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Biomarkers of Oxidative Stress of Sciatic Nerve Tissues in Experimental Diabetic Neuropathy

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Oxidative stress resulting from enhanced free radical formation and/or a defect in antioxidant defenses has been implicated in the pathogenesis of experimental diabetic neuropathy. In the present study, we have investigated the effect of α-lipoic acid a potent free radical scavenger on glycemic control, oxidative stress and antioxidant enzymes of sciatic nerve in streptozotocin (STZ)-induced diabetic neuropathy model in rats. This study was carried out on 120 male rats. All rats were divided into six main equal groups, 20 animals each. Group 1 (control group) received no drugs, group 2 (diabetic group) received a single dose of streptozotocin (STZ) (50 mg kg^{-1} i.p.) for the induction of diabetes, group 3 (normal α-lipoic acid-treated group), group 4 (diabetic alpha-lipoic acid-treated group), group 5 (diabetic insulin-treated group), group 6 (diabetic alpha-lipoic acid and insulin-treated group). Eight weeks after diabetes induction therapeutic treatment with α-lipoic acid (54 mg kg^{-1} b.wt. i.p., daily) and insulin (2 U s.c daily) were given either alone or in combination and continued for six weeks. Equivalent volumes of saline were given subcutaneously to the rats in the other diabetic and non diabetic control groups. Blood samples and sciatic nerve tissues were collected from all animal groups two times at 4 and 6 weeks from the onset of treatment for determination of serum glucose and nitric oxide in addition to sciatic nerve L-malondialdehyde (L-MDA) and antioxidant enzymes (SOD, CAT and GPX) activities. The obtained results revealed that, a significant increase in serum glucose, sciatic nerve L-MDA concentrations and GPX activity with marked reduction in SOD and CAT activities were observed in STZ-induced diabetic neuropathy in rats. Treatment with alpha lipoic acid, insulin and their combination significantly decreased serum glucose and sciatic nerves L-MDA concentrations and significantly increased serum nitric oxide concentration as well as sciatic nerve SOD, CAT and GPX activities. These results suggest that, α-lipoic acid treatment with insulin improved significantly the diabetes-induced deterioration and attenuates the status of antioxidant enzymes and biomarkers of oxidative stress produced by diabetic neuropathy and its complication in diabetes mellitus.

Key words: Diabetic neuropathy, sciatic nerve, oxidative stress, antioxidant enzymes

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INTRODUCTION

Diabetes is a group of metabolic changes which are characterized by high blood sugar levels and defects in insulin secretion, action or both. Chronic high blood sugar diabetes is associated with long-term damage, disruption and ultimately failure of the hardware, especially the eyes, kidneys, nerves and cardiovascular system (Vinik and Vinik, 2003). In addition to hyperglycemia, several other factors such as dyslipidemia or hyperlipidemia are also involved in the development of cardiovascular complications in diabetes which are the major causes of morbidity and mortality (Reasner, 2008). In patients with diabetes and abnormal antioxidant status, auto-oxidation of glucose and glycated proteins were excess (Nawale *et al*., 2006; Nishikawa and Araki, 2007). Oxidative stress incorporation promote the formation of free radicals and/or defect in antioxidant defenses in the pathogenesis of experimental diabetic neuropathy.

Reactive oxygen species (superoxide radical, hydrogen peroxide and hydroxyl radical) and reactive nitrogen species (peroxynitrite) contribute to pathophysiological changes in diabetic neuropathy (Vincent *et al*., 2004). Antioxidant defense system enzymes (superoxide dismutase, catalase and glutathione peroxidase) are attenuated in diabetic peripheral nervous of animals demonstrating the essential role of oxidative stress of diabetic neuropathy (Low *et al*., 1997). Given the known mechanisms leading to diabetic neuropathy, alogical therapeutic approach is to prevent oxidative stress by increasing antioxidant defense. Antioxidant defense arises from antioxidant enzymes that catalyze the removal of ROS antioxidant molecules that prevent the oxidation of other molecules (Dworkin *et al*., 2007), usually because they are readily oxidized molecules that chelate transition metal ions so they cannot catalyze the generation of ROS in a cell (Dworkin *et al*., 2005).

