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The Effects Of Simvastatin On Islets Of Langerhans In The Pancreas Of Rats: A Histological And Biochemical Study

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Abstract

Objective: This study explored the effect of simvastatin on the histomorphology of islets of Langerhans, glucose and insulin levels in rats.

Study Design: The study was a one-year laboratory-based experimental control trial.

Place and duration of study: It was conducted at Army Medical College Rawalpindi, in collaboration with the National Institute of Health Islamabad and Armed Force Institute of Pathology Rawalpindi.

Methods: A one-year, laboratory-based, two-group experimental control trial was conducted. Thirty rats were assigned to each group: a control group receiving saline injections, and a simvastatin group receiving a simvastatin 60 mg/kg/day. Histological analysis of pancreatic islets, and measurements of blood glucose and insulin levels were performed. Statistical analysis was conducted using independent sample t-tests, with significance set at $p < 0.005$.

Results: While simvastatin treatment did not affect the number of islets of Langerhans, The area of pancreatic islets of Langerhans was significantly higher in the simvastatin treatment group compared to control (52,664±38,871 μm2 vs 24,643±16,256 μm2, p=0.001). Serum insulin levels were also significantly elevated with simvastatin treatment (21.49±7.03 μIU/ml) compared to control (16.72±5.38 μIU/ml) (p=0.005). There were no significant differences in weekly fasting blood glucose levels at 4 or 12 weeks between groups (p>0.05). These findings suggest potential modulation of pancreatic islet function by simvastatin without affecting glycemic control in this model.

Conclusions: These findings demonstrate that simvastatin treatment significantly impacts the morphology and function of pancreatic islets in rats, increasing insulin secretion without affecting blood glucose levels. Further research is necessary to elucidate the underlying mechanisms and clinical implications of these observations.

Keywords: Simvastatin, islets of Langerhans, Sprague-Dawley rats, insulin, glucose, pancreas.

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1. Introduction

The pancreas is responsible for regulating blood glucose levels through hormone production in the islets of Langerhans. Insulin is one of the most important hormones produced by islets of Langerhans which is necessary for glucose metabolism. Cholesterol-lowering medications, such as statins, have been linked to pancreatitis. This has been seen in case reports, control studies, and meta-analyses, which suggests a potential association between statins and acute pancreatitis.[1]

It is coherently recognized that simvastatin is the prevailing and familiar medicine for high blood cholesterol levels. Simvastatin has already been investigated and it has been established that it has effects on glucose homeostasis. Nevertheless, there is an insufficient explanation of the histomorphology of the islets of Langerhans in the pancreas after treatment with simvastatin. Also, there is limited study of the biochemical changes exhibited in glucose and insulin levels after the use of simvastatin.[2] Histological changes in the pancreas due to statins are not completely understood. The proposed reasons comprise immune-mediated inflammation, cellular toxicity, and specific metabolic effects.[3] Some studies have suggested that statins may impair insulin secretion and sensitivity by affecting the mevalonate pathway, which is involved in the synthesis of cholesterol and other isoprenoid compounds. These compounds are essential for the regulation of various cellular processes, such as membrane trafficking, signal transduction, and gene expression, in pancreatic β-cells. Statins may also alter the size and number of islets of Langerhans, which could affect their functional mass and insulin production.[4] The histomorphology of the islets of Langerhans, specifically their size and quantity, was studied to observe the impact of simvastatin. Biochemical markers such as glucose and insulin levels were also monitored. Enhancing the existing body of knowledge

on the effects of simvastatin on the pancreas is the primary goal of this research. This study delves into the underlying mechanisms behind changes induced by statins and as such, contributes to a better understanding of them. Modifications to the structures of the islets of Langerhans, the endocrine pancreatic components, can lead to significant alterations in their functionality and results. These islets play a vital role in glucose metabolism. The results of this research may have a vital impact on understanding statin-induced pancreatitis, and its treatment.

