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Beneficial Effect of Camel Milk on Liver and Kidneys Function in Diabetic Sprague-Dawley Rats

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ABSTRACT

Recently, several studies demonstrated the potential health attributes of camel milk. The present study was designed to explore the effect of camel milk - compared to cow and buffalo milks on blood glucose and liver and kidneys function. Thirty male Sprague-Dawley rats were divided into five groups. For comparison, one group was used as a normal control, while the remaining four groups were injected with streptozotocin in order to induce diabetes. One of the diabetic groups were used as a Diabetic Control Group (DCG), whereas, three diabetic groups were fed on diets containing cow (COM), buffalo (BFG) or camel milks (CMG) for six weeks. Insulin content in camel milk ($58.67 \pm 2.01 \text{ U L}^{-1}$) was more than that of cow or buffalo milks (17.01 ± 0.96 and $16.21 \pm 0.95 \text{ U L}^{-1}$, respectively). Feeding diabetic rats on camel milk was showing a higher hypoglycemic effect ($\sim 49.2\%$) than that of either COM or BFG (~ 11.6 and 11.1% , respectively) compared to the DCG group. Giving camel milk led to an improvement in activities of alanine amino-transferase and aspartate amino-transferase by 41 and 38%, respectively; compared to the DCG rats. A significant ($p < 0.05$) reduction effects on uric acid, urea and creatinine levels were observed in the CMG, COG and BFG groups. The present work confirms the hypoglycemic effect of camel milk as well as marked improvements in liver and kidneys function, which was greater than those of COG and BFG groups. Indeed, extensive research on camel milk is still needed to identify the relevance components of these healthy functions.

Key words: Camel milk, hypoglycemic effect, liver function, kidneys function

INTRODUCTION

Diabetes mellitus is a syndrome characterized by metabolism disorders and abnormally high blood sugar (hyperglycemia) resulting from a low level of the hormone insulin with or without abnormal resistance to insulin effects. It is characterized by hyperglycemia in the postprandial and or fasting state. In its severe form, it is accompanied by ketosis and protein wasting. This metabolic disorder can be induced chemically using alloxan (Tirerney *et al.*, 2002; Laleye *et al.*, 2008; Gwarzo *et al.*, 2010) or streptozotocin (Mahesh and Brahatheeswaran, 2007; Arulselvan and Subramanian, 2007; Palsamy and Malathi, 2007). The incidences of diabetes mellitus world wide appear to be increasing (Onkamo *et al.*, 1999). Prevention and early treatment is important because diabetes interrupts normal developments in children and carries the threat of severe complication in more active period of life (Dahlquist, 1999).

Camels live in the vast pastoral areas in Africa and Asia. Dromedary camel (*Camelus dromedarius*, one-humped) is mainly live in the desert areas and considered as an important component of the dry land and desert ecosystem. The dromedary camel widely occurs in the Middle East, East, North and East Africa, South West Asia and Australia. Camels not only representing an important economic means of short distance transport to the rural and urban societies; but also serve as a source for milk (Al Haj and Al Kanhal, 2010).

Camel milk possesses vital role in human nutrition in the hot regions and arid countries. This milk-type contains all the essential nutrients found in bovine milk (El-Agamy *et al.*, 1998; Omer and Eltinay, 2009). Recently, several reviews demonstrate the high potential therapeutic properties of camel milk, such as anti-carcinogenic (Magjeed, 2005), anti-hypertensive (Quan *et al.*, 2008), hepatoprotective effect (Khan and Alzohairy, 2011), hypocholesterolamic effect (Elayan *et al.*, 2008) and has been recommended to be consumed by children who are allergic to bovine milk (El-Agamy *et al.*, 2009). In addition, probiotic lactic acid bacteria have been isolated from camel milk (Yateem *et al.*, 2008).

Besides drugs that being classically used for the treatment of diabetes (insulin, sulphonylureas and biguanides) camel milk is well-known in arid regions and in the wilderness for its usefulness to treat diabetes mellitus (Al Haj and Al Kanhal, 2010). Agrawal *et al.* (2007a) have reported that camel milk could be the responsible for the low prevalence of diabetes in the Raica community in India. Further studies have approved the capability of camel milk consumption to provide effective management for patients with type-1 diabetes (Agrawal *et al.*, 2003) as well as for rats (Sahani *et al.*, 2005).

