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The Anti-Diabetic Effect of One of Nitrile Derivative in the Rabbits Induced with Alloxan

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Abstract

Background: Diabetes Mellitus affects approximately 422 million adults worldwide and is a group of metabolic diseases characterized by hyperglycemia caused by defects in insulin secretion, insulin action, or both. Dipeptidyl-peptidase-IV (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 synthesize 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:

One of the previously prepared nitrile derivatives was used to measure the extent of its effect on rabbits induced by diabetes, where the level of sugar and some enzymes (AST, ALT, ALP) and hormones (Apelin-36, Leptin, insulin like growth factor) related to the induced disease and its side effects, and a group of rabbits was used to achieve this purpose

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Results: The results showed that serum glucose, Apelin, Leptin, Insulin-like growth factor - 1 (IGF-1) levels, Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) activities were significantly lower in the treatment group compared to the diabetic group.
Conclusion: These results show that the newly synthesized agent is effective in improving the sensitivity of cells to insulin and glucose transition by inhibiting the dipeptidyl-peptidase IV (DPP-4) enzyme, activating incretin hormones, increasing the effectiveness of Apelin, Leptin, IGF-1 with low effect on liver enzymes.
Keywords: Apelin, Leptin IGF-1 , Rabbit, Alloxane,

Introduction

Diabetes mellitus is a chronic metabolic disorder associated with a variety of environmental and genetic factors. The incidence of diabetes is increasing worldwide, with a higher rate of accelerating various life-threatening complications such as diabetes-induced retinopathy, nephropathy, neuropathy and other cardiovascular problems such as hypertension, stroke, atherosclerosis. Type 1 diabetes is a disorder that occurs when the beta cells in the pancreas that produce insulin are damaged by an autoimmune process[1]. T2DM is characterized by impaired cell functions and insulin resistance. Type 2 diabetes is much more common in adults overall and accounts for more than 90% of all diabetes cases, and its incidence is increasing worldwide due to the aging of the population and the increasing prevalence of obesity and sedentary lifestyle [2, 3].

Currently available treatments for type 2 diabetes mellitus (T2DM) have several limitations, including less than optimal control of postprandial hyperglycemia, increased risk of hypoglycemia, weight gain, gastrointestinal side effects, and edema. In general, current agents target either relative insulin deficiency or insulin resistance, which defines the specified T2DM. A new approach drawn from its clinical results is the use of agents that appear abnormal in T2DM and have important effects on insulin and glucagon biology, as well as on the central nervous system in relation to appetite, and that strengthen or increase the activities of intestinal hormones called incretins[4]. Today, there are two hormones responsible for the incretin effect, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-I (GLP-1), and both are members of the glucagon peptide family and their amino acid sequences are similar. GLP-1 and GIP are intestinal polypeptides belonging to the incretin family and play a role in glucose homeostasis. In the fasted state, plasma GIP and GLP-I concentrations are extremely low, the half-life in plasma is only 1-2 and 5-7 minutes, respectively, and increases rapidly with meal intake. However, the secreted incretin hormones are rapidly degraded to their inactive metabolites by the enzyme dipeptidyl-peptidase-IV (DPP-4), which is widely found in tissues. DPP-4 activity is found on the surface of the endothelial cells lining the blood vessels through which the intestinal mucosa is shed. DPP-4 is the only enzyme that separates and thus inactivates GLP-1 and GIP under physiological conditions. Therefore, most of the incretin hormones are inactivated before they can reach the systemic circulation[5].

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In addition to being tightly regulated by energy homeostasis, endocrine, paracrine and autocrine factors, it plays an important role in maintaining the energy balance in skeletal muscle, liver, adipose tissue and pancreatic β cells. Changing the energy balance quickly leads to obesity, which is a major cause of insulin resistance. Several mechanisms linking obesity to insulin resistance have been proposed. Among these, adipocyte secreting factors or adipokines have been shown to play an important role. Changing their production (excess or deficiency) can directly stimulate or delay the onset of insulin resistance[6]**.**

Apelin-39, adipocytokine, is a peptide known as the endogenous ligand of the G-protein-coupled receptor Apelin, it is expressed in a variety of tissues, including kidney and endothelial cells. In addition, increased body mass in type 2 diabetic patients might contribute to increase Apelin levels in the blood[7].

