RESEARCH ARTICLE

Effect of Orally Administered Camel Milk in Alloxan® Induced Albino Rats: Long Term Study on Maternal Uterus and Neonates Selected Organs

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A B S T R A C T

This article highlighted the female reproductive problems associated with chronic diabetes and beneficial effects of camel's milk on the gravid uterus of induced diabetic rats. Effects on the liver and kidney of neonatal rats were also observed. Eighteen female rats were divided into 3 groups: Group A was normal. Diabetes was induced with Alloxan® in group B (diabetic control) and C (treated with camel milk @ 40 ml/kg/day for 98 days). On day 60, one adult male was introduced in each group for mating. Fortnightly blood glucose was monitored. Animals were slaughtered postpartum to collect blood for hematology, maternal uterus and liver and kidneys of their neonates. Paraffin embedding technique was used to prepare tissue sections, followed by H&E staining. Slides were examined to measure: uterine epithelium height, endometrial glands and thickness of myometrium. Significant (P<0.05) reduction was observed in hematological values of Group B in comparison to Group A. These parameters were significantly (P<0.05) improved by camel milk treatment. Blood glucose level of Group B was seen significantly (P<0.05) elevated than that of Group A. Camel milk treatment reduced blood glucose level significantly (P<0.05) at 4th week and so on. Diabetes had showed significantly (P<0.05) detrimental impact on uterine histometrical parameters and camel milk therapy had significantly (P<0.05) reversed these changes towards the normal. Same trend was seen in neonate's liver and kidneys. These findings suggest that camel's milk reduces consequences of diabetes on maternal uterine and neonatal liver and kidney. Moreover, it improves the body weight of neonates delivered by camel milk treated diabetic mothers.

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INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder, discriminated by hyper-glycemia and lipedemia coupled with high incidence of microvascular disorders posing a serious threat to global health in this century (Khan *et al.,* 2013). Hyperglycemia encounters carbohydrate, protein and lipid metabolism which have destructive impact on multiple body systems (Baragob, 2015). In 2014, 0.387 billion people suffered from this deadly disease worldwide and this count will reach up to 0.592 billion in 2035 (WHO, 2015). DM, directly or indirectly, is the 7th main cause of human death worldwide and has been ranked at 3rd position for causing incurable complications (Trividi, 2004).

DM scrambles the reproductive system of males as well as females but female are found more resistant to develop DM as compared to males. The perturbative effect of DM on andrology has been extensively studied and recently, consideration has been inclined towards the females' because of the many serious reproductive complications that are associated with DM. It confounds folliculogenesis and ovulation pattern which results in low fertility (Ramalho-Santos *et al.,* 2008; Codner *et al.,* 2012). In chronic hyper-glycaemic pregnancy, there is high incidence of congenital deformities, intrauterine growth constraints and stillbirths. Uterine decidualization phenomenon has been worsening by DM which contributes in the production of pro-inflammatory uterine

condition through generating free radicals leading to embryopathies. Histologically, DM promotes the thinning of uterine myometrium and endometrium (Favaro *et al.*, 2010a, 2013b; Usman *et al.,* 2018). Declined smooth muscles with vanished oxytocin contractive myometrium response was also reported in DM. Recently, allopathic drugs and plant extracts have been studied to explore their anti-diabetic and anti-hyperlipidemic properties to control DM because they are economical and have high safety margin (Ramesh *et al.*, 2003; Orsolic *et al.,* 2012). Camel milk (CM) has gained much importance in this regard due to its medicinal properties.

CM has been known to have anti-hyperglycemic properties due to the presence of high amount of insulin like proteins (45-128 IU/liter) (Kamal *et al.*, 2007; Al-Kanhal, 2010; Sboui *et al.,* 2012), about 5 times higher vitamin C and high amount of Zinc (530-590 µg/100g) (Rahimi *et al.*, 2011; Mullaicharam, 2014; Ali *et al.*, 2017).

