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The effects of long-term diabetes on the haematological and uterine indicators and their association with neonatal nephrogenesis counter-protected by camel milk: A time dependent study

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Abstract: The novelty of this project is to describe how chronic diabetes altered the haematological and uterine indicators in a time dependent-manner that were reversed by camel milk (CM) therapy in pregnant and non-pregnant rat models. Fifty-four female rats were divided into three groups: Placebo (N), diabetic control (DC) and diabetic treated (DT) with CM at 40 ml/kg/24 h for 90 days. A single intact male was introduced into every group for mating at day 60 of the experiment. The sample collection was undertaken at day 30 and 60 of the non-pregnant rats and at day 90 immediately after parturition for the pregnant rats. At every collection, the dam's blood, as well as the uteri and neonatal kidneys were collected and subjected to a paraffin tissue preparation technique for a histological evaluation. The data revealed that at day 30, the uterine endo- and myometrium remained unaffected by diabetes, but at day 60, a significant reduction in the uterine indicators from diabetes was observed. However, the CM restored the uterine histology in the DT. At 90 day, chronic diabetes showed ($P < 0.05$) a harmful effect on the pregnant uterus which was reversed ($P < 0.05$) by the CM. The RBC (red blood cell) indices, platelets, and leucocyte counts were severely affected by the diabetes and protected by the CM at every point of collection. The kidney tissues of the neonate rats, delivered by the dams, in the DC presented a significant ($P < 0.05$) shrinkage in the cortex and glomeruli while the CM potentially reversed these changes. These results will help to understand the chronic diabetes effects on the uterus and neonate's renal genesis, and the role of camel milk in the management of chronic pre-gestational diabetes.

Keywords: female reproductive system; long-term diabetes; pregnancy; camel milk; neonatal kidneys

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The reproductive complications in females associated with diabetes mellitus (DM), a metabolic disorder characterised by elevated blood sugar concentrations, is poorly understood. In diabetic patients, impaired ovarian steroidogenesis can be seen, which alters folliculogenesis and ovulation leading to orgasmic dysfunction, decreased vaginal secretions and low arousability (Amaral et al. 2008; Majeed et al. 2018). These diabetes-induced changes in females eventually cause severe hypogonadism and low fertility (Codner et al. 2012; Ali et al. 2017). Histological studies of uteri of non-pregnant diabetic albino rats revealed that there is a decrease in the smooth muscle cell number along with the impaired contractibility of myometrium to oxytocin (Tatewaki et al. 1989). It is suggested that pre-term labour is associated with poor glycaemic control (Taylor and Davison 2007). Myometrium contractility is crucial for essential events *viz* uterine vascularity for the blood flow, the extension and rearrangement of the outer uterine compartment and proper settlement of the foetus. This contractility is modulated by the endocrine signalling and subsequent stretching of the myometrial smooth muscles (Favaro et al. 2013). Long-term DM promotes oedema in early pregnancy and in late pregnancy promotes collagen deposition in the uterus of rats (McMurtrie et al. 1985; Favaro et al. 2010). The malformations observed in the chronic pre-gestational hyperglycaemia are the defects in the organogenesis in the gestation, such as the improper closure of the neural tube, the caudal regression syndrome, and the defective formation of the urogenital tract which may attribute to renal agenesis (Amri et al. 1999). Arterial hypertension, the main cause of cardiac stroke and kidney problems, is reported in the offspring of hyperglycaemic dams (Aceti et al. 2012; Majeed et al. 2018).

Natural antidiabetic agents are preferred over synthetic drugs because they have a higher safety margin in pregnancy (Baragob 2015; Iftikhar et al. 2018; Mokhtare et al. 2018). Recently, camel milk gained the attention of researchers for its diverse medicinal properties. Many components such as immunoglobulin, lactoferrin, lactoperoxidases and peptidoglycan proteins known for their pharmacological properties are present in camel milk. People who consume camel milk daily have better glycaemic controls and a lower risk of developing diabetes as compared to non-consumers (Agrawal et al. 2007).

