



Evaluation of Arabian Camels' Milk's Ability in Ameliorating Glucose Homeostasis in STZ-induced Diabetes in Adult Male Rats

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Abstract | It is generally known that the milk of Arabian camels helps to maintain good health and is commonly applied in conventional medicine to cure a number of ailments. In this investigation, we delivered fresh milk from Arabian camels to adult male rats that had been given STZ to induce diabetes. Three groups of ten adult male Albino rats each were created at random from the thirty total animals. Control group was given ordinary food and free water, the STZ-Diabetes group received one (ip) injection of streptozotocin (40 mg/kg), and the STZ-diabetes+Camels' Milk group received a STZ injection (40 mg/kg) along with daily (*ad libitum*) bottled camel milk for 28 days. Zero time, one day, one week, two weeks, three weeks, and day 28 following STZ injection were used to monitor fasting blood glucose, insulin, and glucagon. Animals were sacrificed on day 28, and tissues were collected for immunohistochemical identification of pancreatic cells that produce insulin and glucagon. Based on our results, none of the research parameters significantly differed across the groups on day 0. Days 1 through 28 showed that STZ-Diabetes group had the uppermost blood glucose level with lowermost blood insulin among the study groups as a result of the induction of diabetes. Comparing the STZ group to the control and camels' milk groups, the blood glucagon level was the highest in the STZ group. Comparing the diabetes group to other groups, immunohistochemical analysis revealed a significant reduction in insulin secretion cells. In contrast, considerably more glucagon-producing cells were seen in the STZ group. Data from the camel milk group demonstrated its capacity to lessen the effects of STZ, but it did not completely eliminate the consequences of diabetes as there were still some disparities between the camel milk group and the control group. In conclusion, although camel milk didn't completely restore the body's health condition, our research showed that it significantly ameliorated the adverse effects caused by diabetes.

Keywords | STZ, Insulin, Glucose, Glucagon, IHC, Male Rats

Received | July 29, 2023; **Accepted** | August 20, 2023; **Published** | October 20, 2023

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Citation | Al-Yasari AMR, Mohammed ZI, Almnehlawi HS, Bargooth AF, Alsalih NJ, Hasan HF, Alsaadawi MA (2023). Evaluation of arabian camels' milk's ability in ameliorating glucose homeostasis in stz-induced diabetes in adult male rats. *Adv. Anim. Vet. Sci.* 11(11): 1757-1763.

DOI | <http://dx.doi.org/10.17582/journal.aavs/2023/11.11.1757.1763>

ISSN (Online) | 2307-8316



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INTRODUCTION

Diabetes, as one of the most communal endocrine syndrome, affects a respectful percentage of population around the world. It is brought on by a spike or drop in

blood glucose levels, which is brought on by the pancreas' insufficient or ineffective release of insulin. It has been found to harm a number of body functions (Ismail et al., 2009). Localized inflammation in pancreatic tissue is one of the hallmarks of diabetes, which is followed by the se-

lective death of beta cells. (Arora et al., 2009). More than 4,500 years ago, in the Southern region of Arabia, camels (*Camelus dromedarius*), an animal rose in the desert, were first domesticated (Bulliet, 1990). Initially utilized as a mode of transportation, camels also provided communities with meat, milk, and fur as well as opportunities for tourism, agricultural employment, competitions, and beauty pageants (Burger et al., 2019) and (Gebreyohanes and Assen 2017). Consumers can obtain nutrients and health advantages from camel milk (Alhaj and Kanhal, 2010) and (Hammam 2019). Camels' milk is a dense white colour, and it has a little salty flavor (Yadav et al., 2015). Camel milk has a generally similar composition to that of cow's milk in terms of lactose, fat, and protein (Berhi et al., 2017). Camel milk has been found in studies to have promising nutritional benefits that can improve diabetes treatment (Malik et al., 2012). According to (Agrawal et al., 2011) camels' milk is effective and safe for improving blood glucose management, greatly reducing the need for people with type 1 diabetes to take as much insulin. Abdalla, (2014) compared the group consuming camel milk to those receiving standard treatment for juvenile diabetes in a study carried out in India. It was proven that those who drank camel milk had considerably lower HbA1C values. In the present research, we examine the role of camel milk in the treatment of diabetes caused by STZ.

