Effect of Lipoic Acid, Carnitine and *Nigella Sativa* on Diabetic Rat Model

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Abstract

Objectives: Evaluation of therapeutic potentials of α-lipoic acid (α-LA), L-carnitine, *Nigella sativa* (*N. sativa*) or combination of them in carbohydrate and lipid metabolism of DM.

Methods: Rat model of diabetes was induced by single i.p injection of Streptozocin (STZ) 65 mg/kg. The rats were randomly assigned to 6 groups (G): healthy reference (HR), diabetic (DM), DM treated with α-lipoic acid, DM treated with L-carnitine, DM treated with *N. sativa,* and DM treated with combination of the 3 compounds. After 30 days from onset of diabetes, serum and tissue homogenate were obtained for evaluation of glucose metabolism as fasting blood glucose, insulin, insulin sensitivity, HOMA, C-peptide, and pyruvate dehydrogenase (PDH) activity. For lipid metabolism evaluation- of total cholesterol and triacylglycerol (TG) were determined. Markers of antioxidants and oxidative status as total antioxidant capacity (TAC), glutathione-S-transeferase (GST), 8-hydroxy-2-deoxyguanosine (8-OH-dG) were measured.

Results: Either α-LA or N. *sativa* significantly reduced the elevated blood glucose level. The combination of 3 compounds significantly increased the level of insulin and C-peptide. Also, increased the antioxidant activity measured by TAC and decreased the oxidative damage of DNA as measured by 8-OH-dG. HOMA- β increased in G3 and G6 compared to G2. However, the decrease in TG, and total cholesterol levels were non-significant in all groups.

Conclusion: Combination of α-LA, L-carnitine and *N. sativa* will contribute significantly in improvement of the carbohydrate metabolism and to less extent lipid metabolism in diabetic rats, thus increasing the rate of success in management of DM. Also, this combination will have implications in clinical studies and clinical applications.

Key words: lipoic acid - carnitine- *Nigella sativa*- DM

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Introduction

The overall prevalence of DM in adults in KSA is 23.7% in the age group of 30-70-years between 1995 and 2000. [1] According to reports by the American Diabetes Association, people living in Saudi Arabia and the Gulf could face a lifetime risk as high as 60 per cent of developing diabetes. [2]

α-LA is unique natural antioxidants and plays a fundamental role in the metabolism. [3] It is relatively safe, even in patients with renal and liver failure. It targets cellular signal transduction pathways which increased glucose uptake and utilization, antagonizing the oxidative and inflammatory stresses. [4] Acetyl-L-carnitine had a prophylactic and therapeutic potential on the progressive diabetic neuropathy. [5] L-carnitine acts as a carrier for long-chain fatty acids from the cytosol into mitochondria for β-oxidation, hence sustains energy supply. Combination of α-LA, acetyl-L-carnitine, nicotinamide, and biotin effectively improved glucose tolerance, decreased the level of circulating free fatty acid, and prevented the reduction of mitochondrial biogenesis in skeletal muscle of diabetic rats. [6] Acetyl-L-carnitine safely ameliorated arterial hypertension, insulin resistance, impaired glucose tolerance, and hypoadiponetinemia in DM. [7] Several studies showed that extracts from the seeds of N. sativa have antidiabetic effects and may be used as a treatment of diabetes due to their stimulatory effect on beta cell function with consequent increase in serum insulin level. [8]

The aim of this study is to evaluate the isolated effect of α-LA, L- carnitine and *N. sativa* oil on carbohydrate and lipid metabolism of diabetic rats. Also, to investigate the therapeutic potential of combination of the three compounds, hence, improving the rate of success in management of DM.

Materials and Methods
Chemicals and Experimental Design

Streptozocin (STZ) (EC242-646-8), dihydro lipoic acid (α-LA), (T8260), and L-carnitine hydrochloride (EC229) purchased from sigma. *N. sativa* oil purchased from Pharco-Company, Egypt.

The study carried out on healthy adult male Sprague-Dawley rats weighing 150-200g.Their ages ranged from 15-20 weeks. The rats housed conventionally in clean cages under a 12-hour light-dark cycle at 37°C and fed with standard food and water *ad libitum*. The experimental and feeding protocols of the animals approved and performed according to the guidelines of Animal House and ethical standards of college of Medicine, Qassim University, KSA. The experiment carried out on 6 groups. The first group (7 rats) was the healthy reference (H.R) group, injected once with 1 ml saline i.p. The second group (G2) was the diabetic rat model. Rats injected once with 65 mg/kg STZ i.p following overnight fasting. [9] After 3 days, 42 rats that developed diabetes, (sever polyurea, and fasting blood glucose over 200 mg/dl) divided randomly into 4 subgroups received daily oral treatment in the form of α-LA (100 mg/kg) for group (3), [10] L-carnitine (100 mg/kg) for group (4), *N. sativa* oil (500 mg/kg) for group (5) or combination of the 3 compounds for group (6) in the same dose like the above groups for 30 days. The animal fasted overnight, then weighted, blood samples obtained, and sacrificed. Pancreas, kidney, and liver removed, then homogenized separately, in 2,5,20 ml ice cold PBS, respectively, centrifuged at 4000xg for 10 min at 4°C and supernatant kept at -70°C for further biochemical measurements.

