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RESEARCH ARTICLE

Induction of Type-II Diabetes by High Fructose Diet and Low Dose of Intraperitoneal Injection of Streptozotocin in Albino Rats

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ABSTRACT

High fructose diet and streptozotocin (STZ) have been widely used separately to induce type-I and II diabetes in animal models especially rats. The objective of this study is to induce experimental type-II diabetes mellitus by combination of both high fructose and streptozotocin in normal adult wistar rats. Blood and urine glucose level, ketone and blood glucose level during oral glucose tolerance test (OGTT) were found much higher in treated diabetic group animals except plasma insulin in comparison to normal control group rats. Histopathology of pancreas of normal rats showed normal cellular structure of islets of langerhans whereas treated diabetic group rats showed degeneration, necrosis and extensive damage and depleted islets of langerhans. From the present study, it is concluded that, in albino rats, type-II diabetes can be induced by feeding fructose (21%) for 4 weeks followed by a single intra-peritoneal injection of STZ (40mg/kg body weight).

KEYWORDS

Diabetes Mellitus, Fructose, Insulin, Glucose, Streptozotocin, OGTT, Albino Rats

INTRODUCTION

Diabetes affects hundreds of millions of people worldwide. The prevalence of diabetes has increased dramatically during the last two decades and it is expected to increase further, probably due to increased consumption of high calorie and high fat diets and sedentary lifestyle.[1] According to W.H.O report the prevalence of diabetes in adults worldwide has risen and there are now 150 million people with diabetes and this number will rise to 300 million by 2025.[2] The largest numbers of diabetic patients are in India.[3] In 1995, the number of diabetic patients in India was 19.4 million and it is expected to increase by 57.2 million in 2025, which will be the largest population of world diabetics followed by China (16 million), U.S. (13.9 million) and Russian Federation (8.9 million).[4]

Diabetes mellitus is a chronic metabolic disorder and is also a syndrome characterized by a raised glucose level in the blood due to deficiency or diminished effectiveness of insulin with a strong hereditary basis and is usually associated with passage of sugar in the urine. It affects the metabolism of fat and protein.[5] Experimental induction of diabetes mellitus in animal model is essential for the advancement of knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. There are several methods to induce diabetes in laboratory animals. The generally effective method is the surgical removal of pancreas (at least 90-95%) out of the body.[6] The second method is injecting the anterior hypophysis extract to animals to

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induce diabetes with less reliable result.[7] Another popular method is the injection of streptozotocin (STZ) or alloxan. These materials inflate and ultimately degenerate the beta- cells of islets of Langerhans.[8]

Fructose is the principle monosaccharide and it is a ketose isomer of glucose. Free fructose is found together with free glucose. The major source of fructose is sucrose, which when cleaved releases equimolar amount of fructose and glucose. D-fructose changes into glucose in the liver and intestine and so used in the body. It is more rapidly glycolyzed by the liver than glucose. Fructose uptake requires a sodium-independent monosaccharide transporter (GLUT-5) for its absorption. Entry of fructose into cells is not insulin-dependent and in contrast of glucose, fructose doesn't promote the secretion of insulin. High fructose or glucose diet induces non-insulin-dependent diabetes mellitus (Type-II).

Streptozotocin (STZ, N-nitro derivative of glucosamine) is a naturally occurring broad spectrum antibiotic and cyto-toxic chemical synthesized by *Streptomycetes achromogenes* used to induce both insulin dependent (Type-I) and insulin independent (Type-II) diabetes mellitus. It is particularly toxic to the pancreatic beta- cells in mammals.[9,10] STZ injection leads to the degeneration of beta- cells of islets of langerhans. Clinically symptoms of diabetes are clearly seen in rats within 2-4 days following single intravenous or intra-peritoneal injection of 60mg/kg STZ.[11] The present study was carried out to successful induction of Type-II diabetes by single dose of single intra-peritoneal injection of STZ @ 40mg/kg body weight of rats for further study of pharmacological effect of *Terminalia arjuna* Roxb. bark on type-II diabetic albino rats.