MATERIAL AND METHODS

ANIMAL USE

We maintain temperature at (22°C). 30 adult male Albino Wistar rats were kept in light/dark of twelve hours cycles. The treatment of the animals was done in compliance with the Ethical Committee of Animal Care's policy.

EXPERIMENTAL DESIGN

Thirty rats were assigned to three groups of ten animals. The groups were as follows:

Control group had a unrestricted chow and water.

STZ-Diabetes group received one (ip) injection of streptozotocin (40 mg/kg) on day0

STZ-diabetes+Camels' Milk group received a STZ injection (40 mg/kg) on day0 along with daily camel milk for 28 days

Daily samples of camel milk were taken from a farm in the Al-Muthanna Province of Iraq and stored in designated bottles for (*ad libitum*) feeding of STZ-diabetic animals.

LABORATORY ANALYSIS

Blood Glucose, Insulin, And Glucagon: Glucose strips and an AlphaTrak glucometer were used to measure blood glucose levels. Following the manufacturer's instructions, Crystal Chem's Ultra Sensitive Rat Insulin and glucagon kits were used.

IMMUNOHISTOCHEMISTRY

Harvested pancreatic tissues were preserved for fixation in neutral formalin. Tissues from the pancreas were thoroughly treated, including clearing, embedding, and dehydration. Sections were cut at 6 µm and then paraffin was removed by using xylene for 20, 15, and 10 minutes. Finally, the tissue was rehydrated using different ethanol concentrations (from 100% to 50%). The antigens were retrieved after they had been heated to 95°C. Primary antibodies against insulin and glucagon were incubated on pancreatic slices from various groups for an overnight period at 4 °C after dilution with 2% bovine serum albumin in phosphate buffer saline (PBS). The secondary antibody was added and kept for one hour at (22 oC). Slides were mounted using per mount prolong media after being counterstained with DAB (3,3-Diaminobenzidine). Ten random images were captured at a 20X magnification, and ImageJ software was used to calculate staining intensity (Al-Yasari et al., 2022).

STATISTICAL ANALYSIS

GraphPad Prism 8.4.3 and utilizing ordinary one-way-ANOVA and two-way-ANOVA were used to analyze the data collected in this study. P value (<0.05) considered statistically significant.

RESULTS

BLOOD GLUCOSE, INSULIN, AND GLUCAGON ON DAY 0:

Figure (1) demonstrates that there is no difference ($P>0.05$) in any of the measurements of glucose, insulin, or glucagon between the groups when compared to the control group or among themselves. Day 0 of the treatment is expected to show no improvement because STZ has not yet injected.

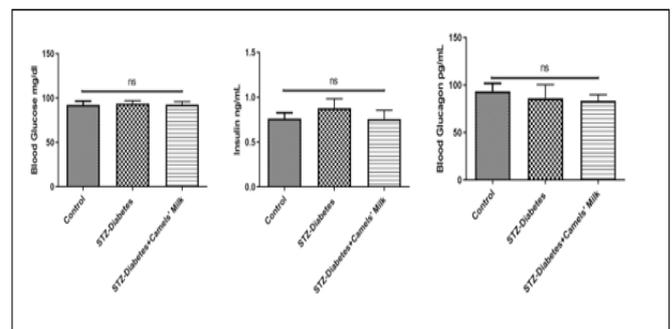


Figure 1: Day 0 shows no change ($P>0.05$) among groups regarding blood (glucose, Insulin, and Glucagon): Control: Chow and water (*ad libitum*), STZ-Diabetes: STZ (40mg/kg) on day0, STZ-Diabetes+Camels' Milk: STZ (40mg/kg) on day0 followed by administration of camels' milk. Ordinary one-wayANOVA and Newman-Keuls post hoc. N=10.

