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EVALUATION OF CAMEL MILK BETA-CASEIN PROTEIN HYDROLYSATES FUNCTIONALITY AGAINST INDUCED DIABETES IN RATS

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ABSTRACT

The current study was aimed to investigate the effect of beta-casein protein (β-CN) hydrolyzate, purified from Iraqi camel milk, on blood glucose levels, oral glucose tolerance test (OGTT), the levels of insulin, insulin-like growth factor 1 (IGF-1), level of liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT)] and the lipid profile status of alloxan-induced diabetes rats. The results indicated that the β-CN protein hydrolysates at concentrations of 50 and 100 mg/kg showed a significant ($p<0.05$) reduction in the level of blood glucose during fasting and in (OGTT). However, there were, significant increasing ($p<0.05$) in the level of insulin, GF-1 and the level of high-density lipoproteins (HDL-C), meanwhile, a significant decreasing were observed on levels of liver enzymes AST and ALT, the levels of cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL-C), and very low-density lipoproteins (VLDL-C). The findings of this study suggest camel milk β-CN protein hydrolysates could be as natural alternative product for the pharmacology medication in controlling the high blood glucose, increasing the concentration of insulin and IGF-1, in addition to controlliny the levels of lipids and liver enzymes at the normal limit.

Key words: alloxan, cholesterol, Glucose, IGF-1, insulin.
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INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by a high level of glucose in the blood due to a complete or relative deficiency in the secretion of the hormone insulin, or the occurrence of insulin resistance. The spread of diabetes has increased significantly in all countries of the world in recent years (Khashan, and Majeed, .2024; Yuen *et al*.,2021). World Health Organization (WHO) reports indicate that the incidence of diabetes continues to rise annually, and according to estimates by the International Diabetes Federation (IDF), the prevalence of diabetes in 2021 is estimated among people between the ages of 20 and 79 years, all over the world by 10.5%, as more than 536.6 million cases of diabetes have been diagnosed. This number is expected to rise to 643 million by 2030. In 2045, the percentage may rise to 12.2%, with a rate of 783 million diabetics (Sun *et al*.,2022). Insulin resistance is a fundamental feature of diabetes, obesity, as well as cardiovascular disease, and contributes to many metabolic syndrome abnormalities. Insulin resistance is defined as an abnormal response to normal insulin levels in the body, or a situation where a greater-than-normal insulin concentration may be necessary for a normal response (Hameed, and Al-Ameri, .2022). Controlling blood glucose levels has become one of the essential effects in the management of diabetes, and there are several pharmacological methods to control blood glucose levels, including stimulating insulin secretion, protecting beta cells, and others (Pappachan *et al*.,2019). However, long-term use of blood glucose-lowering medications and insulin leads to side effects associated with hypoglycemia, renal insufficiency,

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digestive system disorders, as well as hypertension (Abdukarim *et al*.,2019; DeMarsilis *et al*.,2022). Camel milk proteins have received attention from many researchers in the past few years because camel milk and the proteins derived from it possess many functional, therapeutic, and nutritional properties for human health, including anticoagulant, anti-inflammatory, lipid-lowering, anti- hypertensive, immunomodulatory activity, antidiabetic type 2, and hypercholesterolemia (Mudgil, and Maqsood, 2023; Redha *et al*.,2022). This was confirmed by many laboratory studies conducted in vivo using experimental animals, as they found biologically active hydrolyzates and peptides derived from camel milk provide many biological and physiological functions, which have a major role in reducing high blood pressure, high antidiabetic, intestinal pathogens, and others (Althnaibat *et al*.,2024; Su et al.,2024). Several studies have been shown that hydrolyzed camel milk proteins have much stronger and higher antidiabetic properties when compared to intact, non-hydrolyzed camel milk proteins (Kilari *et al*.,2021; Mudgil *et al*.,2018; Wang *et al*.,2020). It was found that the hydrolyzates of β-CN protein of camel milk resulting from the enzymatic hydrolysis using pepsin showed high activity against two Gram-positive strains bacteria, namely *Listeria innocuas* ATCC 33090 and *Escherichia Coli* ATCC, antioxidants, antimicrobials, and ACE inhibition (Almi-Sebbane *et al*.,2018; Ganzorig *et al*.,2020; Mati *et al*.,2017; Tagliazucchi *et al*.,2016). Interestingly, camel milk is one of the main sources that contain bioactive compounds and strong anti-diabetic properties, such as proteins, and insulin-like hormone, containing a low percentage of cholesterol, and lactose that is providing nutrition and many health benefits for humans (Oselu *et al*.,2022; Redha *et al*.,2022). Therefore, the current study aimed to evaluate the role of β-CN protein hydrolyzates isolated from camel milk against diabetes, the concentration of insulin levels, insulin-like hormone, lipids, and liver enzymes in rats with alloxan-induced diabetes.

