# SERUM INSULIN LEVELS AND LIPID PROFILES OF STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS*

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*Part of MVSc. thesis submitted by the first author to KVAFSU, Bidar

## ABSTRACT

The purpose of this study was to determine the serum insulin levels and lipid profiles in experimentally induced diabetes in Wistar rats. Twenty rats were randomly separated into two groups of ten rats each. Group I served as normal control and group II served as diabetic. Experimental diabetes mellitus was induced in groups II rats with single intraperitoneal administration of streptozotocin (STZ) (45mg/kg) dissolved in 0.1M cold citrate buffer (pH 4.5.) The Group I was given citrate buffer alone. The induction of diabetes was confirmed by estimating the blood glucose levels after 72 hours of STZ injection and animals showing the blood glucose level above 300 mg/dL were considered as diabetic. The blood was collected at 15$^{th}$, 30$^{th}$ and 45$^{th}$ days post STZ injection of the present study. The result revealed a significant ($P < 0.001$) increase in the serum level of total cholesterol, triglyceride and blood glucose level of diabetic rats when compared with the normal control rats while a significant ($P < 0.001$) decrease in the serum insulin level(RIA) and body weight was obtained. The present study showed that induction of diabetes using STZ resulted in decreased serum insulin levels, hyperglycemia and hyperlipidemia in rats.

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## INTRODUCTION

Diabetes mellitus (DM) describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO). Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 79.4 million by 2025, making it the country with the highest number of diabetics in the world (King *et al*., 1998).

Experimental induction of diabetes mellitus in animal models is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Streptozotocin (STZ) has been extensively used to induce diabetes for various diabetes studies in laboratory animals (Calabresi and Chabner, 1985). Diabetes is generally accompanied with lipid metabolism abnormality known as diabetic dyslipidaemia which increase the risk for coronary heart disease. The aim of this work is to study the progression of STZ induced diabetes on the serum insulin levels and lipid profiles of adult male Albino Wistar rats.

## MATERIALS AND METHODS

*Animals*

Male Albino Wistar rats weighing 180-260g were obtained from Central Animal Facility, Indian Institute of Science, Bangalore. Rats were maintained under standard laboratory conditions. They were fed with commercially available rat feed (Amruth Feeds, Bangalore) and water *ad libitum* throughout the study. The animals were randomly allocated into two groups, group I as normal control, while group II as diabetic group. The use of animals was approved by the Institutional Animal Ethics Committee of Veterinary College, Bangalore. The experiment was conducted for a period of 45 days.

### Diabetes Induction

Diabetes was induced in group II by intraperitoneal administration of STZ (Sigma Chemicals, USA) at a dose rate of 45 mg/kg of body weight dissolved in citrate buffer (pH 4.5). Control rats were injected with citrate buffer alone. Three days after streptozotocin administration, the glucose levels were determined to confirm diabetes. Rats exhibiting fasting blood glucose levels >300mg/dL were considered for the study.

### Biochemical analysis

The animals were overnight fasted before their blood glucose level was measured. Blood was collected from retro-orbital plexus of the rats under light ether anaesthesia at different time intervals from 3$^{rd}$, 15$^{th}$, 30$^{th}$ to 45$^{th}$ days of experiment. The blood glucose levels and serum lipid levels were measured using commercially available biochemical kits (Span Diagnostics, Bangalore) according to standard procedures (Tietz, 1976). The estimation of serum insulin levels was done by radio-immunoassay (RIA) using iodine labelled insulin assay kit (BARC, Mumbai).

### Statistical Analysis

Statistical analysis was performed using Graph Pad Prism for Windows Version 5.0, 2009. All values are presented as Mean ± Standard Error (SE). The data were analysed using paired sample student t test ($p < 0.001$; two tailed).

## RESULTS AND DISCUSSION

A significant ($p < 0.001$) decrease in the body weight was observed in the diabetic group when compared to the control (Table 1). The blood glucose level was significantly increased ($p < 0.001$) in diabetic group (Table 1). The mean (± SE) values of *serum cholesterol and triglycerides* were found to be progressively increasing from 3$^{rd}$ day ($P<0.001$) to the final day of the experimental study (Table 1). Throughout the experiment, there was a significant reduction ($p < 0.001$) in the serum insulin level of diabetic group (Table 2).

