Anti-hyperglycemic Effects of Diacerein in Alloxan-Induced Diabetes Mellitus in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AU designed the draft and did the data collection. Author SS contributed in the data collection and manuscript writing. Authors RA and YMN managed the analyses of the study and review literature. Authors YMN and NK managed the literature searches and guideline. All authors read and approved the final manuscript.

Short Research Article

ABSTRACT

Objective: To determine the anti-hyperglycemic effects of interleukin-1 inhibitor (diacerein) in alloxan induced diabetic albino wistar rats.
This experimental study was performed at the Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam within 6 months from April 2016 to September 2016. Total of 160 adult Albino Wistar Rats having an average of 200 to 300 grams body weights were selected. Animals were categorized into 4 groups as;

- Group A (n=15): Control rats – receive 0.9% normal saline as placebo
  Experimental Groups
- Group B (n=15): Experimental Control (Diabetic rats) - Alloxan50 mg/kg body weight intraperitoneal.
- Group C (n=15): Diabetic rats + Diacerein (30 mg/kg/day) orally daily.
- Group D (n=15): Diabetic rats + Diacerein (50 mg/kg/day) orally daily.

Animals were kept and treated as per the NIH Guideline for Use and Care of Laboratory Animals.
Diabetes mellitus was induced via a single intraperitoneal injection of 50 milligram/kg alloxan monohydrated dissolved in aseptic 0.9% saline. After 72 hours, blood specimens were taken from

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the caudal vein of the rats and glucose level>200 mg/dL was taken as diabetes. Experimental rats were given diacerein approximately 30 and 50 mg orally for 6 weeks. At the completion of experiment the body weight was measured of each animal by electronic measuring balance and blood sample was taken from each animal of all groups to assess the blood glucose level and HbA1c level. Data were recorded via self-made proforma and analysis was done by using SPSS version 20.

Results: Average body weight of Diabetic control (Group B) was 193.33±22.50 grams, which was lower in contrast to Diacerein treated group C 202.47±25.70 grams and significantly lower as compared to Diacerein treated group D as 212.6±23.43 grams. A significant increase in blood glucose levels 182.07±10.63 mg/dl was noted in the Diabetic control (Group B) compared to Diacerein treated group C (110.13± 8.54 mg/dl) and group D (85.87±8.41 mg/dl) (P=0.001). HbA1c was markedly raised in the Group B- diabetic controls, while diacerein treated diabetic rats (groups C and D) showed a significant decrease in HbA1c (P=0.001).

Conclusion: It was concluded that Diacerein achieves the Euglycemic state by reducing the levels of blood glucose and glycated hemoglobin (HbA1c) in Alloxan-Induced diabetes mellitus in Wistar Albino Rats.

1. INTRODUCTION

Diabetes mellitus (DM) is a common metabolic disorder. DM affects 200 million individuals globally and causes around 32,000,000 deaths yearly [1]. Its prevalence has been on the rise since last thirty years and is increasing exponentially around the world. In 1985 the prevalence of DM was 30 million which rose to 285,000,000 by 2010 and it has been predicted that by 2030, the number will to rise to 439 million [2,3]. Pakistan ranks at 7th position among the largest populations of diabetic cases globally and by 2025 this rank is predicted to take the4th place [4]. As stated by the International Diabetes Federation (IDF), in 2003 the prevalence of DM in Pakistan was 6200,000 individuals, which was anticipated to be more than 14.5 million individuals by 2025 [4]. Around 10% adult population of Pakistan has DM whereas the remaining 10% of individuals are struggling withthe glucose tolerance impairment [5,6]. Patients suffering from Diabetes mellitus usually experience a 10%-30% reduction in life expectancy, primarily because of early death and complications due to DM at an early age [7,8]. These complications have been categorized into chronic and acute. Chronic complications have further been categorized into micro-vascular and macro-vascular complications. Early complications of diabetes mellitus are hyperosmolar hyperglycemic state, diabetic ketoacidosis (DKA), hypoglycemic diabetic coma, periodontal diseases, and respiratory infections. Chronic complications are diabetic retinopathy, diabetic neuropathy (autonomic, motor and sensory) and diabetic kidney disease, while macrovascular complications are peripheral vascular disease partaking in intermittent claudication, coronary artery disease resulting in myocardial infarction or angina, Cerebrovascular incidences such as transient ischemic attack and stroke, diabetic foot and diabetic encephalopathy. The complications of diabetes are markedly reduced if the patient shows good glycemic control [9,10]. DM needs a multidimensional administration plan where subjects make well-versed decisions regarding weight, exercise, diet, blood sugar monitoring, blood pressure regulation, intake of medicines and regulation of various complications. Currently, many drugs are available for its management, but none of them modify the course of the disease. Hence, there is a need for research to overcome the problem of hyperglycemia, and its associated complications [1].

