ORIGINAL ARTICLE

EFFECTS OF INTERLEUKIN 1 INHIBITOR ON INFLAMMATORY CYTOKINES TNF-ALPHA LEVELS IN DIABETIC ALBINO WISTAR RAT MODEL

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Background: Diacerein is a second generation Interleukin-1 inhibitor (IL-1). IL-1 and Tumour Necrosis Factor Alpha (TNF-α), are inhibitors of the pancreatic β-cells secretory activity and proliferation. There is need to test Diacerein on IL-1-TNF-α axis to improves the β-cell functions and proliferation. This study was designed to determine the relation of inflammatory markers and diabetes mellitus in interleukin 1 inhibitors treated albino Wistar rat models. **Methods:** A sample of 60 albino Wistar rats, selected by convenient purposive sampling, was divided into 4 groups: Group A (Controls), Group B (given Alloxan 50 mg/Kg, i.p.), Group C (Diabetic rats given Diacerein 30 mg/Kg for 21 days) and Group D (Diabetic rats given Diacerein 50 mg/Kg for 21 days). The data was analyzed using SPSS-22. **Results:** The Mean±SD of blood glucose in the groups A, B, C, and D was noted as 113.66±10.67, 253.19±63.71, 215.23±36.32 and 178.0±16.52 mg/dl respectively (F=36.77, *p*=0.010). The Mean±SD of HbA1c in the groups A, B, C and D was noted as 4.14±0.86, 9.85±0.97, 7.52±0.78 and 6.71±1.02 mg/dl respectively (F=69.58, *p*=0.018). The Mean±SD of TNF-α in the groups A, B, C, and D was noted as 14.99±4.14, 37.85±3.61, 32.74±4.09, and 26.24±2.64 mg/dl respectively. **Conclusion:** Our findings suggested that inflammatory markers (TNF-α) were decrease and good glycaemic control with interleukin 1 inhibitors (Diacerein) treated diabetic rats.

Keywords: Interleukin-1 inhibitor, Alloxan, Diabetes Mellitus, TNF-α
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INTRODUCTION

Diabetes mellitus (DM) is a major public health problem of both developed and developing countries. It ranks 4th among non-communicable disease of the World and causes 1.5 million mortalities annually around the globe.[1]

Pakistan ranks 6th populous country, suffering the current epidemic of DM.[2] National data on the prevalence of DM is lacking in Pakistan because of no registries. Estimates by the Pakistan Diabetes Association of Pakistan (DAP) and WHO show a prevalence of 6.39–16.5% with mean DM prevalence of 11.47%.[3,4] Projected estimates of IDF reveal a diabetic load of 12.8 million in Pakistan by the year 2035.[5]

β-cells of Islet of Langerhans play a pivotal role in glucose homeostasis. The 'β-cells' are prone to damage by various chemicals. One such 'β-cell' damage is being hypothesized by increasing various inflammatory and immune modulations chemical agents such as cytokines and interleukins. One such cytokine, The Interleukin-1 family (IL-1 family) plays a physiological role in the regulation of immune and inflammatory responses.

The Tumour Necrosis Factor Alpha (TNF-α) is also termed as the cachexin or cachectin is a cell signalling cytokine protein of systemic inflammatory reactions. TNF-α is an acute phase protein, mainly secreted by macrophages followed by natural killer (NK) cells, CD4+ lymphocytes, mast cells, basophils, and neurons.[6,7]

Currently, the IL-1 inhibitors are available for clinical use for various diseases. One of IL-1 inhibitor drug agent is Diacerein which is a semi-synthetic pro-drug. Biochemically, it is 4,5-bis (acetyloxy)-9,10-dihydro-9,10-dioxo-2-anthracene carboxylic acid. It is a purified anthraquinonoid derivative. When taken orally, it passes through liver, and is activated before reaching the systemic circulation.[8,9] Diacerein is converted into active metabolite 'Rhein' by liver and body cells which is the active biological molecule of therapeutic interest. Prescribed dose of Diacerein is 50 mg twice daily in immune and inflammatory conditions.

Diacerein is a semi-synthetic pro-drug of natural source. Rhein exhibits IL-1 inhibitory effects. It improves the IL-1 induced damage of cartilage, bone, synovial membrane and fluid including β-cell of Pancreas. It increases gene expression of transforming growth factor (TGF)[10] which stimulates the extracellular matrix. Release of pro-inflammatory cytokines is decreased by Rhein. It also inhibits phagocytosis.[11,12] Diacerein is a second generation Interleukin-1 inhibitor compared to Anakinra which was the first anti IL-1 receptor inhibitor used clinically. The interleukin-1 and TNF-α are reported to inhibit the pancreatic β-cells secretory activity, inhibition of β-cell proliferation and increased apoptosis of β-cell. There is need of study on IL-1-TNF-α axis for preparing new drugs and testifying the available agents such as Diacerein. IL-1 receptors are found on the β-cells of pancreatic islets.