In addition, diabetes associated with increased production of reactive oxygen species and reduction of antioxidant defense which is partly responsible for diabetic complications, although some minerals and vitamins and cofactors, such as acid, α-lipoic acid is also capable of antioxidant activity. The beneficial effects of α-lipoic acid, both in the prevention and treatment of diabetes, have been suggested by different investigators (Haak *et al*., 2000) and at least one study has shown that α-lipoic acid has beneficial effects on diabetic neuropathy in part because of his activities as an antioxidant, as well as improving blood circulation in small blood vessels that nourish the nervous tissue (Packer *et al*., 2001).

α-lipoic acid has potent antioxidant activity and is used clinically to treat diabetic neuropathy. In addition, lipoic acid α-enhancing insulin stimulated glucose disposal and improves microcirculation and reduces the symptoms of peripheral neuropathy, possibly attenuated oxidative stress (Vessal *et al*., 2003). α-lipoic acid appears to enhance antioxidant capacity in the face of oxidative stress caused by insulin resistance in type 2 diabetes. Also, α-Lipoic acid appears to have a potent therapeutic role in addition to its role in management of diabetic neuropathy in protection of diabetic complications due to oxidative stress (El-Nabarawy *et al*., 2010). These reports suggest that the reduction of oxidative stress may prevent the development of diabetic neuropathy.

Therefore, we have investigated the antioxidant effects of subsequent treatment with α-lipoic acid a potent free radical scavenger, insulin and their combination, on some biomarkers of oxidative stress in sciatic nerve of streptozotocin (STZ)-induced diabetic neuropathy in rats to determine whether α-lipoic acid which has been shown to have antioxidant characteristics, would prevent diabetes-induced changes in biomarkers of oxidative stress in sciatic nerve and beneficial for the treatment of diabetes and diabetic neuropathy.

MATERIALS AND METHODS

Experimental animals: This study was carried out on 120 white male albino rats, 12-16 weeks old and weighted 220-250 g. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied *ad-libitum*. All animals were acclimatized for minimum period of two weeks prior to the beginning of study.

Drugs used

Alpha-lipoic acid (Thiotacid)^{R}: Thiotacid was obtained as pack of five ampoules of 10 mL solution. Each ampoule contains thioctic acid (alpha lipoic acid) 300 mg. Alpha-lipoic acid (Thioctic acid)® manufactured by EVA pharma for pharmaceuticals and Medical Apliances, Egypt.

Human insulin (humulin^{R} U-100): Humulin R presented as regular insulin injection, USP, (recombinant DNA origin) isophane suspension. Humulin R manufactured by LILLY Egypt, under License from ELI LILLY U.S.A.

Diabetes induction: Rats were fasted for 18 h and allowed free access of water. The experimental induction of diabetes in male rats was induced by a single

intraperetinoel (i.p.) injection of 50 mg kg^{-1} b.wt. of streptozotocin (STZ) freshly dissolved in citrate buffer, pH 4.5. A week later, STZ-treated rats were fasted for 12 h and blood samples were collected from the orbital venous sinus for glucose determination. Only those rats in diabetic group with blood glucose levels higher than 250 mg dL^{-1} were considered diabetic (Ramanathan *et al*., 1999).

Eight weeks after diabetes induction therapeutic treatment with alpha-lipoic acid (54 mg kg^{-1} b.wt. i.p. daily) and insulin (2U s.c daily) were given either alone or in combination and continued for six weeks. Equivalent volumes of saline were given subcutaneously to the rats in the other diabetic and non diabetic control groups. Diabetic neuropathy in rats were develop within 8 weeks after induction of diabetes (Kumar *et al*., 2005).

