Congruent assessment of human hypoglycemic effect through STZ-induced diabetic rats fed Dromedary Camel (*Camelus dromedarius*) milk

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Abstract

Diabetes mellitus (DM), a heterogeneous metabolic disorder, affect people at any point of their lives. Globally, a wide variation in the prevalence of diabetes was observed but, showed zero percent within people that regularly consume camel milk because of their highly nutritional and therapeutic potentials to treat many metabolic and autoimmune diseases. The present study validated the efficacy of camel milk by investigating the differential hypoglycemic effects in concurrence with related biochemical tests in rats fed with raw and pasteurized milk of the one-humped Arabian camel (*Camelus dromedarius*). Male Sprague-Dawley rats (60mg.Kg^{-1} body weight) fed separately with raw and pasteurized camel milk for three weeks and injected with streptozotocin induced DM besides the investigation on the levels of blood glucose, cholesterol, triacylglycerol (TAG), and insulin. Results showed a significant hypoglycemic effect with both raw and pasteurized camel milk but, more prominent with the raw milk. Three weeks consumption of raw camel milk significantly increased blood cholesterol levels while consumption of pasteurized camel milk caused the reverse. Both types of camel milk revealed significant decrease in the blood TAG levels and were statistically validated. Thus, the present study recommends the choice of camel milk consumption especially in diabetic patients as well, as an immunity booster in the wake of the present environmental health issues.

Keywords: Camel milk; diabetes mellitus; hypoglycemic effect; streptozotocin; triacylglycerol.

1. Introduction

The incidence of Diabetes mellitus (DM) is increasing worldwide. World Health Organization (WHO) reported more than 420 million diabetic individuals in the world, an estimate that could rise to 570 million by 2030 and to 700 million by 2045 (WHO 2021). Type II DM is a major chronic disease in the population of Kuwait. The prevalence in the adult population is 14.8% (Al-Kandari *et al.,* 2019), and among children aged 0-14 years old, the incidence is 34.9 in every 100,000 children (Shaltout *et al.,* 2017). In addition, the occurrence of type I DM in Kuwait is high compared to other countries and the incidence is 20.1 in every 100,000 children under the age of 14 (Costa-Gouveia *et al.,* 2017; Shaltout *et al.,* 2017). Increasing interest in the constituents of camel milk has developed from the fact that it is a major part of human diet in many arid lands around the world (Hattem 2017). Although most of the camel milk is consumed