As DM is estimated as increasing public health problem in Pakistan, newly available drug agents need scientific analysis of therapeutic and cyto-protective effect on β-cell of Islets of Langerhans. The present study was designed to determine effects of Interleukin-1 inhibitor Diacerein on various inflammatory markers especially TNF alpha, and its ameliorating effects on β-cells and Diabetes mellitus.

METHODOLOGY

Animal Experimentation Ethics Committee of Isra University Hyderabad Campus approved the study. Sixty male Wistar rats, approximately 200–250 g, were used in this experiment. Rats were kept in metal cages with 25 °C with controlled day light cycle and received standard balanced chow for albino rats.

Animals were randomly divided into 4 equal experimental groups: group A (Control Rats, CR,); group B (Diabetics without treatment); group C and D had 15 diabetic rats each, treated with Diacerein 30 mg and 50 mg respectively for 21 days.

Diabetes was induced with single intraperitoneal injection of 50 mg/Kg monohydrated Alloxan (Sigma, St. Louis, MO, USA) dissolved in sterile 0.9% saline. Rats were fasting prior to Alloxan administration. After 12 hours, a 10% glucose solution was given to the animals to prevent hypoglycaemia. After 72 hours, blood samples were collected from the tail vein of the animals for evaluation of plasma glucose levels using Accu-Chek Advantage (glucose-oxidase enzymatic method) (Boehringer, Germany). Animals presenting glucose levels above 200 mg/dL were included in the diabetic group.[13]

Experimental rats were given diacerein orally for 6 weeks duration. The drug was smashed into powder, mixed into water to a concentration of 30 and 50 mg/Kg. This amount of Diacerein was given daily for 6 weeks. All animals were randomly assigned into 4 groups as control (A), and experimental (B, C and D) groups. Rats from each of control group A and experimental groups B, C, D were selected for biochemical analysis. Each rat was deeply anesthetized by an overdose of chloroform; blood was drawn from the tail or through a cardiac puncture and placed in gel tubes. Serum was isolated from the clotted blood by centrifugation. The sera was used to determine the blood glucose, Glycated Haemoglobin A1, TNF-α before and after intervention of drug. Blood Glucose and Glycated Haemoglobin A1c levels were estimated on Hitachi Roche Diagnostics Chemistry Analyzer. The HbA1c measurement was based on the turbid metric inhibition immunoassay (TINIA) haemolysis of whole blood. Tumour Necrosis Factor-α was estimated with ELISA kit assay method. The study was performed at the Postgraduate Laboratory and Clinical Laboratory of Isra University, Hyderabad.

Data analysis was done on SPSS-22. Continuous and categorical variables were analyzed using students *t*-test, ANOVA with post-Hoc testing, and Chi-square tests, and $p \leq 0.05$ was considered significant.

RESULTS

In this study mean blood glucose in groups A, B, C, and D were 113.66±10.67, 253.19±63.71, 215.23±36.32 and 178.0±16.52 mg/dl respectively was significant (F=36.77, p=0.010) (Table-1).

The mean HbA1c in the groups A, B, C, and D was 4.14±0.86, 9.85±0.97, 7.52±0.78, and 6.71±1.02 mg/dl respectively (F=69.58, p=0.018) (Table-2). Mean TNF-α in groups A, B, C, and D was 14.99±4.14, 37.85±3.61, 32.74±4.09, and 26.24±2.64 mg/dl respectively which showed significant changes in TNF-α in animals treated with 50 mg/Kg as compared to 30 mg/Kg (Table-3).

Table-1: Blood glucose levels (mg/dl) among the animal groups

Group	Mean±SD	F	*P*
A	113.66±10.67	36.77	0.010
B	253.19±63.71		
C	215.23±36.32		
D	178.00±16.52		

Table-2: Glycated haemoglobin A1 in animal groups (%)

Group	Mean±SD	F	*p*
A	5.14±0.86	69.58	0.018
B	9.85±0.97		
C	7.52±0.78		
D	6.71±1.02		

Table-3: TNF-α (ηg/ml) in animal groups

Group	Mean±SD	F	*P*
A	14.99±4.14	108.40	0.0001
B	37.85±3.61		
C	32.74±4.09		
D	26.24±2.64		

