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Antidiabetic Activity of Pasteurized Camel Milk; Effect on Pancreatic B-cells

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Abstract

This research was conducted to evaluate the antidiabetic effect of pasteurized camel milk in alloxan diabetic dogs. Diabete was induced by intravenous injection of alloxan (65 mg/kg bodyweight). The effects of camel milk on diabetic dogs were investigated by observing changes in the glycometabolic index (fasting blood glucose, IGTT test), the lipometabolic index (triglycerides, cholesterol), and total proteins and in the degree of injury of β-cells in the pancreatic islets. The group treated with raw camel milk (RCM) produced a significant decrease in fasting plasma glucose (from 9.77±0.19 to 5.4±0.13 mmol/L), cholesterol (from 6.94±0.06 to 5.04±0.8 mmol/L) and total proteins (from 75.31±4.68 to 67.06±3.32 g/L) in blood sample and an improvement on the animal clinical status (normal activity). The same result was showed in animals getting pasteurized camel milk (PCM): fasting blood glucose (from 9.83±0.1 to 5.46±0.22 mmol/L); cholesterol (from 7.25±1.11 to 5.18±0.97 mmol/L) total proteins (from 74.84±5.45 to 67.06±3.32 g/L). Treatment with raw or pasteurized camel milk also induces the renewal of pancreatic b-cells. The curative effect of camel milk on diabetic dogs was approved in this study. This effect was observed using raw or pasteurized camel milk as treatment. Pasteurized camel milk can be used as an alternative for better preservation of nutritional and therapeutic quality of this product.

Keywords

Antidiabetic Effect, Diabetes, Pasteurized Camel Milk, Raw Camel Milk

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1. Introduction

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Diabetes mellitus (DM) is a disease characterized by a high level of blood sugar (i.e. glucose) that results from the failure of the body to produce sufficient insulin (type 1 diabetes) or from the inability to respond properly to the insulin that has been produced by the pancreas (type 2 diabetes) [6].

This metabolic disorder can be caused chemically; alloxan and streptozotocin (STZ) are considered the most potent diabetogenic chemicals used in diabetes research so far [10].

Alloxan has been used to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic beta-islets. Alloxan induces a multiphasic blood glucose response when injected into to an experimental animal, which is accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultrastructural beta cell changes ultimately leading to necrotic

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cell death [4].

Several species were sensitive to alloxan toxicity such as rats, rabbit and dogs [15].

In recent years, besides drugs classically used for the treatment of diabetes (insulin, sulphonylureas and biguanides), there has been renewed interest in natural products (animal or plant) for the treatment against different diseases such as diabetes.

Camel milk was proposed as an alternative treatment for a number of medical problems and to have potential benefits in many diseases. The most important observations remain its anti-diabetic activity observed in type 1 diabetes using both human and animal models [7].

Based to previous research showing the antidiabetic effect of raw camel milk in comparison to cow milk [2, 10]; the present study was conducted to study the effect of pasteurized camel milk (as alternative to store camel milk for several days) treatment on alloxan - induced diabetic dogs by following the variations of the blood glucose levels, and serum chemistry profiles as well as the histological aspect of pancreatic cells.

2. Material and Methods

2.1. Animals and Diet

Twelve clinically normal adult mixed-breed dogs were prepared for this experiment. Their body weight ranged from 12 to 16 kg initially. These dogs were housed individually in the Tunisian Veterinary Medicine School, Sidi Thabet. Animals were fed once daily with 350-400 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- 400 g of beef.

This food was given to all dogs daily in the morning after drinking milk. All animal 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.

2.2. Induction of Experimental Diabetes

All dogs were fasted for 24 h prior to the induction of diabetes mellitus. 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. After one week, dogs showed hyperglycaemia (≥10 mmol/L) and were treated with raw or pasteurized camel milk.

2.3. Experimental Design

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

(i) Group 1: Diabetic dogs treated with 250 mL of raw camel milk daily for 5 weeks;

(ii) Group 2: Diabetic dogs getting 250 mL of pasteurized camel milk daily for 5 weeks;

(iii) Group 3: healthy dogs given 250 mL of raw camel milk.

The quantity of camel milk used (250 mL) was deduced from our previous research [10].

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 were analyzed for two weeks to follow variations by the end of distribution of camel milk.

2.4. 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 fluorure), other for cholesterol, Triglycerides (TG) and total proteins assays.

2.5. Biochemical Analysis

Blood glucose concentration was measured by a glucose oxidase method (Biomaghreb®, Tunis, Tunisia) using a spectrophotometer at 505 nm.

