

Effects of Oxidative Stress in Acute Poisoning in Central India

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Abstract

Background: Tissue inflammation, low food intake of micronutrients due to sleepiness, free radical burst from activated macrophages, and excessive medication cause oxidative stress in acute poisoning. If not neutralised by antioxidants, these free radicals may contribute to tissue inflammation. The word oxidative stress refers to a scenario in which free radical generation and antioxidant defence are out of balance. In humans, oxidative stress is caused by a reduction in bodily antioxidants when free radical generation exceeds endogenous antioxidant levels, resulting in significant cell damage/death.

Free radicals may destroy all major macromolecules, including lipids, proteins, and nucleic acids, but lipids are the most vulnerable.

Aim: To evaluate antioxidant status in acute amitriptyline poisoning (AP) cases undergoing routine standard treatment (RST) after supplementation with Vitamin C and alpha lipoic acid either alone or in combination, and compare it with that of normal healthy volunteers.

Material and Method: A total of 150 people were enrolled in the study, and they were split into five groups. They came from the IMCU and the Toxicology Ward. The consent of the patients' attendants was sought. The patients were chosen at random. To classify the level or grade of coma in poisoned individuals, the Edinburgh scale was utilized. At the time of admission and discharge, the levels of plasma cholinesterase, SOD, catalase, Glut-peroxidase, Glutathione transferase, MDA, Total-Antioxidant, LDH, and CK were measured.

Results: This study offers quantifiable recommendations for vitamin C and alpha lipoic acid intake to aid in rapid recovery and glutathione antioxidant status. On supplementation with vitamin C and alpha lipoic acid, we detected a significant decrease in catalase activity in all groups of acute Amitriptyline poisoning cases. For Gr. V (acute Amitriptyline poisoning cases), we found reduced glutathione activity on admission and higher values on discharge. In light of the preceding discussion, it is worth noting that the higher LDH and CPK in groups that may be attributable to pulmonary infarction rather than cardiac or skeletal muscle involvement could be due to pulmonary infarction.

Conclusion: Supplementation with vitamin C and alpha lipoic acid improved antioxidant levels during therapy. We propose that the antioxidant level of acute amitriptyline poisoning cases be examined for more successful recovery, and that diets poor in antioxidants may cause recovery to be sluggish. Oral supplementation, in our opinion, will be more successful for chronic Amitriptyline poisoning patients for a longer period of time, while IV form of vitamin C and alpha lipoic acid will be more effective for acute Amitriptyline poisoning instances.

Keywords: Amitriptyline, MDA, Total-Antioxidant, LDH, CK, SOD, AP, RST.

Introduction

Acute poisoning is a prevalent and urgent medical concern in all developed countries, as well as many developing ones. It accounts for 15-20% of all acute medical emergency hospital admissions in the United Kingdom. Accidental (10%) and purposeful (10%) acute poisoning are the two types of acute poisoning (90 percent). Only 10% of the purposeful cases are true attempted suicides, whereas the other 80% are self-poisoning cases. The vast majority of older age groups are doing it on purpose. In all age categories, females are more likely than males to suffer from acute poisoning, with a ratio of females to males of roughly 1.4: 1.0. Patients from lower socioeconomic backgrounds have seen a significant increase. Analgesics, antidepressants, and benzodiazepines are currently the most common medicines causing death in self-poisoning patients brought to hospitals across Europe.¹

The term "oxidative stress" refers to a scenario in which the production of free radicals and antioxidant defences are out of balance. When free radical generation exceeds endogenous antioxidant levels, oxidative stress in humans can lead to a reduction in body antioxidant levels, which can cause significant cell damage or death. On a constant basis, a considerable number of reactive oxygen species. Furthermore, when exposed to endotoxin and a range of substances, as well as during reoxygenation following ischemia, reactive oxygen production can be dramatically boosted. A comprehensive network of antioxidant defence systems is required to manage the inescapable reactive oxygen metabolites due to the variety of oxygen metabolites generated, their drastically varying reactivity, and their localisation.²

