

EFFECT OF CAMEL MILK ON THE ACTIVITIES OF ATPases IN NORMAL AND STREPTOZOTOCIN-DIABETIC RATS

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Diabetes mellitus is the world's largest endocrine disorder resulting in multiple aetiologies, involving metabolic disorders of carbohydrate, fat and protein. All forms of diabetes are due to a decrease in the circulating concentration of insulin (insulin deficiency) and a decrease in the response of peripheral tissues to insulin i.e., insulin resistance. According to the World Health Organization projections, the prevalence of diabetes is likely to increase by 35% by the year 2025. In this study, the streptozotocin (STZ)-induced diabetic rats, the activities of membrane-bound adenosine triphosphatases (ATPases) are altered in erythrocytes and in tissues such as liver and kidney. Albino Wistar rats were rendered diabetic by a single intraperitoneal injection of STZ (40 mg/kg body weight). Diabetic rats exhibited significantly ($p < 0.05$) increased levels of plasma glucose and decreased levels of plasma insulin. The activities of total ATPases, ($\text{Na}^+ + \text{K}^+$)-ATPase, Ca^{2+} -ATPase and Mg^{2+} -ATPase were significantly ($p < 0.05$) decreased in diabetic control rats. Control and diabetic rats were treated with camel milk (250 mL/day) for a period of 45 days. A group of diabetic rats were also treated with glibenclamide (600 $\mu\text{g}/\text{kg}$ body weight). After the treatment period, a significant ($p < 0.05$) decrease in the levels of glucose and increase in the levels of plasma insulin and the activities of ATPases in erythrocytes and tissues were observed in diabetic rats treated with camel milk. A similar effect is also observed in the glibenclamide treated rats. But, control rats treated with camel milk did not show any significant ($p < 0.05$) effect in any of the parameters studied. Our study shows that camel milk has the potential to restore the deranged activities of membrane-bound ATPases in STZ-diabetic rats. Further detailed investigation is necessary to find out its mechanism of action.

INTRODUCTION

Diabetes mellitus is the world's largest endocrine disorder resulting in multiple aetiologies, involving metabolic disorders of carbohydrate, fat and protein. All forms of diabetes are due to a decrease in the circulating concentration of insulin (insulin deficiency) and a decrease in the response of peripheral tissues to insulin i.e., insulin resistance. According to the World Health Organization projections, the prevalence of diabetes is likely to increase by 35% by the year 2025 [Boyle *et al.*, 2001]. As the number of people with diabetes multiplies worldwide, the disease has taken an ever-increasing share of national and international health budgets [Njomen *et al.*, 2009]. Saudi Arabia is witnessing a steady increase in the prevalence of diabetes mellitus with the recent estimate of prevalence being as high as 23.7% among adult citizens [Al-Nozha *et al.*, 2004].

Experimentally, streptozotocin (STZ) or alloxan are used to induce diabetes in rodents. STZ is effective in triggering islet cell death by acute oxidative stress. STZ-induced diabetic rats are one of the animal models of insulin-dependent diabetes mellitus characterized by high fasting blood glucose levels and drastic reduction in plasma insulin concentration [Burcelin *et al.*, 1995]. STZ-induced diabetes mellitus is as-

sociated with the generation of reactive oxygen species (ROS) causing oxidative damage [Szkudelski, 2001]. Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation [Baynes & Thorpe, 1997].

The activities of membrane-bound adenosine triphosphatases (ATPases) are altered both in erythrocytes and tissues of streptozotocin (STZ)-induced diabetic rats [Ramesh & Pugalendi, 2007]. In diabetes mellitus, increased ROS activity initiates peroxidation of lipids and MDA accumulation, which in turn can stimulate glycation of proteins in diabetes [Jain & Palmer, 1997]. Plasma membrane and the membranes of intracellular organelles are crucial targets of ROS attack. Diabetes-induced hyperlipidemia and the alterations in membrane phospholipids and fatty acids have been shown to depress membrane-bound enzyme activities, which influence intracellular calcium metabolism resulting in cardiac dysfunction [Kuwahara *et al.*, 1997]. The modification of enzyme molecules either by direct oxidation or by modification mediated by products of lipid peroxidation could also result in the decreased activities of ATPases in diabetes [Siems *et al.*, 1996].