In recent years, insulin-like growth factor- 1(IGF-1) has gained much attention in pathophysiology, especially for diabetic retinopathy. There have been various studies suggesting the role of IGF-1 in diabetic retinopathy, and few studies have shown a direct correlation of IGF-1 with it, whereas some have shown an inverse relationship[8].IGF-1 is closely related to insulin, except that its C chains are not separated and it has an extension of the A chain called the D domain [9].

Leptin was discovered in 1994 and is a hormone containing 167 amino acids that reduces pancreatic insulin secretion, increases lipolysis in adipose tissue and skeletal muscles, is secreted by adipocytes and regulates food intake [10]. The leptin also plays a key role in glucose metabolism [11], In support of this, rodent models of leptin deficiency are characterised by insulin resistance and diabetes [12], and leptin treatment lowers blood glucose and insulin levels [13].

This research aims to investigate the effect of the metformin derivative that contains an effective nitrile group in medicine and the degree of its effects on certain hormones that are important in controlling blood sugar levels as well as determining its effects on liver enzymes.

Materials and methods

Chemicals and instruments

Chemicals: Metformin(sigma Aldrich), methanol(fluka), 3-bromopropanenitrile (sigma Aldrich), alloxane(sigma Aldrich). Instrument:hotplate with stirrore (biotech),

Animals study

A number ofrabbits (30) were taken from the local adult rabbits whose weight ranges between (1700-2000) g and ages between (12-14) months. They were left in the cages for one week to settle and get used to the place. Diabetes was caused by injecting into the peritoneum and in a single dose of (150 mg / kg) of substance alloxan prepared directly [14]. Rabbits were given a 5% glucose solution in drinking water within the first 24 hours after injecting alloxan so that they would not have a low blood sugar level. Diabetes was confirmed as occurring by drawing blood from the outer ear vein to analyze glucose level using a portable blood glucose monitor, and rabbits with fasting blood sugar levels were considered above 170 mg / dL with diabetes[(2,3)]. Thirty adult male rabbits were taken, as they were divided into three groups, in each

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group of 10 male rabbits, and the groups were as follows: (control group) they were given water and food for two weeks, (diabetic control group) were injected with alloxan 150 mg / Kg of body weight and then give them food and water for two weeks. As for the last group (diabetes group + treatment) they were injected with the substance alloxan 150 mg / kg of body weight, then they were given 15.813 mg / kg of bodyweight depending on the dose of one of the diabetic drugs, and used as a blood sugar treatment for two weeks[15].

Biochemical study

Apelin-36 concentration and leptin are determined using (MyBioSource ELISA kit), insulin growth factor-1 by (Fine test ELISA kit), and finally, the liver enzymes aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase(ALP) are determined by the colorimetric method using (Biolabo Kit).

Statistical Analysis

The search results were analyzed using one-way analysis of variance and identified specific differences between groups using the Duncan test at probability level ($p \leq 0.05$)[16]

Results and discussion

Diabetes mellitus is an emerging predator and affects approximately 422 million adults worldwide. Increased pressure on the pancreas to produce higher levels of circulating insulin and insulin has been deduced as the possible etiology for diabetes, leading to a higher risk of pancreatic cancer. Of several drug targets in the hypoglycemic discovery, Dipeptidyl peptidase-IV (DPP-IV) has been recognized as an emerging target. DPP-IV is a protease enzyme that inactivates the incretin hormones Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP). Inhibition of DPP-4 causes GLP-1 and GIP to circulate and act for longer periods of time. Therefore, DPP-4 inhibitors play an important role in maintaining glucose homeostasis. Compared to early oral hypoglycemia, DPP-IV inhibitors are well tolerated and provide better glycemic control over a longer period of time[17].

From the results of our study, we found that the newly synthesized agent improved the passage of glucose from blood to cells and the sensitivity of cells to insulin and decreased blood glucose levels to normal levels by statistically significantly lowering them in the treatment group compared to the diabetes control group (Table 1). These results confirm that the new agent exerts its effect by inhibiting DPP-4 activity, which rapidly converts incretins (GLP-1 and GIP), which play an important role in glucose homeostasis, into inactive metabolites.

