



## MICROANATOMICAL CHANGES OF PANCREAS WITH EFFECT OF ACU-TENS AT ZUSANLI (ST36) ACUPOINTS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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### ABSTRACT

Acupuncture is an alternative and complementary medicine used throughout the World to treat various chronic disorders including diabetes. The aim of this present study is to explain the hypoglycemic effect of non-invasive stimulation with Acu-TENS at bilateral Zusanli (ST36) acupoints in Streptozotocin (45 mg /kg / body weight / IP) induced diabetic rats. The Acu - TENS therapy was given with low frequency 15 HZ, high intensity and width of 5 sec pulse at the Zusanli (ST36) acupoints in diabetic induced rats for 5 minutes every day without anaesthesia for 60 days. A more steady significant ( $p \leq 0.01$ ) decrease in fasting blood glucose level was noted in Zusanli acupoints treated diabetic rats and compared with antidiabetic drug (pioglitazone, 7.5 mg / kg / body weight) treated rats. The acupuncture treated diabetic rats showed more significant ( $p \leq 0.01$ ) decrease in total cholesterol, triglycerides, low density lipid and significant increase in high density lipid. Histopathological examination of pancreas in Zusanli acupoints treated diabetic rats showed near normal architecture and regular shaped islets with regeneration of cells and compared with anti-diabetic drug treated rats pancreas. Stimulation at ST36 acupoints produced antidiabetic effects and improved lipid metabolism may be due to increase in insulin secretion through the mediation of  $\beta$ -endorphin and stimulation of Qi (energy flow) and utilisation of energy due to mild muscle twitching during stimulation.

**KEY WORDS:** *Zusanli, Acupoint, ST36, Acu-TENS, Pancreas, Diabetes, Streptozotocin*



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## INTRODUCTION

Diabetes is a chronic disorder characterized by altered metabolism in carbohydrates, fats and proteins with increased blood sugar levels. Macrovascular diseases (coronary heart disease and peripheral arterial disease) and microvascular diseases (retinal and renal vascular disease) are the long term complications of diabetes mellitus<sup>1</sup>. The Worldwide prevalence of diabetes will expect to increase from 4% in 1995 to 5.4% by the year 2025<sup>2</sup> and will become a growing threat to global health<sup>3</sup>. In India, diabetes is gaining the status of a potential epidemic disease<sup>4,5</sup> and becoming the diabetes capital of the World with a projected 109 million people with diabetes by 2035<sup>4</sup>. Type 2 diabetes (NIDDM), a common type of diabetes, adult-onset with the age group of > 40 years, occurs due to combination of insulin resistance and with reduced insulin secretion. Obesity and physical inactivity is the leading risk factors for type 2 diabetes. Though there is an effective pharmacological management due to some adverse effects of certain drugs, the researchers searched for complementary and alternative medicines to treat diabetes with no side effects and also to prevent its complications. Acupuncture, one of the oldest traditional medicines, is nowadays practice throughout the world mainly in U.S, Japan, Korea and India<sup>7</sup>. The therapeutic effects of acupuncture for diabetes have observed in several studies both experimentally and clinically to reduce symptoms and to prevent complications. According to the theory of traditional medicine, health is a state of balance between yin, yang, Qi, and five elements (metal, wood, water, fire and earth). Qi (life energy) flows within our body through a hypothesized channel called meridians with several acupoints. The ill health or disease in a body occurs due to imbalance in yin and yang, can be corrected with the stimulation of acupoints present along the meridians. Each acupoint has its different therapeutic action. The acupoints can be stimulated either manually using needle or electrically<sup>8</sup>. Acupuncture therapy in diabetes acts on the receptors of pancreas beta cells to increase the insulin secretion, promotes glucose metabolism and reduce the blood sugar level<sup>9</sup>. Several studies explained the beneficial effect of acupuncture on obesity, a major risk factor for type 2 diabetes and also various therapeutic effect of acupuncture on different system<sup>10</sup>. In our study, we selected bilateral Zusanli (ST36) acupoint for the treatment of diabetes based on traditional Chinese medicine<sup>11</sup>.

