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Alpha lipoic acid and diabetes mellitus: potential effects on peripheral neuropathy and different metabolic parameters.

--Manuscript Draft--

Full Title:	Alpha lipoic acid and diabetes mellitus: potential effects on peripheral neuropathy and different metabolic parameters.
Manuscript Number:	TAJM-2021-0005R2
Article Type:	Original Article
Keywords:	Alpha lipoic acid; diabetic peripheral neuropathy; diabetes mellitus.
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	rasha Abd Elmoniem, MD
	amira mohamady elsayed, MD
Response to Reviewers:	Reviewer response Dear reviewer, Greetings, We are appreciating your comments and correction for our manuscript. all notes are responded and corrected according to your recommendations. •Major flaws:-we modify table (2) according to your advice as follows: Old table: Table (2): Comparison between neuropathy parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus. Variable Baseline (N=90)End treatment (N=90)Improvement P (significance) No (%)No (%)No (%) Symptoms of neuropathy 90/90 (100)22/90 (24.4)68/90 (75.6)<0.001 (HS) Impaired Monofilament test74/90 (82.8)54/90 (60.0)20/74 (27.0)<0.001 (HS) Impaired Nerve conduction velocity58/90 (64.4)6/90 (6.7)52/58 (89.7)<0.001 (HS) Impaired cardiovascular autonomic neuropathy tests62/90 (68.9)14/90 (15.6)48/62(77.4)<0.001 (HS) Posterior tibial nerve cross sectional

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	area (cm2)Median (range) 0.16 (0.14-0.17)Median(range) 0.17 (0.13-0.20)Wilcoxon test 0.210.84 (NS) New modified table: Table (2): Comparison between neuropathy parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus. Variable Baseline (N=90)No improvement at end treatment Improvement at end of treatment P (significance) No (%)No (%)No (%) Symptoms of neuropathy 90/90 (100)22/90 (24.4)68/90 (75.6)<0.001 (HS) Impaired Monofilament test74/90 (82.8)54/74 (72.9)20/74 (27.1)<0.012 (S) Impaired Nerve conduction velocity58/90 (64.4)6/58 (10.3)52/58 (89.7)<0.001 (HS) Impaired cardiovascular autonomic neuropathy tests62/90 (68.9)14/ 62 (22.6)48/62(77.4)<0.001 (HS) Posterior tibial nerve cross sectional area (cm2)Median (range) 0.16 (0.14-0.17)Median(range) 0.17 (0.13-0.20)Wilcoxon test 0.210.84 (NS) According to this still the improvement is still significant. Minor flaws: we corrected them according to your recommendations. It was heighlighted in green colour ,while the previous coorections were in red colour.But , simple abbreviations in the abstract failed to be explained in the intial menthioning becauses it make the abstract too long to be submitted(315 words and the required is 300 words only) after this modification.so, we only explain them in the main manuscript, Thank you in advance Corresponding author Dr.Amira M.Elsayed

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Why d waleed salaheldeen was removed?

dr waleed salah sent a letter for editorial, for removing his name from authors as he did not participate in collection of data or manuscript writing and neuromuscular ultrasound was performed as a part of his routine work in rheumatology department.

Response to reviewer comments and corrections:

Dear reviewer,

Greeting

We would like to thank you for your valuable, wise and meticulous review of our manuscript. all notes are responded and corrected with few comments when possible.

- **Reviewer 1:**
- According to reviewer opinion, the following are added to the limitations of the study; further placebo-controlled trial with a multivariate regression analysis are needed; the degree of improvements of peripheral neuropathy symptoms need to be evaluated according to a severity score; serum vitamin B 12 need to be measured; whether the improvements of neuropathy and lipid parameters is related to the drug ALA or it is a sequalae of improvement of glycemic control
- Regarding (the duration of diabetic peripheral neuropathy); It was mentioned in the study that the patient had the neuropathy symptoms for more than 6 months (as inclusion criteria) and the median duration of DM was 6 years (range from 4 to 7).
- Regarding (if the patients were prescribed any other peripheral neuropathy treatments), it was mentioned in the study that (we exclude the medication used in the last 3 months that may affect our results, such as medications used for treatment of diabetic peripheral neuropathy (DPN) vitamin B complexes, antioxidants, or opiates), so, we exclude any medication used for treatment of PN.

- we did not use the first line therapy according to the guidelines ; Both pregabalin and duloxetine have been recommended as the initial approach in the symptomatic treatment. They are not targeting the underlying pathophysiological processes to prevent nerve fibre loss; as

specific treatment of nerve damage other than glycemia control is not available uptill now.

- we have 10 drop out patients, it is unclear for us why they did not complete the study? We are not sure failure response or due to side effects, we missed their follow up.
- at the same time exposure to ALA was only for 3 months, that it can be changed again to medication according to guidelines after the study if clinically indicated.

- Regarding to (there was no discussion of studies with opposite results). It was menthioned that one study , in reference (15) showed opposite result, it was mentioned in the conclusion (However, there were conflicting studies about the suitable dose, duration and route of administration of ALA .

- Regarding to Minor flaws: all corrected and highlighted in red

-"Introduction" at the beginning of the abstract.
-Exclusion Criteria is missing number 2
-There should be references for the laboratory tests used in the study.
-Is it a complete or partial resolution of symptoms? , it was mentioned as limitation of the study.
-table:abbreviations done.
-AGE and GLUT-4, explanation done.

Reviewer 2: all are corrected and highlighted in red in the manuscript.

Alpha lipoic acid and diabetes mellitus: potential effects on peripheral neuropathy and different metabolic parameters.

Abstract:

Introduction: Peripheral neuropathy is a common complication of diabetes mellitus, with a prevalence rate of 5.3–47.6%. Alpha lipoic acid (ALA) is an antioxidant used in the treatment of neuro-inflammation, diabetes and diabetic nephropathy. The current study aiming to gauge the effect of oral ALA on diabetic peripheral neuropathy, glycemic control, LDL-C, and HDL-C. **Patients and Methods:** This is a prospective, interventional study carried out on patients with type 2 diabetes mellitus (DM) who were following at the outpatient internal medicine & diabetes clinics at Benha University Hospital. Treatment with ALA for 3 months was given to patient with diabetic peripheral neuropathy. Data in the form of age, sex, body mass index (BMI), duration & treatment of DM, manifestations of peripheral neuropathy were collected. LDL-C, HDL-C, HbA1c, TSH, ALT, AST were measured before and after intervention. Peripheral neuropathy symptoms, nerve conduction velocities, cardiovascular (CV) tests of autonomic neuropathy, and cross-section area of the posterior tibial nerve were performed before and after treatment intervention. **Results:** 90 adult diabetic patients were recruited in the study, 42.2% were females and 57.8 % were males with a median age of 50-60.3 years (IQR = 52). A statistically significant improvements of neuropathic symptoms, nerve conduction velocity, and cardiovascular autonomic neuropathy were noted after 3 months of administration of ALA ($p < 0.001$). However, the cross-section area of the posterior tibial nerve at baseline and after treatment did not change significantly (p value of 0.84). There was a significant improvement in the BMI, HDL-C, LDL-C, HbA1c ($p < 0.001$). Conclusion: Oral treatment with ALA might cause ameliorations of peripheral neuropathy, HbA1c, and LDL-C & HDL-C levels in diabetic patients. Our result failed to proof effect of ALA on nerve cross-section area. The global data encourage further studies with this medication as an ancillary treatment of DM2.

Keyword: Alpha lipoic acid; diabetic peripheral neuropathy; diabetes mellitus.