MATERIALS AND METHODS

Casein preparation, isolation, and purification of β-CN protein: Raw camel milk was obtained from the local Iraqi breed from Al-Najaf Governorate. The milk was transported under refrigeration, directly to the laboratories of the College of Agricultural Engineering Scfiences / University of Baghdad, the milk fat was separated by refrigerated centrifugation at 2500 RPM for 20 minutes at 4°C, and the fat layer was removed manually. Acid casein was prepared by precipitating the casein with HCL (1M) at pH 4.3, Then the casein was separated using centrifugation at a speed of 4000 RPM for 20 minutes at a temperature of 4°C. The casein was obtained in the form of a white precipitate, and was washed three times with distilled water, then dried and preserved by freezing, the β-CN protein was isolated and purified using ion exchange technique on a cellulose DEAE column with dimensions of 3.5 x 15 cm, and the gel filtration technique Sephadex G-100 column with dimensions of 63 x 1.5 cm, according to the method mentioned by Doosh. (2022).

Confirmation of β-CN protein purity: To confirm the purity of the β-CN protein, the method described by Hadeel and Khalida, (2023) was adopted with some modifications, as the polyacrylamide gel electrophoresis method was used in the presence of (SDS-PAGE) and comparing them with the standard proteins and standard β-CN protein (bovine).

Hydrolysis of β-CN protein: Hydrolysis was carried out according to the method described by Al-Shaikh, and Doosh. (2024) with some modifications by dissolving 0.1 g of β-CN with 10 ml distilled water, and the pH was adjusted to 2 using (0.1 M) hydrochloric acid to suit the working conditions of the pepsin enzyme, then the pH was adjusted to 8 using (1 M) of NaOH to suit the working conditions of trypsin. As for the synergistic 1:1 pH change using NaOH and HCl, the enzymatic reaction was stopped by exposing the mixture to a temperature of 95°C for 10 minutes, and then the samples were centrifuged at 15000 RPM for 10 minutes at 4°C. The supernatant was collected and freeze-dried. The degree of

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hydrolysis was estimated using the method described by Emrerk *et al*., (2021).

Experimental animals: This experiment was conducted using 50 male albino rats aged 2-3 months and weights ranging from 200-250 g, which were obtained from the Faculty of Science / University of Kufa. The rats were placed in metal cages furnished with sawdust, and they were housed in a room with controlled laboratory conditions in terms of humidity 52±3% and temperatures 25°±2, as for the lighting, it was 12 hours of darkness and 12 hours of light, and it was left for seven days to acclimatize, food and water were given continuously and in sufficient quantities throughout the experiment period. After the acclimation period, 40 of experimental rate, fasting for 12 hours, were injected via intraperitonealy with alloxan prepared immediately at a concentration of 80 mg/kg of body weight (B.W) to induces diabetes and left for three days, after examining the blood glucose of the rats through the diabetic examination device and confirming the occurrence of the infection, as the glucose level ranged from 250-430 mg/dl, the rats were randomly distributed into four groups, with 10 rats for each group

Experimental design: Fifty rats were randomly divided into five groups (n=10), as each group contained 10 rats and was designated as follows:

Group 1. Normal control group without diabetes (C-), the rats were given a commercial diet and distilled water.

Group 2. Control group with alloxan-induced diabetes (C^+), were fed a high-fat 6% diet and distilled water.

Group 3 and 4. alloxan-induced diabetes (G1 and G2), were fed a high-fat diet 6% and distilled water, and orally-ingested with 50,100 mg/kg B.W. of β-CN protein hydrolyzates

Group 5. alloxan-induced diabetes (G3), were fed a high-fat diet 6% and distilled water, and orally-ingested with 7.14 mg/kg B.W. of metformin.

Throughout the trial period, weekly monitoring of fasting blood glucose levels was conducted.

Blood samples collection: At the end of the experiment, food and water were pending from rats for 12 hours, the rats were anesthetized, blood samples were drawn from the hearts of all rats placed in anticoagulant test tubes, and left for an hour, central blood was centrifuged, then at 3000 RPM for 15 minutes to obtain the serum and storage at -20 °C for biochemical tests.

Measurement of fasting blood glucose levels

Fasting blood glucose levels were monitored and measured twice a week continuously to confirm hyperglycemia using an Accu-Check blood glucose meter after drawing blood from the tail.

Oral glucose tolerance test: At the end of the 30-day experiment, an oral glucose tolerance (OGTT) was tested after fasting for 12 hours, and then all rats were orally administered glucose (2.5 g/kg B.W), the concentration of blood glucose levels was measured at 0, 30, 60,90, and 120 minutes by drawing a drop of blood from the tail, using an (Accu-Check) glucose meter to (Ghanbari *et al*.,2016; Haddad, and Doosh ,2023).

Estimating the concentration of insulin and IGF-1 in blood serum: The level of insulin in the blood serum was determined by the ELISA method, using BT Lab's Kit and following the manufacturer's instructions.

Estimating the level of liver enzymes (AST) and (ALT): AST and ALT levels in liver and serum samples were determined according to the method prescribed by Ismail *et al*., (2018).

Estimation of blood lipid level

Total cholesterol levels TC, TG, and HDL-C c were estimated by enzymatic method using ready-made diagnostic reagent (Kit) kits prepared by the company and following the manufacturer's manual, while LDL-C proteins and VLDL-C proteins were calculated according to the following equations:

LDL-C concentration (mg/dl) = Total cholesterol - [(HDL-C) + (VLDL-C)].