Cholesterol and Triglycerides concentrations were determined by enzymatic methods (Biomaghreb®, Tunis, Tunisia) using spectrophotometer CECIL (CE 2041) at 505 nm. Total proteins concentrations were analyzed by the same spectrophotometer at 546 nm.

2.6. Milk Samples

Camel milk used during this study was obtained from a camel herd (*Camelus dromedarius*) belonging the Arid Land Institute (Medenine, Tunisia).

Raw camel milk was used fresh without any treatment or dilution and Pasteurized milk was obtained after treatment at 63°C during 30 min.

Before distribution of milk to animals, the pH and acidity of the milk sample was checked to monitor the freshness of milk. The gross composition of milk was determined (fat, total proteins and total solids). Fat content was measured using acidobutyrometric 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 [1].

2.7. Post Mortem Examination

After death or euthanasia, 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 and subjected to autopsy and histopathology. In addition to the three groups getting milk, one healthy dog and one diabetic dog without any treatment were sacrificed to compare with diabetic dogs treated with milk.

2.8. Immunohistochemical Test

In addition to histopathological examinations of major organs and tissues, immunohistochemical staining to demonstrate the presence of insulin – secreting β-cells using an anti-insulin antibody (Anti-insulin antibodies, from Dako Cytomation) was performed in the pancreas. An Anti-Synaptophysine antibody was also used to mark all endocrines cells such as pancreatic α –cells.

2.9. 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 for comparison between treatments. These data were subjected to statistical analysis using SAS computer software (SAS institute, 1998) by using ANOVA on repeated measures and the data were compared among and within the experimental groups.

3. Results of the Research

3.1. Milk Composition

The pH and acidity of raw camel milk provided to the animals were respectively 6.41±0.18 and 16.87±1.035 °Dornic. These characteristics were not affected after pasteurization of camel milk (6.61±0.24 for pH and 17.12±0.64 °Dornic).

The gross composition of raw and pasteurized camel milk didn't show any significant difference between raw and pasteurized camel milk gross composition (table 1).

Table 1. Gross composition of raw and pasteurized camel milk.

chemical composition (g/L)	Raw camel milk	Pasteurized camel milk
Fat	37.5±5	36.4±4.11
Dry matter	119.438±15.34	116.532±10.25
Ash	7.5±1.75	7.9±1.69
Proteins	34.15±3.11	33.25±3.44
Lactose	42.78±2.36	41.61±3.36

3.2. Effect of Camel Milk Treatment on Fasting Blood Glucose, Lipids and Proteins Levels

3.2.1. Fasting Blood Glucose Levels

The treatment of diabetic dogs with raw camel milk significantly restored blood glucose levels (from 9.77±0.19 to 5.4±0.13mmol/L); this significant decrease was observed since week 3 of treatment with camel milk (Figure 1).

The group of animals getting pasteurized camel milk showed a significant decrease in blood glucose levels (from 9.83±0.1 to 5.46±0.22 mmol/L); this decline was shown since the third week of distribution of milk.

Blood glucose variations didn't show any significant difference between animals treated with raw or pasteurized camel milk.

The investigation - in this study- was that by the end of the experiment (weeks 6 and 7), dogs treated with raw or pasteurized camel milk showed normal blood glucose levels (5.29±0.09mmol/L and 5.41±0.04mmol/L respectively) (Figure 1).

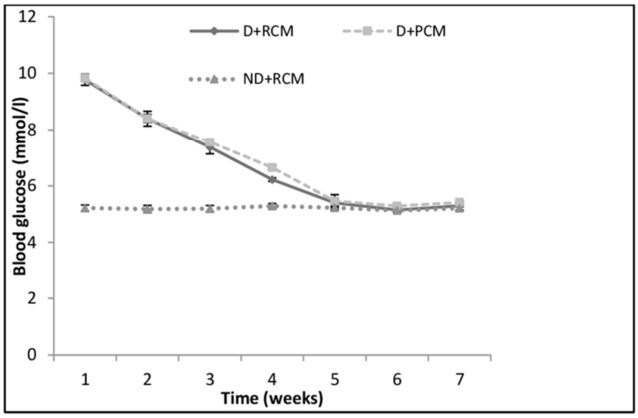

Figure 1. Effect of raw and pasteurized camel milk on Blood Glucose levels.

D+RCM: Diabetic dogs treated with 250mL raw camel milk/day/animal,
D+PCM: Diabetic dogs receiving 250mL pasteurized camel milk./day/animal,
ND+RCM: Healthy dogs getting 250mL raw camel milk/Day/animal.

3.2.2. Blood Lipids and Proteins Levels

Triglycerides (TG) levels were not influenced by the diabetic state (table 2).

The administration of raw camel milk lowered cholesterol (from 6.94±0.06 à 5.04±0.8mmol/L; $p<0.05$) (table 2) and total proteins levels (from 75.31±4.68 à 67.06±3.32 g/L) (table 3).

