ORIGINAL ARTICLE

The Effects of Alpha Lipoic Acid on Oxidative Stress, Lipid Profile, and Blood Glucose Levels in Type 2 Diabetic Male Sprague Dawley Rats

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ABSTRACT

Objective: To determine the effect of Alpha Lipoic Acid (ALA) on blood glucose, lipid profile, and oxidative stress in streptozotocin-induced type II diabetic male Sprague Dawley rats.

Study Design: Laboratory-based experimental study.

Place and Duration of the Study: The study was carried out at the Physiology Research Lab, Army Medical College, Rawalpindi, Pakistan, from 20th January 2021 to 24th June 2021.

Methods: Thirty Sprague Dawley (SD) rats were divided into three equal groups. Group I was given a normal standard rat diet. In Groups II and III after feeding a diet rich in fat and calories for 2 weeks, after feeding a diet rich in fat and calories for 2 weeks, a single low dose of streptozotocin was injected for Type 2 Diabetes Mellitus (T2DM) induction. Alpha Lipoic Acid 30 mg/kg body weight/day was administered intraperitoneally in Group III for 01 weeks after the development of T2DM.

Results: The blood glucose, lipid profile, and serum Malondialdehyde levels were deranged in the diabetic group. After Alpha Lipoic Acid supplementation, the blood glucose levels, Serum MDA, and levels of triglycerides, cholesterol, and Low Density Lipoproteins decreased, whereas High Density Lipoproteins level were significantly raised (p <0.001) in group III as compared to the diabetic group II.

Conclusion: Treatment with Alpha Lipoic Acid reduces oxidative stress and improve reduces oxidative stress and improves glycemic and lipid profiles in T2DM rats.

Keywords: *Alpha Lipoic Acid, Blood Glucose, T2DM, Lipid Profile, Oxidative Stress*.

Introduction

Alpha-Lipoic acid (ALA) is a powerful antioxidant with both hydrophilic and hydrophobic properties: it acts as a strong antioxidant in the cytosol and the plasma membrane.[1] It reduces oxidative stress and increases intracellular glutathione peroxidase, superoxide dismutase, vitamin C, and free radical production.[2] It also increases Glucose transporter 4 (insulin-mediated) translocation and redistribution via AMP-mediated protein kinase, which lowers blood glucose levels and reduces lipid oxidation and catabolism.[3,4]

Type 2 diabetes mellitus (T2DM) caused by decreased insulin sensitivity and increased insulin resistance results in significantly decreased glucose uptake and metabolism, decreased glycogen synthesis due to decreased pyruvate dehydrogenase (PDH) activity[5] causing increased dependence on alternate sources of energy via accelerated lipid and protein catabolism. Consequently, there is an increased plasma concentration of triglycerides and free fatty acids (FFA). Decreased glucose uptake

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leads to hyperglycemia, and associated high levels of FFAs lead to the formation of reactive oxygen species6 such as hydroxyl radical, hydrogen peroxide, and superoxide anion, which causes most of the diabetic complications.

This experimental project was designed to analyze the effects of ALA on oxidative stress, lipid and glucose levels in rat models of streptozotocin-induced T2DM and its further use as antioxidant drug therapy for diabetic diseases.

Methods

This lab -based experimental control trial was carried out at Physiology Research Lab, Army Medical College, Rawalpindi, Pakistan from 20th January 2021 to 24th June 2021 after the approval of institutional review board and ethics committee held on 13th January 2021 vide letter no: 514/Trg. Thirty healthy Sprague-Dawley rats (6-8 weeks old, 240 ± 50 gm weight) were procured from NIH, Islamabad. Pakistan and divided equally into control, diabetic (diseased control) and ALA groups. Plasma glucose levels (PGLs) were assessed to eliminate the possibility of pre-existing diabetes. The rats were kept in temperature-controlled, well-ventilated rooms in 4x4 steel cages following a 12-hour artificial light cycle. Rats were fed on a standard rat diet as per protocols followed by NIH (Table 1). Type 2 diabetes mellitus (T2DM) was induced in both the diabetic

(diseased control) and alpha-Lipoic acid (ALA) groups, following the established rat model protocol outlined by Srinivasan and colleagues. This involved a two-week high-calorie, rich-fat diet, followed by the intraperitoneal injection of a low dose (30-40 mg/kg) of Streptozocin (STZ) in the lower-right quadrant of the abdomen after an additional two weeks (14th day).[6] On the 21st day, the development of T2DM was confirmed in diabetic and ALA groups (Plasma glucose levels >16.65 mmol/l) by measuring plasma glucose after a 12-hour fast and obtaining blood from the rat's tail vein.[7]

Alpha Lipoic acid (ALA) (AstaMedica, Germany) was administered intra-peritoneally (30 mg/kg/day) to the ALA group for one week. ALA was administered intraperitoneally because only 20-40% appears in the plasma due to its high first-pass metabolism.[8] After completion of the study at four weeks all the animals were euthanized under anesthesia after the overnight fast as per the protocol followed at NIH, Islamabad. Terminal blood (4ml) was obtained to evaluate biochemical parameters by cardiac puncture method.