In this study, we used transcutaneous electric nerve stimulation at acupoints (Acu-TENS) therapy, a non-invasive method to stimulate the selected acupoints. This study mainly designed to investigate the antihyperglycemic effect of Zusanli (ST36) acupoint and was proved with the evidence of the histological changes in the pancreatic islets and biochemical parameters (fasting blood glucose level and lipid profile) in Streptozotocin - induced diabetic rats and acupuncture treated diabetic rats.

## MATERIALS AND METHODS

### *Animal*

Male albino wistar rats (200 - 250 gm) were selected for the study. The experimental rat in the animal house was maintained with well ventilation and at a controlled temperature with 12 hours light and dark cycles. The standard laboratory diet and water with ad libitum was given to the animals. The fasting blood glucose, food and water intakes of the rats were periodically measured and compared. This study was approved by Institutional Animal Ethical Committee (Reg.no,889/po /ac /05 / CPCSEA /2011) of Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode.

### *Study design*

The selected experimental rats were divided into four groups with six animals each. The first group rats were normal. Second group rats were induced diabetes with a single dose of Streptozotocin (45 mg /kg / body weight) intraperitoneally. The induction of diabetes was confirmed after 72 hours by estimation of elevated fasting blood glucose level above  $\geq 250$  mg/dl and included for this study. Third Group of streptozotocin induced diabetic rats were treated with pioglitazone (7.5 mg /kg / body weight) given intra orally daily. Fourth Group of streptozotocin induced diabetic rats were treated with bilateral Zusanli acupoints stimulation. Electrical stimulation was carried out for 3 - 5 minutes daily at frequency of 15 Hz, pulse width 5 sec, intensity 15 mA, using Acu -TENS apparatus without anaesthesia. The positive and negative charges are given through pen type surface electrodes to the right and left Zusanli acupoints (ST36), respectively as shown in figure 1. The experiment was carried out for 60 days and blood sample was collected by rat tail and retro orbital venous plexus puncture. Fasting blood glucose and total cholesterol, triglycerides, low density lipid and high density lipid levels were measured and compared periodically between the groups.

Histopathological changes in the pancreatic islets was assessed and compared between the groups. All animals were euthanized by over dose anaesthesia at the end of the treatment, for histopathological observation, the pancreatic tissue specimens were collected and fixed in 10 %

formalin, dehydrated in gradual ethanol (50 - 100%), cleared in xylene and embedded in paraffin sections (4 - 5 $\mu$ ) were prepared, stained with Hematoxylin and Eosin . The body weight, water and food intake of all rats were also measured.



**Figure 1**

*Zusanli(ST36)acupoints was stimulated with Acu-TENS of low- frequency(15HZ), High intensity and the pulse width of 5 sec,for 5 minutes daily without anaesthesia for 60 days.*

## STATISTICAL ANALYSIS

The results were expressed as mean  $\pm$  SD and SE. The fasting blood glucose level were analysed by one-way ANOVA followed by Tukey post hoc test. The value of  $p \leq 0.01$  was considered as statistically significant.

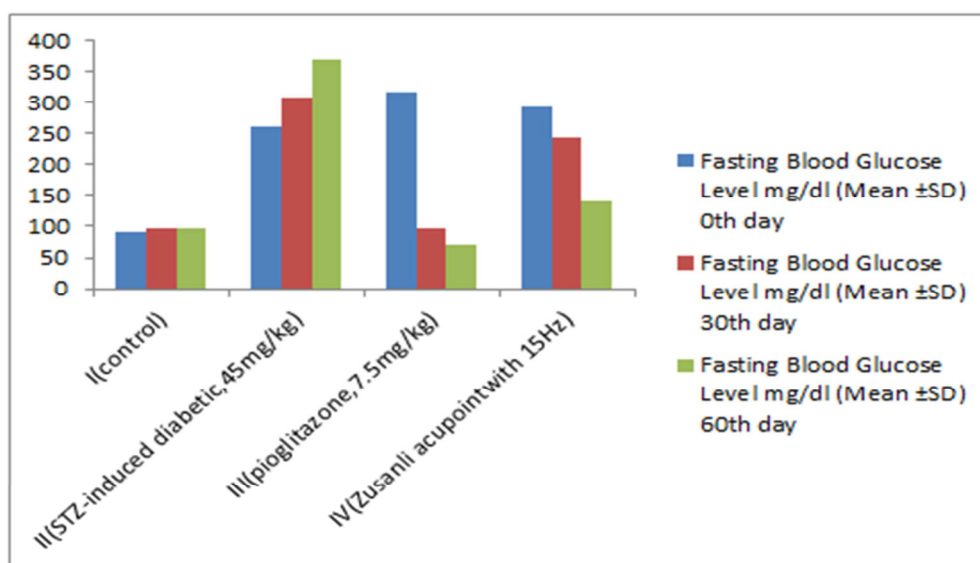
## RESULTS

A steady decrease and significant ( $p \leq 0.01$ ) reduction of fasting blood glucose was observed in