The anti-diabetic property of camel milk is known due to the presence of the high amount of insulin (45–128 IU/1 000 ml) along with zinc which stimulates insulin secretion. The concentration of vitamin C, a strong antioxidant, is five times higher in camel milk in comparison to other ruminants' milk (Rahimi et al. 2011; Mullaicharam 2014; Mirmiran et al. 2017). However, no sufficient literature is available explaining the possible role of the long-term camel milk therapy in the control of chronic pre-gestational diabetes-induced changes in the female reproductive system. We hypothesised that the anti-hyperglycaemic properties of camel milk may be used to control or reverse the deleterious effects of chronic diabetes on the uterus during pregnancy and on the foetal and neonatal nephrogenesis in a time-dependent manner.

Therefore, the primary objective of this study is to evaluate the time-dependent diabetes-induced changes in the female reproductive system in non-pregnant and pregnant rats, and the neonatal renal genesis in their offspring. Apart from that, the counter effect of camel milk in diabetic rats was also evaluated in the long-term study and, especially in the gestational diabetes.

MATERIAL AND METHODS

Animal collection

The experiment was carried out on 54 adult female albino Sprague Dawley rats. The age of the animals was three to four months and the weight ranged between 180–230 g. These animals were kept in a room of the experimental building of the Faculty of Veterinary Science, University of Agriculture Faisalabad, Pakistan at standard room temperatures (25 ± 2 °C) and humidity (50–55%), and also provided with a diet and water *ad libitum.* This experimental trial was carried out in agreement with the guidelines of the Directorate of Graduate Studies and Institutional Animal Ethical Committee.

Induction of diabetes

Diabetes induction was undertaken with a single intraperitoneal injection of Alloxan (Applichem®, Cheshire, USA) at 150 mg/kg to the experimental rats after overnight fasting. The suspension of Alloxan was made in normal saline and injected into the rats

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Table 1. The status of the experimental groups during the trial

Groups	Hyperglycaemic status	Treatment	Mating schedule
Placebo (N*)	2 ml normal saline intraperitoneally as a vehicle	standard diet and water *ad libitum*	at the 61st day of experiment
Diabetic control (DC**)	Alloxan administration intraperitoneally at 150 mg/bwt	standard diet and water *ad libitum*	
Diabetic treated (DT***)		standard diet and water *ad libitum* along with the camel milk at 40 ml/kg	

*The control without any treatment (Negative control). **The diabetic control without the camel milk treatment (Positive control). ***The diabetic control treated with the camel milk

within two minutes after making the suspension. Normal saline was administered to the placebo rats. The fasting glucose concentration using On Call® EZ II (Acon® Laboratories Inc., San Diego, USA) was determined three days post injection for confirmation of the diabetes. Animals with > 13.875 mmol/l glucose concentration were taken as diabetic.

Experimental design

The hyperglycaemic rats were divided into two groups; the diabetic control and the diabetic treated with fresh camel milk at 40 ml/kg of body weight. A group of normal/placebo rats was also taken for comparison among these groups. Each group contained (n = 18) biological replicates. The rats were humanely killed under gaseous anaesthesia at different intervals for blood and tissue collection. The experimental design is given in Table 1.

Camel milk therapy

Fresh raw camel milk was purchased from a local supplier of Anmol Camel Dodh® at the time of milking. The chemical analysis of milk samples was done by the Livestock & Dairy Development Department of Punjab, Pakistan and is given in Table 2. The milk was collected in sterile bottles and transported under chilled conditions. The dose of camel milk was calculated on the basis of live body weight (g) of rats followed by Ali et al. (2017) and administered orally through a nasogastric tube connected to sterile syringes once in 24 hours. This treatment was started from the first day of experiment (confirmation of diabetes) to the end of trial (almost 90 days).

Mating schedule

For mating, healthy male rats were introduced in all the groups on the 61st day after the confirmation of DM to evaluate the long-term effect of the diabetes on the pregnant and non-pregnant uteri. The male rats were removed from the cages after five days of their introduction, except for the diabetic control group because of the late conception. In the diabetic control group, the male was allowed to remain in the cage for ten days. To confirm pregnancy, the females were examined for the presence of a vaginal plug.

