Role of α-Lipoic Acid (ALA) on Oxidative Stress in Sperm of Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Increased oxidative stress and impaired antioxidant defense mechanism are important factors in the pathogenesis and progression of diabetes mellitus and other oxidant-related diseases. Reproductive dysfunction is recognized as a consequence of diabetes mellitus. In this study, the possible protective efficacy of α-lipoic acid (ALA) on sperm characteristics, peroxidative damage and antioxidant levels were assessed in the epididymal sperm as well as in testes of four different groups which includes non-diabetic without ALA, non-diabetic with ALA, diabetic without ALA and diabetic with ALA group. Diabetic groups were induced by a single intravenous injection of STZ (45 mg/kg body weight). ALA treatment (100 mg/kg body weight) was given throughout four weeks by oral gavage while non-treatment groups otherwise received a vehicle. The sperm count was significantly increased in non-diabetic rats treated with ALA as compared to sperm count from the non-treated control group (P<0.05). The epididymal sperm of untreated diabetic rats showed a significant increase in lipid peroxidation for five-fold, along with a significant decrease in vitamin E level as compared to sperm from the control group (P<0.05). These changes were associated with significant decrease in sperm motility of untreated diabetic group (P<0.05). Treatment with ALA showed a significant increase in glutathione peroxidase activity and vitamin C level in epididymal sperm of non-diabetic rats (P<0.05). The level of vitamin C in epididymal sperm was significantly increased in diabetic rats after ALA treatment (P<0.05). In conclusion, the results suggest that diabetes mellitus elicits spermatotoxic effects in male rats through induction of oxidative stress. The treatment of diabetes mellitus with 100 mg/kg body weight of ALA for four weeks may not be effective to reduce oxidative stress in sperm in the STZ-induced diabetic rats.

Keywords: diabetes mellitus, oxidative stress, sperm, α-lipoic acid
INTRODUCTION

Diabetes mellitus is a heterogenous metabolic disorder characterized by hyperglycemia resulting from defective insulin secretion, resistance to insulin action or both (Gavin et al. 1997). Hyperglycemia is widely known to enhance free radical concentrations and decreased antioxidant defense system. This mechanism may occur via at least four different routes: increased glycolysis; intercellular activation of sorbitol (polyol) pathway; auto-oxidation of glucose and non-enzymatic protein glycation (Ahmed 2005).

Previous studies have shown that the increase in oxidative stress in diabetes is due to the increased production of reactive oxygen species (ROS) and decreased antioxidant defense system (Wiernsperger 2003). Studies by Annuziata et al. (2005), showed that tissue injury due to free radical damage acts as an important factor in the pathogenesis and complication of diabetes mellitus. Increase in ROS production causes non-specific modification to nucleic acids, protein and phospholipid structures that lead to DNA, RNA and protein damages as well as changes in antioxidant enzyme levels. The stability and capacity of the antioxidant status during chronic diabetes seriously influences the outcome of the long-term complications caused by oxidative stress (Sasvari & Nyakas 2003).

Diabetes has long been identified to exert a negative effect on male fertility through disruption in normal spermatogenesis process as well as in normal erectile function. According to Jiang (1996), almost 90% of diabetes patients undergo sexual dysfunction due to hyperglycemic induced testicular dysfunction. A recent study showed a significant increase in levels of fragmented sperm DNA in males with diabetes compared against normal non-diabetic males (Agbaje et al. 2007). A six week study on streptozotocin (STZ) induced diabetic rats showed a significant increase in testicular oxidative stress (Shrilatha & Muralidhara 2007). According to Amaral et al. (2006), hyperglycemia has an adverse effect in sperm concentration and motility via changes in energy production and free radical management.