BLOOD GLUCOSE, INSULIN, AND GLUCAGON ON DAY 1 (ONE DAY AFTER STZ INJECTION AND CAMELS' MILK ADMINISTRATION)

Figure 2 displays the data gathered for the STZ-Diabetes and STZ-Diabetes+Camels' Milk groups one day after STZ injection and compares the results with the control and with each individual group. The STZ injection caused blood glucose and insulin levels to rise (****P<0.0001 and *P<0.05) in STZ- Diabetes and STZ-Diabetes+Camels' Milk groups compared to the control group. As demonstrated below, there was no difference in the blood glucagon level across groups. We didn't find any difference between STZ-Diabetes and STZ-Diabetes+Camels' Milk groups regarding all the parameters we have detected in Figure 2.

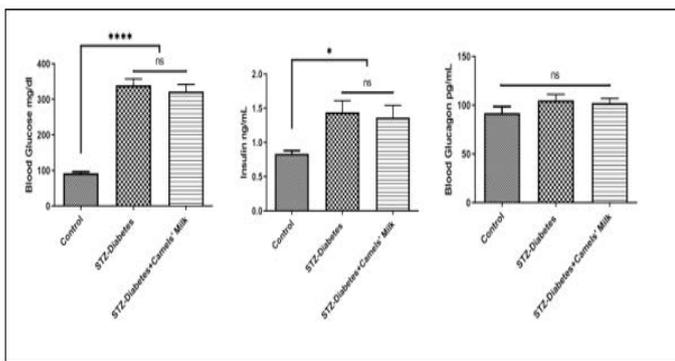


Figure 2: Day 1 shows the effect of STZ injection on blood (glucose, Insulin, and Glucagon): Control: Chow and water (*ad libitum*), STZ-Diabetes: STZ (40mg/kg) on day0, STZ-Diabetes+Camels' Milk: STZ (40mg/kg) on day0 followed by administration of camels' milk. Ordinary one-wayANOVA and Newman-Keuls post hoc. N=10.

BLOOD GLUCOSE, INSULIN, AND GLUCAGON ON DAY 7 (ONE WEEK AFTER STZ INJECTION AND CAMELS' MILK ADMINISTRATION)

Data obtained from research groups revealed that blood glucose levels in treatment groups in contrast with control group one week after STZ injection (*P<0.01 and P<0.05, respectively). In contrast with control animals, blood insulin levels of STZ-Diabetes and STZ-Diabetes+Camels' Milk groups had a dramatic decrement (*** P<0.001). Blood glucagon readings in all groups hasn't shown any difference (Figure 3).

BLOOD GLUCOSE, INSULIN, AND GLUCAGON ON DAY 14 (TWO WEEKS AFTER STZ INJECTION AND CAMELS' MILK ADMINISTRATION)

In all study groups, the impact of camel milk and STZ injection on glucose homeostasis is shown in Figure 4 on day 14. Unlike control and STZ-Diabetes+Camels' Milk groups, there was a substantial (****P<0.0001) rise in glucose levels in the STZ-Diabetes group. STZ-Diabetes+Camels' Milk still has higher blood glucose levels than what is found in control group (****P<0.0001). Blood insulin levels in STZ-Diabetes and STZ-Diabetes+Camels' Milk groups considerably decreased (P<0.0001). Blood glucagon data showed that the STZ-Diabetes group, significantly, had the greatest level (***P<0.001 and *P<0.05).