The anti-hyperglycemic efficacy of CM on microanatomy of chronic diabetic pregnant uterus and neonatal hepato and renal genesis has not been reported yet. Therefore, this project was planned to establish a link between the chronic DM, uterine histometry, maternal hematology and neonatal growth, and antidiabetic effect of camel milk on above said parameters.

MATERIALS AND METHODS

Collection of animals: The current study was employed on 21 adult albino rats including 18 females and 3 males. The weight of animals ranged between 150-200 g. Rats were kept in the animal room of the Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan for 14 days for acclimatization. The animals were offered food and water *ad libitum* and 12 hour light/dark cycle.

Experimental design: The female rats were split into 3 groups (n=6) randomly. Group A was kept as control by administering. Diabetes was induced in group B and C by intraperitoneal injection of Alloxan® @ 160 mg/kg bw and saline solution was injected to the rats of group A. Alloxan® damaged islets of Langerhans in the pancreas which led to Type-1 diabetes. Group C served as diabetic control. Fasting blood glucose level was measured using glucometer (On Call® EZ II) three days after the administration of Alloxan® to confirm induction of diabetes. Rats having blood glucose level >250 mg/dL were considered as diabetic. The day of confirmation of induction of diabetes was considered as day 1 of experiment.

Camel milk: Raw camel milk was purchased in sterile bottles on daily basis from the local farmer. Milk bottles were transported to and stored at room temperature in the laboratory before administration to the rats. Camel milk was administered to group B through gastric tube @ 40 ml/kg/day for a period of 98 days starting from day 1 of treatment. Blood (5ul) was collected from the tail of female rats in all groups to measure blood glucose level during different weeks of experiment.

Induction of pregnancy: On 60th day of treatment, one adult male rat was introduced in each group of females for mating. Pregnancy was confirmed through the formation of vaginal plug. The live body weight gain of female rats during pregnancy was recorded on weekly basis. After parturition, neonatal live body weight was measured with digital weighing scale.

Collection of blood and tissue samples: At the end of the trial, 3 ml of blood was taken from the heart of female rats and preserved in anticoagulant coated vacutainer for hematology. Complete blood count reports were obtained by automated cell counter (Medionic®). Female rats were sacrificed to collect their uteri. After washing with normal saline, uteri samples fixed in Bouin's solution. Neonates were also sacrificed to collect their liver and kidneys. Samples of neonates were fixed in 10% neutral buffered formalin solution after washing with normal saline.

Histological examination: Samples of uterus, liver and kidney were processed by paraffin tissue preparation technique, sections were cut at 5 µm thickness and stained with H&E according to the Bancroft *et al.* (2013). Histological examination of prepared slides was done at 100X to measure the uterine epithelium height (µm), endometrial glands area/region and thickness (µm) of endometrium and myometrium using automated image analysis system image J®. Vacuolation in kidney tubules and degenerative changes in hepatocytes of liver taken from neonates were also studied.

Statistical analysis: The data was analyzed statistically through Mnitab® software in which the groups considered as independent and parameters as dependent variables. One-way analysis of variance (ANOVA) was used to compute parameters means followed by the comparison with Tukey's Honestly Significant test. P- value <0.05 was considered statistically significant.

RESULTS

Hematological parameters: Statistical analysis revealed that chronic diabetes during pregnancy caused significant (P<0.05) reduction in hematological parameters (Red blood cell, Hemoglobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration, White Blood cells, Platelet count, Lymphocyte percentage,) as compared to untreated pregnant rats (Group A). Therapy of camel milk in diabetic pregnant rats caused significant (P<0.05) improvement in these diabetogenic hematological parameters as compared to the pregnant diabetic group B as shown in Table 1.

Blood glucose level: The pattern of fasting blood glucose (mg/dl) level measured fortnightly during the whole project is given in the Table 2. A significant (P<0.05) elevation in the glucose level was found in Group B during the whole period of experiment as compared to group A. The down turn in the glucose level by camel milk therapy in group C remained non-significant (P>0.05) in first fortnight readings while significant (P<0.05) in the successive readings during non-pregnant and pregnant physiological state of rats.