Otherwise, the mammalian sperm cells contain high amount of specific lipid composition which includes polyunsaturated fatty acid, plasmologen and sphingomyelin and lack of antioxidant mechanism. Due to the high quantity of polyunsaturated fatty acid and low antioxidant capacity, mammalian spermatozoa are at a higher risk of peroxidative damage (Vernet et al. 2004). Aitken et al. (1989) stated that high levels of reactive oxygen species (ROS) and free radicals have a damaging effect against sperm motility and fertility. A potent antioxidant is therefore crucial in reducing the damaging effects of oxidative stress on spermatozoa of diabetic patients.

Alpha lipoic acid (ALA), also known as 1,2-dithiolane-3-pentanoic acid, 1,2-dithiolane-3-valeric acid or 6,8-thiocetic acid has generated considerable clinical interest as a cellular thiol-replenishing and redox-modulating agent (Arivazhagan & Panneerselvam 2002). Biologically, ALA functions as a cofactor of oxidative decarboxylation reactions in glucose metabolism to yield energy (Pari & Latha 2005). It has been used for a long time in the western world to treat complications associated with diabetes (Erik 2006). In the cells and tissues, ALA undergoes a reduction process to from dihydrolipoic acid (DHLA). DHLA acts as potent scavengers of reactive oxygen species. Both ALA and DHLA reduces oxidative stress by neutralizing free radicals, making them a more potent antioxidant compared against vitamin C and E. DHLA also influences the reformation of thioredoxin and protects
membranes by interacting with vitamin C and glutathione, which may in turn recycle vitamin E (Packer et al. 1995).

Studies by Bustamante et al. (1998), showed that supplementation of ALA increased free alpha-lipoic acid that has been found to affect cellular metabolic processes in vitro, as it has the ability to alter the redox status of cells and interact with thiols and other antioxidants. Therefore, it appears that this compound has important therapeutic potential in conditions where oxidative stress is involved such as diabetes mellitus. According to Maritim et al. (2003), ALA reduces oxidative stress in diabetes through preventing lipid peroxidation or increasing the antioxidant enzyme activities. Studies by Keegan et al. (1999) showed ALA as a potential antioxidant used in treating infertility due to diabetes. Pre-treatment with ALA has shown to increase semen quality by reducing oxidative stress and DNA damage induced by cyclophosphamide (CF) (Selvakumar et al. 2006).

However, studies on effects of ALA supplementation on oxidative status in diabetic rat reproductive functions are still lacking. Thus, this study is aimed to determine the effects of ALA supplementation against oxidative stress in sperm and testes of diabetic rats.

MATERIALS AND METHODS

Chemicals:
Streptozotocin (STZ) and α-lipoic acid were procured from Sigma-Aldrich Inc. (St. Louis, MO, USA). All other chemicals were of analytical grade.

Animals and care
Forty healthy adult male Sprague-Dawley rats (250 – 280 g) were obtained from the Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. They were acclimatized to the environment for a week prior to experimental use. The animals were kept in plastic cages under 12 h light/dark cycles with 27 ± 9 °C and humidity of 45 – 65%. They were maintained on standard pellet diet and tap water ad libitum. The experimental protocol had been approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) and their guidelines were strictly followed.

Experimental design and sampling
The animals were then randomly divided into four groups consisting of 10 rats in each group; Group I (non-diabetic control); Group II (non-diabetic treated ALA); Group III (diabetic control); and Group IV (diabetic treated ALA group). Diabetes was induced in overnight fasted rats by a single intravenous (i.v.) injection of a normal saline of STZ at a dosage of 45 mg/kg bw (Fonteles et al. 1996). After 72 hours of injection, fasted blood glucose level was measured using reagent strips (Accu-Chek Advantage, Roche Diagnostics Australia Pty. Ltd., Australia) to confirm diabetes. Diabetic rats used in this study showed a blood glucose level more than 15 mmol/l. ALA was dissolve in sterile distilled water with the addition of natrium hydroxide 5N (Cameron et al. 1998). The treatments of ALA were given orally every day for four weeks at a dose of 100 mg/bw according to previous study (Balkis et al. 2008). For control group, 0.25 ml of distilled water was administered via oral gavage. At the end of
the fourth week, animals from all four groups were decapitated after an overnight fasting. Cauda epididymides were necropsy from all animals, cleared of adhering connective tissue and weighed. Epididymides were placed in 4 ml of normal saline and were mechanically minced by using anatomical scissors. The suspension were stirred gently and left for five minutes to allow the sperms to leak out. Part of the sperm suspension was used for sperm characterization analysis while the remaining was kept at -20°C prior to biochemical analysis (Turk et al. 2008).