Biochemical assay/parameters

Serum malondialdehyde (MDA) was estimated by MDA ELISA (rats) kit supplied by Cusabio Technology China (Cat No MD90181). Serum glucose was calculated using glucose oxidase method. TG and

Table: 1: Composition of normal and calorie rich rat feeds

Standard Normal Rat Feed		Calorie-rich High Fat diet	
Composition	Weight (gm/kg)	Composition	Weight (gm/kg)
Flour (wheat)	255	Sunflower oil	185
Dried milk	245	Casein	315
Salt	05	Lard	265
Sunflower oil	10	Cholesterol	130
Chicken meat	90	Vitamin /mineral	90
Vitamins	10	Corn starch	90
Brawn	200	Table salt	15

HDL cholesterol was estimated by enzymatic colorimetric method and a kit supplied by Cusabio Technology China (Cat No. 40334). Total cholesterol was measured by cholesterol esterase method by using the kit of Pioneers Diagnostic, UK (Cat No. 72802).

Statistical analysis

SPSS version 23 software was used by analysis of recorded data. One-way ANOVA (Analysis of Variance) was used for comparisons between the

groups on multiple parameters. Post hoc Tukey's test was applied to confirm the differences among the groups. p value< 0.005 significant.

Results

T2DM induction in diabetic and ALA groups is presented as increased body weight and high Plasma glucose levels (PGLs) in Table: 2 as compared to healthy normal controls. Plasma glucose level >16.65 mmol/l in Sprague Dawley rats confirms the T2DM as per criteria set by Yassin and Mwafy.[9] Increase body

weight is ALA and diabetic group is due to calorie rich high fat diet consumption for three weeks.

Body weight, PGLs and Lipid profile are presented in Table 3 recorded at the end of the study.

PGLs and LP were deranged and body weight increased significantly in the diabetic group compared to controls and ALA treated group. ALA supplementation effects were evident from

reduced blood glucose levels, normalized the lipid profile (low levels of TG's, cholesterol and LDL and

Table 2: Biochemical parameters of all groups on 1ˢᵗ and 21ˢᵗ day

Parameters	Days	Control mean ± SD	Diabetic Mean ± SD	ALA Mean ± SD	*P*-value
Body weight	Day 1	261.70±16.74	261.90 ± 9.26	261.60 ± 9.26	0.023
(gm)	Day 21	293.70±17.93	361.90±25.85	362.80±25.85	<0.001
Blood sugar	Day 1	4.69±0.9	4.79±0.9	4.68±0.9	0.418
(mg/dl)	Day 21	4.79±0.9	18.76±1.31	17.91±1.25	<0.001
Triglyceride	Day 1	0.4 ± 0.6	0.4 ± 0.7	0.4 ± 0.6	0.034
(mmol/L)	Day 21	0.4 ± 0.0	1.9 ± 0.2	1.9 ± 0.2	<0.001
Cholesterol	Day 1	1.24 ± 0.1	1.23 ± 0.1	1.22 ± 0.1	0.044
(mmol/L)	Day 21	1.24 ± 0.6	4.4 ± 0.3	4.3 ± 0.3	<0.001
HDL	Day 1	0.85 ± 0.1	0.86 ± 0.1	0.84 ± 0.1	0.014
(mmol/L)	Day 21	0.86 ± 0.1	0.43 ± 0.1	0.44 ± 0.1	<0.001
LDL	Day 1	0.3 ± 0.1	0.3 ± 0.5	0.3 ± 0.2	0.024
(mmol/L)	Day 21	0.3 ± 0.2	3.1 ± 0.3	3.2 ± 0.6	<0.001

All values have been expressed as mean ± SD (standard deviation)
p-value <0.05 is considered significant

high levels of HDL) in ALA group compared to the diabetic group ($p<0.001$).