Free radicals react with all major biomolecules, including lipids, proteins, and nucleic acids, although lipids are the most vulnerable.³ Lipid peroxidation (oxidative degradation of lipids) is a

damaging self-perpetuating chain reaction that produces malonyldialdehyde as the end result. Acute amitriptyline poisoning cases had higher levels of SOD, MDA, and a lower antioxidant capacity in the blood (FRAP assay). Acute amitriptyline poisoning cases have higher levels of oxidative stress and lower antioxidant status. Based on the aforesaid favorable benefits of alpha lipoic acid on the nervous system, it was determined to supplement alpha lipoic acid to acute amitriptyline poisoning cases with elevated oxidative stress and neurotoxicity, and examine the effect of alpha lipoic acid supplementation.⁴

Ascorbic acid is a reducing agent, which means it may decrease and neutralise reactive oxygen species like hydrogen peroxide.⁵ Aside from its direct antioxidant properties, ascorbic acid provides a substrate for the antioxidant enzyme ascorbate peroxidase, which is vital for plant stress tolerance.⁶ In contrast to enzymes like Aspartate Transaminase (AST), Alanine Transaminase (ALT), and Creatinine Kinase (CK), which exhibit considerable variation in enzyme activity between tissues, the tissue distribution of LDH varies mostly in its isoenzyme composition, not in its quantity of LDH. The most common cause of elevated CK is cardiac or skeletal muscle damage; CK-MB levels are elevated in chronic myopathies⁷ and chronic renal failure cases, CK-MB levels do not surge and fall as they do in myocardial infarction, but remain relatively steady over several days.⁸

Material and Methods

A total of 150 people were enrolled in the study, and they were split into five groups. They came from the IMCU and the Toxicology Ward. The consent of the patients' attendants was sought. The patients were chosen at random. To classify the level or grade of coma in poisoned individuals, the Edinburgh scale was utilised.

The groups were classified as follows:

Group I: Consisted of 34 healthy volunteers mean age 33 years.

Group II: Consisted of 32 patients, mean age 35 years. These patients received only routine standard treatment (RST)

Group III: Consisted of 24 patients, mean age 34 years. These patients received routine standard treatment (RST) + Vitamin C supplementation.

Group IV: Consisted of 32 patients, mean age 33 years. These patients received routine standard treatment (RST) + alpha lipoic acid supplementation.

Group V: Consisted of 28 patients, mean age 34 years. These patients received routine standard treatment (RST) + Vitamin C and alpha lipoic acid supplementation.

Basal level of oxidative stress markers and enzymatic and non-enzymatic antioxidants were measured at the beginning of the treatment and followed up until the day of discharge.

Exclusion criteria:

This study excludes people under the age of 18 and those over the age of 60. This study excludes patients who have taken additional medications in addition to amitriptyline. This study excludes patients who are TLC positive but spectra negative.

Sample Collection

The antecubital vein was used to obtain 5 ml of venous blood from each experimental individual. 3 mL blood in a simple tube for enzyme analysis and 2 mL blood in sterile heparin vacutainer tubes. All of the samples were maintained on cold until they arrived at the lab. Serum/ Plasma was separated by centrifugation at 1500 x g for 5 minutes, then stored in new clean storage vials at -80°C and used for antioxidant and plasma cholinesterase analyses.

Methods:

- Lipid peroxidation levels in plasma was assayed by measuring the TBARS by the method of Ohkawa et al. 1979.⁹
- Catalase was assayed according to the method of Takahara et al., 1960.¹⁰
- SOD was assayed by the method of Misra and Fridovich, 1972.¹¹
- The activity of glutathione peroxidase was determined by the method of Rotruck et al. 1973.¹²
- The enzyme was assayed by the method of Habig et al. 1974.¹³
- The total reduced glutathione content was determined by the method of using Moron et al. 1979.¹⁴
- Ascorbic acid in plasma was oxidized by Cu²⁺ to form dehydroascorbic acid that reacts with acidic 2,4 dinitrophenyl hydrazine to form red bis- hydrazone, which was measured spectrophotometrically by 2,4 dinitrophenylhydrazine method.¹⁵
- Total CK was analysed by semi auto analyser Micro lab 200 by UV kinetic IFCC method.
- Serum total LDH was analyzed by semi auto analyzer Micro lab 200 by Mod. IFCC method.
- Analysis of Isoenzyme of CPK was carried out by HYDRASYS system SEBIA, PN 1210

