Antioxidants Supplementation in Acute Amitriptyline Abuse for Pain

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Abstract
The fundamental aim of this study is to establish the role of antioxidant supplementation in alleviating acute amitriptyline induced oxidative stress. The effect of supplementation was compared on treatment of acute amitriptyline intoxication cases for pain management, with alpha lipoic acid (ALA) alone or with vitamin C, with that of healthy individuals (group I), and those receiving only routine standard treatment (RST) as control (group II). A total of 132 human subjects divided into 5 groups were supplemented with either placebo, RST, RST with vitamin C, RST with ALA, or RST with vitamin C, and ALA. Results of this study revealed that the decrease in the level of oxidative stress and enzyme activity was observed among those supplemented with either alpha lipoic acid alone or along with vitamin C, with a slightly more decrease in the latter group. \( P \) value of <0.001 was considered statistically significant. The percentage of benefit of treatment on supplementation with vitamin C and alpha lipoic acid showed a marked increase in group V cases after supplementation with both in combination. The results provided 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.

Keywords Alpha lipoic acid · Vitamin C · Free radicals · Oxidative stress · Amitriptyline abuse

Abbreviations
ALA Alpha lipoic acid
LDH Lactate dehydrogenase
CK Creatine kinase
SOD Superoxide dismutase
RST Routine standard treatment

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Introduction

Chronic low back pain is ubiquitous in the modern society of predominantly sedentary lifestyle of desk work. The incidence of chronic pain among the US population is recorded to be about 36%, inciting a socioeconomic burden to the health-care industry of about $43 billion per year, while that of 19% in Europe [1]. The unrelenting pain leads to the abuse of pain medications, especially those of the steroidal and non-steroidal anti-inflammatory drugs. The quest for a more potent pain medication has led to the abuse of opioids to a lesser extent due to their not-so-easy availability over-the-counter, and more of the antidepressant analogics like amitriptyline due to their free availability over-the-counter. Low-dose amitriptyline has been a drug of choice for management of chronic low back pain [2, 3].

Acute amitriptyline intoxication caused oxidative stress by induction of free radicals and has profound effect on the structural and functional machinery of major bio-molecules like lipids, protein, and nucleic acids [4]. The American Association of Poison Control Centers (AAPCC) reported 7,430 cases of intoxication due to amitriptyline, with 9% cases in children less than 6 years of age and 32% cases reporting the intoxication as unintentional [5]. The major complications of amitriptyline intoxication noted in adults were that of respiratory insufficiency, hypotension, and arrhythmia, while children commonly suffered from hyperglycemia, leukocytosis, tachycardia, lethargy, and convulsions. Though these initial symptoms resolved with supportive measures, the oxidative stress-induced sequel is vaguely studied.

Amitriptyline exerts its antidepressant and analgesic effect by blocking the neuronal reuptake of nor-adrenaline and serotonin. Nor-adrenaline reuptake inhibition enhances analgesic effects, mainly through α₂-adrenergic receptors in the dorsal horn of the spinal cord [6]. The reason for the increasing efficacy for hypersensitivity of spinal α₂-adrenergic receptors stimulation is that nerve injury changes the function of α₂-adrenergic receptors in the dorsal horn of the spinal cord, while at the same time the interaction with the cholinergic inter-neurons strengthens it. Thus, an increase in nor-adrenaline in the spinal cord plays a crucial role in the inhibitory effects of antidepressants on neuropathic pain [7].

Acute amitriptyline intoxication in subjects abusing it for unrelenting pain induces oxidative stress due to free radical formation [8–10]. This leads to derangement in cellular levels of enzyme activity of lactate dehydrogenase (LDH) [11] and creatine kinase (CK) in metabolically active tissues like skeletal muscle and liver, leading to disruption in activity of protective enzymes in these tissues [12]. In vivo antioxidants like superoxide dismutase (SOD) [13], catalase, and glutathione family play a major role in free radical scavenging [14, 15]. These when supplemented externally by antioxidants such as vitamin C and alpha lipoic acid provide additive benefit in alleviating free radical-induced tissue damage [16, 17]. Hence, this study focused to correlate the effect of various enzyme levels such as LDH, CK, SOD, catalase, and glutathione, the subject after supplementation of alpha lipoic acid (ALA) alone or with vitamin C to prescribe comprehensive medicine.

