BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE
BENHA VETERINARY MEDICAL JOURNAL

BIOCHEMICAL EFFECT OF ALPHA-LIPOIC ACID ON LIPID PROFILES, LIPID PEROXIDATION AND STATUS OF ANTIOXIDANT ENZYMES IN STREPTOZOTOCIN INDUCED DIABETES IN RATS.

Hussein, S.A.; Hassanin M.R. and Amira, R. EL Barky
Biochemistry Department, Faculty of Vet. Med. Moshtohor, Benha University, Egypt.

A B S T R A C T

In the present study, the effects of alpha lipoic acid (ALA) supplementation on glycemic control, lipid profile, vitamin C, lipid peroxidation and antioxidant enzymes in streptozotocin (STZ)-induced diabetic rats have been evaluated. This study was carried out on 80 male rats. The rats were divided into four equal groups of 20 rats each. Group I :(Control group): Injected with citrate buffer only. Group ΠI: (Diabetic group): Injected with a single intraperetinoel (i.p) dose of 50 mg/kg of streptozotocin for diabetes induction. Group III :(diabetic alpha lipoic acid treated group) and Group IV: (control alpha lipoic acid treated group). ALA was injected intrperetinoel in a daily dose of 54 mg/kg bw. Blood samples for serum separation and liver and kidney tissues were collected from all animal groups two times at 4 and 6 weeks from the onset of treatment with α-lipoic acid which begin after five weeks of diabetes induction. All sera were processed directly for determination of glucose, total cholesterol, triacylglycerols, HDL–C, LDL-C, VLDL-C and vitamin C in addition to liver and kidney L- malondialdehyde (L- MDA) and antioxidant enzymes were also determined. The obtained results revealed that, a significant increase in serum glucose, total cholesterol, triacylglycerols, LDL-C, VLDL-C, HDL-C, vitamin C and L-MDA concentrations in liver and kidney as well as marked reduction in CAT, SOD and GpX activites of liver and kidney were observed in STZ-induced diabetic rats. Treatment with ALA to STZ-induced diabetic rats lowered serum glucose, total cholesterol, triacylglycerols, LDL-C, HDL-C concentration and lipid peroxidation of liver and kidney as well as significantly increased serum vitamin C and liver catalase activity. These results suggest that, ALA may be effective in controlling glycemic status and improving dyslipidemia and has the potential in reducing cardiovascular complications due to diabetes mellitus. In addition, treatment with ALA improved significantly the diabetes-induced deterioration of vitamin C and attenuates the status of antioxidant enzymes and biomarkers of oxidative stress produced by diabetes mellitus.

KEY WORDS: alpha lipoic acid (ALA), diabetes mellitus

(BVMJ 23(1): 34-47, 2012**)**

1. I N T R O D U C T I O N

Diabetes mellitus is a group of metabolic alterations characterized by hyperglycemia resulting from defects in insulin secretion, action or both. Chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidney, nerves, heart, and blood vessels [32].

Several distinct types of diabetes mellitus exist and are caused by a complex interaction of genetics and environmental factors [15]. Type I diabetes is the consequence of an autoimmune- mediated destruction of pancreatic β-cells, leading to insulin deficiency. Patients require insulin treatment for survival [44]. While, Type II diabetes mellitus developed by metabolic abnormalities such as impaired insulin secretion, increased hepatic glucose production and decreased insulin-stimulate of glucose uptake in peripheral [38].

In diabetic patients with vascular complications, there are significant changes such as increased lipid peroxidation, dyslipidemia, and irregularity in the metabolism of proteins, carbohydrates and lipids. Increased lipid peroxidation is accepted to be one of the main causes of diabetic complications [24]. Also, the presence of high glucose levels may affect the function of different organs such as liver, pancreas, and retina [56].

Lipoic acid is a unique antioxidant because it has beneficial effects on fuel metabolism and also an essential cofactor of mitochondrial respiratory enzymes, including the pyruvate dehydrogenase complex [63]. ALA can regenerate other antioxidants such as vitamin C and E [14]. Lipoic acid appears to improve the antioxidant capacity in face of the oxidative stress induced by insulin resistance in type 2 DM. Also, LA 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 [20].

Accordingly, this study was performed to investigate the effect of α- lipoic acid on glucose, lipid profile, vitamin C, lipid peroxidation and antioxidant enzymes in streptozotocin (STZ)-induced diabetic rats.

2. MATERIAL AND METHODS

Eighty white male albino rats of 12- 16 weeks old and weighing 220- 250 gm were used in this study. 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.

Diabetes Induction:

Rats were fasted for 18 hour and allowed free access of water. The experimental induction of diabetes in male rats was induced by a single intraperitoneal (i.p) injection of 50 mg / kg of streptozotocin (STZ) freshly dissolved in citrate buffer, PH 4.5. A week later, STZ-treated rats were fasted for 12 hour, 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 were considered diabetic [55]. Five weeks after diabetes induction, therapeutic treatment with alpha lipoic acid (54 mg/kg body weight i.p daily) were given and continued for six weeks. Equivalent volumes of saline were given subcutaneously to the rats in the other diabetic and non diabetic control groups.

Experimental design:

After five weeks of diabetes induction all rats were randomly divided into four main equal groups, 20 rats each, placed in individual cages and classified as follow:

Group I (control normal group): received no drugs, served as control non-treated for all experimental groups.

Group II (diabetic non-treated group): received no drugs and served as STZ-induced diabetic groups.

Group III (diabetic alpha lipoic acid treated group): received alpha lipoic acid i.p daily at a dose level of 54 mg/kg body weight.

Group IV (control alpha lipoic acid treated group): received alpha lipoic acid i.p daily at a dose level of 54 mg/kg body weight.

Sampling:

Random blood samples and tissue specimens (liver and kidney) were collected from all animals groups (control and experimental groups) two times along the duration of experiment at 4 and 6 weeks from the onset of treatment with α-lipoic acid after five weeks diabetes induction.

Blood samples:

Blood samples for serum separation were collected after overnight fasting by ocular vein puncture at the end of each experimental period and serum was separated by centrifugation at 2500 r.p.m for 15 minutes. The clean, clear serum was proceed directly for glucose determination and kept in a deep freeze at 20° C until used for subsequent biochemical analysis.

Tissue samples (liver and kidney):

After four and six weeks of treatment with α-lipoic acid ,the rats were sacrificed, livers and kidneys were removed, rinsed in ice-cold 0.9% sodium chloride solution , quick frozen in a deep freeze at -20 °C for subsequent biochemical analyses.
10 % (w/v) homogenate of liver and kidney were prepared in ice-cold normal saline using a chilled glass-teflon porter-Elvehjem tissue grinder tube, and then centrifuged at 3000 rpm for 15 min. The supernatant was used for the determination of L-malondialdehyde (L-MDA) and antioxidant enzymes.