VLDL-C concentration (mg/dl) = (Triglycerides / 5).

Statistical analysis: The results were analyzed statistically using the GenStat Release 12.1 program a completely randomized design (CRD) was used, and the significant differences between the means were compared

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using Duncan's test at a significance level ($p \geq 0.05$).

RESULTS AND DISCUSSION

B-CN protein purification using ion exchange technology and gel filtration: After the β-CN protein was obtained by primary purification using the urea fractionation method from the total caseinate, it was purified by ion exchange chromatography on the DEAD-Cellulose column and the gel filtration chromatography. The results in Figure (1-A) show the appearance of two protein peaks, the first appeared in the tubes (5-11), and the second peak appeared in tubes (15-28), which represents the β-CN, and the result was consistent with the findings of Ellouze *et al.*, (2021) , Who indicated that purification of β-CN by ion-exchange chromatography method is contaminated with other proteins such as k-CN. to obtain pure β-CN protein, final purification was carried out by gel filtration chromatography method, depending on molecular weight, the results in Figure (1-B) showed the presence of one peak of proteins in pure form, which is the protein β-CN, the tubes containing β-CN were then collected and lyophilized, This result was consistent with the findings of AlKhalidy and Dosh.,(2022) who found only one peak when sheep's milk β-CN protein was fractionated using gel filtration technique

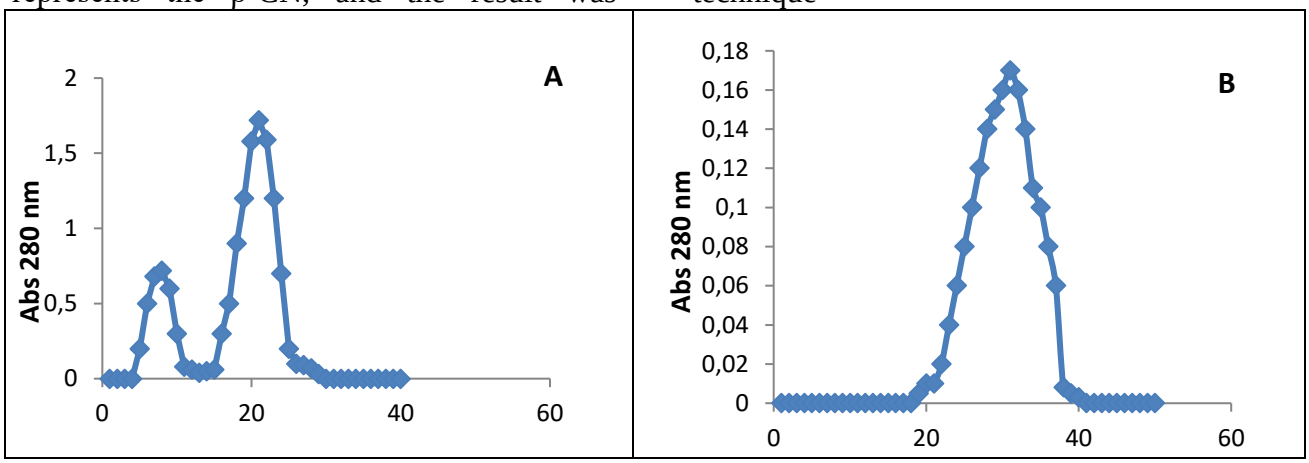

Figure 1. Purification of camel milk β-CN (A) using ion exchange chromatography technique on DEAD-Cellulose column and (B) by gel filtration technique using Sephadex G-100

SDS-PAGE analysis of camel milk β-CN protein. The (SDS-PAGE) was carried out to ascertain the purity of the extracted protein, and the results as shown in Figure (2-A) showed the appearance of two protein bands, the first bands represents the β-CN protein and the second band is traces of K-CN protein associated with the β-CN protein. This result is consistent with the findings of Mohamed *et al.*,(2022) who stated that the protein purified using the ion exchange technique was contaminated with K-CN, Figure (2-B) also shows the appearance of β-CN protein in one pure bands (after gel filtration) in a polyacrylamide gel, and this confirms its purity and the bands of β-CN is free of any traces of the other caseins such as K-CN, This result agreed with what found by Ellouze *et al.*,(2021).