Materials and Methods

The study involved 132 subjects divided into 5 groups based on the type of antioxidant supplementation they received. All the patients admitted to the Intensive Medical Care Unit (IMCU) and toxicology ward were screened for acute intoxication. With a prior
approval from the ethical committee for research and experimental study and consent from the subject or care-taker, the supplementation was initiated orally after a routine supportive care was completed, and a stable general condition was established by the attending physician.

**Inclusion Criteria**

All patients acutely intoxicated with amitriptyline, assessed drowsy but responding to verbal commands as per the Edinburg scale of classification of the grade of coma, were selected. The subjects were divided into four groups with healthy volunteers forming the fifth group. The groups were classified as follows:

- **Group I**: 30 healthy volunteers (15 males and 15 females) with mean age 32 years
- **Group II**: 30 patients (18 males and 12 females) with mean age 34 years, who received only routine standard treatment (RST)
- **Group III**: 21 patients (12 males and 9 females) with mean age 32 years, who received (RST) + vitamin C supplementation
- **Group IV**: 27 patients (13 males and 14 females) with mean age 31 years, who received (RST) + alpha lipoic acid supplementation
- **Group V**: 24 patients (14 males and 10 females) with mean age 34 years, who received (RST) + vitamin C and alpha lipoic acid supplementation

**Exclusion Criteria**

- Unconscious and not responding to verbal commands as per the Edinburg scale of classification of the grade of coma were excluded.
- Age less than 18 years and more than 60 years were not included considering the confounding effect of hormonal and metabolic alterations.
- Have taken other drugs along with amitriptyline were not included, to avoid cross-interference.
- False-positive TLC (thin layer chromatography) and spectra (UV–Vis) negative were excluded.

**Enzyme Levels Estimation**

About 10 ml of venous blood from the ante-cubital vein of each subject and 50 ml of gastric aspirate were collected from all patients who are directly admitted to IMCU for thin later chromatography (TLC).

**Lactate Dehydrogenase (LDH)** Serum total LDH was analyzed by ‘semi-auto-analyzer Micro-lab 200’ by modified IFCC method. LDH catalyzes the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance, which is proportional to the LDH activity in the sample. Initial absorbance \( A_0 \) after 1 min and final absorbance reading after every 1, 2, and 3 min were recorded. The mean absorbance change per minute \( (\Delta A/min) \) is calculated. Total LDH activity in micro-liter at 37 °C = \( \Delta A/min \times 8095 \).
Creatine Kinase (CK) Analysis of isoenzymes of CK was carried out by ‘HYDRASYS system SEBIA, PN 1210.’ The HYDRASYS SEBIA system is a semi-automated multi-parameter electrophoresis system. Commercial kits ‘HYDRAGEL 7 ISO-CK’ are available for the analysis. On HYDRAGEL 7 ISO-CK and HYDRAGEL ISO-CK 15/30 gels, the BB fraction is the most anodic, the MM fraction is the most cathodic, and the MB is intermediary. All CK isoenzymes catalyze the same reaction that is utilized in their visualization.

Catalase Activity Catalase was assayed according to the method of Takahara et al. (1960) [18]. To 1.2 ml of phosphate buffer (0.05 M, pH 7.0), 0.2 ml of the hemolysate was added and the enzyme reaction was started by the addition of 1.0 ml of hydrogen peroxide (0.03 M in phosphate buffer) solution. The decrease in absorbance was measured at 240 nm at 30-s intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity is expressed as µmoles of H₂O₂ decomposed/min/ mgHb.