Table 1: Mean ± SEM values of hematological parameters in normal (A), diabetic control (B) and camel milk treated group (C) @ 40 ml/kg b.w. at the end of 98 days trial (n=6 per group)

Groups	Mean ± SEM							
	RBCs (10^{12}/l)	WBCs (10^9/l)	Platelet count (10^9/l)	Lymphocyte (%)	Hemoglobin (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dL)
Normal (A)	8.40±0.23ᵃ	17.43±0.19ᵃ	8.79±0.04ᵃ	67.08±0.11ᵇ	16.02±0.01ᵃ	55.60±0.28ᵃ	16.07±0.02ᵃ	29.17±0.16ᵃ
Diabetic (B)	4.72±0.56ᵇ	6.31±1.02ᶜ	4.02±1.68ᵇ	60.86±2.74ᶜ	10.50±1.45ᵇ	51.63±1.42ᵇ	13.00±2.08ᵇ	21.36±3.28ᶜ
Treated (C)	6.09±0.46ᶜ	12.03±2.57ᵇ	7.79±0.70ᵃ	76.51±7.66ᵃ	15.01±1.32ᵃ	56.60±0.80ᵃ	16.16±1.91ᵃ	25.73±1.85ᵇ

Values having different alphabets as superscripts in column are different statistically at P<0.05.

Table 2: Mean ± SEM values of blood glucose level (mg/dl) in normal (A), diabetic (B) and camel milk treated group (C) @ 40 ml/kg b.w on weekly basis of 98 days long trial (n=6 per group)

Groups	Mean ± SEM						
	Week-2	Week-4	Week-6	Week-8	Week-10	Week-12	Week-14
Normal (A)	94.6±20.42ᶜ	123±21.54ᶜ	120.3±1.65ᶜ	131.3±22.72ᶜ	130.21±21.14ᶜ	123.7±12.74ᶜ	129.5±11.83ᶜ
Diabetic (B)	485.75±20.64ᵃ	418.47±7.65ᵃ	464.54±21.8ᵃ	420.04±23.32ᵃ	464.57±24.79ᵃ	477.86±23.43ᵃ	489.04±23.4ᵃ
Treated (C)	474.26±14.98ᵃ	350.48±7.34ᵇ	352.73±16.5ᵇ	284.78±17.55ᵇ	254.20±15.40ᵇ	244.77±16.04ᵇ	236.20±7.24ᵇ

Values having different alphabets as superscripts in column are different statistically at P<0.05.

Table 3: Mean ± (SEM) values of uterine histometry in normal (A), diabetic (B) and camel milk treated (C) @ 40 ml/kg b.w groups of adult rats at the end of 98 days trial (n=6 per group)

Groups	Epithelial height (µm)	Endometrium thickness (µm)	Area of endometrial gland ($µm^2$)	Inner circular smooth muscle (µm)	Outer longitudinal smooth muscle (µm)	Total myometrium (µm)
Normal (A)	8.40±0.23ᵃ	17.43±0.19ᵃ	8.79±0.04ᵃ	67.08±0.11ᵇ	16.02±0.01ᵃ	55.60±0.28ᵃ
Diabetic (B)	4.72±0.56ᶜ	6.31±1.02ᶜ	4.02±1.68ᵇ	60.86±2.74ᶜ	10.50±1.45ᵇ	51.63±1.42ᵇ
Treated (C)	6.09±0.46ᵇ	12.03±2.57ᵇ	7.79±0.70ᵃ	76.51±7.66ᵃ	15.01±1.32ᵃ	56.60±0.80ᵃ

Values having different alphabets as superscripts in column are different statistically at P<0.05.

Histometric findings of gravid uterus: Findings of histometric components of uterus like epithelial height, endometrium thickness, area of endometrial glands and myometrium (inner circular and outer longitudinal smooth muscle thickness) had been found badly affected and significantly (P<0.05) declined by diabetes in group B as compared to group A. Camel milk treatment significantly (P<0.05) shifted these diabetic altered parameters towards the normal in Group C as compared to Group B (Table 3).