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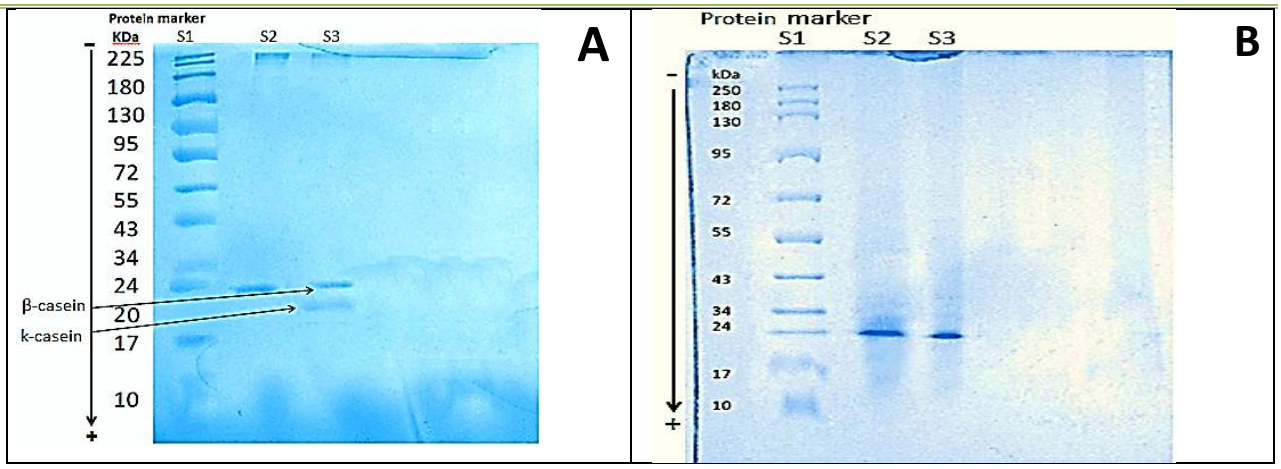

Figure 2. Purification of the β-CN protein by SDS-PAGE (A) by ion exchange chromatography technique (B) by gel filtration chromatography technique, where S1 = Ladder protein, S2 =β -CN standard, S3 in (A) = β-CN resulting from purification by ion exchange, S3 in (B) = β-CN resulting from the purification by gel filtration

Hydrolysis of β-CN protein

Figure (3) shows the degree of hydrolysis (DH%) of the β-CN protein purified from camel milk by the degradation enzymes Pepsin and trypsin individually and indeed the synergism of both enzymes in a ratio of 1:1 for 8 hours to obtain β-CN protein hydrolysates, has been noticed from the figure an increase in DH with time preceding, β-CN protein hydrolysate were obtained by pepsin, trypsin, and Synergistic after 8 hours, as the values of DH reached 52.165,44.563,48.267% respectively, These results were consistent with those found by Taghipour *et al*.,(2023) and Vorob'ev,(2022) who observed an increase in the degree of degradation with the time of decomposition. The highest degree of decomposition after 8 hours was recorded by the synergistic action of the two enzymes, reaching 52.16%. The results agreed with the findings of Akan, (2021), as it was found that the DH value for the synergistic action of the β-CN protein decomposing reached 53.06%, he reported that the peptides he obtained from camel milk caseinate were anti-diabetic and powerful antioxidants.

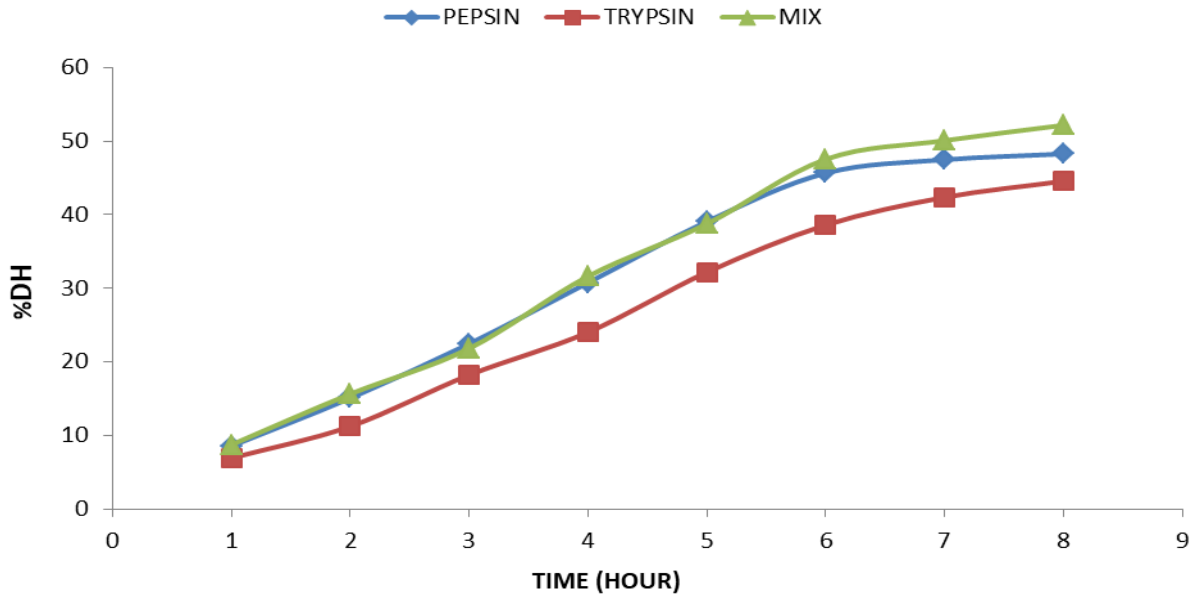

Figure 3. Degree of enzymatic hydrolysis the β-CN protein using the pepsin, trypsin, and their mixture in a 1:1 ratio

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Effect of CN-β protein hydrolysates on blood glucose level : The results in Table (1) show the effect of oral dosing with β-CN protein hydrolyzates isolated from camel milk on blood glucose level, injection rats with alloxan led to an increase in the level of blood glucose level at a high level of significance at ($p<0.05$) at the first week of the experiment for all treatments (C^+, G1, G2, and G3) being 336.3, 327.0, 338.1, 328.9 mg/dl respectively, compared to the negative control treatment C^- which recorded the lowest level of glucose concentration in the adult blood 105.7 mg/dl. The highest concentration of glucose was recorded by G2 group it the first week of experiment, these results were consistent with those obtained by Chen *et al*., (2024) who observed an increase in blood glucose concentration in all STZ-injected treatments to varying degrees at the beginning of the trial.

