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HISTOPATHOLOGICAL STUDY ON CYTOPROTECTIVE EFFECT OF LYCOPENE ON ALLOXAN INDUCED DIABETES IN RATS

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ABSTRACT DM virtually affects every system of the body mainly due to metabolic disturbances caused by hyperglycemia and associated with abnormalities in carbohydrate, fat and protein metabolism and results in chronic complications including microvascular, macrovascular and neuropathic disorders. we planned a study to evaluate the the cytoprotective effect of lycopene in alloxan induced diabetes in rats by doing histopathological examination of pancreas and kidney. The rat was divided in to 5 groups of six each(n=6). 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 diabetes mellitus. GROUP V (n=6) Treated with Lycopene 5mg/kg orally 2 weeks before and3 weeks after induction of diabetic rats. Lycopene at 2.5mg/kg given before and after induction of diabetes showed significant protective effect. Rats treated with 5mg/kg of Lycopene showed regenerating beta cells in the islets of Langerhans in the pancreatic section. The pancreatic section of rats treated with Protamine zinc insulin also showed vacuolated beta cells and moderate infilteration of inflammatory cells in the Islets of Langerhans.. Diabetic Rats treated with Lycopene 2.5mg/kg showed renal tubules with less vacuolation and mild intertubular hemorrhages. But at 5mg/kg showed normal architecture of renal tubules and glomerulus. In diabetic rats treated with Protamine zinc insulin showed , mild vacuolation of renal tubules and normal glomerulus.

KEYWORDS : Diabetes,Alloxan,Lycopene,Histopathology,Cytoprotrective effect.

1. INTRODUCTION:

Diabetes mellitus (DM) 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].I 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 Ahmadvand [4] reported that Coenzyme Q10 a natural antioxidant showed significant nephroprotective effect in diabetic rats compared to untreated diabetic animals. Flavonoids [4] (more than 8000) constitute the largest and most important groups of polyphenolic compounds in fruits, vegetables, wine, tea and cocoa. Recent attention has been focused on the potential use of flavonoids-based drugs for the prevention and treatment of oxidation stress mediated diseases. Flavonoids can exert their antioxidant activity by various mechanisms, eg., by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for free radical generation.

Lycopene extract from tomato is a lycopene-rich extract prepared from the ripe fruits of tomato (Lycopersicon esculentum 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.Hence the present study was done to explore the preventive effect of Lycopene against alloxan induced diabetes in rats and also to evaluate the cytoprotective effect by doing histopathological examination of pancreas and kidney.

2. AIM:

To evaluate the cytoprotective effect of lycopene in alloxan induced diabetes in Rats by doing histopathological examination of pancreas and kidney.

3. MATERIALS AND METHODS:

Study centre:

This study was undertaken at Central Animal House, Rajah Muthiah 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 Muthiah Medical College and Hospital[Registration No.160/1999/(CPCSIA)] Annamalai University, Annamalai Nagar, Tamilnadu, India (Proposal No.1077, dated 17-04-2014).

Materials:

Chemicals and Reagents:

- Lycopene was purchased from La Nutraceuticals,G-40/2 Lawrence Road,Industrial area, Delhi.
- Each 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 Biomedicals India Private Limited, Mumbai, Maharashtra.
- Biomedical and enzymatic kits for measuring antioxidant enzymes were obtained from Aces chemicals and enterpirises pvt Ltd, Chennai.
- Hemoglobin(Hb)A_{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.
- Serum urea and creatinine were determined by biochemical analyser using commercial kits,obtained from microtherapeutic research lab, Chennai.

Lycopene solution:

Lycopene powder insoluble in water, was suspended with sunflower oil 1 ml using clean and dry infant feeding tube.

Insulin:

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±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[61] 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 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 diabetes mellitus.
GROUP V (n=6) Treated with Lycopene 5mg/kg orally 2 weeks before and3 weeks after induction of diabetic rats.

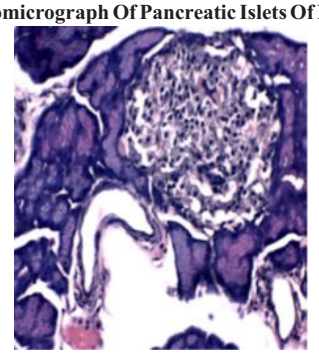
At the end of 7,14 and 21 days of inducing Diabetes mellitus fasting blood glucose levels were estimated in all the seven groups using SD Code free Glucometer.Blood samples were gathered by tail snipping method[5] On the 21st day of the experiment blood samples were taken by retro-orbital puncture under I.M Ketamine [6] from all the groups of rats for biochemical analysis.

The animals were sacrificed by cervical dislocation and the Pancreas and Kidneys from all the groups of rats were dissected out. They were processed for histopathological and biochemical analysis.

Histopathological Study Of Pancreas And Kidney:
Light microscopic study:
For histopathological study the rat tissues were perfused with 10% formalin. The pancreas and kidney of the rats were excised immediately from the abdominal cavity and fixed in 10% neutral formalin The specimens were evaluated with light microscope. All histopathological changes were examined by pathologist.

