INFLUENCE OF CHRONIC TREATMENT OF α-LIPOIC ACID ON THE OXIDATIVE STRESS INDUCED BY OBESITY AND DIABETES MELLITUS IN ALBINO RAT

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Summary

Obesity is a predisposing factor for type 2 diabetes mellitus and is associated with oxidative stress due to increased mitochondrial uncoupling and β oxidation of free fatty acids. Increased oxidative stress and impaired antioxidant defense mechanisms are important factors in the pathogenesis and progression of diabetes mellitus. α-lipoic acid and its reduced form dihydrolipoic acid, reduces oxidative stress by scavenging a number of free radicals in both membrane and aqueous domains. This study was designed to evaluate the effect of α-lipoic acid on oxidative stress induced by diabetes mellitus and obesity. Enzymatic antioxidants and malondialdehyde levels of liver, heart and kidney were estimated as an index of antioxidant status and oxidative stress. The study demonstrates that obese-diabetic rats have highest oxidative stress as compared to diabetic rat. α-lipoic acid significantly lowered the elevated serum glucose, triglycerides, total cholesterol, low-density lipoprotein cholesterol, malondialdehyde levels and increased the high density lipoprotein cholesterol and enzymatic antioxidant levels in obese-diabetic rats. α-lipoic acid decreased the oxidative stress produced by diabetes mellitus and obesity in part by increasing the sensitivity of insulin, thereby maintains glycemic control decreases reactive oxygen species generated by hyperglycemia and dyslipidemia. Further it increases the expression of antioxidants by preventing Reactive oxygen species mediated DNA damage, restores enzymatic antioxidants and quenches reactive oxygen species generated by obesity and diabetes mellitus. α-lipoic acid may prove to be of considerable benefit in the management of obesity and diabetes mellitus in conjunction.

Key Words: α-Lipoic acid, oxidative stress, diabetes mellitus, atherogenic diet.

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Introduction

Chronic hyperglycemia, the hallmark of diabetic state, decreases the antioxidant defenses due to enhanced formation of reactive oxygen species (ROS), advanced glycation end product and lipid peroxidation products [1, 2]. Increased ROS thus generated decreases not only plasma antioxidant levels but also oxidizes protein thiol moieties to produce a variety of sulphur oxidations leading to diminished insulin receptor signaling and inhibition of cellular uptake of glucose and triglycerides (TG) from blood [3, 4]. In addition Type 2 diabetes mellitus is usually accompanied by obesity which elevates the production of ROS [5, 6, 7] by increasing mitochondrial uncoupling [8, 9, 10] and ß oxidation of fatty acids[10]. ß cells of pancreas are highly susceptible to ROS damage leading to decreased insulin production due to characteristic decrease in the concentration of free radical scavengers which can be prevented by antioxidants. Obesity and diabetes in conjugation may add on the production of ROS and thus degenerate ß cells of pancreas at a faster rate.

Alpha lipoic acid (ALA) is readily absorbed from diet, is widely used in various complications of diabetes mellitus[11, 12]. It is rapidly converted to dihydrolipoic acid (DHLA) in many tissues. ALA and its reduced form DHLA, reduces oxidative stress (OST) by scavenging hydroxyl radicals, singlet oxygen and hypochlorous acid in both membrane and aqueous domains, by chelating transition metals in biological systems, by preventing membrane lipid peroxidation and protein damage through regeneration of other antioxidants such as vitamin C, E, ubiquinol (coenzyme Q10) and by increasing intracellular glutathione [11, 12, 13]. The half life of ALA in plasma is approximately 30min, the total plasma clearance is in the same range as the plasma flow of the liver (about 11-17ml/min/kg). The absolute bioavailability is between 20 and 38% depending on the isomer and the formulation. ALA can be reduced to the dithiol DHLA this reduced form greatly contributes to the antioxidant activity of ALA in vivo and its β oxidation products bis nor, and tetra nor lipoic acid also contribute to the antioxidant activity [14]. Both ALA and DHLA are scavengers of several ROS. There are no reports of deleterious effects with ALA treatment, which has LD_{50} of approximately 400-500mg/kg in rats [15].

A detailed review of literature afforded no information on its antioxidant potential in nongenetic animal model of obesity and diabetes mellitus in conjunction. The present study has two fold aims to compare oxidative stress in diabetic, obese and obese diabetic rats and to evaluate the effect of ALA on glycemic control, lipid profile and oxidative stress in obese diabetic rats.

Materials and Methods

Animals

Pathogen free, Wistar strain male albino rats of six months of age (200-220g) were used in the present study. The rats were housed in polypropylene cages (five per cage) under standard laboratory conditions with 12 hour light/dark cycle. The rats were fed with standard laboratory chow (Hindustan Lever Ltd, Mumbai) and water *ad libitum*. Animal Ethical norms were strictly followed during all experimental procedures. The Institutional Animal Ethical Committee approved all experimental protocols. Animals described as fasted were deprived of food for 16hrs but had free access to water.

Induction of obesity

Obesity was induced in the 3 groups of rats (obese, obese diabetic, ALA treatment group of obese diabetic) by giving atherogenic diet [16], which consisted of 0.25% cholesterol, and 25% vegetable fat in addition to normal pellet chow for 12 weeks. The animals which became obese (350-450g) were used for further study.

Induction of diabetes mellitus

Baseline blood glucose, triglyceride and cholesterol levels were determined in all rats before induction of diabetes mellitus. Alloxan monohydrate at a dose of 100mg/kg was administered intraperitoneally to fasted normal diabetic control group rats (200-220g) and obese diabetic control rats and obese diabetic (ALA) treatment rats (350-450g). 10% Dextrose solution was given by oral gavage to combat initial hypoglycemia. Rats with blood sugar levels of 250-400mg/dl were considered diabetic and employed in the study.

