Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 7, Issue 2, 2015

Original Article

NEPHROPROTECTIVE EFFECT OF LYCOPENE IN HYPERGLYCEMIA INDUCED OXIDATIVE STRESS IN MALE WISTAR RATS

AKILANDESWARI. V*[1], SEKKIZHAR. M[2], SYLVIA SANTHAKUMARI. A[3], NIRMALA. P[4]

Postgraduate Department of Pharmacology Rajah Muthaiah Medical College, Annamalai University, Chidambaram-607001, and Tamilnadu, India
Email: akildr57@gmail.com

Received: 16 Mar 2015, Revised and Accepted: 10 Apr 2015

ABSTRACT

Objective: Diabetes mellitus is the chronic metabolic disorder characterized by chronic hyperglycemia associated with absolute or relative deficiency in insulin secretion or insulin action. The most commonly accepted cause of diabetes is the oxidative damage that is caused by free radicals generation. Free radicals have high ability to attract electrons from macromolecules such as carbohydrates, protein, lipid and DNA. Excessive Reactive oxygen species (ROS) can cause structural deterioration and instability of the macromolecules, consequently affecting proper cellular signaling pathways, gene regulation and function. The present study was conducted to investigate the nephroprotective effect of lycopene in alloxan induced type I diabetes.

Methods: Male wistar rats were divided in to 5 groups 6 in each. Group1 as control, Group II. III,IV and V were diabetic groups. Group II diabetic control, Group III treated with protamine zinc insulin 0.9 u/100 gm s. c. Group IV and V treated with 2.5 mg/kg and 5 mg/kg of lycopene. After 3 weeks blood samples were collected from all the groups of animals to measure Lipid peroxidation. Serum glucose, urea and creatinine.

Results: The serum Glucose, urea and creatinine were significantly increased in untreated diabetic rats. In addition, there was significant rise in lipid peroxidation.

Conclusion: In this study, oxidative damage with diabetes was ameliorated with administration of lycopene. The results of this study indicate that lycopene is an effective nutritional component to alleviate or prevent the complications.

Keywords: Male wistar rats oxidative stress, Lycopene, Diabetes, Kidney disease.

INTRODUCTION

Diabetes is one of the most common non-communicable diseases (NCDs). It is the fifth leading cause of death in most countries and there is substantial evidence that it is epidemic in many economically developing and newly industrialized countries. According to Diabetes Atlas 21st century Published by an International Diabetes Federation (IDF), there are 382 million people living with diabetes. [1]. DM is characterized by complete or partial deficiencies in insulin production and/or insulin action coupled with chronic hyperglycemia and disruption in metabolism.[2] The metabolic dysregulation associated with diabetes mellitus causes secondary pathophysiologic changes in multiple organ systems that impose a heavy burden of morbidity and mortality from macrovascular and microvascular complications. Consequent to this, changes in lifestyle occur with westernize dietary pattern leading to increased consumption of dietary fat, sugar and calories. The Diabetes Control and Complications Trial (DCCT) convincingly showed that complications from diabetes can be delayed and reduced by maintaining tight glycemic control.[3] The balance between the rate of free radical generation and elimination is important. Excess cellular radical generation can be harmful however, if there is a significant increase in radical generation, or a decrease in radical elimination from the cell, oxidative cellular stress ensues.[4,5] There is convincing experimental and clinical evidence that the generation of reactive oxygen species (ROS) increases in both types of diabetes and that the onset of diabetes is closely associated with oxidative stress. The possible sources of oxidative stress in diabetes might include auto-oxidation of glucose, shifts in redox balances, decreased tissue concentrations of low molecular weight antioxidants, such as reduced glutathione (GSH) and vitamin E, and impaired activities of antioxidant defence enzymes such as superoxide dismutase (SOD) and catalyse (CAT) [6] ROS generated by high glucose is causally linked to elevated glucose and other metabolic abnormalities important to the development of diabetic complications. However, the exact mechanism by which oxidative stress may contribute to the development of diabetic complications is undetermined.[7]. In type 1 diabetes, β-cell destruction, usually leading to absolute insulin deficiency. This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. In type 2 diabetes, this form, which accounts for 90–95% of those with diabetes, previously referred to as non–insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes ranging from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance.[8] Phenylpropanoids, particularly flavonoids have been recently suggested as playing primary antioxidant functions in the responses of plants to a wide range of abiotic stresses. The significance of flavonoids as scavengers of reactive oxygen species (ROS). the flavonoid concentration in plasma and most tissues is too low to effectively reduce ROS. The present study was taken to understand the antidiabetic effect and therapeutic effect of lycopene on diabetes in alloxan induced diabetic rats. The specific aim of the study was to investigate the effect of lycopene supplementation on blood glucose concentration, lipid peroxidation,superoxide dismutase(SOD),catalase(CAD) and glutathione peroxidase(GSH) levels in the blood.