Table 2. Composition (range) of camel milk used in this study

Chemical contents		Medicinal contents		Minerals contents	
component	concentration	component	concentration	component	concentration
water	86–88%	cholesterol	4.8–5.5 mg/100 g	zinc	0.51–0.56 mg/100 ml
total solids	12–13%	vitamin C	32.3–33.6 mg/l	iron	10–10.3 mg/100 ml
fat	3.6–3.9%	vitamin E	0.5–0.6 mg/l	sodium	0.3–0.55 mg/100 ml
lactose	3.3–3.8%	lactoferrin	0.32–0.44 mg/ml	potassium	149–153 mg/100 ml
protein	3.2–3.5%	immunoglobulin G	1.6–1.7 mg/ml	copper	140–144 mg/100 ml
ash	0.7–1%	insulin	50–52 IU/ml	calcium	110–114 mg/100 ml

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Collection of the samples

The non-pregnant animals were killed on the 30th and 60th day of the experiment whereas the remaining pregnant rats were euthanised under the gaseous anaesthesia immediately on the day of parturition (approximately the 90th day) after the confirmation of the DM. The blood was collected in a vacutainer coated with anticoagulated EDTA (Ethylenediaminetetraacetic acid) before every collection for the haematological evaluation through an Automated cell counter machine (Medionic®, Spanga, Sweden). The uteri were collected and fixed in Bouin's solution after washing with normal saline. The neonates' kidneys were also collected and fixed in a neutral buffered formalin for the histological evaluation of the diabetes-induced changes.

Light microscopy

The tissue samples, uteri and neonate's kidneys, were subjected to a routine paraffin embedding technique for the light microscopic examination. The five micrometre thick sections of tissues cut by a microtome (Microm HM 315; Microm Int., Waldorf, Germany) and were stained with Haematoxylin and Eosin. The stained sections were examined at × 100 and × 400 under a digital camera fitted microscope (Labomed iVu 3100; Labomed Inc., Culver City, USA). The different layers of the endometrium (epithelial height, uterine gland area, and endometrial thickness) and myometrium (the inner and outer smooth muscle layers) were measured using the image analysis software Image J® developed by the National Institute of Health, USA and downloaded from imagej.en.softonic.com/download. The neonatal kidneys were also evaluated for the renal morphometrical changes.

Statistical design

The mean and standard error of the means were computed using the software Minitab® (19.1.1.0). The means of groups were analysed with a one-way analysis of variance (ANOVA) followed by a comparison using Tukey's highly significant test to determine the variation within each group. A two-way analysis was also performed to find the variation among the groups with respect to the duration of the diabetes. A randomised complete model was applied to the data to determine the variation between the variables and time. In comparison, a $P < 0.05$ was considered significant.

RESULTS

Uterine histomorphometry

Two-way ANOVA has shown that the diabetes affects the histological structure including the epithelium height, endometrium gland area and thickness, inner circular and outer longitudinal muscle layers of the non-pregnant and pregnant uterus in a time-dependent manner (Table 3). The diabetic impact on the uterine histological structure at day 30 was not significant ($P > 0.05$) in the diabetic control non-pregnant animals (DC). However, at day 60, the diabetes significantly ($P < 0.05$) reduced the histological indicators of the uterus in the DC group. The camel milk reversed this diabetes-altered value towards being normal ($P < 0.05$). On the day of parturition, approximately day 90, the chronic diabetes showed ($P < 0.05$) a destructive effect on the uterus which was reversed significantly ($P < 0.05$) by the camel milk therapy (Table 3 and Figure 1).

Haematological profile

On juxtaposing with the placebo group (N), the diabetic females (DC) showed ($P < 0.5$) a sharp decline in the red blood cell number and its indices at every point of collection of the samples regardless of the pregnancy status (Figure 2). However, during the pregnancy (day 90), the chronic diabetes caused a more significant ($P < 0.01$) decrease in the aforementioned indices as compared to the values on day 30 and 60. The diabetes led to a significant ($P < 0.05$) decrease in the platelet number on day 30 in the DC and this reduction remained unaltered till day 60 and 90. The short- (30 days) and long-term (60 and 90 days) camel milk therapy in the diabetic females potentially recovered these values towards the physiological values. The camel milk treatment in the diabetic rats (DT) significantly ($P < 0.05$) reversed the diabetogenic platelet count values. The immunological indicators (white blood cells and lymphocyte percentages) were also seen

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Table 3. The uterine histological measurements in the groups of rats varied time intervals