Zusanli (ST36) acupoint and oral anti-diabetic drug (pioglitazone, 7.5mg / kg / body weight) treated diabetic rats after 60 days of treatment. The Zusanli (ST36) acupoints stimulation restored its body weight. Graph 1 shows fasting blood glucose level, Table-1, Graph -2, Table -2 and 3 show total cholesterol, triglycerides, high density lipid and low density lipid level. Diabetic rats treated with drug and Zusanli acupoint stimulation showed significantly ( $p \leq 0.01$ ) steady decrease in the total cholesterol, triglycerides, low density lipid and with significant ( $p \leq 0.01$ ) increase in high density lipid.

**Graph 1**

*Effects of ST36 on Fasting Blood Glucose Level (mg/dl).*



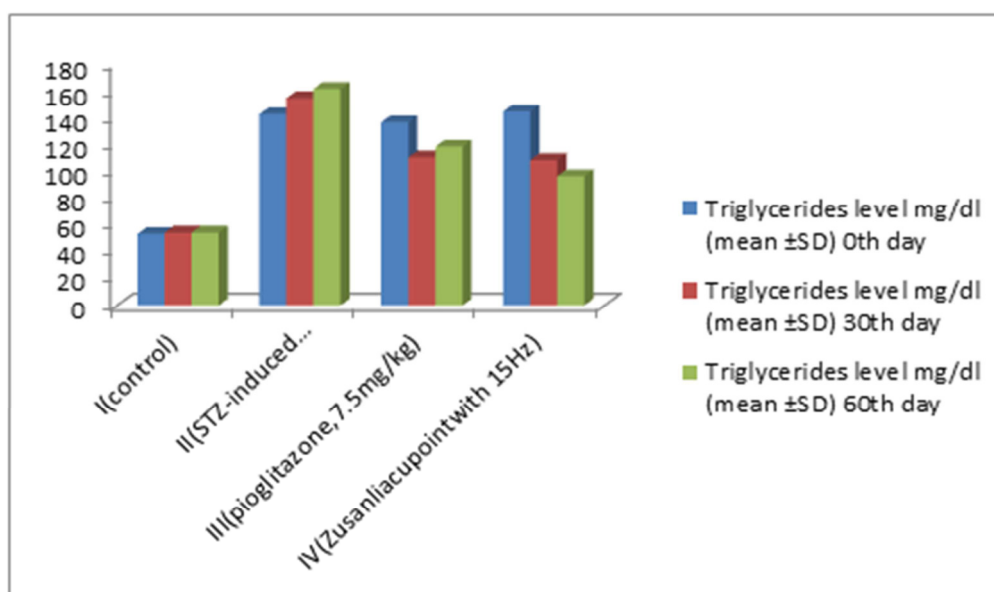
*The results were expressed as Mean  $\pm$  SD. Comparison was done with one way ANOVA and Tukey test with ( $p \leq 0.01$ ) to control and diabetic group.*

**Table 1**  
**Effect of acupuncture on total cholesterol of normal and Streptozotocin - induced diabetes rats**

Groups	Total cholesterol level mg/dl (mean $\pm$ SE)		
	0 <sup>th</sup> day	30 <sup>th</sup> day	60 <sup>th</sup> day
I(control)	87.33 $\pm$ 2.679	91.50 $\pm$ 3.085	96.67 $\pm$ 3.018
II(STZ-induced diabetic, 45mg/kg)	148.50 $\pm$ 4.169	169.17 $\pm$ 4.028	189.83 $\pm$ 3.868
III(pioglitazone, 7.5mg/kg)	156.83 $\pm$ 3.664	132.67 $\pm$ 3.190	96.83 $\pm$ 2.286
IV(Zusanli acupoint with 15Hz)	155.33 $\pm$ 3.827	120.83 $\pm$ 13.484	120.50 $\pm$ 2.604

The results were expressed as Mean  $\pm$  SE. Comparison was done with one way ANOVA and Tukey test with ( $p \leq 0.01$ ) to control and diabetic group.