Superoxide Dismutase Activity SOD was assayed by the method of Misra and Fridovich (1972) [19]. Of hemolysate, 0.1 ml was added to tubes containing 0.75-ml ethanol and 0.15-ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant, added 0.5 ml of EDTA (0.6 mM) solution and 1 ml of carbonate-bicarbonate buffer (0.1 M, pH 10.2). The reaction was initiated by the addition of 0.5 ml of epinephrine (1.8 mM) and the increase in absorbance at 480 nm was measured with UV spectrophotometer. The enzyme activity is expressed as 50% inhibition of epinephrine auto-oxidation/min/mgHb.

Glutathione Peroxidase Activity The activity of glutathione peroxidase was determined by the method of Rotruck et al. (1973) [20]. In brief, 0.4 ml of buffer, 0.1 ml of sodium azide, 0.2 ml of reduced glutathione, an aliquot of hemolysate, 0.1 ml of H₂O₂, and distilled water were taken to make a final volume of 2.0 ml. The tubes were incubated at 37 °C for 10 min. The reaction was stopped by adding 0.56 ml of 10% TCA. To determine the residual glutathione content, the supernatant was removed by centrifugation; 3.0 ml of disodium hydrogen phosphate and 1 ml of DTNB reagent were added and read at 412 nm. A blank was treated with only disodium hydrogen phosphate and 1.0 ml of DTNB reagent. The activity of glutathione was expressed as micrograms of GSH utilized/min/mgHb.

Fig. 1 a The average LDH electrophoresis levels are suggestive of a maximum elevation in the LDH₅ fragment. The total LDH levels were 588u/l, with the individual values of LDH₁, 14.04 (2.4%); LDH₂, 67.8 (11.6%); LDH₃, 70.2 (12.0%); LDH₄, 118.17 (20.2%); and LDH₅, 314.7 (53.8%), respectively. b The average CK electrophoresis levels are suggestive of maximal elevation in the CK–MM fragment. The total CK level was 426u/l, which was attributable only to the CK–MM fragment (100%) while CK–MB and CK–BB were not traceable.
Table 1  Comparison of levels of Superoxide Dismutase in µmoles/mgHb

| Groups                         | On admission | On discharge | Difference (decrease) | Multiple comparison by Bonferroni t test | (One-way ANOVA F test: $F = 7.11$) Significance |
|-------------------------------|--------------|--------------|-----------------------|-----------------------------------------|-------------------------------------------------|
|                               | Mean (% change from baseline) | SD | Mean (% change from baseline) | SD | Mean (% change from baseline) | $t$ | $P$ | Not significant |
| Normal (I)                   | 4.5 (0)      | 0.74         | 4.5 (0)               | 0.74 | 0 (0)                        | $t=0.00$ | $P=1.00$ | Not significant |
| Routine treatment (II)       | 6.33 (40.7)  | 0.87         | 6.21 (38)             | 0.5 | 0.12 (2.7)                   | $t=0.15$ | $P=0.90$ | Not significant |
| Routine treatment + VitC (III)| 6.34 (40.9)  | 0.78         | 5.95 (32.2)           | 0.84 | 0.39 (8.7)                   | $t=2.01$ | $P=0.05$ | Significant     |
| Routine treatment + ALA (IV) | 6.47 (43.7)  | 0.68         | 6.29 (39.7)           | 0.1 | 0.18 (4.0)                   | $t=0.22$ | $P=0.92$ | Not significant |
| Routine treatment + VitC+ALA (V)| 6.52 (44.9)  | 0.94         | 4.85 (33.4)           | 0.57 | 1.67 (11.5)                  | $t=5.11$ | $P=0.001$ | Significant      |
Total Antioxidant Status  Total antioxidant status of the sample was measured by a commercial kit, supplied by the Randox Laboratories®. About 20μL of plasma was added to 1 mL of chromogen and incubated at room temperature for 1 min. The initial absorbance (A1) was measured at 600 nm. Another 200 μl of substrate was added to it and incubated at room temperature for 3 min and the final absorbance (A2) was measured again at 600 nm. A blank and a standard were run simultaneously, and the initial absorbance (A1) and final absorbance (A2) were measured at 600 nm for both the blank and standard, respectively [21].

\[A2-A1 = DA\] of the sample/ blank/ standard were individually determined,

\[\text{Factor} = \frac{\text{Concentration of the standard}}{\text{DA blank} - \text{DA standard}}\]

Total Antioxidant status in m.mol/L = Factor X (DA blank – DA sample)

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, ALA, and both combined were analyzed for each group and expressed as a percentage of change from baseline.

