Antidiabetic effect of camel milk on alloxan-induced diabetic dogs

A. Sboui1,3*, T. Khorchani1, A. Agrebi3, M. Djegham2, M. Mokni4 and O. Belhadj3

1Livestock and Wildlife Laboratory, Arid Land Institute, Medenine, Tunisia.
2Veterinary School, Laboratory of Physiology and Therapeutic, Sidi Thabet, Tunisia.
3College of Science, Biochemistry and Technobiology Laboratory, Tunis, Tunisia.
4Farhat Hached Hospital, Anatomy –Pathology Laboratory, Sousse, Tunisia.

Accepted 8 March, 2012

This research was conducted to evaluate the antidiabetic effect of camel milk in alloxan-induced diabetic dogs. Diabetes was induced by intravenous injection of alloxan monohydrate (65 mg/kg bodyweight). The effects of camel milk on diabetic dogs were investigated by observing changes in the glycometabolic index (fasting blood glucose, intravenous glucose tolerance test (IGTT)) test, the lipometabolic index (triglyceride, cholesterol), total proteins and in the degree of injury of β-cells in the pancreatic islets. A significant decrease in fasting plasma glucose, cholesterol and total proteins in blood sample and an improvement on the animal clinical state (increase of body weight, normal activity) was observed. Camel milk also induces renewal of pancreatic β-cells. A comparison was made between the effect of camel milk and cow milk. Treatment with cow milk does not have any benefit effect on alloxan-induced diabetic dogs.

Key words: Alloxan, diabetes, dogs, antidiabetic effect, camel milk, β-cells.

INTRODUCTION

Diabetes mellitus is one of the endocrine glands diseases in human and animal which involves the gland circulatory system. About 6.3% of world populations live with diabetes. Diabetes creates the following common symptoms during its chronic length: thirst, polyuria, increased appetite and weight decrease, heart and coronary problems, kidneys problems, ketosis, and blood glucose increase among others (Valilou et al., 2007). This metabolic disorder can be induced chemically using alloxan monohydrate or streptozotocine. Alloxan-induced diabetes mellitus is caused by the selective pancreatic beta cell toxicity (Matsuhisa et al., 1997; Rerup, 1970). In order to destroy insulin-producing cells and to induce a state of insulin dependent diabetes mellitus, alloxan, due to its similarity in molecular shape with the glucose molecule, must be taken up into the cell via the low affinity GLUT 2 glucose transporters in the plasma membrane (Sakudelski, 2001; Tyberg et al., 2001). Several species are sensitive to alloxan toxicity such as rats, rabbit and dogs (Tyberg et al., 2001). Besides drugs classically used for the treatment of diabetes (insulin, sulphonylureas and biguanides) camel milk is known, in arid regions and in the wilderness, for its usefulness to treat diabetes mellitus (Sboui et al., 2010; Agrawal et al., 2003).

The purpose of this study was to assess the effects of camel milk treatment on alloxan - induced diabetic dogs by following the variations of the blood glucose levels, and serum chemistry profiles and the histological aspect of pancreatic cells in diabetic dogs treated with camel milk in comparison to cow milk. This research was done in the same conditions of the essay of dose/response effect of camel milk (Sboui et al., 2010). In this study we were interested also in the histopathological findings in pancreatic β-cells.
MATERIALS AND METHODS

As reported in literature, dogs can be useful laboratory animals in studying diabetic deficiencies thereby helping in veterinary and medical research (Hetenyi et al., 1989; Masataka et al., 2000; Valliou et al., 2007).

Animals and diet

Twelve clinically normal adult mixed-breed dogs aged 2 to 3 years were prepared for this experiment. Their body weight ranged from 12 to 16 kg initially. The dogs were housed individually in the Tunisian Veterinary Medicine School, Sidi Thabet. They were fed once daily with 350 to 400 g of commercial dry chow (23% protein, 6% fat, 33% carbohydrates, 4% crude fiber and 3000 kcal/kg as energetic value; (DOGSY) from Tunisian Animal Nutrition Society) and 300 to 400 g of beef.

This food was given to all dogs daily in the morning after drinking milk. All animals were controlled when drinking milk to be sure that all the quantity given was consumed by the dogs. Water was available ad libitum for dogs throughout the duration of the experiment.

