

MODULATING EFFECT OF CAMEL'S MILK ON ALLOXAN-INDUCED EXPERIMENTAL DIABETES IN LABORATORY ANIMAL MODEL

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ABSTRACT

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The present study was conducted to evaluate the hypoglycemic effect of camel's milk in alloxan-induced diabetic animal model compared to control and cow's milk receiving groups. This study was carried out on thirty five female white albino rats and divided into 2 main groups: optimizing and experimental group. Optimizing group (n = 20) was used to determine the optimal dose of alloxan to induce diabetes in a pattern of dose response relationship. This group was divided into four subgroups, sG1 to sG4, (n = 5 each). sG1, sG2, sG3, and sG4 were injected intraperitoneally with alloxan monohydrate at a dose 100, 140, 180 and 200 mg/kg B.W., respectively. Experimental group (n = 15) was divided into three subgroups (n = 5 each) as following: diabetic rats receiving no treatment and acts as a positive control (sG1), diabetic rats receiving camel's milk (sG2) and diabetic rats receiving cow's milk (sG3). Their blood glucose levels (BGLs) were estimated at weekly interval for 5 consecutive weeks. A baseline BGL was determined before conducting the experiment and it was 79.85 ± 4.2 mg/dl. The results showed that 140 mg/kg B.W. of alloxan was the optimal dose to induce stable diabetes in the optimizing group. In the experimental group, the initial mean BGLs for sG1, sG2, and sG3 were 399.5 ± 18.3 , 429.4 ± 66.4 and 404.6 ± 53.6 mg/dl, respectively and after 5th week there were 488.2 ± 20.8 , 308.28 ± 52.2 , 519.8 ± 131.5 mg/dl respectively. Most diabetic animals that received cow's milk exhibits severe signs of diabetes at 4th week of experiment and died by 5th week while animals that received camel's milk showed marked improvement and one of them exhibited normal BGL by the end of experiment. Our findings suggested that administration of camel's milk was effective in improvement of the alloxan-induced diabetic rats and recommended as nutraceuticals supplement in diabetic patient.

Key Words: Diabetes, Alloxan, BGL, Camel's milk, Laboratory animal

INTRODUCTION

Diabetes mellitus is a global major health problem (Macedo *et al.*, 2002), characterized by metabolic disorder and hyperglycemia results from insufficient insulin hormone with or without abnormal resistance to insulin effect (Tirerney *et al.*, 2002). It becomes the third killer of mankind after cancer and cerebrovascular & cardiovascular diseases (Li *et al.*, 2004). It is also one of major endocrine dysfunctions in pet animals (Davison *et al.*, 2005; Catchpole *et al.*, 2008).

In modern medicine, no satisfactory effective therapy is available to cure diabetes mellitus, although it can

be managed by insulin treatment. Pharmaceutical drugs used in diabetic therapy are effective; however they may have undesirable side effects or contraindications. Additionally, they are too expensive and several patients might have needle phobia (Pari and Satheesh, 2004). All these factors force diabetic patients to adopt alternative therapies. Therefore, the research for more effective and safer hypoglycemic agent has continued to be an area of an active research (Lemhadri *et al.*, 2004; Stanely-Prince *et al.*, 2004).

Beside the nutritive value of camel's milk which is extremely well adapted for human requirements, it appears to be an additional factor as medical or natural healing properties. Using camel's milk for

treatment is itself a point of contention. Historically camel's milk has been used for a number of medical problems and according to FAO, camel's milk is the healthiest milk produced by animals (Yagil, 1982). Its consumption may help in reducing the nutritional deficiencies and morbidities in adult communities (Singh *et al.*, 2009). It was found that one of camel's milk proteins has many characteristic similarities with insulin (Beg *et al.*, 1986). Radioimmunoassay tests of camel's milk has revealed high concentration of insulin i.e. 52 micro unit/ml (Agrawal *et al.*, 2003).