Neonatal body weight: The body weight of neonates delivered from the dams of group A and B group were 5.84±0.41 and 4.91±0.71 grams, respectively. Group C which was given camel milk orally had delivered neonates with the body weight of 5.61±0.31 grams. A significant (P<0.05) decline was recorded in the neonates body weight obtained from diabetic dams of group B. Neonates delivered from camel milk treated dams, had significantly (P<0.05) improved body weight compared to group B neonates.

Histopathology of neonates' hepatic tissue: Microscopic evaluation of neonatal hepatic sections of group A, exhibited the normal central vein patterned with hepatocytes in the form of radial arrangement with no hemorrhages and or necrosis. Significant necrosis of hepatocytes with marked hemorrhagic the central vein and disrupted pattern of hepatocytes were seen in hepatic sections of group B. Camel milk treatment in chronic diabetic pregnancy reversed these diabetogenic alteration as demonstrated by hepatic sections of group C.

Histopathology of neonates' renal tissue: Histological examination of neonatal renal sections of group A revealed normal histological features of renal corpuscles with normal glomerulus, proximal and distal convoluted tubules while in group B, damaged glomerulus with massive tubular hemorrhages and degeneration were found.

At the end of trial (98ᵗʰ day), camel milk treatment showed preventive effect on the neonatal hepatic histology on group C. There was less degeneration, hemorrhages and normal hepatocytes structure was observed (Fig. 1).

DISCUSSION

Diabetes mellitus (DM), impaired glucose homeostasis, is the most occurring endocrine disorder that leads multisystem dysfunction (Sharma *et al.*, 2010). Regarding reproductive problems, DM hampers folliculogenesis and ovulation results in low fertility (Ramalho-Santos *et al.*, 2008; Codner *et al.*, 2012). The chronic hyper-glycemic pregnancy enhances the congenital deformities and stillbirth along with uterine histological alteration like myometrium and endometrium thinning (Favaro *et al.*, 2010).

Anti-hyperglycemic effect of camel milk (CM) has been reported that can be attributed to the higher load of insulin like protein than ruminant's milk (Manal and Eman, 2014; Ejtahed *et al.*, 2015). This study was designed to explore beneficial effects of camel's milk on the gravid uterus of albino rats made diabetic by Alloxan. Effects on the liver and kidney of neonatal rats were observed, too.