Study Protocol

The animals were divided into 5 groups of six each, viz. normal, obese, diabetic, obese-diabetic, and obese-diabetic ALA treated group. Obese diabetic treated group received ALA 100mg/kg [17,18,19,20], by gavage daily between 9a.m.-10a.m. for 60 days. In the previous study in our laboratory 60% of the untreated diabetic animals died from 8^{th} day to 10^{th} day (unpublished work). One of the aims of the present tudy is to compare the oxidative stress in vital organs of diabetic, obese and obese diabetic rats, hence diabetic and obese diabetic groups were sacrificed on 7^{th} day and vital organs were collected for the estimation of oxidative stress.

Normal group: Received normal laboratory chow for 12 weeks, from 13th week received 0.5ml of 0.2% CMC (vehicle) daily for 60 days.

Obese group: Received atherogenic diet for 12 weeks, from 13th week received 0.5ml of 0.2% CMC (vehicle) daily for 60 days.

Diabetic group: Received normal laboratory chow for 12 weeks, on 13th week single dose of Alloxan monohydrate 100mg/kg/i.p, 0.5ml of 0.2% CMC (vehicle) daily by gavage for 60 days.

Obese diabetic rats: Received atherogenic diet for 12 weeks, on 13th week single dose of Alloxan monohydrate 100mg/kg/i.p, after induction of diabetes, 0.5ml of 0.2% CMC (vehicle) daily for 60 days.

ALA treated obese diabetic rats: Received atherogenic diet for 12 weeks, on 13th Week single dose of Alloxan monohydrate 100mg/kg/i.p, after induction of diabetes, treating this as 0 day, ALA 100mg/kg suspended in 0.2% CMC by gavage for 60 days.

Estimation of serum glucose, LDL-cholesterol, HDL- cholesterol, total cholesterol and triglycerides

Blood samples were collected from fasted animals, by puncture of retro-orbital plexus under mild ether anesthesia and baseline glucose, cholesterol and TG levels were determined on 0 day of the treatment i.e. after induction of obesity and diabetes mellitus and before starting treatment with ALA. Initial samples after ALA treatment were withdrawn on 7th day of treatment in all groups Serum was separated by centrifuging the samples at 3000 rpm for 10 minutes and analyzed for glucose, total cholesterol, triglycerides using the biochemical kits. Serum glucose was estimated spectrophotometrically using a commercial assay kit (Monozyme, India, Ltd.) 10 µl of serum is used for each assay. Total cholesterol was estimated spectrophotometrically using commercial kit (Span Diagnostics, India, Ltd) one step method of Wybenga and Pilleggi. Enzymatic method, GPO/Trinder, end point colorimetry for triglycerides using commercial kit (Span Diagnostics, India, Ltd). On every 15th day blood samples were collected and processed similarly from normal, obese and ALA treated obese diabetic rats till the end of the study i.e. 60 days.

Enzyme Assays

Diabetic and obese diabetic animals were sacrificed at the end of the 7th day of the treatment schedule by cervical dislocation and the liver, heart and kidney tissues were isolated at 4ºC. The tissues were washed with ice-cold saline and immediately immersed in liquid nitrogen and stored at -80ºC for biochemical analysis and enzymatic assays.

Same procedure was followed at the end of the treatment schedule on the 60th day for normal, obese and ALA treated obese diabetic group. The tissues were thawed, sliced and homogenized under ice-cold conditions. MDA content, SOD, Catalase and GSH-Px activities were estimated by employing standard methods.

Superoxide dismutase (sod-ec: 1.15.1.6)

The liver tissue, heart tissue and kidney tissues were homogenized separately in ice cold 50mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5%(w/v) homogenate. Superoxide dismutase activity was determined according to the method of Misra and Fridovich at room temperature [21]. Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50% is equal to 1 unit.

Catalase (CAT-EC: 1.11.1.6)

Liver, heart, and kidney tissues were homogenized separately in ice cold 50mM phosphate buffer (pH 7.0) containing 0.1Mm EDTA to give 5% homogenate (w/v). Catalase activity was measured by a slightly modified version of Aebi at room temperature [22]. Catalase activity is expressed in moles of H_2O_2 degraded/mg protein/min.

Glutathione peroxidase (GSH-PX-EC: 1.11.1.9)

5% (w/v) of liver, heart and kidney tissue homogenates were prepared in 50m M phosphate buffer (pH 7.0) containing 0.1 mM EDTA. Glutathione peroxidase was determined by a modified version of Flone and Gunzler at 37ºC [23]. The enzyme activity was expressed in µ moles of NADPH oxidized/mg protein/min.

Estimation Of Lipid Peroxidation

The liver, heart and kidney tissues were separately homogenized (5%w/v) in 50mM phosphate buffer (pH 7.0) containing 0.1mM EDTA. MDA levels were determined as described by Ohkawa *et al* 1979 [24]. The values were expressed in µmoles of malondialdehyde formed/ gram wet weight of the tissue.

Statistical analysis

The data is expressed as mean ± SEM. The results were analyzed statistically on 'INSTAT' program by using one way ANOVA followed by Student-Newman-Keuls Multiple Comparisons Test. *P* values <0.05 were considered significant.