Diacereinis a highly purified anthraquinone derivative [11]. Itis a partial chemical prodrug, which is metabolize by the liver into active metabolite before entering the circulatory pathway. It was extracted from the "Aloe Barbadensis" and "Aloe Ferox". The active ingredient was named as the "Aloin", which was used for the preparation of diacerein. The Aloin was acetylated, followed by deglycosylation to produce diacerein [11,12]. Biochemically, it is diacetylrhein and is commonly named "*Diacerein*". In the human body, diacetylrhein is converted into "Rhein" which is the active biological molecule of therapeutic interest [13,14].

Interleukin-1 beta, inhibited by Rhein, is a cytokine protein indicated for inflammatory obliteration of cartilage and contributes to symptoms development of osteoarthritis. Diacerein has a specific mechanism of action i.e. it does not inhibit Prostaglandin synthesis in joint tissues. Diacerein has been proved to have a cartilage sparing and anti-osteoarthritis effects both in-vivo and in-vitro animal prototypes [13,15,16]. Because of its outstanding gastrointestinal tolerance, a polytherapy with NSAID or an analgesic drug can possibly be suggested in the course of the first 2-4 weeks of treatment [11,13]. Diabetes mellitus is a major public health issue in Pakistan and is a newly available agents that must be researched for its anti-hyperglycemic potential and cytoprotective effect on β-cell physiology. There is a need to evaluate newer agents like Diacerein to overcome the problem of Diabetes mellitus. However present study has been conducted to determine the anti-hyperglycemic effects of interleukin-1 inhibitor (diacerein) in alloxan-induced diabetic albino Wistar rats.

2. MATERIALS AND METHODS

This experimental study was performed at the Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam within 6 months from April 2016 to September 2016. A total of 160 adult Albino Wistar Rats of both male and female and having an average of 200 to 300 grams body weights were selected.

All the animals were fed with standard chow comprising of chick feed, wheat flour and milk with a scientifically ratified composition in line with directions of veterinary experts. The chow was provided as raw food. All the sick rats of weight <200 gm and >300 gm were excluded. All the animals were categorized into 4 groups as;

- **Group A (n=15)**: Control rats – receive 0.9% normal saline as placebo
  Experimental Groups
- **Group B (n=15)**: Experimental Control (Diabetic rats) - Alloxan50 mg/kg body weight intraperitoneal.
- **Group C (n=15)**: Diabetic rats + Diacerein (30 mg/kg/day) orally daily.
- **Group D (n=15)**: Diabetic rats + Diacerein (50 mg/kg/day) orally daily.

Administration of Alloxan was done at 50 mg/kg body weight to induce Diabetes mellitus. The dose of Diacerein was based on a previous study.

The animals were kept and treated as per the NIH Guideline for Use and Care of Laboratory Animals.

Rats were housed in cages made of stainless steel and were furnished with feed containers. A well ventilated and hygienic environment was provided for the rats. The rats were given tap water and food *ad libitum*. The dark and light cycle was maintained at the intervals of 12hrs. All measures for animals were carried out under standard animal protocols. The respective group cages of the rats were labeled with tags.

Diabetes mellitus was induced via a single intraperitoneal injection of 50 milligram/kg alloxan monohydrated dissolved in aseptic 0.9% saline. Rats were restricted to feed before alloxan management. After12 hrs, a solution of 10% glucose was given to the rats to avoid hypoglycemia. After 72 hrs, blood specimens were taken from the caudal vein of the rats to assess the levels of plasma glucose by Accu-Chek Advantage (enzymatic glucose-oxidase procedure; Boehringer, Germany). Animals with glucose level>200 mg/dL were considered diabetic.

