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# Brain Oxidative Stress in Diabetic Rats: Role of Lycopene and Linolenic Acid

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***Abstract:*** **Diabetes mellitus is characterized by chronic hyperglycemia which is highly correlates to the initiation and progression of diabetic complications including generation of oxidative stress, neuropathy, nephropathy and cardiovascular diseases. Lycopene, the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and tomato products. The polyunsaturated fatty acids (PUFAs) may have a major role in the prevention of coronary heart disease such as n-3 that have been suggested from epidemiologic and clinical secondary avoidance trials evidences. The aim of this study is to investigate the possible protective effect of lycopene and linolenic acid against oxidative stress in rats injected with STZ. Five groups of rats were included: Normal, Diabetic untreated, diabetic treated with either lycopene or linolenic acid or combination of both. Results obtained showed that In brain, heart and kidney tissues, the levels of malonadialdehyde, and nitric oxide showed highly significant increase in diabetic comparing with control.While, their level extremely significant decreased when treated with lycopene alone or combined with linolenic acid. In conclusion, Lycopene or linolenic acid or combinations of both act as free radical scavenging system that protect the affection of the tissue induced by STZ. The effect of lycopene or linolenic acid varied from tissues to another tissue that depend on bio-availabilities of each to target tissues.**

***Keywords:*** **Diabetic rats- oxidative stress- rats- barin.**

## 1. INTRODUCTION

Diabetes is characterized by chronic hyperglycemia, which is highly a correlate to the initiation and progression of diabetic complications including generation of oxidative stress, neuropathy, nephropathy and cardiovascular diseases (1). Additionally, glucose can undergo auto oxidation, resulting in the generation of intermediates, leading to production of reactive intermediates and may form adducts in proteins to form advanced glycation end products (AGEs) (2). Close association between oxidative stress and inflammation in the pathologies of diabetes was reported ( 3).

Cellular proteins and lipids can be affected via formation of advanced glycation end products (AGEs) such as collagen, hemoglobin, and lipoprotein and causes damage to the eyes, kidney and blood vessels .The development of end-stage renal disease and is characterized by proteinuria, progressive accumulation of glomerular extracellular matrix (ECM) and glomerulosclerosis can be contributed via Diabetic nephropathy (DN) (4).The involvement of free radicals in the pathogenesis of diabetes and more importantly in the development of late diabetic complications can be proposed from Cumulative experimental evidences.the ability to damage cellular molecules, DNA, proteins, and lipids resulting in alteration of many cellular functions is characteristic of Free radicals (5). The antioxidants, which neutralize free radicals, are effective in preventing experimentally induced diabetes in animal models as well as reducing the severity of diabetic (6). Lycopene, the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and tomato products, has received much attention in recent years because of its beneficial effect in the treatment of diseases. It was demonstrated that lycopene provided the best protection against singlet oxygen-induced cell damage.Epidemiologic studies revealed that, lycopene may increasingly be identified as sharing inverse relationships to cancer with other common carotenoids or as being the only carotenoid to show such an association (7).

The associated of increased consumption of tomato and tomato-based foods with a lower risk of some diseases (e.g., cancer and cardiovascular disease) is shown in many studies. It is normally assumed that natural substances (ascorbic acid, tocopherol, and photochemical in tomato) account for the useful effects. Although tomatoes contain an collection of phytochemicals, most attention has been focused on lycopene, thought to be the main contributor to health promotion in human. However, it is worth investigating whether lycopene is the only causative substance of tomato or whether any other substances in tomatoes show additive or synergistic effect for lycopene. The important exogenous defense against oxidative stress may be described via Dietary polyphenols (8). Secondary plant metabolites derived from the phenylpropanoid biochemical pathway are flavonoids. Their basic structure consists of two aromatic benzene rings separated by an oxygenated heterocyclic ring. Flavonoids including flavones, flavonols, flavonones, isoflavones,flavan-3-ols, and anthocyanins are found in plant tissues .Over 4000 naturally occurring flavonoids have been described; most of them conjugate to sugar molecules and are usually located in the upper epidermal layer of leaves. In tomato tissues, primarily contain endogenous flavonols as conjugates. Tomatoes and related products contain rich conjugated quercetin and kaempherol (9).