The progressive increased in the plasma volume of blood has been documented in normal pregnancy. Because of the greater expansion in plasma than the increase in the RBCs mass, there is downfall in Hb concentration and hematocrit (Soma-Pillay *et al.*, 2016). Hematological findings had led us to the significant (P<0.05) declining pattern of RBCs and its indices. Diabetic associated anemic effect during pregnancy might be related with increased non-enzymatic glycosylation of RBCs due to lipid peroxidation (Oyedemi *et al.*, 2011). These chronic diabetogenic alteration were in accordance to the Erukainure *et al.* (2012) and Usman *et al.* (2018). Following CM therapy, the levels of these altered
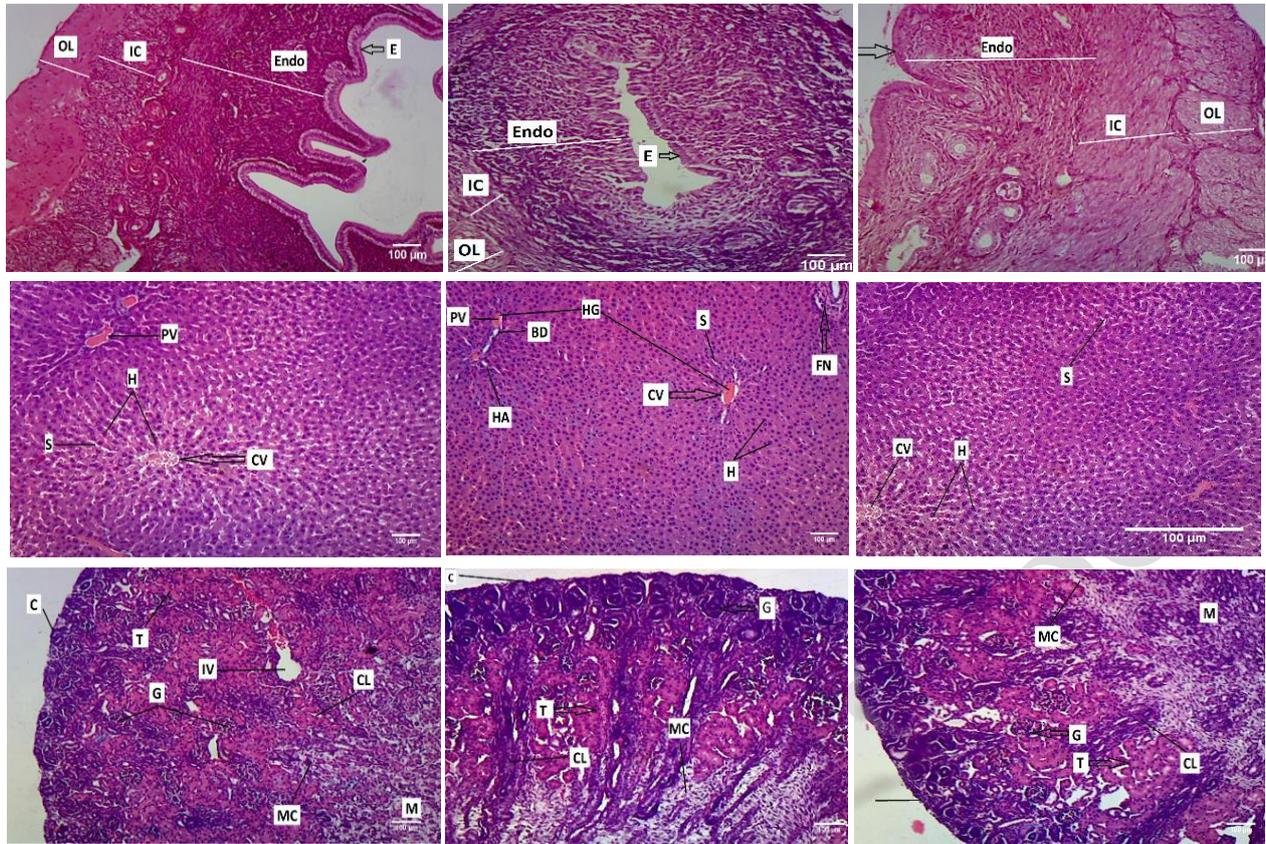
Fig. 1: Photomicrographs showing normal maternal uterus, neonatal liver and kidney (Left), control diabetic maternal uterus, neonatal liver and kidney (Centre) and camel milk treated maternal uterus, neonatal liver and kidney (Right). Hematoxylin and Eosin; 100X. E: Epithelium; H: Hepatocyte; Endo: Endometrium; S: Sinusoid; IC: Inner circular smooth muscle; CV: Central vein; OL: Outer longitudinal smooth muscle; FN: Fat necrosis; C: Capsule; PV: Portal vein; T: Proximal convoluted tubules; BD: Bile duct; G: Glomerulus; HA: Hepatic artery; IV: Intralobular vein; HG: Hemorrhages; CL: Cortical labyrinth; MC: Medullary cord; M: Medulla.

parameters were appreciably improved. This recovery towards normal gave an indication that CM may contain constituent that can enhance erythropoietin production, a hormone which enhances RBCs production in the bone marrow, and high concentration of antioxidant (Vit. C) (Rahimi *et al*., 2011; Mullaicharam, 2014). High levels of vitamin C can be held responsible for reducing diabetes induced blood hemolysis by inhibiting lipid peroxidation of cellular membrane (Torell *et al*., 1986; Faure *et al*., 1991).