**Sperm characteristics:**

The sperm were collected as quickly as possible after dissection. 10 µl of homogenous sperm suspension were placed on a “Makler Counting Chamber” (Sefi-Medical Instruments Sdn. Bhd., St. Santa Ana, CA, USA) and epididymal sperm count and sperm motility were determined under a light microscope at 100x magnification. Sperm count was expressed as million sperm cells per ml of suspension while sperm motility in percentage of motile sperm. For sperm viability and morphology assessment, 10 µl of sperm suspension was added with 10 µl of eosin-nigrosin stain and thick smear were done on the slides. The eosin-nigrosin stain produced a dark background on which the sperm stand out as lightly colored objects. Normal live sperm exclude the eosin stain and appear white in color, whereas "dead" sperm take up eosin and appear pinkish. Morphological abnormalities were described as amorphous and hookless head, double head and coiled or branched tail. In total, 2000 sperm on each slide were evaluated and the result were recorded as percentage of sperm viability and morphological abnormality.

**Lipid peroxidation analysis:**

Lipid peroxidation assay was carried out by measuring malondialdehyde (MDA). MDA assay was performed manually using the principle of adduct formation between TBA according to the method by Hunter and Jamaludin (1986). The reaction yielded a red MDA-TBA adducts which formed a pink complex with absorption maximum at 532 nm.

**Antioxidant status:**

The activity of superoxide dismutase (SOD) enzyme was determined according to the method of Beyer and Fridovich (1987). The method was based on the activation of riboflavin by a proton which further oxidized an electron donor which was L-methionine. Oxidized L-methionine further reduced riboflavin to semiquinone state that lead to reduction of oxygen to $\text{O}_2^-$ which further reacted with nitroblue tetrazolium to form a purple colour. The chromogen formed was then measured spectrophotometrically with a maximum absorbance at 560 nm.

Glutathione peroxidase (GPx) was determined using the method of Paglia and Valentine (1967). Samples were added with cumene hydroperoxide substrate which produced oxidized glutathione which were further reduced to glutathione reductase in the presences of NADPH. Reduction of NADPH in the sample was measured using spectrophotometer at a maximum absorption at 340 nm as a direct indicator of the enzyme activity in the sample.

Vitamin C and E concentrations were determined using the method of Lloyd et al. (1945) and Hansen & Warwick (1969) respectively. Total vitamin C determination method measured
both the oxidized and the reduced form of ascorbic acid which were measured spectrophotometrically at an absorption wavelength of 520 nm. Vitamin E concentration was measured by fluorometric analysis of tocopherol isomers extracted in hexane. Samples were exposed at 295 nm of UV light and its fluorescence measured at 340 nm.

Protein concentration in the samples were determined using the method of Bradford (1976) involving the binding of Coomassie Brilliant Blue G-250 to protein substance. Color changed was observed as an indicator of presences of protein by measuring spectrophotometrically at maximum absorption of 595 nm.

**Statistical Analysis:**

All data are expressed as mean ± standard error (SEM). One-Way Analysis of Variance (ANOVA) using SPSS (version 14) was performed to detect differences between all various groups. Significant differences detected by ANOVA were further analyzed using post-hoc Tukey-HSD. Values of p<0.05 were noted as significant.

**RESULTS**

**Evaluation of epididymal sperm counts**

Non-diabetic group supplemented with ALA showed significant increase from non-diabetic control in total sperm count (P<0.05; Table 1). However, no significant differences were observed in the total sperm count of animals from the diabetes groups treated with ALA and the diabetes control group as well as in non-diabetic group.

