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The effect of antioxidants in acute amitriptyline poisoning

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ABSTRACT

Objective: To study the effect of Antioxidants supplementation in reducing oxidative stress induced in acute amitriptyline poisoning cases.

Design and methods: We compared the effect of supplementation of treatment of acute amitriptyline poisoning cases with alpha lipoic acid alone or with vitamin C, with that of those receiving only routine standard treatment (RST) as a control group.

A total of 132 subjects divided into 5 groups were selected from IMCU (Intensive Medical Care Unit) and Toxicology Ward, Govt. General Hospital, Chennai, India. The study was restricted to grade 1 coma in poisoned subjects per the Edinburg scale. Each of the subjects was in the groups were supplemented with either placebo, RST, RST with vitamin C, RST with ALA, or RST with vitamin C and ALA.

Acute anti-depressant poisoning (especially with amitriptyline) induced oxidative stress caused lipid peroxidation. Plasma cholinesterases (chE) play a major role in combating this effect. A determination of the level of cholinesterase (chE) acts as an indirect indicator of the level of oxidative stress and a measure of the efficacy of antioxidant supplementation. Plasma cholinesterase estimation was done by colorimetric method. The change in color of the indicator bromothymol blue caused by the liberated acetic acid from cholinesterase read by spectrophotometer at 620 nm was used to determine the levels of cholinesterase.

Result: A decrease in the level of oxidative stress was observed among those supplemented with either alpha lipoic acid alone or along with vitamin C, with a slightly more decrease in oxidative stress in the latter group. A p-value of < 0.001 is considered significant statistically. The percentage of the benefit of treatment on supplementation with vitamin C and alpha lipoic acid showed a marked increase in group V (26.9%) cases after supplementation with both in combination.

Conclusion: The results provide evidence that the oxidative stress induced by acute amitriptyline poisoning is comparatively decreased by supplementation with antioxidants like alpha lipoic acid and vitamin C, than those only on routine standard treatment.

1. Introduction

Acute anti-depressant poisoning is a common and urgent medical problem in all developed, and many developing, countries of the world. In the United Kingdom, it accounts for 15–20% of all acute medical emergency admissions to hospital. The different types of acute poisoning are accidental (10%) and intentional (90%). In the intentional type, only 20% are actual attempted suicide cases and 80% are self-poisoning cases. In older age groups the great majority are intentional. Acute poisoning is more common in females than in males in all age groups, the ratio of females to males being about 1.4: 1.0. The increase has been marked in patients of lower social class [1]. In 2004, the Toxic Exposure Surveillance System (TESS) national database of poison center maintained by the American Association of Poison Control Centers (AAPCC) recorded 7,430 exposures to amitriptyline, 4804 to other cyclic antidepressants. Of the 12,234 exposures, there were 1,351 (9%) exposures in children less than 6 years of age and 3,881 (32%) exposures described as unintentional. A total of 9,324 (76%) exposures were treated in healthcare facilities [2]. This increased referral rate shows the potential toxicity of this class of drugs.

The drugs used to treat psychiatric disorders are known collectively as psychotropics [3]. They are classified according to their main mode of action into:

Abbreviations: RST, routine standard treatment; IMCU, intensive medical care unit

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I	IAnti-psychotic drugs (Major Tranquilizers) – Ex: Chlorpromazine
II	II Anti-anxiety drugs – Ex: Alprazolam and diazepam
III	IIIPsychotogenic or Psychodelic drugs – Ex: LSD, cannabis, atropine
IV	IVAntidepressant drugs – Ex: Amitriptyline, Nortriptyline

Drug-induced oxidative stress is a well-studied phenomenon especially those of chlorpromazine-induced phototoxicity, by the generation of reactive oxygen species (ROS) [4]. Antipsychotics are majorly notorious for their severe adverse effects compared to other classes of psychotropic drugs. Anti-depressants and anti-anxiety drugs are more frequently available over the counter and are frequent sources of abuse compared to antipsychotics with no major visibly manifest toxicity. Yet, their damage potential is comparable to that of antipsychotics as far as oxidative stress induced tissue damage is concerned.

Amitriptyline a 3-(10,11-dihydro-5H-dibenzo[a,d]cycloheptene-5-ylidene)-*N, N*-dimethylpropane-1-amine with a chemical formula of $C_{20}H_{23}N$ and a molecular weight of 277.403 g/mol, is a very widely used tricyclic antidepressant. It is metabolized by *N*-demethylation to nortriptyline, latter is an antidepressant in its own right [5]. Amitriptyline is used in the treatment of depression, fibromyalgia, chronic fatigue syndrome, migraine, irritable bowel syndrome, and atypical facial pain. Amitriptyline exerts its antidepressant action by blocking the neuronal reuptake of noradrenaline and serotonin. Amitriptyline has significant anticholinergic activity and some sedative action. Amitriptyline is easily available and commonly abused for suicidal purposes in developing countries.

Amitriptyline can cause serious complications, such as compartment syndrome of abdominal viscera, irreversible damage to the central nervous system and lethal ventricular tachycardia. [6] It also has a key role in tissue damage especially the central nervous and cardiovascular systems via free radical-induced oxidative stress [7]. In a series of 110 cases of overdose by amitriptyline for suicide attempts by adult single women admitted to the emergency department of Dicle University Hospital, Turkey (Güloglu C et al). The most common symptoms were sinus tachycardia (66.3%), altered mental state (78.1%) and hypotension (7.3%). Mechanical ventilator support was required in 9.1% of cases [8].