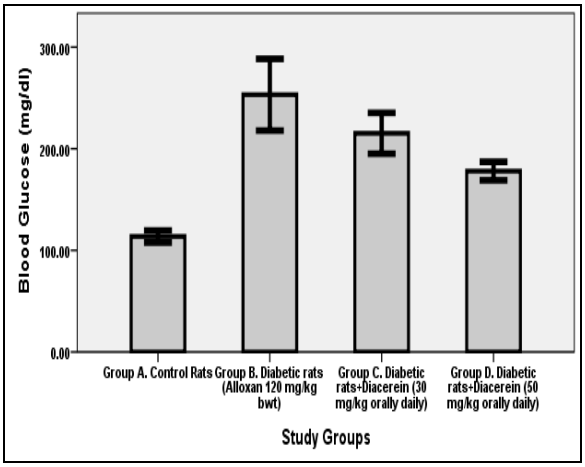

Figure-1: Blood glucose in animal groups

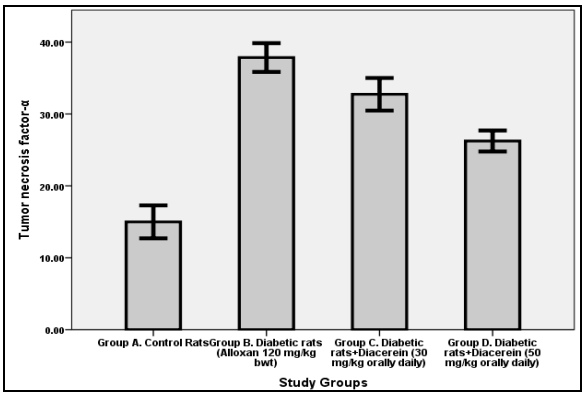

Figure-2: TNF-α (ηg/ml) in animal group

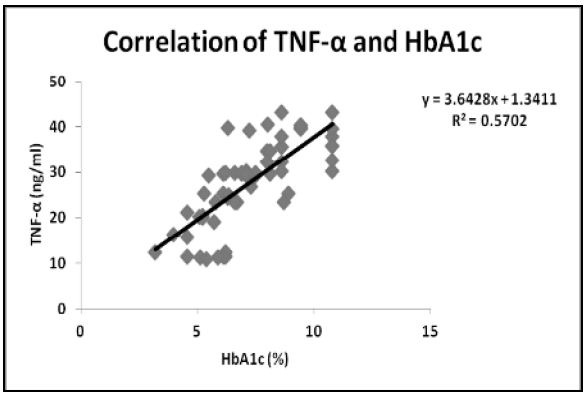

Figure-3: Correlation of TNF-α and HbA1c

DISCUSSION

This study determined the effects of Interleukin-1 inhibitor Diacerein on inflammatory marker as well as its changes on glucose haemostasis and its cyto-protective effects on β-cell of Islets of Langerhan in pancreas. The body weight of rats, blood glucose, HbA1c, serum insulin, and TNF-α were analyzed to evaluate the effects of Diacerein in diabetes induced rat model. IL-I inhibitor, Diacerein was proved of showing anti-diabetic activity through evidence based cytoprotective effect of interleukin-1 inhibitor on β-cells of Islets of Langerhans in Alloxan induced diabetic albino rats. Inhibition of IL-1 receptors improves the β-cell functions, insulin release and proliferation of β-cell, and reduced pro-inflammatory cytokines (TNF-α). These cascades of physiological events are reported to improve the glucose homeostasis in diabetics.[14,15]

In our study serum blood glucose ,HbA1C , TNF-α, were reduced and body weight serum blood glucose, HbA1c, serum insulin and body weight were improved in diacerein treated diabetic rats. The findings of present study are in agreement with previous studies.[15–17] A recent clinical trial of 2 months diacerein therapy in type 2 diabetics reported increased β-cell secretory physiology.[17] The findings of above clinical trial were consistent with present study. In the present study the blood glucose, and glycated HbA1c were reduced to normal in diabetic rats, and anti-insulin cytokine TNF-α was decreased by diacerein therapy. The underlying mechanisms of diacerein could be through direct cytoprotective effects on β-cells and inhibition of pro-inflammatory cytokines. The present study postulates that diacerein may prove a novel agent for DM, similar to conclusion of Du *et al*[18] as they concluded the Rhein/Diacerein would be novel therapeutic agent in near future. However, the present study included TNF-α findings also, which were not studied by Du *et al*[18].

A recent study by Zanchi *et al*[19] has reported conflicting results for diacerein. They studied restoration of physiology of endoplasmic reticulum and INS1-E β-cells activity. They concluded no effects on the research parameters. The above study was advanced in terms of research variables, but the overall results did not support the efficacy of Diacerein. Lack of efficacy of diacerein in above study is controversial to present study and previous studies[20,21]. The reason could be differences in molecular study techniques and research bias.

CONCLUSION

Alloxan induced diabetic rats treated with Diacerein showed amelioration of the blood glucose, HbA1c, serum insulin levels and reduction in pro-inflammatory markers like TNF-α. Diacerein extracts can be used as a choice of drug for type II DM because of its cytoprotective effect on β-cell of Islets of Langerhans in Pancreas.

RECOMMENDATION

The present study recommends further experimental human studies to evaluate the physiological and cytoprotective effects of Interleukin-1 inhibitor (Diacerein) on β-cell of Islets of Langerhans's of Pancreas.

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