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Effect of a Metformin Derivative Containing Nitrile Group on Some Biochemical Variables in Rabbits Induced by Alloxane

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ABSTRACT

Background: Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Dipeptidyl peptidase-4 (DPP-4) degrades some enterocrinins, which are responsible for glucose metabolism, protection in cardiometabolic disease and immune regulation. The aim of this study is to prepare the nitrile derivative of metformin to act as an inhibitor of the DPP-4 enzyme, which is responsible for regulating blood sugar in relation to the secretion of insulin from beta cells in the pancreas. **Methods:** The preparation of the nitrile derivative obtained by the reaction of metformin and 3-bromopropanenitrile in methanol, characterized by 1HNMR and 13CNMR. Alloxan was injected to rabbits to induced diabetes. New synthesized drugs were given to rabbits for two weeks. **Results:** The results of biochemical study show significant decreases in the level of glucose, glucagon, the activity of DPP-4, significant increase in the level of insulin, and no significant difference in C-peptide level in treatment group compared with the diabetic group.

Conclusion: Decrease in the level of glucose, DPP-4 and glucagon were observed in the treatment group compared with the diabetes control group with its return to the normal level due to the effectiveness of the proposed drug in improving the sensitivity of cells to insulin and the transit of glucose molecules from the blood into the cells.

KEYWORDS

Metformin, Rabbit, Alloxane, Dipeptidyl Peptidase-4.

Introduction

Diabetes is a complicated chronic non-communicable disease that that is considered one of the most important public health issues of the 21st century. There are about 400 million people suffering from type 2 diabetes mellitus (T2DM) worldwide, and its prevalence is growing (Zou et al. 2017). Diabetes prevalence is fastly increasing worldwide and that poses a serious threat to human health. Distressingly, by the start of 2040, about 450 million people will be living with the disease (ABDUL-RAZZAKA et al.). T2D is characterized by multiple pathophysiologic defects, including progressive β-cell dysfunction and insulin resistance in the liver and peripheral tissues (Sultan and Abdulrazzak 2019; Wei et al. 2018) . It is well known that macrovascular complications, such as cardiovascular diseases and diabetic kidney disease are the leading cause of death in diabetic patients (Mohammed and Abdul-Razzaq 2020), and among them, atherosclerotic cardiovascular diseases account for up to 75% (Dandona et al. 2008; Defronzo 2010).

Dipeptidyl peptidase-4 (DPP-4) (also known as CD26) is an antigenic enzyme that cleaves dipeptides from the NH_2 terminus of proline or alanine containing polypeptides (Kawasaki et al. 2018). DPP-4 degrades some enterocrinins, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are responsible for glucose metabolism, protection in cardiometabolic disease and immuPPne regulation (Liu et al. 2020; Razzak et al. 2016).

Current pharmacologic agents focus on the multiple pathophysiologic disturbances of T2D aiming at increasing available insulin, reducing resistance to insulin, slowing gastric emptying and absorption of carbohydrates, or promoting urinary glucose excretion. DPP-4 inhibitors improve glucose control through several of these mechanisms, including a glucose-dependent reduction of postprandial glucagon or enhancement of insulin secretion as well as delayed gastric emptying (Al-Tikrity et al. 2020; Gomez-Peralta et al. 2018). DPP4 inhibitors are oral antidiabetic drugs that inhibit the enzyme DPP-4. DPP-4 is a ubiquitous enzyme expressed on the surface of most cell types that deactivates some bioactive peptides, including the incretin hormones GIP and GLP-1 (Holst 2007). Clinical studies to date indicate that DPP-4 inhibitors increase native GLP-1, which effectively stimulates insulin secretion,

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suppresses glucagon release and improves glucose control in patients with T2D. These agents are well tolerated and have a low incidence of adverse effects (Campbell 2007; Gomez-Peralta et al. 2018). The DPP-4 inhibitors have only recently been introduced into clinical use and the full range of adverse events may not be fully known. However, in prelicensure clinical trials, sitagliptin, saxagliptin, linagliptin and alopgliptin, the first four DPP-4 inhibitors to receive Food and Drug Administration (FDA) approval in the United States, were not associated with an increase in serum aminotransferase or alkaline phosphatase elevations above the rates found in controls, and no clinically apparent acute liver injury was reported (Deacon 2016).