Induction of experimental diabetes

The dogs were fasted for 24 h prior to the induction of diabetes mellitus. Blood was collected for baseline data determination including: baseline glucose, cholesterol, triglycerides and total proteins. Diabetes was induced in dogs by intravenous injection of 65 mg/kg bodyweight. Fresh solution of alloxan monohydrate (Sigma, Aldrich Chemicals, St Louis, USA) for diabetes mellitus induction was prepared just prior to injection (Anderson et al., 1993; Black et al., 1980). Because alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, dogs were treated with 10% glucose solution after 4 h. After one week, dogs showed hyperglycaemia (≥10 mmol/L) and were treated with camel or cow milk.

Experimental design

Twelve dogs (8 diabetic dogs; 4 healthy dogs) were used. The animals were divided into 3 groups, of 4 dogs each, as follows:

(i) Group 1: Diabetic dogs treated with 500 ml of camel milk daily for 5 weeks;
(ii) Group 2: Diabetic dogs getting 500 ml of cow milk daily for 5 weeks;
(iii) Group 3: Healthy dogs given 500 ml of camel milk daily for 5 weeks.

The effects of treatment with camel milk in diabetic dogs were evaluated by measuring fast blood glucose (FBG), Triglycerides (TG), cholesterol and total proteins, as well as initial and final bodyweight. Day 7 of induction of diabetes was designated as day 1 for milk treatment in diabetic dogs. After stopping the treatment with milk, all parameters variations were followed for two weeks and completed by intravenous glucose tolerance test (IGTT) trial for each animal.

Blood samples collection

Blood samples were drawn two times per week, for seven weeks on each trial, from the radial vein with Vacutainer system; these samples were divided into two tubes: one for glycemic assay (enclose oxalate fluorurere), other for cholesterol, TG and total proteins assays.

Biochemical measurements

Blood glucose concentration was measured by a glucose oxidase method (Biomaghréb®; Tunis, Tunisia) using a spectrophotometer CECIL (CE 2041) at 505 nm. Cholesterol and Triglycerides concentrations were determined by enzymatic methods (Biomaghréb®, Tunis, Tunisia) using spectrophotometer CECIL (CE 2041) at 505 nm. Total proteins concentrations were analyzed by the same spectrophotometer at 546 nm.

Intravenous glucose tolerance test: IGTT

An IGTT assay was performed to verify the glycemic state of the animals. This test was performed on all animals after the end of the first test. Glucose was injected after an overnight fasting by intravenous administration of 0.5 g of glucose/kg of body weight. Blood sample was drawn at each 30 min during 3 h (Siliart and Garnier, 1995).

Milk samples

Camel milk used during this study was obtained from a camel herd (camelus dromedarius) belonging to the Arid Land Institute (Medenine, Tunisia) and cow milk was obtained from a Tunisian breed of cow housed in the Veterinary School of Medicine, Sidi Thabet, Tunisia. The two types of milk were used fresh without any treatment or dilution. Before distribution of raw milk to the animal, the pH and acidity of the milk sample was determined to monitor the freshness of milk. The gross composition of the two types of milk was determined (fat, total proteins and total solids). Fat content was measured using the neusol method, and the total proteins concentration was determined by the Kjeldahl method using a nitrogen conversion factor of 6.36. Total solids were evaluated after drying at 105°C until a steady weight was achieved (Almor, 1993).

Histopathological examination

After death or euthanasia (using barbituric acid), animals were immediately subjected to autopsy and sampling of histopathological specimens as soon as possible. All dogs receiving camel milk survived during and by after the end of the trial, one from this group was sacrificed by blood withdrawal under deep anaesthesia with pentobarbital and subjected to autopsy and histopathology. In addition, a healthy dog and an alloxan-induced diabetic dog were sacrificed to compare with diabetic dogs treated with camel and cow milk.

Histopathological examinations of major organs and tissues were conducted according to routine procedures. In addition, immuno-histochemical staining to demonstrate the presence of insulin – secreting β-cells using an anti-insulin antibody (Anti-insulin antibodies, from DakoCytomation) was performed. An anti-synaptophysine antibody was also used to mark all endocrines cells such as pancreatic ß-cells.