Biochemical Assays:

Insulin and C-peptide ELISA kits purchased from DRG, Germany. Glucose determined using the glucose oxidase –peroxidase – chromogen from bioMérieux® SA, France. The total antioxidant capacity (TAC), 8-hydroxy-2-deoxyGuanosine (8-OH-dG) and glutathione S-transferase (GST) kits purchased from Cayman's Chemical Company, USA. TAC measured the combined antioxidant activities of all constituents. 8-OH-dG kit quantified both free 8-OH-dG and DNA-incorporated 8-OH-dG. GST assay kit measured the total GST activity (cytosolic and microsomal). Pyruvate dehydrogenase (PDH) enzyme activity determined by kit (MSP18) from MitoSciences. Total protein measured by available commercial kit. Two types of HOMA calculated, HOMA-insulin resistance (HOMA-IR) and HOMA -β-cell function (HOMA- β) which is %β of β-cell function. [11] Triglyceride

glucose index (TyG index) which is a simple measure of insulin sensitivity. [12]

SPSS version 16 for windows used for statistical analysis. Data are expressed as means ± SEM, (n = 7). One way analysis of variance (ANOVA) followed by LSD to judge the difference between different groups. ROC curves, Pearson correlation coefficient (r) used to detect the relation between markers. Significance level was accepted at $p \leq 0.05$.

Results

In this study, the diabetic rat model (G2) developed hyperglycemia that negatively correlate with insulin level, C-peptide and TAC (r = -0.5,-0.6.,-0.6; p=0.01,0.001,0.001, respectively) that clearly explain one of the possible mechanisms of DM. There is significant decrease level of glucose in G3, G5, G6 (p<0.05) compared to G2; however, in G4 (DM+ carnitine), there is significant increase in glucose level of hepatic homogenate (Figure 1) that may be due to increase rate of β-oxidation and gluconeogensis.
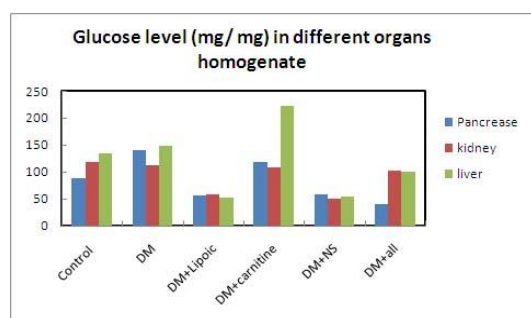

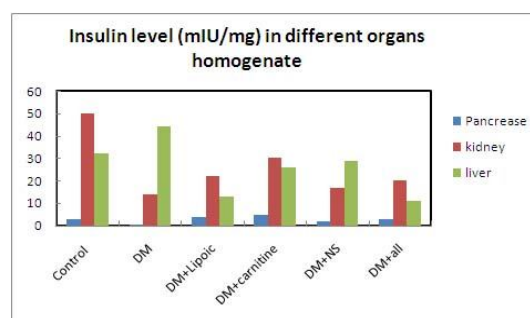**Figure 1: Glucose level in tissue homogenates**

Figure 2: Insulin level in tissue homogenates

In figure 2, G2 showed decreased insulin level of pancreatic and renal homogenate while in liver homogenate increased, but it reached near normal level in pancreatic tissue of treated groups (G3-G6).
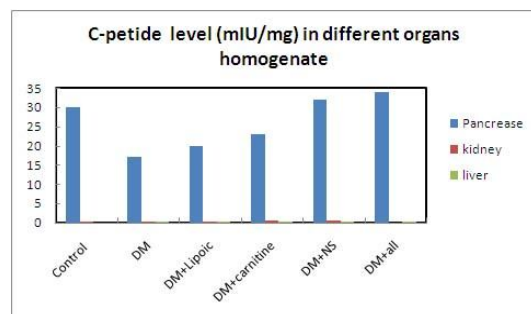