Against this background, the present study was carried out to investigate the therapeutic effect of camel's milk on diabetes mellitus in diabetic-induced animal model and compare its effect with the cow's milk.

MATERIALS and METHODS

Animals

Thirty five female white albino rats of approximately same age group (8-9 weeks old) weighing between 120-150 g were included in this study. All rats were obtained from Laboratory Animals House, Faculty of Medicine, Assiut University. Standard operating procedures for handling of animals in compliance with the guidelines and recommendations for the Institutional Animal Care and Use Committee (IACUC) of Assiut University were adopted. Animals were kept on commercial rat chow and water *ad libitum* for one week before initiating the experiment to acclimatize the laboratory condition (temperature 22 °C, relative humidity 50%, 12 h light and 12 h dark) (Agrawal *et al.*, 2005).

Milk samples

Camel's milk was obtained from she-camels (*Camelous dromedaries*) belonging to individuals, while cow's milk was obtained from Veterinary Teaching Hospital, Assiut University-Egypt.

Both types were used without any treatment or dilution. The clot on boiling and acidity of the milk samples were checked to monitor the freshness.

Experimental Design

1. Animal groups

- a) **Optimizing group:** 20 rats were used to determine the diabetic dose of alloxan monohydrate (EL-Gomhorya Co, Egypt) that develops stable diabetes without toxicity. Dose-response relationship was established at 100, 140, 180 and 200 mg/kg B.W. Each dose was administered into 5 rats.
- b) **Experimental group:** 15 rats were used to test the effect of camel's and cow's milk in diabetic rats.
 - 1) Subgroup 1: Five diabetic rats received no milk (positive control).

- 2) Subgroup 2: Five diabetic rats received camel's milk
- 3) Subgroup 3: Five diabetic rats received cow's milk

Milk was given to them daily by oral cannula (20 ml) in additions to free milk *ad libitum*.

2. Experimental induction of diabetes and studying the antidiabetic effect of camel's and cow's milk.

2.1. Determination of the diabetic dose of alloxan monohydrates in dose response relationship

Alloxan monohydrate has selective destructive cytotoxic effect on the cells of pancreas (Bolaffi *et al.*, 1986). To induce stable diabetes, briefly four different doses (100, 140, 180 or 200 mg/kg B.W.) of alloxan monohydrate dissolved in sterile normal saline were freshly prepared and each dose was injected intraperitoneally (Fig. 1) into overnight (16-18 h) fasted rats (n = 5) at 0.5 ml/rat. Animals were kept on glucose 5% for the next 24 h to avoid hypoglycemia that might be occurred (Tatiya *et al.*, 2010).

2.2. Studying the antidiabetic effect of camel's and cow's milk

Fifteen rats were injected with the optimal dose of alloxan (140 mg/kg B.W.). These animals were divided into 3 subgroups (5 rats each): 1st subgroup received no milk (positive control), 2nd group received camel's milk and 3rd one received cow's milk (Fig. 2). All animals were weekly monitored for their BGLs until the end of the study (5th weeks). Succumbed rats before 5 weeks were recorded as unrecovered diabetic rat.

3. Blood samples

Blood samples were collected (0.5 ml/collection) weekly from the retro-orbital plexus of all animals using heparinized micro-capillary glass tubes into a clean dry plain Epindorf's tubes (Fig. 3). Samples were left to clot at room temperature for 30 min and centrifuged for 10 min at 4500 rpm for separation of the sera. Sera were aspirated by a micropipette into stopper Epindorf's tubes and BGL were determined immediately using glucose test kits.

4. Determination of blood glucose level (BGL)

Glucose oxidase method was adopted to determine BGL spectrophotometrically at 505 nm using a commercially available test kit (Biotechnology, Egypt).

5. Statistical analysis: Generated data of BGL from various groups were subjected to statistical analysis using SPSS (1999) program for windows version 10.0.1 (SPSS Inc., Chicago, IL) for calculation of mean and standard error.