Introduction:

Diabetes mellitus (DM) is estimated as a considerable health issue as a result of its great occurrence (1). Peripheral neuropathy is a widespread problem in diabetic population, with a prevalence rate of 5.3–47.6% (2), (3). Alpha lipoic acid (ALA), an organo-sulphur compound derived from octanoic acid, was studied as an antioxidant agent in the treatment of obstructive nephropathy, neuro-inflammation, diabetes and diabetic nephropathy (4). Clinical trials have shown hopeful outcomes of ALA on neuropathy manifestations (5) with significantly improved nerve conduction velocity (6). Moreover, some studies found a favorable results of ALA supplementation on lipid profile (7). However, other studies did not recognize any considerable relations (8). Furthermore, parenteral treatment with ALA causes improvement in the glucose levels in patients with type 2 DM (9). The aim of the current study was to gauge the effect of oral administration of ALA on diabetic peripheral neuropathy, glycemic control, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C).

Subject and method:

This is a prospective, interventional study on patients with type 2 DM who were following up at the outpatient internal medicine & diabetes clinics in Benha University Hospitals for 3 months. The following criteria were included; [1] patient's consent to participate; [2] patient diagnosed as diabetic peripheral neuropathy and ≥ one classic painful neuropathic symptom as burning, paresthesia, shooting pain, muscle cramps or allodynia, in the feet, for > 6 months, that affect the daily life or sleep; and [3] patients were not allowed to stop ALA during the follow up period. The following criteria were excluded: [1] peripheral neuropathy as a result of chronic liver diseases, chronic alcohol abuse, vitamin B12 deficiency, drug induced neuropathy, hypothyroidism, truncal neuropathy or severe neurological diseases ; [2] severe renal impairment with an estimated glomerular filtration rate (eGFR) < 30 ml/ min/1.73m2 according to the Modification of Diet in Renal Disease formula (MDRD) (10); [3] recent treatment for cancer ; [4] peripheral vascular disease identified by absent peripheral pulse in feet &/or intermittent claudication ; [5] medication used in the last 3 months that may affect our results, such as medications used for treatment of diabetic peripheral

neuropathy (DPN) ,vitamin B complexes, antioxidants, or opiates; [6] pregnant or lactating patients, or female patients without proper contraception method. Participants were prescribed 600 mg of oral ALA once daily before meal, for 3 months, and were advised not to discontinue anti-diabetic drugs, medications used for managing arterial hypertension, and dyslipidemia medications during the study.

Data collection and follow-up:

Two visits were scheduled for data collection, physical examination and laboratory testing of the patients: the first prior to initiation of ALA administration (baseline visit) and the second at the end of the third month following initiation of ALA (2nd visit). Participants were exposed to careful history and clinical examination with special stress on: Age, Gender, Duration of DM; Type of the treatment; manifestations of peripheral neuropathy (sensory & motor); CVS manifestation of autonomic neuropathy; Other manifestations of autonomic neuropathy; BMI.

Laboratory assay:

Blood samples from the patients were withdrawn after overnight fasting before and after intervention. Serum creatinine; hemoglobin A1c (HbA1c); HDL-C; LDL-C; thyroid stimulating hormone (TSH); alanine aminotransferase (ALT), and Aspartate Aminotransferase (AST) were assayed by routine biochemical methods.

Assessment of peripheral neuropathy:

DPN status were evaluated by symptoms of peripheral neuropathy, monofilament test, vibration perception threshold (VPT), ankle reflexes, and nerve conduction studies. NCV (nerve conduction velocity) were carried out using the Neurowerk, EMG (electromyography) (sigma, Germany) machine for both lower limbs. Motor nerve conduction parameters including Compound Muscle Action Potential (CMAP) amplitudes, distal latency and motor conduction velocity was measured in common peroneal and posterior tibial nerves. Sensory conduction studies included measurement of peak latency and amplitude of SNAPs (sensory nerve action potentials). To ensure adequate uniformity during the procedure, we kept the stimulating and recording parameters constant in all subjects. At the end of the twelfth week, an increase of amplitude of ≥1 mv, latency ≥1 m/s, and conduction

velocity ≥10 m/s from baseline were considered an improvement in NCV in motor nerve. While, for sensory nerves; improvement of amplitude ≥5 μV, latency ≥1 m/s, and conduction velocity ≥10 m/s (11). Tests for cardiac parasympathetic neuropathy including Valsalva ratio, and HR (Heart rate) variation during deep breathing. Valsalva ratio of >1.21, and HR response during deep breathing of >15 beats/min were criteria of improvements. Tests for cardiac sympathetic neuropathy including BP response to sudden standing, and BP response to sustained handgrip. BP response to sudden standing of < 10 mm Hg and after exercise hand grip of >16 mmHg were criteria of improvements. Ultrasound on nerve were done by measurement of cross-sectional area of the nerve as assessment of improvement of DPN before& after use of ALA. The patients were examined in supine position, and the foot was bolstered with a pillow to show the lower leg and foot. The transducer was placed over posterior tibial nerve in both transverse (short axis) and in longitudinal (long axis) views. The 5.0–12.0-MHz multifrequency linear array probe was used for posterior tibial nerve scanning. Logiq P9 ultrasound scanner (general electric, USA) was used. The study was approved by the Ethics Committee of Benha Faculty of Medicine, Benha University with written informed consent was obtained from all participants.

Calculation of sample size:

version 16.1 of MedCalc software (© 1993-2016 MedCalc Software) was used to calculate the required sample size using the average percentage of improvement (35%) in neuropathic symptoms according to Agathos et al. (12).The following variables were entered; Level of significance (type I error) =0.05, Type II error (1-level of power) =0.2, Average % of neuropathic improvement =35%,Null hypothesis percentage= 50%. So, the least sample size =85 diabetic patients. It was increased to 100 patients to safeguard against drop out during follow up of the study. Ninety cases have completed the study. Ten dropouts were excluded. The data were analyzed using SPSS version 16 software (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages, Mc-Nemar's test was used for analysis paired proportions. Shapiro-Wilks test were performed for quantitative data assuming normality at (P>0.05). Normally distributed variables were presented as (mean ± standard deviation) and

analyzed by paired "t" test for paired samples. Non-parametric data were expressed as median and inter-quartile range (IQR) and tested by Wilcoxon test considering a significant P value ≤ 0. 05.

Results:

This is a prospective interventional study, conducted at Benha University Hospital, Egypt, between March 2020 and December 2020. The study included 90 type 2 adult diabetic patients. The demographic and laboratory findings of the studied patients at baseline were demonstrated in table (1).The results showed no statistically significant association (P>0.05) among age, sex, type of treatment, duration of DM, and age of onset of diabetes before and after the intervention. In terms of diabetic peripheral neuropathy parameters, neuropathic symptoms were improved significantly at the end of the follow up (p <0.001; table 2). Twenty out of 74 patients showed improvement of the monofilament test. There was a significant improvement of nerve conduction velocity (p <0.001) after intervention treatment by ALA. Additionally, there was a significant improvement in the cardiovascular autonomic neuropathy. However, no significant change in the cross-section area of the posterior tibial nerve at the end of treatment (p value of 0.84) (table 2). Regarding the metabolic parameters, there were a non-significant difference among TSH, ALT, AST levels (table 3). However, HbA1c improved significantly after the intervention (mean± SD; 6. 8± 0. 45%) compared to the baseline (mean± SD; 7. 2± 0. 54 %) (table 3& figure 1). Additionally, there was a significant improvement in the BMI, HDL-C, LDL-C (p value < 0.001) (table 3& figure 2).

Table (1): Baseline clinical data of the studied type 2 diabetic patients.

Variable		N=90	
Age (years)	Median (IQR)	52 (50-60.3)	
Sex (No., %)	Male	38	42.2%
	Female	52	57.8%
Body mass index(kg/m^2)	Median (IQR)	28 (26-32)	
Waist circumference (cm)	Median (IQR)	85 (76-105)	
Hypertension	No	36	40.0%
	Yes	54	60.0%
Age of onset of DM (years)	Median (IQR)	47 (45-49)	
Duration of DM (years)	Median (IQR)	6 (4-7)	
Treatment	Insulin and oral	22	24.4%
	Oral	56	62.2%
	Insulin	12	13.3%
Serum creatinine (mg/dl)	Median (IQR)	0.8 (0.8-0.9)	
Blood Urea (mg/dl)	Median (IQR)	29 (28-31)	

Table (2): Comparison between neuropathy parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus.