Results

In the present study, the serum glucose levels were in the range of 350-600mg/dl in alloxan induced diabetic rats. Peak values (≥550-600mg/dl) were observed on the 7th day in the diabetic groups. This was considered as severe diabetes. Diabetic and obese diabetic rats were sacrificed on the 7th day and the data obtained was compared with ALA treated group (from 0 to 60th day), because in untreated diabetic groups blood glucose and lipid levels will increase proportionately from 7th day to 60th, day, as the increase was linear from 0 to 7th day. Baseline glucose, TGs and cholesterol levels were not same in all groups on 0 day of treatment, as these groups were induced with obesity, diabetes and obesity and diabetes in conjunction, 0 day of treatment indicates before starting treatment with ALA.

Effect of α – Lipoic acid on serum glucose levels

Treatment with ALA produced significant reduction in the serum glucose level with maximum reduction obtained on the 15th day. Serum glucose levels reached the normal value on the 45th day of treatment and were maintained in the normal range till the end of the study (T**able-1**).

Effect of α – Lipoic acid on lipid profile

Effect of ALA on serum TG, TC, LDL-C

Table 2, 3, 4 summarizes respectively the changes in serum TG, TC, LDL-C levels in normal, obese, diabetic, obese diabetic and ALA treated obese diabetic rats. TG, TC, LDL-C levels were significantly (0.001) increased in obese, diabetic, obese diabetic rats on 0 day of treatment and was maintained in obese control group throughout the study. Treatment with AlA significantly (0.001) reduced elevated TG, TC, LDL-C levels in obese diabetic rats on 30th day of treatment and were maintained till the end of the study.

Effect of ALA on serum HDL-C

The changes in serum TC levels in normal, obese, diabetic, and obese diabetic and ALA treated obese diabetic rats are show in **Table 5**. HDL-C levels were significantly (0.001) decreased in obese, diabetic, obese diabetic rats on 0 day of treatment and decrease was maintained in obese control group throughout the study. Treatment with AlA significantly (0.001) elevated HDL-C levels in obese diabetic rats from 30th day of treatment and reached almost normal on 45th day of treatment.

TABLE 1

Effect of alpha lipoic acid on serum glucose levels in obese-diabetic rats.

	Blood glucose level mg/dl					
GROUP	**0 DAY**	**7TH DAY**	**15TH DAY**	**30TH DAY**	**45TH DAY**	**60TH DAY**
NORMAL	75.46$^{@++\bullet\bullet}$ ±3.78	92.99$^{\bullet\bullet++}$ ± 4.67	92$^{\bullet\bullet++}$ ±2.78	98.76$^{\bullet\bullet++}$ ±4.43	101.21$^{\bullet\bullet++}$ ±6.2	89.49$^{\bullet\bullet++}$ ±5.11
OBESE	81.22$^{*++\bullet\bullet}$ ±4.81	78.96$^{\bullet\bullet++}$ ± 5.98	83.61$^{\bullet\bullet++}$ ±4.94	78.85$^{**\bullet\bullet++}$ ±3.45	69.20$^{**\bullet\bullet++}$ ±5.35	72.45$^{*\bullet\bullet++}$ ±4.28
DIABETIC	356.87$^{**@@}$ ±9.63	559.08$^{**\bullet@@}$ ± 24,89	559.08$^{**\bullet@@}$ ± 24,89	559.08$^{**\bullet@@}$ ± 24,89	559.08$^{**\bullet@@}$ ± 24,89	559.08$^{**\bullet@@}$ ± 24,89
OBESE-DIABETIC	369.21$^{**@@++}$ ±8.54	607.08$^{**@@+}$ ± 26.77	607.08$^{**@@+}$ ± 26.77	607.08$^{**@@+}$ ± 26.77	607.08$^{**@@+}$ ± 26.77	607.08$^{**@@++}$ ± 26.77
α – LIPOIC ACID	362.67$^{**@@}$ ±16.7	276.23$^{**\bullet\bullet@@++}$ ± 14.76.	186.5$^{**@@++\bullet\bullet}$ ±11.4	131.06$^{**@@+++\bullet\bullet}$ ±8.4	86.03$^{*@++\bullet\bullet}$ ±7.6	74.4$^{*++\bullet\bullet}$ ±4.6

All values are expressed Mean ± SEM of Six rats

*p< 0.01, **p<0.001 Vs normal rats

$^{@}$p<0.01, $^{@@}$p<0.001 Vs obese rats

$^{+}$p<0.01, $^{++}$p<0.001 Vs diabetic group

$^{\bullet}$p<0.01, $^{\bullet\bullet}$p<0.001 Vs obese-diabetic group

Note: Values showed from 15th to 60th day for diabetic and obese diabetic groups are actual values on day 7.

TABLE 2

Effect of alpha lipoic acid on serum triglyceride levels in obese-diabetic rats.