Groups	Uterine measurements				
	EP. H (μm)	UT. GL (μm^2)	END. THK (μm)	INC (μm)	OUT. L (μm)
Day 30 (non-pregnant rats)					
N*	101.30 ± 1.9^a	30.53 ± 1.6^a	198.86 ± 3.2^a	264.16 ± 13.3^a	374.86 ± 23.2^a
DC**	95.16 ± 2.0^b	29.66 ± 1.8^a	200.66 ± 5.0^a	265.53 ± 5.0^a	378.23 ± 18.5^a
DT***	102.53 ± 2.6^a	32.36 ± 1.2^a	200.20 ± 3.5^a	269.46 ± 7.8^a	387.70 ± 10.9^a
Day 60 (non-pregnant rats)					
N*	101.3 ± 1.9^a	30.53 ± 1.6^a	198.86 ± 1.6^a	264.16 ± 23.3^a	374.86 ± 23.2^a
DC**	82.56 ± 4.2^b	24.66 ± 2.3^b	147.33 ± 4.4^c	154.53 ± 10.0^c	136.56 ± 13.6^c
DT***	108.66 ± 5.0^a	29.26 ± 0.7^a	182.2 ± 8.2^b	222.16 ± 4.5^b	245.40 ± 5.1^b
Day 90 (pregnant rats)					
N*	103.7 ± 5.6^a	37.96 ± 2.2^a	217.16 ± 6.1^a	173.7 ± 10.8^b	387.26 ± 12.0^a
DC**	45.43 ± 3.8^b	15.766 ± 2.5^c	121.10 ± 11.5^b	183.56 ± 4.0^b	207.9 ± 10.8^b
DT***	95.8 ± 4.1^a	29.26 ± 2.1^b	150.8 ± 42.4^b	267.13 ± 15.4^a	407.16 ± 7.1^a

END. THK = endometrium thickness; EP. H = epithelium height; INC = inner circular; OUT. L = outer longitudinal smooth muscles layers; UT. GL = uterine glands area

*The control without any treatment (Negative control). **The diabetic control without the camel milk treatment (Positive control). ***The diabetic control treated with the camel milk

The means having different letters of the alphabet are significantly different at $P < 0.05$