Biochemical analysis:

Seurm glucose, total cholesterol, triacylglycerols, high density lipoprotein cholesterol (HDL–cholesterol), Low density lipoprotein cholesterol (LDL - cholesterol), very low density lipoprotein cholesterol (VLDL- cholesterol), Vitamin C, L- malondialdehyde (L- MDA), superoxide disumatase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were analyzed colorimetric- ally according to the methods described by[74]; [19]; [69]; National cholesterol Education program Recommendation for measurement of High–density Lipoprotein Cholesterol as it was described previously [11, 22, 28, 41, 48, 50, 52, 78].

Statistical analysis:

The obtained data were statistically analyzed and the significant difference between groups was evaluated according to [65].

3. RESULTS AND DISCUSSION

Diabetes mellitus is a syndrome, which is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in enzymes and high oxidative stress induced damage to pancreatic beta cells [61]. Moreover, Diabetes mellitus is a frequent metabolic syndrome initially characterized by loss of glucose homeostasis. The disease is progressive and is associated with a high risk of atherosclerosis [76].
The obtained results demonstrated in (Table 1) revealed that, a significant increase in serum glucose concentration was observed in streptozotocin (STZ)-induced diabetic rats.
The increase in seurm glucose concentration of streptozotocin treated group which came in agreement with [2] who reported that, serum glucose levels was elevated three-fold in the diabetic animals group compared to normal. Who added that, Hyperglycemia, hypoinsulinemia, polyphagia, polyuria and polydipsia accompanied by weight loss were seen in adult rats within three days of Streptozotocin treatment which indicates irreversible destruction of Langerhans islets cells. The developed hyperglycemia have been attributed to the specific toxic effects of STZ uptake through glucose transport–2 (GLUT-2) , these toxic effects lead to end organ damage through activation of the aldose - reductase pathway leading to toxic accumulation of sorbitol in nervous system [27]. Treatment with α-lipoic acid to STZ-induced diabetic rats significantly reduced elevated serum glucose level. These results are nearly

similar to those recorded by [25] 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 cell culture experiments [32]. Some studies suggested that treatment with racemic - lipoic acid improves insulin sensitivity [36] 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 [77].

Regarding, serum lipid profiles the obtained results revealed that, a significant increase in serum total cholesterol, triacylglycerols, LDL-C, VLDL-C and HDL-C concentrations were observed in streptozotocin (STZ)-induced diabetic rats. The obtained results are nearly similar to those reported by [73] who recorded that, abnormal increased in the levels of lipid, lipoproteins and lipid peroxides were observed in plasma of diabetes mellitus which may be due to the abnormal lipid metabolism [71]. Moreover, [12] demonstrated that, Plasma total cholesterol, triacylglycerols and low density lipoprotein levels of STZ group were increased significantly. The most common lipid abnormalities in diabetes are hypercholesterolemia and hyper-triglyceridemia [49]. Increased level of total cholesterol may be due to higher cholesterol biosynthesis and/ or liposysis [45]. Additionally, this atherogenic indexes in agreement with the reported studies of [26] who related the atherosclerosis complications and higher in TG level is predominantly due to reduced lipolysis of triglyceride-rich lipoproteins.

[6] reported that, there was an increase in the serum and cardiac triglyceride levels in diabetic rats.

Table 1 Effect of treatment with α-lipoic acid on Serum Glucose, Total Cholesterol, Triacylglycerol, HDL-C, LDL-C, VLDL-C and Vitamin C concentrations in normal and streptozotocin-induced diabetic male rats**.**

		Animal groups			
Parameter	Time	Group I	Group II	Group III	Group IV
Glucose (mg/dL)	4 weeks	52.42 ± 2.08^{c}	384.00 ± 29.31^{a}	194.84 ± 14.50^{b}	59.92 ± 2.65^{c}
	6 weeks	55.08 ± 3.95^{c}	338.04 ± 12.60^{a}	192.75 ± 28.62^{b}	84.92 ± 3.24^{c}
Total Cholesterol (mg/dL)	4 weeks	80.95 ± 3.25^{b}	117.06 ± 5.42^{a}	79.88 ± 3.11^{b}	60.07 ± 3.40^{c}
	6 weeks	72.77 ± 4.45^{a}	62.33 ± 4.07^{ab}	58.50 ± 4.82^{b}	65.83 ± 4.38^{ab}
Triacylglycerols (mg/dL)	4 weeks	78.13 ± 3.72^{b}	93.54 ± 1.88^{a}	54.86 ± 5.28^{c}	67.67 ± 3.94^{b}
	6 weeks	73.73 ± 3.67^{a}	49.00 ± 5.03^{b}	42.15 ± 4.73^{b}	45.87 ± 2.23^{b}
HDL-C (mg/dL)	4 weeks	52.63 ± 2.37^{b}	68.40 ± 7.65^{a}	51.40 ± 3.29^{b}	37.81 ± 4.43^{b}
	6 weeks	49.42 ± 3.24^{a}	37.07 ± 2.32^{b}	37.86 ± 3.32^{b}	43.53 ± 2.89^{ab}
LDL-C (mg/dL)	4 weeks	13.08 ± 3.68^{b}	31.93 ± 8.50^{a}	17.52 ± 5.45^{ab}	11.32 ± 4.37^{b}
	6 weeks	8.60 ± 1.18^{c}	17.92 ± 1.59^{a}	12.24 ± 1.39^{bc}	13.13 ± 1.57^{b}
VLDL-C (mg/dL)	4 weeks	15.63 ± 0.74^{b}	18.71 ± 0.38^{a}	10.97 ± 1.06^{c}	13.53 ± 0.788^{b}
	6 weeks	14.75 ± 0.734^{a}	9.80 ± 0.01^{b}	8.43 ± 0.95^{b}	9.17 ± 0.45^{b}
Vitamin C (mg/dL)	4 weeks	1.11 ± 0.1^{ab}	0.68 ± 0.07^{c}	0.91 ± 0.03^{bc}	1.3 ± 0.17^{a}
	6 weeks	1.09 ± 0.08^{a}	0.63 ± 0.04^{b}	1.08 ± 0.05^{a}	1.18 ± 0.09^{a}

Groups I, II, III and IV Control group, Diabetic group, Diabetic alpha lipoic acid treated group, and Control alpha lipoic acid treated group. Data are presented as Mean (±S.E) with different superscript letters in the same column are significantly different at ($P<0.05$).

The reported changes in TG could be related to the mild but significant insulin deficiency resulted in mild hypertriglyceridemia, linked to impaired triglyceride removal rather than to an overproduction of VLDL-triglyceride, despite elevated levels of plasma free fatty acids. The obtained results showed that, serum HDL-cholesterol level was increased in diabetic rats. This result is in agreement with these reported by [75] who recorded that, serum cholesterol and HDL cholesterol concentrations of spontaneously diabetic Bio-Breeding (BB) and non diabetic littermate rats did not differ from controls. Concentrations of very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) of spontaneously diabetic Bio-Breeding (BB) and non diabetic littermate rats were higher than those of normal rats. The obtained results showed that, serum total cholesterol levels increased significantly in diabetic group than control one. These results agreed well with [39] who attributed the increase in total cholesterol level to the increased β-oxidation of long chain FA and increased oxidation of ketogenic amino acids producing excess of hepatic acetyle CoA that is used for cholesterol synthesis [1].