Table 1. Effect of β-CN Protein Hydrolyzates on Blood Glucose Concentration Level in rats

Treatments	Blood glucose level (mg/dl) Mean± SD Average of the first week	Average of the second week	Average of the third week	Average of the fourth week
C^-	a 105.7±8.693	a 105.7±7.342	a 108.6±6.973	a 109.9±4.375
C^+	b 336.3±58.43	c 350.4±55.46	d 359.0±56.99	d 350.9±54.86
G1	b 327.0±38.57	b 302.3±19.05	c 208.0±22.05	c 161.3±8.036
G2	b 338.1±50.34	b 286.7±24.94	b 156.9±4.298	ab 127.9±4.451
G3	b 328.9±51.43	b 299.7±41.84	c 193.6±26.84	ab 149.9±10.67
L.S.D. (p<0.05)	49.20	37.39	37.39	27.73

Mean ± SD (n=10). \The different small letters within the Rows indicate a significant difference at (p<0.05)) according to Duncan's test. (C^-) Control natural, (C^+) diabetic control, (G1) diabetic and treated of β-CN protein hydrolyzates (50mg/kg/day), (G2) diabetic and treated of β-CN protein hydrolyzates (100 mg/kg/day), (G3) diabetic and treated group of metformin (7.14 mg/kg/day).

The results of the statistical analysis at the end of the experiment also indicated, that dosing with β-CN protein hydrolyzates isolated from camel milk led to significant decrease ($p<0.05$) in the blood glucose level in the group of diabetic rats, represented by G1 and G2, as it reached 161.3. and 127.9 mg/dl, compared with the positive control C^+, which recorded the highest level of 350.9 mg/dl, the G2 treatment which was dosed with 100 mg/kg β-CN hydrolyzates, recorded the lowest blood glucose level, 127.9 mg/100 ml these results were consistent with Kilari *et al*.,(2021), who found that dosing rats with camel milk protein hydrolyzates was strongly, effective in lowering blood glucose levels at a higher level of significance at the end of the experiment than at the beginning of the experiment. Additionally, these findings agreed with what was found by Chen *et al*., (2024), who observed a significant decrease ($p<0.05$) in blood glucose in group which treated with camel milk protein hydrolysates. This decrease in glucose level may be attributed to the strong role of β-CN hydrolyzates and due to high concentration of insulin in camel milk (three times higher than in cow's milk). In addition, camel milk insulin is a natural protein that leads to reducing glucose levels naturally without affecting the body, it also shows its hypoglycemic effect when taken orally, because it resists breakdown by stomach acidity and enzymes as it is encapsulated in nanoparticles, it is not exposed to digestion and remains available for intestinal absorption (Korish *et al*.,2020).

Effect of β-CN protein hydrolyzates on glucose tolerance test: An oral glucose tolerance test (OGTT) was performed at the end of the experiment, the results showed in Figure (4) the changes occurring in blood glucose levels at 0-120 minutes after fasting in all treatments, it is noted that blood glucose levels in all treatments at time zero (0 minutes)

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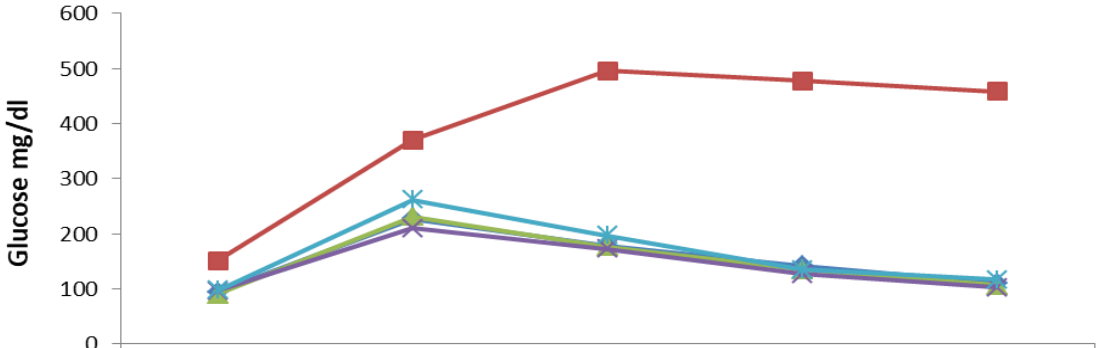
of glucose tolerance were within the normal range, except for the positive control C^+ treatment with diabetes and the untreated one, which reached 151.33 mg/dl, after 30 minutes, the blood glucose concentration increased in all treatments, At the 60th minute, blood glucose levels began to decrease in the group of rats treated with C^-, G1, G2, and G3, and in contrast, its level increased in the group C^+ of rats even at 120 minutes.



