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Modulation of Diabetic Complications by Lycopene and Linolenic Acid in Rats

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Abstract: **Free radicals are capable of damaging cellular molecules, DNA, proteins, and lipids leading to altered cellular functions. Many recent studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models as well as reducing the severity of diabetic complications. The aim of this study is to investigate modulation of diabetic complications by lycopene alone or combined with linolenic acid in diabetic rats. Sixty male Wister rats were used in this experiment. Divided into 6 groups (each 10 rats) . (group 1, normal), which was receive a single dose of 0.1 mol/L citrate buffer. (Group 2-6) rats were injected *i.p* with single dose of STZ (65 mg/kg body weight) ,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 were received 0.1mol/l citrate buffer, Diabetic untreated , and three groups ,diabetic and treated with two different types from the extract lycopene and linolenic acid and third group treated with combination of both above mentioned. Treatments will be continued for 6 weeks. Oxidative stress markers (Nitric oxide, malondialdhyde, glutathione peroxidase and lactate dehydrogenase) were evaluated in all groups. It was found that, lycopene alone or combined with linolenic acid acts as free radical scavenging system that protect the tissue from damage and its complications of diabetic by enhancing antioxidant enzymes activities. Further studies should be done to examine the signalling of diabetic and the action of these nutrients as antioxidant.**

Keywords: **Diabetic complications- rats- Lycopene.**

1. INTRODUCTION

Free radicals are capable of damaging cellular molecules, DNA, proteins, and lipids leading to altered cellular functions. Many recent studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models [1] as well as reducing the severity of diabetic complications [2].Oxidation reaction in biological systems may also occur via radical pathway, for example, by hydrogen peroxide. the products of its action are molecules that are enriched in one or more oxygen atoms that are usually considered to be markers of oxidative stress. According to the concept of oxidative stress, oxygen-centered radicals generated, sequentially, by excessive blood oxygenation cause peroxidation and deprivation of weak biological molecules. In living organisms, two major reactive oxygen species, superoxide radical and hydroxyl radical are being continuously formed in a process of reduction of oxygen to water. Hydroxyl radicals are generated in the presence of hydrogen peroxide and iron ions. Hydroxyl radical (•OH), known to be the most biologically active free radical, is formed in vivo under hypoxic conditions [3].Fibrinogen is the most hydrophobic blood protein thus it is very unstable in aqueous solutions being readily adsorbed on various surfaces [4]. In fact, the conversion of fibrinogen to fibrin by the action of thrombin renders fibrin monomers even more hydrophobic thus facilitating their spontaneous polymerization [5]. Hydroxyl radicals generated in the presence of ferric ions without any redox agent cause dramatic modification of fibrinogen. Formation of insoluble aggregates under the power of hydroxyl radicals is due to a limited reduction of intramolecular disulfide bridges followed by exposure of hidden hydrophobic epitopes leading to the formation of very strong intermolecular bonds.

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 (6). The aim of this study is to evaluate the potential of lycopene and linolenic acid in modulation of diabetic complications in rats.

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2. ANIMALS AND METHODS

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 ± 2oC, 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.

Sixty male Wister rats were used in this experiment. Divided into 6 groups (each 10 rats) . (group 1, normal), which was receive a single dose of 0.1 mol/L citrate buffer intrapertnial. (Group 2-6) rats were intrapretonial injected with STZ (65 mg/kg body weight) [7],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 were received 0.1mol/l citrate buffer, Diabetic untreated , and three groups ,diabetic and treated with two different types from the extract lycopene and linolenic acid and third group treated with combination of both above mentioned. Treatments will be continued for 6 weeks. Oxidative stress markers (Nitric oxide, malondialdhyde, glutathione peroxidase and lactate dehydrogenase) were evaluated in all groups.

3. RESULTS

Results in fig (1) Lactate dehydrogenase (LDH) level showed highly dramatic decreased in diabetic rats in comparing with normal control ($p<0.001$).while, there is highly significant increased in its level with cases treated by lycopene or linolenic acid comparing with diabetic rats ($p<0.001$) for each .But, potassium level showed highly significant decreased in comparing with diabetic ($p<0.001$). Results in fig (2) Results obtained 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 ($p<0.001$)

Results in fig (3) Results obtained showed that tissues glutathione peroxifdase (GPx) was highly significant decreased in diabetic rats as compared with control ($p<0.001$).Lycopene and combined, have more potent effect in reduction of glutathione peroxidase (GPx) level in heart tissues comparing with diabetic ($p<0.001$) for each. While both of linolenic acid and combination of two compounds, have more potent effect in kideny GPx decreasing significantly comparing to diabetic rats ($p<0.001$, $p<0.001$) .GPx in heart, showed highly significant increased compared to diabetic under lycopene extract and combined with linolenic acid treatment ($p<0.001$, $p<0.001$) .Results in fig (4) 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 .

