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Hippocampal injury at celluar level provoked by iminodipropionitrile, neuroprotective effect of alpha lipoic acid

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ABSTRACT:

Iminodipropionitrile, one of the nitrile derivatives causes neurotoxicity is associated with oxidative stress. It has been suggested that mechanism involved can be generation of free radicals 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 Iminodipropionitrile (250mg/kg body weight) and Alpha lipoic acid (100mg/kg body weight) on hippocampal cellular enzyme activities such as SOD (superoxide dismutase), GPx (glutathione peroxidase), CAT (catalase) and GSH (reduced glutathione). Moreover, we also studied the damage on cellular membrane by measuring MDA (malondialdehyde) level. Iminodipropionitrile 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 damage provoked by Iminodipropionitrile.

KEY WORDS: Oxidative stress, Antioxidants, Iminodipropionitrile, Alpha lipoic acid, Neurotoxicity.

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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[1]. 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[2].Free radicals react with lipids and causes peroxidative changes that result in enhanced lipid peroxidative (LPO)[3]. Iminodipropionitrile induces LPO which is an auto-oxidative process initiated by a variety of free radicals to which polyunsaturated fatty acids presented in cell membranes are susceptible[4]. ALA is a derivative of octanoic acid, which forms an intermolecular disulfide bond that can be reduced to form two highly reactive vicinal sulfhydryl groups. As is generally known, ALA act as a cofactor which is covalently attached to the lysine residue forming an essential lipoamide, which is involved in mitochondrial energy metabolism[5]. ALA and its reduced form dihydrolipoic acid, reduce oxidative stress by scavenging a number of free radicals. Previous documentations finger towards nitriles causing cellular toxicity[6]. Iminodipropionitrile 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[7].

Ample literatures suggest that natural compounds with scavenging properties prevent the tissue from the attack of oxidative free radicals[8] generated by Iminodipropionitrile. Antioxidants play a vital role in affecting various neurodegenerative disorders by quenching reactive free radicals[9]. ALA is a potent antioxidant utilized in prevention and cure of various neuronal diseases as proved by previous reports[10]. 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[11]. The goal of our study is to gain insight into the Iminodipropionitrile induced oxidative damage in cytosol of hippocampal region that is counteracted by antioxidant effect of ALA.

MATERIALS AND METHODS

DRUGS AND CHEMICALS:

Iminodipropionitrile 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), 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 Vel's College of Pharmacy, Chennai (Letter No. 290/CPCSEA/PHA-04-08) with CPCSEA Registration No. 290/CPCSEA/12-12-2000. The animals were housed under conditions of control temperature (25±20C) and were acclimatized 12 ± 1 hr day and night rhythm during the experimental period and 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 hr 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 Iminodipropionitrile (250mg/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 Iminodipropionitrile (250mg/kg body weight) for 7 days.

After the 7 days of experimental period (i.e., on the 8th 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 Fridovich12. 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 and Sizer[13] was used to assess the activity of Catalase. The method of Rotruck[14] 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[15].

DETERMINATION OF LIPID PEROXIDES:

The brain mitochondrial lipid peroxide content was determined by the thiobarbituric acid (TBA) reaction described by Ohkawa[16].

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.

RESULT AND DISCUSSION

We observed the effects of IDPN 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 IDPN induced rats (group 2) when compared to control rats (group 1). When ALA was co-treated with IDPN 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).

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

Figure 2 shows the levels of MDA were significantly ($p < 0.05$) increased cellular hippocampus of IDPN induced rats (group 2) when compared to control rats (group 1). The MDA level is reversed by the ALA + IDPN treated rats (group 4) which shows the alteration in the level of when compared to IDPN 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 (group 1).

Table 1: Effect of iminodipropionitrile and alpha lipoic acid on the activities of cellular enzymic antioxidants in hippocampus of control and experimental rats

Groups	Control (Group 1)	Iminodipropionitrile Induced (Group 2)	ALA Alone (Group 3)	ALA+ Iminodipropionitrile (Group 4)
SOD	7.52 ±0.18	3.42 ±0.07 [a],*	7.60 ±0.07 [NS]	6.72 ±0.03 [b],*
CAT	3.14 ±0.02	1.25 ±0.02 [a],*	3.13 ±0.02 [NS]	2.52 ±0.02 [b],*
GPx	4.14 ±0.02	2.13 ±0.02 [a],*	4.15 ±0.02 [NS]	3.75 ±0.18 [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 ($p < 0.05$);