Statistical analysis

The data were expressed as the mean ± SEM and represent the average values for the animals in the same group. Each analysis was repeated three times and the average was used to compare between treatments. These data were subjected to statistical analysis using SAS computer software (SAS institute, 1998) and the data were compared among and within the experimental groups.
Table 1. Effect of camel milk on body weight in alloxan-induced diabetic dogs.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before drinking milk</th>
<th>After the end of the trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.5 ± 1</td>
<td>17.5 ± 1</td>
</tr>
<tr>
<td>2</td>
<td>14 ± 1.5</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>13.8 ± 2</td>
<td>16.5 ± 1</td>
</tr>
</tbody>
</table>

Group 1: Diabetic dogs receiving 500 ml camel milk / dog/ day, Group 2: Diabetic dogs getting 500 ml cow milk / dog/ day, Group 3: Healthy dogs receiving 500 ml camel milk / dog/ day.

Figure 1. Weekly variations of blood glucose levels in groups 1, 2 and 3 during the first trial. Group 1: Diabetic dogs treated with camel milk, Group 2: Diabetic dogs receiving cow milk, Group 3: Healthy dogs getting camel milk, Day 0: first day of the treatment with milk.

using ANOVA test (the level of significance was p=0.05).

RESULTS

Gross chemical composition of milk

The pH and acidity of the camel milk provided to the animals were respectively 6.41 ± 0.18 and 16.87 ± 1.035°Dornic. The characteristics for the cow milk were as follows: 6.61 ± 0.24 for pH and 17.12 ± 0.64°Dornic.

The camel milk used during this study was rich in total protein (34.15 ± 3.11 g/L) and in total solids (119.43 ± 1.84 g/L) compared with bovine milk (30.5 ± 1.95 g/L for total proteins and 104.88 ± 4.39 g/L for total solid amounts). There was no significant difference in fat among the camel and cow milk used (34.5 ± 3.1g/L in camel milk and 32.5 ± 2.12 g/L in bovine milk).

Effect of camel milk on the body weight of alloxan-induced diabetic dogs

There was a significant decrease in bodyweight of animals in diabetic groups getting cow milk compared with those treated with camel milk and with the healthy animals. The dogs treated with cow milk lose two kilos on average during the experiment (from 13.8 ± 1.3 to 11± 1 kg), in this time the animals from group 1 (getting camel milk) showed an increase of theirs bodyweight (1-2 kilos in average) (Table 1).

Fasting blood glucose levels

Figure 1 illustrates the weekly variations of blood glucose levels in normal and diabetic groups treated with camel and cow milk. The diabetic dogs given cow milk showed a significant increase in blood glucose and a stability of this state during the experiment. The treatment with camel milk of diabetic dogs significantly restored blood glucose levels; this significant decrease was observed since week 3 of treatment with camel milk. By the end of the experiments, dogs treated with camel milk showed normal blood glucose levels (values including the normal range).

Blood lipids and proteins levels

Cholesterol, TG and total proteins concentrations in the various experimental groups are given in Tables 2 and 3.
Diabetic dogs treated with cow milk showed a steady high blood glucose level (≈ 10 mmol/L), an increase in cholesterol (from 5.99 ± 0.58 to 7.08 ± 0.98 mmol/L) and total proteins concentrations (from 80.36 ± 0.9 to 87.37 ± 1.21 g/L) (Table 1). TG levels were not influenced by the diabetic state.

The administration of camel milk lowered cholesterol (from 6.17 ± 0.5 mmol/L to 4.35 ± 0.61 mmol/L; p<0.05) and total proteins levels (from 78.16 ± 2.61 g/L to 63.93 ± 2.61 g/L) comparable with diabetic dogs treated with cow milk; a significant decline in weekly variations of these parameters was shown since the third week of treatment with camel milk (Tables 2 and 3).

### Intravenous glucose tolerance test

The results of this test are represented in Figure 2; the difference was mostly observed among the three treatments, especially among the dogs treated with raw cow milk and the two other groups. The diabetic dogs which were treated with cow milk (group 2) showed a steady diabetic state that was confirmed before injection of glucose by a high blood glucose levels (8.61 - 0.22 mmol/L) and a stability of high blood glucose levels during this test (7.78 - 0.55 mmol/L). However, in group 1 - treated with camel milk - blood glucose levels illustrated a large decrease after 60 min (from 9.07 - 0.53 mmol/L (at 10 min) to 6.49-0.9 mmol/L) and a steadiness of this parameter within the normal range (5.94 - 0.94 mmol/L) (Figure 2); variations of blood glucose levels during the IGTG test revealed a non significant difference(p > 0.05) between the animals drinking camel milk (Group 1) and the healthy group (Group 3).