TIME (MIN)	0	30	60	90	120
C-	94	227.3	177.7	141.7	110.3
C+	151.33	369.7	495.7	477	457.7
G1	90.33	230.7	176.7	134.3	106.3
G2	96.67	210	172.7	127	102.3
G3	97.33	261	196.3	134.7	116.3

Figure 4. oral glucose tolerance pattern after 120minet for all treatment

The results indicated a significant decrease in the probability level ($p<0.05$) in blood glucose levels in experimental animals in treatments G1, G2, and G3, blood glucose levels decreased to the normal limit, reaching 106.3, 102.3, and 116.3 mg/dl compared to treatment C^- in which the glucose level reached 110.3 mg/dl, as for treatment C^+, an increase in the level of glucose in the blood was observed, reaching 457.7 mg/dl, these results were consistent with what was reported by Yu *et al*.,(2024), who found a significant increase in the level of blood glucose during fasting in the diabetic and negative control group compared to the control positive group and the other treatments, the findings also agreed with Kilari *et al*.,(2021) found, where a decrease in the level of glucose concentration in the blood was found in rats in which glucose was introduced and treated with camel milk compared to its high level in diabetic rats and left untreated.

Effect of β-CN protein hydrolysates on insulin level and IGF-1: Table (2) shows the effect of oral dosing with different concentration of camel milk β-CN hydrolyzates on insulin levels and IGF-1, in comparison with the negative control treatment C^-, the positive control C^+, and the G3 treatment that was dosed with metformin. The results show an increase in the level of insulin in the group G1, G2, and G3, as the rate of increase reached 3.75, 42.49, and 3.04%, compared to the C^- treatment, as for the group of rats in the G^+ treatment that had diabetes and were left without treatment until the end of the experiment, it was recorded the highest rate of decrease in insulin level reached -44.86% compared to the C^- group. These results agreed with Taher, (Taher,2016) who found a decrease in the level of insulin in alloxan induces diabetes rabbits, this could be attributed to the alloxan, which caused pancreas and liver damage and thus affected the secretion of insulin by beta cells in the islets of lenkerhans. These results agreed with the findings of Jandal and Naji, (2021), as a significant decrease was found in the concentration of insulin levels in the blood of alloxan-induced diabetes rats compared to the negative control group. This result also agreed with the findings of Mansour *et al*., (2027), who found a decrease in the insulin level in diabetic rats that were left without treatment, and an increase in its level in a group of diabetic rats that were treated with camel milk.

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Table 2. Effect of β-CN protein hydrolyzates at different concentrations on the levels of insulin and I, insulin-like growth factor 1 (IGF-1) in experimental animals

Treatments	Mean± SD Insulin(U/ml)	Percentage changes %	IGF-1 (ng/ml)	Percentage changes %
C^-	b 5.779 ±0.132	0	b 59.5±7.392	0
C^+	a 3.186± 0.362	-44.86	a 34.1±2.473	-42.68
G1	b 5.996±0.135	3.75	b 65.1±7.977	9.41
G2	c 8.235±0.207	42.49	b 67.5±12.38	13.44
G3	b 5.955±0.280	3.04	a 42.4±0.774	-28.73
L.S.D. (p<0.05)	0.4366	—	13.57	—

Mean ± SD (n=10). \The different small letters within the Rows indicate a significant difference at (p<0.05)) according to Duncan's test. (C^-) Control natural, (C^+) diabetic control, (G1) diabetic and treated of β-CN protein hydrolyzates (50mg/kg/day), (G2) diabetic and treated of β-CN protein hydrolyzates (100 mg/kg/day), (G3) diabetic and treated group of metformin (7.14 mg/kg/day).

The results in Table (2) indicate that the β-CN protein hydrolyzates isolated from camel milk at different concentrations increased the level of IGF-1 concentration compared to the control treatment. The G2 treatment group of rats recorded the highest percentage of increase, reaching 13.44% followed by group G1, with an increase rate of 9.41%, compared to treatment C^-. This result is consistent with the findings of Hassan, and. Bayoumi, (2010), that camel milk led to a significant increase in IGF-1 in the group of infected rats treated with camel milk compared to the group of untreated rats. The lowest decrease in the level of IGF-1 concentration was recorded by the C^+ treatment, with -42.68% decrease, followed by the G3 treatment, as the decrease rate reached -28.73% compared to the C^- treatment.

Effect of β-CN protein hydrolyzates on liver enzymes (AST and ALT) level: Table (3) illustrates indicate the effect of oral dosing with camel milk β-CN hydrolyzates at different concentration on the level and functions of liver enzymes ALT and AST, as compared with the negative control treatment C^-, the positive control C^+, and the G3 treatment that was dosed with metformin. a significant increase ($p<0.05$) was noticed in the level of ALT and AST enzymes in group C^+ with diabetes, (66.5 and 43.8%) respectively, compared to C^- group. This increase in the concentration of liver enzymes is attributed to damages in liver tissue as a result of injection with alloxan. Consequently, necrosis or damage in liver cells causes the diffusion of the enzymes outside the damaged cells, to blood stream and the high concentration of liver enzymes AST and ALT in blood serum is one of the main indicators of diabetes (Rafaqat *et al.*,2023).