RESULTS

Results of the effect of different doses of alloxan monohydrate on BGL were presented in Table 1 and depicted in Fig. 4 & 5 while the results of the effect

of cow's milk on diabetic group was presented in Table 2 and Fig. 6 compared to the modulating effect of camel's milk (Table 2 and Fig. 7 & 8).

Table 1: The Effect of Different Doses of Alloxan Monohydrate on Blood Glucose Level

| Dose (mg/kg) | No. of rats | Mean ± SE of BGL (mg/dl) | Morbidity rate (%)* | Mortality rate (%)** |
|--------------|-------------|--------------------------|---------------------|----------------------|
| 100 | 5 | 83.69 ± 3.2 | 0 | 0 |
| 140 | 5 | 399.48 ± 18.3 | 80 | 0 |
| 180 | 5 | 746.50 ± 21.9 | 100 | 80 |
| 200 | 5 | Out of range | 100 | 100 |

* Morbidity rate is the number of animals showed high BGL/total number of animals

N.B. Rats gave fasting blood glucose level ≥ 250 mg/dl and remained alive after 2 weeks were considered positive stable BGL and included in the presented study.

** Mortality rate is the number of dead animals/total number of animals

Table 2: Weekly Mean ± SE of Blood Glucose Level (mg/dl) in Different Groups

| Time/Group | Diabetic | | |
|------------|-----------------------------|---------------------------------|-------------------------------|
| | Positive (diabetic) control | Diabetic receiving Camel's milk | Diabetic receiving Cow's milk |
| day 1 | 399.48 ± 18.3 | 429.4 ± 57 | 404.6 ± 53.6 |
| day 7 | 420.3 ± 24.3 | 410.1 ± 66.4 | 469.7 ± 46.3 |
| day 14 | 434.6 ± 25.4 | 362.8 ± 60.5 | 527.6 ± 67.7 |
| day 21 | 447.1 ± 25.9 | 340.1 ± 62.4 | 519.8 ± 131.5 |
| day 28 | 488.2 ± 20.8 | 308.28 ± 52.2 | - |

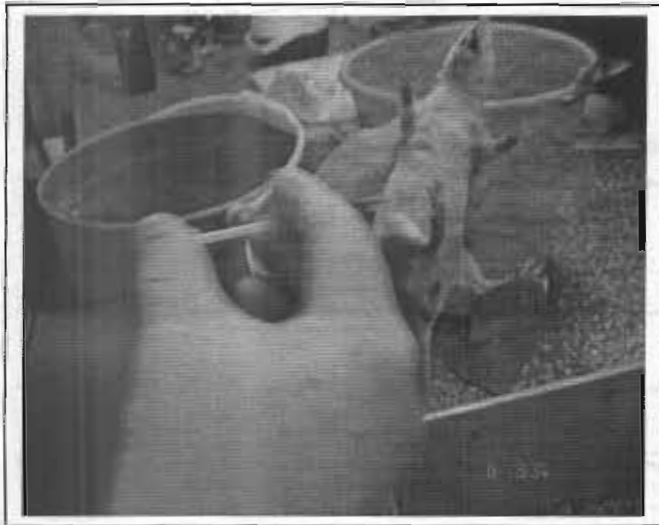


Fig. 1: Intraperitoneal injection of alloxan monohydrate in white albino rat



Fig. 2: Oral feeding of tested milk sample in white albino rat



Fig. 3: Collection of blood sample from the retro-orbital plexus using heparinized microcapillary glass tubes & plain Epindorf's tube in white albino rat



Fig. 4: Female white albino rat of (8- week- old exhibited signs of diabetes, rough coat, polyurea and polydepsia)