The well-established danger factors of coronary artery disease (CAD) are Plasma lipid and its metabolism. the associated of Increased risk of CAD with high triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL-c) and with decreased high-density lipoprotein cholesterol (HDL-c) (10). It was reported that phenolic compounds were associated with antioxidant activity played an important role in stabilizing lipid per oxidation. Flavonoids compounds that present in tomato tissues which is considered as peroxides radical scavenger could be related to their capacities to reduce chelae ferric ion, which induce lipid peroxidation .These dyslipidemic features contribute to the increased risk of developing vascular diseases, such as atherosclerosis, the leading cause of mortality in diabetes type 2. The increased small dense LDL is more liable to oxidation, and readily adheres to and next invade arterial wall leading to atherosclerosis. Furthermore, the small LDL particles present in diabetes are a risk to glycation, which aggravates the oxidative stress associated with diabetes. The development of diabetic complications may caused by reduced antioxidant capacity. To be sure, patients with weakly controlled type 2 diabetes suffering from complications have higher serum lipoperoxidation than patients with complication-free diabetes. The major antioxidants that may protect against the development of diabetic complications via reduction of oxidative stress are Vitamin C, vitamin E, and lycopene . However, the administration of antioxidants to attenuate oxidative stress is still considered a controversial matter in diabetes management (11). The poly unsaturated fatty acids (PUFAs) may have a major role in the prevention of CHD such as n-3 that have been suggested from epidemiologic and clinical secondary avoidance trials evidences. fish oils, rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), along with plants rich in a-linolenic acid are Dietary sources of n-3 PUFAs. Randomized secondary prevention clinical trials with fish oils (eicosapentaenoic acid, docosahexaenoicacid) and a-linolenic acid have demonstrated reductions in risk that compare favorably to those seen in landmark secondary prevention trials with lipid-lowering drugs (12). Due to variation in EPA/DHA content of different source may contribute the anti-inflammatory activity of fish oil., the association were separately of PUFAs, and particularly total n-3 fatty acids with lower levels of proinflammatory markers (IL-6, IL-1ra, TNF-α, C-reactive protein) and higher levels of anti-inflammatory markers(soluble IL-6r, IL-10, TGFα) . The cardio protective effect of the n-3 PUFA can be explained by Several mechanisms. the n-3 PUFA have been suggested including antiarrhythmic and antithrombotic (13).

The vascular calcification can be directly inhibited by. N-3 PUFAs by the use of p38-MAPK and PPAR-gamma pathways, and to reduce gene expression of cyclooxygenase-2, an inflammatory gene involved in plaque angiogenesis an plaque rupture through the activation of some metalloproteinase sand reduction of oxidative stress . The quenching of gene expression of pro-inflammatory proatherogenic by omega-3 fatty acids has consequences on the degree of leukocyte adhesion to vascular endothelium, early atherogenesis and later stages of plaque development and plaque rupture, ultimately yielding a plausible comprehensive explanation for the vasculoprotective effects of these nutrients (14).

## 2. MATERIALS AND METHODS

**Animals:**

Adult male Wistar rats weighing about 185±25 g were used in study. The animals were housed in cages and were received normal rat chow and tap water *ad libitum* in a constant environment (room temperature 22 ± 2$^{o}$C, room humidity 50±5%) with a 14-h light, 10-h dark cycle. The animals were kept under observation for one week prior to the start of the experiments.

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**Induction of Diabetes Model and Study Design:**

One hundred and twenty male Wister rats were used in this experiment. Divided into 6 groups (each 20 rats) . (Group 1, normal control group), which was receive a single dose of 0.1 mol/L citrate buffer intrapertnial. (Group 2-6) rats will be intrapersonal injected with STZ (65 mg/kg body weight) (Sayed and Al-Malki AL, 2013), STZ was freshly prepared in a 0.1 mol/L citrate buffer (pH 4.5).Only rats with blood glucose higher than 250 mg/dL after 5 days will be considered as being diabetic in the fasting state, diabetic rats were randomly divided into 5 groups: healthy group (20rats) were received 0.1mol/l citrate buffer,Diabetic untreated (20 rats), and three groups (60 rats) diabetic and treated with two different types from the extractlycopene and linolenic acid and third group treated with combination of both above mentioned.. Treatments will be continued for6 weeks. Body weight and oxidative stress markers were evaluated in heart, kidneys, and brain.