Statistical Analysis:

Statistical evaluation was carried out using SPSS (Version 14.0) Data obtained from the study groups were compared by the parametric student's t test; correlation analysis between variables were made by Pearson test. All the results were expressed as means with their standard deviation (mean ± SD). Statistical analysis was also performed by using standard deviation and ANOVA.

Result:

Table 1: Comparison of plasma cholinesterase, SOD, catalase, Glut-peroxidase, on admission and on discharge.

On Admission				
Groups	<u>cholinesterase</u> µ/ml	<u>SOD</u> µmoles/mg	<u>catalase</u> µmoles/mg	<u>Glut-peroxidase</u> µg/ mg
Group I	319.4±28.7	5.6±0.78	3.14±0.64	6.62±0.82
Group II	144.4±10.22	7.43±0.80	6.11±0.76	7.56±0.93
Group III	185.9±28.8	7.44±0.91	6.63±0.72	7.60±0.68
Group IV	153.9±34.1	7.33±0.16	5.67±0.27	6.92±0.72
Group V	148.8±11.2	7.55±0.97	5.83±0.75	7.72±0.84

On Discharge				
Groups	<u>Cholinesterase</u> µ/ml	<u>SOD</u> µmoles/mg	<u>catalase</u> µmoles/mg	<u>Glut-peroxidase</u> µg/ mg
Group I	319.4±28.7	5.6±0.78	3.14±0.64	6.62±0.82
Group II	177.4±15.5	6.98±0.87	5.83±0.74	7.13±0.70
Group III	225.4±36.4	7.25±0.58	4.32±0.69	7.18±0.69
Group IV	201.7±45.9	7.51±0.72	4.87±0.66	6.88±0.74
Group V	236.09±49.9	5.88±0.62	5.47±0.57	6.75±0.61

In all groups, the mean plasma cholinesterase levels on admission were significantly greater than the mean plasma cholinesterase levels on discharge. With the addition of antioxidants, the level of SOD was reduced much further in group V. When the mean catalase levels on admission were compared to the mean catalase levels on

discharge, there was a significant difference in groups III, IV, and V, but not in group II. When the mean glutathione peroxidase levels on admission were compared to the mean levels on discharge, all groups showed a drop in levels). In reality, there was no significant difference between the values for group IV cases.

Table 2: Comparison of Glutathione transferase, MDA, Total-Antioxidant, LDH, CK between on admission and on discharge.

On Admission					
Groups	Glutathione transferase	MDA	Total-Antioxidant	LDH	CK
Group I	6.05±0.86	1.89±0.10	1.78±0.22	3.48±0.14	1.22±0.16
Group II	7.18±0.67	2.12±0.96	0.99±0.19	2.14±0.57	2.36±0.62
Group III	6.33±0.68	2.26±0.48	1.65±0.40	2.63±0.47	1.87±0.76
Group IV	6.64±0.93	1.94±0.18	1.38±0.11	1.78±0.83	2.47±0.84
Group V	6.90±0.76	2.19±0.82	0.96±0.18	2.88±0.38	2.89±0.17
On Discharge					

Groups	Glutathione transferase	MDA	Total-Antioxidant	LDH	CK
Group I	6.05±0.86	1.89±0.10	1.78±0.22	3.48±0.14	1.22±0.16
Group II	6.75±0.63	2.48±0.74	1.02±0.14	2.36±0.34	1.69±0.12
Group III	6.14±0.75	2.65±0.66	1.85±0.43	2.39±0.40	1.43±0.50
Group IV	6.34±0.58	2.14±0.27	1.42±0.13	0.98±0.71	1.73±0.44
Group V	6.22±0.57	2.09±0.67	1.77±0.58	2.66±0.29	1.93±0.28