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The management of diabetes mellitus without any side effects is still a challenge to the medical system [Kameswararao *et al.*, 2003]. Camel milk may be a therapeutic adjunctive option for diabetes mellitus in humans [Agrawal *et al.*, 2005]. Agrawal *et al.* [2003a] had reported that camel milk supplementation to type 1 diabetic patients proved effective in reducing glucose levels. Reduction in the occurrence of diabetes mellitus in a population consuming camel milk was also reported by Breitling *et al.* [2002]. A study by Agrawal *et al.* [2004] has shown the hypoglycemic activity of camel milk in STZ-induced diabetic rats. The hypoglycemic activity of camel milk in chemically pancreatectomized rats was also reported by Agrawal *et al.* [2005].

In this study, we have examined the administration of camel milk on the activities of membrane-bound enzymes such as $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$, $\text{Ca}^{2+}\text{-ATPase}$ and $\text{Mg}^{2+}\text{-ATPase}$ in erythrocytes and tissues (liver and kidney) along with the levels of glucose and insulin in STZ-induced diabetic rats.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain of body weight ranging from 180 to 200 g were procured from Central Animal House, King Saud University, and they were maintained in an air conditioned room ($25 \pm 1^\circ\text{C}$) with a 12 h light/12 h dark cycle. The animals were fed *ad libitum* with normal laboratory pellet diet (9 gms/rat/day) and procedures involving animals and their care were accordance with the Policy of Research Centre, King Saud University.

Chemicals

Streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA. All other chemicals were of analytical grade.

Experimental induction of diabetes

The animals were rendered diabetic by a single intraperitoneal injection of streptozotocin (40 mg/kg bodyweight) in freshly prepared citrate buffer (0.1 mol/L, pH 4.5) after an overnight fast [Ramesh & Pugalendi, 2006]. STZ injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. STZ injected animals exhibited massive glycosuria (determined by Benedict's qualitative test) and diabetes in STZ rats was confirmed by measuring the fasting plasma glucose concentration, 96 h after injection with STZ. The animals with plasma glucose above 240 mg/dL were considered diabetic and used for the experiment.

Experimental design

The animals were randomly divided into five groups of eight animals each as given below. Rats of groups II and IV were fed with 250 mL (26 mL/rat/day) of raw camel milk daily for 45 days through watering bottle instead of water. Whereas animals in groups I, III and V were given tap water for 45 days, and rats of group V were given 600 $\mu\text{g}/\text{kg}$ body weight of glibenclamide orally once a day in the morning for 45 days.

- Group I: Control (water)
- Group II: Control + raw camel milk (250 mL/day)
- Group III: Diabetic control
- Group IV: Diabetic rats + raw camel milk (250 mL/day)
- Group V: Diabetic rats + glibenclamide (600 $\mu\text{g}/\text{kg}$ body weight/day).

Glibenclamide is a sulfonylurea antidiabetic agent, a class of drugs used to treat type II diabetes mellitus. This disease is a chronic metabolic illness characterised by a deficiency of insulin, a hormone produced by the pancreas which controls the sugar in the blood. For that, in this study we are using glibenclamide as a standard drug for the comparison of efficacy with camel milk-treated diabetic rats.

Sample collection

After 45 days of treatment, the animals were fasted for 12 h, anaesthetized between 8:00 a.m. to 9:00 a.m. using ketamine (24 mg/kg body weight, intramuscular injection), and sacrificed by decapitation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of plasma insulin and glucose.