Glucose homeostasis depends on the balance between hepatic glucose output and glucose utilization by insulin sensitive (adipose tissue, skeletal muscle) and non-insulin sensitive tissues. Studies have shown that both short and long-term apelin treatment improves insulin sensitivity in insulin-resistant obese mice. In one study, apelin administered in a physiological range was reported to improve glucose metabolism in vivo in mice fed normal and insulin-resistant high-fat diets by

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increasing glucose utilization in insulin-sensitive tissues rather than inhibiting hepatic glucose output [18]. In this study, we found that the Apelin levels of the treatment group were statistically significantly higher compared to the diabetic control group and reached the values of the normal control group (Table 2). This indicates that the new agent also plays a role in the direct or / indirect activation of the synthesis of molecules that play a role in glucose homeostasis by increasing Apelin synthesis in the treatment group. Studies suggest that serum Apelin levels have a parallel relationship with nutritional status and plasma insulin levels in mice and humans. Apelin has been reported to directly increase peripheral glucose utilization, possibly independently of insulin, thus adapting liver glucose output secondary to maintaining normal blood glucose[18].

Leptin is an important adipocytokin that affects both insulin sensitivity and inflammation, plays a close role in the development of T2DM, and is a proinflammatory molecule that plays a key role in the regulation of glucose and energy homeostasis. Experimental studies have suggested that leptin can normalize hyperglycemia independently of insulin[19, 20]. The hormone signals the hypothalamus and is released to reduce the cravings for food and thus control appetite. Leptin is also increased in type 2 diabetes mellitus, which is strongly associated with obesity and insulin resistance. It has been suggested that leptin mediates insulin resistance and thus may be a possible treatment for insulin resistance. In some studies, diet-induced obesity has been reported to cause leptin resistance, so although leptin levels are high, it is ineffective[21]. In the present study, we found that the leptin levels of the treatment group were statistically significantly lower than both the normal control and diabetes control groups (Table 3). This shows that the newly synthesized agent increases its effectiveness in the treatment of Type 2 diabetes by keeping leptin levels low and increasing its sensitivity.

Insulin-like growth factor- 1 is one of the small peptides that generally act locally and stimulate growth in specific cells and resemble human proinsulin. IGF-1 acts as the major mediator of growth hormone in accelerating growth. IGF-1 is synthesized in the liver and secreted into the blood under the control of GH. Insulin and IGF-1 receptors are approximately 38% similar. It is found in the circulation at significant levels during postnatal life and exhibits glucoregulatory and mitogenic properties at doses similar to insulin[22]. In our study, we found that the IGF-1 values of the treatment group were statistically significantly lower than the diabetes control group and were close to the values of the normal control group (Table 4). This suggests that the newly synthesized agent maintains serum IGF-1 levels at normal control group levels and increases its effectiveness in the treatment of Type 2 diabetes.

We would like to point out that the fact that the newly synthesized agent was not applied to the diabetic group in different and lower doses is one of our important limitations. Our research group is planning another study to illuminate this situation by adding more different experimental groups.

Furthermore, the results showed non significant differences in the activity of serum aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) between the treatment group and diabetic group (Table 5-7).

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While there was a significant difference between the control group when compare it with the diabetic group and treatment group, knowing that all the values are within the normal range for the variables. We think this may be the result of the high dose of the newly synthesized agent. Therefore, as we mentioned in the limitations section, we aim to reveal it with other studies we plan to do.

As a result, the findings obtained show that the newly synthesized agent that increases the effectiveness of Apelin, Leptin, IGF-1 by inhibiting dipeptidyl-peptidase IV (DPP-4) enzyme, activating incretin hormones, is effective in improving the cells' sensitivity to insulin and glucose passage. We also plan to conduct more comprehensive studies on the subject by using wider and different parameters.

Table 1: The level of glucose mesuared in control, control diabetic and treatment group.