raw by nomads and desert inhabitants, pasteurized camel milk became available for populations in many African, Asian, and most recently, in some European countries. However, heat treatments have direct influences on the nutritional, biological and functional properties of milk proteins (Hattem 2017; Shori 2015; Atakan *et al.,* 2016). Additionally, the impact of regional and seasonal differences on camel milk composition has been widely suggested, which includes species and breeds, feeding conditions, and seasonal and physiological variations (Bouhaddaoui *et al.,* 2019) as well, the stage of lactation and genetic differences within the species (Pak *et al.,* 2019). The main composition of camel milk (g/100 ml) obtained from meta-analysis of literature data revealed 3.82 of fat, 3.35 total protein, 4.46 of lactose, 12.47 of dry matter and 0.79 for ash (Bouhaddaoui *et al.,* 2019). Camel milk composition is highly affected by water content which ranges from 86% to 91% depending on the accessibility of camels to water (Zibaee *et al.,* 2015). Few regional studies revealed the seasonal effect of camel milk composition with significant variations (Othman 2016). The mean components of camel milk are protein (3.1%), lipid (3.5%), lactose (4.4%), ash (0.79%), and total solids (11.9%) besides, rich source of vitamins (A, E, D, B) (Zibae *et al*., 2015). Casein is the predominant protein (whey protein/casein 0.31) in camel milk (Boughellout *et al.,* 2016). Camel milk whey contains higher contents of some protective antibacterial proteins such as lysozyme, lactoferrin, lactoperoxidase, peptidoglycan, serum albumin, and immunoglobulins (Rasheed, 2017; Koc & Atasever 2016; Nascimento *et al.,* 2016; Sakandar *et al.,* 2018). The average amount of components of camel milk is protein 3.1%; fat 3.5%; lactose 4.4%; ash 0.79%, and total solids 11.9% β-Lactoglobulin protein is however absent suggesting minimal hypersensitivity reactions for camel milk (Nascimento *et al.,* 2016). Moreover, camel milk whey protein is found to contain a small protein that is rich in cystine/half-cystine (Yasmin *et al.,* 2020). In general, camel milk proteins contain an adequate and balanced content of essential amino acids like the cow's milk (Jilo & Tegegne 2016; Rahmeh *et al.,* 2019). Triacylglycerols accounted for 96% of the total lipids in milk. Short chain fatty acids (4:0-12:0) are present in very small amounts in camel milk fat and lower than that of cow milk fat. Camel milk fat showed a higher content of 16:1, 18:0, 18:1 and 18:2*n*-6 but comparable level of 18:3*n*-3 to that of cow milk (Konuspayeva *et al.,* 2009; Karaman *et al.,* 2021; Pak *et al.,* 2019). Although camel milk fat has a higher unsaturated/saturated ratio, the high content of unsaturated fatty acids and the appreciable amount of essential fatty acids contributes to the overall nutritional value of camel milk (Boughellout *et al.,* 2016; Jilo & Tegegene 2016; Bouhaddaoui *et al.,* 2019). Additionally, camel milk contains considerably higher content of manganese, iron (Konuspayeva *et al.,* 2009; Brezovečki *et al.,* 2015), vitamin C and niacin (Costa-Gouveia *et al.,* 2017) than cow's milk. In addition to its nutritional potential, camel milk is also known to have medicinal properties that facilitates the treatment of many metabolic and autoimmune diseases (Urazakov & Baînazarov 1974; Yagil 1982; Agrawal *et al.,* 2005; Agrawal *et al.,* 2007; Aida *et al.,* 2019; Kamlesh & Asha 2020; Karaman *et al.,* 2021) in addition to their role in the prevention and management of DM. Researchers reported that the consumption of camel milk had a hypoglycemic effect in diabetic rats (Shah *et al.,* 2019; Aida *et al*., 2019; Agrawal *et al*., 2005). Similarly, a study performed on streptozotocin (STZ)-induced diabetic rats has reported a significant hypoglycemic effect upon consumption of raw camel milk for three

weeks, an effect which was not as significantly observed for raw cattle milk (Agarwal *et al.,* 2007; Ejtahed *et al.,* 2015). They also established that pasteurization has reduced the hypoglycemic effect suggesting that heating the milk may have affected the biological factors that produced this hypoglycemic effect (Hattem 2017; Kamlesh & Asha 2020). The prevalence of DM is zero among people in the rural Raica community (State of Rajasthan, India), who regularly consume camel milk, as compared to a 5.5% prevalence of the disease among non-Raica subjects from the same area (Urazakov & Baînazarov 1974).

In randomized studies on human subjects, observations revealed improvement of glycemic control in type I DM by standard therapy using camel milk supplementation (Al-Amin *et al.,* 2006; Kappeler *et al.,* 2006). There was a significant reduction (30-35%) in daily doses of insulin in type I diabetic patients fed raw camel milk. In addition, a marked improvement in diabetes quality control of life score (satisfaction score, impact score and worry score) was observed after three months of camel milk treatment. Based on these findings, the present study investigated the differential biochemical effects of feeding raw and pasteurized camel milk to STZ-induced and non-induced diabetic rats on blood levels of glucose, cholesterol, and TAG. This study will pave way not only to researchers in validate the effect of camel milk but, also will facilitate animal husbandry and health sector to promote the yield and consumption of camel milk for better immunity to humans especially, during this pandemic health issues.

