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Study on Antihyperglycemic Effect of Lycopene in Alloxan Induced Diabetes in Rats

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Abstract: ***IntroductionDiabetes affects million people world-wide, and is on the increase. Oxidative stress is also one of the etiological factor for developing diabetes. lycopene is considered to be a potent antioxidant. The present study was done to explore the antihyperglycemic effect of Lycopene against alloxan induced diabetes in rats.Methodology: 36adult male rats of Wistar strain were divided into six groups of six animal in each 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 gms along with normal diet.GROUP IV (n=6) Treated with Lycopen 2.5 mg/kg orally 2weeks before and 3weeks after induction of diabetes mellitus. GROUP V (n=6) Treated with Lycopene 5mg/kg orally 2 weeks before and3 weeks after induction of diabetic rats. Fasting blood glucose levels and HbA1c were estimated. Results: Lycopene(2.5mg/kg and 5mg/kg) before and after induction of diabetes showed significant reduction in blood glucose level. The reduction in blood glucose level was significantly higher in rats with Lycopene(5mg/kg) . The HbA1c level was also significantly lower in Lycopene treated rats when compared to untreated diabetic rats. Conclusion: The present study offer a conclusive evidence that Lycopene has antidiabetic activity***

Keywords: Diabetes mellitus, Lycopene, Antioxidant, Blood sugar, Antihyperglycemic effect.

1. Introduction

Diabetes mellitus is a chronic progressive metabolic disorder characterized by hyperglycemia mainly due to absolute (Type 1 DM) or relative (Type 2 DM) deficiency of insulin hormone [1] DM virtually affects every system of the body mainly due to metabolic disturbances caused by hyperglycemia, especially if diabetes control over a period of time proves to be suboptimal [1]. It is associated with abnormalities in carbohydrate, fat and protein metabolism and results in chronic complications[1] including microvascular, macrovascular and neuropathic disorders [2]

Epidemiological studies, clinical trials and animal experimental models have proved that dietary supplementation of antioxidants[3] like vitamin E, vitamin C, etc., has reduced the incidence of oxidative damage related disorders like ageing, cardiovascular diseases, diabetes, inflammation and neurodegenerative disorder. Hassan Ahmad [4] reported that Coenzyme Q10 a natural antioxidant showed significant nephroprotective effect in diabetic rats compared to untreated diabetic animals. Lycopene extract from tomato is a lycopene-rich extract prepared from the ripe fruits of tomato (Lycopersiconesculentum L.).Lycopene does not have pro-vitamin A properties. Because of the unsaturated nature of lycopene it is considered to be a potent antioxidant and a singlet oxygen quencher. Other mechanisms that include are gene function regulation, gap-junction communication, hormone and immune modulation, carcinogen metabolism and metabolic pathways involving phase II drug-metabolizing enzymes. Few animal studies have proved the anti-diabetic activity of Lycopene

2. Materials and Methods

Study was undertaken at Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University,. 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 Muthiah Medical College and Hospital[Registration No.160/1999/(CPCSEA)] Annamalai University, Annamalai Nagar, Tamilnadu, India (Proposal No.1077, dated 17-04-2014).Lycopene was purchased from La Nutraceuticals,G-40/2 Lawrence Road,Industrial area, DelhiEach 2 mg of capsule contained 2 mg of Lycopene obtained from tomato and red colour fruits.Alloxan monohydrate (2,4,5,6 – tetraoxypyrimidine - 2,4,5, 6 - pyrimidinetetrone) was purchased from MP Biomedical India Private Limited, Mumbai, Maharashtra.HemoglobinHbA_{1C} was determined using glycosylated hemoglobin kit, obtained from Mouli enterprises, puducherry.Blood glucose was determined using glucometer (strip test in one touch),obtained from AVM surgical, Trichy.Lycopene powder insoluble in water, was suspended with sunflower oil 1 ml using clean and dry infant feeding tube .Protamine zinc insulin was purchased from BCP Veterinary pharmacy,Houston, and was administered at a dose 0.9U per 100 gm given subcutaneously.Healthy adult male rats of Wistar strain weighing 230-250 gm were used in the present study.They were purchased from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu, India.Animals were housed in polypropylene cages [28cm x 22cm x 14cm] bedded with husk in groups of six under controlled environmental conditions [Temp-23$\pm 2^0$C, Humidity 65-70% and 12 hrs light/dark cycles] at Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet [VRK Nutritional Solutions, Baramati Agro Limited, Sangli, Maharashtra, India]and water ad libitum.

Alloxan monohydrate powder was dissolved in distilled water to make a solution of 50mg/ml. A pilot study was conducted with different doses (100-150 mg/kg) administered by Subcutaneous(SC) and Intraperitoneal(IP)

routes. A dose of 100mg/kg administered by SC route[5] in overnight fasted rats showed a fasting blood glucose level of more than 150mg/dl, with lowest lethality at 21 days after induction of hyperglycemia, and also led to extensive damage in pancreatic islet cells.