In this context, the present study was conducted to examine the hypoglycemic effects of camel milk, in comparison to cow and buffalo milks, in streptozotocin-induced diabetic rats. Additionally, the effect of camel and cattle milks on the liver and kidneys function of diabetic rats were investigated.

MATERIAL AND METHODS

Materials: During October 2009, buffalo and cow milk samples were collected from the herds of Faculty of Agriculture, Cairo University, Giza, Egypt, whereas, camel (*Camelus dromedarius*) milk samples were collected from areas around Bourg El-Arab, Alexandria, Egypt.

Experimental design: A total of 30 male healthy Sprague-Dawley (SD) rats (obtained from National Research Center, Dokki, Giza, Egypt) has been used in the present work. The animals were of eight weeks old and of body weight in range of 100 and 110 g.

Rats were kept in an air conditioned animal room at National Research center, Dokki, Giza, Egypt. They were housed individually in a well aerated cage under hygienic condition for two weeks before initiating the experiments. The animals were freely allowed to access the tap water and were fed on basal diet composed of 15% casein, 10% corn oil, 5% cellulose, 4% salt mixture, 1% vitamins mixture and starch 65% according to Lane-Peter and Pearson (1971). Following this adaptation period, the rats were divided into five groups of 6 rats for each. The first group was kept as Normal Control Group (NCG) which was continued fed on the basal diet. The remaining groups (24 rats) were subjected to streptozotocin injection to induce hyperglycemia according to the method of Adeghate *et al.* (2001). To induce diabetes in rats, fresh solution of streptozotocin was prepared in phosphate citrate buffer (pH 4.6) and injected to rats at a single dose of 60 mg kg⁻¹ body weight. After 120 h of the injection, a blood sample was taken from each rat for the determination of plasma glucose to ensure the occurrence of diabetes. These diabetic rats (24 animals) were then divided into

four groups: the Diabetes Control Group (DCG) that continued fed on the basal diet, Cow Milk Group (COG) that was fed on basal diet containing cow milk, Buffalo Milk Group (BFG) that was fed on basal diet containing buffalo milk and Camel Milk Group (CMG) that was fed on basal diet containing camel milk. Each of milk-types was mixed adequately to the basal diet within its corresponding group by a proportion of 20 mL milk to each 95 g basal diet. All rat groups were fed on their corresponding diets for 6 weeks.

During the experimental period, blood samples were obtained from tail vein of each rat by capillary tube after fasting for an overnight weekly and blood glucose levels were determined. At the end of the experimental period, rats were killed by decapitation and blood samples were collected from each rat and subjected to centrifugation at 3000 rpm to obtain the plasma which was kept in the deep freezer for the subsequent investigation. The liver and kidneys of each rat were removed, which were kept in screw tube with NaCl saline solution (0.9%) in the deep-freezer.

Methods: The total nitrogen content of milk was measured by the Kjeldahl method (International Dairy Federation (IDF, 1993), where, a nitrogen conversion factor of 6.38 was used to calculate total protein content. Milk fat content was determined by the Gerber method according to Ling (1963). Lactose, milk Total Solids (TS %) and ash contents were determined according to AOAC (1990). pH was measured (WTW 720-inolab, D-82362 Weilheim, Germany). Vitamins (A, C and E) were determined according to procedures outlined in AOAC (2000). All milk samples were analyzed in triplicate.

Commercial enzymatic kits (obtained from Biodiagnostic, Dokki, Giza, Egypt) were used for the determination of: plasma glucose, plasma protein, plasma albumin and activities of aspartate and alanine amino-transferase (ALT and AST). Serum globulin content was calculated as the difference between plasma protein and albumin. Insulin was determined by using commercial ELISA kit (MSDS insulin kit, Calbiotech, Inc., USA).

Determination of uric acid and urea contents in plasma were carried out colorimetrically at 700 and 525 nm, respectively according to the methods of Caraway (1963) as well as determination of creatinine content in plasma was carried out colorimetrically at 520 nm according to the method of Schirmeister (1964). All other chemicals used in this study were of analytical grade.