2. Materials and methods

2.1. Feeding trials and regime

Raw and pasteurized camel milk were obtained from the local farms of Kuwait. Milk samples were transported in polypropylene containers and stored at 4°C. The feeding bottles of the rats were cleaned and refilled with fresh camel milk daily to encourage the rats to drink it. After acclimation with water and camel milk the following regime of milk and water was given to the rats: (a) non-diabetic and diabetic rats (control) were supplied with 300ml water/d, (b) from 1d to 21d, two groups of diabetic induced rats were provided 300ml/d raw and pasteurized camel milk, respectively. The diabetic induced rats were never given water since, this study revealed rats fed with water abstained from consuming camel milk.

2.2 Treatment of rats

Male Sprague-Dawley rats (a strain of *Rattus norvegicus* at F1 generation), bred in our animal house (Life Science Department, Kuwait University). These rats were four weeks old, weighing 145g and maintained on normal pellet diet (VRF1) that followed the standard and validated regime described by Special Diet Services, Essex, UK (SDS, 2022). The ingredients in this feed were fat (2.7%), crude protein (14.4%), crude fiber (4.7%), and Vitamin-A (8000 IU/Kg), D3 (600 IU/Kg), Vitamin-E (62 IU/Kg), Copper (11mg/Kg), and Calcium (0.73%). They were also provided with sufficient filtered water in the experiment. Standard animal care and handling was applied throughout the experiment. The rats were injected with a single dose of 0.5 ml STZ (streptozotocin) intraperitoneally (IP) in saline solution (0.9% NaCl) @ 60 mg/kg body weight to

induce diabetes. These concentrations were standardized in this study after deducing the optimum and non-lethal dose that neither induced stress nor disproportioned the dose against the body weight of the rats. These rats with blood glucose levels higher than 350 mg/dL were selected as severely diabetic rats (Agrawal *et al.,* 2007).

2.3 Biochemical analysis

Blood samples were collected from fasting rats before treatment and after 7, 14, and 21 days (Week 1-3) of treatment. The serum was prepared and stored for later analysis. Serum glucose and cholesterol levels were determined by the glucose oxidase assay and the cholesterol oxidase assay, respectively, using kits provided by WAKO Company, USA. Serum TAG levels were determined using a kit provided by WAKO Company, USA.

2.4 Statistical analysis

Statistical analyses were performed using the ANOVA tests to determine the significance between and within each group. Results were represented with mean and ± standard error. *P* value <0.05 was considered statistically the threshold level for significance.

3. Results

Figure 1 represents mean values of serum levels of glucose, cholesterol, and triacylglycerol (TAG) of the control and experimental rat groups throughout the experiment; normal non-diabetic control group (Ct *n* 7) as a normal baseline, untreated STZ-induced diabetic control group (D-Ct *n* 5) as a diabetic baseline, raw camel milk-treated STZ-induced diabetic group (DT-R *n*5) and pasteurized camel milk-treated STZ-induced diabetic group (DT-P *n*5). In establishing the diabetic status, mean values for D-Ct group were statistically compared to Ct group (Figure 1). It is to note that the results were transformed to logarithmic values to show the wide-ranged differential units and reduce the dispersed numerical data values to visualize or respond to skewness towards large values and express the figure (Figure 1) in compactness. Additionally, analysis of variance (ANOVA) tested the significant differences between the variables and the samples (Table 1).

The significance of changes in blood parameters for every group were calculated by comparing the mean values obtained in each week to the previous week within the same group (Figure 1). In addition, significant changes were also observed for the mean values of DT-R and DT-P groups in comparison to D-Ct (Figure 1).

Treatment with STZ led to several alterations in some of the blood parameters. The mean blood glucose level of Ct rats was 121.00 ±13.12mg/dL (log 2.08) initially, and constantly maintained throughout the duration of the experiment (Figure 1). On the other hand, the D-Ct rats showed significant increase in their mean blood glucose level to 455.21 ±20.21 mg/dL (log 2.66) compared to the Ct rats. Treatment with raw (DT-R) and pasteurized (DT-P) camel milk resulted in a significant reduction in blood glucose levels after the first week and throughout the duration of the experiment (Figure 1).