	Serum triglyceride levels mg/dl					
GROUP	**0 DAY**	**7th DAY**	**15TH DAY**	**30TH DAY**	**45TH DAY**	**60TH DAY**
NORMAL	104.11$^{@\bullet\bullet++}$ ±3.82	99.06$^{@++\bullet\bullet}$ ±5..13	92.63$^{@\bullet\bullet++}$ ±4.60	98.94$^{@\bullet\bullet++}$ ±3.75	105.11$^{@\bullet\bullet++}$ ±5.12	101.35$^{@\bullet\bullet++}$ ±2.63
OBESE	254.83$^{*++\bullet\bullet}$ ±4.46	278.96$^{*++\bullet\bullet}$ ±3.54	293.74$^{*\bullet\bullet++}$ ±3.83	308.06$^{*\bullet\bullet}$ ±3.40	344.16$^{*\bullet\bullet}$ ±5.09	381.62$^{*++\bullet\bullet}$ ±2.84
DIABETIC	310.52$^{*@\bullet\bullet}$ ±5.09	318.76$^{*@\bullet\bullet}$ ±3.05	318.76$^{*\bullet\bullet@}$ ±3.05	318.76$^{*\bullet\bullet}$ ±3.05	318.76$^{*\bullet\bullet}$ ±3.05	318.76$^{*@\bullet\bullet}$ ±3.05
OBESE-DIABETIC	362.7$^{*@++}$ ±4.83	412.46$^{*@++}$ ±5..48	412.46$^{*@++\bullet\bullet}$ ±5..48	412.46$^{*@++}$ ±5..48	412.46$^{*@++}$ ±5..48	412.46$^{*@++}$ ±5..48
α – LIPOIC ACID	290.58$^{*@+\bullet\bullet}$ ±8.6	243.99$^{**@++\bullet\bullet}$ ±8.6	197.83$^{*@++\bullet\bullet}$ ±6.52	145.26$^{*@++\bullet\bullet}$ ±4.28	138.96$^{*@++\bullet\bullet}$ ±4.01	140.35$^{*@++\bullet\bullet}$ ±5.68

All values are expressed as Mean ± SEM of Six rats

*p< 0.001 Vs normal rats

$^{@}$p<0.001 Vs obese rats

$^{+}$p<0.01, $^{++}$p<0.001 Vs diabetic group

$^{\bullet}$p<0.01, $^{\bullet\bullet}$p<0.001 Vs obese-diabetic group

Note: Values showed from 15th to 60th day for diabetic and obese diabetic groups are actual values on day7.

TABLE 3

Effect of alpha lipoic acid on serum total cholesterol levels in obese-diabetic rats.

	Total cholesterol levels mg/dl					
GROUP	**0 DAY**	**7th DAY**	**15TH DAY**	**30TH DAY**	**45TH DAY**	**60TH DAY**
NORMAL	82.30$^{@@++\bullet\bullet}$ ±2.56	87.99$^{@@++\bullet\bullet}$ ±4.01	93.47$^{@@++\bullet\bullet}$ ±3.02	88.12$^{@@++\bullet\bullet}$ ±2.34	92.2$^{@@++\bullet\bullet}$ ±2.90	86.22$^{@@++\bullet\bullet}$ ±1.77
OBESE	150.12$^{**++\bullet\bullet}$ ±3.15	164.22$^{**++\bullet\bullet}$ ±3.22	179.23$^{*++\bullet\bullet}$ ±2.36	196.48$^{**+\bullet\bullet}$ ±3.66	223.42** ±4.13	257.68^{**+} ±4.02
DIABETIC	198.03$^{**@@}$ ±6.33	225.89$^{**@@\bullet}$ ± 7.88	225.89$^{**@@\bullet}$ ± 7.88	225.89$^{**@\bullet}$ ± 7.88	225.89$^{**\bullet}$ ± 7.88	225.89$^{**@\bullet}$ ± 7.88
OBESE-DIABETIC	213.11$^{**@@+}$ ± 5,99	267.98$^{**@@+}$ ±7.08	267.98$^{**@@+}$ ±7.08	267.98$^{**@@+}$ ±7.08	267.98$^{**@+}$ ±7.08	267.98^{**+} ±7.08
α – LIPOIC ACID	239.82$^{**@@++\bullet}$ ±6.7	203.78$^{**@@\bullet}$ ± 5.66	184.5$^{**+\bullet\bullet}$ ±5.14	143.59$^{**@@++\bullet\bullet}$ ±4.65	112.28$^{*@@++\bullet\bullet}$ ±3.96	104.26$^{*@@++\bullet\bullet}$ ±4.42

All values are expressed as Mean ± SEM of Six rats

*p< 0.01, **p<0.001 Vs normal rats

$^{@}$p<0.01, $^{@@}$p<0.001 Vs obese rats

$^{+}$p<0.01, $^{++}$p<0.001 Vs diabetic group

$^{\bullet}$p<0.01, $^{\bullet\bullet}$p<0.001 Vs obese-diabetic group

Note: Values showed from 15th to 60th day for diabetic and obese diabetic groups are actual values on day 7.

TABLE 4

Effect of alpha lipoic acid on serum LDL-C levels in obese-diabetic rats.

	Serum LDL-C levels mg/dl					
GROUP	**0 DAY**	**7TH DAY**	**15TH DAY**	**30TH DAY**	**45TH DAY**	**60TH DAY**
NORMAL	75.55$^{@++\bullet\bullet}$ ±2.24	78.99$^{@++\bullet\bullet}$ ±5.24	82.29$^{@++\bullet\bullet}$ ±3.96	84.33$^{@++\bullet\bullet}$ ±4.65	86.28$^{@++\bullet\bullet}$ ±5.47	89.64$^{@++\bullet\bullet}$ ±5.02
OBESE	91.06$^{*++\bullet\bullet}$ ±3.89	93.22$^{*++\bullet\bullet}$ ±5.33	95.42$^{*+\bullet\bullet}$ ±3.41	109.61$^{*\bullet}$ ±2.82	123.76* ±3.54	147.23$^{*+\bullet\bullet}$ ±4.86
DIABETIC	110.48$^{*@}$ ±5.79	185.34$^{*@++}$ ± 6.98	185.34$^{*@}$ ±6,98	185.34$^{*@}$ ±6,98	185.34$^{*@}$ ±6,98	185.34$^{*@}$ ±6,98
OBESE-DIABETIC	122.27$^{*@+}$ ±4.89	196.89$^{*@}$ ±5.32	196.89$^{*@}$ ±5.32	196.89$^{*@}$ ±5.32	196.89$^{*@}$ ±5.32	196.89$^{*@}$ ±5.32
α – LIPOIC ACID	153.24$^{*@++\bullet\bullet}$ ±8.73	124.17$^{*@++\bullet\bullet}$ ±8.21	124.17$^{*@++\bullet\bullet}$ ±8.21	73.33$^{@++\bullet\bullet}$ ±7.24	71.28$^{@++\bullet\bullet}$ ± 5.83	74.53$^{@++\bullet\bullet}$ ± 6.82

All values are expressed as Mean ± SEM of Six rats.

