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THE PROTECTIVE EFFECT OF DIFFERENT DOSES OF ALPHA LIPOIC ACID AGAINST HEPATOTOXICITY OF SODIUM NITRITE IN RAT

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ABSTRACT

Sodium nitrite ($NaNO_2$) presents in vegetables and is routinely used as a color fixative and as a preservative for meat and fish. Chronic ingestion of $NaNO_2$ induced abnormalities in the biochemical parameters associated with the oxidative stress. The present study was carried out to investigate the protective efficacy of alpha lipoic acid (ALA) on the liver and kidney function tests and the oxidative stress alteration induced by $NaNO_2$ in male albino rats; moreover the microscopical examination was studied. Sixty male albino rats, divided randomly into 6 groups, each of 10 rats were treated for 30 days. Group (1) served as control and fed the basal diet, group (2) fed basal diet and supplemented with $NaNO_2$(100mg/kg diet/day),group (3,4,5 &6) fed basal diet and received ALA at (100,200,300 & 400 mg/kg diet/day), respectively. Rats treated with $NaNO_2$ showed significant elevation of Liver enzymes, bilirubin, urea and creatinine with reduction in albumin levels. In addition to, increased MDA and NO concentrations with reduced GPx and CAT activities. But, all these analytes were reversed and returned near normal upon ALA administration. Finally, nitrite proved to have deleterious impacts on body organs and ALA succeeded to alleviate these bad effects.

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INTRODUCTION

Sodium nitrite ($NaNO_2$) is a pure white or slightly yellowish crystalline powder. It is very soluble in water and hygroscopic. It is also slowly oxidized by oxygen in the air to sodium nitrate ($NaNO_3$). The compound is a strong oxidizing agent. It is used as a color fixative and preservative in meats and fish (Akpabio *et al.,* 2013).Excessive quantities of nitrate and nitrite consumption through food can be harmful to health (Chan, 2011).The $NaNO_2$ and other additives may react with amines of the foods in the stomach and produce nitrosamines and free radicals, like superoxide anion, hydroxyle, peroxynitrite and nitrogen oxide radicals (Hassan *et al.,* 2009 and Marouf *et al.,* 2011). These free radicals, known to cause oxidative stress, an excessive production of ROS above the body's antioxidant capacity, has been implicated in the development of many physiological conditions (Abdul-Ameer and Abed, 2012).

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Alpha lipoic acid (ALA) is a unique antioxidant because it has beneficial effects on energy production, and is also an essential cofactor of mitochondrial complexes. ALA and its metabolites are capable of scavenging a variety of reactive oxygen species (ROS) such as peroxynitrite, nitric oxide, hydroxyl radical, superoxide anion, peroxyl radical and hydrogen peroxide. Furthermore, ALA appears to regenerate other endogenous antioxidants (Morakinyo *et al.,* 2012). Thus, their administration may augment the function of endogenous free radical scavenging and consequently, decrease the deleterious effects of nitrites on the body cells. Antioxidants have been subjected to many epidemiological studies that have related their consumption to a reduction in the incidence of oxidative damage related diseases. Therefore, much attention has been focused on the use of antioxidants (especially natural antioxidants) for improvement of human health (Liu and Ng, 2006). The present study aimed is to investigate the protective effect of Alpha-lipoic acid toward the hepatotoxicity induced by $NaNO_2$ via evaluating some serum biochemical functions of

liver and kidney, analyzing some oxidative stress indices and finally determining the hemogram of male albino rats.

MATERIALS AND METHODS

Chemicals: Alpha lipoicacid (ALA) and Sodium nitrite ($NaNO_2$) were obtained from El-Gomhouria Company for Trading Chemicals, Cairo, Egypt. All the biochemical and oxidative stress parameters were analyzed by ready use kits manufactured by Biodiagnostic Co., Cairo, Egypt, except total and direct bilirubin were analyzed using kits purchased from Spectrum Diagnostics, Cairo, Egypt. Hemogram was determined by MS4e automatic blood cell counter.