**Evaluation of epididymal sperm motility**

Motility of sperm in diabetes control group decreased significantly compared with non-diabetic control group but insignificant decrease between in ALA supplemented diabetes group with diabetes control group (P<0.05; Table 1).

**Evaluation of epididymal sperm viability and sperm morphology**

As for sperm viability and abnormal sperm morphology, no significant differences were noted among the study group. Groups supplemented with ALA did show an increase in the percentage of viable and morphologically normal sperm compared to non diabetic and diabetes control group, however, these differences were not enough to be significant (Table 1).

**Progression of oxidative impairments**

A significant increase in sperm MDA levels were observed in diabetes control animals compared with animals from non-diabetic control (P<0.05; Table 2). Although there was reduction of SOD activity in ALA-treated group differ from control group, but it was not significant differences (Table 2). Otherwise, significant increase in sperm GPx enzyme activity was evident in non-diabetic group supplemented with ALA compared with non-
diabetic control (P<0.05; Table 2). Further, more significant difference was noted in total vitamin C levels in sperm of ALA supplemented groups. Non-diabetic group supplemented with ALA showed a significant enhancement in vitamin C levels compared with non-diabetic and diabetes control group (P<0.05; Table 2). Significant difference was also observed in diabetes treated ALA group from diabetes control group (P<0.05; Table 2). No significant increase in vitamin E levels were observed between untreated and treated ALA group (Table 2). However, a significant difference between normal control groups and diabetes control group was noted (P<0.05; Table 2).

DISCUSSIONS

Present studies showed that increased ROS in hyperglycemic condition plays an important role in formation of diabetes complication (Yue et al. 2005). Chronic hyperglycemia leads to microvascular and macrovascular diabetic complications through intracellular mechanisms, including increased polyol pathway flow, activation of protein kinase C, and increased hexosamine pathway (Aybek et al. 2008).

While the role of oxidative stress in the development of various diabetic complications is well known, the involvement of oxidative stress mechanisms and their contribution in the development of testicular dysfunctions under diabetes is poorly understood (Shrilatha & Muralidharan 2007). In the reproductive system, balance between ROS formation and the antioxidant defense mechanism ensures a normal sperm function (Aitken 1999). Elevated oxidative stress in the testicular milieu is demonstrated to have profound implications on testicular physiology and sperm function. According to Shrilatha & Muralidharan (2007), significant oxidative stress during early diabetic phase is likely to significantly contribute to the development of testicular dysfunction leading to altered steroidogenesis and impaired spermatogenesis. In vitro studies have shown ALA and DHLA as potent ROS scavengers (Dincer et al. 2002). According to Maritim et al. (2003), exogenous ALA has the potential to be used as therapeutic antioxidant agent in prevention and treatment of diabetes mellitus.

Increased oxidative stress in diabetes mellitus causes an adverse effect to the male reproductive system. Hyperglycemic conditions affects the total sperm count as well as sperm motility due to changes in energy production and free radical management (Amaral et al. 2006). A significant decrease in motility of sperms of diabetic rats compared against normal control group observed in this study supports the statement above. Studies by Amaral et al. (2006), showed that STZ induced diabetic rats have decrease in total sperm count and motility. The decrease in sperm concentration is likely due to the influence of severe hyperglycemia in late stages of spermatogenesis, possibly through an increase in ROS damages.

The consequences of such oxidative damage could include loss of motility due lipid peroxidation. Disruption of spermatozoa membrane matrix structure due to lipid peroxidation may contribute to reduction in motility, viability and membrane integrity thus contributing to increase in abnormal morphology of sperms. Reduction in sperm motility may be contributed by the sudden decrease in intracellular ATP concentration, further inducing axonemal damage (Sharma & Agarwal 1996; Sikka 1996). Reduction in energy metabolism and ATP production has been identified as a limiting factor in sperm motility rate (Selvakumar et al.
2006). Studies also show that ROS induced sperm mitochondrial damage contributes to the reduction in sperm motility energy. From the study, a significant increase in total sperm count in normal rats treated with ALA against normal control rats shows the positive effects of ALA supplementation in increasing total sperm count. The results also show an increase in normal morphology of sperm in ALA treated groups compared to STZ induced diabetes group.