2. Materials & Methods

The research was conducted in the Department of Anatomy, Army Medical College Rawalpindi, in collaboration with the National Institute of Health (NIH) Islamabad and Armed Force Institute of Pathology (AFIP) Rawalpindi. This was a laboratory-based experimental control trial of one year. The experiment was conducted after the approval of Ethical Committee on Animal Experiments of Army Medical College, Rawalpindi. Sixty male Sprague-Dawley rats weighing 250 ± 50 g were divided into two equal groups: a control group and experimental group treated with simvastatin. We excluded female rats because they have different hormonal profiles and metabolic responses than males, which could affect the results of the study. The sample was selected based on a power analysis to ensure adequate statistical power to detect potential differences between groups. The approach aligns with standard practices in experimental design. The control group received a standard diet and water for three months through an oral gavage tube, while the simvastatin group received a standard diet supplemented with 60 mg/kg/day [5] of simvastatin daily orally for three months through a gavage tube; at the end of the experiment, the rats were euthanized, and their pancreases were collected for analysis.

The number of islets of Langerhans was counted per slide (three slides per specimen) at X10. Islets containing three or more endocrine cells were considered, and the whole slide was scanned with the help of a pointer.[6] The mean value was obtained for three slides for each specimen.

The area of the islets of Langerhans was calculated using the morphometric computer software "Image J," a software by the National Institute of Health USA for calculating area and other user-defined morphometric parameters.[7] Images of three selected fields were taken from each slide using an Olympus digital camera (10 megapixels). A 5 ml sample of blood was collected through cardiac puncture[8] in a plain test tube for the quantitative measurement of fasting insulin levels from the animals in both groups. While cholesterol levels can be affected by statin use, this study focused on the effects of simvastatin on the pancreas, specifically the islets of Langerhans, and on glucose and insulin levels. The impact of simvastatin on cholesterol levels is well-documented in existing literature, and as such, was not the focus of this investigation. Fasting blood glucose levels were recorded once a week early in the morning by taking blood samples from lateral tail vein of rats with the help of a glucometer after 1 2 hours of overnight fasting of all animals.[9]

Data were analysed using the Statistical Package for the Social Sciences SPSS version 23. Quantitative variables are expressed as the mean ± standard deviation. The analysis of variance (ANOVA) followed by post-hoc Tukey's test was used to determine differences among various groups. Statistical significance was set at $P < 0.05$.

3. Results

The Mean ± SD number of islets of Langerhans in the control group was 12.90±1.34 (Table 1) & (Figure 1), while that of experimental group was 13.46 ± 1.97.

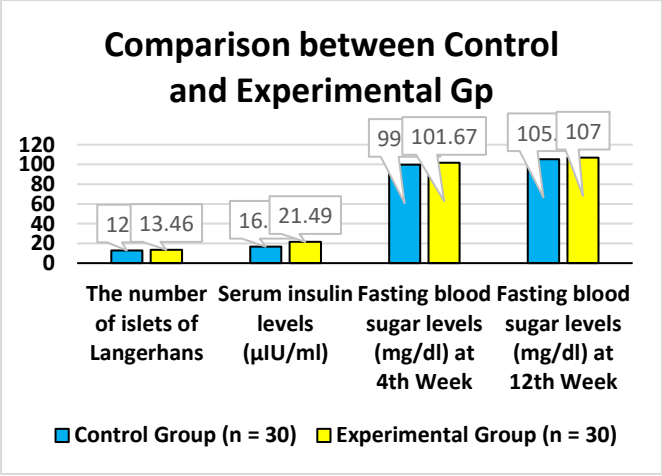

Figure 1: Comparison of mean number of islets of Langerhans, fasting blood sugar and serum insulin levels between the control and experimental groups.

Table 1: Comparison of mean number of islets of Langerhans and median area of islets of Langerhans (µm²), fasting blood sugar and serum insulin levels between the control and experimental groups.

Parameters	Control group (n = 30)	Experimental group (n = 30)	*p-value≤0.05
The number of islets of Langerhans (Mean ± SD)	12.90 ± 1.34	13.46 ± 1.97	0.200
The area of islets of Langerhans (µm²)	24642.85 ± 16256	52663.85 ± 38871	0.001*
Fasting blood sugar levels (mg/dl) at 4th Week (Mean ± SD)	99.68 ± 4.67	101.67 ± 4.77	0.108
Fasting blood sugar levels (mg/dl) at 12th Week (Mean ± SD)	105.40±17.57	107±17.92	0.646
Serum insulin levels (µIU/ml) (Mean ± SD)	16.72±5.38	21.49±7.03	0.005*