MATERIAL AND METHROD

Animals

Only male albino rats of wistar strain (*Ratus norvegicus*) of body weight 125±25gm (about 6-8 weeks old) were taken and housed in cage. All rats were housed in a temperature regulated experimental room under conventional condition maintained at 24±1°C with 55±10% humidity and an inverse artificial light-dark cycle of 12 hours. Rats were randomly divided into two groups- Group (i): Normal control group (n=10) and Group (ii): Treated diabetic group (n=10). Both groups were fed with standard chow (Table-1) and water was supplied *ad libitum*. Total nutrient value of standard chow was calculated as carbohydrate (59.47%), protein (16.64%) and fat (6.39%) determined by Kjeldahl method.

Table 1: Composition of standard chow

Constituents	Standard Chow (gm/kg)
Wheat	259
Bengal gram	463
Groundnut	195
Refined oil	67
Vitamin mixture	01
Mineral mixture	15

Induction of Type-II Diabetes in Rats

All animals were acclimatized for two weeks before onset of experiment in laboratory. Animals of group-ii (treated diabetic group) were fed with 21% fructose with standard chow for four weeks before a single dose of intra-peritoneal injection of STZ @ 40mg/kg body weight. Animals of group-ii were fasted for 12 hours before STZ injection. STZ was freshly prepared in 0.1M citrate phosphate buffer (pH - 6.3). For the I.P. injection, the rat was held in one hand in dorsal position. The injection site was swabbed using antiseptic material like spirit solution and the designated amount of STZ was injected in caudal abdominal cavity using sterile 1ml syringe needle.

Urine Analysis

Urine parameters like urine colour, volume (ml/rat/day), sugar and ketone were observed in rats of both groups at last day of the experiment. Urine sugar and ketone were analyzed by using

Diastix reagent strips for urine analysis (Bayer Polychem India Ltd., Thane, India). Urine volume was measured by using metabolic cage.

Measurement of Blood Sugar

Blood sugar test was performed by using Bayer Contour TS glucometer (Bayer Healthcare Ltd., Hong Kong). Whole blood was collected from tail vein immediately to test strips before and after STZ injection daily until onset of diabetes. Animals whose blood glucose level exceeded 200mg/dl at 24 hours after treatment were considered diabetic.

Measurement of Insulin

Plasma insulin was determined by Mercodia rat insulin ELISA kit (Mercodia developing diagnostic, Sweden). Blood was collected from orbital-venous plexus of overnight fasted anaesthetized rats using heparinized capillary tube and collected in storage vial. The collected plasma was kept at 4^{o}C.

Oral Glucose Tolerance Test

OGTT was performed between 09:00-14:00 h on last week of experimental period. The rats were deprived of food for 12h before the administration of an oral glucose @ 2 gm/kg body weight (200gm/L solution). Blood samples were collected from the tail vein at 0 (before administration), 60 min and 120 min after glucose administration. Glucose levels were determined by using one drop of blood samples by Bayer Contour TS glucometer (Bayer Healthcare Ltd., Hong Kong).

Histopathology of Pancreas

Histological analysis was done in pancreas of both groups of rats. For this, rats were sacrificed at 4^{th} week and pancreas was collected. Tissue was excised of approximately one cm and immediately fixed in Bauin's solution for 48 hours at room temperature. The tissue was washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial sections of 6µm thickness were cut using a rotary microtome. The sections were then deparaffinised with xylene and hydrated with descending grade of alcohol. Sections of pancreas was then stained with haematoxylin & eosin and then mounted with DPX in a slide. The slides were observed in bright field microscope (BX-51, Olympus Singapore PTE Ltd., Singapore) at 200X magnification and images were taken at 300 dpi using ProgRes® Digital Microscopic Camera (Jenoptik AG, Carl-Zeiss).

Statistical Analysis

The data was analysed by statistical method. Each value is expressed as the mean ±SEM and values were analysed by one-way analysis of variance (ANOVA). One way ANOVA was used to analyse the data in SIGMA PLOT 11® software package (Systat software Inc., USA). Differences were considered significant at $p<0.05$.

