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RAW CAMEL MILK PROPERTIES ON ALLOXAN-INDUCED DIABETIC WISTAR RATS

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Abstract

Background and aims*: Diabetes is one of the most frequent and serious chronic diseases in humans all over the world. The aim of our study was to evaluate the antidiabetic activity of camel milk on serum glucose and lipid profile of alloxan-induced diabetic rats.* ***Materials and methods:*** *Diabetes was induced in Wistar albino rats by intraperitoneal injection of alloxan (120 mg/kg BW once). Albino rats each weighing 180-230g were divided into 3 equal groups (n=10) as following: G1- normal rats fed on normal diet, G2 - diabetic rats fed on normal diet, and G3 - diabetic rats were fed with raw camel milk. Fasting blood glucose was measured on days 0, 1, 7, 14, 21 and 30 while lipid profile was assessed at day 30.* ***Results:*** *After four weeks of feeding, data indicated a significant decrease ($p<0.05$) of mean blood glucose in G3 group (133.80±3.22 mg/dL) as compared with G2 diabetic rats (199.6± 7.33 mg/dL). Data also revealed significant lower levels ($p<0.05$) of triglycerides, total cholesterol, LDL and VLDL and higher level of HDL cholesterol in diabetic rats treated with camel milk as compared with diabetic rats fed a normal diet.* ***Conclusion:*** *Raw camel milk improved the glycemic and lipid profile in diabetic rats. These findings indicate that raw camel milk may have potential benefits in the treatment of diabetes. Future studies will be needed to establish its safety and mechanism of action.*

key words: *camel milk, alloxan, diabetes mellitus, hypoglycemic, hyperlipidemia, hyperglycemia*

Background and aims

Diabetes mellitus (DM) (diabetes = overflow, mellitus = honeyed [1]) is a metabolic disorder characterized by the presence of chronic hyperglycemia that follows a defect in insulin secretion, insulin action, or both - either immune-mediated (type 1 diabetes) or resulting from a combination of insulin deficiency and insulin resistance (type 2 diabetes) [2]. Several pathological processes are implicated in the onset and development of diabetes ranging from the autoimmune destruction of beta cells of the pancreas to the insulin resistance associated with obesity [3]. All forms of diabetes are characterized by more or less absolute or relative deficits in insulin secretion, or insulin resistance associated with chronic hyperglycemia and a

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disturbance in the metabolism of carbohydrates, lipids and proteins [4]. Insulin resistance is characterized by a decrease in insulin sensitivity of the whole body, muscle, liver and adipose tissue [5].

Over time, high blood glucose causes chronic complications affecting various organs and tissues: diabetic retinopathy, nephropathy, neuropathy, macrovascular disease, etc. [6]. Insulin deficiency in diabetes mellitus causes lipolysis in adipose tissue and lipoprotein lipase deficiency, resulting in hyperlipidemia and fatty liver disease. Thus, in diabetes, hypercholesterolemia and hypertriglyceridaemia often occur [7].

The global prevalence of diabetes among adults is 6.4%, reaching 285 million adults in 2010 and possibly 439 million adults by 2030. Between 2010 and 2030, there will be an increase of 69% of diabetes prevalence in adults in developing countries, and a 20% increase in developed countries [8]. WHO projects that diabetes will be the 7th leading cause of death in 2030 [9].

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) selectively destroys beta cells in the pancreas if injected in many animal species. This causes "Alloxan Diabetes", a form of insulin-dependent diabetes mellitus similar to type 1 diabetes [10,11]. Alloxan is the most common chemical compound used to induce experimental diabetes due to its selective destruction of beta cells in the pancreatic islets through sequential changes leading ultimately to apoptosis [12]. The dose of alloxan used to induce diabetes varies according to different species of animals such as rat: 40-200 mg/kg intravenously (iv.) or intraperitoneally (ip) [13,14]. Alloxan has two distinct pathological effects: it selectively inhibits insulin secretion induced by glucose, and causes a state of insulin dependence through its ability to generate reactive oxygen species (ROS) resulting in the selective necrosis of beta cells [15].