Parameter	Groups	No	Mean±SD	
Glucose	Control	10	73.20±10.52	c
	Control Diabetic	10	191.19±11,21	a
	Treatment	10	127.64±6.24	bc

The difference in the letters (a,b,c) indicates the presence of significant differences, and the p value is less than 0.05.

Similarity in the letters (a,b,c) indicates that there are no significant differences between the values, and the value of p in them is greater than 0.05.

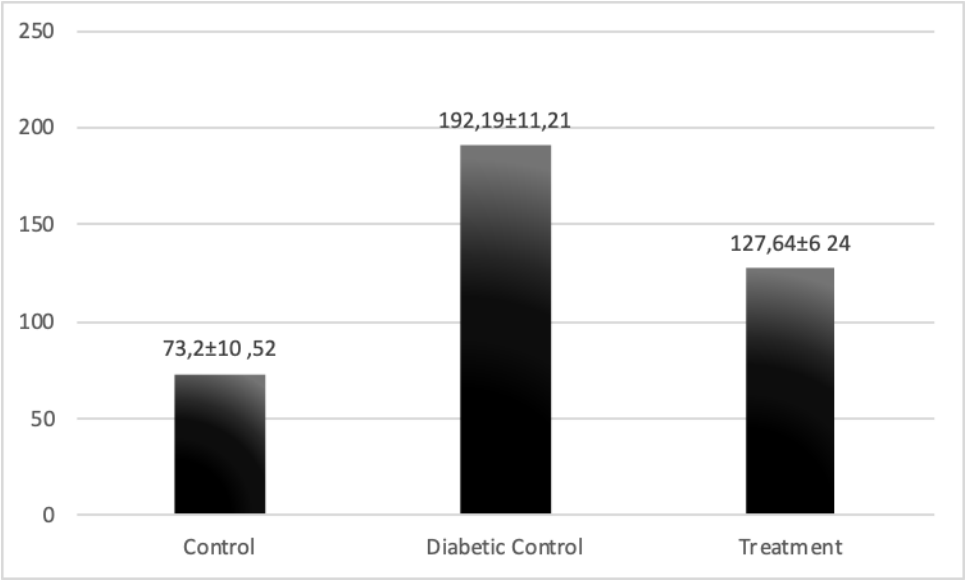

Fig 1: The level of glucose mesuared in control, control diabetic and treatment group.

Table 2: The level of Apelin 36 on control, control diabetic and treatment group

Parameter	Groups	No	Mean±SD	
Apelin 36 ng/mL	Control	10	18.98±2.21	a
	Control Diabetic	10	12.87±4.97	b
	Treatment	10	17,52±1,52	a

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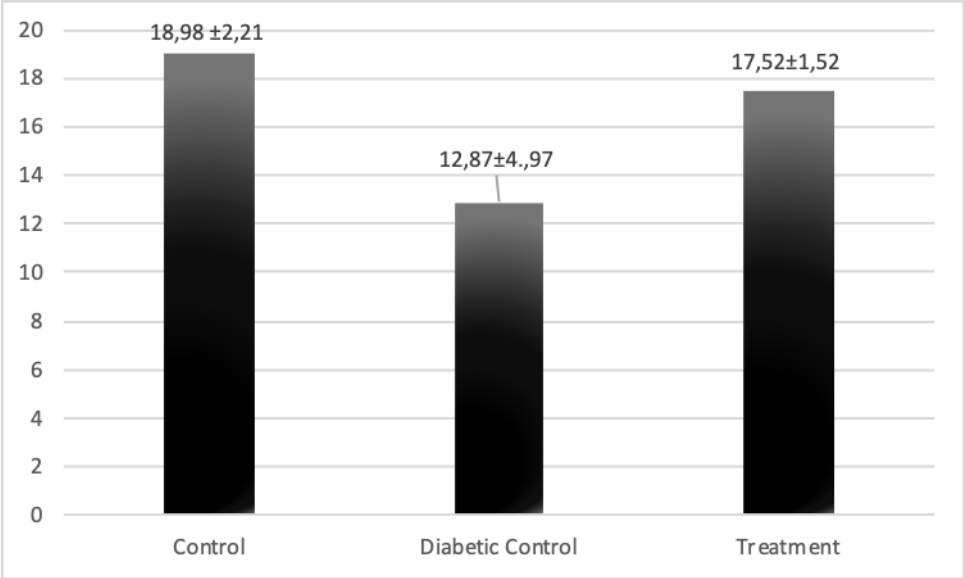

