Investigations on Insulin Levels and Blood Sugar Concentration in *Tinospora cordifolia* Extract Treated Albino Rats

Kinkar Shobha Bhamudas and Patil Kishor Gopal

Department of Zoology, Government Institute of Science, R.T. Road Civil Lines, Nagpur (M.S.) India PIN-440 001

**Abstract:** Experimental animals were divided into three groups (n=6) viz., normal control, diabetic control and *Tinospora cordifolia* extract treated. Insulin levels in albino rats were investigated by collecting the blood from retro orbital plexus of each rat. Healthy animals with 9 months old of both the sexes, weighing 150-190gm were used for experimentation. Rats were treated with *Tinospora cordifolia* whole plant extract 20ml/kg body weight twice a day from day 2 to 30. In normal control group level of insulin become increased. The serum insulin level decreased significantly in the alloxan treated group was noticed. In the *Tinospora cordifolia* whole plant extract treated group the insulin level reverse to normal.

**Key words:** Insulin • Diabetes Mellitus • *Tinospora Cordifolia* • Albino Rats • Blood Sugar

**INTRODUCTION**

Glucose is the major energy source of cells. Stable blood glucose is necessary since energy must be supplied to all cells at all times despite intermittent food intake and variable demands, such as the level of physical activity. The major regulatory hormone for intermediary metabolism is insulin, produced and secreted by the beta-cells of the islets of Langerhans of the pancreas. Impaired control of blood glucose concentrations by insulin leads to diabetes mellitus.

Removing the pancreas from dogs resulted in fatal diabetes, providing the first clue that the pancreas plays a key role in regulating glucose concentrations [1, 2]. Banting and Best actually discovered insulin when they reversed diabetes that had been induced in dogs with an extract from the pancreatic islet cells of healthy dogs; together with Collip and Macleod, they purified the hormone insulin from bovine pancreases and were the first to use it to treat a patient with diabetes [3, 4].

The production of insulin and its therapeutic use quickly spread around the world.

Insulin was the first hormone for which the three-dimensional crystal structure was determined (by Dorothy Hodgkin, who had previously received the Nobel Prize in Chemistry for determining the structure of vitamin B12). Steiner [5] demonstrated that the two polypeptide insulin molecule is derived from a single-chain precursor proinsulin was important not only for our understanding of the biochemistry of insulin but also because it applies to other peptide hormones that are transcribed as single-chain precursors.

Insulin was the first hormone to be cloned [6] and then produced for therapeutic use by means of recombinant DNA technology, which provided an unlimited supply of this important molecule and laid the foundation for the biotechnology industry. The development of the radioimmunoassay for insulin permitted the quantitative measurement of pancreatic beta-cell function in animals and humans and established the radioimmunoassay as a powerful tool for measuring proteins, metabolites and other chemicals present in very low concentrations [7]. Much of our current understanding of diabetes has resulted from the ability to measure serum insulin levels. Number of researches have been accomplished experimental diabetes induce in various mammalian species [8-12].

Over the past two centuries, we have learned that diabetes is a complex, heterogeneous disorder. Type 1 diabetes occurs predominantly in young people and is due to selective autoimmune destruction of the pancreatic beta cell, leading to insulin deficiency. Type 2 diabetes is much more common and the vast majority of people with this disorder are overweight. Increase in body weight in
the general population, a result of high fat, high-calorie diets and a sedentary lifestyle, is the most important factor associated with the increased prevalence of type 2 diabetes. Insulin resistance is essential in the pathogenesis of type 2 diabetes and that the disease results from both insulin resistance and impaired beta-cell function [13].

As diabetes is a multifactorial disease leading to several complications and do not make enough insulin or their cells do not respond to insulin. In case of total lack of insulin, patients are given insulin injections. Whereas in case of those where cells do not respond to insulin many different drugs are developed. Kinkar and Patil [14] investigated the antidiabetic activity of *Tinospora cordifolia* in alloxan treated albino rats. The present study was carried out to study the effect of *Tinospora cordifolia* plant extract to control the insulin level in albino rats *Rattus norvegicus* due to its metabolic relatedness with human.

**MATERIALS AND METHODS**

*Tinospora cordifolia* is a large, glabrous, deciduous climbing shrub native to India. The stems are rather succulent with long filiform fleshy aerial roots from the branches. The plant material was collected from hygienic places from in and around the Nagpur city. Soxhlet apparatus was used for the preparation of concentrated extract. The shade dried whole plant material (roots, stem, leaves and drupe) was grind with mortar and pastel into fine powder. Equal quantity of the powder of plant part was taken. Finely ground crude plant material is placed in a porous bag or “thimble” made of strong filter paper or cotton cloth, which was placed in extractor chamber of the Soxhlet apparatus. Solvent was added until it reaches the siphon point of the extractor. Then, the extract was siphoned out into the distillation still.

Healthy albino rats (9 months old) of both sexes, weighing 150-190 gm were used for the experiment. Animals were free to access drinking water and food. Animals were cared for and used in accordance with the Institutional Animal Ethics Committee (IAEC), P.G.T. Department of Zoology, RTM Nagpur University, Nagpur (Registration number- 478/01/a/CPCSEA). For experimental induction of diabetes, alloxan monohydrate (A7413 Sigma Aldrich) was used.

