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ORIGINAL ARTICLE

Cytoprotective Effects of Pancreatic Cells of Interleukin 1 Inhibitors (DIACEREIN) on Glucose Homeostasis and Beta Cell in Diabetic Rat Model

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ABSTRACT

Objective: This study was conducted to evaluate the pancreatic cells regeneration with interleukin 1 inhibitor drugs (diacerein) of natural source.

Material & Methods: This experimental study was conducted at the Isra University animal house in which 60 albino rats were divided into 4 subgroups of 10 in each. Experimental group of them given alloxan and induced diabetes. interleukin 1 inhibitor (diacerein) was given to 2 experimental groups, diabetic experimental C and D respectively, in dosages of 30 mg/kg and 50 mg/kg for 21 days. After the completion of the experiment, rats were sacrificed by cervical dislocation. The pancreas of rats was removed and set in 10% formalin for fixation after being washed with normal saline. For appropriate fixation, the slides were placed on a hot plate. Hematoxylin and eosin were used to stain the slides, and under the light microscope, stained slides were examined. The study proforma was used to document all of the data.

Results: Diabetic rats showed sustained hyperglycemia, total destruction of beta cells histologically. However, diabetic group rats showed degenerative changes of the pancreatic characterized by ultra-structural changes, shrinkage of beta islet cells, and showing RBCs and fibrosis of regions of endocrine glandular epithelial cells. Beta cells were showing mild regeneration with proliferative changes in the beta cells of islets as well as dilatation of ducts seen in treated groups with interleukin 1 inhibitor with lab findings of improving to euglycemic levels.

Conclusions: Pancreatic tissue of diabetic rats showed ultra-structural changes and regeneration, as well as reduction in inflammatory markers as well as obvious effects on D.M., hence would be used as a cheap, safe drug to control and reduce the oxidative injury of pancreatic cells and its regenerative effects.

Keywords: Diacerein, interleukin 1 inhibitor, regeneration of pancreas hyperglycemia, diabetes mellitus

INTRODUCTION

Currently, 382 million subjects are suffering from DM across the World (40–59 years), and estimated projections for year 2035 are approximately 532 million with a 55% rise in total, has been reported by the International Diabetes Federation (IDF).[1] Diabetes mellitus is a serious public health issue that affects both industrialized and developing nations. It is now the world's fourth most common non-communicable illness. About 1.5 million mortalities annually by the D.M have been recorded around the World globe.[2] The bad news is approximately 80% of DM subjects are living in the developing countries which is a threat for their poor economy. About 175 million Diabetics are reported as un-diagnosed which is an alarming situation. A United States reports revealed 548 billion US dollars were spent on the DM in 2013.[3] Pakistan is the world's 6th most populous nations by population and the world's 36th biggest nation according to geographic region.[4] Because there are no databases in Pakistan, national accurate statistics on the occurrence of DM is unavailable. According to estimates from the Diabetes Association of Pakistan (DAP) and the World Health Organization (WHO), the number of people with diabetes in Pakistan ranges from 6.39 to 16.5 percent, with an average prevalence of 11.47 percent.[5] Estimations of the IDF also show high prevalence of DM in Pakistan.[6] According to IDF projections, Pakistan will have 12.8 million diabetics by 2035.[7] Physiologically, the “β-cells” of the Islet of Langerhans play a critical role in glucose homeostasis in the body. A decrease in the “β-cells” because of any reason causes glucose metabolic disease which is commonly known as the “Diabetes mellitus” (DM).[8,9] The “β-cells” are prone to damage by various chemicals. One such “β-cells” toxic chemical agent used in experimental studies is known as the Alloxan. It is used to induce DM in animal models for analysis and invention of new anti-diabetic drugs.[10,11] Alloxan (2,4,5,6-pyrimidinetetrone) is a pyrimidine derivative. Brugnatelli (1761-1818) prepared the alloxan in 1818 and was named by Wöhler and Liebig (1838). In aqueous solutions, the alloxan exists as the Alloxan hydrate. Alloxan is used for induction of DM in experimental animal models because it is selectively β-cells destructor. Alloxan is a commonly used agent for this purpose. Alloxan induces β-cells destruction by induction of free oxygen radical formation called the reactive oxygen species (ROS),[10] and induces various inflammatory mediators, the mediators include the tumor necrosis factor-α (TNF-α) and the Interleukin-1 (IL-1).[11] The Interleukin-1 family (IL-1 family) is a family of cytokines. IL-1 family plays a physiological role in the regulation of immune and inflammatory responses. IL-1 inhibitors are now accessible in clinical trials for a variety of disorders. Diacerein, a semi-synthetic pro-drug, is one of the IL-1 inhibitor drugs. It is 4,5-bis (acetyloxy)-9,10-dihydro-9,10-dioxo-2-anthracene carboxylic acid in biochemistry. Diacerein is an anthraquinone derivative that has been refined. When taken orally, it is activated by the liver prior accessing the systemic circulation.[14,15] The hepatic and body cells convert diacerein to its active metabolite "Rhein." Rhein is the therapeutically important active biological chemical. Dose of Diacerein is 50 mg two times a day. But, during the first 2- 4 weeks, the Diacerein is prescribed once daily to avoid the adverse reactions.[14-16] Literature reviews showed its regenerative ability and anti-inflammatory effects in Rhematic arthritic patients hence the Diacerein is used now days for clinical trial to prove its efficacy in various degenerative cellular injuries. As diabetes mellitus is considered an ever-raising public health concern in Pakistan, currently added pharmacological agents must be scientifically evaluated for therapeutic and cryoprotective activities on the -cell of the Islets of Langerhans.[14] Although this study has been done to assess the cytoprotective effects of pancreatic cells of Interleukin 1 inhibitors (diacerein) on glucose homeostasis and beta cell in Diabetic rat models.