Table 3. Effect of β-CN protein hydrolyzates at different concentrations on AST and ALT liver Enzyme levels concentration for experimental animals

Treatments	Mean± SD ALT(U/L)	Percentage changes %	AST(U/L)	Percentage changes %
C^-	c 50.43±0.850	-20.45	b 142.10±1.253	-39.94
C^+	d 83.98±4.942	0	e 204.33±3.250	0
G1	b 40.40±1.253	-51.89	c 154.40±2.022	-24.43
G2	a 33.40±1.700	-60.22	a 136.90±2.800	-33.00
G3	c 54.50±3.251	-35.10	d 161.17±2.329	-21.12
L.S.D. (p<0.05)	5.157	—	4.418	—

Mean ± SD (n=10). \The different small letters within the Rows indicate a significant difference at (p<0.05) according to Duncan's test. (C^-) Control natural, (C^+) diabetic control, (G1) diabetic and treated of β-CN protein hydrolyzates (50mg/kg/day), (G2) diabetic and treated of β-CN protein hydrolyzates (100 mg/kg/day), (G3) diabetic and treated group of metformin (7.14 mg/kg/day).

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The results in Table (3) indicate that oral dosing with camel milk β-CN protein hydrolyzates resulted in improved liver function for G1 and G2 group, as a significant decrease ($P<0.05$) in the concentrations of liver enzymes ALT and AST in the G1 group, (-51.89 and -24.43%) compared to positive control G^+. G2 group, is recorded the highest percentage of decrease in liver enzymes levels being (-60.22 and -33.00%) compared to positive control G^+. The group G3 also showed a decrease in the concentration of liver enzymes reaching -35.10 and -21.12%, respectively, these results were consistent with the results of many established studies conducted on experimental animals, which found a significant increase in the concentration of liver enzymes in the group of rats with diabetes induced by alloxan. A significant decrease ($p<0.05$) in the concentrations of liver enzymes ALT and AST in the group of rats treated with hydrolysates camel milk proteins and camel milk proteins (Hussain *et al*.,2021; Kilari *et al*.,2021; Mailam, 2017; Shafiq *et al*.,2024).

Effect of β-CN protein hydrolyzates on Lipid Profile: Table (4) shows the effect of oral dosing with different concentrations of β-CN protein hydrolyzates on the level of T.C, TG, VLDL-C and LDL-C serum in rats under study, and compared with negative control C-, positive control C^+ and G3 group which was dosed with metformin. The results indicated a significant increase at the probability level ($p < 0.05$) in lipid profiles TC, TG, LDL-C, and VLDL-C in the group C^+, by different percentages of 40.84, 84.14, 78.34, and 84.14%. and a decrease in the level of beneficial proteins (HDL-C), with a decrease rate of -52.58% compared to the group of C^-, the reason may be attributed to the lack of insulin, which in turn leads to a change in the lipoprotein synthesis in the liver in diabetic rats, as well as a decline the activity of some enzymes that affected by the absence or lack of insulin, such as lipoprotein lipase (LPL) and cholesterol ester transport protein (CETP), thus reduces the activity of the hepatic lipase enzyme and many stages of biologically active LPL synthesis. These findings are consistent with several studies by researchers who found an increase in lipid profiles and low HDL-C levels in experimental animals with alloxan-induced diabetes or streptozotocin, which were not treated compared to the natural control group (Hauwa'u *et al*.,2023; Mansour,2017; Yu *et al*.,2024).

Table 4. Effect of β-CN protein hydrolysates at different concentrations on Lipid profile in the blood serum of experimental animals

Treatments	Mean± SD T.C mg/dl	T.G mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
C^-	ab 60.47± 3.774	b 32.80±1 .630	c 17.61±0.2 06	b 36.30±3.2 86	b 6.560±0.3 26
C^+	c 85.17± 4.606	d 60.40±0 .854	a 8.35±0.40 9	c 64.74±4.6 85	d 12.080±0. 171
G1	b 62.10± 1.253	b 35.70±3 .148	c 17.41±0.6 12	b 37.55±0.9 69	b 7.140±0.6 30
G2	a 55.20± 2.606	a 28.43±1 .172	d 20.54±0.6 66	a 28.97±2.8 28	a 5.687±0.2 34
G3	b 61.03± 1.716	c 40.87±0 .404	b 14.58±1.5 10	b 38.28±1.8 28	c 8.173±0.0 808
L.S.D.(p< 0.05)	5.564	3.134	1.480	5.459	0.6267

Mean ± SD (n=10). \The different small letters within the Rows indicate a significant difference at ($p<0.05$)) according to Duncan's test. (C^-) Control natural, (C^+) diabetic control, (G1) diabetic and treated of β-CN protein hydrolyzates (50mg/kg/day), (G2) diabetic and treated of β-CN protein hydrolyzates (100 mg/kg/day),(G3) diabetic and treated group of metformin (7.14 mg/kg/day).