*p< 0.001 Vs normal rats

$^{@}$p<0.001 Vs obese rats

$^{+}$p<0.01, $^{++}$p<0.001 Vs diabetic group

$^{\bullet}$p<0.01, $^{\bullet\bullet}$p<0.001 Vs obese-diabetic group

Note: Values showed from 15th to 60th day for diabetic and obese diabetic groups are actual values on day 7.

TABLE 5

Effect of alpha lipoic acid on serum HDL-C levels in obese-diabetic rats

	Serum HDL-C levels mg/dl					
GROUP	**0 DAY**	**7th DAY**	**15TH DAY**	**30TH DAY**	**45TH DAY**	**60TH DAY**
NORMAL	48.63$^{@@++••}$ ±1.82	47.98$^{@@++••}$ ±1.06	43.04$^{@@++••}$ ±1.95	44.56$^{@@++••}$ ±2.35	43.28$^{@@++••}$ ±3.25	44.39$^{@@++••}$ ±3.07
OBESE	28.34* ±2.25	28.75* ±2.44	26.77* ±2.36	24.92* ±2.18	29.34* ±2.91	27.91* ±2.85
DIABETIC	23.75* ±1.08	20.87* ±1.37	20.87* ±1.37	20.87* ±1.37	20.87* ±1.37	20.87* ±1.37
OBESE-DIABETIC	21.88*$^{+}$ ±1.79	21.45* ±1.32	21.45* ±1.32	21.45* ±1.32	21.45* ±1.32	21.45* ±1.32
α – LIPOIC ACID	**24.56* ±1.63**	27.22* ±1.29	28.45* ±1.84	34.68*$^{@++•}$ ±2.04	49.37*$^{@@++••}$ ±1.58	58.96*$^{@@++••}$ ±1.64

All values are expressed as Mean ± SEM of Six rats.

*p< 0.001 Vs normal rats

$^{@}$p<0.01, $^{@@}$p<0.001 Vs obese rats

$^{+}$p<0.01, $^{++}$p<0.001 Vs diabetic group

$^{•}$p<0.01, $^{••}$p<0.001 Vs obese-diabetic group

Note: Values showed from 15th to 60th day for diabetic and obese diabetic groups are actual values on day 7.

Effect of α – Lipoic acid on lipid Peroxidation

Table 6 shows changes in MDA content in liver, heart and kidney tissue of normal, obese, diabetic and obese diabetic rats. MDA content was highest heart tissue obese diabetic rats. Kidney and liver tissue of diabetic and obese diabetic rats had almost same (significantly increased $p<0.001$) MDA content. ALA significantly decreased the lipid peroxidation in the liver tissue compared to the normal group ($P<0.001$). In the heart and kidney tissues of ALA treated group, lipid peroxidation was significantly reduced ($p<0.001$) than obese, diabetic and obese-diabetic groups.

TABLE-6

Effect of α-Lipoic acid on the MDA content of liver, heart and kidney tissues of obese-diabetic rats. (The values are expressed in µ moles of malondialdehyde formed / gm wet tissue/hr).

TISSUE	GROUPS (µ moles of malondialdehyde formed /gm wet tissue/hr)				
	Normal	**Obese**	**Diabetic**	**Obese Diabetic**	**α Lipoic-acid**
Liver	**101.38$^{@@++\bullet\bullet}$ ±3.79**	**129.06$^{**++\bullet\bullet}$ ±3.93**	**141.90$^{**@\bullet}$ ±0.663**	**153.93$^{**\ @@+}$ ±1.48**	**86.340$^{*@@+++\bullet\bullet}$ ±1.45**
Heart	**63.195$^{@@++\bullet\bullet}$ ±0.503**	**160.38$^{**++\bullet\bullet}$ ±2.31**	**191.16$^{**@@\bullet\bullet}$ ±0.255**	**229.31$^{**\ @@++}$ ±0.765**	**95.217$^{@@\ ++\bullet\bullet}$ ±1.63**
Kidney	**79.967$^{@@++\bullet\bullet}$ ±0.949**	**148.31$^{**+\bullet\bullet}$ ±2.51**	**161.37$^{**\ @@\bullet}$ ±0.352**	**178.77$^{**\ @@+}$ ±0.454**	**99.643$^{@@+++\bullet\bullet}$ ±1.86**

All values are expressed as Mean ± SEM of **Six** rats

*p< 0.01, **p<0.001 Vs normal group

$^{@}$p<0.01, $^{@@}$p<0.001 Vs obese rats

$^{+}$p<0.01, $^{++}$p<0.001 Vs diabetic group.

$^{\bullet}$P<0.01, $^{\bullet\bullet}$p<0.001 Vs obese-diabetic group

Note:Values showed for diabetic and obese diabetic groups are actual values on day 7.

TABLE 7

Effect of α-Lipoic acid on Super oxide dismutase activity of liver, heart and kidney tissues of obese diabetic rats. The values are expressed in units of Superoxide anion reduced /mg protein/minute.