Variable	Baseline (N=90)	No improvement at end treatment	Improvement at end of treatment	P (significance)
	No (%)	No (%)	No (%)	
Symptoms of neuropathy	90/90 (100)	22/90 (24.4)	68/90 (75.6)	<0.001 (HS)
Impaired Monofilament test	74/90 (82.8)	54/74 (72.9)	20/74 (27.1)	<0.012 (S)
Impaired Nerve conduction velocity	58/90 (64.4)	6/58 (10.3)	52/58 (89.7)	<0.001 (HS)
Impaired cardiovascular autonomic neuropathy tests	62/90 (68.9)	14/ 62 (22.6)	48/62(77.4)	<0.001 (HS)
Posterior tibial nerve cross sectional area (cm2)	Median (range) 0.16 (0.14-0.17)	Median(range) 0.17 (0.13-0.20)	Wilcoxon test 0.21	0.84 (NS)

Table (3): Comparison between different metabolic parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus.

Variable	Baseline (N=90)	End treatment (N=90)	Wilcoxon test	P (Significance)
	Median (IQR)	Median (IQR)		
BMI (kg/m^2)	31 (27-33.3)	28 (26-32)	3.79	<0.001 (HS)
HbA1C*%	7. 2± 0. 54	6. 8± 0. 45	4.13	<0.001 (HS)
HDL-C mg/dl	46 (38-55)	62 (38-65)	6.01	<0.001 (HS)
LDL-C mg/dl	120 (93-143.2)	110 (89.5-122.5)	8.24	<0.001 (HS)
TSH (mU/L)	2.1 (1.9-2.23)	2.4 (1.9-2.92)	1.01	0.91 (NS)
ALT (IU/L)	19 (18-20)	18.8 (18-20)	1.56	0.12 (NS)
AST (IU/L)	29 (28-30)	30 (28-30)	0.25	0.81 (NS)

BMI= Body mass index, HbA1C= glycated hemoglobin, HDL-C=high density lipoprotein cholesterol, LDL-C= low density lipoprotein cholesterol, TSH= thyroid stimulating hormone, ALT= Alanine transaminase, AST= Aspartate aminotransferase.
*Data were expressed as (mean ± SD) and analyzed by Paired "t" test.

IQR= Interquartile range.

Figure (1): Bar chart showing the mean values of HbA1C pre and post alpha acid lipoic treatment in patients with T2 diabetes mellitus.

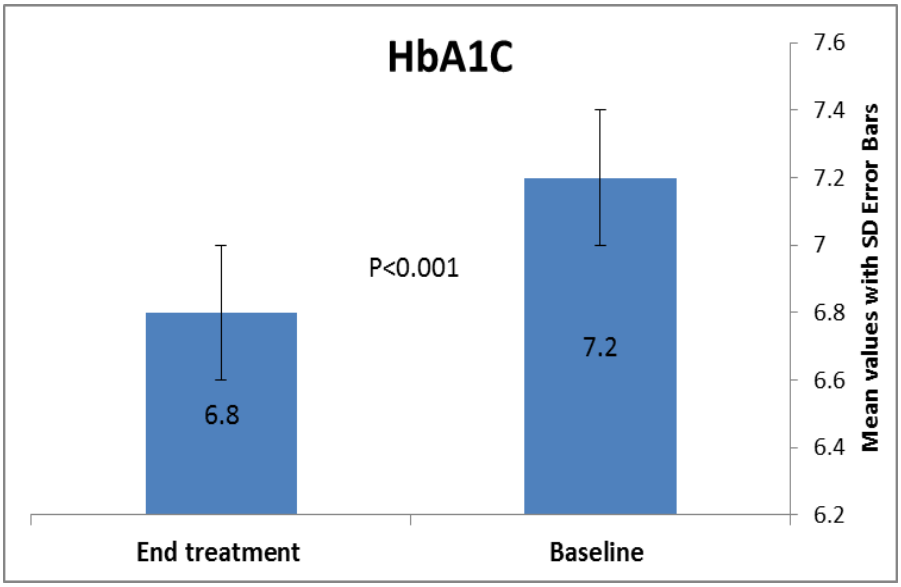
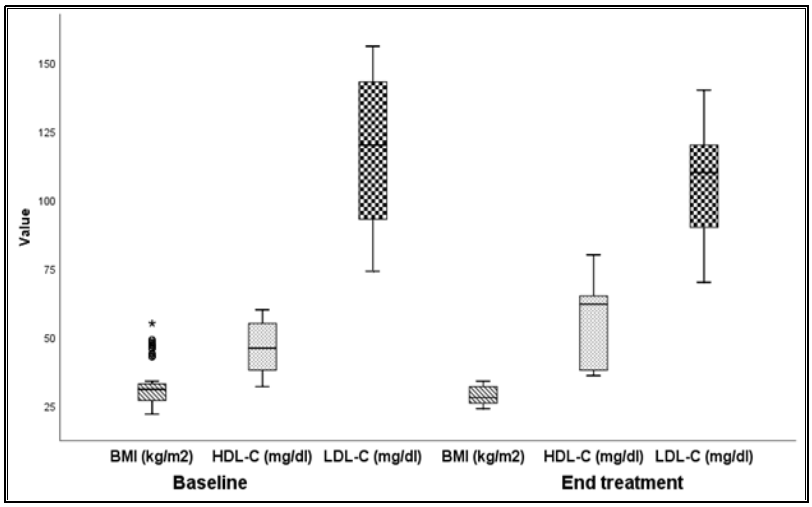


Figure (2): Boxplot presentation for the median(& IQR) of BMI, HDL-C and LDL-C pre and post treatment.



Discussion:

Diabetic neuropathy (DN) is the most widespread sequalae of diabetes. Pathogenesis of DN is linked to chronic hyperglycemia, either by accumulation of free radicals or advanced glycation end products (AGE), which trigger inflammatory cascades causing cell damage and cell death (13). The purpose of the current study was to investigate prospectively the effect of oral ALA on diabetic peripheral neuropathy, glycemic control, and lipid profiles. Our study, concluded that there were a significant improvements of diabetic peripheral neuropathy manifestations, nerve conduction velocity, and cardiovascular autonomic tests after 3 months of intervention. ZIEGLER et al. found similar results, that is to say, a 600 mg /d orally was found to provide the optimal risk-benefit ratio (14). Additionally, significant reductions in neuropathic symptoms were shown at a dose of 600 mg/d of ALA at day 40 versus baseline in another study (12). Mijnhout et al. in a systemic review, concluded that the intravenous daily dose of 600 mg of ALA for 3 weeks, led to a significant improvement in the neuropathic pain (15). However, he found that improvement of the clinical symptoms noted after 3–5 weeks of oral ALA in a dose of 600 mg/day, was unclear (15). It was revealed that, intravenous administration of ALA in patient with diabetic polyneuropathy yielded a quick result on microcirculation (16). It was recognized that, increased vascular oxidative stress seen in diabetics, resulted in impairment of nitric oxide–mediated vasodilation. At this time, intravenous administration of ALA enhanced nitric oxide–mediated endothelium vasodilation (17). The rationale of improvement of