### Histopathological changes

In the present study, an abundant amount of healthy exocrine pancreas, characterized by dense sheets of well-organized acinar cells (Figure 3a), was observed in normal dogs. Atrophy of the exocrine pancreas was noted in the diabetic group. The number of pancreatic β-cells was particularly reduced: almost absent and atrophy of the exocrine pancreas was noted in the diabetic group (Figure 3b and d). The group of animal treated with camel

---

**Table 2. Effect of camel milk treatment on serum lipids on alloxan-induced diabetic dogs.**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Cholesterol (mmol/l)</th>
<th>TG (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>Day 0</td>
<td>6.17 ± 0.5</td>
<td>5.99 ± 0.58</td>
</tr>
<tr>
<td>Week 1</td>
<td>5.93 ± 0.15</td>
<td>6.64 ± 1.31</td>
</tr>
<tr>
<td>Week 2</td>
<td>4.79 ± 0.5</td>
<td>6.81 ± 0.21</td>
</tr>
<tr>
<td>Week 3</td>
<td>4.92 ± 0.36</td>
<td>6.78 ± 1.95</td>
</tr>
<tr>
<td>Week 4</td>
<td>4.4 ± 0.62</td>
<td>6.83 ± 0.58</td>
</tr>
<tr>
<td>Week 5</td>
<td>4.35 ± 0.61</td>
<td>7.08 ± 0.98</td>
</tr>
<tr>
<td>Week 6</td>
<td>5.06 ± 0.64</td>
<td>6.71 ± 1.23</td>
</tr>
<tr>
<td>Week 7</td>
<td>4.58 ± 0.71</td>
<td>6.83 ± 0.42</td>
</tr>
</tbody>
</table>

For each analyzed parameter: Means with the same letter in each line and column are not significantly different: p>0.05. Group 1: Diabetic dogs receiving 500 ml camel milk / dog/ day, Group 2: Diabetic dogs getting 500 ml cow milk / dog/ day, Group 3: Healthy dogs.

---

**Table 3. Effect of camel milk treatment on total proteins concentrations on alloxan-induced diabetic dogs.**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>78.16 ± 2.11</td>
<td>80.36 ± 0.9</td>
<td>68.48 ± 1.11</td>
</tr>
<tr>
<td>Week 1</td>
<td>74.35 ± 7.25</td>
<td>77.35 ± 5.5</td>
<td>68.8 ± 1.25</td>
</tr>
<tr>
<td>Week 2</td>
<td>69.06 ± 5.91</td>
<td>82.87 ± 4.13</td>
<td>67.06 ± 2.27</td>
</tr>
<tr>
<td>Week 3</td>
<td>63.63 ± 4.43</td>
<td>85.79 ± 9.47</td>
<td>65.75 ± 2.27</td>
</tr>
<tr>
<td>Week 4</td>
<td>61.58 ± 3.16</td>
<td>83.28 ± 1.77</td>
<td>64.82 ± 4.11</td>
</tr>
<tr>
<td>Week 5</td>
<td>63.93 ± 2.61</td>
<td>87.36 ± 1.21</td>
<td>65.45 ± 1.03</td>
</tr>
<tr>
<td>Week 6</td>
<td>62.28 ± 3.49</td>
<td>81.92 ± 2.35</td>
<td>64.63 ± 3.2</td>
</tr>
<tr>
<td>Week 7</td>
<td>63.35 ± 3.67</td>
<td>83.25 ± 3.14</td>
<td>65.02 ± 1.04</td>
</tr>
</tbody>
</table>

Data are the mean ± SD. a, b: Values with the same letter in each line and column are not significantly different: p>0.05. Group 1: Diabetic dogs receiving 500 ml camel milk / dog/ day, Group 2: Diabetic dogs getting 500 ml cow milk / dog/ day, Group 3: Healthy dogs receiving 500 ml camel milk / dog/ day.
Figure 2. Blood glucose variation during the IGTT test in group 1, 2 and after the end of the first test. Group 1: Diabetic dogs treated with camel milk, Group 2: Diabetic dogs receiving cow milk, Group 3: Healthy dogs getting camel milk. Day 0: first day of the treatment with milk.