Animal grouping: After eight weeks of diabetes induction indicating development of diabetic neuropathy all rats were randomly divided into 6 main equal groups, 20 animal each, placed in individual cages and classified as follow:

- **Group 1: Non-diabetic control group:** Comprised 20 male rats, received no drugs, served as control for all experimental groups
- **Group 2: Diabetic control group:** Included 20 diabetic male rats received equivalent volumes of saline were given subcutaneously and served as STZ-induced diabetic group
- **Group 3: Normal alpha-lipoic acid-treated group:** consisted of 20 male rats, received alpha-lipoic acid at a dose level of (54 mg kg^{-1} b.wt. i.p daily)
- **Group 4: Diabetic alpha-lipoic acid-treated group:** Comprised 20 diabetic male rats received alpha-lipoic acid at a dose level of (54 mg kg^{-1} b.wt. i.p. daily) after eight weeks of diabetes induction
- **Group 5: Diabetic insulin-treated group:** Included 20 male rats, received subcutaneous injection of insulin at a dose level of 2 U each morning after 8 weeks of induction of diabetes
- **Group 6: Diabetic alpha-lipoic acid with insulin-treated group:** Included 20 male rats received alpha-lipoic acid at a dose level of (54 mg kg^{-1} b.wt. i.p. daily) and insulin at dose of 2 U injected subcutaneously each morning after 8 weeks diabetes induction

Sampling: Random blood samples and sciatic nerve specimence were collected from all animal groups (control and experimental group) 2 times along the duration of experiments at 4 and 6 weeks, from the onset of treatment after eight weeks of diabetes induction:

- **Blood samples:** Blood samples for serum separation were collected after over night fasting by ocular vein puncture at the end of each experimental period and serum was separated by centrifugation at 2500 rpm for 15 min. The clean, clear serum was proceed directly for glucose and nitric oxide concentrations determination
- **Tissue samples (sciatic nerve):** Sciatic nerves from the spin to the peroneal bifurcation were dissected, rinsed in ice- cold saline solution and frozen in liquid nitrogen after removal of adherent tissue. On the day of the homogenate preparation sciatic nerve segments were measured, weighed and rinsed in ice-cold saline solution. Sciatic nerves were cut into small pieces and then homogenized at 4°C in 2 mL of ice-cold saline (11 mmol L^{-1} Tris buffer, pH 7.4) with glass homogenizer, resulting homogenate was passed through a cellulose filter to remove impurities and divided into aliquots for biochemical analysis (Coste *et al*., 2003). All sciatic nerve samples were analyzed for the determination of catalase, superoxide dismutase and Glutathione peroxidase activites in addition to L-malondialdehyde (L-MDA)

Biochemical analysis: Seurm glucose and nitric oxide, sciatic nerve L-malondialdehyde (L-MDA), superoxide disumatase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were analyzed according to the methods described by Trinder (1969), Montgomery and Dymock (1961), Esterbauer *et at*. (1982), Nishikimi *et al*. (1972), Aebi (1984), Fossati *et al*. (1980) and Paglia and Valentine (1967), respectively.

Statistical analysis: The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS 13.0 software, 2009). Values of $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Oxidative stress is involved to play an important role in the pathogenesis of diabetic neuropathy is the most common complication of diabetes that affects more than 50% of patients with diabetes. Clinical studies have shown that oxidative stress plays a major role in the pathogenesis of diabetes. Free radicals formed in diabetes by glucose oxidation, non-enzymatic protein glycation and oxidative degradation of the glycated protein (Mehta *et al*., 2006).

Abnormally high levels of free radicals and this time one of decline in the antioxidant defense mechanism that can lead to damage to cellular organelles and enzymes, increased lipid peroxidation and insulin resistance development. They can cause oxidative stress contributes to diabetic complications (Ghosh and Konishi, 2007).

Blood glucose and nitric oxide concentrations in streptozotocin-induced diabetic neuropathy: The obtained results demonstrated in Table 1 revealed that, a significant increase in serum glucose concentration with mild decrease in serum nitric oxide level were observed in streptozotocin induced diabetic neuropathy in rats allover the period of experiment.

The increase in serum glucose concentration of streptozotocin treated group which came in agreement with Akbarzadeh *et al.* (2007) who reported that, high blood sugar three times in diabetic animals as compared to normal. Who added that, a high blood sugar level, insulin deficiency blood, polyphagia, polyuria and polydipsia with weight loss were seen in adult rats three days after streptozotocin treatment due to irreversible destruction of pancreatic islet cell.