MATERIALS AND METHODS

This study was carried out at the Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University, Annamalai Nagar. All studies were conducted in accordance with the National Institute of Health "Guide for the care and use of Laboratory Animals" (NIH, 1985). The study was approved by the Animal Ethical Committee of Rajah Muthaiah Medical College and Hospital [Registration No.160/1999/(CPCSIA)] Annamalai University, Annamalai Nagar, Tamilnadu, India (Proposal No.1077, dated 17-04-2014).

Chemicals and reagents

Lycopene powder insoluble in water, is suspended with sunflower oil 1 ml using clean and dry infant feeding tube. Protamine zinc insulin was administered at a dose 0.9U per 100 gm given subcutaneouslyAdult male wistar rats weighing 230-250 gm were used in the present study. The animals understudy was maintained at a room temperature of 25±1ºC in a well ventilated animal house under normal photoperiod conditions. they were provided with the balanced diet (carbohydrate 30%, protein 22%, lipids 12%,vitamin 3%) and water and libitum. The rats were fasted over night,alloxan monohydrate powder dissolved in distilled water to make a solution 50 mg/ml. the rat was induced hyperglycemia by single s. c injection of alloxan monohydrate(100 mg/kg),The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. Hyperglycemia in rats was confirmed by estimating fasting blood glucose level>150 mg/dl were considered as diabetic and used in this study. The rat was divided in to 5 groups of six each(n=6). They were housed in the animal house for 6 weeks. GROUP I (n=6) Normal control. GROUP II (n=6) Diabetic rats. GROUP III (n=6) Diabetic rats treated with protamine zinc insulin 0.9 U per 100 gm s. c along with normal diet. GROUP IV (n=6) Treated with Lycopene 2.5 mg/kg orally 2weeks before and 3weeks after induction of diabetic rats. GROUP V (n=6) Treated with Lycopene 5 mg/kg orally 2 weeks before and after induction of diabetic rats. The treatment was given every day morning using intragastric tube.

Blood and organ sampling

After inducing diabetes mellitus, fasting blood glucose levels were estimated in all five groups at the end of 7,14, and 21 days by using glucometer (one touch ultra). Blood samples were collected by tail snipping method. On the 21stday of the experiment,blood samples were taken by retro-orbital puncture from all the groups of rats for biochemical analysis. The whole blood was collected from rats of each group in sterile,covered test tubes and labeled. After collection of the whole blood,centrifuged at 1000-2000 Xg for 10 mins. The supernatant serum was obtained for biochemical analysis.

Statistical analysis

Values of Biochemical analysis were expressed as means±S. D for six rats in each group. The data was analyzed by Duncan's Multiple Range Test (DMRT), by SPSS software. Values not sharing a common superscript differ significantly at p<0.05.