Serum malondialdehyde (MDA) levels recorded after the completion of study to assess the oxidative stress in all groups. Diabetic group depicts high Levels

(7.67±.71) indicating high oxidative stress compared to ALA (4.25±.82) and normal control group (3.61±.66) groups. There was no significant difference among ALA and control group which was further confirmed by applying the Post Hoc Tukey's

Table 3: Biochemical parameters of all groups at day 28

Parameter	Control (n=10) Mean ± SD	Diabetic (n=10) Mean ± SD	ALA (n=10) Mean ± SD
Weight (gm)	269.70±12.55	318.96±17.41	309.90±9.72
Glucose (mmol/L)	4.8 ± 0.8	22.7 ± 1.5	6.2 ± 1.4
Triglyceride(mmol/L)	0.4 ± 0.0	1.9 ± 0.2	0.5 ± 0.1
Cholesterol (mmol/L)	1.2 ± 0.1	4.4 ± 0.3	1.3 ± 0.2
HDL (mmol/L)	0.8 ± 0.1	0.4 ± 0.1	0.9 ± 0.1
LDL (mmol/L)	0.3 ± 0.1	4.1 ± 0.3	0.2 ± 0.2

All values have been expressed as mean ± SD (standard deviation)
p-value <0.05 is considered significant

Test. Control vs diabetic ($p<0.001$) control vs ALA ($p=0.02$) diabetic vs ALA ($p<0.001$)

Discussion

Most commonly used method in scientific research to study diabetes mellitus, test possible treatments, and comprehend the underlying causes of the

disease is the creation of a diabetic rat model. Sprague Dawley rats were used because of their easily operated, convenient low price and similarity with human metabolic attributes.[10] Diet with high quantity of fats increased body lipids which causes resistance to actions of insulin burdening β cells of

rat's pancreas. STZ was injected to induce T2DM by causing β cell death in the pancreas islet of Langerhans and grossly reduced β cells mass which led to the development of frank hyperglycemia.[11] The blood glucose level >11.11mmol/L was used as the "cut off value" for confirming the presence of diabetes mellitus; the criteria laid down by Maged and Saleh for Sprague Dawley rats.[12] This is the same T2DM rat model as used by Srinivasan and his colleagues (high calorie fat rich diet and single injection of low dose streptozotocin (30-40mg/kg body weight).[13] This protocol conveniently developed diabetes mellitus in 3 weeks reducing the overall cost and duration of the study. Reed *et al* initially developed the fat fed-streptozotocin rat's model of type II diabetes mellitus in which he used high dose of streptozotocin (50 mg/kg, intravenous) for inducing diabetes. This caused extreme insulin deficiency and overt hyperglycemia in rats and their characteristics were similar to type T1DM-1 than to T2DM.[14]

Hyperglycemia in diabetic and ALA groups as shown by high blood glucose levels was mainly because of Insulin resistance, decreased glucose utilization in the peripheral tissues, increase breakdown of stored glycogen and increase gluconeogenesis. Decreased glucose uptake and its utilization in peripheral tissues caused increased dependence on alternate sources of energy. With increase lipolysis there is more production of VLDL which causes increased LDL levels and decreased HDL levels.[15] This ultimately deranged blood lipid levels in diabetic and ALA groups.

ALA was injected 200mg/kg intraperitoneally in this study for effective plasma levels and optimal bioavailability, this concentration of ALA was safe and produced desired metabolic effects in Sprague Dawley rats effectively without any side effects.[16] ALA is a powerful antioxidant by oxidizing the reactive oxygen species and nitrogen species and it also help regenerate oxidized antioxidants like vitamin E vitamin C glutathione and ascorbic acid.[17] Serum MDA levels indicate oxidative stress. Serum MDA levels in control group of Sprague Dawley rats was 3.6 µmol / L which was consistent with the published data of different studies. Ruifen Zhang *et al* measured the serum MDA levels in healthy Sprague Dawley rats as 3.9 ± 0.44 µmol / L.[18] Markedly decrease serum MDA levels in ALA group indicate reduced oxidative stress (Figure 1) as compare to diabetic group proving its antioxidant effects as studied by Pınar N et al and Kim MY et al.

ALA also decreases plasma glucose levels in ALA treated group as compare to (diseased) diabetic group (*p*<0.001).[8,19] ALA increases glucose uptake peripheral tissues via increase glucose transporters (GLUT4) redistribution in the cell membrane, increase phosphorylation of insulin receptor it also increases glycogen synthesis and increase oxidation of glucose normalizing glucose levels in ALA treated group. ALA also decrease gluconeogenesis by inhibiting pyruvate carboxylase further decreasing the PGLs.[20] Garkuwa UA et al studied the anti-glycemic effects of ALA in Wister rats and it showed that ALA administration lowers glucose levels in Wister rats as well.[21]