Streptozotocin is a naturally occurring product produced by *Streptomyces achromogenes* which has been extensively used to induce diabetes for various diabetes studies in laboratory animals. Streptozotocin was observed to cause a massive reduction of the β- cells of the islets of Langerhans and induce hyperglycaemia as reported by a number of workers (Babu and Prince, 2004). STZ has been reported to be capable of generating reactive oxygen species resulting in oxidative stress and cell death (Szkudelski, 2001).

Table 1: Effects of STZ on the body weight (g), blood glucose (mg/dL), serum cholesterol (mg/dL) and serum triglyceride (mg/dL)

| Days | Parameter | Group I | Group II |
|---|---|---|---|
| 3rd day | Body weight (g) | 213.16±8.02[a] | 241.83±3.34[b] |
| | Blood glucose(mg/dL) | 106.00± 5.18[a] | 428.50±6.74[b] |
| | Cholesterol(mg/dL) | 43.03±1.82[a] | 74.98±4.25[b] |
| | Triglyceride(mg/dL) | 98.85 ± 2.18[a] | 211.10±3.41[b] |
| 15th day | Body weight (g) | 228.16±6.63[a] | 212.66±2.30[a] |
| | Blood glucose(mg/dL) | 103.83± 3.60[a] | 474.66±5.57[b] |
| | Cholesterol(mg/dL) | 42.71±1.86[a] | 91.90±5.53[b] |
| | Triglyceride(mg/dL) | 98.51±1.20[a] | 248.95±3.16[b] |
| 30th day | Body weight(g) | 250.33±4.47[a] | 189.66±3.16[b] |
| | Blood glucose(mg/dL) | 106.83±4.24[a] | 513.66±7.09[b] |
| | Cholesterol(mg/dL) | 42.16±1.93[a] | 105.60±5.24[b] |
| | Triglyceride(mg/dL) | 100.86±1.22[a] | 285.35±2.46[b] |
| 45th day | Body weight(g) | 281.83± 3.38[a] | 169.00±2.93[b] |
| | Blood glucose(mg/dL) | 107.13±3.21[a] | 557.83±5.71[b] |
| | Cholesterol(mg/dL) | 41.48±1.78[a] | 119.28±4.19[b] |
| | Triglyceride(mg/dL) | 99.48±1.29[a] | 328.22±4.35[b] |

Values are given as Mean (± SE) for ten rats in each group.

For each parameter, means bearing the same superscript do not differ significantly at $P < 0.001$.

Table 2: Effects of STZ on the serum insulin (μU/mL) level

| Days | Parameter | Group I | Group II |
|---|---|---|---|
| 15th day | Serum insulin(μU/mL) | 56.86±1.73[a] | 16.03±0.39[b] |
| 30th day | Serum insulin(μU/mL) | 56.51±1.80[a] | 12.29±0.33[b] |
| 45th day | Serum insulin(μU/mL) | 56.75±1.78[a] | 11.22±0.44[b] |

Values are given as Mean (± SE) for ten rats in each group.

For each parameter, means bearing the same superscript do not differ significantly at $P < 0.001$.

In the present study, diabetes was induced in rats by administration of STZ which was characterized by polyuria, polydipsia, weight loss and decreased physical activities. The present findings appear to be in consonance with the findings of many earlier workers (Shenoy and Ramesh, 2002).

The elevation in the serum glucose level and decline in serum insulin level of diabetic control animals may be attributed to the specific destruction of β- cells by STZ which produces the hormone insulin for normal glucose homeostasis

(Kumar et al., 1999). Insulin enables the cells to absorb glucose from the blood and also helps in the utilization of the glucose in the cells by glycolysis, tricarboxylic acid cycle, hexose monophosphate shunt, and glycogenesis. In STZ induced diabetes, â cells fail to produce insulin which causes excess glucose accumulation in the blood instead of being utilized or stored. The decline in the mean insulin values observed in the present study has also been reported by many earlier workers (Punitha et al., 2005 and Wadood et al., 2007).

