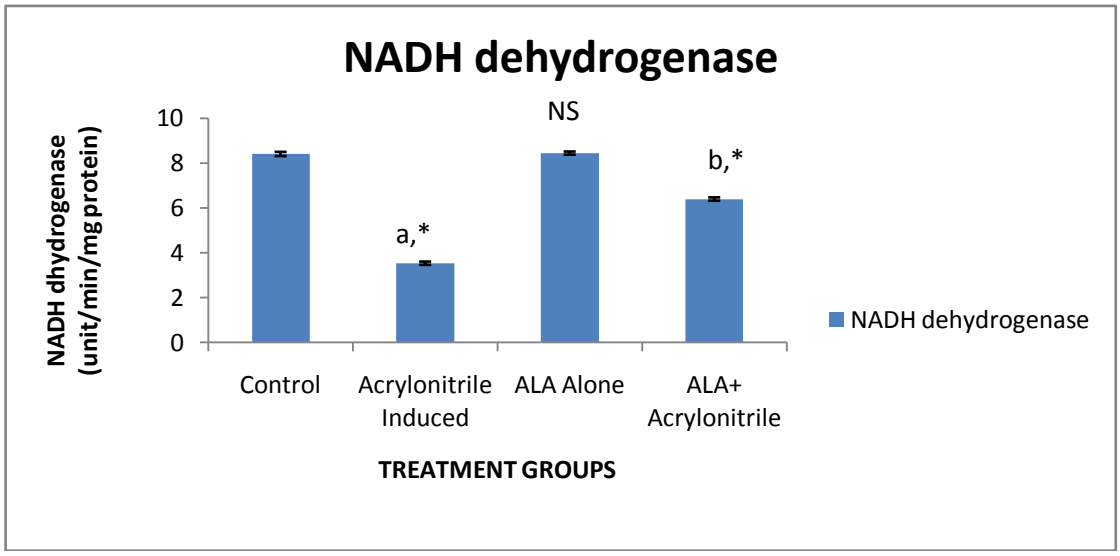
Graph 2:- Levels of MDA (malondialdehyde) in the mitochondrial hippocampus of the experimental rats. Results are given as mean ±S.D. for 6 rats.

Comparisons are made between the following: a. Group I and Group II; b. Group II and Group IV; NS. Group I and Group III. *statistically significant ($p < 0.05$); NS, non-significant.



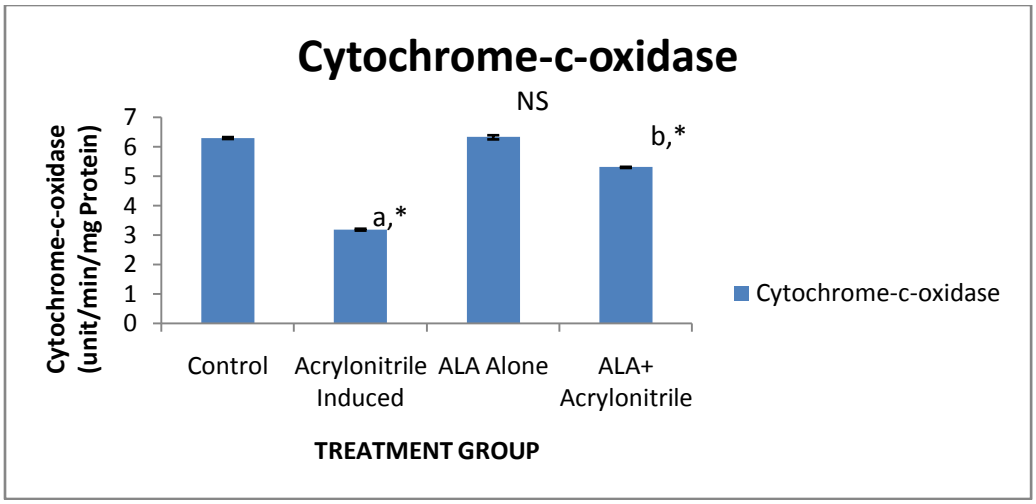
Graph 3:- Levels of GSH (Glutathione) in the mitochondrial hippocampus of the experimental rats. Results are given as mean ±S.D. for 6 rats.

Comparisons are made between the following: a. Group I and Group II; b. Group II and Group IV; NS. Group I and Group III. *statistically significant ($p < 0.05$); NS, non-significant.



Graph 4:- Levels of NADH dehydrogenase in the mitochondrial hippocampus of the experimental rats. Results are given as mean ±S.D. for 6 rats.