**Graph 2**  
**Effect of acupuncture on triglycerides of normal and Streptozotocin -induced diabetes rats**



The results were expressed as Mean  $\pm$  SD. Comparison was done with one way ANOVA and Turkey Test with ( $p \leq 0.01$ ) to control and diabetic group.

**Table 2**  
**Effect of acupuncture on HDL of normal and Streptozotocin -induced diabetes rats**

Groups	HDL level mg/dl (mean $\pm$ SE)		
	0 <sup>th</sup> day	30 <sup>th</sup> day	60 <sup>th</sup> day
I(control)	47.83 $\pm$ 1.869	48.83 $\pm$ 1.682	50.5 $\pm$ 1.432
II(STZ-induced diabetic, 45mg/kg)	33 $\pm$ 2.324	24.83 $\pm$ 2.982	20.33 $\pm$ 1.783
III(pioglitazone, 7.5mg/kg)	27 $\pm$ 2.309	43.33 $\pm$ 1.606	52.17 $\pm$ 0.792**
IV(Zusanli acupoint with 15Hz)	27.5 $\pm$ 2.232	35 $\pm$ 1.880	41.5 $\pm$ 1.408**

The results were expressed as Mean  $\pm$  SE. Comparison was done with one way ANOVA and Turkey test with ( $p \leq 0.01$ ) to control and diabetic group.



**Table 3**  
**Effect of acupuncture on LDL of normal and Streptozotocin**  
**-induced diabetes rats**

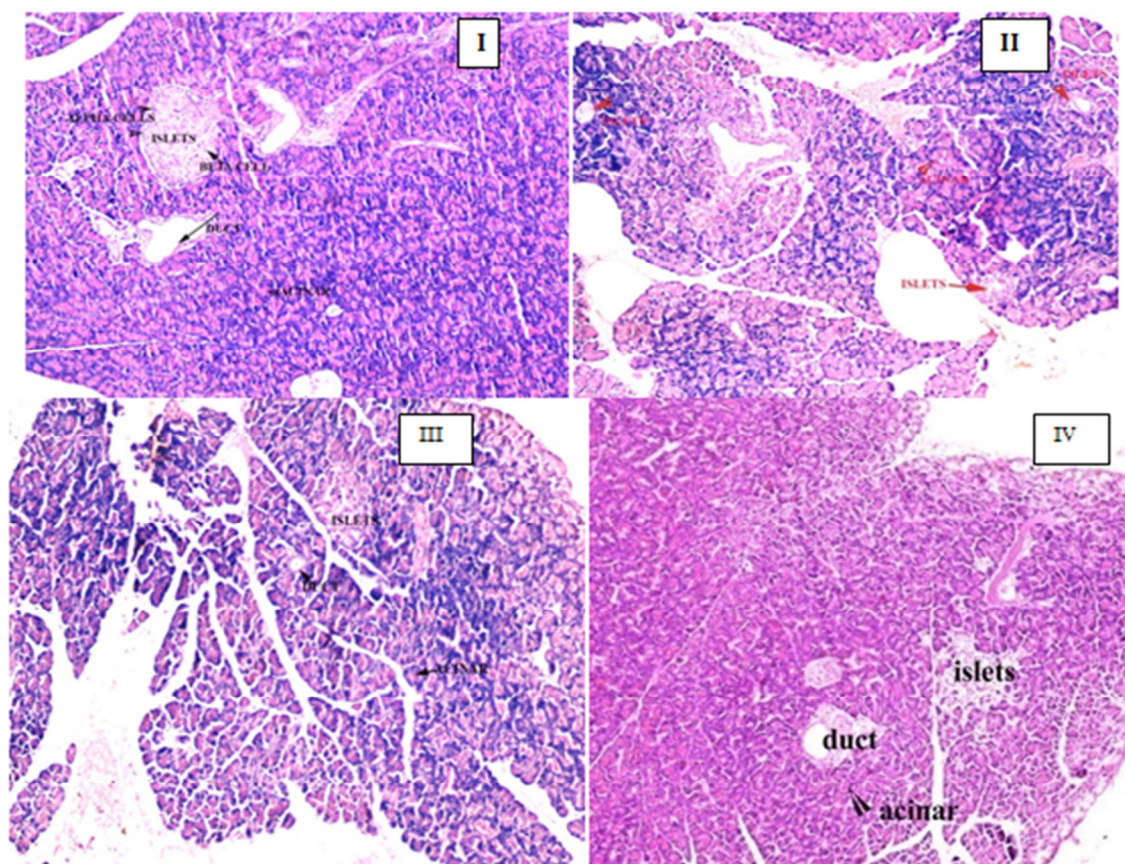
Groups	LDL level mg/dl (mean $\pm$ SE)		
	0 <sup>th</sup> day	30 <sup>th</sup> day	60 <sup>th</sup> day
I(control)	30.48 $\pm$ 4.276	31.9 $\pm$ 3.802	37.97 $\pm$ 3.117
II(STZ-induced diabetic,45mg/kg)	86.93 $\pm$ 5.728	96.87 $\pm$ 18.617	137.3 $\pm$ 4.807
III(pioglitazone,7.5mg/kg)	102.5 $\pm$ 4.365	67.37 $\pm$ 3.583	28.87 $\pm$ 2.268**
IV(Zusanli acupoint with 15Hz)	99 $\pm$ 4.779	77.53 $\pm$ 4.794	59.73 $\pm$ 3.169**