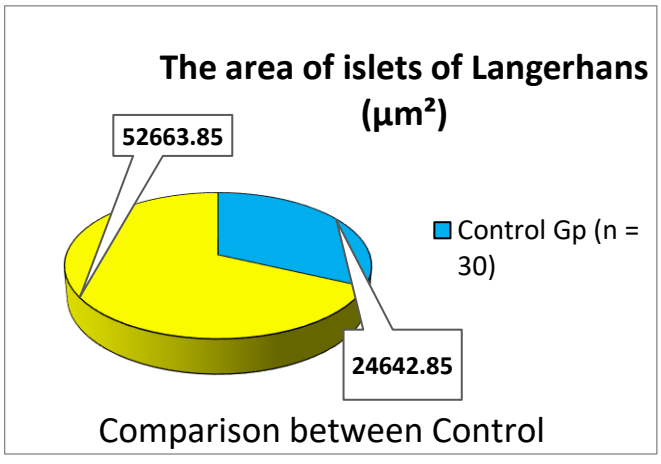

Figure 2: Comparison of median area of islets of Langerhans between the control and experimental groups.

The Median area of islets of Langerhans in the control group was 24642.85±16256 µm2) (Table 1) & (Figure 2).

The weekly fasting blood sugar levels from the first week to the twelfth week are shown in (Table 1) & (Figure 1). The serum insulin level at the end the study was 16.72±5.38 µIU/ml.

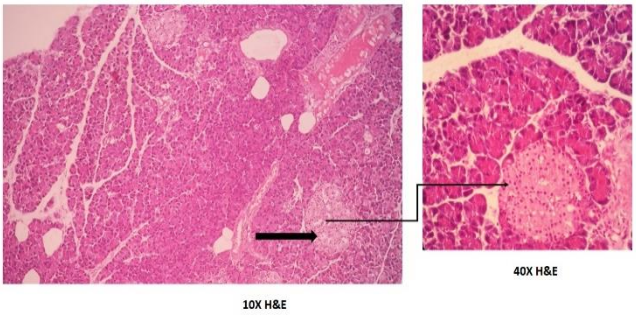

Figure 3: Photomicrograph of histological section of pancreas of rat in control group showing islets of Langerhans (Arrow)

The median area of islets of Langerhans was 24642.85 µm2. The area of islet of Langerhans was significantly higher in the experimental group than in the control group (p = 0.001) (Table 1) & (Figure 2). The weekly fasting blood sugar level from first week to 4th week and at 12 weeks between the groups was insignificant, p value = 0.108.

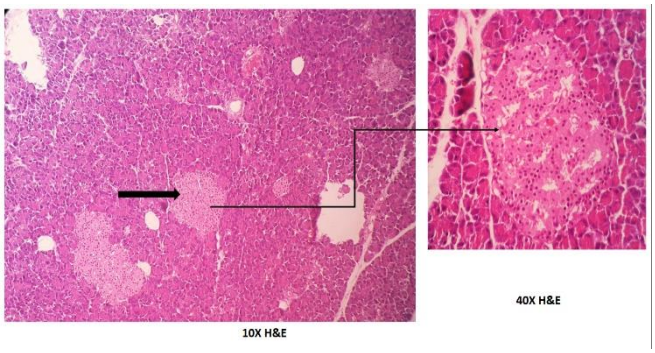

Figure 4: Photomicrograph of histological section of pancreas of rat in B group showing islets of Langerhans (Arrow).

The serum insulin level at the end of the study was 21.49±7.03 µIU/ml. The difference was statistically significant compared with control group (p = 0.005) (Table 1). Our results showed that the area of islets of Langerhans were significantly increased in the simvastatin group compared to the control group (p<0.001). In addition, insulin levels in the pancreas were significantly higher in the simvastatin group than in the control group (p<0.005).