Comparisons are made between the following: a. Group I and Group II; b. Group II and Group IV; NS. Group I and Group III. *statistically significant ($p < 0.05$); NS, non-significant.



Graph 5:- Levels of Cytochrome-c-oxidase in the mitochondrial hippocampus of the experimental rats. Results are given as mean ±S.D. for 6 rats.

Comparisons are made between the following: a. Group I and Group II; b. Group II and Group IV; NS. Group I and Group III. *statistically significant ($p < 0.05$); NS, non-significant.

Reference:-

1. Arivazhagan P, Panneerselvam C, 2002. Neurochemical changes related to ageing in the rat brain and the effect of DL-α-lipoic acid. ExpGerontol. 37:1487-92.
2. Arivazhagan P, Ramanathan K, Panneerselvam C., 2001. Effect of DL-alpha-lipoic acid on mitochondrial enzymes in aged rats. ChemBiol Interact. 138: 189-98.
3. Beers RF Jr and Sizer IW, 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J BiolChem 195: 133-140.
4. Celik S, Ozkaya A. Effects of intraperitoneally administered lipoic acid, Vitamin E, and Linalool on the level of total lipid and fatty acids in guinea pig brain with oxidative stress induced by H_2O_2. Journal of Biochemistry and molecular biology. 2002; 35: 547-52.
5. El-Sayed el-SM, Abo-Salem OM, Abd-Ellah MF, Abd-Alla GM., 2008. Hesperidin, an antioxidant flavonoid, prevents acrylonitrile-induced oxidative stress in rat brain. BiochemMolToxicol. 22: 268-73.
6. Garcia-Estrada J, Gonzalez-Perez O, Gonzalez-Castaneda RE, Martinez-Contreras A, Luquin S, de la Mora PG, Navarro-Ruiz A, 2003. An alpha-lipoic acid-vitamin E mixture reduces post-embolism lipid peroxidation, cerebral infarction, and neurological deficit in rats. Neurosci Res. 47: 219-24.
7. Habig WH, Pabst MJ and Jakoby WB, 1974. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J BiolChem 249: 7130-7139.
8. Johnson D and Lardy H, 1967. Isolation of liver or kidney mitochondria. Methods Enzymol 10: 94-96.
9. Kamendulis LM, Jiang J, Xu Y, Klaunig JE., 1999. Induction of oxidative stress and oxidative damage in rat glial cells by acrylonitrile. Carcinogenesis. 20: 1555-60.
10. King J, 1965a. The hydrolases-acid and alkaline phosphatases. In: Practical clinical enzymology (King JC, ed.), Van D Nostrand Company, London pp.199-208.
11. Kristal BS, Koopmans SJ, Jackson CT, Ikeno Y, Park BJ, Yu BP., 1997. Oxidantmediated repression of mitochondrial transcription in diabetic rats. Free RadicBiol Med. 22: 813-22.
12. Mehler AH, Kornberg A, Grisolia S and Ochoa S, 1948. The enzymatic mechanism of oxidation-reductions between malate or isocitrate and pyruvate. J BiolChem 174: 961- 977.
13. Minakami S, Ringler RL and Singer TP, 1962. Studies on the respiratory chain linked dihydrodiphosphopyridine nucleotide dehydrogenase. I. Assay of the enzyme in particulate and in soluble preparations. J BiolChem 237: 569-576.
14. Misra HP and Fridovich I, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J BiolChem 247: 3170-3175.
15. Moreira PI, Santos MS, Seiça R, Oliveira CR., 2007. Brain mitochondrial dysfunction as a link between Alzheimer's disease and diabetes. J Neurol Sci. 257: 206-14.