## 3. RESULTS

Results in table (1) Results obtained showed that serum malondialdehyde (MDA) was highly significantly increased in diabetic rats as compared with control ($p<0.001$). Administration of lycopene or linolenic acid or combined showed a significant reduction of malonyldialdehyde (MDA) as compared with untreated group ($p<0.001$ ) for each. Lycopene effect showed potent reduction on MDA than linolenic acid or combined in kideny and heart. While,linolenic acid is more potent effect in protection of brian tissues against oxidative damage of free radicals .Showed that serum nitric oxide (NO) was highly significantly increased in diabetic rats as compared with control ( $p<0.001$). Administration of lycopene or linolenic acid or combined showed a significant reduction of Nitric oxide (NO) as compared with untreated group ($p<0.001$ ) for each in brain tissues. Linolenic acid has more potent effect in reduction of nitric oxide (NO) in heart tissues with lycopene or combination ($p<0.001$). While, combination of lycopene and linol;enic acid have more potent effect in reduction of nitric oxide in kidney ($p<0.001$).

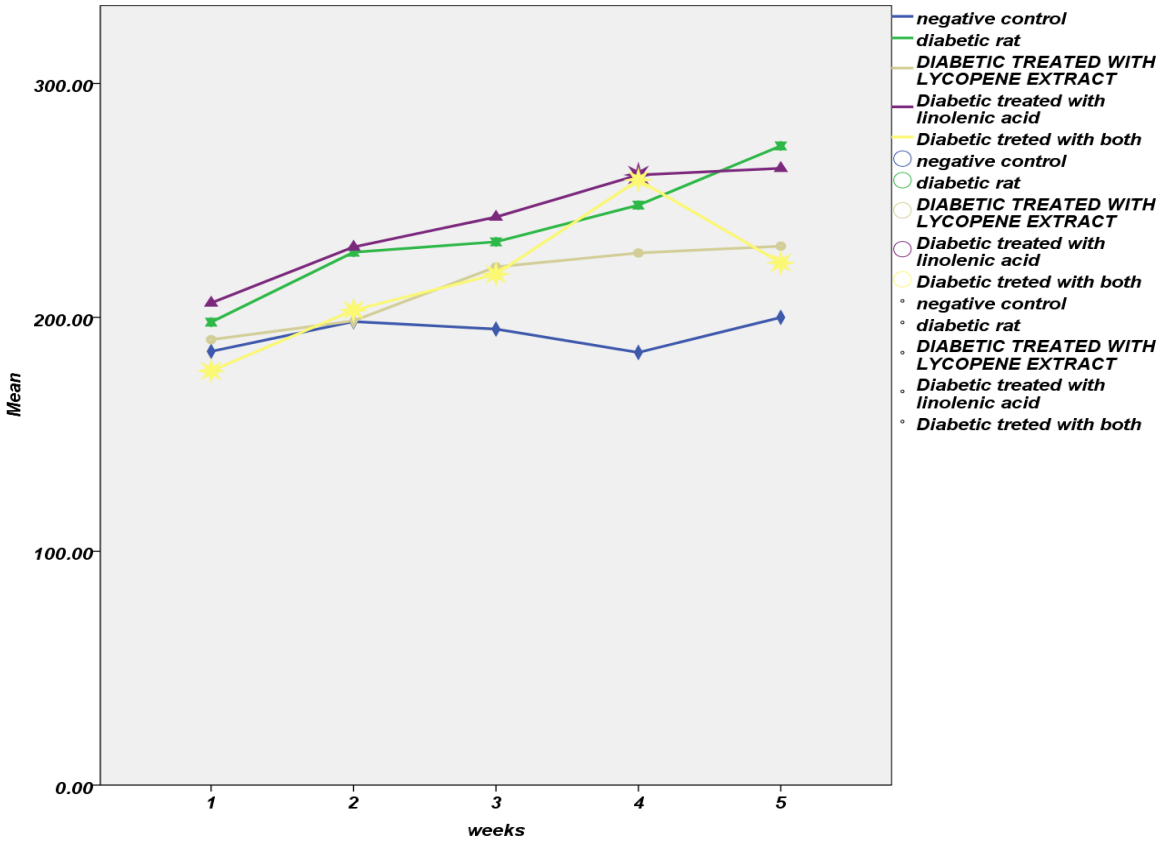


**Fig.1 Body weight during five weeks in control, diabetic, diabetic treated with lycopene, diabetic with linolenic acid, and cases treated with both respectively**