The same effect was shown when animals were treated with pasteurized camel milk (from 6.17±0.5mmol/L to 4.35±0.61mmol/L for cholesterol levels and from 78.16±2.61g/L to 63.93±2.61g/L for total proteins levels during the experiment).

The significant decline in weekly variations of these

parameters was shown since the third week of treatment with camel milk (tables 2 and 3).

Table 2. Effects of raw and pasteurized camel milk treatment on cholesterol and TG levels.

	Cholesterol (mmol/L)			TG (mmol/L)		
	D+RCM	D+PCM	ND+RCM	D+RCM	D+PCM	ND+RCM
Day 0	$75.31^{a}\pm4.68$	$74.84^{a}\pm5.45$	$65.34^{b}\pm0.86$	$1.11^{a}\pm0.15$	$0.94^{a}\pm0.22$	$1.13^{a}\pm0.22$
W1	$71.26^{a}\pm2.46$	$71.37^{a}\pm4.66$	$64.29^{b}\pm1.29$	$0.93^{a}\pm0.15$	$0.94^{a}\pm0.17$	$0.99^{a}\pm0.13$
W2	$71.21^{a}\pm3.39$	$72.10^{a}\pm4.4$	$65.6^{b}\pm1.28$	$0.65^{a}\pm0.01$	$0.73^{a}\pm0.23$	$0.66^{a}\pm0.16$
W3	$70.71^{a}\pm2.8$	$71.87^{a}\pm2.15$	$66.33^{b}\pm2.36$	$0.96^{a}\pm0.31$	$0.74^{a}\pm0.11$	$0.96^{a}\pm0.31$
W4	$67.08^{b}\pm0.15$	$67.06^{b}\pm3.32$	$63.55^{b}\pm4.55$	$0.92^{a}\pm0.28$	$0.83^{a}\pm0.24$	$0.92^{a}\pm0.28$
W5	$65.44^{b}\pm0.76$	$66.03^{b}\pm3.36$	$64.77^{b}\pm3.96$	$1.04^{a}\pm0.68$	$0.96^{a}\pm0.33$	$1^{a}\pm0.56$
W6	$64.19^{b}\pm1.79$	$64.95^{b}\pm4.43$	$63.32^{b}\pm2.66$	$0.8^{a}\pm0.11$	$0.91^{a}\pm0.08$	$0.88^{a}\pm0.16$
W7	$65.2^{b}\pm1.58$	$62.77^{b}\pm3.96$	$64.12^{b}\pm1.66$	$1.04^{a}\pm0.08$	$0.93^{a}\pm0.04$	$1.01^{a}\pm0.08$

Mean ± standard deviation. Values sharing same letters differ non-significantly ($p>0.05$).
W1 to W5: week 1 to week 5: during the treatment with camel milk; W6 and W7: week 6 and Week 7: After stopping distribution of camel milk.
D+RCM: Diabetic dogs treated with 250 mL raw camel milk/day/animal.
D+PCM: Diabetic dogs receiving 250 mL pasteurized camel milk./day/animal.
ND+RCM: Healthy dogs getting 250 mL raw camel milk/Day/animal.

Table 3. Effect of camel milk treatment on total proteins concentrations.

	Total Proteins (g/L)		
	D+RCM	D+PCM	ND+RCM
Day 0	$6.94^{a}\pm0.06$	$7.25^{a}\pm1.11$	$4.86^{b}\pm1.23$
W1	$6.96^{a}\pm0.43$	$7.52^{a}\pm0.51$	$5.12^{b}\pm1.03$
W2	$6.41^{b}\pm0.51$	$6.62^{b}\pm1.14$	$5.25^{b}\pm1.33$
W3	$5.42^{b}\pm0.48$	$4.78^{b}\pm1.6$	$5.32^{b}\pm1.44$
W4	$5.11^{b}\pm0.72$	$5.4^{b}\pm0.24$	$5.2^{b}\pm0.85$
W5	$5.04^{b}\pm0.8$	$5.18^{b}\pm0.97$	$4.91^{b}\pm1.02$
W6	$4.66^{b}\pm1.17$	$5.57^{b}\pm0.66$	$5.16^{b}\pm0.96$
W7	$4.86^{b}\pm0.79$	$5.51^{b}\pm0.19$	$5.42^{b}\pm1.05$

Mean ± standard deviation. Values sharing same letters differ non-significantly ($p>0.05$).
W1 to W5: week 1 to week 5: during the treatment with camel milk; W6 and W7: week 6 and Week 7: After stopping distribution of camel milk.
D+RCM: Diabetic dogs treated with 250 mL raw camel milk/day/animal.
D+PCM: Diabetic dogs receiving 250 mL pasteurized camel milk./day/animal.
ND+RCM: Healthy dogs getting 250 mL raw camel milk/Day/animal.