Results

The samples along with the controls were run on the thin layer chromatograph, and the following results were obtained based on the $R_f$ values. The presence of oxidative stress was confirmed by the elevation of the LDH$_5$ and CK-MM fractions of LDH and CK enzymes, respectively (Fig. 1). The mean levels of superoxide dismutase on admission compared to that on discharge in all the groups showed a significant reduction in the activity in groups

![Fig. 2 Comparison of levels of superoxide dismutase in μmoles/mgHb](image-url)
| Groups                        | On admission | On discharge | Difference (decrease) | Multiple comparison by Bonferroni t test | (One-way ANOVA F test: $F=9.45$) Significance |
|------------------------------|--------------|--------------|------------------------|------------------------------------------|-----------------------------------------------|
|                              | Mean (% change from baseline) | SD | Mean (% change from baseline) | SD | Mean (% change from baseline) |                                          |
| Normal (I)                   | 2.98 (0)     | 0.58         | 2.98 (0)               | 0.58 | 0 (0)                        | $t=0.00 \ P=1.00$ Not significant         |
| Routine treatment (II)       | 5.09 (71)    | 0.71         | 4.97 (67)              | 0.73 | 0.12 (4)                     | $t=0.14 \ P=0.89$ Not significant         |
| Routine treatment + VitC (III)| 5.28 (59)    | 0.67         | 4.43 (49)              | 0.63 | 0.85 (10)                    | $t=4.08 \ P=0.001$ Significant           |
| Routine treatment + ALA (IV)  | 4.98 (67)    | 0.22         | 3.82 (39)              | 0.55 | 1.16 (28)                    | $t=4.80 \ P=0.001$ Significant           |
| Routine treatment + VitC+ALA (V)| 5.58 (89)  | 0.69         | 3.27 (52)              | 0.49 | 2.31 (37)                    | $t=15.11 \ P=0.001$ Significant          |
supplemented with either of the antioxidant or their combination. The reduction in levels was highest in the latter (Table 1). The return to levels closer to baseline values was observed in group V supplemented with a combination of vitamin C and alpha lipoic acid, viz., 4.85µmoles/mg hemoglobin. Though supplementation with vitamin C alone was beneficial, the additive effect was more beneficial (Fig. 2).

The mean levels of catalase enzyme levels on admission compared to that on discharge showed clearly in group V that the combination was much more effective (Table 2). The combination provided a dramatic reduction in the enzyme level in group V to 2.31µmoles/mg hemoglobin (Fig. 3).

Furthermore, the mean level of the glutathione on admission and discharge showed combined supplementation has maximal effect. The levels of glutathione peroxidase showed maximum reduction of 0.97 µg/mg hemoglobin, with a percentage difference of 17.6% (Table 3) (Fig. 4). The level of total serum antioxidant levels was maximal in group V followed by group III (Table 4) (Fig. 5).

The percentage change from baseline values of all the enzymes showed that maximum benefit of decrease in enzyme activity was found in group V (Fig. 6). The demographic distribution showed a slight male preponderance of 55.3% against 44.7% female population, with a mean age distribution of 33 for the males and 32 for the females.