Statistical analysis: Data are expressed as the Mean \pm SD. Data were analyzed by one-way Analysis of Variance (ANOVA), followed by assessment of differences by LSD post-hoc test. All statistical calculations were performed using MSTAT-C (ver. 2.10, Michigan state university, USA). Results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Milk composition: The average gross composition of cow, buffalo and camel milks used in the present study are given in Table 1. As may be seen from Table 1, the camel milk composition was of compatibility with that of cow milk. In agreement with this finding, Al Haj and Al Kanhal (2010) stated that the main components of camel milk were relatively closed to that of bovine milk.

Regarding the milk minor components, camel milk possessed significantly higher ($p < 0.05$) insulin content than both cow and buffalo milks. It is clear from the data presented in Table 1 that the insulin content in camel milk ($58.67 \pm 2.01 \text{ U L}^{-1}$) was of more than three folds as that of cow or buffalo milks (17.01 ± 0.96 and $16.21 \pm 0.95 \text{ U L}^{-1}$, respectively). This finding concurs with the results of Shehadeh *et al.* (2001) who have found a high concentration of insulin ($\sim 52 \text{ U L}^{-1}$) in camel milk. The obvious increased insulin content in camel milk compared with that in cow or buffalo

Table 1: Chemical composition (major and minor components), physicochemical and insulin content of cow, buffalo and camel milks

Chemical composition	Cow milk	Buffalo milk	Camel milk
Total solids (%)	12.30±1.01 ^b	16.02±1.14 ^a	12.84±1.12 ^b
Fat (%)	3.58±0.22 ^b	6.59±0.29 ^a	4.01±0.25 ^b
Total protein (%)	3.30±0.17 ^a	4.09±0.20 ^a	3.31±0.19 ^a
Lactose (%)	4.59±0.23 ^a	4.44±0.20 ^a	4.80±0.21 ^a
Ash (%)	0.80±0.05 ^a	0.80±0.03 ^a	0.83±0.04 ^a
Vita. A (mg/100 mL)	0.36±0.02 ^a	0.06±0.002 ^b	0.04±0.002 ^b
Vita. C (mg/100 mL)	1.58±0.81 ^b	1.24±0.70 ^b	4.51±0.24 ^a
Vita. E (mg/100 mL)	0.11±0.01 ^b	0.12±0.01 ^b	0.26±0.03 ^a
Na (mg/100 mL)	39.00±2.67 ^a	41.00±2.29 ^a	40.00±3.01 ^a
K (mg/100 mL)	126.00±7.24 ^a	96.00±4.16 ^b	61.00±3.51 ^c
Fe (mg/100 mL)	0.45±0.03 ^a	0.37±0.02 ^a	0.35±0.03 ^a
Zn (mg/100 mL)	2.01±0.14 ^a	2.00±0.11 ^a	2.21±0.17 ^a
Cu (mg/100 mL)	0.11±0.01 ^a	0.18±0.01 ^a	0.17±0.02 ^a
Mg (mg/100 mL)	13.12±1.00 ^a	12.67±0.86 ^a	12.12±1.00 ^a
P (mg/100 mL)	83.21±5.11 ^a	77.11±4.11 ^a	46.11±3.21 ^b
Cl (mg/100 mL)	120.00±9.99 ^a	118.00±7.76 ^a	123.00±8.31 ^a
Ca (mg/100 mL)	110.00±5.23 ^a	107.00±7.01 ^a	95.00±6.66 ^a
pH value	6.69±0.10 ^a	6.70±0.11 ^a	6.72±0.12 ^a
Insulin (U L ⁻¹)	17.01±0.96 ^b	16.21±0.95 ^b	58.67±2.01 ^a

Values are means of 6 rats±SD. Means with different superscripts in the same row are significantly differ (p<0.05)

milks may be attributed to one of the camel milk protein-types that possessed many characteristics similar to insulin as concluded by Beg *et al.* (1986).

Furthermore, camel milk showed a significantly higher (p<0.05) content of vitamins C and E, whereas, camel milk possessed the significantly lowest (p<0.05) levels of K and P among the three types of milk.

However, it is worthwhile to note that there are some differences between the composition of camel milk in the current study and those stated in earlier findings; i.e., Khaskheli *et al.* (2005), Konuspayeva *et al.* (2009) and Omer and Eltinay (2009). Disparities in the composition of camel milks mainly correlated with the geographical origin and seasonal variations and go further with the different breeds, stage of lactation, age, calving number and water availability as reported by Al Haj and Al Kanhal (2010).