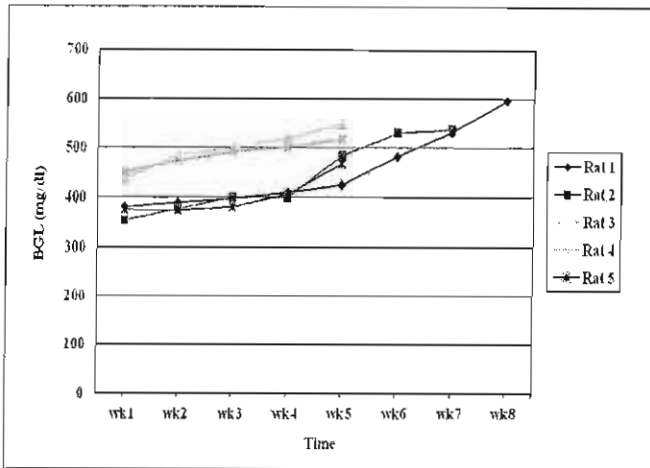


Fig. 5: Time-course evaluation of blood glucose level at 140 mg/dl Alloxan monohydrate in 5 rats (Positive control group)

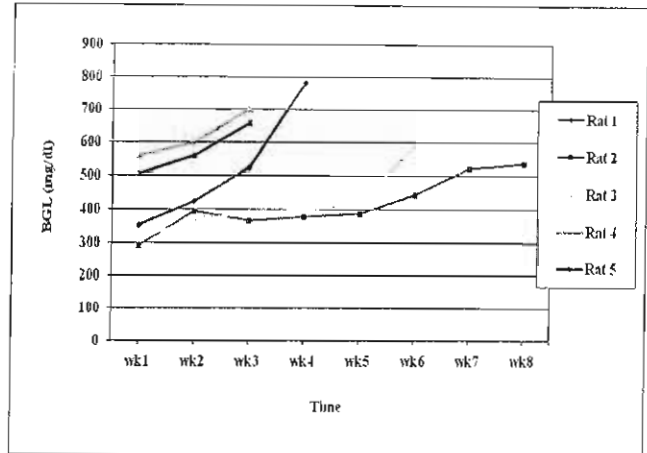


Fig. 6: Time-course evaluation of anti-diabetic effect of cow's milk on 5 rats

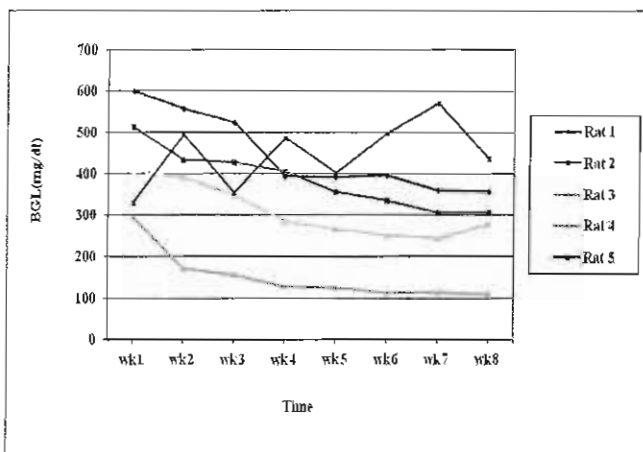


Fig. 7: Time-course evaluation of anti-diabetic effect of camel's milk on 5 rats

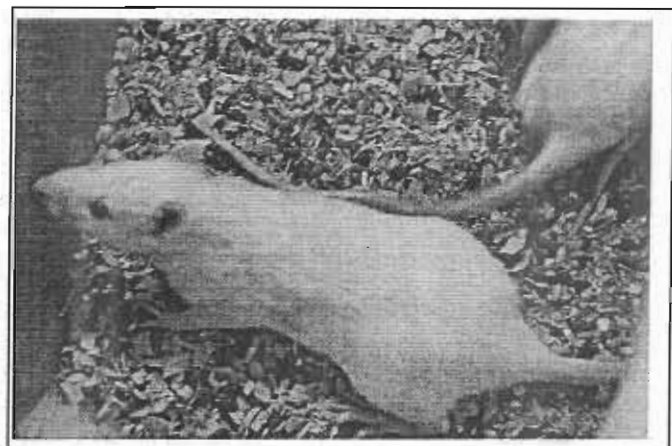


Fig. 8: White albino rat showed sign of recovery from diabetes after 5 weeks of receiving camel's milk