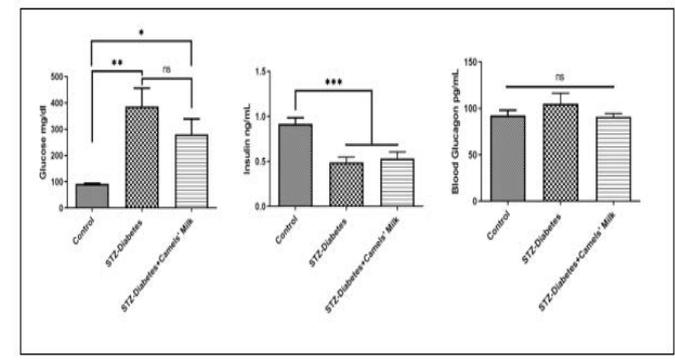


Figure 3: Day 7 shows the effect of STZ injection and Camels milk on blood (glucose, Insulin, and Glucagon): Control: Chow and water (*ad libitum*), STZ-Diabetes: STZ (40mg/kg) on day0, STZ-Diabetes+Camels' Milk: STZ (40mg/kg) on day0 followed by administration of camels' milk. Ordinary one-wayANOVA and Newman-Keuls post hoc. N=10.

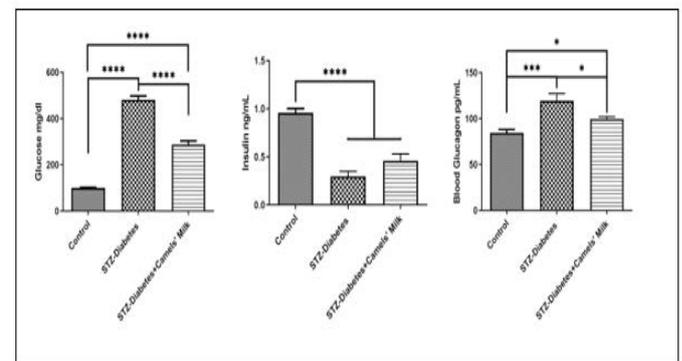


Figure 4: Day 14 shows the effect of STZ injection and Camels milk on blood (glucose, Insulin, and Glucagon): Control: Chow and water (*ad libitum*), STZ-Diabetes: STZ (40mg/kg) on day0, STZ-Diabetes+Camels' Milk: STZ (40mg/kg) on day0 followed by administration of camels' milk. Ordinary one-wayANOVA and Newman-Keuls post hoc. N=10.

BLOOD GLUCOSE, INSULIN, AND GLUCAGON ON DAY 21 (THREE WEEKS AFTER STZ INJECTION AND CAMELS' MILK ADMINISTRATION)

All study groups had their blood glucose, insulin, and glucagon levels tested. Comparing STZ-Diabetes group to the control and STZ-Diabetes+Camels' Milk groups, the blood glucose level in STZ-Diabetes group was considerably higher (****P<0.0001). Compared to STZ-Diabe-

tes group, camel milk significantly ($****P<0.0001$) lowers blood glucose levels. Insulin measurements were statistically ($****P<0.0001$) lower in STZ-Diabetes and STZ-Diabetes+Camels' Milk groups compared to the control group. In comparison to the control and STZ- Diabetes+Camels' Milk groups, STZ-Diabetes group has the highest blood glucagon. Interestingly, camel milk increased blood glucagon levels to the point where there was no change ($P>0.05$) between the control and STZ-Diabetes+Camels' Milk groups (Figure 5).

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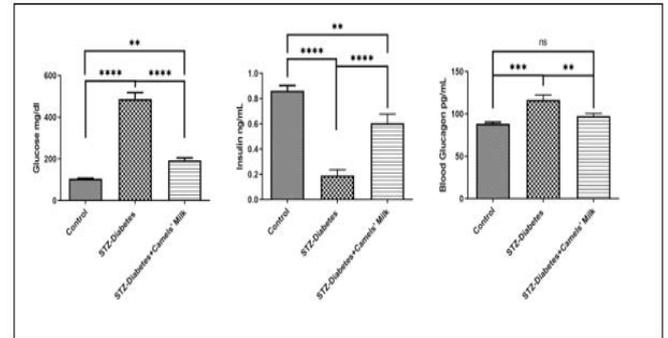


Figure 6: Day 28 shows the effect of STZ injection and Camels milk on blood (glucose, Insulin, and Glucagon): Control: Chow and water (*ad libitum*), STZ-Diabetes: STZ (40mg/kg) on day0, STZ-Diabetes+Camels' Milk: STZ (40mg/kg) on day0 followed by administration of camels' milk. Ordinary one-wayANOVA and Newman-Keuls post hoc. N=10.