Blood was also collected in tubes with EDTA and erythrocytes were separated by washing with 0.15 mol/L sodium chloride solution. Erythrocytes and tissues (liver and kidney) were collected for the measurement of membrane-bound ATPases such as total ATPases, $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$, $\text{Ca}^{2+}\text{-ATPase}$ and $\text{Mg}^{2+}\text{-ATPase}$. Tris buffer (pH 7-8) was prepared using redistilled water and tissues were homogenized in Tris buffer. Redistilled water was used throughout the experiment to avoid interference prior to phosphorous estimation in the assay of ATPases.

Tissue sampling for biochemical study

For the biochemical study, the liver and kidney was immediately dissected and washed with cold physiological saline, followed by formalin (10% formaldehyde). Liver and kidney was excised immediately and fixed in 10% formalin.

Estimation of plasma glucose and insulin

Plasma glucose was estimated by the method of Trinder [1969] using a reagent kit purchased from Biotron Diagnostics, Inc, U.S.A. Plasma insulin was measured by the method of Burgi *et al.* [1988].

Estimation of the activities of ATPases

The activity of total ATPases was measured by the method of Evans [1969]. The phosphate liberated was estimated by the method of Fiske & Subbarow [1925]. The activities of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$, $\text{Ca}^{2+}\text{-ATPase}$ and $\text{Mg}^{2+}\text{-ATPase}$ were measured by the methods of Bonting [1970], Hjerten & Pan [1983] and Ohnishi *et al.* [1982], respectively.

Statistical analysis

Values were given as means \pm SD for eight rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 11 software (SPSS, Chicago, IL). The limit of statistical significance was set at $p(0.05)$. The Student's t-tests were followed by diabetic and glibenclamide *versus* diabetic and camel milk groups ($p < 0.001$).

RESULTS

Table 1 shows the effect of camel milk on the levels of plasma glucose and insulin in control and diabetic rats. Diabetic control rats exhibited significantly ($p < 0.05$) higher levels of glucose and decreased levels of insulin. Treatment with camel milk (250 mL/day) to diabetic rats resulted in a significant ($p < 0.05$) decrease in glucose levels and increased insulin levels. Control rats treated with camel milk (250 mL/day) did not show any significant effect on glucose and insulin and levels.

Table 2 shows the activity of total ATPases in erythrocytes, liver and kidney in control and diabetic rats. A significant ($p < 0.05$) decrease in the activity of total ATPases in erythrocytes, liver and kidney were observed in diabetic rats. Treatment with camel milk (250 mL/day) to diabetic rats resulted in a significant ($p < 0.05$) increase in the total ATPases activity in erythrocytes and tissues. Control rats treated with camel milk (250 mL/day) did not show any significant effect on the activity of total ATPases in erythrocytes, liver and kidney.

Tables 3-5 show the activities of ($\text{Na}^+ + \text{K}^+$)-ATPase, Ca^{2+} -ATPase and Mg^{2+} -ATPase in erythrocytes, liver and kidney re-

spectively in control and diabetic rats. Significantly ($p < 0.05$) decreased activities of these enzymes were observed in erythrocytes and tissues of diabetic rats. Camel milk (250 mL/day) treatment to diabetic rats resulted in a significant ($p < 0.05$)

TABLE 3. Effect of camel milk on ($\text{Na}^+ + \text{K}^+$)-ATPase in the erythrocytes and tissues of control and STZ-diabetic rats.