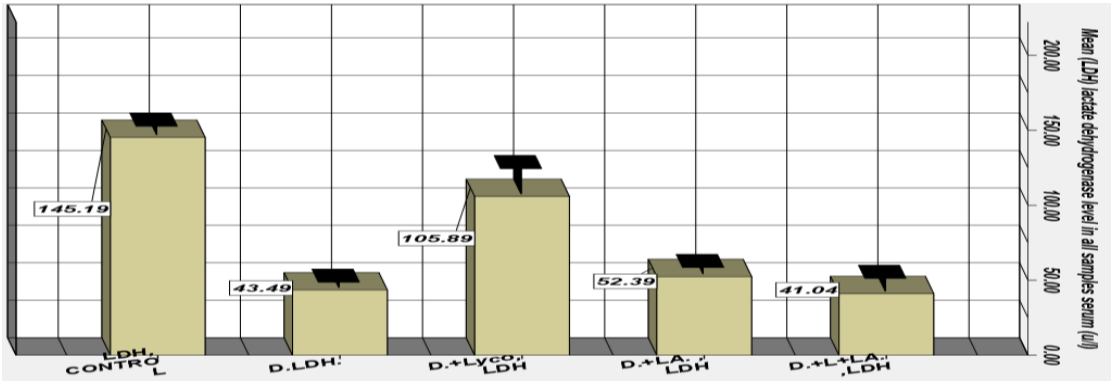

Fig.1. Lactate dehydrogenise in serum of control, diabetic, diabetic treated with lycopene,diabetic with linolenic acid, and cases treated with both respectively

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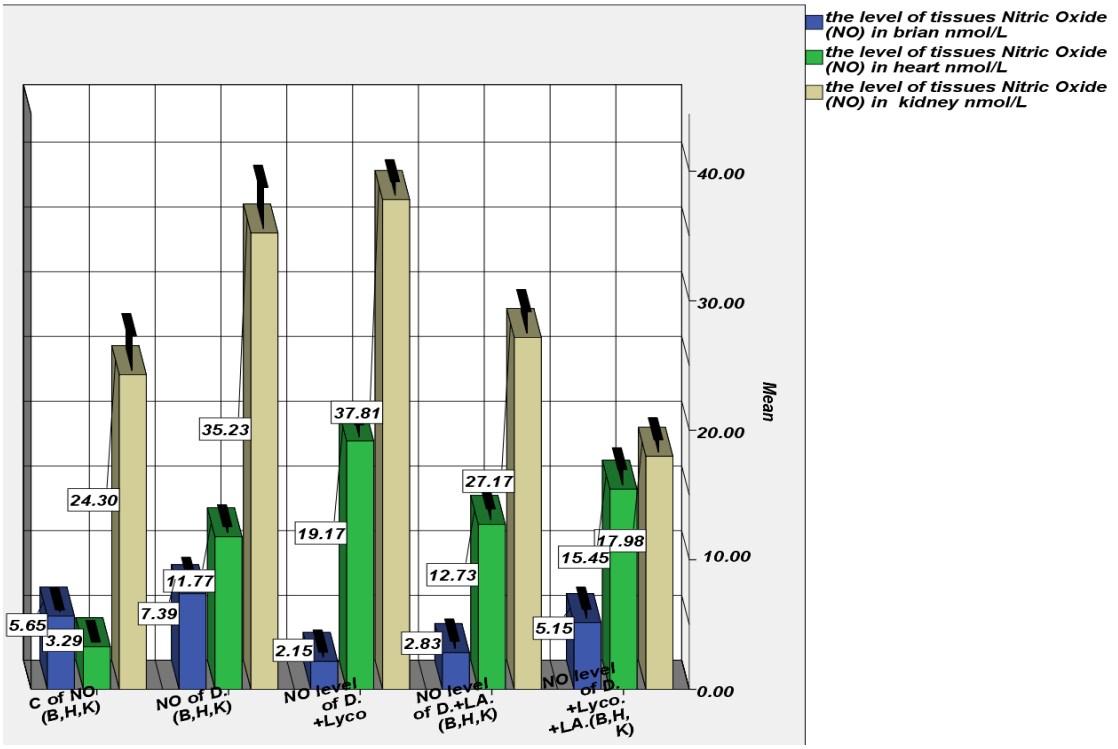