*The results were expressed as Mean  $\pm$  SE. Comparison was done with one way ANOVA and Tukey test with ( $p \leq 0.01$ ) to control and diabetic group.*

### Histopathological examination

The group I control rats showed normal rounded or spherical shaped islets with different types of cells with capillaries, markedly separated by exocrine part made of closely packed acinar cells. In Group II, Streptozotocin induced diabetic rats; pancreas showed reduced size of islets with decrease in cellular density, numerous vacuoles and with degenerative changes in exocrine part. The pioglitazone treated group III rats showed near normal architecture of lobules with irregular shaped

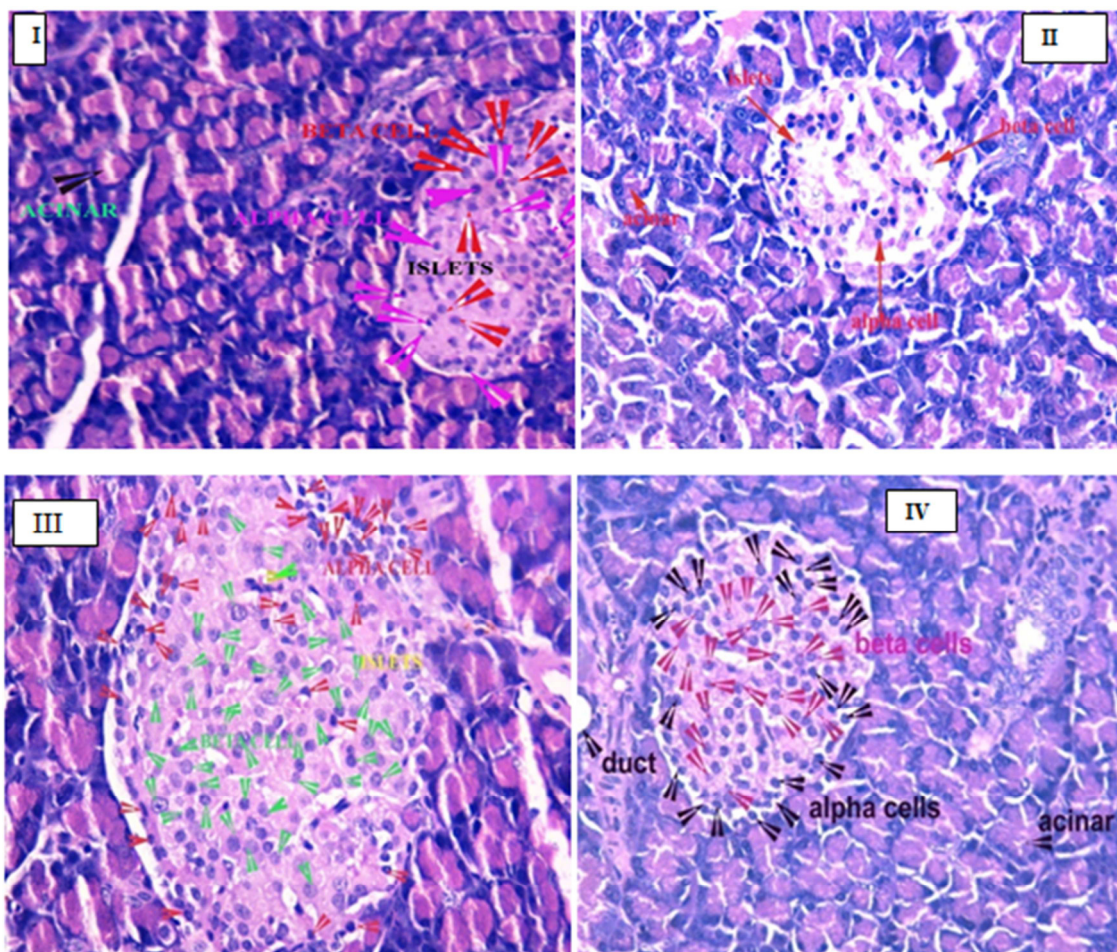
islets with increase in cellular density and reduced number of vacuoles. Zusanli acupoints treated group IV rats showed recovery of near normal architecture of pancreas with regular shaped islets, increase in cellular density and reduced vacuole and compared with the standard antidiabetic treated group. It was observed using LEICA DMD 360 MICROIMAGING CAMERA, GERMAN, PHOTOMICROSCOPY. All the images of pancreas were captured at 400X and 100X, illustrated in figure 2 & 3.