1.1. Oxidative stress

The term oxidative stress refers to the situation of an altered interplay between the production of free radicals and antioxidant defense [9]. Oxidative stress in human can result in diminished antioxidant efficacy, when the free radical production overwhelms the endogenous antioxidant levels like glutathione, thereby causing considerable cell damage or death via apoptosis. It is responsible for various systemic disorders like Parkinsonism, Alzheimer's disease, myocardial infarction, depression, sickle cell disease, and autism and ADHD (Attention-deficit/Hyperactivity Disorder) in children.

All major biomolecules like lipids, proteins, and nucleic acids react with free radicals, but lipids are probably the most susceptible [10]. The polyunsaturated fatty acids, like arachidonic acid and linoleic acid, are the major targets for free radical oxidation. The oxidative destruction of lipids (lipid peroxidation) is a destructive self-perpetuating chain reaction releasing malonyldialdehyde (MDA) as the end product [11].

Antioxidants such as catalase, superoxide dismutase (SOD), glutathione, sulfiredoxin glutathione-S transferases, and aldehyde dehydrogenases are potent free radical scavengers. Unlike in-vivo antioxidants, certain dietary antioxidants not synthesized by human bodies like vitamin A (beta-carotene), vitamin E (tocopherol), vitamin B12 (cholecalciferol) and alpha lipoic acid potentiate the free radical scavenging ability of these in vivo antioxidants.

Studies have shown that in acute amitriptyline poisoning cases, there is an increased level of SOD, MDA and reduced antioxidant capacity of blood (FRAP assay) [12]. An increased production of reactive oxygen species due to excessive intake of amitriptyline leading to an increase in tissue oxidative stress and tissue structural and functional damage has been documented [13].

1.2. Vitamin C

Ascorbic acid (vitamin C) is a monosaccharide antioxidant found in both animals and plants, [14] is vital for the conversion of procollagen to collagen, the formation of norepinephrine from dopamine, carnitine synthesis and metabolism of tyrosine. Vitamin C is one of the popular, extensively studied, and the widely supplemented antioxidant. It works even better in conjunction with other antioxidants, such as alpha lipoic acid and carotenoids, by establishing a peculiar recycling system with a synergic effect.

In its reduced form with glutathione, it acts as a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide [15]. Ascorbate acts as an electron donor in all these reactions thereby itself getting oxidized. Ascorbic acid has shown promising results in the management of drug inducted oxidative stress damage to tissue in various animal models [16].

Apart from the aforementioned antioxidant properties of vitamin C, it does also posses anti carcinogenic effect such as those in the prevention of cigarette smoking and its major derivative p-Benzoquinone induced oxidative damage in urothelial cancer of the renal pelvis, ureter and bladder [17,18]. It also act as a hepato-protectant in various pesticide toxicity like carbofuran induced free radical damage and increased lipid per-oxidation in rat liver models at low concentration intoxication [19]. Furthermore, vitamin C also protects from chloroquine induced oto-toxicity, a drug commonly used as anti-lupus and anti-malarial agent [20]. Such versatile is the action of vitamin C in biological tissue protection from oxidative damage.

1.3. Alpha lipoic acid

Alpha lipoic acid initially classified as a vitamin was discovered three decades earlier and possessed potent antioxidant properties. It is more potent than the old guard antioxidants like vitamin C and E; it even recycles these vitamins and enhances their effectiveness. In addition to functioning as an antioxidant, it also assists B vitamins in producing energy from the proteins, carbohydrates, and fats consumed through foods. It has properties of both lipid and water solubility, thereby facilitating its reach into tissues composed of fat, such as the nervous system as well as those mainly of water such as the cardiovascular system.

A study conducted with 600 mg of alpha lipoic acid given daily to nine Alzheimer's patients, on an average for 80 days lead to stabilization of cognitive functions in the Alzheimer's study group. This was the first indication that alpha lipoic acid might be a successful neuroprotective therapy option for Alzheimer diseases and related dementias [21].

In animal studies, alpha lipoic acid has been seen to increase the blood flow to the nerves and improved transmission of nerve impulses. Parkinson's disease, a disorder of the central nervous system, is characterized clinically by spontaneous movements, gait difficulties, postural instability, rigidity, and tremor. Oxidative stress plays a major role in neuronal degeneration associated with Parkinson's disease. Depletion of glutathione (GSH) in the brain is the earliest indicator of oxidative stress in presymptomatic Parkinson's disease [22].

Studies in both in-vitro and in-vivo models have suggested that pretreatment with alpha lipoic acid increase cellular levels of GSH, probably by preventing its depletion thereby protecting mitochondria integrity. Results with previous studies suggest that alpha lipoic acid may be an effective neuroprotective agent in age-associated neurodegeneration utilizing the PC12 cell model system.

Based on the above beneficial effects of alpha lipoic acid on nervous system it has been decided to supplement alpha lipoic acid in acute

amitriptyline poisoning cases, which have increased oxidative stress and neurotoxicity and study the effect after supplementation.