Fig 2: The level of Apelin 36 on control, control diabetic and treatment group

Table 3: The level of leptin in control, control diabetic and treatment group

Parameter	Groups	No	Mean±SD	
Leptin ng/mL	Control	10	24,39±8,13	a
	Control Diabetic	10	23,98±4,89	a
	Treatment	10	14,33±5,47	b

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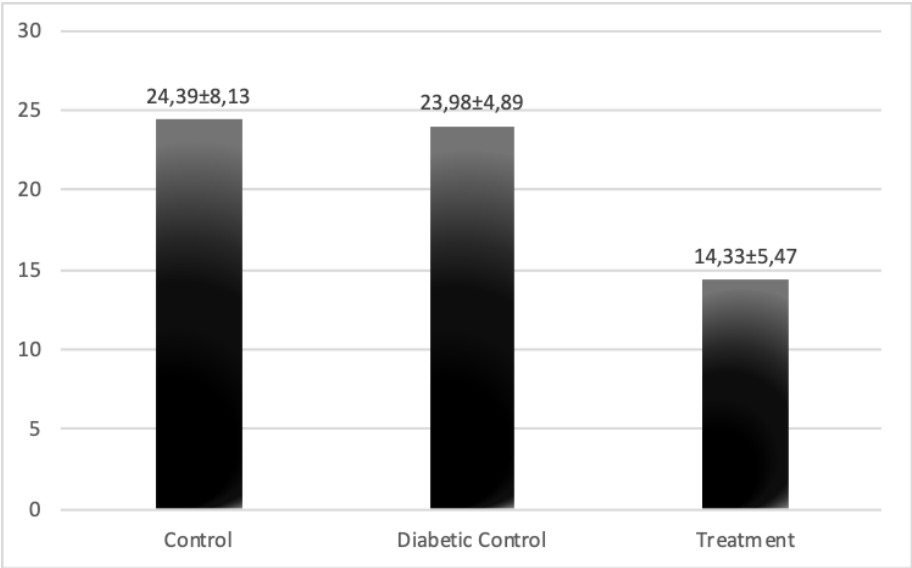

Fig 3: The level of leptin in control, control diabetic and treatment group

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Table 4: The level of insulin growth factor in control, control diabetic and treatment group

Parameter	Groups	No	Mean±SD
Insulin-like growth factor ng/mL	Control	10	14,97±1,38 b
	Control Diabetic	10	18,15±2,44 a
	Treatment	10	12,77±2,29 c

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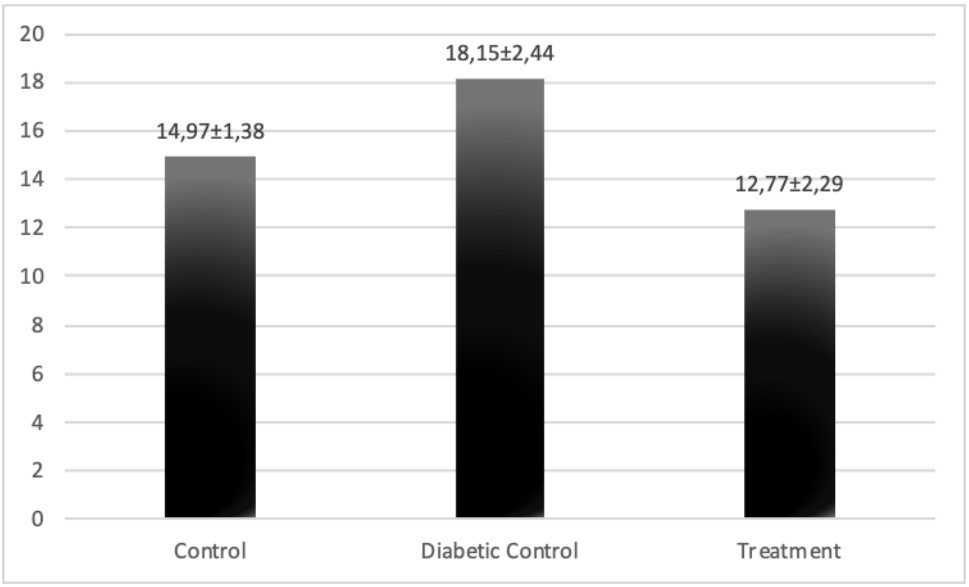