Animals and Experimental design: Sixty, healthymale adult rats of albino Sprague-Dawely strain, weighing 150 ±5g, supplied from the Animal House of *National-Cancer-Institute,* Egypt were used throughout the study. The rats were adapted for 1 week, fed on a balanced diet and housed individually in plastic cages fitted with a wire mesh bottoms and fronts in a room maintained at 25-30° C with about 50%relative humidity. The room was lighted on a daily photo period of 12hrs light and dark. Then, they were divided into 6 homogenous groups, 10 rats for each group, and were allocated to the various experimental diets for 30 days.

Group 1: Fed on balanced diet (control group).
Group 2: fed on balanced diet containing sodium nitrite ($NaNO_2$) at 100mg/kg diet/day (to induce toxicity).
Group 3: Fed on balanced diet containing $NaNO_2$ and supplemented by ALA at a dose 100mg/kg diet/day.
Group 4: Fed on balanced diet containing $NaNO_2$ and supplemented by ALA at a dose 200mg/kg diet/day.
Group 5: Fed on balanced diet containing $NaNO_2$ and supplemented by ALA at a dose 300mg/kg diet/day.
Group 6: Fed on balanced diet containing $NaNo_2$ and supplemented by ALA at a dose (400mg/kg diet/day).

Blood samples: At the end of the 30thday of the experiment, blood samples were collected from retro-orbital venous plexus of rats after anesthesia then divided into two parts; the first one was put in tubes of potassium salt of EDTA for determination of hemogramand the other part was put in clean, plain centrifuge tube and left in inclined position at room temperature for clot formation, samples were centrifuged at 3000 rpm for 10 minutes then the clear sera were collected carefully and stored at -20 °C until being used for the estimation of biochemical and different oxidative stress parameters.

Serum biochemical assay: Serum hepatic tranaminases (ALT and AST) were performed according to Reitman and Frankel (1957).Alkaline phosphatase (ALP) activity in serum was performed following colorimetric method as described by Belfield and Goldberg (1971). Albumin was determined according to Doumas *et al.* (1971). Total and direct bilirubin was assessed following the method of Tietz (1995).Serum creatinine and urea were performed according to Bartles *et al.,* (1972) and Fawcett and Soctt (1960) respectively. Plasma catalase (CAT) and Glutathione peroxidase (GPx) activities were assayed as Aebi (1984) and Paglia and Valentine (1967). Serum Malondialdehyde (MDA) and nitric oxide (NO) levels were carried out as Ohkawa *et al.* (1979) and Montgomery and Dymock (1961).

Hemogramassay: Hemogram was determined as described by Jain (1986) including; erythrocytic count (RBC), hemoglobin concentration (Hb), hematocrite value (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were carried out using automated blood cell counter (MS4e).

Statistical analysis: Data of serum biochemical and hemogram analysis of treated groups of rats were statistically analyzed in comparison to the control group for the mean and standard error using statistical software program (SPSS for Windows, version 16, USA). Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests at $(p\leq0.05)$

RESULTS

The obtained data of hepatic (Table, 1)and renal (Table, 2)function evaluation revealed significant$(p\leq0.05)$ increment in serum liver transaminases (ALT and AST) and ALP activities, total and direct bilirubin, creatinine and urea levels, with significant decrement in serum albumin in the $NaNO_2$ treated group when compared with the control untreated one. While, supplementation of ALA at different doses to the $NaNO_2$ intoxicated rats ameliorated significantly the deleterious impacts of $NaNO_2$ on the various analyzed parameters, especially the 400 mg/kg diet ALA supplementation returned the values near normal. Concerning the oxidative stress indices, there were significant $(p\leq0.05)$ elevation in the serum concentration of MDA and NO with significant $(p\leq0.05)$ reduction in the plasma activities of GPx and CAT in the $NaNO_2$ intoxicated rats in comparison to the healthy control group. But, these analytes were reversed upon ALA supplementation to the $NaNO_2$ intoxicated groups (Table, 3). The hemogram of the $NaNO_2$ intoxicated rats showed microcytic hypochromic anemia in correspondence to the healthy rats. Whereas, the ALA supplemented groups manifested significant $(p\leq0.05)$ alleviation of the anemic effect of $NaNO_2$ (Table, 4).