diabetic neuropathy symptoms, following treatment with ALA, is mostly due to its antioxidant action. ALA, and its reduced form (dihydrolipoic acid) act as antioxidants by neutralization of reactive oxygen species, inhibition of reactive-oxygen generators, and restoration of damage caused by other oxidants (12). Furthermore, studies support that ALA increases glutathione levels, and hampers lipid peroxidation (18) & (19). In our results, the body mass index decreased significantly at the end of the third month. These findings are consistent with a previous study which showed that ALA produced a reduction in BMI, and fasting blood sugar levels in patients suffering from chronic spinal cord injury after administration of 600 mg of alpha lipoic acid daily (20). In another study, a dose of 1800 mg daily produced a moderate weight loss in obese patients (21). Li et al. disclosed that an oral dose of 1200 mg daily of ALA for 8 weeks induced mild weight loss (22).Udupa et al. found that , a dose of 300 mg of alpha lipoic acid resulted in a significant reduction in the BMI in type 2 DM after 90 days of the intervention (23). Further studies will be required to gauge the dose of alpha-lipoic acid that can cause weight loss, and the long-term safety of this agent. The current study revealed that HbA1c decreased significantly at the end of the treatment. In line with our result, one study concluded that, ALA improved peripheral insulin sensitivity in type 2 diabetes mellitus (9). One meta-analysis showed a significant lower levels of serum glucose after ALA supplementation in patients with stroke (24). The beneficial role of alpha lipoic acid in lowering the fasting plasma glucose may be connected to its effect in modulating adenosine monophosphate-activated protein kinase (AMPK) (25) in skeletal muscle and beta-cells (26)&(27) ,which subsequently potentiates the insulin-secretory response of β cells to glucose (28). Consistent with previous studies, it was declared that ALA can decrease FBS and HbA1C level, possibly by increasing Glucose transporter type 4 (GLUT-4) transportation to fat and muscle cell membranes (20), and increasing the skeletal muscle glucose transport activity (29). In addition, ALA appears to suppress gluconeogenesis in the liver (30). It is stated that ALA augments the activity of some proteins of the insulin signalling pathway such as insulin receptor (IR), insulin receptor substrate 1 (IRS1), protein kinase B (AKT), and phosphatidylinositide 3-kinase (PI3K) (31). According to this, ALA is considered

an insulin-mimetic agent (32). In the current study, HDL-C & LDL-C improved significantly at the end of follow up. One study found, a significant decrease in total cholesterol, and LDL-C and higher HDL-C following 12 weeks of ALA (33). One meta-analysis concluded that ALA supplementation might be beneficial in lowering total cholesterol levels in subjects with stroke (23). In contrast, another study did not detect any change in lipid profile following intake of 600 mg ALA/day for 8 weeks in subjects with ESRD (34). In addition, another study, did not reveal a significant improvement of serum total cholesterol, triglyceride, HDL, and LDL after alpha lipoic acid (35). In our study the improvement of LDL-C& HDL-C might be related to the improvement of BMI and glycemic control. Different study design, sample size, dosages of ALA, and the participants characteristics might clarify the inconsistencies among studies. The probable lipid-lowering effects of alpha lipoic acid; firstly, the beneficial effects of ALA on β oxidation of fatty acids in the mitochondria via activation of AMP-activated protein kinase (36). Secondly, it might be related to the role of ALA in lowering blood glucose levels (37). Thirdly, ALA administration might decrease the expression of acetyl-CoA carboxylase and fatty acid synthase (enzymes in fatty acid synthesis) (38). Other possible mechanisms of lowering TC or LDL after administration of ALA include: (i) augmented activity of lipoprotein lipase, (ii) increased synthesis of LDL receptors in the liver which transfer cholesterol to the hepatic system (39), (iii) increase plasma adiponectin levels which enhanced FFAs β-oxidation (40). We did not report any significant reduction in the cross-section area of the posterior tibial nerve after the intervention. Singh et al. found that, morphological changes of the tibial nerves in diabetic subjects can be detected by ultrasonography, even before the clinical onset of peripheral neuropathy(41).Watanabe and colleagues, indicated the possibility of using US for the diagnosis of DPN with sensitivity of 80% and specificity of 94% (42). A study performed by Riazi et al. detected lower sensitivity and specificity (69 and 77%, respectively) (43). To our knowledge, there is little studies comparing the effect of ALA on the cross-sectional area of the nerve. It may need longer duration and larger numbers of participants to assess the cross-section area in a follow up prospective study after treatment intervention with alpha lipoic acid.

Conclusion: Oral supplements of ALA improved peripheral neuropathy, glycemic control, and LDL-C & HDL-C levels in diabetic patients. However, there were conflicting studies about the suitable dose, duration and route of administration of ALA. Our result failed to proof effect of ALA on the nerve cross-section area.

Limitations of the study: Firstly, it was unclear whether the improvements of neuropathy and lipid parameters is related to the drug ALA or it is a sequalae of improvement of glycemic control. Secondly, serum levels of vitamin B12 should be measured to exclude the true cases of vitamin B 12 deficiency. Thirdly, the degree of improvements of peripheral neuropathy symptoms need to be evaluated according to a severity score such as Neuropathy Symptoms Score (NSS). Fourthly, additional research, placebo-controlled trials with a multivariate regression analysis are needed, with longer duration, different doses of ALA to judge the effective dose on body weight, glucose control, and different lipid fractions as well as safety on long term before using this agent as an ancillary treatment of DM2.

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Alpha lipoic acid and diabetes mellitus: potential effects on peripheral neuropathy and different metabolic parameters.

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Abstract:

Introduction: Peripheral neuropathy is a common complication of diabetes mellitus, with a prevalence rate of 5.3–47.6%. Alpha lipoic acid (ALA) is an antioxidant used in the treatment of neuro-inflammation, diabetes and diabetic nephropathy. The current study aiming to gauge the effect of oral ALA on diabetic peripheral neuropathy, glycemic control, LDL-C, and HDL-C. **Patients and Methods:** This is a prospective, interventional study carried out on patients with type 2 diabetes mellitus (DM) who were following at the outpatient internal medicine & diabetes clinics at Benha University Hospital. Treatment with ALA for 3 months was given to patient with diabetic peripheral neuropathy. Data in the form of age, sex, body mass index (BMI), duration & treatment of DM, manifestations of peripheral neuropathy were collected. LDL-C, HDL-C, HbA1c, TSH, ALT, AST were measured before and after intervention. Peripheral neuropathy symptoms, nerve conduction velocities, cardiovascular (CV) tests of autonomic neuropathy, and cross-section area of the posterior tibial nerve were performed before and after treatment intervention. **Results:** 90 adult diabetic patients were recruited in the study, 42.2% were females and 57.8 % were males with a median age of 50-60.3 years (IQR = 52). A statistically significant improvements of neuropathic symptoms, nerve conduction velocity, and cardiovascular autonomic neuropathy were noted after 3 months of administration of ALA ($p < 0.001$). However, the cross-section area of the posterior tibial nerve at baseline and after treatment did not change significantly (p value of 0.84). There was a significant improvement in the BMI, HDL-C, LDL-C, HbA1c ($p < 0.001$). Conclusion: Oral treatment with ALA might cause ameliorations of peripheral neuropathy, HbA1c, and LDL-C & HDL-C levels in diabetic patients. Our result failed to proof effect of ALA on nerve cross-section area. The global data encourage further studies with this medication as an ancillary treatment of DM2.

Keyword: Alpha lipoic acid; diabetic peripheral neuropathy; diabetes mellitus.

Introduction:

Diabetes mellitus (DM) is estimated as a considerable health issue as a result of its great occurrence (1). Peripheral neuropathy is a widespread problem in diabetic population, with a prevalence rate of 5.3–47.6% (2), (3). Alpha lipoic acid (ALA), an organo-sulphur compound derived from octanoic acid, was studied as an antioxidant agent in the treatment of obstructive nephropathy, neuro-inflammation, diabetes and diabetic nephropathy (4). Clinical trials have shown hopeful outcomes of ALA on neuropathy manifestations (5) with significantly improved nerve conduction velocity (6). Moreover, some studies found a favorable results of ALA supplementation on lipid profile (7). However, other studies did not recognize any considerable relations (8). Furthermore, parenteral treatment with ALA causes improvement in the glucose levels in patients with type 2 DM (9). The aim of the current study was to gauge the effect of oral administration of ALA on diabetic peripheral neuropathy, glycemic control, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C).