Immunity of animal is suppressed by administration of alloxan monohydrate following establishment of Chronic DM. This decline in WBCs count may be linked to the hampered leukocytosis from the bone marrow. WBCs count had been observed to be significantly (P<0.05) below the normal values during experiment which is in accordance with Baskar *et al.*, (2006) and Akomas *et al*. (2014). CM therapy during chronic DM had showed significant (P<0.05) elevation in WBCs count which gives a clue that CM may contain some immune-boster component like zinc that are responsible for significant elevation of these values. Platelet count was seen reduced significantly (P<0.05) in hyper-glycemic rats. Platelets or thrombocytes are involved in blood clotting by forming a fibrin fiber meshwork at the site of vascular opening and forbid the blood loss (Jarald *et al*., 2008; Oyedemi *et al*., 2010). During normal pregnancy in women platelets count tends to fall progressively (Soma-Pillay *et al.,* 2016). Diabetes induced contraction in platelet count may lead to extensive hemorrhages in body

and death. However, after CM treatment, there was significant (P<0.05) enhancement in the platelet count of diabetic animals indicating that camel milk may have the potency to activate the biosynthesis of clotting factors in chronic diabetic pregnancy in rats.

Vascular complications in diabetic uterus are correlated with the level of glucose, hypoinsulemia and duration of hyperglycemia which reproduces an animal model characteristically comparable to diabetic women (Favaro *et al*., 2013). As reported by Favaro *et al*. (2010), duration of diabetes 50-70 day has no perturbative impact on uterus so we extended the duration of this project up to 98 days. Diabetic pregnant uterine histometric constituent including epithelium height, thickness of endometrium and myometrium significantly (P<0.05) shrinked during long term DM as compared to normal pregnant rats. The myometrium undergoes many histological alterations during pregnancy that influenced by the elevated synthesis of extracellular matrix (ECM) proteins (Shynlova *et al*., 2004). The changes in the ECM synthesis and deposition during DM are thought to case loss of contractility of myometrium. These diabetic induced alterations are significantly (P<0.05) shifted towards normal after camel milk therapy. Long term exposure of hyperglycemia, under the experimental conditions, resulted in reduced weight gain of neonates. Similar results were described by Sinzato *et al*. (2012). Abnormal fetal growth could be linked to the synthesis of hyperglycemia and hyperketonemia (Ramalho-Santos *et al*., 2008). CM was

used as antihyperglycemic agent, has some unknown elements which facilitate the fetus to accommodate in diabetic uterus.

DM caused many feto-maternal complications but regarding the histological changes in renal and hepatic tissues of neonates, the literature is silent. Chronic DM in pregnancy can deteriorate the hepatic and renal structure of neonates. Tubular degeneration along with the glomerular degeneration was seen in neonates' kidney. In liver sections, disturbance in the radial pattern of hepatocytes and sinusoidal cords were found. Hemorrhages and congestion in central vein and portal vein were also there. These histological findings might be due to diabetic oxidative stress which affect or negatively influence the normal physiological decidualization and ECM remodeling process and reduction of platelets during long term diabetes (Favaro *et al.*, 2013). CM therapy during chronic diabetic pregnancy showed protective effect which depicts that CM has potential to compensate the diabetic maternal glycemic control, decidualization and ECM remodeling during pregnancy.

Fasting blood glucose level was found significantly (P<0.05) low after CM therapy in pregnant diabetic rats. The reason behind this change that camel milk is a rich source of insulin like protein which can tolerate stomach acidity (Kamal *et al.*, 2007; Al Kanhal, 2010) and also IGF-1 which promotes energy substrate by suppressing lipolysis and stimulating glucose oxidation (Hassan and Bayoumi, 2010).

Conclusions: Based on the results obtained in this study, CM has potential to control glucose, diabetic induced uterine histopathies and embryopathies during pregnancy and improves the neonatal growth. Dibetogenic hematological parameters can be improved by CM. These results may have implications in the clinical management of diabetic associated complications during pregnancy in human if CM is to be considered as part of the dietary plan.

Authors contribution: ASQ and WAK conceived the idea and finalized manuscript: ASQ and RUS supervised the lab work. MKK and MU conducted lab work, applied statistics and prepared rough draft.

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