DISCUSSION

Liver function tests were used as important biomarkers for the detection of hepatotoxicity. The increased activities of liver enzymes are mainly due to the leakage from the liver cytosol into the bloodstream. Any disturbance in the activity of these enzymes leads to biochemical impairment ultimately causes hepatocellular necrosis (Dwivedi *et al.,* 2014). In chronic liver diseases, the serum albumin levels are reduced due to protein synthesis disruption in liver (Shin and Moon, 2010). The results of the present study revealed significant increases in serum ALT, AST, ALP activities and bilirubin levels with a significant decrease in serum level of albumin in group of rats fed on $NaNO_2$which reflects damage of hepatocytes. These results are in accordance with those reported byHelal *et al.* (2008) who concluded a highly significant elevation in serum AST, ALT activities with $NaNO_2$ administration. Elevated activity of liver transaminases in blood has been considered as an indicator of tissue damage. However, other factors are considered for this process such as alteration in permeability of cell membrane, increasing the synthesis of the enzyme or decreasing the rate of degradation of the enzyme. Similarly, Helal *et al.,* (1997) recorded a significant increase of serum AST and ALT activities in rats treated with $NaNO_2$.

Table 1. The effect of alpha lipoic acid (ALA) at different doses on serum ALT, AST, ALP activities, bilirubin (total & direct) and albumin concentrations in healthy and intoxicated rats by sodium nitrite (NaNO2)at 30 days

Parameter	Experimental groups					
	Control	$NaNO_2$	$NaNO_2$-LA100	$NaNO_2$-LA200	$NaNO_2$-LA300	$NaNO_2$-LA400
AST (U/L)	20.70^{f} ±0.33	82.40^{a} ±0.80	72.00^{b} ±0.67	67.00^{c} ±0.63	53.20^{d} ±0.77	44.20^{e} ±1.13
ALT (U/L)	20.20^{f} ±0.41	90.70^{a} ±1.24	79.00^{b} ±0.71	71.60^{c} ±0.54	56.30^{d} ±0.98	46.90^{e} ±0.43
ALP (IU/L)	114.90^{d} ±1.93	142.00^{a} ±0.81	133.10^{b} ±0.59	131.90^{b} ±0.57	127.00^{c} ±1.02	130.00^{bc} ±0.84
Alb (g/dl)	3.56^{a} ±0.04	2.70^{cd} ±0.04	2.59^{d} ±0.03	2.77^{c} ±0.03	2.94^{b} ±0.04	3.00^{b} ±0.03
T.Bil. (mg/dl)	0.32^{f} ±0.01	0.89^{a} ±0.01	0.78^{b} ±0.00	0.68^{c} ±0.01	0.64^{d} ±0.01	0.59^{e} ±0.01
D.Bil. (mg/dl)	0.08^{d} ±0.02	0.21^{a} ±0.01	0.13^{b} ±0.00	0.14^{b} ±0.01	0.11^{bc} ±0.00	0.10^{cd} ±0.00

Values are means ± SE (n=10). Means in the same row with different superscript letters are statistically significant (*P ≤0.05*).
Na nitrite ($NaNO_2$), Lipoic acid (LA),aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (Alb), total bilirubin (T.Bil.), direct bilirubin (D.Bil.).

Table 2. Theeffect of alpha lipoic acid (ALA) at different doses on serum urea and creatinine concentrations in healthy and intoxicated rats by sodium nitrite (NaNO2) at 30 days

Parameter	Experimental groups					
	Control	$NaNO_2$	$NaNO_2$-LA100	$NaNO_2$-LA200	$NaNO_2$-LA300	$NaNO_2$-LA400
Urea (mg/dl)	27.60^{e} ±1.32	48.10^{a} ±0.90	47.40^{a} ±0.34	44.30^{b} ±0.63	39.00^{c} ±0.36	31.80^{d} ±0.63
Cr (mg/dl)	0.77^{c} ±0.03	1.06^{a} ±0.04	0.92^{b} ± 0.02	0.89^{b} ±0.01	0.71^{cd} ±0.02	0.65^{d} ±0.02

Values are means ± SE (n=10). Means in the same row with different superscript letters are statistically significant (*P ≤0.05*).
Na nitrite ($NaNO_2$), Lipoic acid (LA) and creatinine (Cr).