Subject and method:

This is a prospective, interventional study on patients with type 2 DM who were following up at the outpatient internal medicine & diabetes clinics in Benha University Hospitals for 3 months. The following criteria were included; [1] patient's consent to participate; [2] patient diagnosed as diabetic peripheral neuropathy and ≥ one classic painful neuropathic symptom as burning, paresthesia, shooting pain, muscle cramps or allodynia, in the feet, for > 6 months, that affect the daily life or sleep; and [3] patients were not allowed to stop ALA during the follow up period. The following criteria were excluded: [1] peripheral neuropathy as a result of chronic liver diseases, chronic alcohol abuse, vitamin B12 deficiency, drug induced neuropathy, hypothyroidism, truncal neuropathy or severe neurological diseases ; [2] severe

renal impairment with an estimated glomerular filtration rate (eGFR) < 30 ml/ min/1.73m2 according to the Modification of Diet in Renal Disease formula (MDRD) (10); [3] recent treatment for cancer ; [4] peripheral vascular disease identified by absent peripheral pulse in feet &/or intermittent claudication ; [5] medication used in the last 3 months that may affect our results, such as medications used for treatment of diabetic peripheral neuropathy (DPN) ,vitamin B complexes, antioxidants, or opiates; [6] pregnant or lactating patients, or female patients without proper contraception method. Participants were prescribed 600 mg of oral ALA once daily before meal, for 3 months, and were advised not to discontinue anti-diabetic drugs, medications used for managing arterial hypertension, and dyslipidemia medications during the study.

Data collection and follow-up:

Two visits were scheduled for data collection, physical examination and laboratory testing of the patients: the first prior to initiation of ALA administration (baseline visit) and the second at the end of the third month following initiation of ALA (2nd visit). Participants were exposed to careful history and clinical examination with special stress on: Age, Gender, Duration of DM; Type of the treatment; manifestations of peripheral neuropathy (sensory & motor); CVS manifestation of autonomic neuropathy; Other manifestations of autonomic neuropathy; BMI.

Laboratory assay:

Blood samples from the patients were withdrawn after overnight fasting before and after intervention. Serum creatinine; hemoglobin A1c (HbA1c); HDL-C; LDL-C; thyroid stimulating hormone (TSH); alanine aminotransferase (ALT), and Aspartate Aminotransferase (AST) were assayed by routine biochemical methods.

Assessment of peripheral neuropathy:

DPN status were evaluated by symptoms of peripheral neuropathy, monofilament test, vibration perception threshold (VPT), ankle reflexes, and nerve conduction studies. NCV (nerve conduction velocity) were carried out using the Neurowerk, EMG (electromyography) (sigma, Germany) machine for both lower limbs. Motor nerve conduction parameters including Compound Muscle Action Potential (CMAP) amplitudes, distal latency and motor conduction velocity was measured in common peroneal and posterior tibial nerves. Sensory conduction studies included measurement of peak latency and amplitude of SNAPs (sensory nerve action potentials). To ensure adequate uniformity during the procedure, we kept the stimulating and recording parameters constant in all subjects. At the end of the twelfth week, an increase of amplitude of ≥1 mv, latency ≥1 m/s, and conduction velocity ≥10 m/s from baseline were considered an improvement in NCV in motor nerve. While, for sensory nerves; improvement of amplitude ≥5 μV, latency ≥1 m/s, and conduction velocity ≥10 m/s (11). Tests for cardiac parasympathetic neuropathy including Valsalva ratio, and HR (Heart rate) variation during deep breathing. Valsalva ratio of >1.21, and HR response during deep breathing of >15 beats/min were criteria of improvements. Tests for cardiac sympathetic neuropathy including BP response to sudden standing, and BP response to sustained handgrip. BP response to sudden standing of < 10 mm Hg and after exercise hand grip of >16 mmHg were criteria of improvements. Ultrasound on nerve were done by measurement of cross-sectional area of the nerve as assessment of improvement of DPN before& after use of ALA. The patients were examined in supine position, and the foot was bolstered with a pillow to show the lower leg and foot. The transducer was placed over posterior tibial nerve in both transverse (short axis) and in longitudinal (long axis) views. The 5.0–12.0-MHz multifrequency linear array probe was used for posterior tibial nerve scanning. Logiq P9 ultrasound scanner (general electric, USA) was used.

The study was approved by the Ethics Committee of Benha Faculty of Medicine, Benha University with written informed consent was obtained from all participants. It was registered in clinical trial website; ClinicalTrials.gov Identifier (NCT number): NCT04322240.

Calculation of sample size:

version 16.1 of MedCalc software (© 1993-2016 MedCalc Software) was used to calculate the required sample size using the average percentage of improvement (35%) in neuropathic symptoms according to Agathos et al. (12).The following variables were entered; Level of significance (type I error) =0.05, Type II error (1-level of power) =0.2, Average % of neuropathic improvement =35%,Null hypothesis percentage= 50%. So, the least sample size =85 diabetic patients. It was increased to 100 patients to safeguard against drop out during follow up of the study. Ninety cases have completed the study. Ten dropouts were excluded. The data were analyzed using SPSS version 16 software (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages, Mc-Nemar's test was used for analysis paired proportions. Shapiro-Wilks test were performed for quantitative data assuming normality at (P>0.05). Normally distributed variables were presented as (mean ± standard deviation) and analyzed by paired "t" test for paired samples. Non-parametric data were expressed as median and inter-quartile range (IQR) and tested by Wilcoxon test considering a significant P value ≤ 0. 05.

Results:

This is a prospective interventional study, conducted at Benha University Hospital, Egypt, between March 2020 and December 2020. The study included 90 type 2 adult diabetic patients. The demographic and laboratory findings of the studied patients at baseline were demonstrated in table (1).The results showed no statistically significant association (P>0.05) among age, sex, type of treatment, duration of DM, and age of onset of diabetes before and after the intervention. In

terms of diabetic peripheral neuropathy parameters, neuropathic symptoms were improved significantly at the end of the follow up ($p < 0.001$; table 2). Twenty out of 74 patients showed improvement of the monofilament test. There was a significant improvement of nerve conduction velocity ($p < 0.001$) after intervention treatment by ALA. Additionally, there was a significant improvement in the cardiovascular autonomic neuropathy. However, no significant change in the cross-section area of the posterior tibial nerve at the end of treatment (p value of 0.84) (table 2). Regarding the metabolic parameters, there were a non-significant difference among TSH, ALT, AST levels (table 3). However, HbA1c improved significantly after the intervention (mean± SD; 6. 8± 0. 45%) compared to the baseline (mean± SD; 7. 2± 0. 54 %) (table 3& figure 1). Additionally, there was a significant improvement in the BMI, HDL-C, LDL-C (p value < 0.001) (table 3& figure 2).

Table (1): Baseline clinical data of the studied type 2 diabetic patients.

Variable		N=90	
Age (years)	Median (IQR)	52 (50-60.3)	
Sex (No., %)	Male	38	42.2%
	Female	52	57.8%
Body mass index(kg/m^2)	Median (IQR)	28 (26-32)	
Waist circumference (cm)	Median (IQR)	85 (76-105)	
Hypertension	No	36	40.0%
	Yes	54	60.0%
Age of onset of DM (years)	Median (IQR)	47 (45-49)	
Duration of DM (years)	Median (IQR)	6 (4-7)	
Treatment	Insulin and oral	22	24.4%
	Oral	56	62.2%
	Insulin	12	13.3%
Serum creatinine (mg/dl)	Median (IQR)	0.8 (0.8-0.9)	
Blood Urea (mg/dl)	Median (IQR)	29 (28-31)	

Table (2): Comparison between neuropathy parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus.