MATERIAL AND METHODS

This experimental study was done at Physiology department of Isra university Hyderabad after approval of the Ethics Committee, with collaboration of animal house of Tandojam. In this study Sixty male Wistar rats, approximately 200-250 g, were selected for the experiment. Sick rats and rats less than 200gm and rats more than 300gm were excluded. Rats were kept in metal cages with 25◦C with controlled day light cycle received standard balanced chow for albino rats.

Animals were randomly assigned to 4 groups:

Group A= 15 control rats were kept on normal diet

Group B: 15 diabetic induced rats

Group C: 15 diabetic induced rats were diacerein 30 mg as a treatment

Group D: 15 diabetic induced rats were diacerein 50 mg as a treatment

A single intraperitoneal injection of 50 mg/kg monohydrated alloxan (Sigma, St. Louis, MO, USA) dissolved in sterile 0.9 percent saline was used to induce diabetes. Prior to getting alloxan, the rats were fasting. To avoid hypoglycemia, the animals were given a 10% glucose solution after 12 hours. Blood samples were obtained from the animals' tail veins after 72 hours to assess plasma glucose levels using the Accu-Chek Advantage system (glucose-oxidase enzymatic method). The diabetes group consisted of animals with blood glucose levels more than 200 mg/dL.

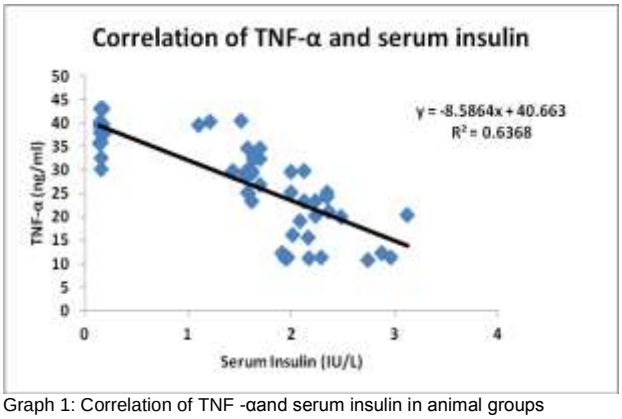
Diacerein was administered to the rats orally for a period of 6 weeks. The medicine was ground into a powder, combined with water to a concentration of 30 and 50 mg/kg, and administered to experimental groups C and D for 6 weeks. After completion of experiment body weight was measured on electronic measuring balance and all the rats were sacrificed by cervical dislocation. Pancreas of rats were removed and following washing with normal saline, any gross abnormalities and gross morphological parameters (weight & size) were recorded and pancreas were set in 10% formalin for fixation. Tissue was passed in ascending grades of ethyl alcohol (70%, 80%, 90%, 100%). Tissue was passed in xylene for clearing. Tissue was embedded in paraffin wax and hardened paraffin blocks were obtained. 4 micrometer sections were cut on Microtome. For appropriate fixation, the slides were placed on a hot plate. Hematoxylins and eosin were used to stain the slides and under the light microscope, stained slides were examined. The research proforma was used to document all of the data, and SPSS version 26 was applied to analyze it.