In arid and semi-arid areas, heat waves, water scarcity and feed, dromedaries remain the best supplier of milk, meat. Camel milk is also known for its medicinal therapeutic indications, which are often exploited for human health [16]. Nowadays, consumers are increasingly paying attention to foods that have a health advantage, safety and quality beyond basic nutrition [17]. The unique characteristics of camel milk make it commonly used in the field of therapy for its antimicrobial, antidiabetic and hepatoprotective properties [18]. The richness of camel's milk in vitamin C stimulating immunity makes it a main source of vitamin C for the desert inhabitants. Its acidity is unfavorable to bacterial growth, allowing milk to be stored under difficult conditions of high temperature for several hours [19]. It is well known that the value of camel milk is found in its high concentrations of linoleic acid and polyunsaturated acids, which are beneficial to human nutrition [20].

Camel milk is different from other ruminant's milk - low in cholesterol and sugar, but with a higher content of minerals (sodium, potassium, iron, copper, zinc and magnesium) and vitamin C. It is a potential remedy, with anti-hypertensive, anti-diabetic and anti-carcinogenic properties [21]. Thus, an epidemiological study revealed that diabetes prevalence is lower in Raica community subject's regularly consuming camel milk than non-Raica subjects living in the same environment, having the same lifestyle except that they do not drank camel milk [22]. Continuous consumption of camel milk can therefore be considered as a useful complement to the administration of parenteral insulin in the treatment of type 1 diabetes [23]. Camel milk contains about 52 micro-units of insulin in addition to its richness in zinc, which plays a key

role in both insulin secretion and action, allowing it to be associated with insulin therapy and being a good adjuvant for blood glucose control [24].

Insulin contained in milk has specific characteristics, making it more absorbable, taking advantage of the milk that resists coagulation. In addition, insulin in camel milk is covered by nanoparticles allowing its protection in the stomach and its passage in the blood, explaining part of its antidiabetic effects [25].

The present study was carried out to evaluate the therapeutic efficiency of camel milk on alloxan-induced diabetes in rats.

Materials and methods

Camel milk samples: Every other day milk samples were collected early in the morning from a camel (*Camelus dromedaries)* herd in the South of Algeria: (El Khaiter) EL Bayadh. The samples were collected in sterile screw bottles and kept in cool boxes until transported to the laboratory.

Chemicals: Alloxan monohydrate (10 g) was purchased from Sigma® Chemical Company (St Louis Mo, USA).

Experimental animals: Thirty healthy Wistar albino rats of both sexes between 3 and 4 months of age and weighing between 180-230g were used for the study. The rats were procured from the animal house of the Department of Biological Science, Djillali Liabés University, Sidi Bel Abbes, Algeria.

Induction of experimental diabetes: The animals were housed individually in large, spacious, hygienic stainless steel cages during the course of the experimental period under standard conditions (house was well ventilated and animals had 12 ± 1 hours day and night schedule; at room temperature 25 ± 5°C; 40–60% relative humidity). The animals were fed with standard rat pellet diet and water ad libitum during the experimental period.

Diabetes was induced in Wistar rats by a single intraperitoneal injection of alloxan monohydrate in sterile normal saline to overnight fasted animals at a dose of 120 mg/kg body weight (bw). Four days after alloxan injection, diabetes was confirmed by measurement of blood glucose from tail vein blood using a Glucometer (On Call Plus®). Rats with fasting glycemia > 180 mg/dl were considered diabetic and selected for the experiment [26,27]

Experimental design: Experimental animals were randomly assigned to three groups, each consisting of ten animals: Group (G1) – healthy control rats (negative control group); Group (G2) - diabetic rats fed a normal diet (positive control group); and Group (G3) - diabetic rats fed with raw camel milk. Rats in Group G3 were fed camel milk 50 ml/rat/day for four weeks [28] using a feeding bottle instead of water, whereas animals in Group 1 and 2 were given tap water. The diabetic animals were allowed free access to pellet diet, and were maintained at room temperature in large, spacious, hygienic cages.