**Discussion**

Amitriptyline abuse either with suicidal intention for unrelenting pain or want of better pain relief has been widely observed. Though routine treatment in an intensive medical care unit does provide symptomatic relief and recovery, the long-term sequel of this intoxication in the form of oxidative stress needs to be addressed. Though in vivo antioxidants and free radical scavenging mechanisms do provide considerable protection against free radical-induced tissue damage, their effects fall well short of the amount of protection needed for the tissues, culminating in measurable tissue damage. The external supplementation of antioxidants directly provides for free radical scavenging and potentiating of in vivo protection mechanisms. A highly water-soluble antioxidant like vitamin C along with a versatile lipid soluble antioxidant like alpha lipoic acid do adequately provide for...
| Groups                        | On admission (Mean (% change from baseline), SD) | On discharge (Mean (% change from baseline), SD) | Difference (decrease) (Mean (% change from baseline), SD) | Multiple comparison by Bonferroni t test | Significance (One-way ANOVA F test: $F=3.78$) |
|-------------------------------|-----------------------------------------------|-----------------------------------------------|----------------------------------------------------------|---------------------------------------|--------------------------------------------|
| Normal (I)                   | 5.53 (0), 0.74                               | 5.53 (0), 0.74                                 | 0 (0)                                                   | $t=0.00$ $P=1.00$                       | Not significant                            |
| Routine treatment (II)        | 5.88 (6.3), 0.65                              | 5.83 (5.4), 0.61                               | 0.05 (0.9)                                              | $t=0.25$ $P=0.80$                       | Not significant                            |
| Routine treatment + VitC (III)| 5.58 (0.9), 0.65                              | 5.1 (4.7), 0.61                                | 0.48 (3.8)                                              | $t=2.20$ $P=0.02$                       | Significant                               |
| Routine treatment + ALA (IV)  | 6.46 (16.8), 0.98                             | 6.04 (9.2), 0.65                               | 0.42 (7.6)                                              | $t=2.21$ $P=0.03$                       | Significant                               |
| Routine treatment + VitC+ALA (V)| 6.62 (19.7), 0.74                         | 5.65 (2.1), 0.51                               | 0.97 (17.6)                                             | $t=5.69$ $P=0.001$                       | Significant                               |
Vitamin C as a free radical scavenger has been widely studied [22, 23]. Alpha lipoic acid on the other hand, in addition to having its own antioxidant effect, also replenishes other antioxidants like vitamins C and E.

The proof of oxidative stress in amitriptyline intoxication was established by the rise of acute phase reactants such as lactate dehydrogenase and creatine kinase. We observed that muscle- and liver-specific LDH fraction, LDH5, and CK-MM were predominantly seen on gel electrophoresis. It was also observed that supplementation with vitamin C and alpha lipoic acid produced a dramatic decrease in the activity of enzymes like superoxide dismutase, catalase, and glutathione, indicating that free radical damage was well counteracted by these ex vivo antioxidants leaving only a little role for these in vivo antioxidants to act upon. Though oral supplementation was initiated immediately upon completion of the routine standard treatment, it is not clear as to whether an intravenous administration of antioxidants could be more beneficial.

A rise in serum total antioxidant levels also supported the fact that antioxidants potentiate each other. Despite these, a direct cause-effect relationship could not be established between the levels of amitriptyline that could trigger the oxidative stress and cause derangement in the levels of free radical scavenging enzymes. Though the supplemented antioxidants potentiated their in vivo counterparts, a dose–effect relationship could not be established, to cap the maximum permissible doses of these supplements. The effect of rebound in the oxidative stress on discontinuation of the antioxidant supplementation also needs to be studied. Yet another vaguely understood part is the role of chronic intoxication with deranged drug clearance leading to drug build-up in actively metabolizing tissues such as the muscles and liver.

**Conclusion**

Antioxidant supplementation is an inevitable need in drug intoxication-induced oxidative stress. Vitamin C and alpha lipoic acid have profound effect in protecting against free radical-induced tissue damage, especially in acute intoxication with pain medications such as amitriptyline. A long-term supplementation is recommended to protect against free radical damage sequel. Yet again, drug-induced systemic derangements should be cautioned in persons on supplementation like any other medication.
Table 4  Comparison of levels of total antioxidants in m.mol/L