Table 3. The effect of alpha lipoic acid (ALA) at different doses on serum NO and MDA levels and plasma GPx and CAT activities in healthy and intoxicated rats by sodium nitrite (NaNO2) at 30 days

Parameter	Experimental groups					
	Control	$NaNO_2$	$NaNO_2$-LA100	$NaNO_2$-LA200	$NaNO_2$-LA300	$NaNO_2$-LA400
NO (µmol/L)	23.44^{b} ±0.26	40.19^{a} ±0.39	24.29^{b} ±0.49	22.89^{b} ±0.52	17.48^{c} ±0.52	14.60^{d} ±0.58
MDA (nmol/ml)	3.41^{e} ±0.26	8.91^{a} ±0.39	6.18^{b} ±0.49	5.45^{c} ±0.52	4.32^{d} ±0.52	3.87^{de} ±0.58
GPx (mU/ml)	65.70^{a} ±0.96	30.70^{f} ±0.51	36.40^{e} ±0.67	39.20^{d} ±0.61	44.60^{c} ±0.90	55.90^{b} ±0.60
CAT (U/ml)	808.23^{b} ±12.34	464.70^{e} ±18.57	597.67^{d} ±12.88	680.15^{c} ±6.76	818.77^{b} ±9.68	928.74^{a} ±7.21

Values are means ± SE (n =10). Means in the same row with different superscript letters are statistically significant (*P* ≤0.05).
Na nitrite ($NaNO_2$), nitric oxide (NO),malondialdehyde (MDA), glutathione peroxidase (GPx) and catalase (CAT).

Table 4. The effect of alpha lipoic acid (ALA) at different doses on erythrogramin healthy and intoxicated rats by sodium nitrite (NaNO2) at 30 days

Parameter	Experimental groups					
	Control	$NaNO_2$	$NaNO_2$-LA100	$NaNO_2$-LA200	$NaNO_2$-LA300	$NaNO_2$-LA400
RBC (10^6/µl)	4.33^{a} ±0.02	2.68^{e} ±0.03	3.38^{d} ±0.04	3.65^{c} ±0.01	3.89^{b} ±0.05	4.27^{a} ±0.10
Hb (g/dl)	12.17^{a} ±0.06	5.09^{f} ±0.06	7.67^{e} ±0.15	9.09^{d} ±0.06	10.02^{c} ±0.15	11.58^{b} ±0.14
Hct (%)	37.25^{a} ±0.30	16.06^{f} ±0.20	23.75^{e} ±0.38	27.82^{d} ±0.17	30.74^{c} ±0.37	35.65^{b} ±0.60
MCV (fl)	86.03^{a} ±0.33	59.91^{f} ±0.17	70.26^{e} ±0.36	76.21^{d} ±0.27	78.88^{c} ±0.62	81.60^{b} ±0.99
MCH (pg)	27.96^{a} ±0.06	18.98^{f} ±0.05	22.69^{e} ±0.20	24.91^{d} ±0.09	25.78^{c} ±0.18	26.49^{b} ±0.20
MCHC (%)	32.56^{a} ±0.15	31.68^{b} ±0.05	32.27^{a} ±0.14	32.68^{a} ±0.07	32.70^{a} ±0.18	32.50^{a} ±0.17

Values are means ± SE (n=10). Means in the same row with different superscript letters are statistically significant (*P ≤0.05*).
Na nitrite ($NaNO_2$), Lipoic acid (LA),Red blood cell count (RBC), Hematocrite value (Hct), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and MCHC (Mean corpuscular hemoglobin concentration).