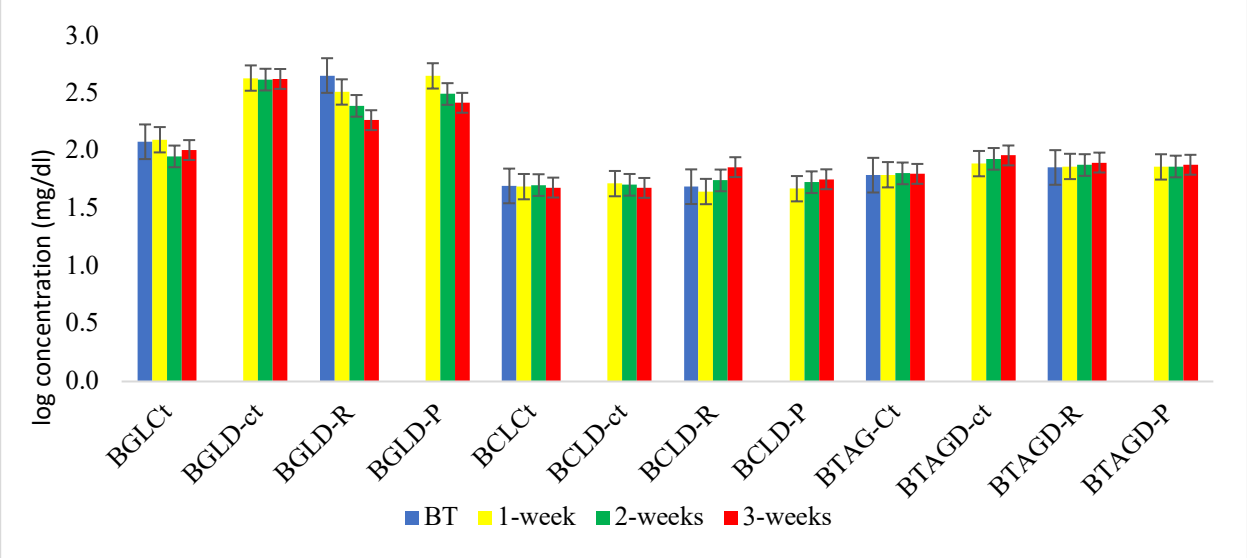

Fig .1. Serum levels of glucose, cholesterol, triacylglycerol (TAG) and control group in rats treated with raw and pasteurized camel milk Ct: control, D-Ct: STZ-induced diabetic rats, DT-R and DT-P: STZ-induced diabetic rats treated with raw and pasteurized camel milk, BCL: blood cholesterol level, BGL: blood glucose level, B-TAG: blood triglyceride, BT: before treatment

Initially, the mean blood cholesterol levels of Ct and D-Ct rats were 50 ±7.32 mg/dL (log 1.70) and 49.25±5.92 mg/dL (log 1.69) respectively and there were no significant changes in these levels throughout the duration of the experiment (Figure 1). In the case of the DT-R and DT-P groups, noticeably there were no significant changes in the mean blood cholesterol levels (p=0.424 and p=0.29 respectively) after one week of treatment. However, by the end of the third week of treatment, DT-R showed a significant increase in the mean blood cholesterol levels while DT-P showed insignificant increase (p=0.117). By the end of the experiment, treatment with raw camel milk resulted in 47% elevation in blood cholesterol compared to 16% exerted by pasteurized camel milk. A significant increase was also observed when the mean blood cholesterol levels of DT-R rats were compared to that of the D-Ct rats (Figure 1).

The mean blood TAG level of Ct rats was 62.02 ± 0.96 mg/dL (log 1.79) at the beginning of the experiment. There were no significant changes in this level throughout the duration of the experiment (Figure 1). However, diabetic status induced by STZ has caused a significant increase in blood TAG level seen for D-Ct rats up to 72.35 ±1.13 mg/dL (log 1.86). The mean blood TAG levels of the D-Ct group remained at significant levels compared to the Ct rats throughout the duration of the experiment. In the first and second weeks of treatment, there were no significant changes in the blood TAG levels of STZ-induced diabetic rats treated with raw camel milk (DT-R) or with pasteurized camel milk (DT-P). However, after the third week of treatment observations revealed significant increase in TAG levels of both DT-R and DT-P rats. Noticeably, the blood TAG levels of both treated groups (DT-R and DT-P) were lower than the diabetic control group (D-Ct) throughout the three weeks of treatment.