16. Moron MS, Depierre JW and Mannervik B, 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. BiochimBiophysActa 582: 67-68.
17. Mukherjee PK, Das J, Saha K, Giri SN, Pal M and Saha BP, 1969. Antipyretic activity of Nelumbonucifera rhizome extract. Indian J ExpBiol 34: 275-276.
18. Mythili Y, Sudharshan PT, Varalakshmi P, 2005. DL-α lipoic acid ameliorates cyclophosphamide induced cardiac mitochondrial injury. Toxicology 215: 108-114.
19. Nita DA, Nita V, Spulber S, Moldovan M, Popa DP, Zagrean AM, Zagrean L, (2001). Oxidative damage following cerebral ischemia depends on reperfusion – a biochemical study in rat. J. Cell.Mol. Med. vol 5 no. 2 163-170.
20. Ohkawa H, Ohishi N and Yagi K, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351-358.
21. Ozdemir D, Uysal N, Gonenc S, Acikgoz O, Sonmez A, Topcu A, Ozdemir N, Duman M, Semin I, Ozkan H., 2005. Effect of melatonin on brain oxidative damage induced by traumatic brain injury in immature rats. Physiol Res. 54: 631-7.
22. Pearl W, Cancercao J and Zweifach BW 1963. Microdetermination of cytochrome oxidase in rat tissues by the oxidation of N-phenyl-p-phenylene diamine of ascorbic acid. J HistochemCytochem 11: 102-104.
23. Poon HF, Farr SA, Thongboonkerd V, Lynn BC, Banks WA, Morley JE, Klein JB, Butterfield DA. Proteomic analysis of specific brain proteins in aged SAMP8 mice treated with alpha lipoic acid: implications for aging and age related neurodegenerative disorders. Neurochemistry international. 2005; 46 (2): 159-68.
24. Prabhakaran K, Li L, Borowitz JL, Isom GE., 2006. Inducible nitric oxide synthase upregulation and mitochondrial glutathione depletion mediate cyanide-induced necrosis in mesencephalic cells. J Neurosci Res. 84: 1003-11.
25. Preston TJ, Abadi A, Wilson L, Singh G., 2001. Mitochondrial contributions to cancer cell physiology: potential for drug development. Adv Drug Deliv Rev. 49: 45-61.
26. Rajamani R, Muthuvel A, Manikandan S, Srikumar R, Sheeladevi R., (2007). Efficacy of DL-alpha-lipoic acid on methanol induced free radical changes, protein oxidative damages and hsp70 expression in folate deficient rat nervous tissue. ChemBiol Interact. 1; 167(3):161-7.
27. Rotruck JT, Pope AL, Ganther H, Swanson AB, Hafeman DG and Hoektsra WG, 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science 179: 588- 590.
28. Sagara Y, Ishige K, Tsai C, Maher P., 2002. Tyrphostins protect neuronal cells from oxidative stress. J Biol Chem. 277: 36204-15.
29. Sas K, Robotka H, Toldi J, Vecsei L., (2007). Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. J Neurol Sci.; 257(1-2):221-39.
30. Savitha S, Panneerselvam C., 2006. Mitochondrial membrane damage during aging process in rat heart: potential efficacy of L-carnitine and DL alpha lipoic acid. Mech Ageing Dev. 127: 349-55.
31. Shanmugarajan TS, Sivaraman D, Somasundaram I, Arunsundar M, Krishnakumar E, Balaji R, Ravichandiran V, 2008. Influence of alpha lipoic acid on antioxidant status in D-galactosamine-induced hepatic injury. ToxicolInd Health 24: 635-42.
32. Sharma AB, Sun J, Howard LL, Williams AG and Mallet RT, 2007. Oxidative stress reversibly inactivates myocardial enzymes during cardiac arrest. Am J Physiol Heart CircPhysiol 292: H198–H206.
33. Slater EC and Bonner WD, 1952. The effect of fluoride on succinic oxidase system. Biochem J 52: 185-196.
34. Tariq M, Khan HA, Al Moutaery K, Al Deeb S., 2002. Attenuation of iminodipropionitrile induced behavioral syndrome by sodium salicylate in rats. PharmacolBiochemBehav. 73: 647-54.
35. Tretter L and Vizi VA. Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress. Phil. Trans. R. Soc. B 2005; 360: 2335–2345.
36. Trushina E, McMurray CT., 2007. Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases. Neuroscience 145: 1233-48.
37. Unnisa AZ, Devaraj SN. Brain Lipid Peroxidation and Antioxidant Status after Acute Methacrylonitrile Intoxication. Drug and Chemical Toxicology. 2005; 28 (2): 187-95.
38. Vali S, Mythri RB, Jagatha B, Padiadpu J, Ramanujan KS, Andersen JK, Gorin F, Bharath MM. Integrating glutathione metabolism and mitochondrial dysfunction with implications for Parkinson's disease: a dynamic model. Neuroscience. 2007; 149 (4):917-30.
39. Zhang J, Keyang M, Wang H. Cyclophosphamide suppresses thioredoxin reductase in bladder tissue and its adaptive response via inductions of thioredoxin reductase and glutathione peroxidase. Chemico-Biological Interactions. 2006; 162 (1): 24-30.