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**Table 1: The level of tissues MDA (malonyldialdehyde) in brian, heart, and kidney respectively nmol/gm**

| Groups | Control | Diabetic | Diabetic+Lyc | Diabetic+LA | Diabetic+LA+Lyco |
|---|---|---|---|---|---|
| **Brain** | | | | | |
| Mean±SD | 5.57±0.75 | 20±3.3 | 10±2.1 | 6±1.1 | 14±4.2 |
| P value | --- | <0.001 | <0.01 | <0.001 | <0.001 |
| P* | | | <0.001 | <0.001 | <0.001 |
| Tuky test | | 0.9[a] | 0.9[a] | 0.9[a] | 0.9[a] |
| **Heart** | | | | | |
| Mean±SD | 8±0.9 | 23±3.3 | 7±0.5 | 8±1.8 | 14±2 |
| P value | --- | <0.001. | <0.001 | <0.001 | <0.001 |
| P* | | -- | <0.01 | <0.01 | <0.001 |
| Tuky test | | 0.1[a] | 0.1[a] | 0.9[a] | 0.1[a] |
| **Kidney** | | | | | |
| Mean±SD | 15±4.3 | 19±4.0 | 16±2.5 | 16±1.6 | 17±2.4 |
| P value | | <0.001 | <0.001 | <0.001 | <0.001 |
| P* | | 0.9[a] | <0.01 | <0.01 | <0.01 |
| Tuky test | | 0.9[a] | 0.1[a] | 0.1[a] | 0.9[a] |

a: highly response to treatment Lyco.or L. = lycopene LA.= Linolenic acid

**P**=Control VS Diabetic, 99% Confidence Interval of the Difference, $\alpha$=0.01

**P***= Diabetic VS (D. +L., D. +LA., +D. +L. +LA) respectively

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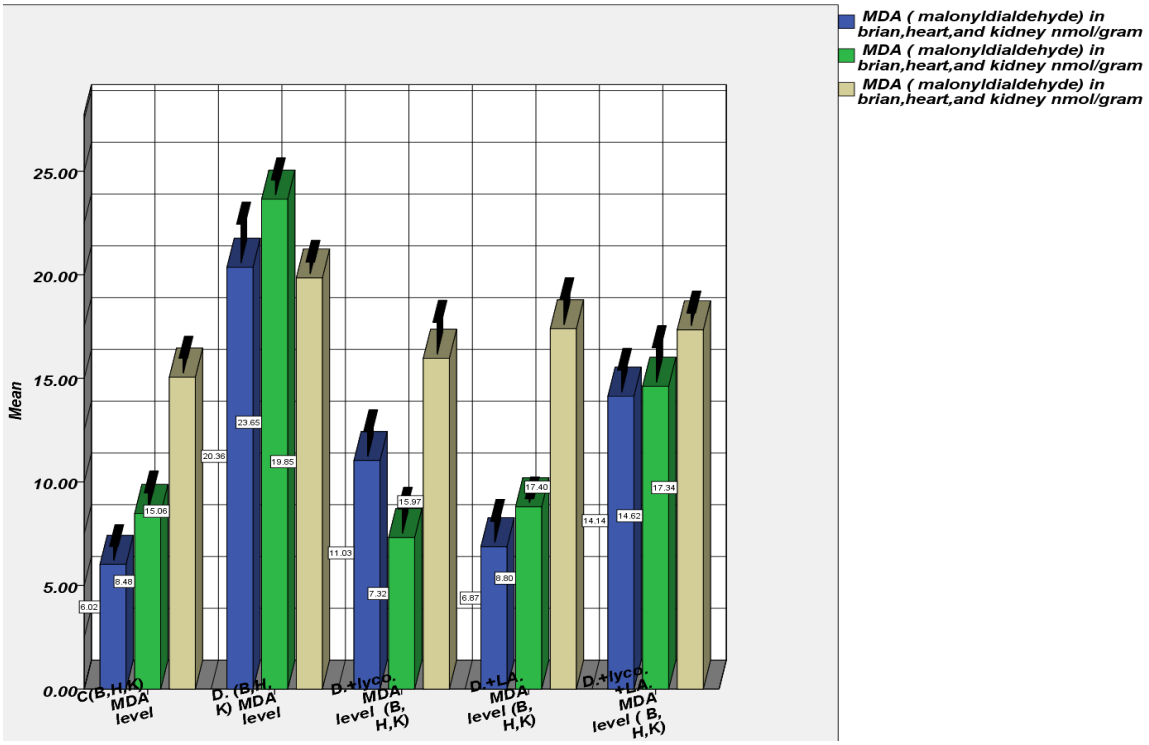


**Fig. 2**

## 4. DISCUSSION

STZ is rapidly taken up by the ,β-cells and although it is not toxic by itself, the metabolites of STZ are toxic to the , β -islet cells of pancreas (15). STZ inhibits glucose-stimulated insulin release (16) and the generation of glucose-derived energy by inhibiting glycolytic flux and pyruvate oxidation (17). Superoxide radicals generated during the redox cycling of STZ resulted in the formation of $H_2O_2$ which forms OH. Radicals toxic to the pancreatic , β -cells (18). IT was found a significant association between the use of CLO during the first year of life and a lower risk of type 1 diabetes, suggesting that CLO may reduce the risk of type 1 diabetes, perhaps through the effects of long-chain fatty acids. CLO or individual fatty acids such as DHA may be candidates for preventive intervention trials.