Table 1. ANOVA test on serum levels of glucose, cholesterol and triacylglycerol (TAG) in rats fed with untreated and treated group camel's milk

SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
BGL:Ct	3	318.81	106.27	329.47		
BGL:D-ct	3	1277.59	425.86	43.54		
BGL:DT-R	3	762.14	254.05	5010.53		
BGL:DT-P	3	1030.58	343.53	9485.42		
BCL:Ct	3	148.55	49.52	1.55		
BCL:D-ct	3	151.58	50.53	4.90		
BCL:DT-R	3	173.37	57.79	200.34		
BCL:DT-P	3	158.33	52.78	25.14		
BTAG:Ct	3	190.51	63.50	0.77		
BTAG:D-ct	3	256.14	85.38	48.75		
BTAG:DT-R	3	229.63	76.54	9.19		
BTAG:DT-P	3	223.31	74.44	2.95		
After one week	12	1819.8	151.65	24465.63		
After two weeks	12	1584.42	132.04	15415.35		
After three weeks	12	1516.32	126.36	12911.46		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	554617.97	11	50419.81	42.50	<0.0001	2.25852
Columns	4226.15	2	2113.07	1.78	0.19	3.44336
Error	26098.95	22	1186.31			
Total	584943.07	35				

* Statistically significant $p<0.05$; Control rats (Ct), control STZ-induced diabetic rats (D-Ct), STZ-induced diabetic rats treated with raw camel milk (DT-R), STZ-induced diabetic rats treated with pasteurized camel milk (DT-P), TAG: triglyceride, B: blood

4. Discussion

Studies (Urazakov & Baînazarov 1974; Shori 2015) revealed DM is very uncommon in communities that consume camel milk, and this knowledge has led to an interest in studying the beneficial health and therapeutic effects of camel milk consumption to protect from DM. Previous studies in camel milk on diabetic patients as well as rats with induced diabetes proved that daily consumption of camel milk can lower the blood glucose levels considerably (Shori, 2015; Shah *et al.,* 2019; Kamlesh & Asha 2020). Additionally, it was shown that patients who consumed camel milk had to lower their daily insulin requirement to achieve a balanced blood glucose level. However, observations showed the reverse of glycemic control when camel milk consumption was discontinued (Ejtahed *et al.,* 2015; Shori, 2015; Shaltout *et al*., 2016; Shah *et*

al., 2019). In the present study, the blood glucose, cholesterol, and TAG levels of the normal, non- diabetic rats (Ct) were used as a normal baseline, while those of the diabetic, untreated rats (D-Ct) were used as a diabetic baseline. The D-Ct group maintained a diabetic status throughout the duration of the experiment, and any changes in blood levels of glucose, cholesterol, and TAG of the DT-R and DT-P groups were observed from the treatment with raw or pasteurized camel milk and not from other unrelated factors.

The present study on the hypoglycemic effect while consuming the local dromedary camel milk was found comparatively in line with the effect observed with other camel milk elsewhere the globe (Al-Amin 2006; Shori, 2015; Costa- Gouveia *et al*., 2017; Kamlesh & Asha 2020). Treatment with camel milk, whether raw or pasteurized, showed apparent and significant hypoglycemic effects in STZ-induced diabetic rats. Comparatively, the raw camel milk indicated prominent effect than pasteurized camel milk. During the three weeks experimental study, the reduction of blood glucose levels in DT-R and DT-P rats were 59% and 42% respectively and were in line with the earlier findings (Shori 2015; Kamlesh & Asha 2020). The reduced hypoglycemic activity of the pasteurized camel milk could be because of heating on the bioactive component(s) of the camel milk that facilitates the hypoglycemic effect.