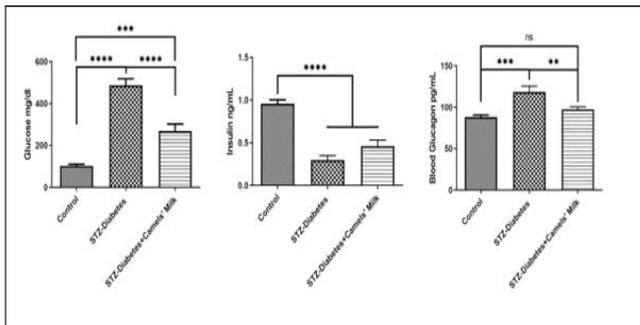


Figure 5: Day 21 shows the effect of STZ injection and Camels milk on blood (glucose, Insulin, and Glucagon): Control: Chow and water (*ad libitum*), STZ-Diabetes: STZ (40mg/kg) on day0, STZ-Diabetes+Camels' Milk: STZ (40mg/kg) on day0 followed by administration of camels' milk. Ordinary one-wayANOVA and Newman-Keuls post hoc. N=10.

BLOOD GLUCOSE, INSULIN, AND GLUCAGON ON DAY 28 (FOUR WEEKS AFTER STZ INJECTION AND CAMLES' MILK ADMINISTRATION)

To assess the effectiveness of camel milk in treating STZ-induced diabetes, blood samples were taken on day 28 and tested for glucose, insulin, and glucagon levels. In contrast to the STZ-Diabetes group, the camel milk-treated group (STZ-Diabetes+Camels' Milk) had considerably lower blood glucose levels ($****P<0.0001$) than the STZ-Diabetes group. Amazingly, compared to the STZ-Diabetes group, camel's milk considerably ($****P<0.0001$) increased blood insulin levels. As predicted by the data gathered on day 21, camel milk entirely improved the blood glucagon level, and as can be seen in the histogram, no change between STZ-Diabetes+Camels' Milk and control groups (Figure-6). To summarize, Figure 7 shows the overall influence of camels' milk in diminishing the effects of STZ-induced diabetes. STZ-Diabetes group (red-square) had the highest blood glucose level, whereas the control group (black-dotted) had the lowest. On the other hand, the STZ-Diabetes group had the greatest glucagon curve and the lowest insulin curve. The fact that STZ-Diabetes+Camels' Milk was in the middle of all curves indicates that camel milk has a favorable effect on minimizing the negative effects of STZ-induced diabetes on overall body