3.2.3. Histopathological Changes

An abundant amount of healthy exocrine pancreas, characterized by dense sheets of well-organized acinar cells (Figure 2b), was observed in normal dogs. Atrophy of the exocrine pancreas was noted in the diabetic group. The number of pancreatic b-cells was particularly reduced: almost absent and atrophy of the exocrine pancreas was shown in the diabetic group (Figure 2a). The group of animal treated with raw camel milk was found to have moderate amount of healthy exocrine pancreas and induce the pancreatic b-cells regeneration (Figure 2c). Similar result was shown in exocrine pancreas from diabetic dogs treated with pasteurized camel milk (Figure 2d). The present study demonstrates that, in diabetic dog, there is atrophy of pancreatic exocrine tissue and islets in many areas. But treatments with raw or pasteurized camel milk induce beta- cell regeneration.

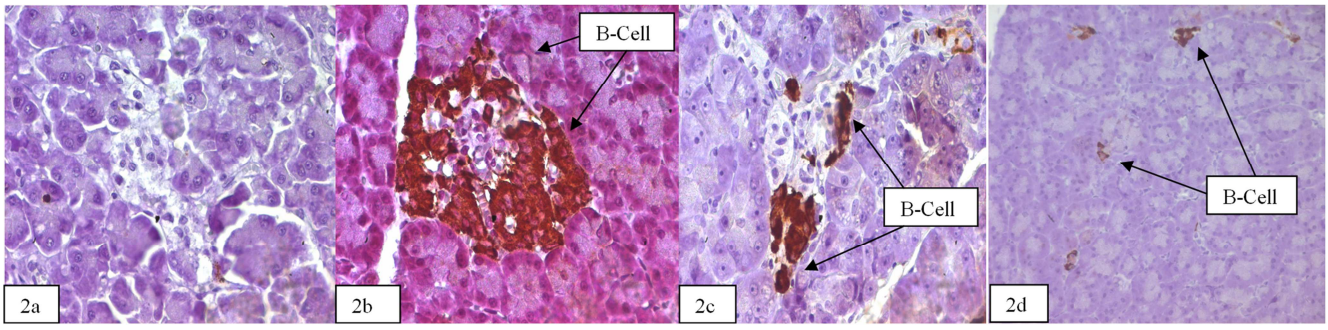

Figure 2. Pancreatic histological findings of raw and pasteurized camel milk treatment on diabetes induced by alloxan (ALX; 65 mg/kg); (H-E stain; original maginification × 200). (a) Alloxan alone (H-E stain; original maginification ×200); (b) healthy dogs treated with raw camel milk (haematoxylin and eosin (H-E) stain; original maginification×200); (c) Alloxan+ 250 ml of raw camel milk during five weeks (H-E stain; original maginification ×200); (d) Alloxan + 250 ml of pasteurized camel milk during five weeks (H-E stain; original maginification × 200).

4. Discussion

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 [7, 8].

The diabetic dogs treated with raw or pasteurized 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. 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 micro unit/mL) in camel milk in comparison with cow milk [2, 13].

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 ($C_{18:1}$-$C_{18:3}$), and the high amount of vitamin B3 [9, 13] and also some particularities of camel immunoglobulin, such as their small size and weight which offers enormous potential to camel milk.

Alloxan induces diabetes by damaging the insulin-secreting cells of the pancreas, leading to hyperglycaemia. In the present study, it was 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 b-cells of the islets of Langerhans or the release of insulin from its bound form [14].

Thus, the significant antidiabetic effect of raw and pasteurized camel milk in the present study may be attributed to a renewal of b-cells of the pancreatic islets to release insulin as well as a correction of the metabolic disorders of lipids and proteins. These findings can be further corroborated with histopathological studies. Histopathological examinations reveal that the pancreatic islet cells are almost normal in dogs treated with pasteurized or raw camel milk in comparison with diabetic animal which approve the stability of analyzed biochemical parameters (blood glucose, cholesterol, TG, total proteins) after stopping camel distribution (weeks 6 and 7).

5. Conclusion

Pasteurized camel milk can be a very interested alternative to preserve nutritional and therapeutic qualities of camel milk.

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.

6. Practical Applications

Antidiabetic effect of raw camel milk was confirmed in our previous studies, but in this form (raw) camel milk couldn't be preserved for long time.

This research aimed to test the antidiabetic effect of pasteurized camel milk as alternative to preserve the nutritional quality of raw milk having in view to provide people distant of the areas of production of this milk.

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