Experimental rats were given diacerein orally for 6 weeks duration. The drug was powdered, mixed with water to make a solution. The final drug administered was approximately 30 and 50 mg. This amount of diacerein was given daily for six weeks.

At the completion of experiment the body weight was measured of each animal by electronic measuring balance and blood sample was taken from each animal of all groups to assess the blood glucose level and HbA1c level. Blood sugar was estimated by the glucose oxidase method on HRDC Analyzer (Roche Diagnostics, USA). Data were recorded in the self-made proformat and analyzed using SPSS version 20. The mean and standard deviation were calculated for numerical variables like body weight, blood glucose level and HbA1C level. ANOVA was applied and a p-value ≤ 0.05 was considered as significant.

3. RESULTS

The mean body weight in the groups A, B, C and D was noted as 243.73±29.74, 193.33±22.50, 202.47±25.70 and 212.6±23.43 grams, respectively. Significant weight loss was noted in Diabetic control (Group B). Diacerein treated Diabetic rats (group C and D) also revealed a decrease in body weight but it was less compared to the Diabetic controls (Group B).This reveals that Diacerein prevented a significant weight loss (Table 1).

The mean of blood glucose in the group's A, B, C, and D was noted as 111.33±10.97, 182.07±10.63, 110.13±8.54, and 85.87±8.41 mg/dl, respectively. A significant increase in blood glucose levels was noted in the Diabetic control (Group B) compared to Diacerein treated Diabetic rats (group C and D) which revealed a decrease in blood glucose. This reveals that Diacerein prevented hyperglycemia significantly (Table 2).

The mean of HbA1c in the group's A, B, C, and D was noted as 6.08± 0.66, 8.98± 1.105, 6.72±0.57 and 6.63±1.04 mg/dl respectively (P=0.0001). HbA1c was markedly raised in the Group B-diabetic controls. While diacerein treated diabetic rats (groups C and D) showed a significant decrease in HbA1c for diacerein treated rats was indicated by bar height p-values were quite significant (Table 3).

4. DISCUSSION

The present research is describing the role of Diacerein, an interleukin-1 inhibitor, in alloxan-induced Wistar rat from the Pakistan. The study evaluated the outcome of β-cell physiology by estimating and comparing the blood sugar and insulin in controls, diabetic controls and diacerein treated diabetic rats. The experimental and clinical trials have reported that diacereinis effective in treating diabetes mellitus [17], left ventricular dysfunction [18] and relieving the visceral pain [19]. A previous study reported its use in breast cancer [20]. A recent study reported that Diacerein was effective in protecting kidneys against drug-induced nephrotoxicity in animal model [21]. Diacerein is a semi-synthetic pro-drug, converted into "rhein" by the liver. Rheininhibits the activity of IL-1 in various tissues such as superficial and deep layers of the cartilage, the synovial membrane, synovial fluid and other body tissues including the β-cell of Pancreas. Rhein stimulates transforming growth factor (TGF) expression and extracellular matrix [14-16]. Rhein inhibits the synthesis of proinflammatory cytokines and phagocytosis by macrophages [13,14].

The present study reveals body weight, blood glucose, HbA1c, serum insulin and GPX were significantly improved in diacerein treated diabetic rats. The findings of current study are

Table 1. Body weight in animal groups (grams) (n=60)

Animal groups	Mean+SD		P-value
Group AXB	243.73+29.74	193.33+22.50	0.001
Group AXC	243.73+29.74	212.60+23.43	0.001
Group AXD	243.73+29.74	202.47+25.70	0.001
Group BXC	193.33+22.50	202.47+25.70	0.374
Group BXD	193.33+22.50	212.60+23.43	0.006

Group A= Controls, Group B= Diabetic controls;
Group C= Diabetic rats + Diacerein (30 mg/kg bwt)
Group D= Diabetic rats + Diacerein (50 mg/kg bwt)

Table 2. Blood glucose in animal groups (mg/dl) (n=60)