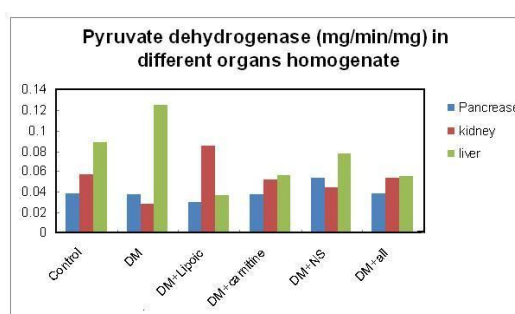**Figure 3: C-peptide level in tissue homogenates**

Figure 4: Pyruvate dehydrogenase level in tissue homogenates

There is increase in C-peptide level in pancreatic tissue homogenate in treated groups (G3-G6) but, the highest increment in G6 compared to G2 (Figure 3). In Figure 4, significant increased level of PDH activity in liver of DM (G2) and (G5) (DM+*N. sativa*), kidney homogenate of G3 (DM + α-LA). That is because; α-LA is essential cofactor for mitochondrial enzymes, including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. Figure 5 showed decreased level of TAC in pancreatic and renal homogenates of G2 compared to G1, while the other groups (G3-G6) showed increased level of TAC

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except pancreatic homogenate of G6 was decreased. Figure 6 showed normalization of GST level in various organs of different groups (except G5). There is significant increase in 8-OH-dG in pancreas of G2 compared to G1, and significant decrease in pancreatic homogenate of G3 and renal homogenate of G4 (Figure 7). A positive correlation between 8-OH-dG and blood glucose level (r= 0.45, p=0.05) and negative correlation with s. insulin level (r = -0.6, p=0.01) were detected. That can explain hyperglycemia due to decrease insulin level leads to increase DNA oxidative damage.
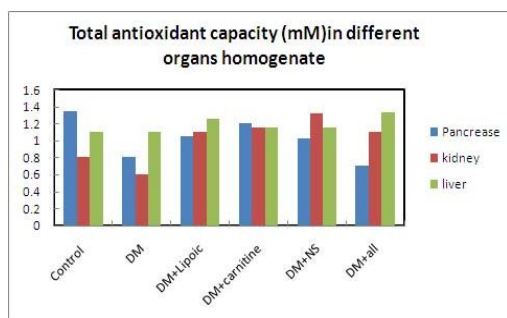
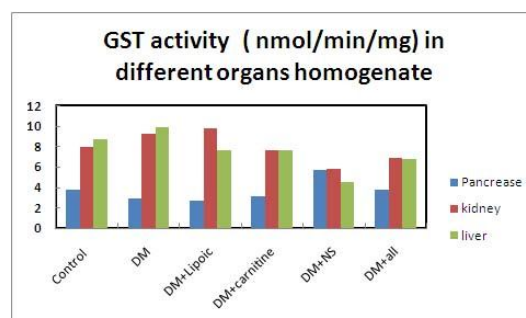**Figure 5: Total antioxidant level in tissue homogenates**

Figure 6: Glutathione –S- transferase level in tissue homogenates
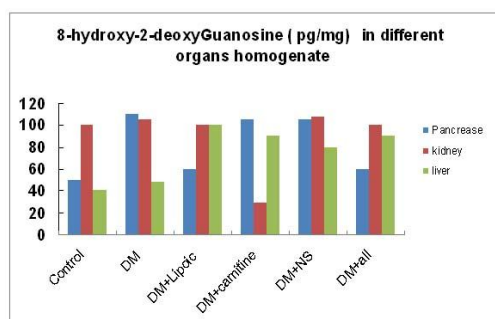
Figure 7: DNA oxidative damage level in tissue homogenates

Table1: Biochemical markers of glucose homeostasis in serum of diabetic mice and control groups

	G1	G2	G3	G4	G5	G6
Glucose mg/dl	78.17 ± 7.97	247.31 ± 11.74	181. 1±1 .2	182.1 ± 7.58	191.17 ± 1.13	164.61± 1. 5
p	0.000		0.000	0.000	0.001	0.000
Insulin (mIU/ml)	4.2　±　1.8	0.5±0.01	3.01±0.1	2.1　± 0.3	1.17± 0.1	3.75±0.25
p	0.01		NS	NS	NS	0.04
C-peptide (ng/ml)	0.42±0.15	0.08±0.01	0.4±0.01	0.2±0.05	0.2±0.05	0.35± 0. 05
p	0.001		0.006	0.04	NS	0.01
Pyruvate dehydrogenase (mg/min)	0.04±0.004	0.04±0.002	0.05±0.002	0.09±0.04	0.03±0.002	0.04±0.007
p	NS		NS	NS	NS	NS
HOMA-IR	0.80	0.3	1.35	0.94	0.55	1.52
HOMA- β (insulin)	99.67%	0.97%	9.18%	6.35%	3.29%	13.29%
HOMA- β (C-peptide)	9.96%	0.15%	1.22%	0.60%	0.56%	1.25%