Groups	Erythrocyte (U*/mg protein)	Liver (U*/mg protein)	Kidney (U*/mg protein)
Control	0.90±0.07 ^a	0.90±0.08 ^a	0.60±0.04 ^a
Control + camel milk (250 mL/day)	0.90±0.08 ^a	1.00±0.08 ^a	0.60±0.05 ^a
Diabetic control	0.40±0.03 ^b	0.40±0.03 ^b	0.30±0.03 ^b
Diabetic + camel milk (250 mL/day)	0.60±0.05 ^{c,*}	0.70±0.05 ^{c,*}	0.40±0.03 ^{c,*}
Diabetic + glibenclamide (600 µg/kg b.wt)	0.80±0.06 ^d	0.80±0.07 ^d	0.50±0.04 ^d

Values are means ±SD for eight rats. Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT). U*—µmol of Pi liberated per hour. * $p < 0.001$ significantly different between the diabetic and glibenclamide versus diabetic and camel milk (Student's *t*-test).

TABLE 1. Effect of camel milk on plasma glucose and insulin levels in control and STZ-diabetic rats.

Groups	Glucose (mg/dL)		Insulin (µU/mL)
	Day 0	Day 45	
Control	69.00±02.07	76.30±07.59 ^a	15.40±1.31 ^a
Control + camel milk (250 mL/day)	68.00±04.94	75.40±07.76 ^a	15.30±1.30 ^a
Diabetic control	251.30±15.80	292.40±19.20 ^b	5.50±0.41 ^b
Diabetic + camel milk (250 mL/day)	248.00±17.92	141.60±12.82 ^{c,*}	10.00±0.80 ^{c,*}
Diabetic + glibenclamide (600 µg/kg b.wt)	255.10±14.60	106.20±8.68 ^d	14.90±1.26 ^a

Values are means ±SD for eight rats. Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT). * $p < 0.001$ significantly different between the diabetic and glibenclamide versus diabetic and camel milk (Student's *t*-test).

TABLE 2. Effect of camel milk on total ATPases in the erythrocytes and tissues of control and STZ- diabetic rats.

Groups	Erythrocytes (U*/mg protein)	Liver (U*/mg protein)	Kidney (U*/mg protein)
Control	2.80±0.16 ^a	3.30±0.20 ^a	2.70±0.15 ^a
Control + camel milk (250 mL/day)	2.80±0.16 ^a	3.30±0.21 ^a	2.80±0.16 ^a
Diabetic control	1.90±0.07 ^b	2.00±0.08 ^b	1.70±0.06 ^b
Diabetic + camel milk (250 mL/day)	2.20±0.11 ^{c,*}	2.50±0.13 ^{c,*}	2.90±0.08 ^{c,*}
Diabetic + glibenclamide (600 µg/kg b.wt)	2.60±0.14 ^d	3.00±0.18 ^d	2.20±0.11 ^d

Values are means ±SD for eight rats. Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT). U*—µmol of Pi liberated per hour. * $p < 0.001$ significantly different between the diabetic and glibenclamide versus diabetic and camel milk (Student's *t*-test).

TABLE 4. Effect of camel milk on Ca^{2+} -ATPase in the erythrocytes and tissues of control and STZ-diabetic rats.

Groups	Erythrocyte (U*/mg protein)	Liver (U*/mg protein)	Kidney (U*/mg protein)
Control	0.60±0.04 ^a	0.60±0.05 ^a	0.50±0.05 ^a
Control + camel milk (250 mL/day)	0.60±0.05 ^a	0.70±0.05 ^a	0.50±0.04 ^a
Diabetic control	0.40±0.03 ^b	0.30±0.02 ^b	0.20±0.01 ^b
Diabetic + camel milk (250 mL/day)	0.50±0.04 ^{c,*}	0.40±0.03 ^{c,*}	0.40±0.03 ^{c,*}
Diabetic + glibenclamide (600 µg/kg b.wt)	0.50±0.04 ^d	0.60±0.05 ^d	0.40±0.03 ^d

Values are means ±SD for eight rats. Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT). U*—µmol of Pi liberated per hour. * $p < 0.001$ significantly different between the diabetic and glibenclamide versus diabetic and camel milk (Student's *t*-test).

TABLE 5. Effect of camel milk on Mg^{2+} -ATPase in the erythrocytes and tissues of control and STZ- diabetic rats.