Variable	Baseline (N=90)	No improvement at end treatment	Improvement at end of treatment	P (significance)
	No (%)	No (%)	No (%)	
Symptoms of neuropathy	90/90 (100)	22/90 (24.4)	68/90 (75.6)	<0.001 (HS)
Impaired Monofilament test	74/90 (82.8)	54/74 (72.9)	20/74 (27.1)	<0.012 (S)
Impaired Nerve conduction velocity	58/90 (64.4)	6/58 (10.3)	52/58 (89.7)	<0.001 (HS)
Impaired cardiovascular autonomic neuropathy tests	62/90 (68.9)	14/ 62 (22.6)	48/62(77.4)	<0.001 (HS)
Posterior tibial nerve cross sectional area (cm2)	Median (range) 0.16 (0.14-0.17)	Median(range) 0.17 (0.13-0.20)	Wilcoxon test 0.21	0.84 (NS)

Table (3): Comparison between different metabolic parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus.

Variable	Baseline (N=90)	End treatment (N=90)	Wilcoxon test	P (Significance)
	Median (IQR)	Median (IQR)		
BMI (kg/m^2)	31 (27-33.3)	28 (26-32)	3.79	<0.001 (HS)
HbA1C*%	7. 2± 0. 54	6. 8± 0. 45	4.13	<0.001 (HS)
HDL-C mg/dl	46 (38-55)	62 (38-65)	6.01	<0.001 (HS)
LDL-C mg/dl	120 (93-143.2)	110 (89.5-122.5)	8.24	<0.001 (HS)
TSH (mU/L)	2.1 (1.9-2.23)	2.4 (1.9-2.92)	1.01	0.91 (NS)
ALT (IU/L)	19 (18-20)	18.8 (18-20)	1.56	0.12 (NS)
AST (IU/L)	29 (28-30)	30 (28-30)	0.25	0.81 (NS)

BMI= Body mass index, HbA1C= glycated hemoglobin, HDL-C=high density lipoprotein cholesterol, LDL-C= low density lipoprotein cholesterol, TSH= thyroid stimulating hormone, ALT= Alanine transaminase, AST= Aspartate aminotransferase.

*Data were expressed as (mean ± SD) and analyzed by Paired "t" test.

IQR= Interquartile range.

Figure (1): Bar chart showing the mean values of HbA1C pre and post alpha acid lipoic treatment in patients with T2 diabetes mellitus.

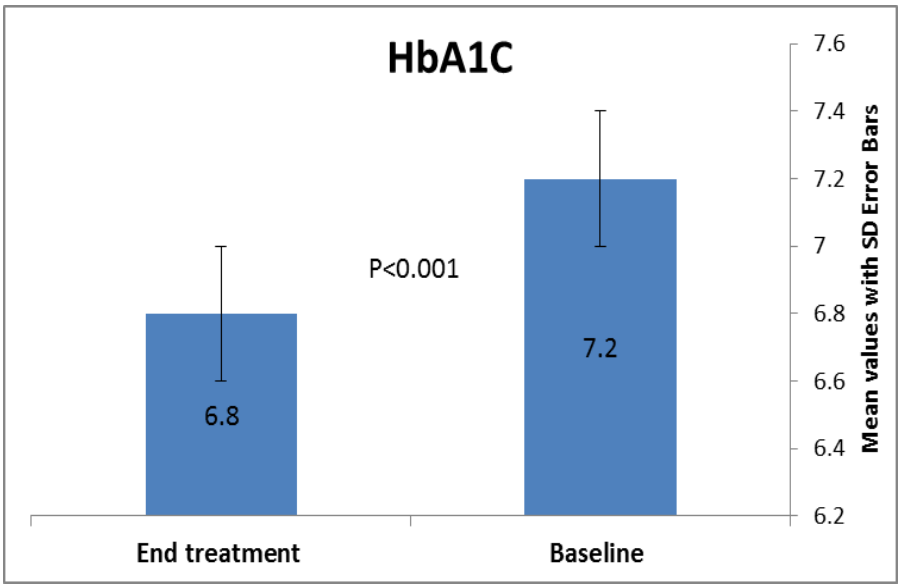
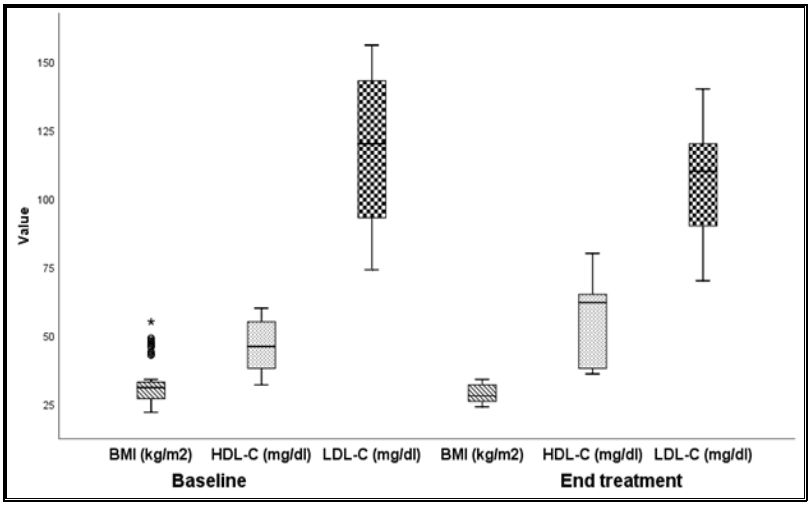


Figure (2): Boxplot presentation for the median(& IQR) of BMI, HDL-C and LDL-C pre and post treatment.



Discussion:

Diabetic neuropathy (DN) is the most widespread sequalae of diabetes. Pathogenesis of DN is linked to chronic hyperglycemia, either by accumulation of free radicals or advanced glycation end products (AGE), which trigger inflammatory cascades causing cell damage and cell death (13). The purpose of the current study was to investigate prospectively the effect of oral ALA on diabetic peripheral neuropathy, glycemic control, and lipid profiles. Our study, concluded that there were a significant improvements of diabetic peripheral neuropathy manifestations, nerve conduction velocity, and cardiovascular autonomic tests after 3 months of intervention. ZIEGLER et al. found similar results, that is to say, a 600 mg /d orally was found to provide the optimal risk-benefit ratio (14). Additionally, significant reductions in neuropathic symptoms were shown at a dose of 600 mg/d of ALA at day 40 versus baseline in another study (12). Mijnhout et al. in a systemic review, concluded that the intravenous daily dose of 600 mg of ALA for 3 weeks, led to a significant improvement in the neuropathic pain (15). However, he found

that improvement of the clinical symptoms noted after 3–5 weeks of oral ALA in a dose of 600 mg/day, was unclear (15). It was revealed that, intravenous administration of ALA in patient with diabetic polyneuropathy yielded a quick result on microcirculation (16). It was recognized that, increased vascular oxidative stress seen in diabetics, resulted in impairment of nitric oxide–mediated vasodilation. At this time, intravenous administration of ALA enhanced nitric oxide–mediated endothelium vasodilation (17). The rationale of improvement of diabetic neuropathy symptoms, following treatment with ALA, is mostly due to its antioxidant action. ALA, and its reduced form (dihydrolipoic acid) act as antioxidants by neutralization of reactive oxygen species, inhibition of reactive-oxygen generators, and restoration of damage caused by other oxidants (12). Furthermore, studies support that ALA increases glutathione levels, and hampers lipid peroxidation (18) & (19). In our results, the body mass index decreased significantly at the end of the third month. These findings are consistent with a previous study which showed that ALA produced a reduction in BMI, and fasting blood sugar levels in patients suffering from chronic spinal cord injury after administration of 600 mg of alpha lipoic acid daily (20). In another study, a dose of 1800 mg daily produced a moderate weight loss in obese patients (21). Li et al. disclosed that an oral dose of 1200 mg daily of ALA for 8 weeks induced mild weight loss (22).Udupa et al. found that , a dose of 300 mg of alpha lipoic acid resulted in a significant reduction in the BMI in type 2 DM after 90 days of the intervention (23). Further studies will be required to gauge the dose of alpha-lipoic acid that can cause weight loss, and the long-term safety of this agent. The current study revealed that HbA1c decreased significantly at the end of the treatment. In line with our result, one study concluded that, ALA improved peripheral insulin sensitivity in type 2 diabetes mellitus (9). One meta-analysis showed a significant lower levels of serum glucose after ALA supplementation in patients with stroke (24). The beneficial role of alpha lipoic acid in lowering