Fig 4: The level of insulin growth factor in control, control diabetic and treatment

Table 5: The level of AST on control, control diabetic and treatment group

Parameter	Groups	No	Mean±SD
AST IU/L	Control	10	29,44±19,97 b
	Control Diabetic	10	75,38±28,54 a
	Treatment	10	71,30±37,30 a

The difference in the letters (a,b,c) indicates the presence of significant differences, and the p value is less than 0.05.
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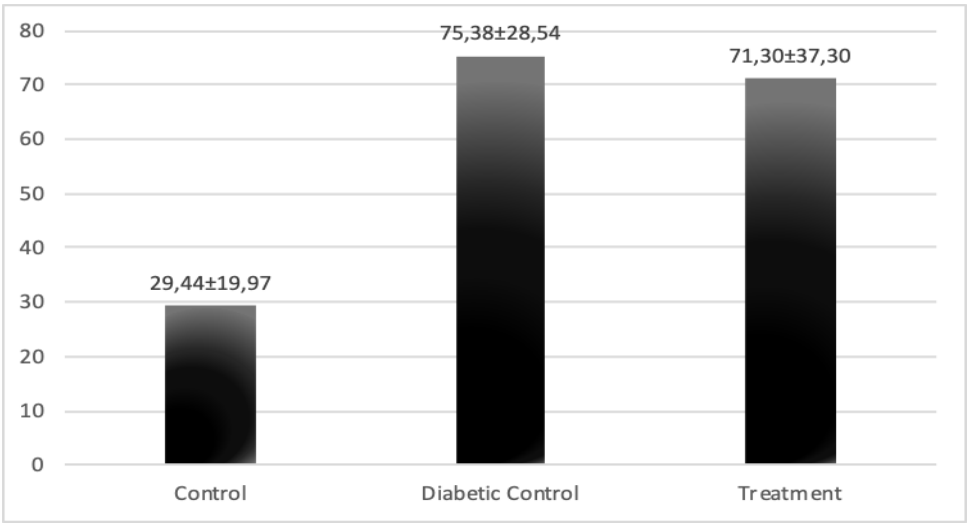

Fig 5:The level of AST on control, control diabetic and treatment group

Table 6: The level of ALT in control, control diabetic and treatment group

Parameter	Groups	No	Mean±SD
ALT IU/L	Control	10	23,94±10,22 b
	Control Diabetic	10	69,18±13,27 a
	Treatment	10	50,12±12,50 a

The difference in the letters (a,b,c) indicates the presence of significant differences, and the p value is less than 0.05.
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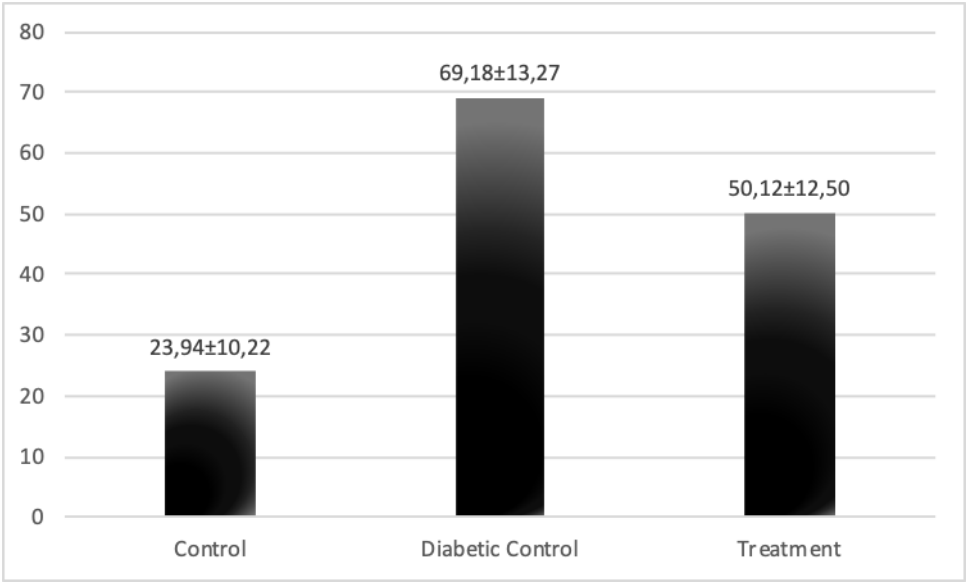