The developed hyperglycemia have been attributed to the specific toxic effects of STZ uptake through glucose transport-2 (GLUT-2) , These effects lead to an end to toxic damage by activating aldose-reductase pathway that leads to the toxic accumulation of sorbitol in the nervous system (Green *et al.*, 1987). Furthermore, Yilmaz *et al.* (2004) reported that, the concentration of glucose in blood plasma in the rats injected streptozotocin- much higher than the control group.

The accumulated polyols may cause oxidative stress in diabetes and may inhibit glycolytic pathway enzymes (Hammes, 2003) as evident in the reduced G6PDH activity which derives substrate from glycolysis. Fructose; whose accumulation in diabetic nerve is a slow process is a more effective glycating agent than glucose (Sharma *et al.*, 2002), generating AGEs, also plays an important role in its contribution to diabetic complications (Brownlee, 1995).

Total Nitric Oxide (NO), an nitrosative stress index is increased in the experimental model of diabetic neuropathy (Kuhad *et al.*, 2008). The increase in NO production in the spinal cord following peripheral injury was confirmed by a rise in NO metabolites production (Guedes *et al.*, 2009). Cho (2010) reported that, oxidative stress may reduce the bioavailability of NO, yielding low NO levels, so the reduction of serum NO in this study could be explained by the oxidative stress in diabetic patients. This suggestion was supported by Stevens *et al.* (2002) who suggesting that many of the factors that will eventually come together, causing the mitochondria dysfunction, free radicals overproduction in addition to oxidative and nitrosative stress leading to NO depletion, impaired nerve perfusion which would provide a common mechanism underlying the genesis of diabetic peripheral neuropathy.

Nitric oxide may interact with ROS, such as superoxide-radical in order to obtain highly active oxidant peroxynitrite species that cause oxidative and nitrosative stress (Llorens and Nava, 2003). In addition, depletion of intracellular NADPH also cause other adverse effects and it is reduced NO synthesis, NADPH as cofactors of a NO-synthesis which synthetize from L-arginine. Besides, NO metabolism can also be change by the abnormal production of superoxide anions derived from elevated intracellular glucose concentration. Superoxide anions are involved in the physiological inactivation of NO: They react with NO to form peroxinitrite (OONO) which is a potential oxidizing agent due to its decomposition into nitrogen dioxide (NO_2) and OH (Tesfamariam, 1994).

Treatment with α-lipoic acid, insulin and their combination significantly reduced elevated serum glucose concentration but significantly reduced serum nitric oxide concentration in streptozotocin (STZ)-induced diabetic neuropathy in rats allover the periods of the experiments. These results are nearly similar to those recorded by El-Hossary *et al.* (2010) who reported that, after treatment of diabetic rats with α-lipoic acid serum glucose level slightly improved at the 2nd and 4th months when compared with control diabetic non treated group. This effect can be explained by the ability of α-lipoic acid to increase cellular uptake of glucose by recruiting glucose transporter-4 to the cell membrane which is evidenced in

Table 1: Effects of treatment with alpha-lipoic acid, insulin and their combination on serum glucose and nitric oxide concentrations in streptozotocin-induced diabetic neuropathy in male rats

Parameters	Glucose (mg dL^{-1})		Nitric oxide (μmol dL^{-1})	
Animals groups	4 weeks ($\bar{X}\pm SE$)	6 weeks ($\bar{X}\pm SE$)	4 weeks ($\bar{X}\pm SE$)	6 weeks ($\bar{X}\pm SE$)
Normal control	117.5 ± 11.09^{d}	123.25 ± 3.54^{d}	$2.68\pm0.24^{a,b}$	$3.33\pm0.240^{a,b}$
Diabetic control	582.75 ± 6.75^{a}	446.50 ± 3.88^{a}	1.50 ± 0.20^{d}	$2.33\pm0.470^{b,c}$
Normal+α-Lipoic acid	$131.50\pm5.58^{c,d}$	132.50 ± 2.33^{d}	$2.68\pm0.24^{a,b}$	2.00 ± 0.410^{c}
Diabetic+α-Lipoic acid	156.50 ± 11.86^{c}	126.00 ± 3.49^{d}	3.65 ± 0.85^{a}	4.33 ± 0.240^{a}
Diabetic+insulin	$142.50\pm8.21^{c,d}$	153.50 ± 23.45^{d}	1.68 ± 0.24^{b}	4.00 ± 0.410^{a}
Diabetic+α-Lipoic acid+insulin	192.00 ± 20.51^{b}	180.75 ± 16.48^{c}	$2.50\pm0.20^{a,d}$	2.00 ± 0.410^{c}