The rats were fasted overnight and hyperglycemia was induced by single SC injection of Alloxan monohydrate (100mg/kg). The rats were maintained on 5% glucose solution for next 24 hours to prevent hypoglycemia. The animals had access to food and water. The development of hyperglycemia in rats was confirmed by estimating fasting blood glucose at 48 hrs after Alloxan monohydrate injection. The rats with fasting blood glucose level >150mg/dl were considered as diabetic and were included in the study.

The rat was divided in to 5 groups of six each(n=6).They were housed in the animal house for 5 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 gms along with normal diet,GROUP IV (n=6) Treated with Lycopene 2.5 mg/kg orally 2weeks before and 3weeks after induction of diabetes mellitus. GROUP V (n=6) Treated with Lycopene 5mg/kg orally 2 weeks before and3 weeks after induction of diabetic rats.At the end of 7,14 and 21 days of inducing Diabetes mellitus fasting blood glucose levels were estimated in all the seven groups using SD Code free Glucometer.Blood samples were gathered by tail snipping method [6].Ionexchange Resin Method was used to estimate the HbA1C.Values of Biochemical analysis were expressed as means ± S.D for six rats in each group. The data were analyzed by Duncan's Multiple Range Test (DMRT), by SPSS(Version 16) software

3. Results

Effect of Lycopene on Blood Glucose Levels:The mean fasting blood glucose levels of rats treated with Lycopene before and after induction of Diabetes mellitus, measured every week, for 3 weeks . The glucose values were compared to values obtained for Diabetic control rats and Protamine zinc insulin treated rats. In Alloxan treated Diabetic rats, the blood glucose levels remained significantly high on all the three weeks. Rats treated with Lycopene(2.5mg/kg and 5mg/kg) before and after induction of Diabetes showed significant reduction in blood glucose levels every week ($p<0.05$), with the maximum hypoglycemic effect on 21st day in a dose dependent manner.

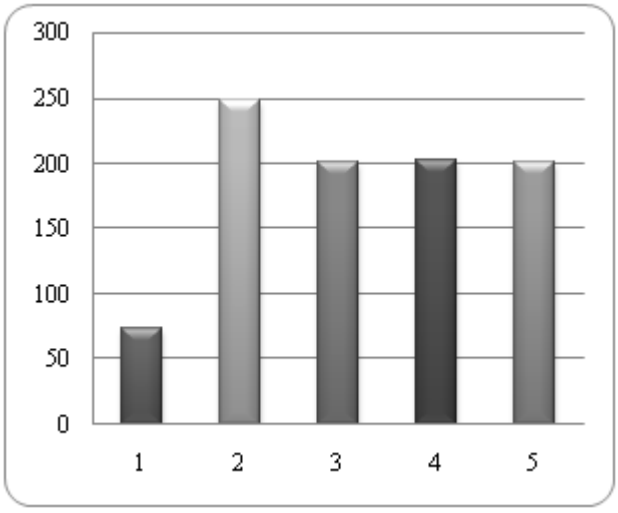

Figure 1: Effect of Lycopene on Blood Glucose on Day 1 In Diabetic Rats

The reduction in blood glucose level was significantly higher in rats with Lycopene(5mg/kg) after induction of diabetes on the first week. But in the subsequent weeks the reduction in blood glucose levels was same in both the groups. Diabetic rats treated with Lycopene showed significant reduction in blood glucose levels on all the three weeks in a dose dependent manner. The maximum hypoglycemic effect was seen on 21st day in Group V (Lycopene 5mg/kg before and after diabetes)In Protamine zinc insulin treated rats, blood glucose levels were significantly lower in all the three weeks compared to Lycopene treated rats

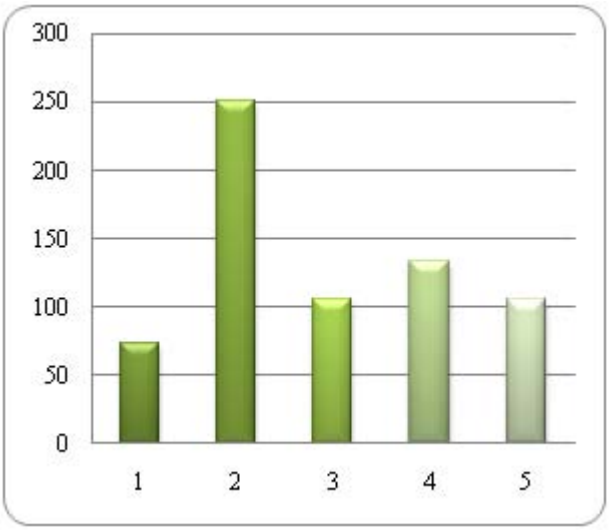

Figure 2: Effect of Lycopene on Blood Glucose Levels On Day21 In Diabetic Rats

Effect of Lycopene on Glycosylated Hemoglobin(HbA_{1c}):It was observed that at the end of third week after induction of Diabetes, the HbA_{1c} was significantly higher in Alloxan treated Diabetic rats ($p<0.05$), compared to normal Control rats. Diabetic rats treated with Lycopene showed significant decrease in HbA_{1c} at both the doses(2.5mg/kg & 5mg/kg), when compared to untreated Diabetic rats. The HbA_{1c} level was significantly lower in rats treated with Lycopene (2.5mg &5mg /kg) after induction of Diabetes (Group IV & V). The

effect of Lycopene at 5mg/kg on HbA_{1c} was more significant than the effect of Protamine zinc insulin.

