Antidiabetic Effects of Camel Milk in Streptozotocin-induced Diabetic Rats

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ABSTRACT
This study was conducted to analyse the possible antidiabetic effects of camel milk in streptozotocin (STZ)-induced diabetic rats by assaying liver and kidney clinical function parameters. Administration of streptozotocin (55 mg kg$^{-1}$ b.wt.) to the experimental groups of rats resulted in marked detectable changes. The rats were fed daily with fresh camel milk by feeding bottles for 30 days. The effects of camel milk on blood glucose, serum proteins, urea, uric acid, creatinine, lipid profile and the activities of diagnostic marker enzymes of liver function and Alkaline Phosphatase (ALP) were examined in the plasma/serum of control and experimental groups of rats. Camel milk feeding to diabetic rats significantly reduces the levels of blood glucose, urea, uric acid and creatinine and increases the activities of albumin, albumin/globulin ratio and restores all liver function marker enzymes and lipid profile to near control levels. The present study shows that feeding of camel milk to diabetic rats has antihyperglycemic effects and consequently may alleviate liver and renal damage associated with streptozotocin-induced diabetic rats.

Key words: Camel milk, diabetes mellitus, aminotransferases, liver function profile, kidney function profile, lipid profile

INTRODUCTION
Diabetes Mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion and/or insulin action, which results in hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism (Hovens et al., 2005). The incidence of DM has increased dramatically in recent decades, predominantly because of changes in life style and increase in the prevalence of obesity. Recent estimates indicate that there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 360 million in the year 2030 (Wild et al., 2004). Management of DM without any side effects is still a challenging task for the medical system. So there is an increasing demand by patients to use natural products with antidiabetic activity, because insulin and other oral hypoglycaemic drugs have undesirable side effects (Rao and Rao, 2001). Currently available synthetic antidiabetic agents produce serious side effects such as hypoglycaemic coma and hepatorenal disturbances (Suba et al., 2004). Uses of some medicines which act as insulin secretagogues or sensitizers are associated with weight gain (Hays et al., 2008). Moreover, such anti-diabetic products are not safe for use during pregnancy (Rahman and Zaman, 1989). Hence, the search for natural, safer and more effective hypoglycaemic agents has
continued tremendously. Following the WHO's recommendation for research on the beneficial uses of medicinal plants in the treatment of diabetes mellitus (World Health Organization, 1980), investigations on other hypoglycaemic agents have also gained momentum.

Camel milk is known for its medicinal properties since ancient times and recently camel milk has been deeply studied for its special properties because of higher hepatoprotective, insulin like, antibacterial and antiviral activities (Khan and Alzohairy, 2011). Camel milk is considered to have anti-cancer (Magied, 2005), hypoallergenic (Shabo et al., 2005) and anti-diabetic properties (Agrawal et al., 2003). Other components such as lactoferrin, immunoglobulins, lysozyme and vitamin C are in good quantity in camel milk, were reported to play a crucial role in the determination of these properties (Konuspayeva, 2007). Compared to the other bovine species, camel whey contains a high concentration of anti-microbial factors such as lysozyme, lactoferrin and immunoglobulins (Elagamy et al., 1996).

In a preliminary study, Agrawal et al. (2004) reported the low prevalence of diabetes in Raica community of Rajasthan, India and attributed the low prevalence level to consumption of camel milk. The low prevalence of impaired fasting glucose, impaired glucose tolerance and diabetes in camel milk consuming groups is due to various factors like high concentration of insulin like substance in camel milk, immunomodulatory effect of camel milk on beta cell function and lack of coagulum formation in acidic medium. Although, patients treated with camel milk needed less insulin to achieve better control than the controlled group (Agrawal et al., 2005).

Moreover, camel milk is different from other ruminant milk as it is low in cholesterol, sugar and protein but high in minerals, vitamins and contains higher concentration of insulin and immunoglobulins (Kamal et al., 2007; Al-Hashem, 2009). Furthermore, camel milk has a higher storage capacity at room temperature as compared to milk of other animals (Ömer and Eltinay, 2009). It has no allergic properties and can be consumed by lactose-deficient individuals and those with a weakened immune system (Inayat et al., 2003; Yateem et al., 2008).

The present study, we investigated the anti-diabetic effects of fresh camel milk in streptozotocin-induced diabetic rats by assaying kidney function parameters as urea, uric acid and creatinine, liver function test enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), protein, albumin and lipid profile.