Their results suggested that, the observed stimulation of ALT activity was due to $NaNO_2$ interaction with the enzyme molecule rather than with the tissues. While, Krishnamoorthy and Sangeetha (2008) reported that, the increase in the marker enzymes such as AST and ALT in $NaNO_2$ treated rats indicated hepatic damage due to significant increase of lipid peroxidation (LPO) in the liver. Moreover, Usunomena *et al.* (2011) who reported that, the toxicological evaluation of the liver showed a steady elevation of the enzymes in rats treated with $NaNO_2$, as these enzymes are mainly found in the liver at high concentration and whenever these enzymes found in high amounts in the serum, it signifies that the liver has problem. The high values of the activities of serum transaminases and ALP relatively to the control values are indicative of severe hepatic cell damage. Helal *et al.,* (2008) reported a highly significant decrease in serum total proteins and albumin levels in $NaNO_2$ treated groups. This reduction may be due to depression of protein synthesis by the liver which may be attributed to an alteration in the intracellular protein synthesis mechanism and that the oxidative enzyme change was probably secondary in altering proteins. Also, the decrease in serum albumin level may result from the loss of protein from the alimentary tract, or due to decreased albumin formation in the liver. Our results are in harmony with those obtained by Saiyed *et al.* (1992) who found that the decrease in serum albumin level was due to hepatic necrosis as a result of $NaNO_2$ administration to male albino rats. Furthermore, Abdul-Ameer and Abed (2012) concluded that $NaNO_2$ has an inhibitory effect on the biosynthesis of protein; this may be attributed to a stimulation of the thyroid and the adrenal glands by $NaNO_2$ which can lead to a blockade in protein synthesis, fast breakdown, increased rate of free amino acids, and decreased protein turnover (Liu *et al.,* 2005). In addition, nitrite interactions result in nitric oxide release, which can inhibit total protein synthesis (Eidi *et al.,* 2006). However, the increase in bilirubin concentration as well as the activity of AST, ALT and ALP enzymes in the serum of $NaNO_2$treated rats could be attributed to the toxic effect of the formed

nitroso-compounds causing severe hepatic necrosis (Kalantari and Salehi, 2001). Fortunately, the current study showed that ALA significantly reduced the toxic effects of $NaNO_2$ expressed by altered hepatic enzyme activities and thus can be considered a potential protective agent in conditions of liver toxicity.Our results came in agreement with Anandakumar *et al.* (2007); Saad *et al.* (2010); Morakinyo *et al.* (2012) andSomi *et al.* (2013). The liver is the most sensitive organ to oxidative damage. The increment of oxidative stress on the hepatocytes and the consequent decrease in the antioxidant ability of the cells result in the occurrence of aggressive cellular damage with destruction of their membranes and the release of the enzymes into the blood stream. The more severe the liver damage, the higher the release of the liver enzymes (El-Khayat *et al.,* 2009). Dwivedi *et al.,* (2014) recordeda significant recovery in liver function tests induced by the supplementation of ALA,thus reducing hepatotoxicity due to the free radical scavenging activity of ALA. Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase in serum urea and creatinine, confirms an indication of functional damage to the kidney (Garba *et al.,* 2007). Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases the serum creatinine level (Nwanjo *et al.,* 2005).

The results of the current study revealed that, a significant increase in serum level of urea and creatinine in group of rats fed on $NaNO_2$. These results are in accordance with those obtained by Hassan *et al.* (2009) and Abdul-Ameer and Abed (2012) who concluded that, serum urea and creatinine were increased upon $NaNO_2$ treatment suggesting an impairment of kidney functions. On the other hand, a significant decrease in serum level of urea and creatinine was recorded in groups of rats treated by ALA.These results were in accordance with Malarkodi *et al.* (2003); Abdel-Zaher *et al.* (2008); Motawi *et al.* (2010) and Morakinyo *et al.* (2012)who found thatALA administration had a beneficial effect in renal toxicity. Oxidative stress has been defined as a disturbance in the equilibrium status of oxidant/anti-oxidant systems in intact cells. It results from the metabolic reactions that use oxygen, the molecular oxygen undergoes a series of reactions that ultimately lead to the generation of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and H_2O (Valko *et al.,* 2007). An excessive amount of reactive oxygen/nitrogen species (ROS/RNS) leading to an imbalance between antioxidants and oxidants can cause oxidative damage (Ceconi *et al.,* 2003).However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, protein and lipid membrane. Where, Goel *et al.* (2005) reported that highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of membrane phospholipids producing MDA, a lipid peroxidation product leading to severe damage to organs of the body. Herein,$NaNO_2$ has been reported to induce oxidative stress, by means of increased MDA and NO levels and reduced activities of CAT and GPx. Similarly, Krishnmoorthy and sangeetha (2008) concluded that, $NaNO_2$ induced a significant increase in lipid peroxide (LPO) level. Also, Shahjahan *et al.* (2005) and Hassan *et al.* (2009) reported that, there was a significant increase in lipid peroxidation in $NaNO_2$treated rats due to a significant decrease in antioxidant enzymes activities (CAT, GSH, SOD), thus inducing oxidative stress. Over and above, Glebova (1998) demonstrated that $NaNO_2$ in rats induced NO production which acts as a factor resulting in accumulation of peroxidation products in the liver. Overproduction of NO was directly linked to liverdamage due to the reactive nature of NO with oxygen free radicals which promote oxidative stress-induced cell injury (Gardner *et al.,* 2002). Free radicals can be prevented or reduced by dietary natural antioxidants through their capacity to scavenge these products (Aruma, 1998).Antioxidants prevent diseases by various mechanisms including, scavenging free radicals and inhibiting lipid per-oxidation (Miller and Rice-Evans, 1997). Scientific literature abounds with examples of the multiple protective actions displayed by LA including its activity as a metal chelator, ROS/RNS scavenger, and its role in the preservation of other antioxidant molecules (Holmquist *et al.* 2007). This fact is of interest from the clinical point of view, suggesting that LA could be considered as a supplement to be used in cases of suspected sub-clinical free radical formation, for example exposure to environmental pollutants causing oxidative stress (Astiz *et al.* 2011).