Data are represented as ($\bar{X}\pm SE$), $\bar{X}$: Mean values, SE: Standard error. Means value with different superscript letters in the same column are significantly different at $p\geq0.05$

cell culture experiments (Henriksen, 2006). Some studies suggested that treatment with α-lipoic acid improves insulin sensitivity (Jacob *et al*., 1999) and glucose effectiveness by increasing pyruvate transportation into the mitochondria, increases pyruvate oxidation and in turn, allows glucose to enter the cytoplasm, thereby decreasing insulin resistance (Walgren *et al*., 2004). The blood glucose level was significantly lower than that of untreated diabetics, though all of the insulin-treated diabetic rats were still hyperglycemic (Izbeki *et al*., 2008). Additionally, insulin treatment blocked blood glucose increase and decreases in brain and b.wt. These observations are consistent with insulin therapy's overall amelioration of complications associated with diabetes (Nathan *et al*., 2009). Streptozotocin-injected mice had significantly higher blood glucose level. Insulin alone corrected the hyperglycemia and partially reversed the neuropathic pain in diabetic rats (Kuhad and Chopra, 2009).

Oxidative stress significantly decreased in diabetic rats treated with α-lipoic acid. These results implied that antioxidative treatment is capable of reversing changes in the NO-cGMP system and may, therefore, be an important therapeutic option for preventing vascular damage in diabetes mellitus (Bojunga *et al*., 2004). Also, Du *et al*. (1999) illustrated that, incubation of endothelial cells with α-lipoic acid protected the cultured cells against oxidative stress induced by hyperglycemia. Furthermore, in diabetic animal models, α-lipoic acid has been demonstrated to have beneficial effects on vascular and endothelial function as a result of its antioxidant potency (Heinisch *et al*., 2010). These experimental findings provide the rationale for a potential therapeutic value in diabetic patients with neuropathy. Also, insulin controls expression of NO-synthase and, thus, via availability of NO, controls skin and skeletal muscle blood flow and relaxation of the distal colonic smooth muscle (Middleton *et al*., 1993; Clark *et al*., 2003).

L-malondialdehyde (L-MDA) concentration, superoxide dismutase, catalase and glutathione peroxidase activities of sciatic nerve in streptozotocin-induced diabetic neuropathy: The obtained results presented in (Table 2) revealed a significant increase in L-malondialdehyde (L-MDA) concentration; a significant decrease in superoxide dismutase (SOD) and catalase (CAT) activities with a non significant increase in glutathione peroxidase activity were observed in sciatic nerve of streptozotocin-induced diabetic neuropathy in male rats when compared with control normal rats.

In diabetes mellitus, oxidative stress is correlated with the development of complications in both type 1 and 2 diabetic patients (Vincent *et al*., 2004) and with a decreased antioxidant potential as well as increased lipid peroxidation and DNA oxidation accompanying disease progression. The lipid content of nerve is likely dominated by myelin lipids and oxidative damage to myelin may plausibly contribute to nerve disorders in diabetic polyneuropathy. The DNA within a nerve trunk derives from Schwann cells, endothelial cells, perineurial cells and assorted stromal cells, as well as from mitochondria within all cell types. The extent to which oxidative damage to DNA can alter protein expression has not yet been widely explored but all of these cell types show some dysfunction during diabetes. Damage to mitochondrial DNA also has the potential to impact many aspects of nerve metabolism and function (Manfredi and Beal, 2000) and there is emerging evidence that mitochondrial dysfunction is an early pathogenic event in diabetic neuropathy (Srinivasan *et al*., 2000; Huang *et al*., 2003, 2005). Lipid peroxides may cause oxidative damage to the myelin sheath surrounding the nerve. Therefore, oxidative stress may predispose diabetic patients to the development of neuropathy by a mechanism involving increased lipid peroxidation (Dickinson *et al*., 2002).