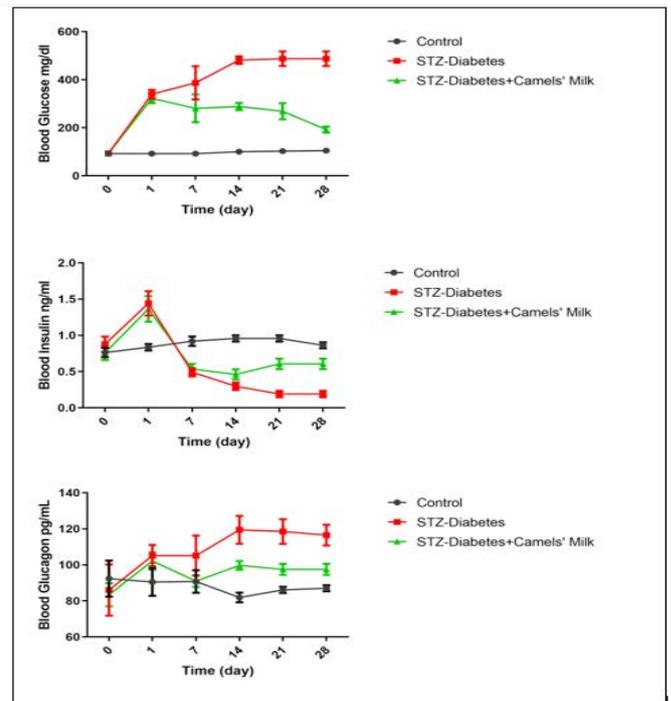


Figure 7: Curves represent blood glucose, insulin, and glucagon levels during the experiment. Glucose; Interaction: $F(10,60)=9.97, P<0.0001$, Time: $F(5,60)=22.65, P<0.0001$, and Treatment: $F(2,12)=187.49, P<0.0001$, - Insulin; Interaction: $F(10,75)=16.27, P<0.0001$, Time: $F(5,75)=38.25, P<0.0001$, and Treatment: $F(2,15)=7.96, P=0.0044$, and Glucagon, Interaction: $F(10,60)=3.52, P=0.0011$, Time: $F(5,60)=4.08, P=0.0030$, and Treatment: $F(2,12)=3.71, P=0.0558$, respectively (two-way ANOVA) and Bonferroni posttests. N=10.

IHC detection of insulin and glucagon in pancreatic tissues is shown in IHC sections and histograms. In comparison to the control group, the insulin staining intensity was lowest in the STZ-Diabetes group. Compared to STZ-Diabetes, the insulin intensity in the STZ-Diabetes+Camels' milk group considerably increased (**P<0.01). STZ-Diabetes group had the greatest levels of glucagon (*P<0.05) compared to the control and STZ-Diabetes+Camels' Milk groups. Remarkably, control and STZ-Diabetes+Camels' Milk groups showed no difference between them (Figure-8).

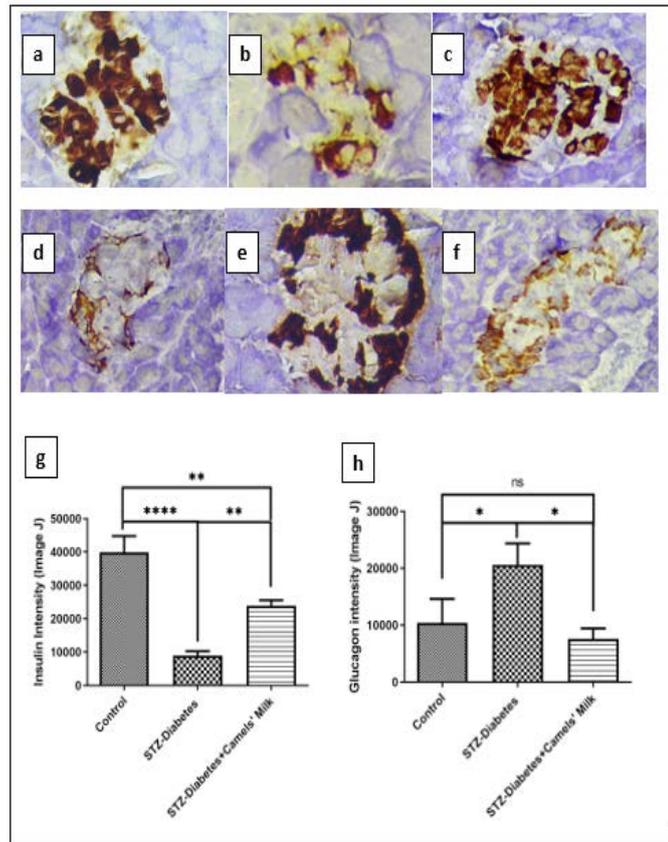


Figure 8: IHC of pancreatic tissues showed that the intensity of insulin and glucagon staining in Control: Chow and water (ad libitum), STZ-Diabetes: injected with single dose of STZ 40mg/kg) on day0, STZ-Diabetes+Camels' Milk: STZ)40mg/kg) on day0 followed by administration of camels' milk. . a. Control-insulin, b. STZ-Diabetes-insulin, c. STZ-Diabetes+Camels' Milk-insulin, d. Control-glucagon, e. STZ-Diabetes-glucagon, f. STZ-Diabetes+Camels' Milk-glucagon, g and h: Histograms showing means and SEM of insulin and glucagon, respectively. One way ANOVA and Newman-Keuls Multiple Comparison Test, N=10 *P<0.05 and **P<0.01. (20X magnification)