Blood glucose levels: Data of blood glucose levels of the five rat groups (NCG, DCG, CMG, COG and BFG) are presented in Table 2. It can be noticed at the first experiment week that Camel Milk fed Group (CMG) has shown significant difference (p<0.05) from Diabetic Control Group (DCG) with an obvious reduction in blood glucose levels of about 19.4% (from 146±9.8-158±9.9 mg dL⁻¹). While, both cow and buffalo milks treated groups (COG and BFG) have shown significant reduction (p<0.05) in blood glucose levels after four weeks, compared to the Diabetic Control Group (DCG). A closer observation of the data revealed that giving camel milk to streptozotocin-treated rats was associated with a continuous decrease in blood glucose levels throughout experiment period which in turn has shown higher hypoglycemic effect (~49.2%) than that of both cow or buffalo milks treated groups (~11.6 and 11.1%, respectively) compared to the DCG.

Indeed, the potential hypoglycemic effect of camel milk observed in the current work was not out of expectation with respect to the highest insulin content obtained for camel milk as revealed from

Table 2: Influence of camel and cattle's milk administration on blood glucose levels of diabetic rats

Groups	Blood glucose concentration (mg dL ⁻¹)						
	Zero time	1 weeks	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
NCG	89±7.11 ^b	86±7.0 ^c	84±6.4 ^c	97±5.6 ^c	87±4.0 ^d	87±6.11 ^d	86±4.4 ^d
DCG	202±11.2 ^a	204±12.1 ^a	198±9.9 ^a	199±10.2 ^a	205±12.4 ^a	200±11.1 ^a	199±9.8 ^a
CMG	146±9.8 ^a	158±9.9 ^b	151±10.0 ^b	146±12.0 ^b	130±10.0 ^c	120±9.2 ^c	101±9.7 ^c
COG	200±11.1 ^a	191±12.0 ^a	185±13.2 ^a	186±11.2 ^a	176±10.2 ^b	175±9.8 ^b	176±8.9 ^b
BFG	197±10.1 ^a	192±12.1 ^a	190±13.0 ^a	182±12.0 ^a	180±9.9 ^b	178±9.8 ^b	177±9.0 ^b

Values are means of 6 rats±SD. Zero time that was after 6 days of streptozotocin ingestion into rats to induce diabetes. Means with different superscripts in the same column are significantly differ (p<0.05)

Table 3: Influences of camel and cattle's milk administration on liver function

Groups	ALT (U L ⁻¹)	AST (U L ⁻¹)	Total protein (g dL ⁻¹)	Albumin (A) (g dL ⁻¹)	Globulin (G) (g dL ⁻¹)
NCG	19.21±1.04 ^d	24.11±1.12 ^d	6.60±0.42 ^a	4.72±0.27 ^a	1.88±0.11 ^a
DCG	48.80±2.46 ^a	50.21±3.21 ^a	4.02±0.24 ^c	2.66±0.16 ^c	1.36±0.13 ^b
CMG	29.00±2.03 ^c	30.91±2.22 ^c	6.02±0.37 ^a	3.99±0.21 ^b	2.03±0.09 ^a
COG	34.00±2.41 ^b	35.32±1.92 ^b	5.00±0.41 ^b	3.58±0.20 ^b	1.49±0.12 ^b
BFG	34.09±2.14 ^b	34.91±1.99 ^b	4.89±0.30 ^b	3.10±0.20 ^c	1.79±0.11 ^a

Values are means of 6 rats±SD. Means with different superscripts in the same column are significantly differ (p<0.05)

the data in Table 1. This finding is consistent with the observations of Agrawal *et al.* (2003), Sahani *et al.* (2005) and Agrawal *et al.* (2007a) for hypoglycemic effect of camel milk. It should be noted that camel milk does not form coagulum in acidic environment of the stomach, which may in turn provides a rapid pass of camel milk with its specific like protein/insulin through stomach and remains available for absorption in intestine (Beg *et al.*, 1986; Wangoh, 1993). A further later on study has found a potential effect of small size immunoglobulins, which presented naturally in camel milk, on β -cell of the pancreas (Agrawal *et al.*, 2007b). All these factors may combine and contributed to the observed hypoglycemic effect of camel milk in the present work.