The obtained data showed that, after 6 weeks of the experiment there is a non significant decrease of cholesterol value was observed, and significant decrease of HDL–cholesterol, triglyceride and VLDL, but LDL-C still increase. Such variations in plasma lipid and lipoprotein profiles in STZ-diabetic rats may be due to impaired liver function as a result of diabetes complications. The liver helps maintain normal blood glucose concentration in the fasting and postprandial states. Loss of insulin effect on the liver leads to glycogenolysis and an increase in hepatic glucose production. Abnormalities of triglycerides storage and lipolysis in insulin-sensitive tissues such as the liver are an early manifestation of conditions characterized by insulin resistance and are detectable earlier than fasting hyperglycemia. The precise genetic, environmental, and metabolic factors and sequence of events that lead to the underlying insulin resistance, however, is not fully understood [43]. However cholesterol is synthesized mainly in the liver, so its value is decreased as a result of liver dysfunction and diabetic rats cannot use glucose for take energy so it depend mainly on lipids as a supply for energy.

Treatment with α- Lipoic acid to STZ-induced diabetes in rats resulted in significant reduction of elevated serum total cholesterol, HDL-cholesterol, triacylglycerols, VLDL-cholesterol and significantly decreased LDL-cholesterol concentrations. These results are nearly similar to those reported by, [64] who reported that, Alpha-Lipoic acid (α-LA) is a unique antioxidant and has beneficial effects on fuel metabolism. Moreover, they added that, following eight weeks supplementation of Alpha-Lipoic acid (α-LA) in diabetic rats were significantly lower plasma total cholesterol (TC), triglycerides and low density lipoprotein-cholesterol (LDL-C) levels and increased high density lipoprotein-cholesterol (HDL-C) levels when compared with control group. These results suggest that, ALA has the potential in improving dyslipidemia and may exert some protective effects on atherosclerotic vascular changes in diabetic rats. These results suggest that alpha-Lipoic acid (α-LA) has the potential in improving dyslipidemia and may exert some protective effects on atherosclerotic vascular changes in diabetic rats. Lipoic acid-supplemented aged rats showed a reduction in the levels of triglycerides, and this may be attributed to an increase in the activity of lipoprotein lipase [31]. The mechanism by which ALA improves the dyslipidemia is still unclear. One of the actions which might attribute to this finding is by decreasing the non-esterified fatty acid levels. The mechanism of action is also believed to be through the

controlling activities of enzymes that involved in lipid metabolism. ALA was reported to reduce HMG-CoA reductase activity as well as increases the lipoprotein lipase and Lecithin Cholesterol Acyl Transferase (LCAT) activities [64]. The decrease in serum triacylglycerols and total cholesterol after administration of LA comes in agreement with [66]. This result could be attributed to the effect of LA in reducing triacylglycerols accumulation in skeletal muscles, pancreatic islets as well as in adipose tissue where, LA increases fatty acid oxidation by activating the AMP-activated Protein Kinase (AMPK) in skeletal muscles [42]. The anti-oxidative action of LA may suggest its preventive effect on LDL-cholesterol oxidation and thus, enhancing its catabolism. From the previous results, it could be concluded that, LA has an anti-hyperlipidemic effect on diabetic rats.

Regarding, vitamin C the obtained results showed that, a significant decrease in serum vitamin C concentration was observed in streptozotocin-induced diabetic rats all over the periods of the experiments. These results were nearly similar to those reported by [4] who reported that, hypovitaminosis among diabetics is wide spread , 32%, 13% and 37% of the subjects had severe vitamins A, E and C deficiencies, respectively. This pronounced decrease in vitamin C levels in diabetic rats is in agreement with the results reported previously by [53] who reported that , stress in diabetes is corroborated by the fact that diabetic patients have significantly lower cellular and plasma levels of antioxidants, particularly GSH, ascorbate (vitamin C) and tocopherol (vitamin E) in comparison with healthy people . Increased oxidative stress and low vitamin C levels are correlated with severity of diabetic neuropathy [79]. Low vitamin C levels may be a consequence of diabetes because cellular uptake of vitamin C is regulated by glucose and insulin [17]. Diabetic subjects may also have a higher turnover of vitamin C due to increased oxidative stress and oxidation of ascorbate to dehydroascorbic acid in mitochondria [62]. Patients with diabetic nephropathy have increased renal clearance of vitamin C [33]. Meanwhile, treatment with α-Lipoic acid significantly increased vitamin C concentrations in streptozotocin (STZ)-induced diabetic rats after 6 weeks of the experiment when compared with control diabetic non treated group. These results are nearly similar to those reported by [25] who reported that, serum vitamin C and erythrocyte reduced glutathione levels reached values that were significantly higher than the untreated diabetic model after treatment with ALA. In addition, α-lipoic acid can reduces oxidative stress in the body and indirectly spares or recycles or regenerates the other major antioxidants, raising their levels in the blood stream [23]. Vitamin C can be regenerated through reaction with alpha-lipoic acid [51]. In fact, alpha-lipoic acid has been shown to protect against the symptoms of vitamin E or vitamin C deficiency in animals fed diets deficient in those nutrients ([57]. Also, dihydrolipoic acid is able to reduce GSSG to GSH and dehydroscorbic acid to vitamin C. [10]. LA is the only antioxidant that is both fat- and water-soluble. Consequently, his property has made it a perfect electron transporter for both oxidized ascorbic acid (water-soluble) and vitamin E (fat-soluble); it is easily absorbed and transported across cell membranes. Whereas many antioxidants provide protection only outside of cells, LA is broken down inside cells to dihydrolipoic acid (a more potent antioxidant). It also regenerates other antioxidants like vitamin C, vitamin E and GSH. LA alleviates free radical damage in both fatty and aqueous regions of cells and helps to recycle other antioxidants in what is described as "antioxidant synergism". When LA is missing, other antioxidants do not interact well, thereby reducing their ability to protect cells [40].

The obtained results demonstrated in (Table 2) revealed that, a significant increase in liver and kidney L-MDA concentrations were observed in streptozotocin-induced diabetic rats when compared with control group. However, treatment with α-Lipoic acid resulted in significant decrease in both liver and kidney L-Malondialdehyde concentrations all over the periods of the experiments when compared with diabetic non-treated group. Lipid peroxidation is a marker of cellular oxidative damage initiated by reactive oxygen species [21]. These results are nearly similar to those reported by [59] who reported that, the levels of TBARS and hydroperoxides in liver and kidney diabetic rats were significantly higher than control rats. The increased lipid peroxidation in the diabetic animals may be due to the observed remarkable increase in the concentration of TBARS and hydroperoxides (lipid peroxidative markers) in the liver and kidney of diabetic rats [68]. [30] reported that, in diabetes mellitus, oxygen free radicals are generated by stimulating H_2O_2 in-vitro, as well as in-vivo and in the pancreatic β-cells. In the present study, the increased tissue malondialdehyde in liver and kidney of STZ- induced diabetic rats served as an index of elevated lipid peroxidation in diabetic condition. The increase in lipid peroxidation indicates an increased oxidative stress as a result of excessive generation of free radicals.