Fig 6:The level of ALT in control, control diabetic and treatment group

Table 7: The level of ALP on control, control diabetic and treatment group

Parameter	Groups	No	Mean±SD
ALP	Control	10	58,21±28,43 a
	Control Diabetic	10	68,98±29,45 a

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IU/L	Treatment	10	61,18 ±20,04 a

The difference in the letters (a,b,c) indicates the presence of significant differences, and the p value is less than 0.05.

Similarity in the letters (a,b,c) indicates that there are no significant differences between the values, and the value of p in them is greater than 0.05.

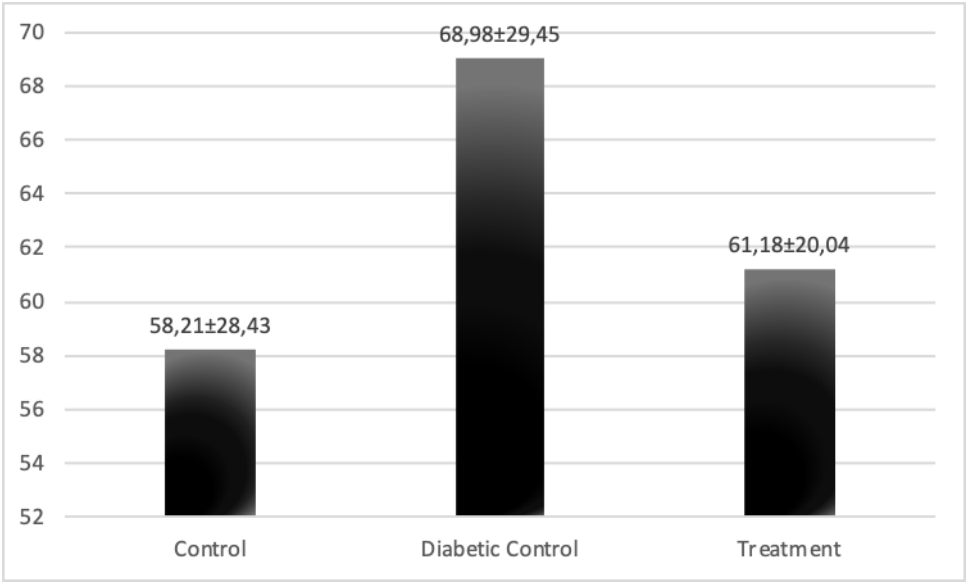

Fig 7: The level of ALP on control, control diabetic and treatment group

References

1. Singh, R., et al., *Epigenetic modification and therapeutic targets of diabetes mellitus.* Biosci Rep, 2020. **40**(9).
2. Huang, J., et al., *Adverse event profiles of dipeptidyl peptidase-4 inhibitors: data mining of the public version of the FDA adverse event reporting system.* BMC Pharmacol Toxicol, 2020. **21**(1): p. 68.
3. Abdul-Razzak, F., S., M. Mohammed, J., and E. Mustafa, M., *Synergistic effect of l-carnitine, omega-3 with metformin on the level of apelin-36 and some hormones in rabbits induced with diabetes.* Asian J Microbiol Biotech Env Sc, 2019. **21**(1): p. 38-45.
4. Rosenstock, J. and B. Zinman, *Dipeptidyl peptidase-4 inhibitors and the management of type 2 diabetes mellitus.* Curr Opin Endocrinol Diabetes Obes, 2007. **14**(2): p. 98-107.
5. Çolak, R., *Tip 2 diabetes mellitus tedavisinde inkretinler.* J Exp Clin Med, 2012. **29**: p. 30-38.
6. Bertrand, C., P. Valet, and I. Castan-Laurell, *Apelin and energy metabolism.* Front Physiol, 2015. **6**: p. 115.
7. Elsehmawy, A.A.E.W., et al., *Apelin and chemerin as promising adipokines in children with type 1 diabetes mellitus.* Diabetes, metabolic syndrome and obesity : targets and therapy, 2019. **12**: p. 383-389.
8. Raman, P., A.K. Singal, and A. Behl, *Effect of Insulin-Like Growth Factor-1 on Diabetic Retinopathy in Pubertal Age Patients With Type 1 Diabetes.* Asia Pac J Ophthalmol (Phila), 2019. **8**(4): p. 319-323.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 14587 - 14598
Received 05 March 2021; Accepted 01 April 2021.

9. Barrett, K.E., et al., in *Ganong's Review of Medical Physiology, 25e*. 2018, McGraw-Hill Education: New York, NY. p. 382-383.