From the study, a non-significant increase in percentage of abnormal morphology of sperm in diabetic rats compared to normal rats were observed corresponding to the total sperm count and motility of sperm. According to studies by Shrilatha & Muralidhara (2007), STZ has been shown to have genotoxic effects towards the spermatogenic cycle as well as potential mutagenic effect towards germ cells thus increasing the number of abnormal sperm. Abnormal morphology of sperm could also be caused by possible synergetic effects of reduction in Leydig cell function (Tanaka et al. 2001) and diabetic oxidative stress. Oxidative stress plays a critical role in formation of abnormal sperms induced through denaturation and fragmentation damage to sperm DNA structure (Agarwal & Saleh 2002).

Reactive oxygen metabolites such as singlet oxygen, hydroxyl radicals, superoxide and \( \text{H}_2\text{O}_2 \) are known to be cytotoxic agents because of their ability to induce lipid peroxidation in tissues and membranes (Farombi et al. 2008). Determination of malondialdehyde (MDA) levels can be used as an important indicator of lipid peroxidation (Qujeq et al. 2004). According to Selvakumar et al. (2006), lipid peroxidation is an important manifestation of ROS induced oxidative damage that is highly related to changes in membrane structures and enzyme denaturation. Excessive production of ROS contributes towards peroxidation of sperm acrosome membrane structure and further weakens sperm acrosin activity (Zalata et al. 2004). From this study, MDA levels in sperms of diabetic rats were found to be significantly higher compared with normal rats. This finding corresponds to the findings of an earlier study by Shrilatha & Muralidhara (2007).

Increase in MDA levels in diabetic rat sperm may be contributed by increased production of free radical or due to decrease in the antioxidant status. Additionally, because of the extraordinarily high content of polyunsaturated fatty acids in the plasma membrane, which is an essential requirement for the male germ cell to maintain sperm functions, and owing to the very low content of protective systems, spermatozoa are highly susceptible to oxidative stress (Turk et al. 2008). According to Tavilani et al. (2007), the plasma membrane of the human spermatozoa is particularly vulnerable to lipid peroxidation because their plasma membranes are enriched with polyunsaturated fatty acids, particularly docosahexaenoic acid with six double bonds. Sperm membrane has been reported to be adversely affected by peroxidation which contributes to accumulation of organic hydroperoxides as well decrease ability to absorb antioxidant enzymes form the seminal plasma (Tavilani et al. 2007).

Studies have shown increased ROS to contribute to the disruption of cell function and its life cycle. Living organisms possess antioxidant defense systems against ROS. These defense systems include endogen antioxidants, which can be classified as enzymatic (SOD, GSH) and nonenzymatic (vitamin E, uric acid, bilirubin) defense systems (Aybek et al. 2008). SOD is an important class of enzyme that plays a crucial role in the bodies’ antioxidant defense system. Its main function is to catalyze the dismutation of superoxide (first product in oxygen radical formation) into oxygen and hydrogen peroxide thus reducing the toxic effect.
of these radical as well as other forms from the secondary reaction (Sen & Hanninen 1994). Previous studies have proven the presences of SOD in sperm and seminal plasma (Mennella & Jones 1980; Zini et al. 1993).