Hyperlipidemia is a recognized complication of DM characterized by elevated levels of cholesterol, triglycerides and phospholipids and changes in lipoprotein composition. One of the major pathogenesis of lipid metabolism disturbances in diabetes is the increased mobilization of free fatty acids from adipose tissue and secondary elevation of free fatty acid level in the blood due to insulin deficiency or insulin resistance. The excessive lipolysis in diabetic adipose tissue may lead to increased free fatty acids in circulation which enter the liver and are esterified to form triglycerides. The fatty acid compositions of various tissues are altered in both experimental and human diabetes (Tilvis and Miettinen, 1985). The finding in the present study is in correlation with the findings of Pepato *et al*. (2005) and Sharma *et al*. (2008).

In conclusion, the present study demonstrated that administration of STZ had both potential hyperglycemic and hyperlipidemic activity in male Wistar rats.

**REFERENCES**


Babu, P.S. and Prince, P.S.M. 2004. Antihyperglycaemic and antioxidant effect of hyponidd, an ayurvedic herbomineral formulation in streptozotocin-induced diabetic rats. *J. Pharm. and Pharmacol.* 56: 1435-1442

Calabresi, P. and Chabner, B.A. 1985. Antineoplastic agents. *In*: The pharmacological basis of therapeutics. *Edt*. Goodman, A. and Rall, J.W. *Edn*. 8th., Pergmann Press, New York. *pp* 1209-1263

King, H, Aubert R.E. and Herman, W.H. 1998. Global burden of diabetes 1995-2025: Prevalence, numerical estimates and projections. *Diabet. Care*. 21: 1414- 31

Kumar, K. K., Augusti, K.T. and Vijayammal, P. L. 1999. Hypoglycaemic and anti-oxidant activity of *Salacia oblonga* Wall. extract in streptozotocin induced diabetic rats. *Ind. J. Physiol. Pharmacol*. 43(3): 510-514

Pepato, M.T., Mori, D.M., Baviera, A.M., Harami, J.B., Vendramini, R.C. and Brunetti, I.L. 2005. Fruit of the jambolan tree (*Eugenia jambolana* Lam.) and experimental diabetes. *J. Ethnopharmacol*. 96: 4348

Punitha, I.S.R., Shirwarikar, A. and Shirwarikar A. 2005. Antidiabetic activity of benzyl tetra isoquinoline alkaloid alkaloid beriberine in STZ nicotinamide induced type 2 diabetic rats. *Diabetologia Croatica*. 34(4): 117-128

Sharma, B., Balomajumder, C. and Roy, P. 2008. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. *Food Chemi. Toxicol*. 46: 2376-2383

Shenoy, A.G. and Ramesh, K. G. 2002. Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and streptozotocin-diabetic rats. *Ind. J. Pharmacol*. 34: 156-164

Szkudelski, T. 2001. The mechanism of alloxan and streptozotocin action in cells of the rat pancreas. *Physiol. Res. 50: 536-546*

Tietz. 1976. Fundamentals of clinical chemistry. W.B.Saunders Co., Philadephia.

Tilvis, R.S. and Miettinen, T.A. 1985. Fatty acid composition of serum lipids, erythrocytes and platelets in insulin-dependent diabetic women. *J. Clin. Endocrinol. Metab*. 61: 741745

Wadood, N., Nisar, M., Rashid, A., Wadood, A., Nawab, G. and Khan, A. 2007. Effect of a compound recipe (medicinal plants) on serum insulin levels of alloxan induced diabetic rabbits. *J. Ayub. Med. Coll*. 19(1):32-38

World Health Organization, "Definition, diagnosis and classification of diabetes mellitus and its complications," Report of WHO Consultation, Geneva, Switzerland, 1999


## ARANYAKAM

The annual state convention for the year 2012 of the Indian Veterinary Association, Kerala, Kerala Veterinary Surgeons Service Association and Animal Husbandry Officer's Association Kerala nomenclatured as ARANYAKAM is being held at Lakkidi, Wayanad, during 28th, 29th and 30th December 2012.