Fig.2. Nitric oxide level in control, diabetic, diabetic with lycopene, diabetic with linolenic acid ,and diabetic treated respectively

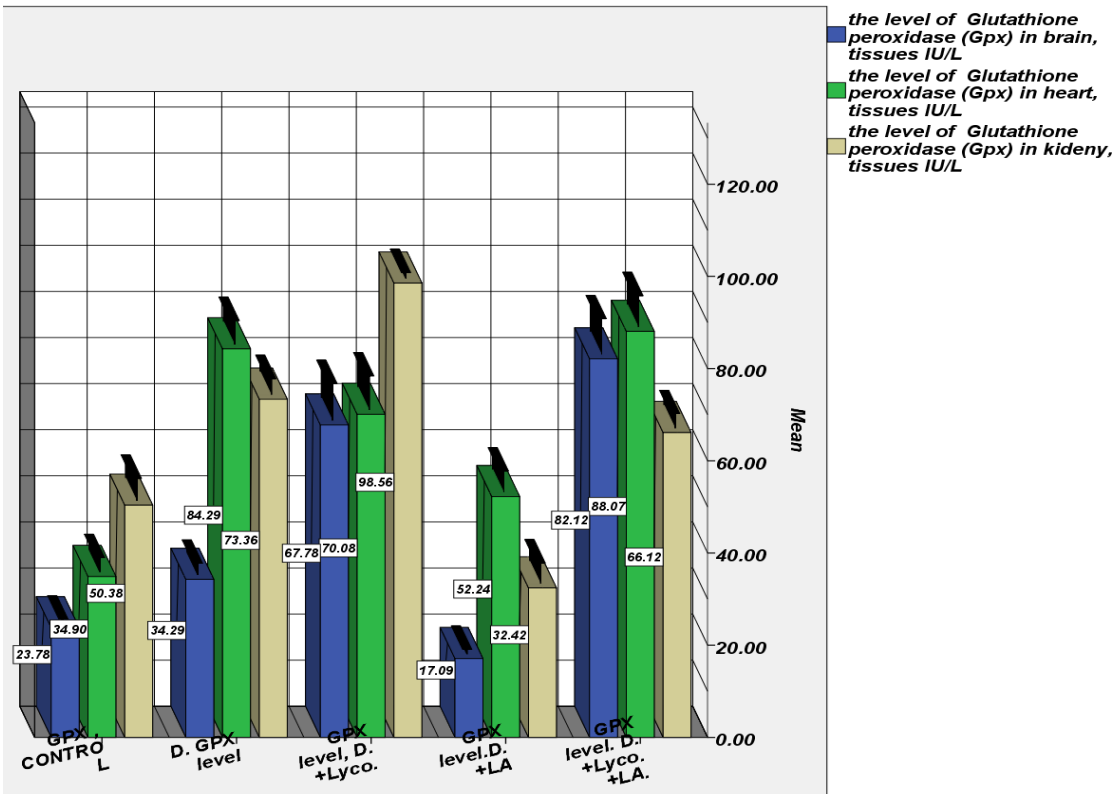

Fig.3 Glutathione Peroxidase in control, diabetic, diabetic treated with lycopene,diabetic with linolenic acid, and cases treated with both respectively.