the fasting plasma glucose may be connected to its effect in modulating adenosine monophosphate-activated protein kinase (AMPK) (25) in skeletal muscle and beta-cells (26)&(27) ,which subsequently potentiates the insulin-secretory response of β cells to glucose (28). Consistent with previous studies, it was declared that ALA can decrease FBS and HbA1C level, possibly by increasing Glucose transporter type 4 (GLUT-4) transportation to fat and muscle cell membranes (20), and increasing the skeletal muscle glucose transport activity (29). In addition, ALA appears to suppress gluconeogenesis in the liver (30). It is stated that ALA augments the activity of some proteins of the insulin signalling pathway such as insulin receptor (IR), insulin receptor substrate 1 (IRS1), protein kinase B (AKT), and phosphatidylinositide 3-kinase (PI3K) (31). According to this, ALA is considered an insulin-mimetic agent (32). In the current study, HDL-C & LDL-C improved significantly at the end of follow up. One study found, a significant decrease in total cholesterol, and LDL-C and higher HDL-C following 12 weeks of ALA (33). One meta-analysis concluded that ALA supplementation might be beneficial in lowering total cholesterol levels in subjects with stroke (23). In contrast, another study did not detect any change in lipid profile following intake of 600 mg ALA/day for 8 weeks in subjects with ESRD (34). In addition, another study, did not reveal a significant improvement of serum total cholesterol, triglyceride, HDL, and LDL after alpha lipoic acid (35). In our study the improvement of LDL-C& HDL-C might be related to the improvement of BMI and glycemic control. Different study design, sample size, dosages of ALA, and the participants characteristics might clarify the inconsistencies among studies. The probable lipid-lowering effects of alpha lipoic acid; firstly, the beneficial effects of ALA on β oxidation of fatty acids in the mitochondria via activation of AMP-activated protein kinase (36). Secondly, it might be related to the role of ALA in lowering blood glucose levels (37). Thirdly, ALA administration might decrease the expression of acetyl-CoA carboxylase and fatty acid synthase

(enzymes in fatty acid synthesis) (38). Other possible mechanisms of lowering TC or LDL after administration of ALA include: (i) augmented activity of lipoprotein lipase, (ii) increased synthesis of LDL receptors in the liver which transfer cholesterol to the hepatic system (39), (iii) increase plasma adiponectin levels which enhanced FFAs β-oxidation (40). We did not report any significant reduction in the cross-section area of the posterior tibial nerve after the intervention. Singh et al. found that, morphological changes of the tibial nerves in diabetic subjects can be detected by ultrasonography, even before the clinical onset of peripheral neuropathy(41).Watanabe and colleagues, indicated the possibility of using US for the diagnosis of DPN with sensitivity of 80% and specificity of 94% (42). A study performed by Riazi et al. detected lower sensitivity and specificity (69 and 77%, respectively) (43). To our knowledge, there is little studies comparing the effect of ALA on the cross-sectional area of the nerve. It may need longer duration and larger numbers of participants to assess the cross-section area in a follow up prospective study after treatment intervention with alpha lipoic acid.

Conclusion: Oral supplements of ALA improved peripheral neuropathy, glycemic control, and LDL-C & HDL-C levels in diabetic patients. However, there were conflicting studies about the suitable dose, duration and route of administration of ALA. Our result failed to proof effect of ALA on the nerve cross-section area.

Limitations of the study: Firstly, it was unclear whether the improvements of neuropathy and lipid parameters is related to the drug ALA or it is a sequalae of improvement of glycemic control. Secondly, serum levels of vitamin B12 should be measured to exclude the true cases of vitamin B 12 deficiency. Thirdly, the degree of improvements of peripheral neuropathy symptoms need to be evaluated according to a severity score such as Neuropathy Symptoms

Score (NSS). Fourthly, additional research, placebo-controlled trials with a multivariate regression analysis are needed, with longer duration, different doses of ALA to judge the effective dose on body weight, glucose control, and different lipid fractions as well as safety on long term before using this agent as an ancillary treatment of DM2.

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Table

Table (1): Baseline clinical data of the studied type 2 diabetic patients.

Variable		N=90	
Age (years)	Median (IQR)	52 (50-60.3)	
Sex (No., %)	Male	38	42.2%
	Female	52	57.8%
Body mass index(kg/m^2)	Median (IQR)	28 (26-32)	
Waist circumference (cm)	Median (IQR)	85 (76-105)	
Hypertension	No	36	40.0%
	Yes	54	60.0%
Age of onset of DM (years)	Median (IQR)	47 (45-49)	
Duration of DM (years)	Median (IQR)	6 (4-7)	
Treatment	Insulin and oral	22	24.4%
	Oral	56	62.2%
	Insulin	12	13.3%
Serum creatinine (mg/dl)	Median (IQR)	0.8 (0.8-0.9)	
Blood Urea (mg/dl)	Median (IQR)	29 (28-31)	

Table (2): Comparison between neuropathy parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus.

Variable	Baseline (N=90)	No improvement at end treatment	Improvement at end of treatment	P (significance)
	No (%)	No (%)	No (%)	
Symptoms of neuropathy	90/90 (100)	22/90 (24.4)	68/90 (75.6)	<0.001 (HS)
Impaired Monofilament test	74/90 (82.8)	54/74 (72.9)	20/74 (27.1)	<0.012 (S)
Impaired Nerve conduction velocity	58/90 (64.4)	6/58 (10.3)	52/58 (89.7)	<0.001 (HS)
Impaired cardiovascular autonomic neuropathy tests	62/90 (68.9)	14/ 62 (22.6)	48/62(77.4)	<0.001 (HS)
Posterior tibial nerve cross sectional area (cm2)	Median (range) 0.16 (0.14-0.17)	Median(range) 0.17 (0.13-0.20)	Wilcoxon test 0.21	0.84 (NS)

Table (3): Comparison between different metabolic parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus.

Variable	Baseline (N=90)	End treatment (N=90)	Wilcoxon test	P (Significance)
	Median (IQR)	Median (IQR)		
BMI (kg/m^2)	31 (27-33.3)	28 (26-32)	3.79	<0.001 (HS)
HbA1C*%	7. 2± 0. 54	6. 8± 0. 45	4.13	<0.001 (HS)
HDL-C mg/dl	46 (38-55)	62 (38-65)	6.01	<0.001 (HS)
LDL-C mg/dl	120 (93-143.2)	110 (89.5-122.5)	8.24	<0.001 (HS)
TSH (mU/L)	2.1 (1.9-2.23)	2.4 (1.9-2.92)	1.01	0.91 (NS)
ALT (IU/L)	19 (18-20)	18.8 (18-20)	1.56	0.12 (NS)
AST (IU/L)	29 (28-30)	30 (28-30)	0.25	0.81 (NS)

BMI= Body mass index, HbA1C= glycated hemoglobin, HDL-C=high density lipoprotein cholesterol, LDL-C= low density lipoprotein cholesterol, TSH= thyroid stimulating hormone, ALT= Alanine transaminase, AST= Aspartate aminotransferase.
*Data were expressed as (mean ± SD) and analyzed by Paired "t" test.