RESULTS

After 24 h of STZ treatment the blood glucose level (mg/dl) was significantly ($p<0.05$) increased to 292.46±13.96 in rats of treated diabetic group (group-ii).

Urine colour, volume (ml/rat/day), sugar and ketone bodies were observed in rats of both groups at last day of the experiment. Rats of normal control group (group-i) showed clear urine and absence of sugar and ketone bodies throughout the experimental period. Animals of treated diabetic group (group-ii) showed yellow coloured urine, increased urine volume and presence of sugar and ketone bodies. At last day, urine volume was found to be significantly ($p<0.05$) high in rats of treated diabetic group (36±1.06) as compared to rats of normal control group (23±1.65).

Blood glucose concentration (mg/dl) was measured in rats of both groups at last day of experiment. Blood glucose was 78.00±4.04 and 273.33±7.21 in rats of normal (group-i) and treated diabetic group (group-ii), respectively. The blood glucose of group-i rats showed significantly ($p<0.05$) lower blood glucose as compared to treated diabetic group (Fig.-1.1).

Plasma insulin was measured at last day of experiment. Plasma insulin (µU/ml) was found to be significantly ($p<0.05$) higher in rats of

normal group (17.81±1.25) as compared to rats of treated diabetic group (7.28±1.03).

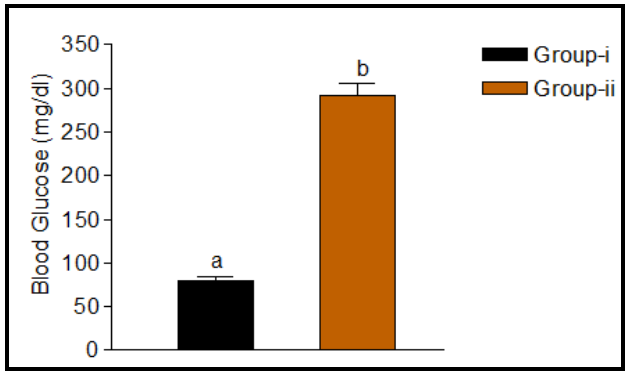

Figure 1.1: Blood glucose level of both normal control and treated diabetic group rats at first day of diabetes induction (values are Mean±S.E.M. of three experiments, Means with different letters are significantly different at ($p<0.05$)

OGTT was performed in rats of all groups at last day of experiment. Blood glucose concentration (mg/dl) was back to normal (79.00±6.72) after 120 min of administration of glucose in rats of normal control group. In rats of treated diabetic group (group-ii), blood glucose remains high (316.66±7.21) after 120 min of glucose administration which was 273.00±12.52 mg/dl before administration (Fig.-1.2).

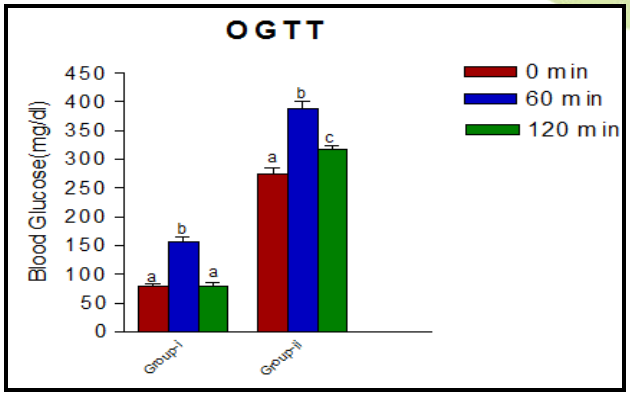

Figure 1.2: Performance of normal control and treated diabetic group rats for oral glucose tolerance test (OGTT) on last day of experiment. (values are Mean±S.E.M. of three experiments, Means with different letters are significantly different at ($p<0.05$)

The histological examination of pancreas showed normal size cells, normal lobules of exocrine acini, intracellular ducts and normal cellular population of islets of langerhans without atrophy and vacuolation of connective tissues in islets in normal control group (group-i) rats pancreas sections (Fig.-1.4) which was not seen in treated diabetic group rats (group-ii). Significant reduction in number of β-cells and apparent atrophia in pancreatic islets, extensive damage and depleted islets of langerhans, reduced cellular population with decreased cell size, vacuolation and invasion of connective tissue in parenchyma of pancreatic islets were detected in treated diabetic group (group-ii, Fig.-1.5).