TISSUE	GROUPS units of Superoxide anion reduced /mg protein/minute				
	Normal	**Obese**	**Diabetic**	**Obese Diabetic**	**α Lipoic-acid**
Liver	$8.575^{@+\bullet\bullet}$ ±0.059	$7.17^{*\ +\bullet\bullet}$ ±0.200	$6.385^{*\ @\bullet\bullet}$ ±0.024	$4.96^{*\ @@\ ++}$ ±0.268	$12.195^{*@@++\bullet\bullet}$ ±0.013
Heart	$13.685^{@++\bullet\bullet}$ ±0.171	$10.54^{*+\bullet\bullet}$ ±0.142	$9.453^{*\ @\bullet\bullet}$ ±0.371	$5.18^{*\ @@\ ++}$ ±0.259	$15.637^{*@@++\bullet\bullet}$ ±0.076
Kidney	$8.356^{@++\bullet\bullet}$ ±0.011	$7.493^{*\ +\bullet\bullet}$ ±0.056	$6.265^{*\ @\bullet\bullet}$ ±0.119	$5.33^{*\ @@\ +}$ ±0.244	$12.587^{*@@++\bullet\bullet}$ ±0.073

All values are Mean ± SEM of **Six** rats

*p<0.001 Vs normal group

$^{@}$p<0.01, $^{@@}$p<0.001 Vs obese rats

$^{+}$p<0.01, $^{++}$p<0.001 Vs diabetic group.

$^{\bullet}$p<0.01, $^{\bullet\bullet}$p<0.001 Vs obese-diabetic group

Note:Values showed for diabetic and obese diabetic groups are actual values on day 7.

Effect of α – Lipoic acid on enzymatic antioxidants

Both SOD and CAT activities were significantly decreased in obese-diabetic group compared to other groups ($P<0.01$) that were indicative of increased oxidative stress present in obese diabetic rats. Obese-diabetic rats treated with ALA showed significant increase in SOD (Table 7), Catalase activities ($P<0.001$) in all the three tissues, when compared to all the control groups (Table 8).

TABLE 8

Effect of α-Lipoic acid on Catalase activity of liver, heart and kidney tissues of obese diabetic rats. The values are expressed in μ moles of H_2O_2 degraded / mg protein/ minute.

TISSUE	GROUPS μ moles of H_2O_2 degraded / mg protein/ minute				
	Normal	**Obese**	**Diabetic**	**Obese Diabetic**	**α Lipoic-acid**
Liver	$0.6438^{@++\bullet\bullet}$ ±0.024	$0.4675^{*\bullet\bullet}$ ±0.001	$0.4777^{*\bullet\bullet}$ ±0.002	$0.3067^{*\ @@+}$ ±0.001	$0.9163^{*@@++\bullet\bullet}$ ±0.007
Heart	$0.2767^{@+\bullet\bullet}$ ±0.004	$0.1917^{*\ ++}$ ±0.002	$0.2268^{*\bullet}$ ±0.004	$0.1917^{*\ ++}$ ± 0.002	$0.5065^{*++\bullet\bullet}$ ±0.016
Kidney	$0.5143^{@@++\bullet\bullet}$ ±0.002	$0.3172^{*\bullet\bullet}$ ±0.001	$0.3172^{*\bullet\bullet}$ ±0.001	$0.1910^{*\ @@\ ++}$ ±0.001	$0.7292^{*@@++\bullet\bullet}$ ±0.021

All values are expressed as Mean ± SEM of **Six** rats

$^{*}p<0.001$ Vs normal group

$^{@}p<0.01$, $^{@@}p<0.001$ Vs obese rats

$^{+}p<0.01$, $^{++}p<0.001$ Vs diabetic group

$^{\bullet}P<0.01$, $^{\bullet\bullet}p<0.001$ Vs obese-diabetic group

Note: Values showed for diabetic and obese diabetic groups are actual values on day 7.

The activity of glutathione peroxidase in the liver and kidney tissues of ALA treated group increased significantly compared to the normal control group (P<0.01). The heart tissue GSH-Px activity was comparable to the activity of the normal group (Table 9).

TABLE 9

Effect of α-lipoic acid on Glutathione Peroxidase activity of liver, heart and kidney tissues of obese diabetic rats. The values are expressed in μ moles of NADPH oxidized / mg protein/ minute.

TISSUE	GROUPS μ moles of NADPH oxidized / mg protein/ minute.				
	Normal	**Obese**	**Diabetic**	**Obese Diabetic**	**α Lipoic acid**
Liver	$0.8283^{@++\bullet\bullet}$ ±0.001	$0.4390^{*\ +\bullet}$ ±0.088	$0.5170^{*\ @\bullet\bullet}$ ±0.020	$0.3808^{*\ ++@}$ ± 0.019	$0.9113^{*\ +@\bullet\bullet}$ ±0.010
Heart	$0.9942^{@++\bullet\bullet}$ ±0.004	$0.5568^{*\ ++\bullet\bullet}$ ±0.0113	$0.3847^{*\ @\bullet}$ ±0.012	$0.2612^{*\ @\ ++}$ ±0.024	$0.9942^{@++\bullet\bullet}$ ±0.008
Kidney	$0.6067^{@++\bullet\bullet}$ ±0.016	$0.3153^{*\ +}$ ±0.004	$0.3867^{*\bullet}$ ±0.010	$0.3153^{*\ +}$ ±0.004	$1.111^{*@++\bullet\bullet}$ ±0.002

All values are expressed as Mean ± SEM of **Six** rats

*p< 0.001 Vs normal group

$^{@}$p<0.001 Vs obese rats

$^{+}$p<0.01, $^{++}$p<0.001 Vs diabetic group.