Previous studies on SOD enzyme activities in diabetic rats have produced results which are inconsistent. According to Yue et al. (2005), SOD activities in diabetic rats were found to be significantly higher compared to normal rats. During diabetes, ROS may be produced through autooxidation of glucose and auto oxidative glycosylation of proteins (Hunt et al. 1988). Increase SOD activities in diabetic condition corresponds to the increased production of superoxide anion radical (Yue et al. 2005). However, studies by Aybek et al. (2008) contradicts these findings in which lower SOD activities were reported in diabetic rats compared to normal rats. According to that study hyperglycemia together with an increase in free radical formation eventually leads to reduction in endogenous antioxidant capacity as noted with the reduced SOD activity. Increased lipid peroxidation levels cause reduction in SOD antioxidant enzyme activity (Selvakumar et al. 2006) which further contributes to excessive formation of ROS eventually leading to denaturation of antioxidant enzymes (Pigeolet et al. 1990).

In the present study, SOD enzyme levels were noted to be lower in both normal and diabetic groups treated with ALA compared with groups not supplemented with ALA although these findings were not significant. This may be contributed by the prooxidant activity of ALA observed in the presences of iron. DHLA can also exert pro-oxidant properties, both by its iron ion-reducing ability and probably by its ability to generate reactive sulphur-containing radicals that can damage certain proteins, such as alpha 1-antiproteinase and creatine kinase (Scott et al. 1994). Studies by Dincer et al. (2002) proved that supplementation of ALA in diabetic rats can decreased SOD activities in the liver of the animals. This reduction may be a consequence of the scavenging effects of DHLA on superoxide anion radical.

GPx is a cytosol enzyme that complements with CAT in order to detoxify hydrogen peroxide and hydroperoxide organic (Sen & Hanninen 1994). Reduction of hydroperoxide by glutathione in the presence of GPx has been shown to protect mammalian cells from oxidative damages. GPx is an important antioxidant enzyme present in the seminal plasma. In the male reproductive system, GPx is found concentrated in the testes, prostate, seminal vesicle, vas deferens, epididymis, seminal plasma and spermatozoa (Vernet et al. 2004). Reduction in GPX enzyme activity in seminal plasma (Giannattasio et al. 2002) and spermatozoa (Garrido et al. 2004) is a contributing factor for male infertility. The results of this study shows a significant increase in GPx enzyme activity in ALA supplemented normal group compared to normal group without ALA supplement. It can be suggested here that supplementation of ALA decreases the need and use of cellular GPx enzyme during an oxidative stress respond. This is due to the fact that ALA and DHLA has the potential to counteract the effect of various ROS such as superoxide, hydroxyl and singlet oxygen species (Packer et al. 1995).

Supplementation of antioxidants has been shown to reduce oxidative stress and diabetic complications in diabetic animal models (Kocak et al. 2000) and human patients. According to Schwedhelm et al. (2003), vitamin C, vitamin E and other micronutrients can protect humans from various diseases including diabetes and aging. Vitamin C together with vitamin E has been proven to disrupt the formation of hydroperoxides (Maritim et al. 2003). ALA is
known to function in the recycling of vitamin C and according to Cakatay et al. (2000),
DHLA also has the potential to recycle other antioxidants such as vitamin E and ascorbic
acid. Vitamin C is a potent antioxidant that has been shown to protect normal
spermatogenesis in animal models (Raymond & Costabile 1998) as well as being used to treat
male infertility problems (Irvine 1996). From this study, vitamin C levels in sperm and testis
of diabetes rats supplemented with ALA showed a significant increase compared to diabetes
control rats. This finding correlates with the finding from the studies by Selvakumar et al.
(2006) in which increase in vitamin C levels with ALA supplementation can prevent
reduction in general antioxidant level. Similarly rats of normal group supplemented with
ALA also showed a significant increase in vitamin C levels compared to normal control.