Group (1) healthy reference (HR), group (2) received (STZ) only; diabetic rat model. Group (3) received lipoic acid (100 mg/kg orally) in diabetic rat, daily, group (4) received L-carnitine daily (100 mg/kg orally) in diabetic rat, group (5) received N*. sativa* oil daily (500 mg/kg orally) in diabetic rat, group (6) received daily combination of lipoic acid, carnitine and *N. sativa* oil in diabetic rat. ANOVA test followed by LSD for the significant values ($p > 0.05$), compared all groups to group 2. HOMA-IR i.e Homeostatic Model Assessment-insulin resistance, HOMA- β i.e Homeostatic Model Assessment %β of β-cell function.

Table 2: Oxidative and antioxidant status in serum of diabetic mice and control groups

	G1	G2	G3	G4	G5	G6
TAC (mM)	1.27± 0.10	0.66 ±0 .07	1.1± 0.01	0.79 ± 0.09	0.73± 0.19	1.07±0.01
p	0.002		0.03	NS	NS	0.04
GST activity (nmol/min/ml)	3.67± 0.29	9.57± 0.35	4.79±0.57	7.13± 1.01	6.57 ± 0.87	4.83 ± 0.18
p	0.002		0.01	NS	NS	0.01
8-OH-G (pg/ml)	33.67±8.57	57.12±7.68	31.5±3.5	37.14±2.8	35.67±8.78	30.09±2.1
p	0.01		0.02	0.01	0.02	0.01

Group (1) healthy reference (HR), group (2) received (STZ) only; diabetic rat model. Group (3) received lipoic acid (100 mg/kg orally) daily in diabetic rat, group (4) received L-carnitine daily (100 mg/kg orally) in diabetic rat, group (5) received *N. sativa* oil daily (500 mg/kg orally) in diabetic rat, group (6) received daily combination of the 3 compounds. TAC; total antioxidant capacity, GST; glutathione –S-transeferase 8-OH-dG; 8-hydroxy-2-deoxyguanisone.

Table 3: Biochemical markers of lipid metabolism in serum of diabetic mice and control groups

	G1	G2	G3	G4	G5	G6
Total cholesterol (mg/dl)	86.27±22.4	98.33± 10.7	80.35±0.1	101.04 ± 7.4	88.43± 6. 8	105.5± 6.5
P	NS		NS	NS	NS	NS
Triacylglcerol (mg/dl)	90.3± 10.9	117.5 ± 5.9	100.75 ± 24.2	108.19 ± 12.6	137.4± 32.1	125.5± 1.5
P	NS		NS	NS	NS	NS
TyG index	3529.37	14529.46	9122.91	9850.69	13133.37	10329.27

Group (1) healthy reference (HR), group (2) received (STZ) only, Group (3) received lipoic acid (100 mg/kg orally) daily in diabetic rat, group (4) received L-carnitine daily (100 mg/kg orally) in diabetic rat, group (5) received *N. sativa* oil daily (500 mg/kg orally) in diabetic rat, group (6) received daily combination of the 3 compounds. TyG i.e triglycerides and glucose index.

Discussion

In this study, the diabetic rat model (G2) showed significant increase in glucose level while insulin, C-peptide and PDH decreased (Table 1, Fig.3-6), also, TAC decreased (Table 2,Fig.5), while, GST and 8-OH-dG increased (Table 2, Fig.6,7), this could be explained by the body response to overcome the production of reactive oxygen species and/ or increased insulin degradation by glutathione insulin transhydrogenases enzyme which used reduced glutathione and GST enzyme. [13] 8-OH-dG increased especially in pancreatic homogenates (Fig. 7) indicating destruction of pancreatic tissue. HOMA-IR is decreased because STZ caused destruction of β cell, so, insulin resistance decrease but, %β of β-cell function is very low due destruction of β cell Table (1) and the triglycerides and glucose index was the highest in all groups indicate impaired lipid and carbohydrate metabolism Table (3).