The present study revealed that, administration of ALA significantly inhibited MDA and NO levels, and significantly increased the activities of CAT and GPx. Our results are coincided with Anandakumar *et al.* (2007); Ghibu *et al.* (2012) and Somi *et al.* (2013).The decreased levels of MDA observed in the ALA groups may be attributed to its capacity to regenerate the reduced glutathione and / or may be directly react with ROS (Shila *et al.,* 2005). Omar *et al.* (2012) found that the level of NO was reverted back to near normal by ALA treatment as it inhibits NO synthesis by down regulating NOS expression. Abdel-Zaher *et al.* (2008) explained the direct scavenging effect of NO by sulphydryl group of ALA.ALA can restore GPx activity by donating electrons and this increased GPx activity use synthesized GSH as a substrate to up-regulate its activity (Dwivedi *et al.,* 2014). Similarly, Saad *et al.* (2010) demonstrated that, co-administration of ALA restored CAT activity and maintained the imbalance in the glutathione level. Rats exposed to $NaNO_2$ showed microcytic, hypochromic anemia which is reverted by ALA administration. The obtained results of decreased Hb, Hct and RBC count with $NaNO_2$ treatment were similar to Baky *et al.* (2010) and Helal *et al.* (2008). The decreased RBC count may be relevant to the observed oxidative stress or may be a sequel of $NaNO_2$ toxic effect on the bone marrow, liver and spleen (Aboel-Zahab *et al.,* 1997). $NaNO_2$ induced methemoglobinemia (ferric iron) in rat's blood that result from the oxidation of hemoglobin by free radicals, and the oxygen binding ability is lost(Hirneth and Classen, 1984).The reduction of methemoglobin to its oxygen transporting ferrous form is catalyzed by red blood cell methemoglobinreductase (Stolk and Smith, 1966). CAT is a hemoprotein that requires NADPH for its regeneration to active form. LA is able to increase glucose uptake by the cells which serves as a fuel for both the pentose phosphate pathway and oxidative phosphorylation thus bringing up cellular levels of NADPH thereby significantly enhancing CAT activity. GPx, the seleno-cystenine containing antioxidant enzyme is the main scavenger of H_2O_2. GPx is required to repair LPO initiated by superoxide in the membrane to maintain its integrity. The observed increase in the GPx activity may be due to its GSH sparing action (Saraswathi and Devaraj, 2013).

Conclusion

In conclusion, Nitrite is an environmental concern due to its harmful effects to human health. So, the intake of high amount

of food stuff which contains $NaNO_2$ must be avoided. ALA supplementation prevents $NaNO_2$ toxicity by reducing oxidative stress and enhancing antioxidant status. Also, ALA markedly decreased tissue inflammation of the liver and brings it nearly to the normal. Thus ALA is a potential antioxidant for chronic diseases associated with oxidative stress.

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