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The results in Table (4) indicate a significant decrease in the level of TC, TG, LDL-C, and VLDL-C and a significant increase in HDL levels in the blood serum of the G1 and G2 group, which were dosed with β-CN protein hydrolysates at a concentration of 50 and 100 mg/kg B.W compared to group C^+ as the highest reduction percentage in lipid profile was recorded in G2 group, being (-35.19, -52.93, -55.25, -52.93%), respectively, The decreasing percentage for the G1 treatment was (-27.08, -40.89, -42.00, and -40.89%) respectively compared to the G^+ group, while the increasing percentages in the HDL level in the blood serum of the G1 and G2 group were 108.50 and 145.98% respectively compared to the C^- treatment, the reason is due to the positive role of β-CN protein hydrolysates in lowering the levels of TC, TG, LDL-C, and VLDL-C and raising the level of HDL-C insulin and IGF-1 as well, the presence of insulin and IGF-1 at a normal level in the blood is one of the necessary factors for regulating lipoproteins metabolism, which can inhibit lipoprotein lipase activity, Therefore, any disorder in the insulin level (absence or decrease) is related in one way or another to the proportion of HDL-C in the blood, and therefore its presence is important in increasing the level of HDL-C in the blood (Alhamid, and Mousawi, 2022; Alshuniaber *et al*.,2022). These results agreed with the findings of Chen e*t al*.,(2024) that there was a significant decrease ($p < 0.05$) in the levels of TC, TG, LDL-C, and VLDL-C, In negative control group compared with STZ-induced diabetes, group agreed with what El-Bahr *et al*.,(2023) findings who observed a significant decrease in the group of rats fed camel milk and a diet containing 1% cholesterol compared to the group of rats that were fed a diet containing 1% cholesterol. Kilari *et al*.,(2021) stated that camel milk protein hydrolysates led to a significant reduction in T.C, TG, LDL-C, and VLDL-C, in the diabetic rats group that was fed camel milk protein hydrolysates compared to positive group, It also agreed with Khalid *et al*.,(2023) that camel milk can be a useful complementary therapy in the treatment of patients suffering from type 1 and type 2 diabetes, This is due its high ability to reduce the levels of TC, TG, and LDL in the blood, and increase the level of HDL.

CONCLUSION

Camel milk β-CN protein hydrolyzates was significantly effective in lowering blood glucose to the normal limit, increasing the level of insulin and IGF-1, reducing the levels of liver enzymes ALT and AST, and decreasing the levels of T.C, TG, and LDL-C. VLDL-C and making it within normal limits, and increasing the high-density proteins (HDL) in alloxan-induced diabetes rats as compared to the C^+ positive control, Therefore, it can be considered an supportive treatment for diabetic and health promotion.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, no figures or images that do not belong to us have been incorporated into the manuscript, and the required permissions for re-publication are included with the manuscript. -Author/s signature on the Ethical Approval Statement.

AUTHOR'S CONTRIBUTION STATEMENT

Sh. A. H. designed the projectdata collection, analysis, and paper writing. Author k.s.d review the article and participates in its design and analysis

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تقييم فاعلية متحللات بروتين بيتا كازين حليب الإبل ضد مرض السكري المستحث في الجرذان

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المستخلص

هدفت الدراسة الحالية الى التعرف على تأثير متحللات بروتين البيتا كازين (β–CN) المنقى من حليب الابل العراقي على مستويات الكلوكوز في الدم، واختبار تحمل الكلوكوز عن طريق الفم (OGTT), ومستوى الأنسولين والبروتين الشبيه بالأنسولين (IGF–1) Insulin–like Growth Factor 1 ووظائف انزيمات الكبد (aspartate aminotransferase AST) و(ALT) alanine aminotransferase, وصورة الدهون في مصل الدم لدى الجرذان المصابة بداء السكري من النوع الثاني المستحدث بالألوكسان, بينت النتائج أن متحللات بروتين β–CN بتركيز 50 و 100 ملغم/كغم سببت انخفاضاً معنوياً (P <0.05) في مستوى الكلوكوز في الدم أثناء الصيام (OGTT)، كما بينت النتائج ارتفاع معنوي (P <0.05) في مستوى الانسولين و IGF–1 ومستوى البروتينات الدهنية عالية الكثافة HDL–C , في حين لوحظ انخفاض عالي في مستويات إنزيمات الكبد AST وALT, ومستويات الكوليسترول TC الكليسيريدات الثلاثية TG والبروتينات الدهنية واطئة الكثافة LDL–C والبروتينات الدهنية منخفضة الكثافة جدا–VLDL. تقترح نتائج هذه الدراسة إلى أن متحللات بروتين –β CN حليب الإبل يمكن ان تكون منتجا طبيعيا بديلا للأدوية الصيدلانية في السيطرة على مستوى نسبة الكلوكوز في الدم، وزيادة تركيز الانسولين و IGF–1 بالإضافة الى السيطرة على مستويات الدهون و انزيمات الكبد عند الحد الطبيعي.

الكلمات المفتاحية: الالوكسان، كوليسترول، الكلوكوز، IGF–1, الانسولين.

*** جزء من اطروحة دكتوراه للباحث الأول**