10. Facey, A., L. Dilworth, and R. Irving, *A Review of the Leptin Hormone and the Association with Obesity and Diabetes Mellitus.* Journal of Diabetes and Metabolism, 2017. **8**.
11. Morton, G.J. and M.W. Schwartz, *Leptin and the central nervous system control of glucose metabolism.* Physiol Rev, 2011. **91**(2): p. 389-411.
12. Dubuc, P.U., *The development of obesity, hyperinsulinemia, and hyperglycemia in ob/ob mice.* Metabolism, 1976. **25**(12): p. 1567-74.
13. Pelleymounter, M.A., et al., *Effects of the obese gene product on body weight regulation in ob/ob mice.* Science, 1995. **269**(5223): p. 540-3.
14. Razzak, F.S.A., Z.M.M. Al-Rubaei, and Y. Mohammed, *Synthesis of Novel Acetylinc Derivative of Metformin as a DPP-4 Inhibitors and Study its Effects on Sera of Rabbits with induced Diabetes.* Journal of Natural Sciences Research, 2016. **6**: p. 143-153.
15. FIRAS S. ABDUL-RAZZAK, M.J.M.A.E.M.M., *SYNERGISTIC EFFECT OF L-CARNITINE, OMEGA-3 WITH METFORMIN ON THE LEVEL OF APELIN-36 AND SOME HORMONES IN RABBITS INDUCED WITH DIABETES.* Asian Journal of Microbiology, Biotechnology & Environmental Sciences Paper, 2019. **21**(1): p. 38-45.
16. Duncan, D.B., *Multiple Range and Multiple F Tests.* Biometrics, 1955. **11**(1): p. 1-42.
17. De, S., et al., *Critical Role of Dipeptidyl Peptidase IV: A Therapeutic Target for Diabetes and Cancer.* Mini Rev Med Chem, 2019. **19**(2): p. 88-97.
18. Dray, C., et al., *Apelin stimulates glucose utilization in normal and obese insulin-resistant mice.* Cell Metab, 2008. **8**(5): p. 437-45.
19. Liu, W., et al., *Serum leptin, resistin, and adiponectin levels in obese and non-obese patients with newly diagnosed type 2 diabetes mellitus A population-based study.* Medicine, 2020. **99**(6).
20. Schwartz, M.W., et al., *Cooperation between brain and islet in glucose homeostasis and diabetes.* Nature, 2013. **503**(7474): p. 59-66.
21. Facey, A., L. Dilworth, and R. Irving, *A Review of the Leptin Hormone and the Association with Obesity and Diabetes Mellitus.* Journal of Diabetes & Metabolism, 2017. **8**(3).
22. Çolak, R., *İnsülin Benzeri Büyüme Faktörleri ve İnsülin Benzeri Büyüme Faktörü Bağlayıcı Proteinler.* Turkiye Klinikleri J Int Med Sci, 2007. **3**(37): p. 10-17.

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Consent for publication: Authors give permissions for publication.

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Code availability: Not applicable

Authour Contribution

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