The hypoglycemic activity of camel milk attributes to several factors. Few studies suggest that camel milk does not coagulate in the acidic media of the stomach (Othman 2016; Aida *et al*., 2019). Milk coagulation in the stomach takes place when the acid stable peptidases act on a sensitive sequence in the κ-casein proteins in the milk (Sakandar *et al*., 2018; Nascimento *et al*., 2016; Ejtahed *et al*., 2015; Al-Amin, 2006). Camel milk coagulates two to three times slower than cow milk (Shori, 2015). There is only a small quantity of κ-casein in camel milk (Konuspayeva *et al*., 2009) and it has structural differences from other species. Hence, it is not sensitive to coagulation (Konuspayeva *et al.*, 2009; Eitahed *et al.,* 2015; Shori 2015; Hattem, 2017; Kasmlesh & Asha 2020). Moreover, calcium plays an important role in the coagulation of proteins. Casein (65%) are linked to calcium in many dairy products while camel milk indicates 35% of the casein link with calcium (Brezovečki *et al*., 2015). Furthermore, one among the contributing factor is that of the moisture richness in camel milk during the summer season with fewer solids constituents that were observed to prevent earlier and complete coagulation (Brezovečki *et al.,* 2015). This also suggested the faster mobility of camel milk into the small intestine. Additionally, camel milk was found to have a high content of insulin (45-128 units/l) that reaches the intestinal mucosa unharmed with minimal digestion. Such diabetes can either be obtained as insulin present in camel milk or by beta cells stimulation through secretion. Earlier studies showed that camel milk augment insulin absorption and act within the body in a similarly to that of the endogenous insulin (Kamlesh 2020, Aida *et al.*, 2019; Shah *et al.,* 2019). Likewise, the high levels of antioxidants, especially vitamin C present in camel milk is found to improve insulin sensitivity to insulin receptors (Agrawal 2007).

Figure 1 showed seldom effect of STZ on blood cholesterol levels throughout the three weeks after induction of diabetes. Observations on the blood cholesterol levels in camel milk-treated groups (DT-R and DT-P) indicated significantly increase blood cholesterol levels when raw camel milk for three weeks was consumed while, this was not observed with the treatment

with pasteurized camel milk. Although this study has shown that the raw camel milk has more prominent hypoglycemic effect than the pasteurized camel milk, it could be a preferred option of diabetic patients to consume pasteurized rather raw camel milk to avoid the unwanted increase in blood cholesterol levels. Shori *et al.* (2015) revealed the consumption of camel milk does not affect total blood cholesterol levels in humans. However, the present study showed an increase in total cholesterol that was significant for raw camel milk. The possible attributes for these results could be related to the free form of cholesterol in the camel milk which makes it easier for the absorption in the intestine (Al-Amin *et al.,* 2006; Zibaee *et al.*, 2015). Camel milk is relatively high in cholesterol (31.32 mg/100 g) when compared to other kinds of milk, such as cow milk (25.63 mg/100 g), goat milk (13.0 mg/100 g), sheep milk (23.0 mg/100 g), and human milk (20mg/100g) (Brezovečki *et al*., 2015; Bouhaddaoui *et al*., 2019). Furthermore, there are many seasonal and regional differences that may reflect different concentrations of cholesterol and fatty acids in the camel milk (Bouhaddaoui *et al.*, 2019; Rahmeh *et al.*, 2019).

Conclusion

In the present study, both the raw and pasteurized camel milk has favorable TAG-lowering (hypotriglyceridemic) effect on DT-R and DT-P rat groups. Statistical comparison between these two treated groups revealed no significant differences between the impacts of the two camel milk types on blood TAG levels of the diabetic rats. Such TAG-lowering effects of consumption of camel milk may be attributed to the regulation of metabolism and improvised body functions (Kamlesh & Asha 2020). Thus, this study recommends the consumption of camel milk since, it not only increases the general body immunity but also a preferable option to diabetic patients due to their low glucose levels when compared to the cow or goat milk.

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