Baseline (t = 0) blood samples were taken to measure fasting blood glucose (FBG) before intraperitoneal injection of alloxan. Blood samples were drawn from the tail vein of each rat and the fasting blood glucose determination was done on day 0, 1, 7, 14, 21 and 30 using a Glucometer (On Call Plus®, ACON Laboratories, Inc San Diego USA). On day 30 the rats were sacrificed under ether anesthesia and blood was collected by cardiac puncture for the analysis of total cholesterol (TC), triglyceride (TG), high and low density lipoprotein (HDL and LDL) using commercial kits obtained from Roche® Diagnostics Gmbh Laboratories, Germany. Serum very low-density lipoprotein cholesterol (VLDL-C) concentration

was calculated according to Friedewald et al. (1972) by the following equation: Serum VLDL-C (mg/dl) = TC – HDLc - Triglycerides/5, and atherogenic index (AI) was determined according to the equation: AI = (TC – HDL-C) / HDL-C [29]. In addition, LDL/HDL ratio as well as the HTR (HDL-cholesterol/Total-cholesterol Ratio) were calculated.

Statistical analysis The data was analyzed using SPSS version 20.0 (IBM). Statistical tests performed were Student's paired *t*-test for comparison between numerical variables. The results are presented as mean and standard deviation (SD) and were considered significant when the p-value was less than 0.05.

Results

The serum glucose level before alloxan diabetes induction was similar in all the three groups as detailed in table 1. After diabetes induction with alloxan, groups G2 and G3 became diabetic on 4th day as determined by serum glucose levels. The group G1 (negative control group) showed almost similar serum glucose levels on days 0, 1, 7, 14, 21 and 30. The diabetic group G3 that was fed with raw camel milk showed a significant decrease in serum glucose levels compared to G2 and G1 groups as detailed in Table 1.

Table 1. Serum glucose levels (mg/dL) in apparently healthy, diabetic and camel milk treated rats.

Day	G1 (Negative Control)	G2 (Diabetic+ standard diet)	G3 (Diabetic+camel milk)
T0	96,40 ± 6.63	98.9± 12.11	97.10± 6.54
T1 (D1)	97,40 ± 8.44	191.5± 5.40*	193.20± 10.50*
T2(D7)	97.10± 5.60	192.2± 3.49*	172.10± 4.70*
T3(D14)	97.80± 5.20	198.9 ±9.19*	158.40± 8.75*
T4(D21)	97.70 ± 7.80	199.2± 9.02*	148.90± 5.84*
T5(D30)	98.50 ± 4.74	199.6± 7.33*	133.80± 3.22*
Mean serum glucose	97.48 ± 6.40	180.05 ±7.75*	150.58± 6.59*

All results are reported as mean ±SD; *p<0.05; T1 was 1st day after alloxan administration when the rats became diabetic (day 4th).

Table 2. Effect of feeding camel milk on lipid profile parameters.

Rat	TG (mg/dL)$^{\#}$	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
(G1) (negative control group)	84.10±2.60	69.30±1.49	35.50±1.69	16.60 ±0.84	16.82±0.52
(G2) Diabetic (positive control group)	182.30±1.82*	113.30±1.41*	28.70±1.49*	47.70±0.48*	36.46±0.36*
(G3) Diabetic + raw camel milk	122.80±3.52**	74.50±1.50**	33.80±1.81**	15.80±0.78**	24.56±0.70**

All results are reported as mean ±SD; *p<0.05 G2 vs. G1; ** p<0.05 G3 vs. G2.

Table 3. Effect of feeding camel milk on AI, LDL-C/HDL-C and HTR.