DISCUSSION

Test 1: Optimizing the dose of alloxan monohydrate

Before initiating the experiment, the baseline for BGL was estimated in healthy animals to be 79.58 ± 2 mg/dl. Alloxan monohydrate at dose of 100, 140, 180 and 200 mg/kg B.W. were injected intraperitoneally to the experimental rats. The data presented in Table 1 demonstrated that BGL at 100 mg/kg B.W. of alloxan monohydrate was 90-120

mg/dl and considered as suboptimal to induce diabetes BGL at 100 mg/kg B.W. was lower than what was previously recorded by Sushruta *et al.* (2006) and Dhasarathan and Theriappan (2010). The other 3 doses of alloxan monohydrate showed varying degree of severity of hyperglycemia. In the present study, alloxan monohydrate at dose 200 mg/kg B.W. was lethal and led to death of all rats within 3 days from injection. These animals suffered from severe loss of body weight, polyurea, diabetic coma and death. On contrary, Tripathi and Chandra (2009) found that 140-200 mg/kg B.W. alloxan doses were reported as

non lethal. Our results also revealed that animals injected with alloxan at dose 180 mg /kg B.W. exhibited high fasting BLG with high mortality, while at dose 140 mg/kg B.W. resulted in a diabetic state (80 % morbidity) and no mortality. Alloxan monohydrate at dose 140 mg/kg B.W. was selected as optimal dose to induce stable diabetes in rats.

Test 2: Effect of camel's milk in comparison with cow's milk on alloxan-induced diabetic rats

This study was performed to observe the role of camel's milk in achieving glycemic control in alloxan-induced diabetic rats. The results of this experiment were presented in Table 2. During the experimental period, the initial mean BGL in diabetic control group was 399.5 ± 18.3 mg/dl and increased by the 5th week to 488.2 ± 20.8 mg/dl. In camel's milk receiving group, diabetic rats had marked reduction in weekly BGL and one rat showed complete improvement after 8 weeks of treatment (BGL). The mean BGL came down from 429.24 ± 57 to 308.28 ± 52.2 mg/dl by the end of 5th week. On contrary, diabetic rats received cow's milk, showed increase in their mean BGL and 2 of them developed severe diabetes and succumbed. The mean BGL was increased from 404.6 ± 53.6 to 519.8 ± 131.5 mg/dl. The effect of cow's milk in our results is similar to the result recorded by Agrawal *et al.* (2005). This improvement may be due to presence of high concentration of insulin-like protein in camel's milk (Beg *et al.*, 1986 and Yagil *et al.*, 1994), According to Wangoh (1993) camel's milk does not form coagulum in acidic environment of the stomach. This lack of coagulum formation allows the camel's milk to pass through the stomach together with the specific insulin-like protein and remain available for absorption in the intestine. Also camel's milk is rich in mineral content and antioxidant (Farah, 1993). High concentrations of antioxidants may also make the insulin receptors to respond better to available insulin (Agrawal *et al.*, 2004). Moreover, El-Said *et al.* (2010) reported that the pancreas of alloxan-induced rabbits receiving camel's milk showed high restoration number of islets of Langerhans among the pancreatic acini. Time-course evaluation of antidiabetic effect of camel's milk on 5 rats is depicted in Fig. 6.

In the present study, we kept continue measuring the BGL after the testing period found that the diabetic group receiving camel's milk has long survival period. All diabetic rats in positive diabetic groups succumbed after the 5th week while in diabetic group treated with cow's milk died before 5th week (2 rats developed severe diabetes and died at the 3rd weeks). The data in Fig. 7 revealed that cow's milk had adverse effect on diabetic rats. This diabetogenicity of cow's milk may be due to low concentration of insulin-like protein in cow's milk (16.32 ± 5.98 micro

unit/ml) (Shehadeh *et al.*, 2001) or it might be due to milk clot in acid environment of the stomach (Breitling, 2002). The retention of milk clots in the stomach helps to accelerate the degradation of insulin. Vaarala *et al.* (1999) reported that bovine insulin acts as an immunogen and the IgG anti-bovine insulin may react with human insulin and damage β cells (Breitling, 2002).