Treatment with α-Lipoic acid to STZ-induced diabetic rats showed significant decrease in both liver and kidney L-malondialdehyde concentrations all over the periods of the experiments. Similarly, [60] reported that, MDA levels have been found to be increased in the brain, liver, and kidney in STZ-induced diabetic rats. However, treatment with Alpha-Lipoic acid (α-LA) significantly decreased the MDA level, which may be partly due to the ability of Alpha-Lipoic acid (α-LA) to scavenge free radicals.

Table 2 Effect of treatment with alpha lipoic acid on L-MDA concentrations, Catalase (CAT), Superoxide dismutase (SOD) and Glutathione peroxidase (GPX) activities in liver and kidney of normal and streptozotocin-induced diabetic rats**.**

Parameter	Organ	Time	Group I	Group II	Group III	Group IV
L – MDA (nmol/gm tissue)	Liver	4 weeks	76.95± 7.22^{c}	106.15±13.48^{b}	72.05±8.10^{c}	141.70±4.43^{a}
		6 weeks	57.98±5.39^{c}	71.72±6.46^{b}	37.85±2.99^{d}	147.27±2.61^{a}
	kidney	4 weeks	36.43± 1.26^{b}	50.48±2.63^{a}	34.58±2.88^{b}	22.31±1.78^{c}
		6 weeks	12.75±0.935^{c}	36.36±3.66^{a}	19.78±2.01^{c}	26.96±2.21^{b}
CAT (mmol/min/μg protein)	Liver	4 weeks	5.57±0.276^{a}	1.36±0.161^{c}	2.16±0.147^{b}	2.10±0.221^{b}
		6 weeks	3.73±0.527^{a}	1.51±0.136^{b}	1.04±0.070^{b}	0.950±0.120^{b}
	kidney	4 weeks	2.66±0.289^{a}	1.43±0.084^{b}	1.15±0.177^{b}	1.21±0.090^{b}
		6 weeks	1.43±0.072^{b}	4.07±0.237^{a}	0.354±0.017^{c}	1.47±0.208^{b}
SOD (U/μg protein tissue)	Liver	4 weeks	0.625±0.084^{a}	0.516±0.068^{ab}	0.370±0.052^{bc}	0.276±0.062^{c}
		6 weeks	0.440±0.019^{a}	0.422±0.028^{a}	0.248±0.023^{b}	0.383±0.036^{a}
	kidney	4 weeks	1.40±0.115^{a}	1.10±0.155^{a}	1.45±0.218^{a}	1.01±0.119^{a}
		6 weeks	1.22±0.304^{a}	0.940±0.073^{a}	1.17±0.257^{a}	0.971±0.066^{a}
GPx (mg/min/mg protein)	Liver	4 weeks	0.046±0.003^{a}	0.027±0.005^{b}	0.018±0.001^{b}	0.022±0.002^{b}
		6 weeks	0.041±0.004^{a}	0.023±0.003^{b}	0.022±0.002^{b}	0.023±0.004^{b}
	kidney	4 weeks	0.135±0.018^{a}	0.127±0.021^{a}	0.137±0.017^{a}	0.120±0.004^{a}
		6 weeks	0.087± 0.012^{a}	0.088±0.006^{a}	0.062±0.008^{a}	0.077±0.008^{a}

Groups I, II, III and IV Control group, Diabetic group, Diabetic alpha lipoic acid treated group, and Control alpha lipoic acid treated group. Data are presented as Mean (±S.E) with different superscript letters in the same column are significantly different at ($P<0.05$).

Alpha-lipoic acid improves other markers of oxidative stress in the diabetic kidney, including glutathione and malondialdehyde [47]. Moreover, alpha lipoic acid has the unique ability to neutralize free radicals within aqueous and lipid regions of the cells, as well as in intracellular and extra cellular environments [29]. This ability allows alpha lipoic acid to be easily transported across cellular membranes to neutralize free radicals. Furthermore, [8] reported that, supplementation (DL- α -lipoic acid (100 mg/kg body wt/day) intraperitoneally for 7 and 14 days in aged rats prevents the elevated levels of TBARS and lipids. Lipid peroxidation is characteristically a free radical chain reaction initiated by the abstraction of a hydrogen atom from a polyunsaturated fatty acid side chain. The free radical-mediated lipid peroxidation has been proposed to be critically involved in several disease states including cancer, cardiovascular diseases, and cataracts as well as the degenerative processes associated with ageing [5].

Regarding, antioxidant enzymes the obtained data (Table 2) showed that, a significant decrease in liver and kidney catalase activities were observed in STZ-induced diabetic rats followed by significant increase in kidney catalase after six weeks of the experiment. On the other hand, a non significant decrease in liver and kidney SOD activities were observed in streptozotocin-induced diabetic rats all over the periods of the experiment. Also, GPx activity showed a significant decrease in liver of diabetic rats while, kidney GPx activity showed a non significant decrease. The recorded data are nearly similar to those reported by [54] who reported that, reduced activities of SOD and CAT in liver and kidney tissues have been observed in diabetes rats, and this decrease may result in a number of deleterious effects due to accumulation of superoxide radicals ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). Also, such decrease in the activities of (GPx, GST and GR) enzymes results in the involvement of deleterious oxidative changes and also insufficient availability of GSH. [58]. Also, the obtained results is agreement with [72] who reported that , an increase in the concentration of thiobarbituric acid reactant species (TBARS), and reduced activities of glutathione reductase, glutathione peroxidase and superoxide dismutase were observed in alloxan induced diabetic rats when compared with controls. Furthermore, [80] reported that, oxidative stress means disturbing dynamic balance between prooxidants and antioxidants, either due to the increased production of oxygen free radicals or the decreased antioxidant activity. Consequences of oxidative stress are damage to DNA, lipids, proteins, disruption in cellular homeostasis and accumulation of damaged molecules [37]. SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen, [46] hence diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of enzymes [67].