MATERIALS AND METHODS
Chemicals and kits: Streptozotocin was purchased from Sigma Chemical Company (St Louis, MO, USA). Diagnostic kits for serum ALT, AST, ALP, albumin, total protein and Cholesterol, urea, uric acid and creatinine were purchased from Human diagnostic kits (Human GmbH, Wiesbaden Germany). Insulin was purchased from Sanofi aventis (Deutschland GmbH, Germany). All other chemicals and solvents were of highest grade commercially available.

Camel milk: Camel milk samples were collected daily for one month, early in the morning from Alsalman camel milk farms in the Buraidah area of Qassim (central Saudi Arabia). Milk was collected from camels by automated milking machines. The milk samples were collected in sterile screw bottles and kept in cool boxes until transported to the laboratory. The rats were fed daily with fresh camel milk (400 mL/24 h/cage) as such without any further treatment by the feeding bottles. This whole study was conducted in four months from October, 2011 January, 2012.
Animals and treatment: Male albino Wistar rats (200-250 g) were obtained from College of Pharmacy, King Saud University, Riyadh and acclimated for at least 7 d before starting the experiment. All animals were housed in standard aluminium cages (4 rats\(^{-1}\) cage), feeding with standard laboratory diet and tap water \textit{ad libitum}. The experimental animals were housed in air-conditioned rooms at 21-23°C and 60-65% of relative humidity and kept on a 12 h light/dark cycle. The animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health.

Experimental groups and protocol: The rats were divided randomly into five groups comprising eight rats in each group as follows:

- **Group 1**: Normal control rats (vehicle treated)
- **Group 2**: Normal rats fed with camel milk for 30 days
- **Group 3**: Diabetic control group injected with STZ (55 mg kg\(^{-1}\) b.wt.)
- **Group 4**: Diabetic rats fed with camel milk
- **Group 5**: Diabetic rats treated with insulin by i.p. injection (6 units kg\(^{-1}\) b.wt. day\(^{-1}\))
  (Gupta \textit{et al.}, 2004)

Induction of diabetes with streptozotocin: The animals were fasted overnight and diabetes was induced in rats by a single intra peritoneal (i.p.) injection of freshly prepared STZ, 55 mg kg\(^{-1}\) b.wt. of rats in 0.1 M citrate buffer (pH 4.5) (Khan and Alzohairy, 2011). The animals were allowed to drink 5\% glucose solution overnight to overcome the drug induced hypoglycaemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg dL\(^{-1}\) on third day after STZ injection. The treatment was started on the fourth day after STZ injection and this was considered as the first day of treatment. The treatment was continued for 30 days.

Blood and tissue collection: At the end of day 30, the animals were sacrificed and the blood samples were divided for plasma and serum collection. Potassium oxalate and sodium fluoride containing sterile tubes were used for plasma collection and tubes without any anticoagulant were used to collect blood and it was allowed to clot at room temperature for 30 min. The serum was separated by centrifugation at 1000xg for 15 min at 4°C and were saved in aliquots and stored at -20°C for further analysis. The liver, kidney and pancreas were also quickly removed and washed with cold normal saline, cut and preserved in 10\% neutral formalin for further pathological studies for microscopy.

Serum biochemistry: Blood glucose, liver function parameters (ALT, AST, ALP, protein, albumin and cholesterol) and kidney function tests (urea, uric acid and creatinine) were performed by using the diagnostic kits as mentioned earlier. All spectrophotometric measurements were carried out in a Shimadzu UV-Visible spectrophotometer.

Statistical analysis: Results were expressed as Means±standard deviation of three replicates. The significance of differences was calculated by using student t-test. The \(p<0.05\) was considered statistically significant.
RESULTS

The administration of STZ (55 mg kg\(^{-1}\) b.wt.) to the experimental groups of rats resulted in marked detectable changes. A significant decrease in body weight (24.8%) was observed in diabetic rats as compared to control rats. Treatment of diabetic groups of rats with camel milk increased body weight to near normal level. A significant increase in the level of blood glucose was observed in diabetic rats (530 mg dL\(^{-1}\)) when compared to control and insulin treated rats. Feeding of diabetic rats with camel milk significantly decreased the levels of blood glucose to about 235 mg dL\(^{-1}\) (Table 1).

The level of protein, plasma albumin and albumin/globulin ratio in control and STZ-DM rats were also assayed for comparative study. The level of protein in plasma was found to be reduced in diabetic animals (p<0.05) when compared to control animals. The lowered level of protein after camel milk feeding increased to near control. The levels of albumin and albumin/globulin (A/G) ratio in plasma were also found to be decreased in diabetic animals. These lowered levels of plasma albumin and A/G ratio levels were restored significantly in camel milk treated diabetic rats (Table 1).