RESULTS

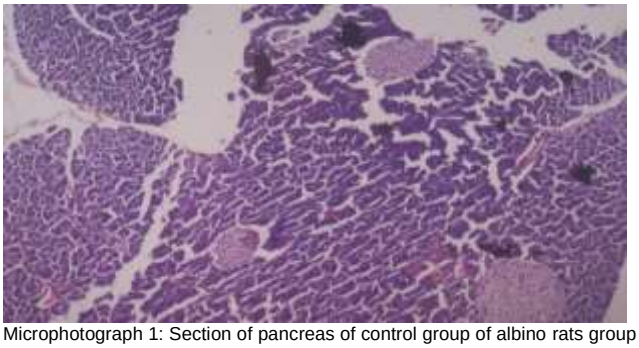
The average body weight of all the groups A, B, C and D was noted as 241.53±28.64, 192.13±22.40, 202.07±25.50 and 211.50±23.21 grams respectively (P=0.043). Significant weight loss was noted in the Diabetic control (Group B). Experimental Diabetic rats (group C and D) showed a decrease in body weight but it was not as much severe as in the Diabetic controls (Group B). This reveals the Diacerein prevents the body weight loss. Diabetic control group B showed severe decline in insulin secretion i.e. 0.29±0.35 IU/L. The Diacerein treated Diabetic experimental rats (group C and D) shows an increase of insulin to 1.38±0.51 and 1.90± 0.32IU/L respectively (P=0.003). This points towards the diacerein stimulating the β- cell functioning because the insulin secretion is exclusive function of the cells. TNF-α show significant increase in the Diabetic control (37.85± 3.61 ng/ml) compared to Diacerein treated Diabetic experimental rats. This reveals the Diacerein exerts anti-inflammatory activity because the TNF-α levels were found low in the diacerein treated experimental rats. Table.1There was a significant negative correlation between Tumor necrosis factor-α and insulin level (r=- 0.798, P=0.0001). fig.1 Histological effects of induced diabetes and cytoprotective effects of diacerein shown in microphotograph 1-4

Table 1: Serum Insulin (IU/L) and Tumor necrosis factor-α (ng/ml) in animal groups (n=60)

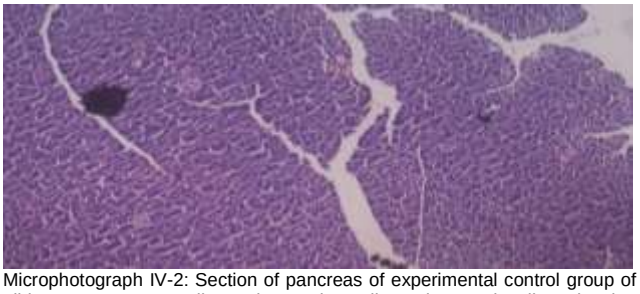
Variables		Mean	SD	P-value
Serum Insulin (IU/L)	Group A	2.35	0.40	0.003
	Group B	0.29	0.35	
	Group C	1.38	0.51	
	Group D	1.90	0.32	
Tumor necrosis factor-α (ng/ml)	Group A	14.99	4.14	0.0001
	Group B	37.85	3.61	
	Group C	32.74	4.09	
	Group D	26.24	2.64	



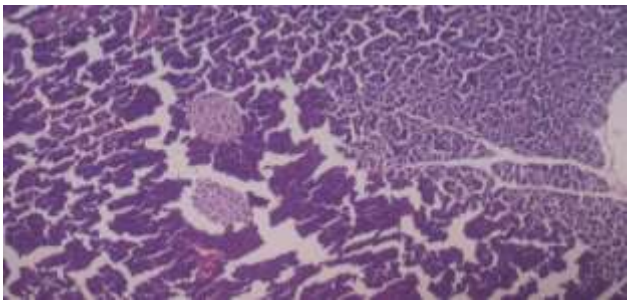
Graph 1: Correlation of TNF -αand serum insulin in animal groups



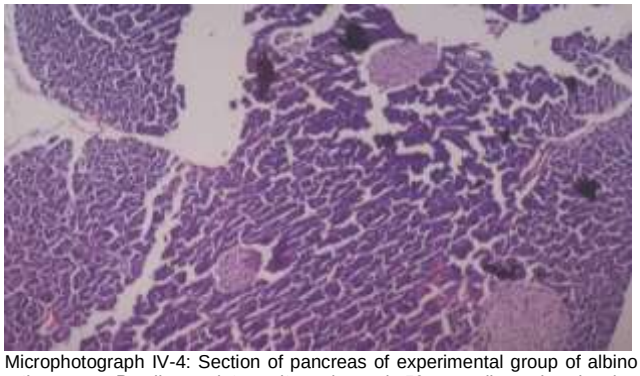
Microphotograph 1: Section of pancreas of control group of albino rats group A (controls) showing normal structure of pancreas composed of normal size and shape of islets of Langerhans, exocrine glands, normal pancreatic ducts and blood vessels (H&E) (x100).