Glutathione peroxidase (GPx) plays a primary role in minimizing oxidative damage. GPx, an enzyme with selenium, and Glutathione-s-transferase (GST) works together with glutathione in the decomposition of H_2O_2 or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione [13]. Reduced activities of GPx may result from radical–induced inactivation and glycation of the enzyme [34]. Reduced activities of GPx and GST in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of toxic products. In this context, other workers also reported a decrease in the activity of these antioxidant enzymes (SOD, CAT, GPx and GST) in the liver and kidneys of

diabetic rats [7]. Treatment with α-lipoic acid to STZ-induced diabetic rats significantly increased liver catalase activity after four weeks of the experiment. However, kidney catalase showed a significant decrease after six weeks of treatment. Also, α-lipoic acid significantly decreased liver SOD after six weeks. While kidney SOD was none significantly increased all over the period of the experiment. On the other hand, α-lipoic acid administration to STZ-diabetic rats significantly decreased GPx in liver after four weeks and non significantly decrease kidney GPx after six weeks of treatment. [3] found that, LA contributes to antioxidant defense by increasing CAT activity in the stress group. The decline in CAT can be attributed to ineffective scavenging of H_2O_2 resulting in increased H_2O_2 levels, which can react with O_2.- to give OH. radical and thus increased lipid peroxidation. Catalase requires NADPH for its regeneration from its inactive form [9]. [70] reported that, α-Lipoic acid treatment significantly reduced MDA levels and increased antioxidant enzyme (SOD and catalase) activity 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. On the other hand, increased oxidative stress in diabetic animals was reversed with α–lipoic acid treatment. In the meantime, SOD scavenger significantly decreased in diabetes, but α–lipoic acid increased again. This increment was similar to that reported by [16] who suggested that, α–lipoic acid recycles other antioxidants.

Lipoic acid inhibited the free radical mediated lipid peroxidation, preserved the antioxidant enzymes and maintained the non-enzymic antioxidant concentrations. This ameliorative effect of LA on tissue lipid peroxidation might also be attributed to its ability to increase glucose disposal [35] thereby abolishing the consequences of hyperglycaemia. In the present study, no significant effect of ALA on the activity of G-Px was observed, this results are similar to those reported by [18].

From the obtained results it could be concluded that, experimental induced diabetes mellitus in rats extensively alters and induced disturbances in lipid metabolism. Moreover, vitamin C and lipid peroxidation which may contribute to the development of diabetic complications. This study suggest that, ALA may be effective in controlling glycemic status and improving dyslipidemia in streptozotocin-induced diabetic rats and has the potential in reducing cardiovascular complications due to diabetes mellitus. Also, ALA improved significantly the diabetes-induced deterioration of vitamin C and maintain the increase in the lipid peroxidation prevent oxidative damage.

5. REFERENCES

1. Abdel-Azim, S.A.; Bader, A.M. and Barakat, M.A. 2002. Effect of metformin, glyburide, and/or selenium on glucose homeostasis, lipid peroxidation, glutathione levels and changes in glutathione peroxidase activity in streptozotocin-induced diabetic rats. *Egypt. J. Biochem.* **20**: 393-411.
2. Akbarzadeh, A., Norouzian, D., Mehrabi,M.R., Jamshidi, S.H., Farhangi, A., Allah Verdi, A., Mofidian, S.M. and LameRad,,B 2007. Induction of diabetes by streptozotocin in rats. *Indian J. Clinical Biochem*, **22**: 60-64.
3. Akpinar, D., Yargiçoğlu, P., Derin, N.; Alicigüzel, Y. and Ağar, A. 2008. The Effect of Lipoic Acid on Antioxidant Status and Lipid Peroxidation in Rats Exposed to Chronic Restraint Stress. *Physiol. Res.* **57**: 893-901.
4. Aliyu, M.; Lawal, M.; Mojiminiyi, F.; Saidu, Y. and Bilbis, L.S. 2005. Serum antioxidant vitamins levels in non- insulin-

dependent diabetes mellitus subjects in Sokoto, Nigeria. *Biokemistr*. **17**:107-114.

5. Ames, B.N.; Shigenaga, M.K. and Hagel, T.M. 1993. Antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci.* **90**: 7915-7922.
6. Anandh Babu, P. V.; Sabitha, K. E. and Shyamaladevi, C. S. 2006. Green tea extract impedes dyslipidaemia and development of cardiac dysfunction in streptozotocin- diabetic rats. *Clinical and Experimental Pharmacology and Physiology.* **33**: 1184-1189.
7. Anuradha, C.V. and Selvam, R. 1993. Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan induced diabetic rats. *J Nutr Biochem,* **4**: 212.
8. Arivazhagan, P.; Ramarathan, K. and Panneerselman, C. 2003. Effect of DL-α-Lipoic acid on the status of lipid peroxidation and antioxidants in mitochondrion of aged rats. *J. Nutr Biochem* **12**: 2-6.
9. Arivazhagan, P.; Thilakovathy, T. and Panneerselvam, C. 2000. Antioxidant lipoate and tissue antioxidants in aged rats. *J Nutr Biochem* **11**: 122-127.
10. Bast, A. and Haenen, G.R. 1988, Interplay between lipoic acid and glutathione in the protection against microsomal lipid-peroxidation. *Biochem.Biophys Acta.* **963**: 558-561.
11. Bauer, J. D. 1982. Clinical laboratory methods" 9th Ed, the C.V. Company Waistline Industrial Missouri 63116 Chapter 33. Pp. 555.
12. Baydas, G.; Canatan, H. and Turkoglu, A. 2002. Comparative analysis of the protective effects of melatonin and vitamin E on streptozocin-induced diabetes mellitus. *J. Pineal Res.* **32**: 225–230.
13. Bruce, A.; Freeman, D. and James, C. 1982. Biology of disease – free radicals and tissue injury. *Lab Invest* **47**: 412.
14. Cakatay, U.; Kayali, R.; Sivas, A. andTekeli, F. 2005. Prooxidant activities of alpha-lipoic acid on oxidative protein damage in the aging rat heart muscle. *Arch. Gerontol. Geriatr.*, **40**: 231-40.
15. Chitra, V.; Varma, P.V.; Raju, M.V. and Prakash, K.J. 2010. Study of antidiabetic and free radical scavenging activity of the seed extract of Strychnos nuxvomica. *Int J Pharm Pharm Sci*. **2**:106-110.
16. Cho, Y.S.; Lee, J.; Lee, T.; Park, J. and Moon, H. 2004. α-Lipoic acid inhibits airway inflammation and hyper responsiveness in a mouse model of asthma. *J. Allergy and Clinical Immunology* **114**: 429- 435.
17. Cunningham, J.J. 1998. The glucose/insulin system and vitamin C: implications in insulin-dependent diabetes mellitus. *J Am Coll Nutr* **17**: 105–108.
18. Dincer, Y.; Telci, A.; Kayali, R.; Yilmaj, I.A.; Cakatay, U. and Akcay, T. 2002. Effect of alpha-lipoic acid on lipid peroxidation and antioxidant enzyme activities in diabetic rats. *Clin. Exp. Pharmacol and Physiol.* **29**: 281- 284.
19. Ellefson, R.D. and Caraway, W.T. 1976. Fundamentals of clinical chemistry. Tietz NW. Pp. 506.
20. El-Nabarawy, S.K.; Mohamed, M.A.; Mohamed, M.A. and El-Arabi, G.H. 2010. α-Lipoic Acid Therapy Modulates Serum Levels of Some Trace Elements and Antioxidants in Type 2 Diabetic Patients. *American Journal of Pharmacology and Toxicology* **5**: 152-158.
21. Farber, J.L.; Kyle, M.E. and Coleman, J.B. 1990. Mechanisms of cell injury by activated oxygen species. *Lab Invest* **62**: 670-679.
22. Friedewald, W. T. 1971. Colorimetric method for determination of cholesterol LDL activity.
23. Fuchs, J.; Schofer, H.; Milbradt, R.; Freisleben,H.J.; Buhl,R.; Siems,W. and Grune,T. 1993. Studies on lipoate effects on blood redox state in human immunodeficiency virus infected patients. *Arzneimittel-Forschung / Drug Research* **43-2**: 1359-1362.
24. Gallou, G.; Ruelland, A.; Campion, L. Maugendre; D.; Le Moullec, N.; Legras, B.; Allannic, H. and Cloarec, L. 1994. Increase in thiobarbituric acid-reactive substances and vascular complications in type 2 diabetes mellitus. *Diabetes Metab,* **20**:258–264.
25. Ghada, G. El-Hossary; Amany, H.M. El-Shazly; Nadia S. Ahmed; Amal A. El-Gohary; Fatma G. Metwally and Sawsan H. Karam 2010. Efficacy of Alpha-Lipoic Acid against Diabetes-Induced