The discovery and development of new drug is a long and complicated process. It is estimated that a typical drug discovery process initiating from lead identification to clinical trials can take up to 14 years with a cost of 800 million US dollars (Song et al. 2009). In this study, we aimed to synthesize a new drug based on the metformin used in T2D, to avoid these problems. The new drug obtained by adding functional groups to the metformin molecule acted as a DPP-4 inhibitor.

Materials and Methods

Preparation of the Proposed Treatment

Metformin, methanol, 3-bromopropanenitrile and alloxane were purchased from Sigma Aldrich (USA).
A solution of metformin (3 g, 23.226 mmole) in 100 ml of methanol was cooled to 0° C and added dropwise with stirring to (0.65 mole) of 3-bromopropanenitrile. The temperature was maintained below 5° C during the addition. When the addition was complete, the reaction mixture was stirred at 5° C for 90 minutes and thereafter allowed to stand at room temperature overnight. The solvent was then removed and the residue precipitate by diethyl ether to obtain 5N-(acetonitrile)-1,1-dimethylbiguanide (2.867gm, 73.4%). The products were characterized by ^{1}HNMR and ^{13}CNMR.

Animals Study

The experiment was performed on 30 adult male domestic rabbits weighing between 1700-2000 g and their age ranges between 12-14 months. Diabetes was induced by single dose (150 mg/kg) of freshly prepared injection of alloxan (Indradevi et al. 2012). The rabbits gave a 5% glucose solution in drinking water throw the first 24 hours after injection of alloxan to avoid hypoglycemia. The induction of diabetes was confirmed by collecting blood from the external ear vein for glucose analysis by using portable blood glucose monitor every day for 7 days, and then rabbits with fasting blood glucose levels above 150 mg/kg were considered diabetic (Akhtar et al. 2011; Misra and Aiman 2012). Thirty adult male rabbits were used, divided into three groups: nine male rabbits were included in each group, equal weight of each group was taken into consideration as much as possible before the start of the study, (control group) given water and food for two weeks, (control diabetic group) they have been injected alloxan 150 mg/kg body weight injection then given food and water for two weeks and (Diabetic + treatment) they have been injected alloxan 150mg/kg body weight, then given 15.813 mg/kg body weight the prepared treatment orally concomitantly for two weeks.

Biochemical Study

Glucose are determined using Randox enzymatic kit, while the serum activity of DPP4 was measured using commercial DPP4 Activity Fluorometric Assay Kit (BioVision Incorporated, USA), insulin and glucagon were measured by using commercial enzyme linked immunosorbent assay (ELISA) kit (MyBioSource, USA).

Statistical Analysis

All data collected were analyzed by one-way ANOVA. Difference between mean values were determined using Duncan's multiple range test (Kerridge et al. 1984).

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Results

The proposed drug was prepared by the reaction of the N-terminal of metformin at position 5 with 2-bromoacetonitrile in methanol as a solvent at 0^{o}C for 90 minutes and then allowed to stand at room temperature overnight. The results precipitated by diethyl ether to obtain the final product (Eq 1).

The synthesized compound was characterized by ^{1}HNMR and ^{13}CNMR, as shown in Figure1 and Figure2 respectively, and Table 1 explain the results.

After completing the treatment preparation, he was tried invivo by using 30 male rabbits. The results of the tests were as follows.

The effectiveness of the prepared treatment on glucose level and dipeptidyl peptidase-4 activity has shown in Table 2. Table 2 show the level of glucose and DPP-4 (mean±SD) on control, control diabetic and treatment groups.

The result of Table 2 show significant increase in the level of control diabetic group (187.22±10.21) compared with the control group (71.21±11.56), and the effect of treatment group clearly show decreasing in the level of glucose (128.67±6.84) when compare it with the control diabetic group.

Table 3 results show significant decrease in the level of control diabetic group (38.30±8.16) compared with the control group (64.17±6.15), the low level of insulin refers to the damage of beta cells of pancreas because of the effect of alloxan.

Table 3 demonstrates an adjustment to the glucagon due to the treatment used, so the level of glucagon in treatment group was (1.15±0.07) and in control diabetic group was (1.63±0.17), while the glucagon level in control group was (1.31±0.11).