2. Subjects and methods

A total of 132 subjects were enrolled for the study and were divided into 5 groups. They were selected from IMCU and Toxicology Ward, Govt. General Hospital between Sept. 2005 and March 2008. Consent was obtained from the attendants of the patients. The study was approved by the Ethical committee of Madras Medical College, Chennai.

2.1. Inclusion criteria

The Patient selection was done randomly. The Edinburgh scale was used to classify the depth or grade of the coma of poisoned patients, graded as under:-

Grade 1:Patient drowsy but responding to verbal commands.
Grade 2:Patient unconscious but responding to minimal stimuli (For example, shaking, shouting)
Grade 3:Patient unconscious and responding only to painful stimuli (For example, rubbing the sternum)
Grade 4:Patient unconscious with no response to any stimuli
The study was restricted only to the grade 1 patients from IMCU.
The groups were classified as follows:

- **Group I:** Consisted of 30 healthy volunteers (15 males and 15 females) mean age 32 years.
- **Group II:** Consisted of 30 patients (18 males and 12 females) mean age 34 years. These patients received only Routine Standard Treatment (RST)
- **Group III:** Consisted of 21 patients (12 males and 9 females) mean age 32 years. These patients received (RST) + vitamin C supplementation.
- **Group IV:** Consisted of 27 patients (13 males and 14 females), mean age 31 years. These patients received (RST) + alpha lipoic acid supplementation.
- **Group V:** Consisted of 24 patients (14 males and 10 females), mean age 34 years. These patients received (RST) + vitamin C and alpha lipoic acid supplementation.

The 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 from IMCU.

2.2. Exclusion criteria

- Subjects with age less than 18 years and more than 60 years were not included in this study, considering the confounding effect of hormonal and metabolic alterations.
- Patients those who have taken other drugs along with amitriptyline were not included in this study, due to cross interference.
- Patients with TLC (Thin Layer Chromatography) positive and spectra (uv–vis) negative were not included in this study.

2.3. Sample collection

10 ml of venous blood was drawn from the antecubital vein of each experimental subject. 5 ml of blood was collected in a plain tube for enzyme analysis and 5 ml in a sterile heparin vacutainer tubes. The plasma was separated by centrifugation at 1500 x g for 10 min and stored in new clean storage vials at - 80 °C to be used for analysis of antioxidants and plasma cholinesterase. The cells were separated and washed with normal saline and RBCs were subjected to lysis and used for RBC cholinesterase estimation.

50 ml of gastric aspirate was collected from all patients who are directly admitted to IMCU and poison center GGH, Chennai-3. This gastric aspirate was taken for TLC identification.

2.4. Methods

2.4.1. Cholinesterase estimation

2.4.1.1. Plasma cholinesterase. The cholinesterase levels were determined from both the centrifuge derived plasma and the substrate from RBC lysate. Estimation was done by colorimetric method using acetylcholine as substrate (Venkataraman et al) [23]. Both true and pseudo cholinesterase would hydrolyze the substrate and produce choline and acetic acid. The change in color of the indicator, bromothymol blue caused by the liberated acetic acid from cholinesterase was read by spectrophotometer at 620 nm.

Bromothymol blue 0.5 ml solution was diluted with 3.8 ml of distilled water and 0.2 ml of 15% acetylcholine chloride was added. About 100 µl of plasma was added to it and the change in color was read at 620 nm at 37-degree c after 30 min. A standard graph was plotted using acetic acid 0.15 N in the concentration of 10, 20, 50,100 and 200 µmol.

2.4.1.2. RBC cholinesterase. The RBCs were extracted by adding 3 ml of distilled water followed by precipitation of hemoglobin with acetone 2 ml and centrifugation at 3000 rpm. The supernatant was used for estimation of RBC Cholinesterase in a similar fashion.

2.4.2. Vitamin C estimation

Ascorbic acid in plasma was oxidized by Cu2+ to form a dehydroascorbic acid that reacts with acidic 2, 4 dinitrophenyl hydrazine to form red bis-hydrazone, which was measured spectrophotometrically by 2,4 dinitrophenylhydrazine method. The concentration of ascorbic acid in plasma was determined using a standard curve [24].

2.4.3. Total antioxidant status

Total antioxidant status of the sample was measured by a commercial kit, supplied by the Randox Laboratories.

Principle: $ABTS^+$ (2-2′ – Azino-di-[ethylbenzthiazoline sulphonate] is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^+$. This has a relatively stable blue-green color, when measured at 600 nm Antioxidants in the added sample cause suppression of this color production to a degree which was proportional to their concentrations [25].

20 µl of plasma was added to 1 ml of chromogen and incubated at room temperature for 1 min. The initial Absorbance (A_1) was measured at 600 nm Another 200 µl of substrate added to it and incubated at room temperature for 3 min and the final absorbance A_2 was measured again at 600 nm A Blank and a Standard were run simultaneously, and the initial absorbance A_1 and final absorbance A_2 was measured at 600 nm for both the blank and standard respectively.