Deterioration of Blood Antioxidants and Diabetic Retinopathy in Experimental Animals. *Australian Journal of Basic and Applied Sciences* **4**: 127-134.

26. Goldfard, A.S. and Passas, L. 2002. Prediction of renal insufficiency in pima. Indians with nephropathy of type-2 diabetes mellitus. *Am. J. Kidney Dis.* **40**: 252-264.
27. Greene, D.A.; Lattimer, S.A. and Sima, i. A. 1999. Sorbitol, phosphoinositides, and sodium-potassium- ATPase in the pathogenesis of diabetic complications. N. *Engl. J. Med.* **316**: 599-606.
28. Gross, R.T.; Bracci, R.; Rudolph, N.; Schroeder, E. and Kochen, J.A. 1967. Hydrogen peroxide toxicity and detoxification in the erythrocytes of newborn infants. *Blood* **29**: 481–493.
29. Gurer, H.; Ozgunes, H.; Oztezcan, S. and Ercal, N. 1999. Antioxidant role of alpha lipoic acid in lead toxicity. *Free Radic Biol Med.* **27**: 75-81.
30. Halliwall, B. and Gutteridge, J.M. 1989. Free radicals in biology and medicine 2nd ed. Clarendern Press, Oxford.
31. Hemila, H.. 1992. Vitamin C and plasma cholesterol. *Crit Res Food Sci Nutr*. **32**: 33-57.
32. Henriksen, E.J. 2006. Exercise Training and the antioxidant alpha-lipoic acid in the treatment of insulin resistance and type 2 diabetes. *Free Radic. Biol. Med.* **40**: 3-12.
33. Hirsch, I.B.; Atchley, D.H.; Tsai, E.; Labbe, R.F. and Chait, A. 1998. Ascorbic acid clearance in diabetic nephropathy. *J Diabetes Complications* **12**: 259–263.
34. Hodgson, E.K. and Fridovich, I. 1975. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: inactivation of the enzyme. *Biochemistry* **24**: 5294.
35. Jacob, S.; Henriksen, E.J.; Schiemann, A.L.; Simon, I.; Clancy, D.E. and Tritschler, H.J. 1995. Enhancement of glucose disposal in patients with type 2 diabetes by α-lipoic acid. *Arzneimittelfor schung,* **45**: 872-874.
36. Jacob, S.; Ruus, P.; Hermann, R.; Tritschler, H.J.; Maerker, E.; Renn, W.; Augustin, H.J.; Dietze, G.J. and Rett, K. 1999. Oral administration of RAC-alpha-lipoic acid modulates insulin sensitivity in patients with type 2 diabetes mellitus: a placebo-controlled pilot trial. *Free Radio Biol Med* **27**: 309-314.
37. Jakus, V. 2000. The Role of Free Radicals, Oxidative Stress and Antioxidant Systems in Diabetic Vascular Disease. *Bratisl Lek Listy*. **101**: 541-551.
38. Kakadiya, J.; Shah, M. and Shah, N. 2010. Glimepiride reduces on experimentally induced is chemical reperfusion in diabetic rats. *IJABPT* **1**: 276-285.
39. Kandeil, M.A.; Amin, K.A.; Hassanin, K.M.A.; Ali, K.M. and Eman T. Mohammed. 2007. Serum levels of insulin and leptin in lipoic acid- treated and nontreated experimentally diabetic rats. BS. VET. MED. J. 5TH Scientific Conference. Pp. 87-95.
40. Kim, M.S.; Park, J.Y.; Namkoong, C.; Jang, P.G.; Ryu, J.W.; Song, H.S.; Yun, J.Y.; Namgoong, I.S.; Ha, J.; Park, I.S.; Lee, I.K.; Viollet, B.; Youn, J.H.; Lee, H.K. and Lee, K.U. 2004. Anti-obesity of a-lipoic acid-mediated by suppression of hypothalamic AMP activated protein kinase. *Nat. Med.* **10**: 727-733.
41. Kyaw, A. 1978. A simple colorimetric method for ascorbic acid determination in blood plasma. *Clin. Chim. Acta* **86**: 153-157.
42. Lee, W.J.; Song, K. H.; Koh, E.H.; Won, J.C.; Kim, H.S.; Park, H. S.; Kim, M. S.; Kim, S.W.; Lee, K. U. and Park, J.Y. 2005. α-Lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle. *Biochem. Biophys. Res. Communi.* **332**: 885–891.
43. Lewis, G.F.; Carpentier, A.; Khosrow, A. and Giacca, A. 2002. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* **23**: 201-229.
44. Malaisse, W.J. 1983. Insulin release: the fuel hypothesis. *Diab. Metab.* **9**: 313-320.
45. Mallick, C.; Rajkumar, M. and Gosh, D. 2006. Comparative study on anti hyperglycemic and anti hyperlipidemic Effects of separate and composite extract of seed of Eugenia jambolana and root of musa paradisiacal in streptozotocin-induced diabetic male Alibino Rat. *Iranian J pharmacol Therap*. **5**: 27-33.
46. Mc Crod, J.M.; Keele, B.B. and Fridovich, I. 1976. An enzyme based theory of obigate anaerobiosis, the physiological