Microphotograph IV-2: Section of pancreas of experimental control group of albino rats group B allaxon intraperitoneally and normal saline showing degenerative changes shrinkage of islets of pancreas islets of Langerhans, exocrine glands, shrinked pancreatic ducts and blood vessels are visible. (H&E) (x100)



Microphotograph IV-3: Section of pancreas of experimental group of albino rats group C alloxan intraperitoneally and 30 mg diacerein showing regenerative changes of islets of pancreas. Islets of Langerhans, exocrine glands, regenerated pancreatic ducts and blood vessels are visible (H&E) (x100).



Microphotograph IV-4: Section of pancreas of experimental group of albino rat's group D alloxan intraperitonealy and 50 mg diacerein showing regenerative changes of islets of pancreas. Islets of Langerhans, exocrine glands, regenerated pancreatic ducts and blood vessels are visible (H&E) (x100).

DISCUSSION

Diacerein is a drug that is often used in the treatment of articular ailments due to its anti-inflammatory properties.[17] Diacerein reduces cytokine levels, particularly TNF-a and IL-1b, by an unknown mechanism. As a result, if diacerein is administered to obese individuals having type II diabetes, it may lower cytokines, enhance insulin production and, most likely, insulin release, and therefore enhance glucose control.[17] Present study was the first, in which evaluated the cytoprotective effects of pancreatic cells of Interleukin 1 inhibitors (diacerein) on glucose homeostasis and beta cell in Diabetic rat models. In this study allaxon intraperitoneally and normal saline showing degenerative changes shrinkage of islets of pancreas, while with alloxan intraperitoneally administration of 30 mg diacerein showing regenerative changes of islets of pancreas and alloxan intraperitoneally administration with 50 mg diacerein showing more regenerative changes of islets of pancreas. On other hand Burke SJ et al[18] observed that the pancreatic IL-1R has a key physiological function in glucose homeostasis by decreasing Aldh1a3 expression, maintaining MafA abundance, and promoting glucose-stimulated insulin secretion in vivo. Recently it have been showed that knocking down the IL-1R antagonist protein results in increased signaling through the IL-1R pathway, which inhibits -cell growth.[19] In vitro studies have shown that when the IL-1 pathway is activated, the incorporation of radiolabeled nucleotides into DNA (a measure of proliferation) is reduced.[20] Postprandial rises in IL-1 enhance glucose elimination from a physiological standpoint, limiting physiological IL-1 signaling diminishes glucose tolerance without compromising insulin sensitivity, but too much IL-1 signaling is deleterious to -cell health.[18-21] Endogenous pancreatic β - cell restoration might be used to treat diabetes by cellular growth or neogenesis. The encouragement of current cell replication or the conversion of additional pancreatic cells into cells can both lead to regeneration. Several methodologies and procedures for stimulating endogenous cell regeneration have recently been studied, but none of them are acceptable for therapeutic use.[22] Furthermore it is suggested that the to increase flexibility in clinical applications, researchers recommend concentrating on the functioning and immunogenicity of fresh pancreatic cells.[22] Following that, efforts should be made to better understand the properties of pancreatic islets, islet cells, and new insulin-producing cells.[22] Interleukin-1 (IL-1)–induced IL-1 signaling has recently been proposed as a possible target for -cell regeneration. Studies have indicated that IL-1 expression is substantially increased in the pancreatic islets of individuals having type II diabetes, and that human -cells are susceptible to both IL-1–induced apoptosis and functioning impairment, implying that IL-1 may play a role in T2DM development.[23,24] In this experiment, alloxan-induced diabetic rats administered using diacerein had enhanced histological exams and tissue reduction. Similar findings were reported by Du et al[25], who found that pancreatic histology was enhanced by preserving cell mass and inhibiting cell death through an immunohistochemistry analysis. The current research's histoprotective results are consistent with the previous study. The product's implementation is still controversial due to various limitations of the current study, however more large-scale studies are suggested before confidential usage.

CONCLUSION

Pancreatic tissue of diabetic rats showed ultra-structural changes regeneration, as well as reduction in inflammatory markers as well as obvious effects on D.M hence would be used as cheap, safe drug to control and reduce the oxidative injury of pancreatic cells and it regenerative effects. Although the Diacerein exerts can be used as a choice of drug for type II DM for its cytoprotective effect on β-cell of the Islets of Langerhans of the pancreas, Further experimental human studies to evaluate the physiological and cytoprotective effects of Interleukin-1 inhibitor (Diacerein) on β-cell of the Islets of Langerhan's of the pancreas.

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