The dromedary, also known as the one-humped or Arabian camel (*Camelus dromedarius*), is found in the arid and desert regions of Africa and Asia, where it serves as a source of meat and milk for the Bedouin people who live in the desert. These people live in harsh climates with limited access to water and food (Omer & Eltinay, 2008). In contrast to other milk-producing animals, camels can survive in harsh environments with high temperatures, draughts, and a shortage of grasses while still producing milk with excellent nutritional value (Omer & Eltinay, 2008). On day 0, there was no any difference among the groups as expected since the experiment has not yet begun. The data collected on day1, one day after injection of STZ (40 mg/kg) showed high level of blood glucose as a sign of STZ-induced diabetes and that comes in an agreement with (Deeds et al., 2012) and (Al-Yasari et al., 2022). STZ is a broad-spectrum antibiotic that is especially harmful to the pancreatic beta-cells that make insulin. It is also utilized to treat -cell pancreatic tumors because to its selective toxicity (Szkudelski, 2001). The high blood insulin that was observed along with hyperglycemia on day 1 was agreed with Lenzen, 2008. STZ (streptozotocin), STZ damages pancreatic cells and results in hypoinsulinemia and hyperglycemia. The STZ can result in diabetes in one of two ways, depending on the dosage. Due to its structural similarity to glucose, STZ can attach to the GLUT2 glucose transporter receptor, which helps explain why some cell types preferentially accumulate the molecule (Lenzen, 2008). We found that STZ leads to high glucagon levels as it is demonstrated previously. Dusaulcy and his colleagues show that the primary causes of the higher glucagonemia in diabetic animals was due to an increase in glucagon release and biosynthesis. Additionally, some genes that encode proteins essential for glucagon production and secretion, beta cell differentiation, and putative stress signals like the glucagon receptor are found to be related to glucagon level (Dusaulcy et al., 2016). We discovered that camel milk has the effect to mitigate the STZ-induced alterations in blood glucose, insulin, and glucagon in addition to histological detection of insulin and glucagon. Camel milk may offer beneficial nutritional qualities that can help with diabetes treatment, according to several studies (Kaskous, 2016, Hadeef et al., 2022, Malik et al., 2012). According to previous work, camels' milk is effective and safe to improve long-term blood glucose management, greatly reducing the need for people with type 1 diabetes to take as much insulin (Agrawal et al., 2011). Additionally, camel milk insulin can be encapsulated into micelles and guarded against proteolysis (Jilo and Jilo, 2016). Because it is enclosed in nanoparticles, the human body can easily absorb it and transfer it to the bloodstream. Camel milk is beneficial in improving glucose homeostasis among diabetics according

to Khan and Alzohairy (2011). Treatment with camel milk improved the lipid profile, serum insulin, and blood sugar levels in diabetic animals. The high concentration of insulin and insulin-like proteins, which might boost the activation of insulin receptors, may play a possible beneficial function in the management of diabetes. It might be due to fewer carbohydrates, which is advantageous for those with diabetes, as well as a lesser amount of lactose, which is responsible for the rise in blood sugar levels.

CONCLUSION

Our research showed that camel milk has powerful, secure anti-diabetic properties. Those discovering promising a future application of camel milk as a supplementary or even substitute natural product to enhance the diabetes-related health status markers. More investigation is required to determine the precise mechanism through which the amelioration effects are obtained.

ACKNOWLEDGMENTS

We would like to express our gratitude to Al-Muthanna University for the support and direction during the execution of this research.

CONFLICT OF INTEREST

No any conflict of interest.

NOVELTY STATEMENT

The specific objective and novelty of this work is to explore the medical uses of Arabian camels' milk as a natural product, which has a wide range of practical applications. Along with its accessibility and approachability.

AUTHOR CONTRIBUTION

All authors contributed equally.

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