functions of superoxide dismutase. *Pro Natl Acad Sci USA* **68**: 1024.
47. Melhem, M.F.; Craven, P.A.; Liachenko, J. and Derubertis, F.R. 2002. Alpha-lipoic acid attenuates hyperglycemia and prevents glomerular mesangial matrix expansion in diabetes. *J Am Soc Nephrol*, **13**: 108–116.
48. Mesbah, L.; Soraya, B.; Narimane, S. and Jean, P.F. 2004. Protective effect of flavonides against the toxicity of vinblastine cyclophosphamide and paracetamol by inhibition of lipid – peroxydation and increase of liver glutathione. *Haematol*. **7**: 59-67.
49. Mitra, S.K.; Gopumadhavan, S.; Muralidhar, T.S.; Anturlikar, S.D. and Sujatha, M.B. 1995. Effect of D-400, ahebomineral preparation on lipid profile, glycated hemoglobin and glucose tolerance in STZ- induced diabetes in rats. *Indian J Exp Biol*. **33**: 798-800.
50. National Cholesterol Education program Recommendation 1995. For Measurement of High- Density Lipoprotein Cholesterol: Executive Summary. *Clin Chem.* **41**: 1427-1433.
51. Packer, L.; Witt, E.H. and Tritschler, H.J. 1995. Alpha-lipoic acid as a biological antioxidant. *Free Radical Biology and Medicine* **19**: 227-250.
52. Paoletti, F. and Macali, A. 1990. Determination of superoxide disumutase activity by purely chemical system based on NAD (P) H oxidation. *Methods Enzymol* **186**: 209–220.
53. Patel, M.S. and Vettakkorumakankav, N.N. 1995. Lipoic acid-requiring proteins: recent advances. In: Biothiols in Health and Disease. Ed. Packer L, Cadenas E, Marcel Dekker Inc., New York. Pp. 373–388.
54. Raghavan, B.; and Kumari, S.K. 2006. Effect of terminalia arjuna stem bark on antioxidant status in liver and kidney of alloxan diabetic rats. *Indian J Physiol Pharmacol*. **50**: 133–142.
55. Ramanathan, M.; Jaiswal, A.K. and Bhattacharya, S.K. 1999. Superoxide dismutase, catalase jand glutahiones peroxidase activities in the brain of streptozotocin induced diabetic rats. Indian. *J. Exp. Biol.* **37**: 182-183.
56. Ramkumar, K.; Rajaguru, P.; Latha, M. and Ananthan, R. 2007. Ethanol extracts of Gymnema montanum leaves reduces glycoprotein components in experimental diabetes. *Nutr.Res.* **27**: 97-103.
57. Rosenberg, H.R. and Culik, R. 1959. Effect of "α-lipoic acid on vitamin C and vitamin E deficiencies. *Archives of Biochemistry and Biophysics* **80**: 86-93.
58. Satheesh, M.A. and Pari, L. 2004. Antioxidant effect of Boerhavia diffuse L in tissues of alloxan induced diabetic rats. *Indian J Exp Biol.* **42**: 989-992.
59. Sathishsekar, D. and Subramanian, S. 2005. Antioxidant properties of Momordica Charantia (bitter gourd) seeds on Streptozotocin induced diabetic rats. *Asia Pac J Clin Nutr*, **14**: 153-158.
60. Sena, C.M.; Nunes, E.; Louro, T.; Proencoa, T. and Seica, R.M. 2007. Endothelial dysfunction in type 2 diabetes effects of antioxidants. *Rev. port. Cardia*. **26**: 609 – 619.
61. Sharma, V.K.; Kumar, S.; Patel, H.J. and Hugar, S. 2010. Hypoglycemic activity of Ficus glomerata in alloxan induced diabetic rats. *IJPSRR* **1**: 18-22.
62. Sinclair, A.J.; Girling, A.J.; Gray, L.; Le Guen, C.; Lunec, J. and Barnett, A.H. 1991. Disturbed handling of ascorbic acid in diabetic patients with and without microangiopathy during high dose ascorbate supplementation. *Diabetologia* **34**: 171–175.
63. Siti, B.B.; Khairul, O.; Wan Nazaimoon, W. M.; Faizah, O.; Santhana, R. L.; Mokhtar, A. B.; Megat, R. and Jamaludin, M. 2008. Alpha lipoic acid reduces plasma glucose and lipid and the Ultra-Microscopic vascular changes in streptozotocin induced diabetic rats. *Annals of Microscopy* **8**: 58-65.
64. Siti, B.B.; Kwa, P.K.; Monica, Y.S.; Khairul, O.; Mokhtar, A.B. and Jamaludin, M. 2007). Alpha-lipoic acid prevents pancreatic islet cells damage and dyslipidemia in streptozotocin-induced diabetic rats. *Malaysian J. Med. Sci.* **14**: 47-53.
65. Snedecor, G. W. and Cochoran W. G. 1969. Statistical methods 6th ed. The Iowa state Univ. Press, Iowa, USA.
66. Song, K.H.; Lee, W.J.; Koh, J.M.; Kim, H.S.; Youn, J.Y.; Park, H. S.; Koh, E.H.;

Kim, M.S.; Youn, J.H.; Lee, K.U. and Park, J. Y. 2005. Alpha-lipoic acid prevents diabetes mellitus in diabetes-prone obese rats. *Biochem. Biophys. Res. Commun.* 326: 197-202.

67. Sozmen, E.Y.; Sozmen, B.; Delen, Y. and Onal, T. 2001. Catalase\superoxide dismutase and catalase paraoxonase ratios may implicate poor glycemic control. *Arch. Med. Res.* **32**: 283-287.
68. Stanely, P.; Prince, M. and Menon, V.P. 2001. Antioxidant action of Tinospora cordifolia root extract in alloxan diabetic rats. *Phytother Res* **15**: 213.
69. Stein, E.A. (1987): lipids, lipoproteins, and apolipoproteins. In NW Tietz, ed. Fundamentals of clinical chemistry, 3rd ed. Philadelphia: WB Saunders. Pp. 448.
70. Stevens, M.J.; Obrosova, X.; Cao, X.; van Huysen, C. and Greene, D.A. 2000. Effects of DL-alpha-lipoic acid on peripheral nerve conduction, blood flow, energy metabolism, and oxidative stress in experimental diabetic neuropathy. *Diabetes* 1006–1015.
71. Suckling, K.E. and Brian, J. 1993. Animal Models of Human lipid Metabolism. *Prog lipid. Res.* **32**: 124.
72. Sukalski, K.A.; Pinto, K.A. and Berntson, J.L. 1993. Decreased Susceptibility of Liver Mitochondria from Diabetic Rats to Oxidative Damage and Associated Increase in Alpha-Tocopherol. *Free Radic Biol. Med.* **14**: 57-65.
73. Suryawanshi, N.P.; Bhutey, A.K.; Nagdeote, A.N.; Jadhav, A.A. and Manoorkar, G.S. 2006. Study of lipid peroxide and lipid profile in diabetes mellitus. *Indian Journal of Clinical Biochemistry* **21**: 126-130.
74. Tietz, N.W. 1995. Clinical guide to laboratory tests. 3rd ed. Philadeiphia. WB Saunders. Pp. 268-273.
75. Tsutsumi, Y.;Matsubara, H.; Masaki,H.; Kurihara, H.;Murasawa,S.; Takai, S.; Miyazaki, M.; Nozawa, Y.; Ozono, R.; Nakagawa, K.;Miwa, T., Kawada, N.; Mori, Y.; Shibasaki, Y.; Tanaka, Y., Fujiyama, S.; Koyama, Y.; Fujiyama, A.; Takahashi, H.; Iwasaka, T. 1999. Angiotensin II type 2 receptor overexpresion activates the vascular kinin system and cause vasodilation . *J Clin Invest.* **104**: 925-935.
76. Wakabayashi, I. and Masuda, H. (2004): Relationship between obesity and atherosclerotic risk in elderly type 2 diabetic patients. *Nippon Ronen Igakkai Zasshi* **41**: 223-7.
77. Walgren, J.L.; Amani, Z.; McMillan, J.M.; Locher, M. and Buse, M.G. 2004. Effect of R (+) α- lipoic acid on pyruvate metabolism and fatty acid oxidation in rat hepatocytes. *Metabolism* **53**: 165-173.
78. Xu, J.B.; Yuan, X.F. and Lang, P.Z. 1997. Determination of catalase activity and catalase inhibition by ultraviolet spectrophtometry. Chinese Environ. *Chem*. **16**: 73-76.
79. Ziegler, D.; Sohr, C.G. and Nourooz-Zadeh, J. 2004. Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneuropathy and cardiovascular autonomic neuropathy. *Diabetes Care* **27**: 2178–2183.
80. Zivic, S.; Vlaski, J.; Kocic, G.; Pesic, M.; Cirić, V. and Durić, Z. 2008. The Important of Oxidative Stress in Pathogenesis of Type 1 Diabetes-Determination of Catalase Activity in Lymphocytes of Diabetic Patients. *Med. Pregl.* **61**: 458-63.