Animal groups	Mean+SD		P-value
Group AXB	111.33+10.97	182.07+10.63	0.001
Group AXC	111.33+10.97	110.13+8.54	0.996
Group AXD	111.33+10.97	85.87+8.41	0.001
Group BXC	182.07+10.63	110.13+8.54	0.001
Group BXD	182.07+10.63	85.87+8.41	0.001

Group A= Controls, Group B= Diabetic controls
Group C= Diabetic rats + Diacerein (30 mg/kg bwt)
Group D= Diabetic rats + Diacerein (50 mg/kg bwt)

Table 3. Glycated hemoglobin A1 in animal groups (n=60)

Animal groups	Mean+SD		P-value
Group AXB	6.08+0.66	8.98+1.105	0.001
Group AXC	6.08+0.66	6.72+0.56	0.013
Group AXD	6.08+0.66	6.63+1.04	0.045
Group BXC	8.98+1.105	6.72+0.56	0.001
Group BXD	8.98+1.105	6.63+1.04	0.001

Group A= Controls, Group B= Diabetic controls
Group C= Diabetic rats + Diacerein (30 mg/kg bwt)
Group D= Diabetic rats + Diacerein (50 mg/kg bwt)

consistent with earlier studies [17,22-24]. A double-blind randomized placebo control trial was concluded that the diacerein has an effect on β- cell secretory physiology [22]. Results of the present study are in agreement with results of previous study on the effects of diacereinto the restoration of β- cell secretory physiology was observed. Du H et al. [23] conducted experimental study on effects of diacerein/rhein in db/db mice to analyze β- cell physiology. They observed an improvement in glucose tolerance and the early-phase secretion of insulin in db/db mice. Both findings suggest that glucose improvement and insulin secretion are consistent with the present study.

Malaguti et al. [25] conducted a study to evaluate the efficacy of diacerein in non-obese diabetic mice. The mice were of type 1 DM. The Diacerein was administered to analyze the blood glucose levels, and pro-inflammatory cytokines. Diacerein was used in doses of 5, 10 and 50 mg/kg per day. The IL-1β, IFN-γ, IL-12, and TNF-α levels decreased significantly in T1DM mice [25].

Although the findings - IL-1β, IFN-γ, IL-12 and TNF-α level by Malaguti et al. not consistent with the present study, they indirectly supports the present study through cytokines which are involved in the β- cell dysfunction.

Ramos-Zavala et al. [26] conducted a clinical trial of diacerein on the insulin secretion and metabolic effects in the Drug-NaïveT2DM subjects. This previous study reported first- and late phases of insulin secretion were increased without alteration of insensitivity of insulin after administration of diacerein. The fasting blood glucose was improved after diacerein administration. They concluded that diacerein such therapy increases the insulin secretion and ameliorates the metabolic control in T2DM [26]. The findings of Ramos-Zavala et al are consistent with the present study. However, they did not

report the glycated HbA1 and Glutathione Peroxidase levels in their study.

Tobar N et al. [27] conducted a study on high-fat diet-fed mice to analyze the effects of diacerein on glucose tolerance and sensitivity of insulin. High fat diet was used to induce obesity and resistance of insulin. Tobar N et al observed that diacerein improved insulin sensitivity in obesity through the insulin-signaling pathway in the target tissues of the liver, muscle and adipose tissue in mice [27]. These findings regarding glucose and insulin are comparable to this study.

Turner N et al. [28] reported the effects of diacereinon the blood glucose and insulin-induced diabetes in animal models. They reported Diacerein ameliorated the blood glucose and insulin secretion, but the glycated HbA1 did not significantly decrease. The findings of blood glucose and insulin levels are consistent with our study, but the glycated HbA1 level showed inconsistency with this finding. The reason might be attributable to several unknown factors.

5. CONCLUSION

The present study concludes that Diacerein achieves the Euglycemic state by reducing blood glucose levels and glycated hemoglobin (HbA1c) in diabetes-induced Wistar rats. Diacerein may prove a novel therapeutic agent for the treatment of diabetes in the future, though this can be made accessible by conducting experimental and clinical trials to further prove the Diacerein significance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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