Table 1: Effect of lycopene on blood glucose levels

Group	Day 1 (mg/dl)	Day 7 (mg/dl)	Day 14 (mg/dl)	Day 21 (mg/dl)
1. Normal control	74.00±3.09	73.33±2.42	73.00±1.79	73.33±1.96
2. Diabetic Rats	248.50±4.59	248.00±5.09	250.83±6.88	250.67±6.74
3. Diabetic Rats Treated with Protamine Zinc 0.9U/100 gm S. C	201.00±3.16	152.50±2.07	128.67±1.63	106.17±2.04
4. Treated with Lycopene 2.5 mg/kg	202.00±2.28	182.50±2.42	165.00±3.52	133.50±2.81
5. Treated with Lycopene 5 mg/kg	201.67±1.63	172.50±1.76	142. 33±1.51	106.00±1.79

Legend 1: In alloxan induced diabetic rats the blood glucose levels significantly high on all three weeks. Lycopene treated rats the blood glucose levels shows significant reduction in blood glucose levels from 2nd week, after induction on 21st day Lycopene treated rats shows maximum reduction in blood glucose on 21st at 5 mg/kg. Values are expressed as means±SD for Five rats in each group. Values not sharing the common superscript differ significantly at p≤0.05. (Dunen's test)

Table 2: Effect of lycopene on antioxidants in kidney tissues

Group	SOD (unit/mg protein)	GSH (µg/mg protein)	CAT (µmol/mg protein)	LPO (mmole/100g tissue)
1. Normal control	16.31±0.17	31.25±0.82	19.84±0.41	3.91±0.15
2. Diabetic Rats	5.03±0.05	9.28±0.38	3.99±0.10	17.33±0.30
3. Diabetic Rats Treatedwith Protamine Zinc0.9U/100 gm S. C	14.29±0.42	31.11±0.51	18.97±0.05	3.83±0.05
4. Treated with Lycopene 2.5 mg/kg	10.95±0.43	19.58±0.36	17.80±0.10	7.78±0.13
5. Treated with Lycopene 5 mg/kg	15.70±0.81	30.80±0.16	18.57±0.44	4.40±0.16

Legend 2: In this study SOD higher in normal rats than diabetic rats. Diabetic rats treated with lycopene significantly higher in SOD. GSH level is higher in normal rats than diabetic rats. Lycopene treated with diabetic rats nearly normal to normal control rats. CAT level significantly higher in normal control than diabetic control. Lycopene treated with diabetic rats shows nearly normal to normal control rats. LPO level significantly higher in diabetic rats compared to normal control rats. Lycopene treated diabetic rats shows significant reduction in LPO. T he antioxidants levels significantly (P<0.05) higher in lycopene treated diabetic rats

Table 3: Effect of lycopene on serum urea, creatinine, hba$_1$c

Group	Urea(mg/dl)	Serum Creatinine(mg/dl)	Glycosylated haemoglobin gmo/ol
1. Normal control	25.50±1.64	0.64±0.02	6.23±0.27
2. Diabetic Rats	72.67±2.06	1.71±0.03	10.90±0.13
3. Diabetic Rates Treated with Protanine Zinc 0.9U/100 gm S. C	33.67±1.03	0.69±0.01	7.38±0.24
4. Treated with Lycopene 2.5 mg/kg	45.67±2.33	1.56±0.03	8.76±0.42
5. Treated with Lycoene 5 mg/kg	33.50±1.38	0.66±0.02	7.03±0.25

Legend 3: The serum urea and creatinine levels increased significantly in alloxan induced diabetic rats at the end of the 3rd week. Lycopene treated with diabetic rats showed significant(P<0.05) reduction in serum urea and creatinine in a dose dependent manner compared to untreated diabetic rats. Protamine zinc insulin treated rats showed significant (P<0.05) reduction in serum urea and creatinine compared to diabetic rats. Values are expressed as means±SD for FIVE rats in each group. Values not sharing a common superscript differ significantly at p *≤ 0.05*. (Dunken's test)

RESULTS AND DISCUSSION

Diabetes has become that most common single cause of end stage renal disease [10]. The metabolic dysregulation associated with diabetes mellitus causes secondary pathophysiologic changes in multiple organ systems that impose a heavy burden of morbidity and mortality from macrovascular and microvascular complications.(American diabetes association 2005),while micro vascular complication of diabetes mellitus include nephropathy. The Diabetes Control and Complications trial (DCCT) demonstrated that