$^{\bullet}$P<0.01, $^{\bullet\bullet}$p<0.001 Vs obese-diabetic group

Note: Values showed for diabetic and obese diabetic groups are actual values on day 7.

Discussion

Cholesterol feeding has been often used to assess hypercholesterolemia-related metabolic disturbances in different animal models [25, 26]. Obesity was induced by giving atherogenic diet, which consisted of 0.25% cholesterol, and 25% vegetable fat in addition to normal pellet chow for 12 weeks. This diet has been employed previously for inducing obesity [16]. We have attempted to simulate the natural history of Type 2 diabetes by injecting alloxan to fat-fed rats. The occurrence of type II diabetes is indicated by the elevated free fatty acid which your study has not done. The data in Table 1 & 2 indicate that significant hyperglycaemia and hypertriglyceridaemia was present in fat-fed, alloxan injected rats indicating the development of diabetes mellitus and obesity. Based upon this, we believe that the rat model of Type 2 diabetes used in this study mimics both the natural history and the pathophysiological characteristics of patients with this syndrome.

In the present study obese rats had normal glucose utilization as blood glucose levels of these animals were not significantly different from that of control, indicating obesity was induced in the rats without production of insulin resistance where as diabetic and obese diabetic rats had initial glucose levels of 356mg/dl, 369mg/dl and on 7th day it reached 559mg/dl, 607mg/dl respectively indicating significant decrease in glucose utilization of obese diabetic rats. (It indicates the lack of insulin due free radicals induced damage to beta cells by alloxan).

The increase in LDL-C and decrease in HDL-C have been pointed out as risk factors for the development of atherosclerosis and related cardiovascular diseases, the higher the LDL-C and lower the HDL-C promoted by a dietary animal model, the better it must be considered. The data in Table 4 and 5 indicate a significant dyslipidaemia in obese, diabetic and obese diabetic rats. LDL-C receptors are down regulated by the cholesterol and saturated fatty acids included in the atherogenic diet leading to increased serum LDL-C levels [27, 28]. In the present study decreased uptake of LDL-C by hepatic cells of the animals fed on atherogenic might have contributed for higher serum LDL-C concentrations. In this study it was observed that there was a decrease in serum HDL-C levels with a similar hypercholesterolemic dietary pattern. As per other investigators it may be due to the occurrence of an abnormal apoprotein (apo A-I) in HDL-C [29,30]leading to decreased lecithin cholesterol acyltransferase activity [31].

In the present study obese diabetic rats had higher oxidative stress compared to obese or diabetic rats as shown by the significant increase in MDA content and decreased SOD, CAT and GSH-Px activities. Auto-oxidation of glucose and nonenzymatic glycation of proteins including GLUT 4 [1, 2] in diabetic rats might be in part responsible for highly significant decrease in enzymatic antioxidants in diabetic rats. In addition acetyl-CoA derived either from glucose through pyruvate or from beta-oxidation of FFA, combines with oxaloacetate to form citrate, which enters the citric acid cycle and is converted to isocitrate. NAD^+- dependent isocitrate dehydrogenase generates NADH. In hyperglycemia and obesity excessive NADH cannot be dissipated by oxidative phosphorylation (or other mechanisms), the mitochondrial proton gradient increases and single electrons are transferred to oxygen, leading to the formation of free radicals, particularly superoxide anion[32] and in turn to H_2O_2 by SOD [33]. The decreased activities of CAT, GSH-Px and SOD may be a response to increased production of H_2O_2 and superoxide anion by mitochondrial proton gradient induced transfer of electrons to oxygen due to hyperglycemia and obesity in diabetic and obese rats respectively or both might have contributed in obese diabetic rats These enzymes have been suggested to play an important role in maintaining physiological levels of oxygen radicals and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated from hyperglycaemia [1,2], and thereby curtailing the quantity of cellular destruction inflicted by lipid peroxidation [32,34,35]. The decreased level of antioxidant enzymes SOD, CAT, GSH-Px may be responsible for the inadequacy of the antioxidant defenses in combating ROS mediated damage to beta cells of pancreas, as these cells are highly susceptible to ROS damage due to their low concentrations of free radical scavengers [36, 37]. In the present study significant increase in glucose, TGs levels on 7th day itself indicates ROS induced damage of ß cells of pancreas and decreased release of insulin. In previous study as well as reported by other investigator [17], 60% of diabetic animals died from 8th day onwards may be due to persistently severe hyperglycemia and increase in ketone bodies, lactate levels and oxidative stress mediated cell damage. One of the aims of our study is to compare the oxidative stress in obese, diabetic and obese diabetic rats; diabetic animals were sacrificed on 7th day for the estimations of enzymatic antioxidants in vital organs. We assume sacrificing untreated diabetic animals on 7th day is appropriate, as animals suffering can be reduced. On the 7th day itself in the untreated diabetic rats blood glucose, LDL-C, TG levels were very high and all vital organs antioxidant enzyme levels were low indicating oxyradical mediated damage to these organs or development of diabetic complications.

We selected a dose of ALA (100mg/kg), as this dose was effective in increasing insulin signaling, glucose utilization and decreasing oxidative stress in genetic models of obese diabetic rats [19, 20, 21, 38, 39]. In the present study, ALA reversed the elevated serum glucose, TG, TC, LDL-C levels. Notable restoration of normal serum glucose levels was possible in obese diabetic rats treated with ALA, which may be attributed to the retention of partial beta cell activity. ALA is a potent biological antioxidant[15] and when administered exogenously, significantly improves insulin-stimulated glucose utilisation in insulin-resistant animal models [40] and in humans [41, 42]. Studies using cultured cells [31, 39, 43], isolated diaphragm [44] have demonstrated that ALA stimulates the glucose uptake by activating critical elements of the insulin signaling pathways, including tyrosine phosphorylation of IR and IRS-1.