The transition metal Linolenic acid serves as a cellular signaling molecule, present at high concentrations in the central nervous system, particularly in the hippocampal mossy fibres, where it is stored in synaptic vesicles and released upon depolarisation from central nerve terminals (19). Linolenic acid produces a wide variety of neuromodulatory effects, the most prominent of which may be upon glutamate receptors (20).

Ninety percent of those with diabetes have type-2 diabetes, characterized by insulin resistance, hyper insulinaemia, β-cell dysfunction and subsequent β–cell failure (21). Insulin is stored as a hexamer containing two Linolenic acid ions in β-cells of the pancreas and released into the portal venous system at the time of β-cells de-granulation (22). The Linolenic acid which are co-secreted with insulin suppress inherent amyloidogenic properties of monomeric insulin (23). It was showed that high concentrations of glucose and other secretagogues decrease the islet cell labile Linolenic acid and video fluorescence analysis showed Linolenic acid concentrated in the islet cells was related to the synthesis, storage and secretion of insulin (21). In vitro data suggests that insulin binds to isolated liver membranes to a greater extent and that there is less degradation when co-administered with Linolenic acid (22). Linolenic acid is important in insulin action and carbohydrate metabolism (23). Oxidative stress plays an important role in the pathogenesis of diabetes and its' complications. Linolenic acid is a structural part of key anti-oxidant enzymes such as superoxide dismutase, and Linolenic acid deficiency impairs their synthesis, leading to increased oxidative stress (24). Studies have shown that diabetes is accompanied by hypoLinolenicacidemia (25) and hyper Linolenic aciduria .In addition Linolenic acid deficiency is more common in developing countries (26), where diabetes is also showing an exponential increase in prevalence.

Animal studies have shown that Linolenic acid supplementation improves fasting insulin level and fasting glucose in mice. Human studies have also shown the beneficial effects of Linolenic acid supplementation in both type-1and type-2 diabetes However, results of isolated randomized controlled trials are frequently contradicted by subsequent studies (27). Especially, in type-1 diabetes studies have reported a negative effect of Linolenic acid supplementation on glucose homeostasis (22). Even under the most rigorous study design conditions, a well-planned single study, rarely provides definitive results and changing clinical practices relying on a single high-profile clinical trial can be harmful to patients' health (23). Well-designed randomized controlled trials are excellent when looking at effectiveness, though many fall short in reporting of safety and adverse events associated with an intervention. Systematic reviews often have increased power and decreased bias as compared with the individual studies they include, and the careful pooling of treatment effects can provide the most accurate overall assessment of an intervention (24). Presently there are no systematic reviews exploring the therapeutic efficacy of Linolenic acid supplementation in humans with diabetes. The study aims to systematically evaluate the literature and Meta-analyze the effects of Linolenic acid supplementation in humans with diabetes and evaluate potential toxic effects advocating against regular supplementation.

In conclusion, MDA analysis used for detecting the changes in metabolism in diabetic rats and diabetic treated with different nutrient supplement as Lycoor Linolenic acid or both. This will reflect the impact of these nutrients and aid the physician to design new regime for treatment of diabetes and to prevent of its complications. The present study showed that long-term Linolenic acid treatment significantly improves hepatic insulin sensitivity in diabetic rats. Further studies in which this treatment is prolonged are necessary to confirm these preliminary results. Further metabolic pathways are recommended to be explored to investigate the other metabolic changes in diabetic research.

## 5. CONCLUSION

Via generation of free radicals that partially damage β-cell of pancreas that decrease in efficiency in insulin release. Lycopene or linolenic acid or combinations of both acts as free radical scavenging system that protect the damage of pancreas induce by STZ. The effect of lycopene or linolenic acid varied from tissues to another tissue that depends on bioavailability of each to target tissues. Further studies should be done to explore the mechanism action of these nutraceutical as antioxidant.The effect of lycopene or linolenic acid varied from tissues to another tissue that depends on bioavailability of each to target tissues.

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