tight control of blood glucose is effective in reducing clinical complications significantly, but even optimal control of blood glucose could not prevent complications suggesting that alternative treatment strategies are needed.[11] Since numerous studies demonstrated that oxidative stress, mediated mainly by hyperglycemia-induced generation of free radicals, contributes to the development and progression of diabetes and related contributions, it became clear that ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complications.[12-14] Nonenzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate •OH radicals.[15] In addition, glucose reacts with proteins in a nonenzymatic manner leading to the development of Amadori products followed by formation of AGEs. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of superoxide ($\cdot O_2$). In this study diabetic rats showed decrease in antioxidants and increase in LPO in renal tissues. Both experimental and clinical studies reported that naturally occurring antioxidants, especially vitamins C, E and α-lipoic acid, in order to delineate the role of oxidative stress in the development of vascular complications of diabetes.[16-18]Treatment of diabetic animals with Lycopene, increase the levels of superoxide dismutase (SOD),Glutathione reductase (GSH) and catalase(CAT) and reduced LPO. Impaired activities of antioxidant defence enzymes such as superoxide dismutase (SOD) and catalase (CAT).[19]ROS generated by high glucose is causally linked to elevated glucose.[20]Increase in serum urea and creatinine was confirmed by due to oxidative damage to renal cells. Lycopene treated diabetic animal showed decrease in serum urea and creatinine which confirms its nephroprotective effect.

CONCULSION

From the study we concluded that prolonged lycopene administration reduces the incidence of nephrotoxicity among oxidative stress induced diabetes mellitus. This study shows that lycopene had significant nephroprotective effect against the hyperglycemia induced oxidative damage.

ACKNOWLEDGEMENT

I am thank full to Professors, Assistant Professors and co-Postgraduates of the Department of Pharmacology Raja Muthaiah Institute of Medical Sciences for their inspiration to take up this study and they guided me at each & every step of this research work by giving useful suggestions and made me complete this work successfully.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Duckworth WC. Hyperglycemia and cardiovascular disease. Curr Atheroscler 2001;3:383–91.
2. Varzi HN, Esmailzadeh S, Morovvati H, Avizeh R, Shahriari A, Givi ME. Effect of Silymarin and vitamine E on gentamicin-induced nephrotoxicity in dogs. J Vet Pharmacol Ther 2007;30:477-87.
3. DCCT. The diabetes control and complications trial research group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–86.
4. Rice EC, Miller N, Paganaga G. Antioxidant properties of phenolic compounds. Trends Plant Sci 1997;2:152–9.
5. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44–84.
6. Haskins K, Bradley B, Powers K. Oxidative stress in type 1 diabetes. Ann N Y Acad Sci 2003;1005:43–54.
7. Kowluru RA, Chan PS. Oxidative stress and diabetic retinopathy. Exp Diabetes Res 2007;4:43603.
8. The expert committee on the diagnosis and classification of diabetes mellitus: Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997;20:1183–97.
9. Anurag kuhad, Richa S, Dethi, Kanwaljit chopra. Lycopene attenuates diabetes associated cognitive decline in rats. Life Sci 2008;83:128-34.
10. American diabetes association standards of medical care in diabetes (position statement). Diabetes Care 2004;27(Suppl 1):S15-S35.
11. The diabetes control and complications trial research group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329(14):977-86.
12. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996;19(3):257-67.
13. Ceriello A. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care 2003;26(5):1589-96.
14. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004;24(5):816-23.
15. Turko IV, Marcondes S, Murad F. Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA: 3-oxoacid CoA-transferase. Am J Physiol: Heart Circ Physiol 2001;281(6):H2289-94.
16. Cuzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, Channon KM. Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors. Circ Res 2000;86(9):E85-90.
17. Cuzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, *et al*. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 2002;105(14):1656-62.
18. Aliciguzel Y, Ozen I, Aslan M, Karayalcin U. Activities of xanthine oxireductase and antioxidant enzymes in different tissues of diabetic rats. J Lab Clin Med 2003;142(3):172-7.
19. Haskins K, Bradley B, Powers K. Oxidative stress in type 1 diabetes. Ann N Y Acad Sci 2003;1005:43–54.
20. Kowluru RA, Chan PS. Oxidative stress and diabetic retinopathy. Exp Diabetes Res 2007;4:43603.