A_2-A_1 = ΔA of the sample/ blank/ standard were individually determined,

$$\text{Factor} = \frac{\text{Concentration of the standard}}{\Delta A \text{ blank} - \Delta A \text{ standard}}$$

Total Antioxidant status in m.mol/l = Factor X (ΔA blank-ΔA sample)

2.4.4. Drug extraction from stomach wash contents for TLC

The stomach wash contents obtained from the patient who has been suspected to have consumed drug was taken for TLC analysis, 10 ml of the collected stomach wash contents was taken in the vortex tube of 11 cm length and 2.5 cm diameter and equal volume of 10 ml of chloroform: propane-2-ol (9:1) mixture which was allowed to stand for 5 min was added. The solution was thoroughly mixed by the vortex mixer (cyclomixer) for about 5–10 minutes.

The mixed solution was poured into a separating funnel and allowed to stand for 30–45 min for the aqueous and organic layer to separate. The drug compound suspected get extracted into the chloroform layer,

which remained at the bottom of the separating funnel. The separated organic layer containing chloroform and drug was removed from the separating funnel by slowly opening the knob; whereby the lower layer was allowed to run into a funnel with a filter paper containing 2 gm of sodium sulfite, which removes the water molecules and polar substances from the layer. The rest of the solution is poured into a beaker and evaporated to near dryness in a water bath at 60°c. Few drops of chloroform were added in the breaker and the extract was ready for application on a TLC.

2.4.5. TLC (thin layer chromatography) application

About 5 µl of the extracted sample was spotted on to a TLC plate at a position of 2.0–2.5 cm from the bottom edge of the plate with a help of a specialized capillary tube. The circular spot about 2–6 mm in diameter was spotted on a line parallel to the standard drug substance. The two spots of standard and test sample were plotted at a distance of more than 1.5 cm to prevent any smearing [26,27].

2.4.6. Calculation of Rf (relative front or ratio front)

The distance traveled by the solvent mixer is marked and measured in cm. Next, the distance traveled by the solute is marked as a rounded spot. The distance from the center of the spot to the point of its spotting, measured in cm is the distance traveled by the solute.

Rf value for the drug was calculated using the following formula.

$$\text{Rf} = \frac{\text{Distance travel by solute in cm}}{\text{Distance travel by solvent in cm}}$$

3. Statistical analysis

Statistical evaluation was carried out using SPSS (14.0). Data obtained from the study groups were compared by the parametric student's t-test. A correlation analysis between the variables was made by Pearson test and a P value of < 0.001 was considered statistically significant.

The effect of vitamin C, alpha lipoic acid and both combined were analyzed for each group and expressed as a percentage of benefit with and without supplementation of vitamin C, alpha lipoic acid and both combined. Multiple comparisons of each group with the normal were carried out using the Bonferroni corrected t-test [14].

4. Results

Identification of amitriptyline overdoses in gastric aspirate in all cases admitted directly to Intensive Medical Care Unit (IMCU) and toxicology ward, Government General Hospital between September 2005 and March 2008 are carried out by TLC. The samples along with controls were run simultaneously and based on their Rf values in the TLC chromatogram, the level of amitriptyline overdose and antioxidant effect were evaluated in all the groups.

The mean levels of plasma Cholinesterase on admission compared with those on discharge in all groups, showed significantly higher values in the latter. (Table 1) The percentage of benefit of treatment before and after supplementation with vitamin C showed a significant decrease in group III (39.67 (22.8%)) when compared with group II (45.40(26.1%)). Group V showed a marked improvement (70.0(40.3%)) when compared with group II (45.40(26.1%)). (Chart 1 and 5) Even though the values of plasma Cholinesterase increased on discharge it was not up to the levels of normal individuals (i.e. group I).

The mean levels of RBC Cholinesterase on admission compared with those on discharge in all groups, showed significantly higher values on discharge. (Table 2) Though the levels of RBC Cholinesterase showed an increase at the time of discharge it was not up to the level of normal individuals. The percentage of the benefit of treatment on supplementation with vitamin C and alpha lipoic acid showed a slight increase in group III (40.57(12.8%)) and group IV (47.7(15.0%)) when compared with group II (33.13(10.5%)) without supplementation (Chart 2 and 5). But the marked increase was observed in group V (85.38(26.9%)) cases after supplementation with vitamin C and alpha lipoic acid in combination.

The mean levels of MDA in all groups on admission compared with those on discharge, showed an increase in MDA levels on admission and the increase continued till discharge in all groups except in group V cases (Table 3, Chart 3). In group V cases though the levels at the time of discharge decreased, the decrease though was meager, was significant. The benefit of treatment on MDA showed improvement in group V cases and the levels of MDA decreased on combined supplementation with antioxidants in this group (Chart 3 and 5 i.e. 2.10 to 2.01(−0.08)).

The mean levels of total antioxidants on admission compared with those on discharge, showed a decrease in levels on admission in all groups and the levels improved significantly in group III(0.19(11.3%)) and group V(0.52(31.1%)) cases after supplementation of vitamin C and the combination (vitamin C + AlA) respectively (Table 4, Chart 4 and 5). Group II and group IV showed no significant improvement.