| Groups                        | On admission | On discharge | Difference (decrease) | Multiple comparison by Bonferroni t test | (One-way ANOVA F test: $F = 20.8$) Significance |
|-------------------------------|--------------|--------------|-----------------------|-----------------------------------------|-------------------------------------------------|
|                               | Mean (% change from baseline) | Mean (% change from baseline) | Mean (% change from baseline) | $t$ | $P$ |
| Normal (I)                    | 1.67 (0)     | 0.16         | 1.67 (0)              | 0(0)                                   | $t=0.00$ $P=1.00$                               | Not significant                                 |
| Routine treatment (II)        | 0.97 (58.9)  | 0.15         | 0.99 (59.3)           | 0.16                                   | $t=1.90$ $P=0.07$                               | Not significant                                 |
| Routine treatment + VitC (III)| 1.36 (81.4)  | 0.30         | 1.55 (76.0)           | 0.30                                   | $t=9.30$ $P=0.001$                              | Significant                                     |
| Routine treatment + ALA (IV)  | 1.27 (76.0)  | 0.09         | 1.27 (76.0)           | 0.09                                   | $t=0.01$ $P=0.98$                               | Not significant                                 |
| Routine treatment + VitC+ALA (V)| 0.92 (55.1) | 0.12         | 1.44 (86.2)           | 0.47                                   | $t=4.89$ $P=0.001$                              | Significant                                     |
This study provides a quantitative recommendation of supplementation with vitamin C and alpha lipoic acid for an accelerated recovery in subjects with pain medication abuse. Oral supplementation has considerably reduced oxidative stress-induced systemic damage and could be provided as an adjunct. A delay in supplementation could cause lack of harnessing the maximum potential of these antioxidants in providing superior protection to these intoxicated subjects.

**Author Contribution** S. Hameed Kadar Ali—conceptualized the work. K. Wasim Ali Raja—performed the work. N. Irfan—wrote the manuscript. Mohammad Habeeb—statistical analysis. Y. Ismail—guidance and analysis.

**Data and Materials Availability** The authors confirm that the data analyzed and generated from this research findings are provided within this article.

**Declarations**

**Ethical Approval** DME office ref.no.18509/E5/1/2005 Dated: 20.12.05.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Competing Interests** The authors declare no competing interests.
References

1. Turk, D. C. (2002). Clinical effectiveness and cost-effectiveness of treatments for patients with chronic pain. *Clinical Journal of Pain, 18*(6), 355–365. doi:10.1097/00002508-200211000-00003

2. Steiner, T. J., Stovner, L. J., Al Jumah, M., et al. (2013). Improving quality in population surveys of headache prevalence, burden and cost: Key methodological considerations. *The Journal of Headache Pain, 14*(1), 87. doi:10.1186/1129-2377-14-87

3. Urquhart, D. M., Wluka, A. E., Sim, M. R., et al. (2016). Is low-dose amitriptyline effective in the management of chronic low back pain? Study protocol for a randomised controlled trial. *Trials, 17*(1), 514. doi:10.1186/s13668-016-1637-1

4. Kadar Ali, S. H., & Raja, W. A. (2019). The effect of antioxidants in acute amitriptyline poisoning. *Toxicology Reports, 6*, 380–388. https://doi.org/10.1016/j.toxrep.2019.04.002

5. Mowry, J. B., Spyker, D. A., Cantilena, L. R., Bailey, J. E., & Ford, M. (2013). 2012 Annual Report of the American Association of Poison Control Centers’ National Poison Data System (NPDS): 30th Annual Report. *Clinical Toxicology (Philadelphia, Pa.), 51*(10), 949–1229. doi:10.3109/15563650.2013.863906

6. Obata H (2017) Analgesic mechanisms of antidepressants for neuropathic pain. *International Journal of Molecular Sciences. 18*(11). doi:10.3390/ijms18112483

7. Chen, M., Hoshino, H., Saito, S., Yang, Y., & Obata, H. (2017). Spinal dopaminergic involvement in the antihyperalgesic effect of antidepressants in a rat model of neuropathic pain. *Neuroscience Letters, 649*, 116–123. https://doi.org/10.1016/j.neulet.2017.04.017

8. Grant, M. M., & Weiss, J. M. (2001). Effects of chronic antidepressant drug administration and electroconvulsive shock on locus coeruleus electrophysiologic activity. *Biological Psychiatry, 49*(2), 117–129. doi:10.1016/s0006-3223(00)00936-7