For standardization of dose four batches of experiment was carried out. Each batch include three groups of rats (n=6). Group I included young rats of less than 6 month old, group II included the rat of age group 6-12 month old and group III was included rats of more than 12 month age group. Diabetes was induced in 16hrs fasted albino rats with single intraperitoneal dose of alloxan monohydrate. Alloxan injection was prepared in 0.9% normal saline. Rats with fasting blood glucose more than 220 mg/dl was considered for study. The batch I was injected with 120mg/kg bw, batch II with140mg/kg, bw, batch III with 160mg/kg bw and batch IV with180 mg/kg bw. During dose standardization study it was found that 180mg/kg intraperitoneal dose of alloxan monohydrate was suitable for diabetes induction with the 6-12 month old rats.

For this study rats were divided into three groups (n=6),

**Group-I (NC):** Kept as normal control (NC) the animals of this group was free to access drinking water and food they neither injected by alloxan nor fed on plant extract.

**Group- II (DC):** These group animals were injected with alloxan monohydrate (180 mg / kg bw) and kept as diabetic control. They were not fed on extract.

**Group III (DC+TCE):** This group was injected with alloxan monohydrate (180 mg/kg bw) and from day 2 to 30 half an hour prior to feeding, orally administrated with TCE (20 ml/kg bw) twice a day.

**Estimation of Insulin:** For insulin estimation blood was collected from retro orbital plexus of each rat. Insulin estimation was carried out by Chemiluminescent Microparticle Immunoassay (CMIA) in automated analyzer (ARCHITECHT) at Nagpur reference pathology laboratory (NRPL). During transportation (5 km distance) blood was stored in vials covered with ice bag.

**RESULTS AND DISCUSSION**

The rate of glucose transport as well as transport of some other monosaccharides is greatly increased by insulin. When large amount of insulin secreted by the pancreas, the rate of glucose transport into most cells increases to 10 or more times the rate of transport when no insulin is secreted. Conversely, the amounts of glucose that can diffuse to the insides of most cells of the body in the absence of insulin, with the exception of liver and brain cells, are far too little to supply the amount of glucose normally required for energy metabolism.
Table 1: Estimation of Insulin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Insulin (µU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>6.23±0.85</td>
</tr>
<tr>
<td>DC</td>
<td>2.37±1.25b</td>
</tr>
<tr>
<td>DC+TCE</td>
<td>5.64±0.30b</td>
</tr>
</tbody>
</table>

NC: Normal control  
DC: Diabetic control  
TCE: Tinospora cordifolia extract  
(Value are expressed as Mean ± SEM (n=6), paired t-test was performed to compared between groups. * P<0.05 when DC compared with NC and DC+TCE compared with DC.)

Daisy and Rajathi, [15] observed that the aqueous extracts of Clitoria ternatea leaves and flowers significantly (P<0.05) reduced serum glucose increased serum insulin, alloxan causes a massive reduction in insulin release by the destruction of beta-cells of the islets of Langerhans, thereby inducing hyperglycemia. Daily administration of the aqueous extracts of Clitoria ternatea leaf and flower extract for 84 days resulted in decrease in the blood glucose levels of alloxan-induced diabetic rats. They predict the possible hypoglycemic mechanism of this plant part may be through potentiation of pancreatic secretion of insulin from beta-cell of islets or due to enhanced transport of blood glucose to the peripheral tissues. Significant decreases in blood sugar level of animals was noticed after the oral doses of extract of Tinospora cordifolia showing antidiabetogenic and possess hypoglycemic effects [14].

Dewalkar et al.[16] reported that the active principal of the medicinal plant may have insulin like activity or the healing activity on the alloxan diabetes. In their study they observed that the hypoglycemic activity of fruit squash of L. speciosa had better insulin like activity than fresh etiolated wheat grass juice. On the basis of insulin and histological data they conclude that, fresh etiolated wheat grass juice possesses hypoglycemic activity due to its stimulating effect on regeneration of alloxan induced damage to pancreas while hypoglycemic activity of L. speciosa fruit squash may due to its insulin mimicking bioactive compound.

In present investigation it is observed that the serum insulin level decreased significantly (P<0.05) in the alloxan treated group. In contrast to other studies in present study the Tinospora Cardifolia whole plant extract treated group the insulin level reverse to normal significantly (P<0.05). This contradictory result compared to previous studies of the various authors may be due to the additive or synergistic effect of the Tinospora Cardifolia root, stem, leave and fruit bioactive compounds. This increase in the insulin level in the treated group confirms that Tinospora Cardifolia whole plant extract do not bears insulin mimicking activity instead it activates the insulin secretion from beta cell.

CONCLUSION

The serum insulin level decreased significantly in the alloxan treated group. In the Tinospora cardifolia whole plant extract treated group the insulin level reverse to normal. This contradictory result compared to previous studies of the various authors may be due to the additive or synergistic effect of the Tinospora cardifolia root, stem, leave and fruit bioactive compounds. This increase in the insulin level in the treated group confirms that Tinospora cardifolia whole plant extract do not bears insulin mimicking activity instead it activates the insulin secretion from beta cell.

The hike in insulin secretion observed in this study from diabetic control to the normal as observed in Tinospora cardifolia treated group indicates that the whole plant extract may exerts its healing effect on the islets of Langerhans. It is confirm by the histological staining of pancreatic section with aldehyde fuschin.

REFERENCES