The percentage of benefit of treatment on Plasma and RBC Cholinesterases, Malonlydialdehyde and total antioxidant levels is indicated above. Group V showed an overall increase in all the parameters (plasma and RBC cholinesterase being 40.3% and 26.9% respectively and total antioxidant gain being 31.1%), with a decrease in malonyldialdehyde (a negative indicator of oxidative damage). MDA showed improvement in group V cases and their levels decreased on combined supplementation with antioxidants in this group i.e. from 2.10 on admission to 2.01(-0.08, 6.3%) on discharge.

Out of 132 acute amitriptyline poisoning cases studied, the age distribution of male and female showed an almost similar pattern and the mean average age in all groups for the male was 33 and female was 32. The number of male subjects outnumbered the female, with males being 73(55.3%) against 59(44.7%) females. This distribution shows that amitriptyline poisoning is seen more in males (Table 5).

The results also showed that the regeneration or reactivation of plasma Cholinesterase was more rapid than RBC Cholinesterase (Chart 1, Chart 2). The base levels of both RBC and plasma Cholinesterase remained higher in all groups (Tables 1,2, Chart 1, 2). In our study, we observed that the effect of supplementation of vitamin C was higher in group III and the effect of supplementation of the combination (i.e. vitamin C + AlA) was greatest in group V (Chart 5).

5. Discussion

The main objective of the present study was to determine the efficacy of vitamin C and alpha lipoic acid supplementation to routine standard treatment in acute amitriptyline poisoning cases. The presence of an increased oxidative stress was noticed in these acutely poisoned cases during the study. We chose to supplement vitamin C and alpha lipoic acid for all poisoned patients, vide their major role in ameliorating in vivo free radical damage. Antioxidants broadly fall into 2 classes; those of the Preventive antioxidants, which reduce the rate of chain initiation, (e.g.) catalase and other peroxidases; and the Chain breaking antioxidants which interfere with chain propagation (e.g.) vitamin C and E. Vitamin C reacts rapidly with oxygen free radicals, and are widely accepted antioxidant compounds for their biological activity [28,29]. Alpha lipoic acid in addition to its own antioxidant properties also recycles vitamin C and E thereby enhancing their availability in both aqueous phase (cytosol) and lipid phase (cell membrane). Also alpha lipoic acid dissolves both in lipid and water and acts effectively in both phases.

The mechanism of action of amitriptyline on acetylcholinesterase (AchE) indicates that it interacts with a moiety of hydroxyl group on serine in the active (esteric) site of the enzyme, thereby impeding the action of the latter in physiological substrates. This leads to a profound reduction in their levels by conversion to a lesser active form. RBC and

Table 1
Comparison of levels (in μ/ml) and the percentage of benefit of different methods of treatment on plasma Cholinesterase.

Groups	On Admission		On Discharge		Pl. ChE gain	Students Paired t-test
	Mean	SD	Mean	SD		
Normal (I)	174.00	8.93	174.00	8.93	0.00	t = 0.00 p = 1.00 Not significant
Routine treatment (II)	70.07 (40.30%)	8.31	115.47 (66.40%)	11.59	45.40 (26.10%)	t = 16.73 p = 0.001 Significant
Routine treatment + VitC (III)	90.71 (52.10%)	13.71	130.38 (74.90%)	23.39	39.67 (22.80 %)	t = 7.81 p = 0.001 Significant
Routine treatment + ALA (IV)	82.81 (47.60%)	7.39	116.07 (66.70%)	13.01	33.26 (19.1%)	t = 12.16 p = 0.001 Significant
Routine treatment + ALA + VitC (V)	62.54 (35.90%)	12.18	132.54 (76.20%)	23.24	70.00 (40.30%)	t = 12.89 p = 0.001 Significant

Plasma cholinestrase showed significant improvement in all groups after supplementation with antioxidants, with maximum being in Group V. Hence the increase in RBC and plasma cholinesterase may be due to the effect of treatment with oximes, in all groups of acute poisoning cases. The supplementation of vitamin C and alpha lipoic acid may not have had a direct effect in the improvement of RBC and plasma cholinestrase enzymes, and it would be interesting to further study whether the reduction in oxidative stress and increase in total antioxidant status of acute amitriptyline poisoning patients have any influence in reactivation or de novo synthesis of RBC and plasma cholinesterase.

We observed that the lipid peroxidation (LPO) activity was high in all groups of acute poisoning cases, when compared to normal individual indicating oxidative damage in them. Even after supplementation of vitamin C and alpha lipoic acid alone, the MDA levels remained high, except in group V cases. These results suggests that there is considerable oxidative damage occurring in all acute amitriptyline poisoning cases, our results are in agreement with earlier studies which reported increased LPO activity in acute amitriptyline poisoning. It has been established in animals as well as in humans that serum concentrations of lipid peroxidation products (LPPs) increase in pulmonary inflammation [30]. The clinical manifestation of acute amitriptyline poisoning were pulmonary edema, pulmonary infarction, hence the increased MDA may be due to the pulmonary disorder manifestations of the poisoning.

The results for total antioxidants showed significant increase in Group III and Group V acute amitriptyline poisoning cases who received maximum supplementation with alpha lipoic acid. The increase in total antioxidant levels in these patients is a good sign as these antioxidants are believed to neutralize the ROS, and thereby reduce the oxidative stress. The exact mechanism for increase in total antioxidants after supplementation of vitamin C and alpha lipoic acid is unclear, further study is required to investigate whether vitamin C and alpha lipoic acid enhances, or recycles the endogenous antioxidants or the increase in total antioxidant levels were due to the direct supplementation effect of vitamin C and alpha lipoic acid. As the total antioxidant levels significantly improve, this in turn will improve the faster recovery of these patients, and also reduce long term side effects such as Amitriptyline induced delayed polyneuropathy (AIDP).