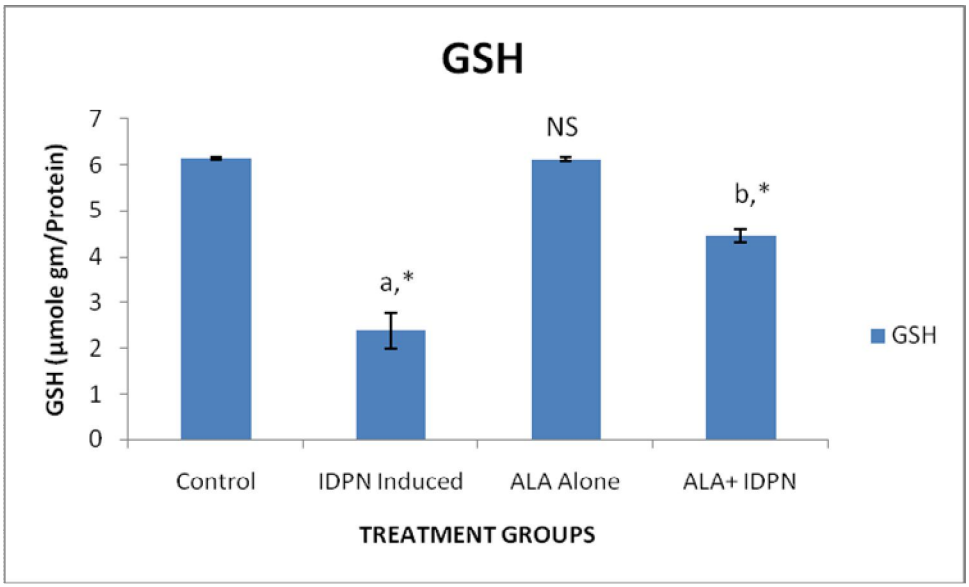

Figure 1: Levels of cellular GSH (Glutathione) in hippocampus of the experimental rats. Results are given as mean ±S.D. for six rats

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.

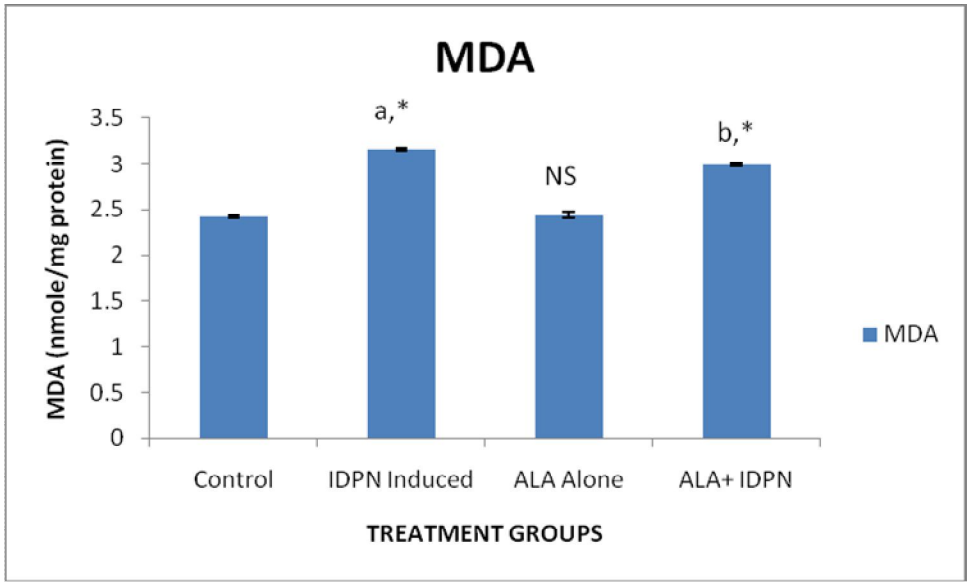

Figure 2: Levels of MDA (Malondialdehyde) in the cellular hippocampus of the experimental rats. Results are given as mean ±S.D. for 6 rats

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.

Nitrile derivatives are extensively acquired in day-to-day life, among which Iminodipropionitrile exhibits behavioral syndrome in rodents, which is depicted by hyperactivity and repetitive head movements. Several preceding explorations revealed that Iminodipropionitrile and its metabolite (i.e. N-hydroxyl Iminodipropionitrile) exposure augmented synthesis of reactive species thereby culminating in oxidative stress[17]. Massive free radical generation evoked cellular damage as culminated by previous literatures[18, 19]. 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[20]. Our present investigation is rationalized on the effect of ALA on Iminodipropionitrile induced hippocampal oxidative damage on cellular level, owing to its antioxidant effect and its profound ability to regenerate cellular 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[21, 22].

Previous studies depicted that oxidative stress due to nitrile intoxication caused marked reduction in the activity of antioxidants SOD, CAT, GPx and GSH[23]. In our study, Iminodipropionitrile 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[24] which is in harmony with our present study. For the further confirmation of Iminodipropionitrile provoked oxidative hippocampal damage, we laid emphasis on its damaging effects on cellular level.

Earlier reports revealed that nitriles initiate lipid peroxidation (LPO) by generating free radicals, which cause deleterious effect on cellular membrane thereby leading to distortion and loss of membrane integrity.[25] Mitochondrial membrane repercussions provoked by generation of lipid peroxyl radicals ($LOO^{\cdot}$) give rise to degradation product MDA (malondialdehyde)[6], which can be applied to assess lipid peroxidation in hippocampal mitochondria. Iminodipropionitrile 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.[26] In our study, ALA treatment demonstrated decreased levels of MDA due to its free radical quenching effect and membrane stabilizing effect.

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

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