Chronic in vivo treatment with ALA was shown to improve whole- body glucose tolerance and insulin sensitivity, as well as insulin action on skeletal muscle glucose transport in insulin–resistant obese Zucker rats [20, 40, 45, 46], prevented the age-dependent development of hyperglycemia, hyperinsulinemia, dyslipidemia, and plasma markers of oxidative stress in diabetes-prone Otsuka Long- Evans Tokushima Fatty (OLETF) rats [47]. Alternatively, ALA might increase insulin sensitivity through its tissue-specific effects on AMPK, which have been described recently to decrease obesity [48]. Our results also confirm this, as the anti-hyperglycemic and lipid lowering effects were significant only after the 30th day indicating the lag time required for the synthesis and functioning of ALA stimulated expression of insulin signaling proteins. Restoration of serum glucose levels and lipid profile to normal by ALA may be due to its antioxidant properties and by direct translocation of insulin dependent GLUT 4 to plasma membrane, resulting in a better glycemic control and its possible action on the lipid metabolism.

The ability of ALA or its reduced form DHLA, to scavenge hydroxyl radicals and chelate transition metals in Fenton reaction restricts the molecular damage by hydroxyl radicals and so reduces cellular need for CAT. Hyperglycemia can not only generate more ROS but can also attenuate the auto-oxidative mechanisms through glycation of the scavenging enzymes [49]. For example, the glycation of specific lysine residues of Cu-Zn SOD can inactivate the enzyme [38]. ALA or DHLA has been shown to quench various reactive oxygen species such as superoxide, hydroxyl radicals and singlet oxygen, prevent oxygen induced DNA damage, exhibit chelating activity, reduces lipid peroxidation and prevent glycation of proteins [14, 15]. Restoration of enzymatic antioxidant activity i.e., (SOD, Catalase, and GSH-Px) in the ALA treated obese-diabetic group is consistent with previous studies showing that ALA or DHLA scavenges various reactive oxygen species such as superoxide, hydroxyl radicals and singlet oxygen [11, 14, 15, 17, 18, 19, 33].

ALA functions as a powerful antioxidant, recycling vitamins C and E and elevating tissue glutathione [13]. ALA and its derivative, the dihydrolipoic acid, act as antioxidants for ascorbic acid and tocopherol [50]. ALA might possibly react with free radicals that are pro-oxidants for ascorbic acid and tocopherol. Significantly lower levels of lipid peroxides in obese diabetic rats and increased activities of enzymatic antioxidants suggest that the ALA reduces oxidative stress by quenching free radicals and by preventing ROS mediated DNA damage might reverse the decreased expression of antioxidant enzymes. The antioxidant responsiveness mediated by ALA may be anticipated to have biological significance in eliminating reactive free radicals and restoring antioxidant enzymes that may otherwise affect the normal functioning, especially vital organs. The dysfunction of these antioxidant enzymes has been implicated in several disorders including diabetes mellitus. Further ALA treated rats were healthy throughout the study for 60days indicating the dose of ALA (100mg/kg) is well tolerated by obese diabetic rats. To the best of our knowledge this is first report on the effect of chronic administration of ALA for 60days on oxidative stress induced by hyperglycemia and obesity in nongenetic obese diabetic rat.

Previous investigators studied the antioxidant potential, hypoglycemic activity and insulin sensitivity of ALA in diabetic patients [41, 51, 52] or in cells lines [41, 43], or in isolated tissues [44, 53] or combined with exercise on insulin signaling and glucose transport in Obese Zucker rats [45, 46] or on the development of diabetes in diabetes prone obese genetic rats i.e. prophylactic effect [47]. Isolated cells or tissues will give an indication of effect but they will not reflect the in vivo effect totally, the other studies utilized genetic animal models. Jacob et al. and streeper et al. [20, 40] studied independently effect of parenteral administration of ALA (1hr and 14 days) on insulin stimulated glucose transport and non-oxidative and oxidative glucose metabolism. Obrosova et al. [19] investigated prophylactic effect of 100mg/kg ALA on streptozotocin induced diabetes in rats. Thus Obrosova et al. [19] examined the effect ALA on the development of the diabetes. Same investigators also studied antioxidant effect of chronic administration ALA (100mg/kg/p.o) for 3 weeks on streptozocin induced diabetic kidney [18]. Maritim et al. [17], also studied the effect of treatment of ALA (100 mg/kg for 14 days) on streptozocin induced diabetes in rats, these rats were only diabetic. Our present study examined the effect of ALA on ameliorating preexisting obesity and diabetes mellitus in conjunction in nongenetic animal model.

Obesity is a major predisposing factor to insulin resistance and further to type 2 diabetes mellitus. The present study was conducted in an animal model presenting with both the conditions. The results of the present study indicate that obese diabetic rats had higher oxidative stress as compared to obese or diabetic rats, due to obesity and hyperglycemia induced ROS and enzymatic antioxidant are utilized in scavenging ROS and thereby leading to decreased enzymatic antioxidant defense.

ALA decreases oxidative stress induced by hyperglycemia and dyslipidaemia by increasing insulin sensitivity, thereby increasing the utilization and transportation of glucose and TGS, cholesterol and by scavenging ROS spares enzymatic antioxidants, in addition it prevents ROS induced DNA damage thereby increasing the expression of antioxidant enzymes.

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