Though each case of acute amitriptyline poisoning is unique, the onset, severity and duration of poisoning, are determined by the dose, rate of exposure, physical and chemical properties of amitriptyline. In this study Group V cases showed maximum effect, the reasons probably would be the combination of antioxidant and stay of these patients in IMCU and poison centre was maximum (5–13 days). Hence they received the symptomatic treatment more than other groups, and also the supplementation of vitamin C and alpha lipoic acid was greatest in this group. A major drawback of the study was the number of subjects included in each group, and the exact levels and time since consumption being unknown. A long term follow of these subjects for any rebound in

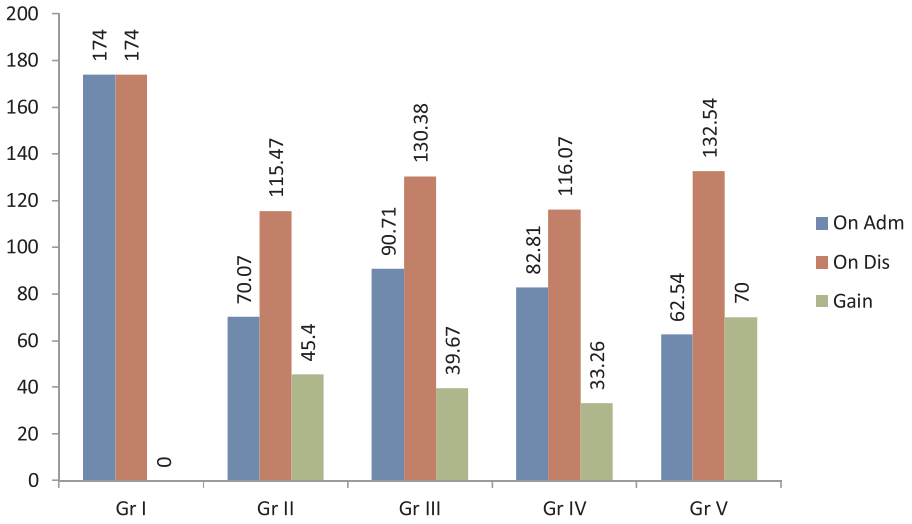

Chart 1. Comparison of levels of plasma Cholinesterase on different methods of treatment.

Table 2
Comparison of levels (in μ/ml) and the percentage of benefit of different methods of treatment on RBC Cholinesterase.

Groups	On Admission		On Discharge		RBC ChE gain	Students Paired t-test
	Mean	SD	Mean	SD		
Normal (I)	317.33	27.65	317.33	27.65	0.00	t = 0.00 p = 1.00 Not significant
Routine treatment (II)	142.23 (44.80%)	9.71	175.37 (55.30%)	13.39	33.13 (10.50%)	t = 11.69 p = 0.001 Significant
Routine treatment + VitC (III)	182.62 (57.50%)	25.79	223.19 (70.30%)	34.31	40.57 (12.80%)	t = 6.46 p = 0.001 Significant
Routine treatment + ALA (IV)	150.81 (47.50%)	32.09	198.52 (62.50%)	42.98	47.70 (15.00%)	t = 5.88 p = 0.001 Significant
Routine treatment + ALA + VitC (V)	146.71 (46.20%)	9.88	232.08 (73.10%)	47.89	85.38 (26.90%)	t = 9.06 p = 0.001 Significant

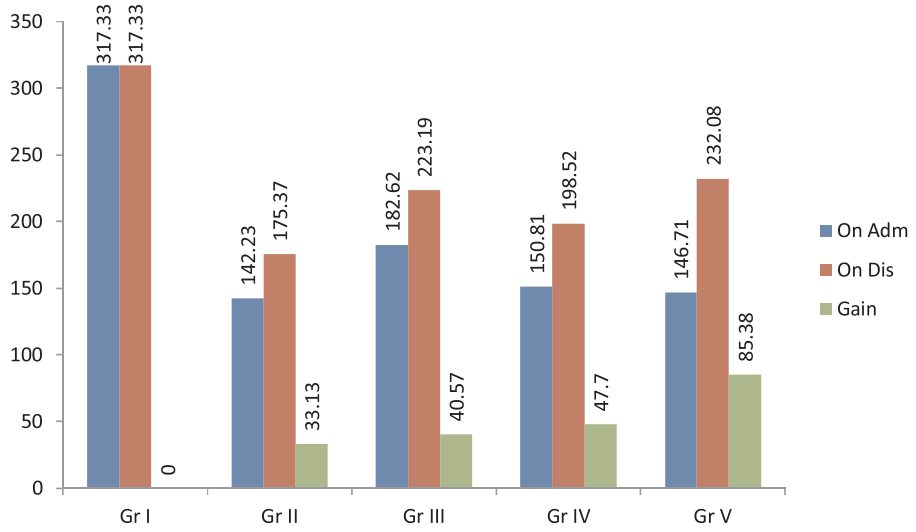

Chart 2. Comparison of levels of RBC Cholinesterase on different methods of treatment.