Groups	Erythrocyte (U*/mg protein)	Liver (U*/mg protein)	Kidney (U*/mg protein)
Control	0.50±0.03 ^a	0.70±0.05 ^a	0.40±0.03 ^a
Control + camel milk (250 mL/day)	0.50±0.04 ^a	0.60±0.05 ^a	0.40±0.03 ^a
Diabetic control	0.20±0.02 ^b	0.30±0.02 ^b	0.20±0.01 ^b
Diabetic + camel milk (250 mL/day)	0.30±0.02 ^{c,*}	0.40±0.03 ^{c,*}	0.30±0.02 ^{c,*}
Diabetic + glibenclamide (600 µg/kg b.wt)	0.40±0.03 ^d	0.60±0.05 ^d	0.30±0.02 ^d

Values are means ±SD for eight rats. Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT). U*—µmol of Pi liberated per hour. * $p < 0.001$ significantly different between the diabetic and glibenclamide versus diabetic and camel milk (Student's *t*-test).

increase in the activities of these enzymes in erythrocytes and tissues. Treatment with camel milk (250 mL/day) to control rats did not exhibit any significant effect on the activities of these enzymes in erythrocytes and tissues.

For all the parameters studied, diabetic rats treated with camel milk (250 mL/day) exhibited a similar effect to glibenclamide (600 µg/kg body weight).

DISCUSSION

Administration of camel milk to diabetic rats resulted in reduction of glucose levels and increased insulin levels in this study. This could be due to presence of high concentrations of insulin like protein in camel milk. Earlier studies show that camel milk supplementation reduces the insulin requirement in type 1 diabetic patients [Agrawal *et al.*, 2003a,b]. It is found that one of the camel milk proteins has many characteristics similar to insulin [Beg *et al.*, 1989] and it does not form coagulum in acidic environment [Wangoh, 1993]. This lack of coagulum formation allows the camel milk to pass rapidly through the stomach together with the specific insulin like protein/ insulin and remains available for absorption in intestine. Radioimmunoassay of camel milk has revealed high concentration of insulin, *i.e.* 52 units/L [Singh, 2001]. Camel milk also contains high amount of zinc [Mehaia *et al.*, 1995]. Zinc plays a major role of in pancreatic beta cells. Richards-Williams *et al.* [2008] had reported that, extracellular ATP and zinc are co-secreted with insulin and activate multiple P2X purinergic receptor channels expressed by islet beta-cells to potentiate insulin secretion. Endocrine pancreatic islets, secreted ATP and zinc have profound autocrine regulatory influence on insulin secretion via ATP-gated and zinc modulated P2XR channels.

Determination of membrane associated enzyme activity like ATPases indicate alterations in membrane under pathological conditions. ATPases are intimately associated with the plasma membrane and participates in the energy requiring translocation of sodium, potassium, calcium and magnesium [Mourelle & Franco, 1991]. Diabetes-induced hyperlipidemia and the alterations in membrane phospholipids and fatty acids have been shown to depress membrane-bound enzyme activities [Kuwahara *et al.*, 1997]. Total ATPases consist of (Na⁺+K⁺)-ATPase, Ca²⁺-ATPase and Mg²⁺-ATPase. The ubiquitous cellular enzyme (Na⁺+K⁺)-ATPase is responsible for the maintenance of intracellular sodium and potassium concentrations [McDonough *et al.*, 1990]. Ca²⁺-ATPase is the major active calcium transport protein responsible for the maintenance of normal intracellular calcium levels in a variety of cell types. It is also considered to be responsible for the shape and deformability of the erythrocyte membranes [La Celle & Kirkpatrick, 1975]. Mg²⁺-ATPases are all cell surface ATPases, which hydrolyze intracellular ATP [Sabbadini & Dahms, 1989].