When the mean glutathione transferase levels on admission were compared to the levels on discharge, all groups showed a substantial drop in values. When the mean MDA levels on admission were compared to the mean levels on discharge, all groups demonstrated an increase in MDA levels on admission, which maintained until discharge, with the exception of group V patients. When the mean total antioxidant levels on admission were compared to the mean levels on discharge, all groups indicated a drop in levels on admission, whereas the levels improved significantly in group III and group V cases after vitamin C and combination supplementation, respectively. In groups of acute amitriptyline poisoning cases, the LDH exhibited an increase in Total LDH activity. This was consistent with previous research. Isoenzymes were found in these samples. For groups of acute amitriptyline poisoning cases, the results of the CK isoenzyme showed an increase in the 100 percent CK-MM percentage.

Discussion

The primary goal of this study was to see how effective Vitamin C and alpha lipoic acid were in treating acute AP patients. In this investigation, there was evidence of increased oxidative stress. All AP patients receive vitamin C and Alpha lipoic acid supplements. Preventive antioxidants, such as catalase and other peroxidases, limit the rate of chain initiation; chain breaking antioxidants, such as Vitamin C and Vitamin E, interfere with chain propagation. Vitamin E and Vitamin C are well-known antioxidants that react quickly with oxygen free radicals and have a wide range of biological applications.^{16,17}

Cu-Zn SOD is commonly thought to be a non-inducible enzyme. In the face of a variety of potentially triggering situations, such as oxidative stress and cytokines like tumour necrosis factor-Alpha (TNF-Alpha) and interleukin I, the protein and mRNA levels in mammalian tissue stay stable (IL-I). However, oxidative stress has been shown to induce the enzyme in endothelial cells and Chinese hamster fibroblasts in several studies.¹⁸⁻²⁰ In humans, catalase and GPx detoxify H₂O₂. Despite the fact that both remove the same substrate, only GPx can effectively remove organic hydroperoxides, which are the primary source of protection against oxidative stress at low levels.²¹

These findings show that all acute AP cases have significant oxidative damage; our findings are consistent with previous research that found higher LPO activity in acute AP cases. When compared to RBC cholinesterase, plasma cholinesterase recovered and regenerated quickly in all groups of acute Amitriptyline poisoning cases. On supplementation with vitamin C and alpha lipoic acid, we detected a significant decrease in catalase activity in all groups of acute Amitriptyline poisoning cases.²²

Oral alpha lipoic acid intake for a longer period of time will increase overall antioxidant levels and reduce oxidative stress. Vitamin C and alpha lipoic acid supplementation in chronic Amitriptyline poisoning cases would be interesting to research.

According to the literature on LDH enzymes, patients with pulmonary infarction have high LDH levels within 24 hours of beginning of pain.

Within one to two days after an episode of chest discomfort, the pattern of normal AST and high LDH levels suggests pulmonary infarction. The presence of pulmonary edema and pulmonary infarction is also suggested by the signs and symptoms of acute amitriptyline overdose. Because acute amitriptyline poisoning patients also show signs and symptoms of respiratory and pulmonary problems, we believe the rise in CPK is caused by respiratory and pulmonary disorders rather than cardiac or skeletal muscle disorders.²³

Conclusion:

This study reveals that oral supplementation with Vitamin C and alpha lipoic acid reduces oxidative stress in acute Amitriptyline poisoning cases while also increasing total antioxidant levels in these individuals. This will assist patients with acute Amitriptyline poisoning recover faster and spend less time in intensive care. Supplementing with vitamin C and alpha lipoic acid also lowers long-term side effects such as Amitriptyline-induced delayed neuropathy. To have the most positive effect, supplementation should be done for a longer period of time (reduced oxidative stress and increased total antioxidant status). Oral supplementation, in our opinion, will be more successful for chronic Amitriptyline poisoning patients for a longer period of time, while I.V. form of vitamin C and alpha lipoic acid will be more effective for acute Amitriptyline poisoning instances.

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