From the result presented in our study, it could be concluded that raw camel's milk reduced BGL in diabetic rats. Thus, the therapeutic efficacy of camel's milk on alloxan-induced diabetic rats may have an importance implication for the clinical management in treatment of diabetes in target population.

Authors' contribution

Authors have formulated the research plan and conducted the study equally. Authors discussed the results, read and approved the final manuscript.

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Competing interests

None of the authors have any conflict of interest to declare.

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التأثير المعادل للبن الجمال علي داء السكري المستحث بمادة الألوكسان في نموذج لحيوان التجارب

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أجريت هذه الدراسة لتقييم التأثير الخافض لحليب الإبل علي سكر الدم في داء السكري المستحث بمادة الألوكسان في نموذج لحيوان التجارب. أجريت هذه الدراسة على 35 من إناث الفئران البيضاء التي قسمت الى مجموعتين رئيسيتين: مجموعة تمهيدية (الاولي) ومجموعة تجريبية (الثانية). تم استخدام المجموعة الاولى (N = 20) لتحديد الجرعة المثلى من مادة الألوكسان للحث على وجود نمط مستقر من داء السكري باستخدام علاقة الاستجابة للجرعة. تم تقسيم هذه المجموعة إلى أربع مجموعات فرعية (N = 5 لكل منهما). تم الحقن البريتوني لجرعة الألوكسان بمعدل 100، 140، 180، 200 ملغ / كغ وزن جسم في كل مجموعة علي حده. علي الناحية الاخرى، تم تقسيم المجموعة الثانية (N = 15) إلى ثلاث مجموعات فرعية (N = 5 لكل منهما) علي النحو التالي: جرذان مصابة بداء السكري لا تتلقي أي معاملة (SG1)، جرذان مصابة بداء السكري تتلقي ألبان الإبل (SG2) وجرذان مصابة بداء السكري تتلقي حليب الأبقار (SG3). قدرت مستويات السكر في الدم (BGLs) على فترات أسبوعية لمدة 5 أسابيع متتالية. تم تحديد مستويات السكر في دم جميع الحيوانات قبل البدء في التجربة، وكانت 79.85 ± 4.2 ملغ % . أظهرت النتائج أن 140 ملغ / كغ وزن جسم من مادة الألوكسان كانت الجرعة المثلى للحث على وجود نمط مستقر من داء السكري في المجموعة الاولى. علي الناحية الاخرى، كانت مستويات السكر في الدم للمجموعات الفرعية SG1، SG2، و SG3 للمجموعة الثانية في بداية التجربة 399.5 ± 18.3 ، 429.4 ± 66.4 ، 404.6 ± 53.6 و ملغ % علي التوالي. بينما كانت مستويات سكر الدم في هذه الحيوانات في الأسبوع الخامس 488.2 ± 20.8 ، 308.28 ± 52.2 ، 131.5 ± 519.8 ملغ % علي التوالي. أظهرت الحيوانات المصابة بداء السكري والتي تلقت حليب الأبقار علامات شديدة من المرض في الأسبوع 4 من التجربة، وتوفي معظمها خلال الأسبوع 5، بينما أظهرت الحيوانات المصابة بداء السكري والتي تلقت ألبان الإبل تحسنا ملحوظا كما اظهر حيوان واحد مستويات طبيعية من السكر في الدم بنهاية التجربة. اقترحت النتائج التي توصلنا إليها الي ان استخدام ألبان الإبل كان فعالا في تحسين داء السكري في الجرذان المستحثة بالألوكسان ويوصى به في كتنغذية علاجية لمرضى السكري.