On the other hand, G3 that received α-LA showed significant decrease in blood glucose, increase in insulin, C-peptide and PDH activity (Table1). In organ homogenates, there are significant decrease (p=0.05) in blood glucose (Fig.1) non-significant increase in insulin level, C-peptide, PDH activity and TAC (Fig.2-5) but with changes in GST level (Fig.6). However, there is significant decrease in 8-OH-dG in pancreatic tissue homogenate (Fig. 7). The changes in TG, and total cholesterol levels were non-significant in all groups (Table 3). Also, HOMA- β is increased in G3 compared to G2. In another studies α-LA treatment induced a significant decrease in glucose, TG, and total cholesterol levels and significant increase in serum insulin and leptin levels in diabetic animal. [14] The hypoglycemic and beta cell protective effects of LA occurred most probably by stimulating glucose transport protein subtype 4 (GLUT4) membrane translocation. LA is referred to as an insulin mimetic agent; hence, it is a potential new anti-diabetic agent. [15] For the treatment of diabetes, the recommended dosage of LA is 300–600 mg daily but for general antioxidant support, the dosage is 20–50 mg daily. Intravenous and oral LA approved for the treatment of diabetic neuropathy in Germany. LA speeds the removal of glucose from the bloodstream at least partly by enhancing insulin function, and reduces insulin resistance. [16] When LA, 400mg/day was given to NIDDM patients over a period of 4 weeks with oral hypoglycemic drugs, it reduced significantly the concentration of ROS and improve HDL-C value, especially in men with metabolic syndrome. [17] Also, LA-supplementation may correct the dyslipidemia by increasing hydrxoy-menthyl-gluteryl-Co-A (HMG-CoA) reductase activity, lipoprotein lipase and lecithin cholesterol acyl transferase (LCAT). [18]

During evaluation of lipid metabolism in children with DM, they found decreased plasma free carnitine level and increased fatty acids. That had negative effects on pancreatic beta-cell functions, and enhanced mitochondrial transportation of fatty acids. [19] L-carnitine promotes the improvement of some aspects of the diabetes such as body weight loss and glycemic level after fasting, [20] that is why we tried carntine in DM. In this study, G4 showed significantly decrease of glucose level, increase in insulin, c-peptide, PDH (Table 1), but it decreased significantly the level of 8-OH-dG (Table 2). There is no effect of L-carnitine on cholesterol and TG levels as shown in table 3, Here in, administration of L-carnitine alone lead to increase in glucose level in liver homogenate (Fig. 1). This could be explained by excessive hepatic glucose production through gluconeogenesis in diabetic patients. [21] Increased insulin level in pancreatic and kidney homogenates (Fig. 2), with increase C-peptide in pancreas (Fig.3). TAC increased in pancreatic and renal homogenates (Fig.5), but, there are non-significant changes in GST (Fig.6). Also, there is increase of 8-OH-dG in pancreatic tissue, while decrease in renal tissue (Fig.7). In agreement with our study- L-carnitine orally administered for a period of 4 weeks did not modify insulin sensitivity or the lipid profile. [22] There is a remarkable abnormality in lipid and carnitine metabolism in Korean diabetic patients. Both exercise training and supplementation of carnitine and antioxidants improved lipid profiles and carnitine

metabolism in human. [23] Also, creatine, choline and carnitine decreased in diabetic mice. [24] Therapies for diabetic neuropathy may include replacement of acetyl-L-carnitine and replenishment of C-peptide in type 1 diabetic neuropathy. [25]

Thymoquinone at 80mg/kg b.w was associated with beneficial changes in hepatic enzyme activities, the altered activities of carbohydrate metabolic enzymes were restored to near normal, and thereby exerts potential antihyperglycemic effects. [26] Treatment of diabetic rats with N. sativa extract and oil significantly decreased the serum glucose and significantly increased serum insulin [27] hence, the designed fifth group received N .sativa. There was significant decrease in glucose level while insulin and c-peptide increased (Table 1). In organs homogenate, glucose level was significantly decreased (Fig.1), while insulin, c-peptide PDH and TAC increased (Fig.2-5). There was decrease of GST with variable changes in 8-OH-dG (Fig.6).

G6 that received combination of the 3 compounds showed significant decrease in glucose level while, insulin level, c-peptide, PDH and TAC were significantly increased (Table 1, 3). GST and 8-OH-dG levels decreased as shown in table 3 may be due to decrease degradation of insulin hormone by insulinase enzyme that use GST. These finding suggested that the combination of LA, carnitine and *N. sativa* oil have a potential therapeutic role in treatment of DM. There is significant negative correlation between glucose level, insulin and C-peptide (r = -0.5, -0.6; p=0.05, 0.01, respectively). Percentage of β cell function is the highest one in all treated groups (Table1). Moreover, LA, carnitine, or *N.sativa* has been used separately in treatment DM and/or its complications. [6-8, 16, 17, 23, 25-27]

Acknowledgment:

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