Lipid profile was also normalized (TGs, cholesterol and LDL levels decreased and increase levels of HDL in the ALA group as compare to diabetic group. ALA supplementation effects on glucose metabolism stimulate fatty acid oxidation and lipolysis in mitochondria which decreases the blood triglycerides and cholesterol levels.[22] Enhanced glucose uptake and inhibition of pyruvate carboxylase and stimulation of pyruvate dehydrogenase reduces the levels of acetyl-CoA which ultimately enhances the glycolysis and further improves the lipid profile.[23] Seo EY and his colleagues studies the effects of alpha Lipoic acid on lipid profile of obese Sprague Dawley rats having HFD for two weeks and it shown that oral administration of ALA for 04 weeks significantly corrects the lipid profile. on contrary to his study we used ALA for two weeks only and showed the same results as we give ALA I/V as compare to Seo *et al* which shows its better bioavailability.[24]

Our study explored the metabolic derangements of diabetes mellitus and beneficial effects of ALA on oxidative stress, blood glucose levels and lipid profile status in diabetic rats. The ALA mechanism of action on glucose transporters and insulin receptors at molecular levels could not be assessed in this study due to financial constraints and time limitation. Our study was animal based and uses of ALA as an ad-

junct therapy in treating T2DM and its associated complications and its beneficial effects in humans can further be explored in our clinical setups

Conclusion

Alpha Lipoic acid supplementation has antioxidant, improvement of plasma glucose levels and serum lipid profile measures in T2DM rats. ALA supplementation in humans as trial in Pakistan is recommended for therapeutic use to verify its efficacy as adjunct therapy for antidiabetic and anti-dyslipidemic agent.

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REFERENCES

1. Ramos CSCB, Silva VAPd, Corrêa LBNS, Abboud RdS, Boaventura GT, Chagas MA. Long-Term Alpha-Lipoic Acid (ALA) Antioxidant Therapy Reduces Damage in the Cardiovascular System of Streptozotocin-Induced Diabetic Rats. Journal of Dietary Supplements. 2023; 20: 444-58. doi: 10.1080/19390211.2021.2004290

2. Maciejczyk M, Żebrowska E, Nesterowicz M, Żendzian-Piotrowska M, Zalewska A. α-Lipoic acid strengthens the antioxidant barrier and reduces oxidative, nitrosative, and glycative damage, as well as inhibits inflammation and apoptosis in the hypothalamus but not in the cerebral cortex of insulin-resistant rats. Oxidative Medicine and Cellular Longevity. 2022; 2022: 7450514. doi: 10.1155/2022/7450514

3. Diane A, Mahmoud N, Bensmail I, Khattab N, Abunada HA, Dehbi M. Alpha lipoic acid attenuates ER stress and improves glucose uptake through DNAJB3 cochaperone. Scientific Reports. 2020; 10: 20482. doi: 10.1038/s41598-020-77621-x

4. Yang J, Shi L, Xu D, Liu J, Zhang L, Liu X, et al. Aminolaevulinic acid photodynamic therapy suppresses lipid secretion of primary sebocytes through AMPK/SREBP-1 pathway. Photodiagnosis Photodyn Ther. 2021; 36: 102537. doi: 10.1016/j.pdpdt.2021.102537

5. Gopal K, Al Batran R, Altamimi TR, Greenwell AA, Saed CT, Dakhili SAT, et al. FoxO1 inhibition alleviates type 2 diabetes-related diastolic dysfunction by increasing myocardial pyruvate dehydrogenase activity. Cell Reports. 2021; 35: 108935. doi: 10.1016/j.celrep.2021.108935

6. Adki KM, Kulkarni YA. Neuroprotective effect of paeonol in streptozotocin-induced diabetes in rats. Life Science. 2021; 271: 119202. doi: 10.1016/j.lfs.2021.119202

7. Altındağ F, Rağbetli MÇ, Özdek U, Koyun N, Alhalboosi JKI, Elasan S. Combined treatment of sinapic acid and ellagic acid attenuates hyperglycemia in streptozotocin-induced diabetic rats. Food and Chemical Toxicology. 2021; 156: 112443. doi: 10.1016/j.fct.2021.112443

8. Pınar N, Çakırca G, Hakverdi S, Kaplan M. Protective effect of alpha lipoic acid on cisplatin induced hepatotoxicity in rats. Biotech Histochem. 2020; 95: 219-24. doi: 10.1080/10520295.2019.1667025

9. Yassin MM, Mwafy SN. Protective potential of glimepiride and Nerium oleander extract on lipid profile, body growth rate, and renal function in streptozotocin-induced diabetic rats. Turkish Journal of Biology. 2007; 31: 95-102.