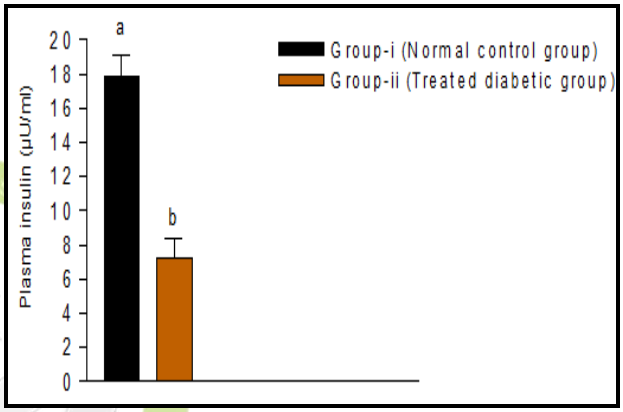

Figure 1.3: Performance of normal control and treated diabetic group rats for plasma Insulin level at last day of experiment (values are Mean ± S.E.M. of three experiments, Means with different letters are significantly different at ($p<0.05$)

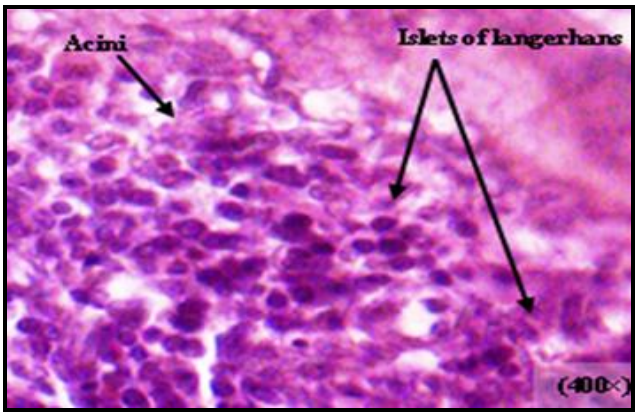

Figure 1.4: Histopathology of pancreas of normal control rats (group-i) showing normal cellular structure, normal acini and normal cellular population of islets of langerhans in pancreas (400X)

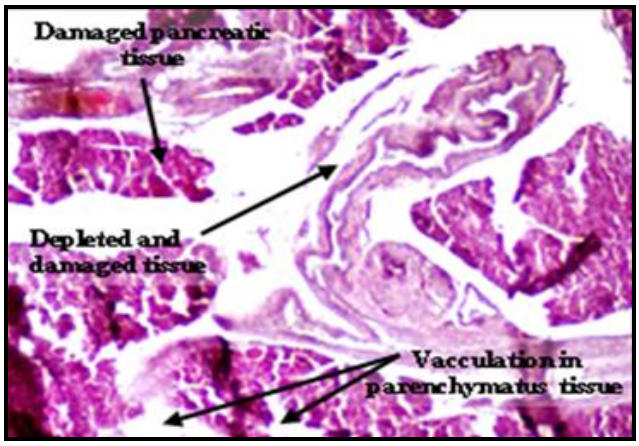

Figure 1.5: Histopathology of pancreas of treated diabetic rats (group-ii) showing degeneration, necrosis and extensive damage and depleted islets of langerhans due to type-II diabetes (400X)

DISCUSSION

Diabetes is a chronic metabolic disease affecting a large part of world's population. It is a major lifestyle disorder and the prevalence of which is increasing globally. Asian countries contribute to more than 60% of the world's diabetic population as the prevalence of diabetes is increasing in these countries.[12]