Figure 3. Pancreatic histological findings of milk treatment on diabetes induced by alloxan (ALX; 65 mg/kg); (H-E stain; original magnification × 200). (a) Normal group (haematoxylin and eosin (H-E) stain; original magnification×200); (b) Alloxan alone (H-E stain; original magnification ×200); (c) Alloxan + 500 ml of camel milk during five weeks (H-E stain; original magnification ×200); (d) Alloxan + 500 ml of cow milk during five weeks (H-E stain; original magnification × 200).

milk was found to have moderate amount of healthy exocrine pancreas and induce the pancreatic β-cells regeneration (Figure 3c). The present study demonstrates that, in diabetic dog, there is atrophy of
pancreatic exocrine tissue and islets in many areas in addition to the classic findings of ductal dilatation and intraductular calculi.

**DISCUSSION**

Diabetes in dogs is generally associated, in addition to high blood glucose levels, with an increase of total protein concentrations (Toulon, 1986) which was illustrated in this study; diabetes was clearly induced in all dogs one week after injection of alloxan, this is caused by the alloxan toxicity on kidney and liver as well as to the pancreas as investigated by our immunohistopathological finding and other reported study on alloxan induced-diabetes in dogs (Kim et al., 2006).

The diabetic dogs treated with camel milk showed—after three weeks- a significant decrease of blood glucose levels. This improvement in glycemic control was shown during the experiment and 2 weeks after stopping drinking camel milk. This stability was well illustrated by an IGTT test (Figure 2). Some previous study reported that this improvement in blood glucose level caused by camel milk treatment may be due to the high level of insulin (52 µU / ml) in camel milk in comparison with cow milk (Agrawal et al., 2003; Farah, 1993; Shehadeh et al., 2001). In this context also, Beg et al. (1986) reported that camel milk whey protein is rich in half-cystine which has superficial similarities with the insulin family of peptides.

This effect may be explained by the particularity and properties of camel milk in comparison with milk from other species, such as the high amount of polyunsaturated fatty acids (C18:1-C18: 3), and the high amount of vitamin B3 (Farah, 1993; Shehadeh et al., 2001) and also some particularities of camel immunoglobulin, such as their small size and weight which offers enormous potential to camel milk. Also camel milk immunoglobulins, of relatively small size and weight, might offer interplay with host cell protein leading to an induction of regulatory cells and finally leading to a downward regulation of immune system and β-cell salvage (Hamers-Casterman et al., 1993; Rajendra et al., 2007).

Alloxan induces diabetes by damaging the insulin-secreting cells of the pancreas, leading to hyperglycaemia. In the present study, we have found that administration of camel milk to diabetic dogs reverses their elevated blood glucose levels. A possible mechanism by which camel milk brings about its hypoglycaemic action in diabetic dogs may be by potentiating the insulin effect by increasing either the pancreatic secretion of insulin from β-cells of the islets of Langerhans or the release of insulin from its bound form (Stanely Mainzen et al., 1998).

The obvious increase cholesterol observed in diabetic dogs untreated with camel milk is mainly due to the increase in the mobilization of free fatty acids (FFA) from peripheral depots because insulin inhibits the hormone sensitive lipase. Therefore, the marked hyperlipidemia that characterizes the diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Fengjie and Wutong, 2005).

A decrease of bodyweight has been demonstrated in alloxan diabetic dogs. When camel milk was administered to diabetic animals, the weight loss was reversed. The ability of camel milk to protect against bodyweight loss seems to be the result of its ability to reduce hyperglycaemia. Alloxan produces oxygen radicals in the body, which cause pancreatic injury (Jhon, 1991) and could be responsible for the increased blood sugar seen in animals. Thus, the significant antidiabetic effect of camel milk in the present study may be attributed to the clearing away of free radicals, inhibition of lipid peroxidation and a correction of the metabolic disorders of lipids and proteins, as well as protection of β-cells of the pancreatic islets to release insulin. These findings can be further corroborated with histopathological studies. Histopathological examinations in the present study reveal that the pancreatic islet cells are almost normal in dogs treated with camel milk in contrast with the group that received cow milk.

In conclusion, camel milk possesses anti-diabetic effect on alloxan diabetes. However, further pharmacological and histological investigations are necessary to identify this effect, as well as to confirm its mechanism of action and its antidiabetic potential.

**ACKNOWLEDGEMENT**

Authors are grateful to Dr. Barhoumi kamel (ENMV, Sidi Thabet) for his serious help when inducing diabetes to the animals and Dr. Rejeb Ahmed (ENMV, Sidi Thabet) for his help during the histopathological test.

**REFERENCES**