Rat	AI	LDL/HDL ratio	HTR ratio
(G1) Control (negative control group)	0.95±0.05	0.46± 0.03	51.22±1.37
(G2) Diabetic (positive control group)	2.95±0.15	1.66± 0.09	25.31±1.01
(G3) Diabetic + raw camel milk	1.20±0.08*	0.46± 0.03*	45.34±1.77*

All results are reported as mean ±SD; *p<0.05; HTR ratio = (HDL-C/TC) x 10.

The results in Table 2 show significantly (p<0.05) lower levels of total cholesterol, triglycerides, LDL and VLDL cholesterol and significantly higher levels of HDL cholesterol in rats fed with camel milk (G3) in comparison

with the diabetic group treated a standard diet (G2).

As shown in Table 3, treatment of diabetic rats with raw camel milk for four weeks led to significant lower values of the Atherogenic index (AI) and LDL/HDL ratio and a significant higher value of the HTR ratio.

Discussion

The results of our study showed a significant effect of raw camel milk on blood glucose and lipid profile parameters in alloxan induced diabetic rats. This is in agreement with the results of Manal et al. [30]. The hypoglycemic effect of camel milk could be probably due to the high levels of insulin or insulin-like proteins in camel milk. These results are in agreement with those of Agarwal et al. who used a radioimmunoassay to measure insulin in camel milk and revealed that it contains a high concentration of insulin at 52.03 U/l [31].

The camel's milk resistance to coagulation allows it to pass rapidly through the stomach, thus ensuring that specific proteins, including insulin, remain available for absorption into the intestine. This unique property gives it the advantage of being a support and protector, which facilitates the absorption of intact insulin molecules through the small intestine [32]. It has been reported that camel milk contains high levels of vitamins A, B2, C and E and high mineral content (sodium, potassium, iron, zinc, copper and magnesium). These vitamins play the role of antioxidants, thus eliminating free radicals, useful in the prevention of tissue damage caused by toxic agents [33]. Vitamin C has been found to play a significant role in decreasing the high levels of blood hydroperoxide, glucose, cholesterol, triglycerides and low-density lipoprotein (LDL) in diabetic rats [34]. The antidiabetic action of camel milk and the enhancement of β-cell activity may be due also to its role in regulating the immune system and preserving β-cell destruction [35].

Our results showed a significant increase in total cholesterol (TC), total triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) in the diabetic group compared to the control group, while HDL-C was significantly lower. A significant alteration of the lipid profile may be due to the lack of insulin under diabetic condition. It is well known that in uncontrolled diabetes mellitus there will be an increase in total cholesterol, triglycerides and LDL cholesterol associated with a decrease in HDL cholesterol which is often linked with hyperlipidemia, all major risk factor for cardiovascular diseases [36].

In our study, the diabetic rats treated with camel milk showed an elevation in HDL-C and reduction in TC, TG, LDL-C and VLDL-C. These results are supported by those of Khan et al. [37], showing that the high insulin concentration of camel milk can cause the activation of lipoprotein lipase enzyme. Therefore, it is likely that raw camel milk induces favorable changes in the lipid profile of diabetic rats not only through a better glycemic control (secondary), but also by its direct action on lipid metabolic pathways. Thus, raw camel milk could alleviate the risk of cardiovascular diseases [38].

In our study, the atherogenic index was markedly decreased in diabetic rats fed with raw camel milk, causing a significant reduction in LDL/HDL ratio. We also recorded a significant increase in HTR ratio as compared with the diabetic control group. These results are in agreement with those of Isa et al. [39], and Barakat et al. [40] who stated that the increase in HDL-C or HTR ratio is one of the most important criteria of an anti-hypercholesterolemic agent.

Conclusions

Our study indicated the positive effect of camel milk on blood glucose and lipid profile in alloxan induced diabetic Wistar rats. This suggests that camel milk could be used in the treatment of diabetes in humans and may be helpful in controlling diabetes. However, more studies are needed to assess its safety, as well as its mechanism of action.

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