Liver function: A comparison of the liver function parameters data for the camel, cow and buffalo milks administration at the end of the experiment is shown in Table 3. A significant elevation (p<0.05) in the levels of liver enzymes (alanine transaminase: ALT and aspartate aminotransferase: AST) appeared in Diabetic rat Control Group (DCG). This elevation reflected the generally recognized detrimental effect of hepatocyte damage, which represented in the leakage of ALT and AST from damaged hepatic cells. However, it is worthwhile to note that ALT is more commonly used for screening of liver problems, as the AST level may also be increased as a result of deficiencies or diseases in other body organs (Bujanda *et al.*, 2008).

As it can be seen from Table 3, a significant (p<0.05) overall improvement in liver function parameters appeared within diabetic rat groups of camel, cow and buffalo milks feeding, with a particular respect to the highest refinement effect in CMG rats (Table 3). Accordingly, it is interesting to note that giving camel milk led to an improvements in both ALT and AST activities by 41 and 38%, respectively; compared to the DCG rats. This finding is consistent with the observations of Magjeed (2005) and Khan and Alzohairy (2011) who found that giving camel milk improved the levels of ALT and AST activities in intoxicated rats.

Table 4: Influence of camel and cattle's milk administration on kidneys function

Groups	Uric acid		Urea		Creatinine	
	mg dL ⁻¹	%	mg dL ⁻¹	%	mg dL ⁻¹	%
NCG	3.97±0.21 ^c	100	17.97±1.11 ^c	100	0.79±0.08 ^c	100
DCG	8.88±0.66 ^a	224	50.20±2.46 ^a	279	1.80±0.09 ^a	228
CMG	5.17±0.24 ^b	130	20.20±1.09 ^c	112	1.00±0.07 ^b	127
COG	5.78±0.36 ^b	146	30.11±1.94 ^b	168	1.10±0.09 ^b	139
BFG	6.00±0.42 ^b	151	33.20±1.97 ^b	185	1.17±1.01 ^b	148

Values are means of 6 rats±SD. *% from normal control. Means with different superscripts in the same column are significantly differ (p<0.05)

A closer observation of the data in Table 3 revealed that the highest elevation in blood total protein, albumin and globulin levels were obtained in CMG rats. Additionally, no significant differences (p>0.05) observed between CMG rats and Normal Control rats Group (NCG) in blood total protein and globulin levels. These findings are confirming the significant improvement in the liver functions of diabetic rats as a result of giving camel milk. According to Abu-Lebdeh and Nair (1996) insulin enhanced the short-side-chain amino acid intracellular uptake, stimulated transcription and translation of RNA increased the gene expression of albumin and other proteins and inhibited liver protein breakdown enzymes *in vivo* studies. From this point of view, the highest improvement effect of giving camel milk on diabetic rats could be explained. The present results concur with those of Magjeed (2005) for intoxicated rats with aflatoxin B1 fed on camel milk.

Kidneys function: In order to determine the impact of camel and cattle's milk administration on the kidneys function of diabetic rats, the levels of uric acid, urea and creatinine in blood were performed. A comparison of the data is given in Table 4. It is clearly noticed that the uric acid, urea and creatinine levels in blood of Diabetic Control Group rats (DCG) significantly increased (p<0.05) by 2.2, 2.8 and 2.3 folds, respectively, reflecting the great deficiency occurred in kidneys function as a result of diabetic disease. In contrast, a significant (p<0.05) improvement effect on these kidneys function parameters were obtained in the CMG, COG and BFG rat groups. It is worthwhile to note that there were no significant differences (p>0.05) between CMG, COG and BFG rat groups, except that of urea levels (Table 4), where giving camel milk showed the highest improvement and has reached no significant difference (p>0.05) with that of Normal Control rat Group (NCG). These results are inline with those of Magjeed (2005) who found that camel milk has improved the kidneys function of intoxicated rats.

CONCLUSION

This study demonstrated that camel milk possesses a potential hypoglycemic effect and was higher than that obtained by cow or buffalo milks. This action is presumed to be due to the presence of insulin/insulin like substances. Its therapeutic efficacy has rather explained by the lack of coagulation in acidic condition of the human stomach. Besides, the health beneficial effects of camel milk extended to the liver and kidneys function with a markedly improvement impact and was even higher than cow or buffalo milks. Indeed, further investigation on camel milk is needed to identify the relevant healthy components.

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