**Fig 2**

*Islets of pancreas after 60 days of treatment:H&E:X100: I (Control group)- islet appear normal; II(Diabetic group)- shrunken islets; III(Pioglitazone treated group)-islets with increase cellular density; IV( Zusanli acupoints treated group)-regular shaped islets with increase cellular density.*





**Fig 3**

*Islets of pancreas after 60 days of treatment:H&E:X400: I (Control group)-normal round islets with alpha & beta cells;II(Diabetic group)- shrunk islets with few surviving beta cells;III(Pioglitazone treated group)-islets with regeneration of beta cells(green arrow);IV(Zusanli(ST36)acupoints treated group)-regular islets with regeneration of beta cell(pink arrow).*

## DISCUSSION

Diabetes is a significant public health problem throughout the World. Daily use of medicines and insulin therapy for diabetic patients are challenging. For the past few years, patients showing interest towards the alternative and complementary medicines especially acupuncture therapy due to its high therapeutic effect and low cost. This study was aimed mainly to find out the histopathological changes in pancreatic islets and the hypoglycemic effect of Acu-TENS at bilateral Zusanli acupoints in diabetes. Histology of control rats' pancreas showed normal lobular architecture with rounded islets consisting of lightly stained cells separated by blood capillaries. The pancreas of Streptozotocin induced diabetic rats showed marked histopathological changes with reduced islets size and numerous vacuoles compared to normal islets. In antidiabetic drug treated rats, pancreas shows lobules with light stained islets and increased cellular density. In Zusanli acupoints treated rats,

pancreas shows near normal architecture and islets with lightly stained beta cells and peripheral alpha cells are noted. In our study the increase in cellular density in islets of pancreas may be due to  $\beta$ -endorphin activation on the specific receptors in the pancreatic  $\beta$  cells. According to Skau M et al<sup>12</sup>, new beta cells stimulate the growth of beta cells in the islets and increase the total volume. Seaberg in his study showed that the adult precursor cells in the pancreas differentiated into islets  $\beta$  cells<sup>13</sup>. In a previous study made by the authors, it was already explained that Acu-TENS stimulation at bilateral Zusanli acupoints result in a significant reduction in fasting blood glucose level with increase in insulin secretion through the mediation of  $\beta$ -endorphin<sup>14</sup>. Hui-Ching Pai et al study explained hypoglycemic effect of EA(15HZ) stimulation at bilateral Zusanli (ST36) acupoint with rosiglitazone due to an increase in insulin secretion through mediation of  $\beta$ -endorphin in normal and Streptozotocin-induced type 2 diabetic rats<sup>15</sup>. In this study, we stimulated bilateral Zusanli acupoints with Acu-TENS unit