4. Discussion

The endocrine function of the pancreas is performed by the islets of Langerhans. They comprise distinct cell clusters which are responsible for the production and secretion of insulin. Any structural or functional abnormality in the islets of Langerhans can result in impaired insulin secretion and succeeding hyperglycemia.[10] Hence, any intervention that can enhance the number of functional islets of Langerhans may increase insulin levels, this can be beneficial in individuals with type 2 diabetes mellitus.[11] Our research established that the administration of simvastatin to Sprague Dawley rats for 12 weeks lead to a significant increase in the area of the pancreatic islets of Langerhans i.e. indicating simvastatin induced increase in islet mass. This increase in size of islets suggests simvastatin may induce proliferation of pancreatic beta cells.[2] Previous research in rodents supports this, having shown that statins can increase beta cell area and mass, potentially by inhibiting apoptosis in islet cells.[12] The area of islets was substantially higher in the simvastatin-treated group compared to controls. It is known that in humans and animals, increased size of pancreatic islets and total islet area is directly proportional to increased insulin levels.[13] Thus, whenever insulin demand rises, there is a compensatory increase in the number and size of islets. Researchers have demonstrated the direct relationship between statins and insulin release.[14] It has also been observed that potential endocrine progenitor cells exist in the pancreatic duct walls.[15] These progenitor duct cells can form endocrine tissue not just during embryonic development but throughout life.[16] Any pancreatic insult, whether pathological or experimental, can lead to the efficient formation of new islets from these progenitor cells, even in adults. In the current study, pancreatic islet area was increased in the experimental group compared to controls. The likely explanation is that simvastatin increased insulin release, which resulted in a compensatory increase in islet area. It is known that increased insulin release directly correlates with increased islet area. Multiple studies confirm statins affect glucose metabolism and insulin secretion, causing an increase in insulin release.[17] This increased demand for insulin is compensated by expansion of the pancreatic islet area. Another concept is that pancreatic beta cells and islets of Langerhans have an inherent ability to regenerate in order to maintain homeostasis.[18] This regenerative capacity could also lead to increased islet area. While simvastatin altered islet morphology and insulin levels in rats, there were no significant effects on blood glucose regulation. This suggests intact counter regulatory mechanisms which prevented development of hypoglycemia despite elevated insulin. Catecholamine such as epinephrine potently counteract insulin activity, while other counter regulatory hormones participate in balancing glucose homeostasis.[19] Although simvastatin may modulate insulin secretion, redundant physiological mechanisms likely maintained glucose homeostasis. The mechanisms by which simvastatin induced islet cell proliferation and increased insulin secretion are incompletely understood. Proposed mechanisms include activation of AMPK, which regulates beta cell mass and insulin release.[19] Simvastatin may also inhibit the mevalonate pathway, thereby modulating insulin secretion.[14] Further studies are required to elucidate the molecular pathways involved in this process. This research makes unique contributions by specifically focusing on simvastatin's effects on islet morphology, unlike broader statin research. Methodologically, we employed innovative quantitative morphometric techniques to precisely analyze islet size and number. Moreover, we identified novel proliferative effects of simvastatin on beta cells, suggesting mechanisms for the observed islet changes. These insights extend understanding of statin-induced pancreatitis, informing clinical use. Although built on prior work, our focused examination, novel methods and findings offer distinctive value, significantly advancing knowledge on simvastatin's impacts on pancreatic islets.

Limitations: Our study provides initial insights into simvastatin's effects on rat pancreatic islets. However, as a single animal model, the findings may not extrapolate to humans. Further diverse model and human research is needed to validate and extend the results. Though foundational, uncontrolled factors like age, diet, and genetics may influence outcomes. Addressing such confounders is crucial for refining understanding of simvastatin’s complex impacts on pancreatic function across species. Overall, this study serves as a stepping stone for broader investigations of simvastatin’s effects on pancreatic function.

5. Conclusion

This study found simvastatin administration increased pancreatic islet area in rats, likely reflecting compensatory expansion in response to increased insulin demand. The islet changes did not disrupt glucose homeostasis, indicating intact counter-regulatory mechanisms. The results suggest simvastatin may induce beta cell proliferation and insulin release through mechanisms like AMPK activation, although molecular pathways need further elucidation. Overall, this study provides evidence that simvastatin can influence

pancreatic islet morphology and insulin dynamics through pathways warranting further investigation.

CONFLICTS OF INTEREST- None

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Potential competing interests: None to report

Contributions:

A.Q, H.G.K - Conception of study/ Designing /Planning
A.Q, H.G.K, H.K - Experimentation/Study Conduction
H.K, A.A - Analysis/Interpretation/Discussion
A.Q, S.B - Manuscript Writing
S.B, T.K - Critical Review
A.A, H.K, T.K - Facilitation and Material

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