Urea, uric acid and creatinine levels were significantly elevated in STZ-DM rats (p<0.05) when compared to control animals (Table 2). Feeding of diabetic rats with camel milk for 30 days significantly lowered urea, uric acid and creatinine levels in STZ-DM rats.

Table 2 further shows the activities of AST, ALT and ALP in plasma of control and STZ-DM rats. The activities of these enzymes were found to be significantly increased (p<0.05) in the plasma of diabetic rats. Feeding of rats with camel milk for one month resulted in dramatic shift towards normal value of these liver function diagnostic enzymes in STZ-DM rat groups.

Table 1: Effect of camel milk treatment on blood glucose and serum proteins of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
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<tbody>
<tr>
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<tr>
<td>Glucose (mg dL(^{-1}))</td>
<td>115.6±5.6</td>
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<tr>
<td>Protein (g dL(^{-1}))</td>
<td>6.83±0.95</td>
</tr>
<tr>
<td>Albumin (g dL(^{-1}))</td>
<td>3.86±0.45</td>
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<tr>
<td>A/G ratio</td>
<td>1.32±0.18</td>
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Values are given as ±SD for groups of eight animals. Values are statistically significant. *p<0.05 compared to control, †p<0.05 compared to diabetic, nd: Not determined

Table 2: Effect of camel milk treatment on liver and kidney diagnostic markers on control and experimental groups of rats

<table>
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<tr>
<td>ALT (U L(^{-1}))</td>
<td>45.29±4.2</td>
</tr>
<tr>
<td>AST (U L(^{-1}))</td>
<td>75.76±5.4</td>
</tr>
<tr>
<td>ALP (mg dL(^{-1}))</td>
<td>92.76±4.7</td>
</tr>
<tr>
<td>Urea (mg dL(^{-1}))</td>
<td>21.23±2.3</td>
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<tr>
<td>Uric acid (mg dL(^{-1}))</td>
<td>2.71±0.6</td>
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<tr>
<td>Creatinine (mg dL(^{-1}))</td>
<td>0.91±0.08</td>
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</tbody>
</table>

Values are given as ±SD for groups of eight animals. Values are statistically significant. *p<0.05 compared to control, †p<0.05 compared to diabetic, nd: Not determined, AST: Aspartate aminotransferase, ALP: Alamine aminotransferase, ALT: Alamine transaminase
Table 3: Effect of camel milk treatment on lipid profile on control and experimental groups of rats

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>Cholesterol (mg dL⁻¹)</td>
<td>131.20±10.4</td>
<td>138.45±8.7</td>
<td>293.31±12.4*</td>
<td>195.27±11.9*</td>
<td>153.21±4.8</td>
</tr>
<tr>
<td>LDL-C (mg dL⁻¹)</td>
<td>70.21±4.5</td>
<td>71.25±5.3</td>
<td>191.31±8.4*</td>
<td>128.34±5.9*</td>
<td>106.34±6.3</td>
</tr>
<tr>
<td>HDL-C (mg dL⁻¹)</td>
<td>41.37±5.1</td>
<td>42.43±6.2</td>
<td>58.43±6.8*</td>
<td>52.37±5.6*</td>
<td>49.31±5.3</td>
</tr>
<tr>
<td>TG (mg dL⁻¹)</td>
<td>74.22±7.1</td>
<td>76.43±6.5</td>
<td>167.43±5.8*</td>
<td>109.22±6.3*</td>
<td>84.36±6.1</td>
</tr>
</tbody>
</table>

Values are given as ±SD for groups of eight animals. Values are statistically significant, *p<0.05 compared to control, **p<0.05 compared to diabetic, nd: Not determined, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, TG: Triglycerides

Changes in the levels of lipid profile were also assayed to assess the STZ-induced diabetic impairment and the protective role of camel milk against dyslipidemia. The results of this study showed that levels of cholesterol, low density lipoprotein and high density lipoprotein-cholesterol and Triglycerides (TG) were significantly higher (p<0.05) in diabetic control groups of rats and these levels were significantly decreased in the group of rats treated with camel milk (Table 3).

DISCUSSION

Diabetes mellitus is a serious illness with multiple complications and premature mortality, accounting for at least 10% of total health care expenditure in many countries (King et al., 1998). Global postulates that three fourth of the world population cannot afford the products of allopathic medicine and thus have to rely upon the use of traditional medicines, which are largely derived from natural products of animals and plants (Hayes et al., 2008). Some of these substances have shown antidiabetic effects by directly influencing β-cells to stimulate insulin and restore insulin sensitivity (Lombardo and Chicco, 2006).