Oxidative stress results from an imbalance between ROS (e.g., superoxide anion, hydroxyl radicals,

Table 2: Effects of treatment with alpha-lipoic acid, insulin and their combination on L-Malondialdehyde concentration, superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPX) activities of sciatic nerve in streptozotocin-induced diabetic neuropathy in male rats

Parameters	L-Malondialdehyde (mmol g^{-1} tissue) ($\bar{X} \pm SE$)		Superoxide dismutase activity (U g^{-1} tissue) ($\bar{X} \pm SE$)		Catalase (U g^{-1} tissue) ($\bar{X} \pm SE$)		Glutathione peroxidase (nmol NADPH min^{-1} g^{-1} tissue) ($\bar{X} \pm SE$)	
Animals groups	4 weeks	6 weeks	4 weeks	6 weeks	4 weeks	6 weeks	4 weeks	6 weeks
Normal control	$8.14\pm0.65^{b,c}$	$7.93\pm0.52^{a,b,c}$	$542.76\pm60.43^{c,d}$	1401.32 ± 23.26^{b}	$1.16\pm0.22^{a,b}$	1.39 ± 0.15^{a}	0.24 ± 0.02^{b}	0.56 ± 0.13^{a}
Diabetic	12.17 ± 0.31^{a}	9.55 ± 0.31^{a}	417.77 ± 31.77^{d}	1090.83 ± 2.83^{c}	0.51 ± 0.29^{d}	$1.24\pm0.53^{a,b}$	$0.39\pm0.09^{a,b}$	0.62 ± 0.07^{a}
Normal+α-lipoic acid	6.07 ± 0.31^{d}	$6.07\pm0.81^{c,d}$	$625.00\pm61.55^{a,b,c}$	1394.73 ± 80.58^{b}	$0.55\pm0.14^{c,d}$	1.46 ± 0.08^{a}	$0.35\pm0.03^{a,b}$	0.32 ± 0.08^{b}
Diabetic+α-Lipoic acid	$6.83\pm1.95^{c,d}$	5.64 ± 0.81^{d}	$723.67\pm83.87^{a,b}$	1566.45 ± 90.76^{b}	1.42 ± 0.14^{a}	0.98 ± 0.04^{b}	0.29 ± 0.04^{b}	0.30 ± 0.04^{b}
Diabetic+insulin	9.22 ± 0.27^{b}	$8.37\pm0.56^{a,b}$	749.99 ± 16.11^{a}	$1825.49\pm90.14^{b,c}$	0.91 ± 0.04^{b}	0.99 ± 0.08^{b}	0.32 ± 0.08^{b}	0.55 ± 0.04^{a}
Diabetic+α-lipoic acid+ insulin	9.22 ± 0.27^{b}	$7.19\pm0.39^{b,c,d}$	$578.28\pm36.97^{b,c,d}$	1493.42 ± 40.29^{b}	$0.86\pm0.06^{b,c,d}$	1.33 ± 0.13^{a}	0.52 ± 0.07^{a}	0.58 ± 0.03^{a}

Data are represented as ($\bar{X} \pm SE$), $\bar{X}$: Mean values, SE: Standard error. Means value with different superscript letters in the same column are significantly different at $p \geq 0.05$

peroxynitrite, hydrogen peroxide) and antioxidants such as superoxide dismutase (SOD), catalase, glutathione, vitamin C, Vitamin E and α-lipoic acid. Therefore, increased oxidative stress which contributes to the pathogenesis of diabetic complications, is the consequence of either enhanced ROS production or attenuated ROS scavenging capacity, resulting in tissue damage that is most easily assessed by measuring lipid peroxide levels (Dickinson *et al*., 2002). Mitochondrial damage occurs due to excess formation of ROS and Reactive Nitrogen Species (RNS) (Obrosova *et al*., 2007). ROS, such as superoxide and hydrogen peroxide, are produced under normal conditions through the Mitochondrial (Mt) electron transport chain and are normally removed by cellular detoxification agents such as superoxide dismutase, catalase and glutathione (Leinninger *et al*., 2006). Guedes *et al*. (2009) reported that, GPx activity was increased 3 days after the peripheral nerve axotomy might be crucial to protect the nervous tissue against ROS. Aldose reductase is the firest and rate limiting enzyme in the polyol pathway.