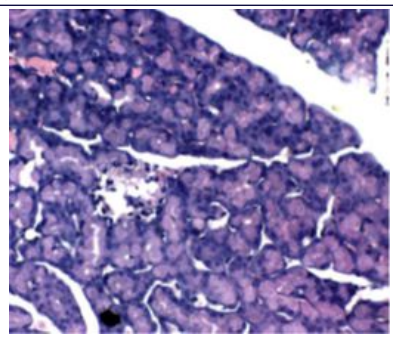
4.RESULTS:
Histopathology Of Rat Pancreas:

Figure1: Photomicrograph Of Pancreatic Islets Of Normal Rats



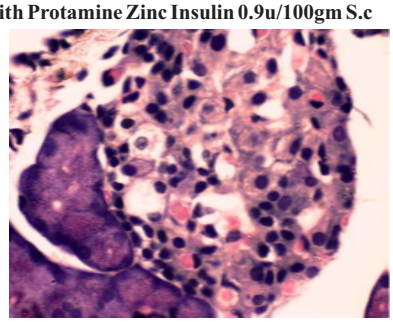
Pancreatic section showing the normal architecture of Islets of Langerhans embedded within the strongly stained acinar cells surrounded by a fine capsule. (H&E: 400x)

Figure 2: photomicrograph Of Pancreatic Islets Of Diabetic Rats



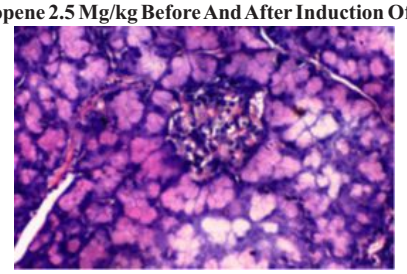
Pancreatic tissue showing vacuolated and atrophied Islets of Langerhans (H&E: 400x).

Figure 3: Photomicrograph Of Pancreatic Islets Of Diabetic Rats Treated With Protamine Zinc Insulin 0.9u/100gm S.c



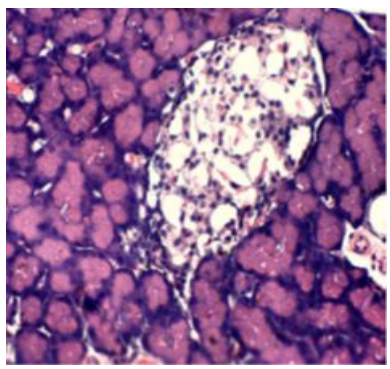
Pancreatic section showing vacuolated beta cell and moderate infiltration of inflammatory cells in the Islets of Langerhans. (H&E: 1000x).

Figure 4:photomicrograph Of Pancreatic Islets Of Rats Treated With Lycopene 2.5 Mg/kg Before And After Induction Of Diabetes



Pancretic Section showing normal cellularity in the Islets of Langerhans within the exocrine pancreas (H&E: 400x).

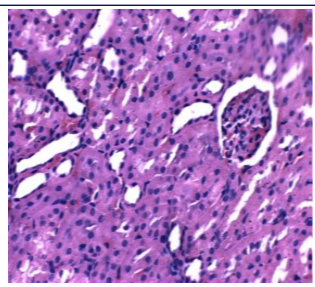
Figure 5.photomicrograph Of Pancreatic Islets Of Diabetic Rats Treated With Lycopene 5 Mg Before And After Induction Of Diabetes



Pancretic Section showing regenerating beta cells in the islets of Langerhans. (H&E: 1000x).

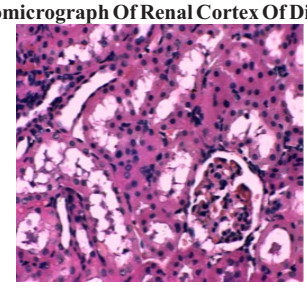
Histopathology Of Rat Kidneys:

Figure6: Photomicrograph Renal Cortex Of Normal Rats



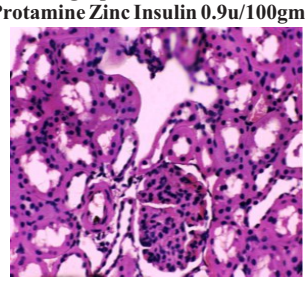
Section showing normal architecture of renal tubules and glomerulus . (H&E: 40)

Figure7: Photomicrograph Of Renal Cortex Of Diabetic Rats



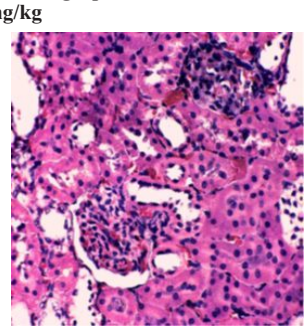
Section showing vacuolated renal tubules, hemorrhagic glomerulus with mild to moderate inflammatory cell infiltration. (H&E: 400x)

Figure8 : Photomicrograph Of Renal Cortex Of Diabetic Rats Treated With Protamine Zinc Insulin 0.9u/100gm S.c



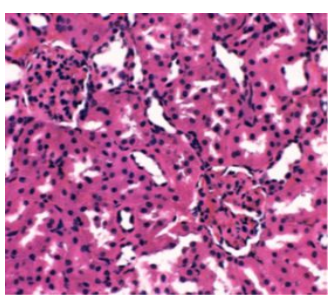
Kidney: Section showing mild vacuolation of renal tubules and normal glomerulus.(H&E: 400x).