and found the significant reduction of fasting blood glucose with increase in serum  $\beta$ -endorphin and compared with standard drug (pioglitazone) group. Shih-Liang Chang investigated the hypoglycemic effect of EA(2HZ) stimulation at bilateral Zusanli acupoints(ST36) with increase in insulin and plasma  $\beta$ -endorphin in Streptozotocin induced diabetic rats<sup>16</sup>. Dyslipidemia in diabetes is a major cause for morbidity. Various studies showed that acupuncture regulates the lipid metabolism. Cabioglu MT & Ergene N, 2005, studied electroacupuncture therapy in obesity decreased serum total cholesterol, triglyceride and LDL cholesterol<sup>17</sup>. Richter et al and Vetter et al explained in their study about the lipolytic effect of  $\beta$ -endorphin on rodents and human fat tissues<sup>18,19</sup>. Dr.Mehmet and Cho Sha et al explained the influence of electroacupuncture in lowering blood glucose levels, cholesterol level, free-fatty acid, body mass index and eliminating diabetic neuropathy in type 2 diabetes<sup>20,21</sup>. Through this present study, it was found that stimulation of Zusanli acupoints produced beneficial lipolytic effect may be due to the role of  $\beta$ -endorphin. Zhi-Qi-Zhao et al mentioned that electroacupuncture can also be given with surface electrode on the skin instead of acupuncture needles for stimulating the acupoints<sup>7</sup>. Hence, in this study, Acu-TENS apparatus with pen type electrode was selected to stimulate the Zusanli acupoint in diabetic induced rats. P.V.Ingle et al, stated the hypoglycemic effect of Accustimulator and lowered the level of TC, TG, LDL and increase HDL with stimulation of ST36 acupoint in type 2 diabetes<sup>22</sup>. In our study we stimulated the Zusanli acupoints with Acu-TENS and proved the hypoglycemic effect and also noted histological changes with increase in cellular density in islets of pancreas. Wu Zhiyuan et al explained the effect of TENS at specific acupoints (LI11, LI4, ST36, SP6) on type 2 diabetes patients for 30 minutes for each session and 5 times a week for 2 months. In this study, the stimulation of only bilateral Zusanli acupoints were for 5 minutes daily for 60 day treatment in diabetic rats showed a significant decrease in fasting blood glucose (FBG) level. The non-invasive Acu-TENS therapy, is beneficial especially to the diabetic patients due to

its low risk of infection. We selected Acu-TENS stimulator due its combined effect of both electrical and acupoints stimulation. In this study Acu-TENS with the frequency of 15 HZ (low frequency) produced a positive effect in reducing blood glucose level in type 2 diabetes and increased cellular density with near normal architecture in pancreatic islets and also with improvement in lipid metabolism. Hence, the result of the present study suggests that the non-invasive acupuncture therapy is beneficial and cost-effective treatment for the elderly type 2 diabetes mellitus patient.

## CONCLUSION

This microanatomical study concluded the effect of Acu-TENS therapy at Zusanli acupoints on pancreatic islets of Langerhans in streptozotocin induced diabetic treated rats. The Zusanli acupoints stimulation produced beneficial hypoglycemic effect and improved lipid metabolism in type 2 diabetes. Low frequency and high intensity Acu-TENS also produced mild muscle twitching during the stimulation of the specific acupoint which is similar to that of physiological process of physical exercise. Hence, this therapy will be useful for elderly diabetes patients who are not able to perform physical exercise. Moreover, the Acu-TENS application is non-invasive, applied only with skin electrodes to stimulate the acupoints. This helps more in preventing infection or inflammation than with needle acupuncture therapy especially in diabetic patients. Here we suggested that Acu-TENS therapy is a prior adjunct therapy for diabetic patients.

## AUTHORS CONTRIBUTION STATEMENT

Dr.Girija sivakumar my research guide, encouraged and supervised the project and findings of this work. Dr.Sathiya narayana murthy helped the shape of the manuscript.

## CONFLICTS OF INTEREST

Conflict of interest declared none.

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