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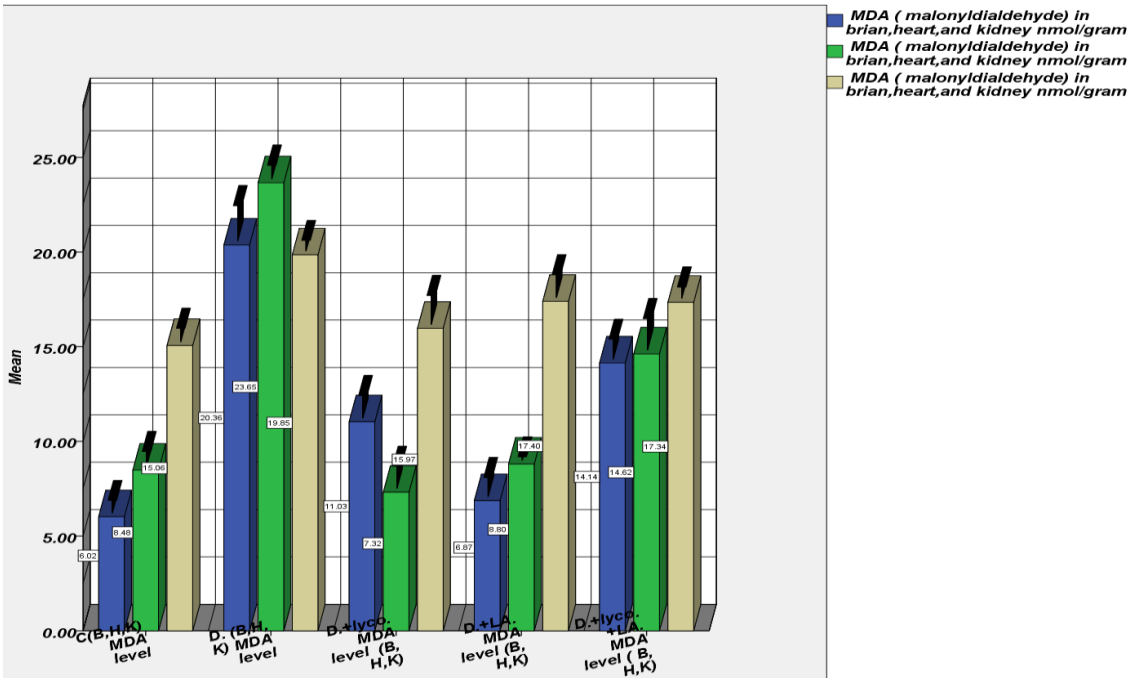

Fig.4. Levels of MDA in control, diabetic ,diabetic treated with lycopene,diabetic with linolenic acid, and cases treated with both respectively.

4. DISCUSSION

hyperglycemia is the initiating cause of the diabetic tissue damage, Although this process is modified by both genetic determinants of individual susceptibility and by independent accelerating factors such as hypertension, [8]. When I pass on to the tissue-damaging effects of hyperglycemia, I stand for damage to a particular subset of cell types: capillary endothelial cells in the retina, mesangial cells in the renal glomerulus, and neurons and Schwann

Diabetic nephropathy (DN) is characterized by structural abnormalities including hypertrophy of both glomerular and tubular elements, increase in the thickness of glomerularbasement membranes, and progressive accumulation of extracellular matrix components [9]. The early increase in the glomerular filtration rate with intraglomerular hypertension, following proteinuria, and final loss of renal function. These of functional alterations associated with. The development of irreversible renal change in diabetes mellitus such as glomerulosclerosis and tubulointerstitial fibrosis results ultimately in end stage renal disease [9]. even though enough control of blood glucose levels may prevent the development of complications, it is difficult to achieve strict blood glucose control, leading to a year-by-year increase in the number of patients with diabetes [10].The chronic hyperglycemia destroys function and structure the kidney, leading to albuminuria which in turn further damages the renal tubular structure [11].In diabetes, the kidney is a direct target to the enhanced glucose levels. Advanced glycation end products (AGE) are heterogeneous products formed by the nonenzymatic reactions between reducing sugars and free amino groups of proteins, lipids, and nucleic acids [12] .many factors can be considered for DM complexity including the activation of the renin—angiotensin system, activation of protein kinase Cβ ,activation of nuclear factor kaba B (NF-κB) , enhanced formation of advanced glycation end products (AGEs) , and acceleration of oxidative stress [13], Free radicals are able of damaging cellular molecules, DNA, proteins, and lipids leading to altered cellular functions. Many current studies tell that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models [14],as well as reducing the severity of diabetic complications [15]. Many experimental evidences propose the involvement of free radicals in the pathogenesis of diabetes [16] and more importantly in the development of diabetic complications [15].therefore; it is not clarified mechanism of DN.

Animal studies have shown that lycopene alone or combined with Linolenic acid supplementation improves the oxidativew stress markers as NO,MDA and GsPx in diabetic rats compared with control.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 [16]. Especially, in type-1 diabetes studies have reported a negative effect of Linolenic acid supplementation on glucose homeostasis . 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 . 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, MDAanalysis used for detecting the changes in metabolism in diabetic rats and diabetic treated with different nutrient supplement as Lyco or Linolenic acidor both. This will reflect the impact of these nutrients and aid the physician to design new regime for treatment of diabetes and to prevented of its complications.The present study showed that long-term Linolenic acidand lycopene treatment significantly improves LDH in diabetic rats.

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