Figure 9: Photomicrograph Of Renal Cotex Of Rats Treated With Lycopene 2.5mg/kg



Section showing renal tubules with less vacuolation and mild intertubular hemorrhages. (H&E: 400x).

Figure 10: Photomicrograph Of Pancreatic Islets Of Diabetic Rats Treated With Lycopene 5 Mg/kg



Section showing normal architecture of renal tubules and glomerulus (H&E: 400x).

Histopathological study of Pancreatic Islets:

In alloxan treated rats, the photomicrograph showed extensive destruction of beta cells. Islets showed extensive necrosis, vacuolation, degeneration and shrinkage. Lycopene at 2.5mg/kg given before and after induction of diabetes showed significant protective effect. Section showed normal cellularity in the islets of Langerhans within the the exocrine pancreas. Rats treated with 5mg/kg of Lycopene showed regenerating beta cells in the islets of Langerhans in the pancreatic section. The pancreatic section of rats treated with Protamine zinc insulin also showed vacuolated beta cells and moderate infilteration of inflammatory cells in the Islets of Langerhans.

Histopathological study of Renal Cortex:

The renal cortex of diabetic rats showed renal cellular injury, necrotic tubular epithelium with widening of Bowman's Capsule and glomerular atrophy. Diabetic Rats treated with Lycopene 2.5mg/kg showed renal tubules with less vacuolation and mild intertubular hemorrhages. But at 5mg/kg showed normal architecture of renal tubules and glomerulus. In diabetic rats treated with Protamine zinc insulin showed , mild vacuolation of renal tubules and normal glomerulus.

5.DISCUSSION AND CONCLUSION:

Diabetes mellitus is a disease due to abnormality of carbohydrate metabolism and it is mainly linked with low blood insulin level or insensitivity of target organs to insulin(Urger and Foster,1998). Diabetes causes disturbances in the uptake of glucose as well as glucose metabolism.Liver one of the central metabolic organs in the body,plays a pivotal role in glucose homeostasis and is severely affected during diabetes.Persistent hyperglycaemia is a major contributor to alterations in enzymes of glucose metabolism,which leads to pathogenesis of diabetic complications.

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. Every six seconds [7]a person dies from diabetes. Diabetes imposes a large economic burden on individuals, families, national health systems and countries. These statistics illustrate the importance of identifying and preventing the onset of diabetes, preventing the complications of diabetes mellitus and thus reducing the burden on the community and the nation as a whole.

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 known diabetogenic 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[61] 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.

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. The cytoprotective activity of Lycopene was confirmed by the histopathological examination of beta cells. Epicatechin [8]and Vinca Rosea [9]extracts had been shown to act by Beta cell regeneration in alloxan induced diabetic rats. In the present

study, alloxan treated rats showed extensive necrosis of beta cells. Lycopene treated diabetic rats not only showed significant restoration of beta cells but also regeneration of Islet cells.

Chronic hyperglycemia is an important etiological factor leading to microvascular and macrovascular complications of DM. Hyperglycemia leads to increased production of ROS or superoxide in the mitochondria from glucose oxidation, protein glycosylation and glucose metabolism via sorbital pathway. The findings of DCCT [10], UKPDS [10] and Kumamoto [10]study support the idea that chronic hyperglycemia plays a causative role in the pathogenesis of diabetic microvascular complication. DM is the leading contributor to end stage renal disease. In the present study diabetic rats showed significant increase in serum urea and creatinine at 21 days after induction of diabetes. The oxidative damage induced by hyperglycemia was also evidenced by significant rise in LPO and decrease in antioxidants in renal tissue of diabetic rats. There are reports that natural antioxidants such as vitamin E [11], Caffeic acid [12], lipoic acid [13], quercetin, melatonin [14]and natural phenolic compounds have protective effects against hyperglycemia induced oxidative stress. Lycopene treated diabetic rats showed enhanced antioxidant activity in a dose dependent manner. Hence serum urea and creatinine levels were normal. The DCCT [10] demonstrated that improvement of glycemic control reduced microalbuminuria (39%) and clinical nephropathy (54%). The UKPDS [10] demonstrated that each percentage point reduction in HbA1c was associated with a 35% reduction in microvascular complications. In the present study Lycopene by its antioxidant and antidiabetic activities had protected the renal tissue of diabetic rats from oxidative damage. The histopathological study of renal tissue of diabetic rats showed glomerular atrophy with necrosis of tubular epithelium. This damage was due to hyperglycemia. In Lycopene treated diabetic rats there was restoration of glomerular structure, with only mild congestion in tubular epithelium.

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