.

مجلة بنها للعلوم الطبية البيطرية
BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

التأثيرالكيميائى الحيوى لحمض ألفا ليبويك على نمط الدهون والأكسدة الفوقية للدهون و حالة الإنزيمات المضادة للأكسدة في الفئران المستحدث فيها البول السكري بالأستربتوزوتوسين

سامي علي حسين، محمد رجائى رجب حسانين ، أميرة رجب البرقي

قسم الكيمياء الحيوية– كلية الطب البيطري بمشتهر– جامعة بنها

الملخص العربى

في هذه الدراسة تم تقييم التأثير الواقي لحمض ألفا ليبويك على التغيرات في مستوى جلوكوز ودهون الدم ، فيتامين ج ، الأكسدة الفوقية للدهون والإنزيمات المضادة للأكسدة في دم وأنسجة الفئران المستحدث فيها البول السكري بالأستربتوزوتوسين. هذا وقد أستخدم لأجراء هذه الدراسة عدد 80 من ذكور الفئران البيضاء أعمارهم تتراوح من 12–16 أسبوع و أوزانها من 220–250جرام وقد قسمت إلي أربعة مجموعات متساوية اشتملت كل مجموعة على عدد عشرون فأر وتم توزيعها كالآتي: المجموعة الأولى: (المجموعة الضابطة): اشتملت على 20 فأر لم تعطى أي أدوية واستخدمت كمجموعة ضابطة للمجموعات الأخرى. المجموعة الثانية: (المجموعة المحدث بها مرض البول السكري): تكونت من 20 فأر تم حقنهم بمادة الأستربتوزوتوسين في التجويف البريتونى بجرعة مقدارها 50 ميللي جرام لكل كيلوجرام من وزن الجسم. المجموعة الثالثة:(مجموعة حمض ألفا ليبويك والمحدث بها مرض البول السكري): اشتملت على 20 فأر تم حقنهم بحمض ألفا ليبويك يومياً في التجويف البريتونى بجرعة مقدراها 54 ملليجرام لكل كيلوجرام من وزن الجسم طوال فترة التجربة وذلك بعد 5 أسابيع من حقنهم بمادة الأستربتوزوتوسين المسببة لمرض البول السكري تجريبياً. المجموعة الرابعة:(مجموعة حمض ألفا ليبويك الطبيعية): اشتملت على 20 فأر تم حقنهم بحمض ألفا ليبويك يومياً في التجويف البريتونى بجرعة مقدراها 54 ملليجرام لكل كيلوجرام من وزن الجسم طوال فترة التجربة. وقد تم تجميع عينات الدم على فترات بعد أربعة وستة أسابيع من بدء العلاج بحمض ألفا– ليبويك وذلك بعد خمسة أسابيع من الاصابة التجريبية بالداء السكرى في أنابيب نظيفة ، جافة ومعقمة وقد تم فصل مصل الدم واستخدم مباشرة لقياس تركيز الجلوكوز والكولستيرول الكلى والدهون الثلاثية والدهون عالية الكثافة والدهون منخفضة الكثافة وفيتامين ج ثم تم ذبح الفئران واخذ كلا من الكبد والكلى ودلك لتقدير الأكسدة الفوقية للدهون والأنزيمات المضادة للأكسدة بها مثل إنزيم سوبر أكسيد ديسميوتيز والكتاليز والجلوتاثايون بيروكسيديز. وقد أسفرت نتائج التحليل البيوكيميائى عن وجود زيادة فى كلا من جلوكوز الدم ونمط الدهون والليبوبروتنات بالمصل بالأضافة إلى الأكسدة الفوقية للدهون فى نسيج الكبد والكلى مع حدوث انخفاض معنوى فى مستوى فيتامين ج بالمصل وأيضا نقص معنوى فى نشاط الأنزيمات المضادة للأكسدة خاصة فى نسيج الكبد. كما أوضحت النتائج أن مجموعة الفئران المحدث بها مرض البول السكري والتي تم علاجها بحمض ألفا ليبويك تسبب في خفض كلا من مستوى الجلوكوز والدهون بالدم بالأضافة إلى نسبة الأكسدة الفوقية للدهون بالكبد والكلى في حين ارتفع مستوى فيتامين ج في الدم وزاد نشاط إنزيم الكتاليز. وخلصت الدراسة أن حمض ألفا– ليبويك له تأثير جيد في خفض مستوى سكر الدم وتحسين نسبة الدهون العالية لذلك لديه القدرة من الحد من مضاعفات أمراض القلب التي تنتج من زيادة الدهون .كذلك حمض ألفا– ليبويك لديه القدرة للحد من أمراض الأوعية الدموية وذلك برفع مستوى فيتامين ج وأيضا الحد من المضاعفات الناتجة من زيادة الأكسدة الفوقية للدهون بتخفيضها وإعادة نشاط الإنزيمات المضادة للأكسدة لطبيعتها ثانية .لذلك ينصح بتناول حمض ألفا– ليبويك لمرضى السكرى في حدود الجرعة الطبية الموصى بها وذلك للوقاية من التغيرات المصاحبة لمضاعفات مرض البول السكري.

(مجلة بنها للعلوم الطبية البيطرية: عدد 23 (1)، يونيو 2012: 34–47)