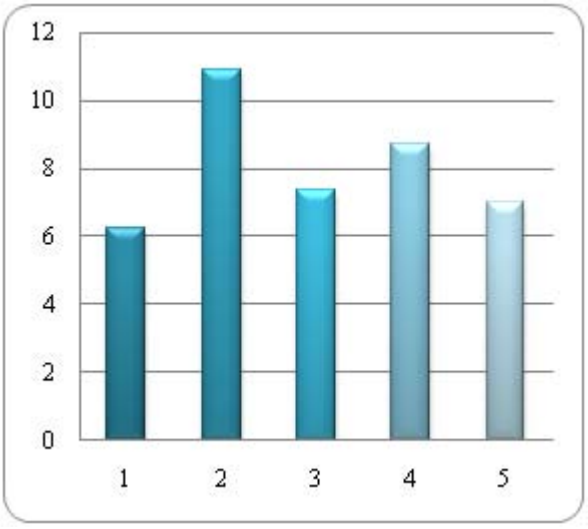

Figure 3: Effect of Lycopene on Glycosylated Haemoglobin Percentage In Diabetic Rats

4. Discussion and Conclusion

Diabetes mellitus is one of the most common non-communicable diseases. It is the 4th or 5th leading [7] cause of death in most high income countries. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. India leads the world with the largest number of diabetic subjects. The morbidity and mortality associated with Diabetes mellitus are staggering.Numerous animal models, epidemiological studies and clinical trials have been developed for understanding the pathophysiology of Diabetes mellitus and its complications in order to design and develop drugs for treatment. One of the most potent, most reliable and easily reproducible methods to induce experimental Diabetes mellitus is chemical induction by Alloxan. It is a well-knowndiabetogenic agent that is used to induce Type I diabetes in animals. Rodents are sensitive to the diabetogenic action of alloxan. Hence in the present study diabetes was induced in male wistar rats by injecting alloxan monohydrate. As the potency of [5]the drug is very much lower in fed than in starved animals, the animals were made to fast overnight before injecting alloxan monohydrate. Alloxan selectively accumulates in beta cells through uptake via (GLUT 2) glucose transporter and cause selective necrosis of beta cells in 24-48 hrs after administration. In the pancreatic beta cells, alloxan is reduced to dialuric acid in the presence of reducing agents like reduced glutathione (GSH). Dialuric acid is then re-oxidised back to alloxan establishing a redox cycle for the generation of ROS and superoxide radicals. The superoxide radicals, hydroxyl radicals and H_2O_2 cause beta cell necrosis. The damage to Beta cells by alloxan was evidenced by significant rise in fasting blood glucose levels and glycosylated Hemoglobin (HbA1c) in alloxan treated rats. The blood glucose levels remained significantly high on all the three weeks of study.

Lycopene rich extract from tomato is prepared from the ripe fruits of tomato (Lycopersiconesculentum L.).Lycopene does not have pro-vitamin A properties. Because of the unsaturated nature of lycopene it is considered to be a potent antioxidant and a singlet oxygen quencher.Antioxidant treatment using N-acetyl cysteine, vitamin C and vitamin E [8] (Hideaki Kaneto et al., 1999) decreased the blood glucose level with preservation of pancreatic Beta cell function in diabetic mice. In the present study, Lycopene treated diabetic rats showed significant decrease in blood glucose levels on all the three weeks in a dose dependent manner. The HbA1c was also significantly reduced. The anti-diabetic activity of Lycopene could be because of its powerful antioxidant property.In alloxan treated diabetic rats (Group II) the blood glucose levels remained significantly high on all three weeks. Lycopene treatment both before and after induction of diabetes (Goups IV & V) showed significant reduction in blood glucose levels on all three weeks in a dose dependent manner. The maximum reduction in blood glucose level was seen on 21st day. The HbA1c level was also significantly lower in Lycopene treated rats when compared to untreated diabetic rats. The reduction was better in groups IV & V (Pretreated with Lycopene before induction of diabetes) when compared to Groups IV & V (Treated with Lycopene after induction of diabetes).Our study offer a conclusive evidence that Lycopene has effective antidiabetic activity Its safety profile, easy availability and low cost are added advantages. Hence Lycopene can be added as adjuvant therapy for controlling the blood sugar

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