Table 3
Comparison of levels (in moles/ml) and the percentage of benefit of different methods of treatment on Malonyldialdehyde (MDA).

Groups	On Admission		On Discharge		MDA level	Students Paired t-test
	Mean	SD	Mean	SD		
Normal (I)	1.26	0.08	1.26	0.08	0.00	t = 0.00 p = 1.00 Not significant
Routine treatment (II)	2.03 (161%)	0.89	2.37 (188%)	0.63	0.34 (26.90%)	t = 3.27 p = 0.003 Significant
Routine treatment + VitC (III)	2.20 (175%)	0.41	2.54 (201%)	0.56	0.34 (26.90%)	t = 3.63 p = 0.002 Significant
Routine treatment + ALA (IV)	1.87 (148%)	0.11	2.10 (167%)	0.23	0.23 (18.30%)	t = 6.05 p = 0.001 Significant
Routine treatment + ALA + VitC (V)	2.10 (167%)	0.74	2.01 (159%)	0.56	−0.08 (6.30%)	t = 1.74 p = 0.09 Not Significant

the oxidative stress or drop in antioxidant levels is warranted. Furthermore, the exact pharmacological mechanism by which the antioxidant supplementation with vitamin C and alpha lipoic acid help in reducing the oxidative stress also needs to be studied.

Oral supplementation of alpha lipoic acid for longer period will enhance the total antioxidant levels, and decrease the oxidative stress. It would be interesting to study the supplement of vitamin C and alpha lipoic acid to chronic amitriptyline poisoning cases. Also we suggest that I.V. administration of vitamin C and alpha lipoic acid would be more beneficial to acute amitriptyline poisoning cases.

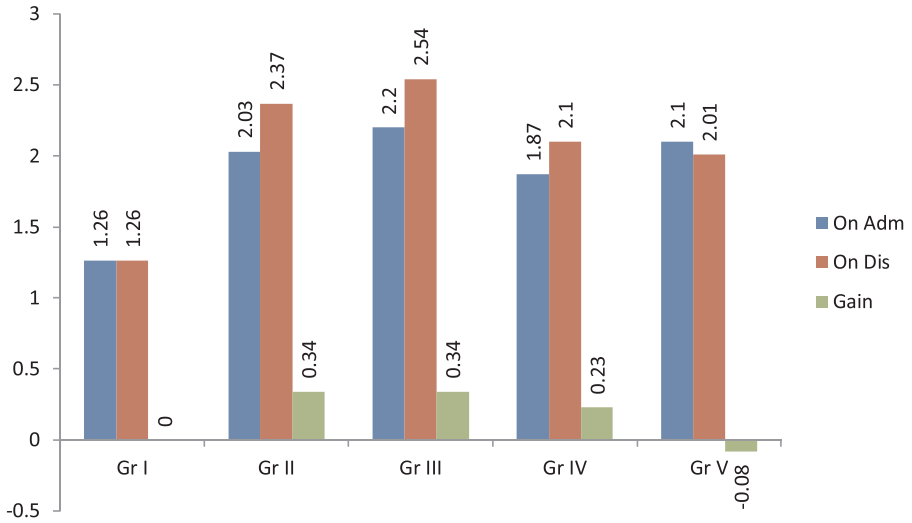

Chart 3. Comparison of levels of Malonyldialdehyde (MDA) on different methods of treatment.

Table 4
Comparison of levels (in m. moles/l) and the percentage of benefit of different methods of treatment on total anti-oxidant level.

Groups	On Admission		On Discharge		Total gain	Students Paired t-test
	Mean	SD	Mean	SD		
Normal (I)	1.67	0.16	1.67	0.16	0.00	t = 0.00 p = 1.00 Not significant
Routine treatment (II)	0.97 (58.9%)	0.15	0.99 (59.30%)	0.16	0.02 m(1.10%)	t = 1.90 p = 0.07 Not significant
Routine treatment + VitC (III)	1.36 (81.4%)	0.30	1.55 (76.00%)	0.30	0.19 (11.30%)	t = 9.30 p = 0.001 Significant
Routine treatment + ALA (IV)	1.27 (76.0%)	0.09	1.27 (76.0%)	0.09	0 (0%)	t = 0.01 p = 0.98 Not significant
Routine treatment + ALA + VitC (V)	0.92 (55.1%)	0.12	1.44 (86.20%)	0.47	0.52 (31.10%)	t = 4.89 p = 0.001 Significant