A number of non-genetic models have been developed in last three decades for diabetes research including adult alloxan or streptozotocin (STZ) models, partial pancreatectomy model, high-fat (HF) diet-fed models, fructose-fed models, HF diet-fed STZ models, nicotinamide-STZ models, monosodium-glutamate (MSG) induced models, and intrauterine growth retardation.[12] In the present study, HF diet-fed STZ model were used to induce type-II diabetes in male albino wistar rats and found constantly high blood glucose level after induction. Similarly, Islam and Wilson[13] used Fructose-fed streptozotocin-injected model for type-II diabetes induction in male Sprague-Dawley rats. Authors have concluded that 10% fructose-fed followed by 40 mg/kg of body weight STZ injection can be a new and alternative model for type-II diabetes in Dawley rats. This indicates in our study rats were induced to type-II diabetes and sowed their complications.

At the last day of experiment, rats of diabetic groups showed change in urine colour, increased urine volume and presence of sugar and ketone bodies. Present results are in line with result of Nakhoul, *et. al.*[14], reported increased urine volume, urine sugar[15], urine ketone bodies[16] in diabetic rats. Recently, Eleazu *et al.*[17] reported feeding unripe Plantain (*Musa paradisiacae*) to diabetic rats improved urine quality and decreased urine sugar and ketone bodies.

High fructose diet followed by STZ injection constantly increased in blood glucose in rats. Similar results were obtained by Wilson and Islam[13] and Ragavan and Krishnakumari[18] showed increased blood sugar in normal rats after STZ treatment. Similarly, Nagappa *et al.*[19] reported that administration of alloxan (150 mg/kg, I.P.) to wistar albino rats led to 1.5-fold elevation of fasting blood glucose levels.

In this study, glucose administered to rats of normal control and type-II treated diabetic groups to test their effects on blood glucose regulation, using the OGTT. Rats of type-II treated diabetic as compared to normal control group showed lower tolerance towards oral administration of glucose and higher blood glucose even after 120 min of glucose administration. In a similar line of study, Murali *et al.*[20] and Govinda *et al.*[21] reported improved OGTT of STZ induced diabetic male albino wistar rats.

Plasma insulin (µU/ml) was found to be significantly ($p<0.05$) higher in rats of normal control group (17.81±1.25) as compared to rats of treated diabetic group (7.28±1.03). The present results are in close agreement with report of Steffes *et al.*[22] that diabetic rats were significantly ($p<0.05$) lower non fasting insulin level before administration of glucose as compared to non-diabetic rats. After equal glucose feeding to both diabetic and non-diabetic rats, diabetic rats showed significantly ($p<0.05$) lower plasma insulin (µU/ml) and high blood glucose as compared to non-diabetic rats. Latha and Daisy[23] found significant increase in plasma insulin level in *Terminalia bellerica*

gallic acid fed diabetic rats and this increase in plasma insulin were comparable with diabetic rats fed with synthetic gallic acid.

The pancreas plays a primary role in the metabolism of glucose by secreting the hormones insulin and glucagon. The islets of langerhans secrete insulin through endocrine part of pancreas and glucagon directly into the blood. Insulin is a protein that is essential for proper regulation of glucose and for maintenance of proper blood glucose levels.[24] In the treated diabetic group rats, decrease of pancreatic islets numbers and their size, atrophy and vacuolation and invasion of connective tissues in parenchyma of pancreatic islets were detected but these abnormal histological signs were dramatically decreased in all treated group rats compared to that of normal control group rats. Same findings of result were observed by Ragavan and Krishnakumari.[18]

Table 2: Effect of HFD and STZ on the blood glucose, plasma insulin, urine volume, urine sugar and ketone bodies in group-i (normal control group) and group-ii (treated diabetic group) rats at the last day of experiment

Parameters	**Normal control group**	**Treated diabetic group**
Blood glucose (mg/dl)	78.00±4.04	273.33±7.21
Plasma insulin (µU/ml)	17.81±1.25	7.28±1.03
Urine volume (ml/rat/day)	23±1.06	36±1.65
Urine sugar	-ve	+4
Urine ketone bodies	-ve	Trace

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