IQR= Interquartile range.

Figure

Figure (1): Bar chart showing the mean values of HbA1C pre and post alpha acid lipoic treatment in patients with T2 diabetes mellitus.

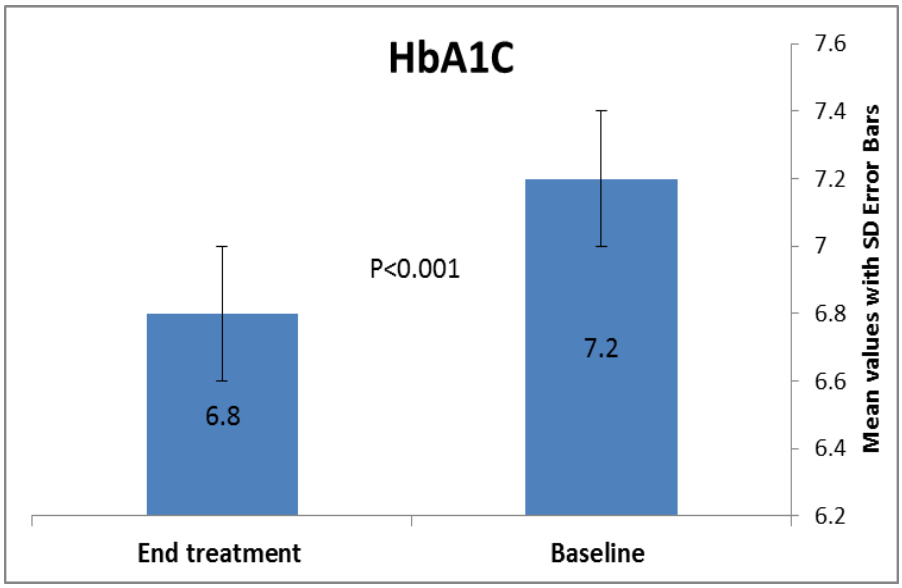
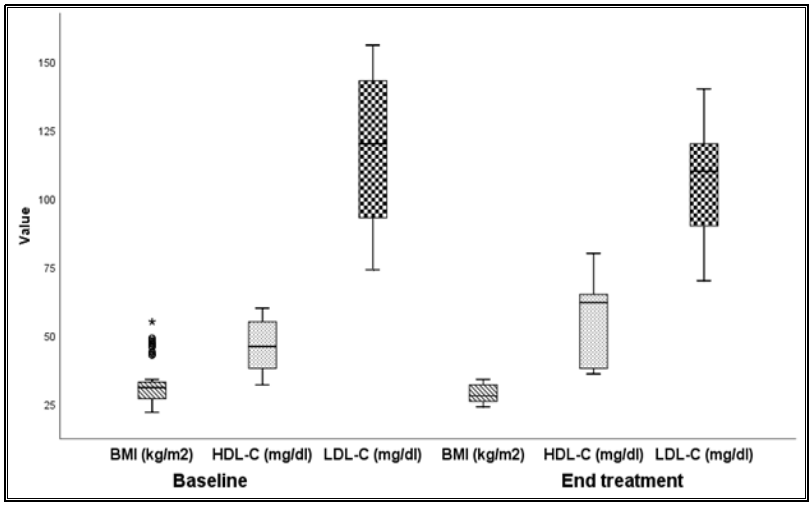


Figure (2): Boxplot presentation for the median(& IQR) of BMI, HDL-C and LDL-C pre and post treatment.



Comment : this sentence was removed from anonymous manuscript temporary: (It was registered in clinical trial website; ClinicalTrials.gov Identifier (NCT number): NCT04322240).

But it is present in the whole manuscript with author.

Reviewer response

Dear reviewer ,

Greetings,

We are appreciating your comments and correction for our manuscript. all notes are responded and corrected according to your recommendations.

- Major flaws:-we modify table (2) according to your advice as follows:

Old table: **Table (2): Comparison between neuropathy parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus.**

Variable	Baseline (N=90)	End treatment (N=90)	Improvement	P (significance)
	No (%)	No (%)	No (%)	
Symptoms of neuropathy	90/90 (100)	22/90 (24.4)	68/90 (75.6)	<0.001 (HS)
Impaired Monofilament test	74/90 (82.8)	54/90 (60.0)	20/74 (27.0)	<0.001 (HS)
Impaired Nerve conduction velocity	58/90 (64.4)	6/90 (6.7)	52/58 (89.7)	<0.001 (HS)
Impaired cardiovascular autonomic neuropathy tests	62/90 (68.9)	14/90 (15.6)	48/62(77.4)	<0.001 (HS)
Posterior tibial nerve cross sectional area (cm2)	Median (range) 0.16 (0.14-0.17)	Median(range) 0.17 (0.13-0.20)	Wilcoxon test 0.21	0.84 (NS)

New modified table:

Table (2): Comparison between neuropathy parameters before and after alpha lipoic acid treatment intervention in patients with T2 diabetes mellitus.

Variable	Baseline (N=90)	No improvement at end treatment	Improvement at end of treatment	P (significance)
	No (%)	No (%)	No (%)	
Symptoms of neuropathy	90/90 (100)	22/90 (24.4)	68/90 (75.6)	<0.001 (HS)
Impaired Monofilament test	74/90 (82.8)	54/74 (72.9)	20/74 (27.1)	<0.012 (S)
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Impaired cardiovascular autonomic neuropathy tests	62/90 (68.9)	14/ 62 (22.6)	48/62(77.4)	<0.001 (HS)
Posterior tibial nerve cross sectional area (cm2)	Median (range) 0.16 (0.14-0.17)	Median(range) 0.17 (0.13-0.20)	Wilcoxon test 0.21	0.84 (NS)

According to this still the improvement is still significant.

Minor flaws: we corrected them according to your recommendations. It was heighlighted in green colour ,while the previous coorections were in red colour.But , simple abbreviations in the abstract failed to be explained in the intial menthioning becauses it make the abstract too long to be submitted(315 words and the required is 300 words only) after this modification.so, we only explain them in the main manuscript,

Thank you in advance

Corresponding author

Dr.Amira M.Elsayed

COVER LETTER FOR SUBMISSION OF MANUSCRIPT

Dear Editor,

We would like to submit our manuscript entitled " **Alpha lipoic acid and diabetes mellitus: potential effects on peripheral neuropathy and different metabolic parameters.**" for publication in Alexandria Journal of Medicine**.**

Authors of this manuscript are:

1. Ayman M Elbadawy MD.

2. Rasha O. Abd Elmoniem MD.

3.Amira M. Elsayed MD.

With the submission of this manuscript, we confirm that

- All authors of this research paper have directly participated in the planning, execution, or analysis of this study;
- All authors of this paper have read and approved the final version submitted.
- The contents of this manuscript have not been copyrighted or published previously;
- The contents of this manuscript are not now under consideration for publication elsewhere;
- The contents of this manuscript will not be copyrighted, submitted, or published elsewhere, while acceptance by the Journal is under consideration;

We are thankful in advance for your time and attention to our original manuscript.

Submitted manuscript is a (original article).

Yours sincerely,

Dr.Amira M Elsayed

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Title page

(Alpha lipoic acid and diabetes mellitus: potential effects on peripheral neuropathy and different metabolic parameters)
(original article)

Running title: Effects of alpha lipoic acid on peripheral neuropathy and metabolic parameters.

Ethical approval: The study was approved by the Ethics Committee of Benha Faculty of Medicine, Benha University with written informed consent was obtained from all participants.

Clinical trial registration: It was registered in clinical trial website; ClinicalTrials.gov Identifier (NCT number): NCT04322240.

Key words: Alpha lipoic acid; diabetic peripheral neuropathy; diabetes mellitus.

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