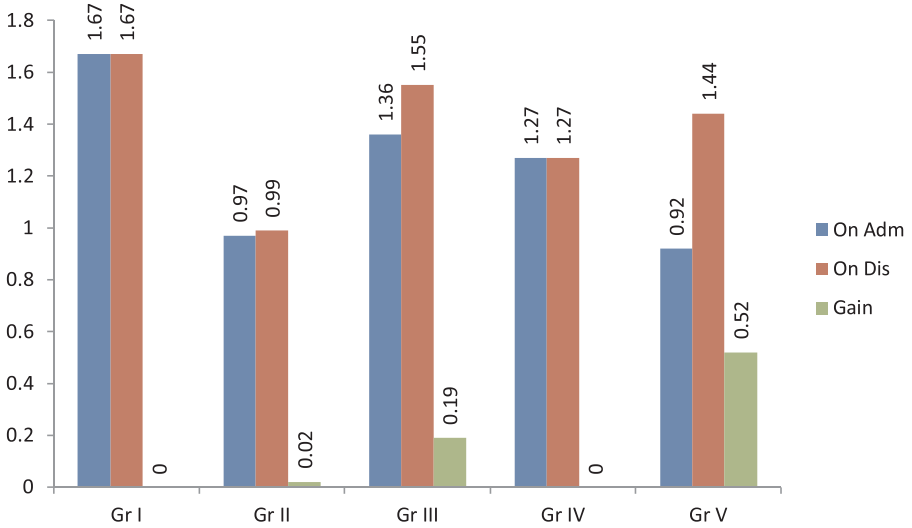

Chart 4. Comparison of levels of total antioxidants on different methods of treatment.

6. Conclusion

Antioxidants levels were increased with treatment after supplementation with vitamin C and alpha lipoic acid. We suggest that antioxidant status of acute amitriptyline poisoning cases should be considered for more effective recovery and that diets low in antioxidants may render the recovery slow [31].

This study provides quantitative recommendations for the intake of

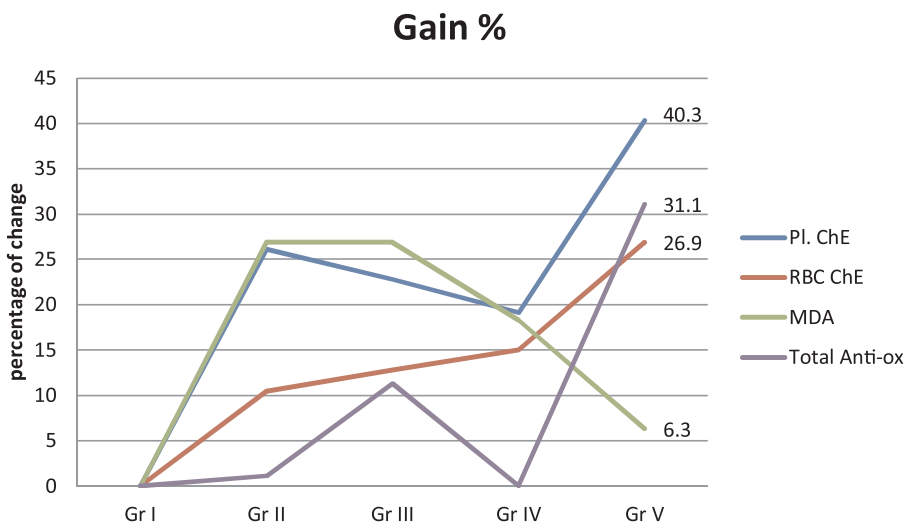

Chart 5. Percentage of benefit (gain) of different method of treatment on Plasma and RBC Choline esterase, Malonlydialdehyde and total antioxidant levels.

Table 5
Demographic Characteristics.

Groups	Male			Female			Total Subjects
	Number	Age Distribution		Number	Age Distribution		
		Mean	SD		Mean	SD	
Normal (I)	16	30.92	8.46	14	33.56	16.87	30
Routine treatment (II)	18	34.13	9.54	12	30.00	6.53	30
Routine treatment + VitC (III)	12	31.85	10.96	9	30.93	12.40	21
Routine treatment + ALA (IV)	13	33.00	11.41	14	29.40	11.25	27
Routine treatment + ALA + VitC (V)	14	32.67	11.87	10	34.75	15.62	24
Total	73 (55.30%)			59 (44.70%)			132

vitamin C and alpha lipoic acid for fast recovery in acute amitriptyline poisoning cases. It also showed that oral supplementation with vitamin C and alpha lipoic acid to acute amitriptyline poisoning cases considerably reduced the oxidative stress, and it also enhanced the total antioxidant levels of these patients. This will also facilitate in reducing the number of days of stay in intensive care units of these poisoning patients, and also supplementation of vitamin C and alpha lipoic acid reduces the long-term side effects of the drug such as Amitriptyline induced delayed Neuropathy (AIDN).

The maximum effect of supplementation of vitamin C and alpha lipoic acid was in Group V (Routine standard treatment + vitamin C + alpha lipoic acid), who received the maximum dosage of vitamin C and alpha lipoic acid (5–13 days). It is clear that the supplementation should be for a longer period to have the maximum beneficial effect (reduced oxidative stress and increased total antioxidant status). In our opinion oral supplementation will be more effective for chronic amitriptyline poison cases for a longer duration and for acute amitriptyline poisoning cases intravenous route will be more effective.

The recovery and regeneration of plasma Cholinesterase were rapid when compared to RBC Cholinesterase in all groups of acute amitriptyline poisoning cases. The average age of acute amitriptyline poisoning cases were 33 for male and 32 for female, and this study shows that the amitriptyline poisoning is seen more in males than in females.

Conflict of interest statement

We would like to mention that there is no any conflict of interest, no financial disclosures to make and no any non-financial interest and relationship, with regard to the below mentioned research work.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2019.04.002.

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