Camel milk is gaining more popularity nowadays because of its high nutritional qualities and therapeutic value (Strasser et al., 2006). Camel milk has been used for the diabetes treatment in many parts of the world. Camel milk has powerful antibacterial and antiviral properties which could remodelate the immune system. Drinking non-pasteurized camel milk has been observed to be beneficial to people with infection of the alimentary canal and autoimmune diseases (Shabo et al., 2008).

Literature overview shows that camel milk is a high quality drink and since ancient times people have been using this product for curing a number of diseases (Shabo et al., 2005; Agrawal et al., 2007; Redwan and Tabll, 2007). This study evaluates the effect of camel milk against diabetes in albino male Wistar rats by assessing the liver and kidney functions of control and streptozotocin-induced diabetes rats. Streptozotocin is a selective β-cell genotoxicant and when administrated in a single high dose it induces a rapid onset of diabetes by generating sufficient levels of DNA adducts to cause over activation of poly adenosine diphosphate ribose synthetase in the base excision repair pathway (Burns and Gold, 2007).

In the current study, we observed significant increase in blood glucose level in diabetic rats (Table 1). This may be due to the destruction of pancreatic beta cells by STZ, reinforcing the fact that STZ induces diabetes, probably through the generation of oxygen free radicals (Gupta et al., 2004). The elevation of glucose in STZ treated rats is due to an oxidative stress produced in the pancreas, due to a single strand break in pancreatic islets DNA (Yamamoto et al., 1981). Sboui et al. (2010) also observed that alloxan-induced diabetic dogs showed a significant decrease in blood glucose level after treatment with camel milk.

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The protective effects of camel milk could be attributed to its antioxidant activity and it may possibly have chelating effects on toxicants (Al-Humaid et al., 2010). It has been reported that camel milk contains high levels of vitamins (A, E, C and E) and is rich in magnesium (Knoess, 1979). These vitamins are antioxidants that have been found useful in preventing tissue injury caused by toxic agents like CCl₄, AlCl₃ and STZ etc. Additionally, camel milk is rich in Zinc (Knoess, 1979), a trace element essential for living organisms. More than 300 enzymes require Zn for their activity and it has a relationship with many enzymes in the body and can prevent cell damage through activation of antioxidant system (Powell, 2000; Ozturk et al., 2003; Ozdemir and Inanc, 2005).

Our results are in agreement with Al-Humaid et al. (2010) who observed that treatment of rats with camel milk that were poisoned with lead acetate, the raised levels of liver function enzymes (ALT and AST), urea and triglycerides restores to normal levels after treatment. Moreover, camel milk has been proven to be antigenotoxic and anticytotoxic.

In addition to this we recently observed that the rats poisoned with CCl₄, the level of aminotransferases, alkaline phosphatase, proteins and cholesterol levels restore to almost normal levels after treatment with camel milk. Histopathological studies of the liver markedly showed the reduction in fatty changes, inflammatory cell infiltration and necrosis after treatment with camel milk (Khan and Alzohairy, 2011).

Urea, creatinine and uric acid levels were significantly elevated in STZ-DM rats as compared to control groups. Feeding of diabetic rats with camel milk for 30 days significantly lowered these kidney function parameter (Table 2). Al-Hashem (2009) also showed that rats poisoned with AlCl₃ suffered with increased serum levels of urea, creatinine, bilirubin, AST, ALT, ALP, LDH (Lactate Dehydrogenase), cholesterol and TG. The total amount of albumin and protein were also significantly decreased with AlCl₃ poisoning. However, these parameters were within normal levels in rats given camel milk prior to AlCl₃ toxicity.

Agarwal et al. (2009) further proved that there was a significant improvement of microalbuminuria after receiving camel milk and the mean dose of insulin was significantly reduced for obtaining glycemic control. A significant change in lipid profile was also observed after camel milk supplementation of camel milk.

CONCLUSION
The therapeutic efficacy of the fresh camel milk on streptozotocin-induced diabetic rats was found in our study. Camel milk shows a significant therapeutic role in treatment of diabetes induced metabolic disorders possible due to the presence of insulin/insulin-like proteins. This may have important implication for the clinical management of diabetes mellitus in humans. So camel milk seems to be a beneficial health drink, although further studies are necessary.

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REFERENCES