Insulin and catecholamines are the principal mediators of acute hormonal control of Na⁺/K⁺-ATPase [Clausen & Everts, 1989]. Insulin is a major regulator of potassium homeostasis and has multiple effects on sodium pump activity. Within minutes of elevated insulin secretion, pumps containing alpha-1 and 2 isoforms have increased affinity for sodium

and increased turnover rate. Sustained elevations in insulin cause upregulation of alpha-2 synthesis. In skeletal muscle, insulin may also recruit pumps stored in the cytoplasm or activate latent pumps already present in the membrane. The activities of total ATPases, (Na⁺+K⁺)-ATPase, Ca²⁺-ATPase and Mg²⁺-ATPase were significantly decreased in erythrocytes, liver and kidney of diabetic rats in this study, which resembles the previous report [Kjeldsen *et al.*, 1987]. This might be associated with the deficiency of insulin as insulin administration partially restored (Na⁺+K⁺)-ATPase [Gupta, 1996]. The oxidative damage of tissue lipids and proteins might have caused (Na⁺+K⁺)-ATPase inactivation. (Na⁺+K⁺)-ATPase is rich in thiol groups and oxidation of thiol groups has been reported to inhibit enzyme activity [Unlucerci *et al.*, 2001]. Treatment with camel milk and glibenclamide restored (Na⁺+K⁺)-ATPase. This may be due to insulin secretory effect along with decreasing peroxidative damage to membrane lipids as reported earlier [Ramesh & Pugalendi, 2006; 2005a,b]. Hyperglycemia can cause glycosylation of proteins and cellular lipid peroxidation, which, in turn, can cause inhibition/reduction in the activities of (Na⁺+K⁺)- and Ca²⁺-ATPases. This, in turn, can affect the intracellular concentrations of Na⁺, K⁺, and Ca²⁺, alter the signal transduction pathways, and affect contractility and excitability and cellular dysfunctions [Jain & Lim, 2000]. Glycosylation of proteins may also alter their physiological properties, particularly their binding affinities [Yagihashi, 1995]. Several alterations in structural and dynamic properties of erythrocyte membrane have been reported in type 1 and type 2 diabetes [Ver *et al.*, 1997].

A direct interaction between enzyme and phospholipids was shown by Muczynsky & Stahl [1983]. Lipid alteration in the erythrocyte membrane was reportedly related to the reduced (Na⁺+K⁺)-ATPase activity in type 1 and type 2 diabetic patients [Baldini *et al.*, 1989]. The decreased activity of (Na⁺+K⁺)-ATPase in both erythrocyte and tissues was restored to near normal in diabetic rats treated with camel milk. This could be due to the restoration of insulin levels in diabetic rats administered with camel milk.

It has been reported that diabetic rats had decreased activity of Ca²⁺-ATPase as a consequence of interaction of this enzyme with glucose in a hyperglycemic state [Rajeswari *et al.*, 1991]. A decrease in the activity of this enzyme was observed in erythrocytes and tissues in this study. The decreased activity could be attributed to the insulin deficiency in the diabetic state as insulin is the regulator of this enzyme [Hope-Gill & Nanda, 1979]. Increased lipid peroxidation diminish the activities of Ca²⁺-ATPase and Mg²⁺-ATPase in erythrocyte membrane when exposed to a higher glucose concentration-containing medium [Jain & Lim, 2000]. Oral administration of camel milk to diabetic rats had a significant effect on the activities of these enzymes. The activities of these enzymes were restored to near normal in diabetic rats when treated with camel milk may be due to the insulin like protein.

CONCLUSIONS

In conclusion, our study has shown that administration of camel milk to diabetic rats significantly increased the activities of ATPases in erythrocytes, liver and kidney. Camel milk

also decreased the glucose levels and increased insulin levels in diabetic rats. This could be due to the presence of higher concentrations of insulin like protein and also zinc in camel milk. Further detailed investigation is necessary to find out its mechanism of action and to establish its therapeutic potential in the treatment of diabetes and diabetic complications.

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