The result of table 3 show significant decrease in the level of C-peptide in control diabetic group (2.01 ±0.71) compared with the control group (6.47±1.27), because of the partial damage in pancreatic beta cells that effected by alloxan. and the treatment group show increasing in the level of c-peptide (3.93± 0.82) when compare it with the control diabetic group so this result proves the repairing in pancreatic beta cells by the treatment.

Discussion

The results of the level of glucose in table 2 for the treatment group show that the treatment proposed in this study is the reason for the low level of glucose in the blood when compared with the control diabetic group, that is, the proposed treatment works as an anti-hyperglycemic by reducing the level of glucose and achieving the desired goal that appears also in table results for the DPP-4 activity Level. This effect contributes to the regulation of high blood glucose levels in type 2 diabetes (Chyan and Chuang 2007; White 2008).

Inhibition of DPP-4 by inhibitor materials used as drugs enhances the hormonal activity of GLP-1 and other biologically active peptides (GIP, release of gastrin peptides), thus causing stimulation of insulin secretion from beta cells and reducing glucagon secretion from alpha cells in the pancreas which causes Low level of glucose in the blood (Chyan and Chuang 2007).

The effect of treatment showed an increase in the level of insulin in the treatment group (55.33 ± 3.74) when compared to that of the diabetic group. As is known, the group of diabetics has damage to the pancreatic beta cells due to the effect of toxic alloxan, and that the increase in the level of insulin in the treatment group indicates that there has been a repair of beta cells, which caused an increase in insulin secretion and a high percentage of blood in the blood, that is, the treatment has taken place with two processes in one, the first is the repair of beta cells (the direct responsible for insulin secretion), and the second is the inhibition of the action of the DPP-4 (the direct stimulus of the beta cells that secrete insulin). Furthermore, the suggested drug has an effect in improving cell sensitivity to insulin and the transit of glucose molecules from the blood into the cells.

The normal metabolic process of glucagon in general is to raise the concentration of glucose in the blood by promoting the development of sugar and glycogenolysis, where the liver stimulates the degradation of the stored

glycogen into free glucose, which is released into the bloodstream, causing a high level of blood glucose (Bryan 1996). The compound suggested in this study causes low levels of glucagon, and as shown in the results table 2, it will prevent high blood sugar levels, it will prevent high blood sugar levels, so it can be adopted as a treatment for diabetics after conducting the necessary additional studies on it.

Conclusions

From the research results, a decrease in the level of glucose was observed in the treatment group compared with the diabetes control group with its return to the normal level due to the effectiveness of the proposed drug in improving the sensitivity of cells to insulin and the transit of glucose molecules from the blood into the cells.

A decrease in the activity of DPP-4 in the treatment group compared with the control group of diabetes and the return of the level of effectiveness to the normal level due to the inhibition of the drug proposed to the enzyme, which is the direct responsible for stimulating pancreatic beta cells in the secretion of insulin and a mantle to the blood.

An increase in the level of insulin in the treatment group compared to the diabetes control group due to the effectiveness of the proposed drug in the restoration of pancreatic beta cells responsible for insulin secretion and a thrush to the blood.

A high decrease in the concentration of glucacone in the treatment group compared to the control group of diabetes and the return of the level of effectiveness to the normal level, that this decrease causes a reduction in the hydrolysis of clycogen in the liver and the release of free glucose into the blood. That is, the treatment proposed here has closed the possibility of a high level of glucose in the blood by the decomposition of glycogen.
An increase in the concentration of C-peptide in the treatment group compared to the control group of diabetes. This increase occurred as a result of the restoration of beta cells in the pancreas, which also caused an increase in the concentration of insulin as well.

Declarations

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Code availability: Not applicable

Authour Contribution

FSA: Conceptualization, Methodology, Validation, Investigation, Writing-Original Draft, **NYA:** Conceptualization, Formal Analysis, Resources, Writing-Original Draft, **OAA:** Conceptualization, Methodology, Validation, Writing-Reviews Editing, : Investigation, Resources, Writing-Reviews Editing, **ST:** Methodology, Resources, Writing-Reviews Editing,

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