10. Sihota P, Yadav RN, Poleboina S, Mehandia V, Bhadada SK, Tikoo K, et al. Development of HFD-fed/low-dose STZ-Treated female Sprague-Dawley rat model to investigate diabetic bone fragility at different organization levels. JBMR plus. 2020; 4: e10379. doi: 10.1002/jbm4.10379

11. Li X, Jayachandran M, Xu B. Antidiabetic effect of konjac glucomannan via insulin signaling pathway regulation in high-fat diet and streptozotocin-induced diabetic rats. Food Research International. 2021; 149: 110664. doi: 10.1016/j.foodres.2021.110664

12. Yassin MM, Mwafy SN. Protective potential of glimepiride and Nerium oleander extract on lipid profile, body growth rate, and renal function in streptozotocin-induced diabetic rats. Turkish Journal of Biology. 2007; 31: 95-102.

13. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: an overview. Indian Journal of Medical Research. 2007; 125: 451-72.

14. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, et al. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism. 2000; 49: 1390–4. doi: 10.1053/meta.2000.17721

15. Francis D, Ani C, Nworgu C, Pamela O, Uzoma I, Uzoigwe J, et al. The effect of ethanolic seed extract of Citrullus lanatus (watermelon) on blood glucose level and lipid profile of diabetic Wistar rats. European Journal of Medicinal Plants. 2019; 26: 1-6. doi: 10.9734/ejmp/2018/v26i4735

16. Ghelani H, Razmovski-Naumovski V, Nammi S. Chronic treatment of (R)-α-lipoic acid reduces blood glucose and lipid levels in high-fat diet and low-dose streptozotocin-induced metabolic syndrome and type 2 diabetes in Sprague-Dawley rats. Pharmacology research &

perspectives. 2017; 5: e00306. doi: 10.1002/prp2.306

17. Theodosis-Nobelos P, Papagiouvannis G, Tziona P, Rekka EA. Lipoic acid. Kinetics and pluripotent biological properties and derivatives. Molecular Biology Reports. 2021; 48: 6539-50. doi: 10.1007/s11033-021-06643-z

18. Zhang R, Ma J, Xia M, Zhu H, Ling W. Mild hyperhomocysteinemia induced by feeding rats diets rich in methionine or deficient in folate promotes early atherosclerotic inflammatory processes. The Journal of nutrition. 2004; 134: 825-30. doi: 10.1093/jn/134.4.825

19. Kim MY, Kim EJ, Kim YN, Choi C, Lee BH. Effects of α-lipoic acid and L-carnosine supplementation on antioxidant activities and lipid profiles in rats. Nutrition research and practice. 2011; 5: 421-8. doi: 10.4162/nrp.2011.5.5.421.

20. Ko CY, Xu JH, Lo YM, Tu RS, Wu JS, Huang WC, et al. Alleviative effect of alpha-lipoic acid on cognitive impairment in high-fat diet and streptozotocin-induced type 2 diabetic rats. Frontiers in Aging Neuroscience. 2021; 13: 774477. doi: 10.3389/fnagi.2021.774477.

21. Garkuwa UA, Saleh MI, Alhassan A, Kawu MU. Alpha lipoic acid improved blood glucose level and lipid profile in type-2 diabetic Wistar rats. Gadau Journal of Pure and Allied Sciences. 2023; 2: 106-12. doi: 10.54117/gjpas.v2i2.107

22. Najafi N, Mehri S, Ghasemzadeh Rahbardar M, Hosseinzadeh H. Effects of alpha lipoic acid on metabolic syndrome: A comprehensive review. Phytotherapy Research. 2022; 36: 2300-23. doi: 10.1002/ptr.7406

23. Yue H, Qiu B, Jia M, Liu W, Guo XF, Li N, et al. Effects of α-linolenic acid intake on blood lipid profiles : A systematic review and meta-analysis of randomized controlled trials. Critical Reviews in Food Science and Nutrition. 2021; 61: 2894-910. doi: 10.1080/10408398.2020.1790496

24. Seo EY, Ha AW, Kim WK. α-Lipoic acid reduced weight gain and improved the lipid profile in rats fed with high fat diet. Nutrition Research and Practice. 2012; 6: 195-200. doi: 10.4162/nrp.2012.6.3.195

Authors Contribution

BUK: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proof reading

SA: Idea conception, study designing

FI: Study designing, manuscript writing and proof reading

IY: Data collection, data analysis, results and interpretation, manuscript writing and proof reading

IN: Data collection, data analysis, results and interpretation, manuscript writing and proof reading

MI: Data collection, manuscript writing and proof reading

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