Administration of α-lipoic acid to normal rats significantly decrease L-malondialdehyde (L-MDA) concentration and catalase activity in sciatic nerve after four weeks of treatment followed by significant increase after six weeks as compared to the normal control group. Also, treatment with α-lipoic acid, insulin and their combination in streptozotocin-induced diabetic neuropathy in rats caused a marked decrease in L-malondialdehyde (L-MDA) concentration, significantly increased superoxide dismutase (SOD) and catalase activities in sciatic nerve after four weeks of treatment. However, administration of α-lipoic acid to normal and diabetic groups significantly decreased glutathione peroxides activity in sciatic nerve after six weeks of treatment in comparison with control normal rats and diabetic non-treated group, respectively. Cunha *et al*. (2008) concluded that, lipid and DNA but not protein, oxidation adducts are increased in sciatic nerve of STZ-diabetic rats. Both increased lipid peroxidation and DNA oxidation were prevented by insulin, oxidative stress in experimental diabetes is induced by hyperglycemia and is not a toxic effect of STZ. Because accumulation of lipid oxidation adducts is sensitive to aldose reductase inhibition in nerves from STZ-diabetic rats and is not present in nerves from galactose-fed rats, lipid peroxidation is likely a downstream consequence of flux through aldose reductase. A significant reductions in serum levels of SOD, GSH and vitamin C were observed in diabetic patients. However, oral administration of α-lipoic acid improved significantly the different antioxidant defense systems in these patients (El-Nabarawy *et al*., 2011). It is also said that α-lipoic acid offer advantages over other antioxidants as it increases the level of reduced glutathione not only by regenerating the existing glutathione but also by increasing its de novo synthesis (Osfor *et al*., 2010). Stevens *et al*. (2000) reported that, α-lipoic acid treatment significantly reduced MDA levels and increased antioxidant enzyme (SOD and catalase) activities in diabetic rats. α-lipoic acid, an antioxidant, has also been reported to improve levels of these antioxidant enzymes and restore deficits in diabetic neuropathy. There might be possibility that α-lipoic acid enhance antioxidant enzyme expression, there by increasing antioxidant enzyme levels and decrease the formation of free radicals leading to inhibition of lipid peroxidation. Akpinar *et al*. (2008) found that, α-lipoic acid of antioxidant defense by increasing CAT activity in the stress group. On the other hand, it was observed that α-lipoic significantly decreased levels of brain and retina CAT enzyme in the α-lipoic group compared with the control group. Thus, the increase in GSH content may be explained by the need to provide a substrate for GPx activity. Furthermore as GPx is a major enzyme involved in H_2O_2 metabolism, these results match the increase in H_2O_2 found earlier by our group (Guedes *et al*., 2009). GSH-Px activity in the retina and brain of stressed rats was restored to a normal level by the administration of α-lipoic acid. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2) to H_2O and O_2 at the expense of reduced glutathione (GSH) (Arivazhagan *et al*., 2000; Devi and Kiran, 2004). Therefore, the increment of glutathione peroxidase activity in α-lipoic acid groups indicated that α-lipoic acid increased intracellular glutathione levels. Since, GSH has an important role in the protection of vitamin E and C levels (Fang *et al*., 2002). Moreover, Mao *et al*. (2009) recorded that, treatment using free radicals scavengers reverts and or prevents sensory alterations caused by nerve injury suggesting that endogenous antioxidant mechanism alone is not enough to prevent neuropathic pain. Diabetes-induced alterations in glutathione peroxidase activity are reversed by treatment with α-lipoic acid (Kocak *et al*., 2000). In conclusion, treatment with α-lipoic acid and insulin showed significant protection in diabetic neuropathy as evident from improvement in antioxidant enzyme activities, reduction in blood glucose and lipid peroxidation in treated diabetic rats. This study suggests the beneficial effects of α-lipoic acid in diabetic neuropathy and its protective effect may be mediated through reduction in oxidative stress.

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