9. Moreno-Fernández, A. M., Cordero, M. D., Garrido-Maraver, J., et al. (2012). Oral treatment with amitriptyline induces coenzyme Q deficiency and oxidative stress in psychiatric patients. *Journal of Psychiatric Research, 46*(3), 341–345. https://doi.org/10.1016/j.jpsychires.2011.11.002

10. Bautista-Ferrufino, M. R., Cordero, M. D., Sánchez-Alcázar, J. A., et al. (2011). Amitriptyline induces coenzyme Q deficiency and oxidative damage in mouse lung and liver. *Toxicology Letters, 204*(1), 32–37. https://doi.org/10.1016/j.toxlet.2011.03.033

11. Clinical Chemistry Concepts And Applications: Revised Edition (Pb 2015 | 9788123926629 | Anderson. http://www.cbsdp.co.in/clinical-chemistry-concepts-and-applications-revised-edition-pb-2015-9788123926629-anderson.html. Accessed 6 May 2020.

12. Puleo, P. R., Meyer, D., Warthen, C., et al. (1994). Use of a rapid assay of subforms of creatine kinase MB to diagnose or rule out acute myocardial infarction. *New England Journal of Medicine, 331*(9), 561–566. https://doi.org/10.1056/NEJM199409013310901

13. Stralin, P., & Marklund, S. L. (1994). Effects of oxidative stress on expression of extracellular superoxide dismutase, CuZn-superoxide dismutase and Mn-superoxide dismutase in human dermal fibroblasts. *The Biochemical Journal, 298*(2), 347–352. https://doi.org/10.1042/bj2980347

14. Atalla, S. L., Toledo-Pereyra, L. H., Mackenzie, G. H., & Cederna, J. P. (1985). Influence of oxygen-derived free radical scavengers on ischemic livers. *Transplantation, 40*(6), 584–589. https://doi.org/10.1097/00007890-198512000-00002

15. Burk, R. F., Lawrence, R. A., & Lane, J. M. (1980). Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration. Effect of selenium deficiency. *Journal of Clinical Investigation, 65*(5), 1024–1031. https://doi.org/10.1172/JCI109754

16. Vitamin C - Health Encyclopedia - University of Rochester Medical Center. https://www.urmc.rochester.edu/content.aspx?contenttypeid=19&contentid=VitaminC. Accessed 6 May 2020.

17. Hager, K., Marahrens, A., Kenklies, M., Riederer, P., & Münch, G. (2001). Alpha-lipoic acid as a new treatment option for Alzheimer [corrected] type dementia. *Archives of Gerontology and Geriatrics, 32*(3), 275–282. https://doi.org/10.1016/s0167-4943(01)00104-2

18. Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y., & Nishimura, E. T. (1960). Hypocatalasemia: A new genetic carrier state. *Journal of Clinical Investigation, 39*, 610–619. https://doi.org/10.1172/JCI104075

19. Misra, H. P., & Fridovich, I. (1977). Superoxide dismutase: “Positive” spectrophotometric assays. *Analytical Biochemistry, 79*(1–2), 553–560. https://doi.org/10.1016/0003-2697(77)90429-8

20. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hokestra, W. G. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science (80-), 179*(4073), 588–590. https://doi.org/10.1126/science.179.4073.588
21. Miller, N. J., & Rice-Evans, C. A. (1997). Factors influencing the antioxidant activity determined by the ABTS.+ radical cation assay. Free Radical Research, 26(3), 195–199. https://doi.org/10.3109/10715769709097799

22. Smirnoff, N. (2001). l-Ascorbic acid biosynthesis. Vitamins and Hormones, 61, 241–266. https://doi.org/10.1016/s0083-6729(01)61008-2

23. Bielski, B. H. J., Comstock, D. A., & Bowen, R. A. (1971). Ascorbic acid free radicals. I. Pulse radiolysis study of optical absorption and kinetic properties. Journal of the American Chemical Society, 93(22), 5624–5629. https://doi.org/10.1021/ja00751a006

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