Findings on vitamin E levels in both sperms and testes showed a decrease in diabetes control
rats compared with normal control which significantly reduced in sperms. However, vitamin
E levels in sperms of ALA supplemented diabetes rats were found to be almost similar with
sperms and testes from diabetes control group. Previous studies have also produced
contradicting results in vitamin E levels in diabetic human and animal subjects (Maritim et al.
2003). Plasma and/or tissue vitamin E levels were reported as unchanged (Maritinoli et al.
1993), increased (Asayama et al. 1994) or decreased (Cinar et al. 2001) due to diabetes.
Increase lipid peroxidation contributes to disruption of membrane function and deactivation
of enzymes and membrane receptors that further contribute to the loss of vitamin E (Sikka
2004). Reduction in vitamin E levels in sperms of diabetes rats may be due to increase use of
vitamin E for oxygen radical trapping during hyperglycemia (Palmeira et al. 2001) or due to
reduction in oxygen radical detoxification enzyme activity (MacPherson 1994). Supplementation
with ALA did not produce significant changes as it might have been used
for free radical trapping rather than recycling of antioxidants such as vitamin E itself (Borcea
et al. 1999).

From the study it can be concluded that supplementation of 100 mg/kg of ALA for four
weeks was not sufficient to reduce oxidative stress as well as to improve the quality of
sperms in STZ induced diabetes animals. However supplementation of ALA did increase
vitamin C levels in sperms proving an important role of ALA in vitamin C recycling. Further
studies with longer treatment periods with nucleotide analysis and sperm DNA analysis is
required to further confirm the beneficial effects of ALA.

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REFERENCES


and S.E. Lewis, 2007. Insulin dependent diabetes mellitus: implication for male


### TABLES

#### Table 1. Effects of diabetes and ALA supplementation on total caudal sperm count, sperm motility, viability and morphology.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total sperm count (10^6/ml)</th>
<th>Sperm Motility (%)</th>
<th>Sperm Viability (%)</th>
<th>Abnormal Morphology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>62 ± 9</td>
<td>69 ± 5</td>
<td>85 ± 6</td>
<td>4.8 ± 3.1</td>
</tr>
<tr>
<td>Normal + ALA</td>
<td>116 ± 7^a</td>
<td>66 ± 3</td>
<td>88 ± 2</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>Diabetes Control</td>
<td>38 ± 7</td>
<td>37 ± 6^a</td>
<td>70 ± 5</td>
<td>8.0 ± 1.4</td>
</tr>
<tr>
<td>Diabetes + ALA</td>
<td>74 ± 26</td>
<td>54 ± 5</td>
<td>83 ± 3</td>
<td>5.5 ± 1.4</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM of 10 animals in each group.

a: significantly different against non-diabetic control group at P<0.05.

#### Table 2. Effects of diabetes and ALA supplementation on MDA, SOD, GPx, Total vitamin C and E concentrations in sperm.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/g protein)</th>
<th>SOD (U/mg protein)</th>
<th>GPx (mU/mg protein)</th>
<th>Total Vitamin C (mg/L)</th>
<th>Total Vitamin E (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>7.18 ± 1.46</td>
<td>3.19 ± 0.87</td>
<td>0.011 ± 0.001</td>
<td>3.27 ± 0.28</td>
<td>3.15 ± 0.36</td>
</tr>
<tr>
<td>Normal + ALA</td>
<td>9.07 ± 1.18</td>
<td>1.63 ± 0.30</td>
<td>0.034 ± 0.004^a</td>
<td>9.80 ± 0.54ab</td>
<td>2.84 ± 0.13</td>
</tr>
<tr>
<td>Diabetes Control</td>
<td>34.97 ± 11.75^a</td>
<td>3.38 ± 0.22</td>
<td>0.028 ± 0.004</td>
<td>2.33 ± 0.31</td>
<td>2.09 ± 0.13^a</td>
</tr>
<tr>
<td>Diabetes + ALA</td>
<td>17.49 ± 9.81</td>
<td>2.05 ± 0.47</td>
<td>0.026 ± 0.008</td>
<td>5.60 ± 0.58^b</td>
<td>2.31 ± 0.21</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM of 10 animals in each group.

a: significantly different against non-diabetic control group at P<0.05.

b: significantly different against diabetes control group at P<0.05.