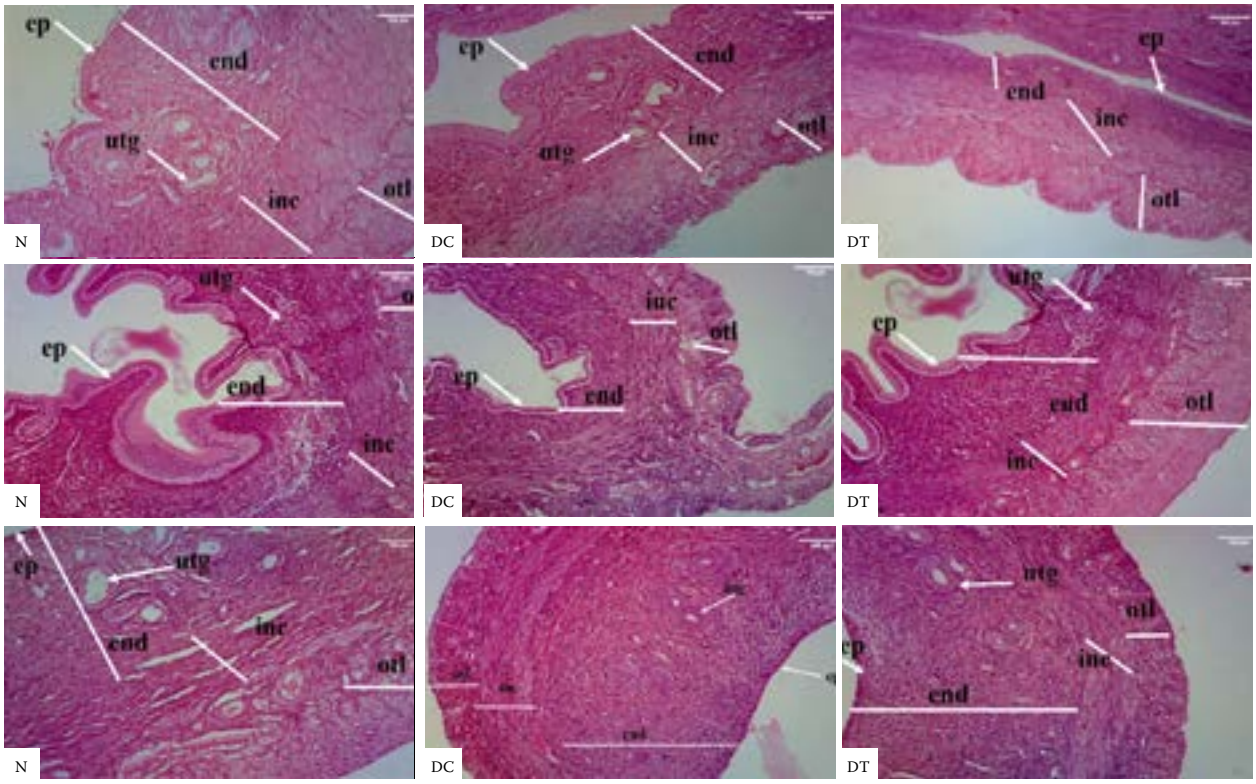

Figure 1. The representative uterine sections of the normal (N), diabetic control (DC) and camel milk diabetic treated (DT) groups at the different intervals; the first row slides represent the sampling at day 30 (non-pregnant), the second row represents day 60 (non-pregnant) and the third row represents day 90 (pregnant rats) (H&E, 100 ×)

End = endometrium; ep = epithelium; inc = inner circular; otl = outer longitudinal smooth muscles; utg = uterine glands

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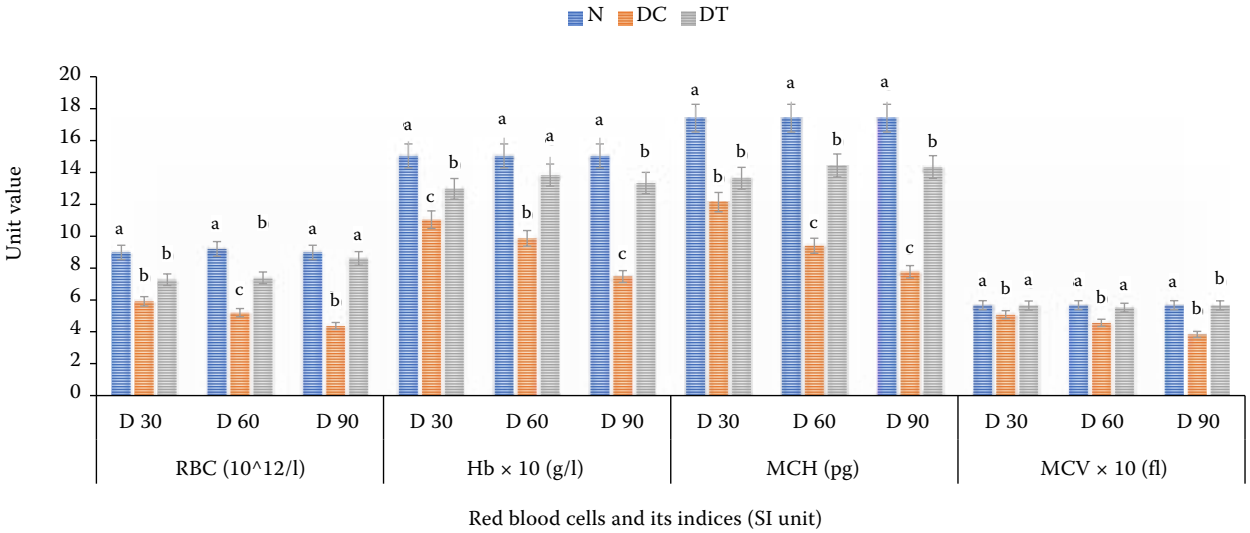

Figure 2. The graphical representation of the various erythrocyte counts and its indices in the different groups of rats at varied time intervals

DC = the diabetic control without the camel milk treatment (Positive control); DT = the diabetic control treated with camel milk; N = the control without any treatment (Negative control, Placebo)

($P < 0.05$) to be affected with the diabetes. The lymphocyte (%) followed the same pattern as that of the platelet count, but in the case of the WBCs (white blood cells), a more significant ($P < 0.05$) reduction was seen at day 60 and 90 and the camel milk potentially ($P < 0.05$) recovered these diabetes-altered values towards normal (Figures 2 and 3).

Neonatal indicators

In this long-term study, diabetes affected ($P < 0.05$) the nephrogenesis in the foetuses during their intrauterine development. The cortical regions of the kidneys of the neonates delivered by diabetic dams, were significantly ($P < 0.05$) reduced

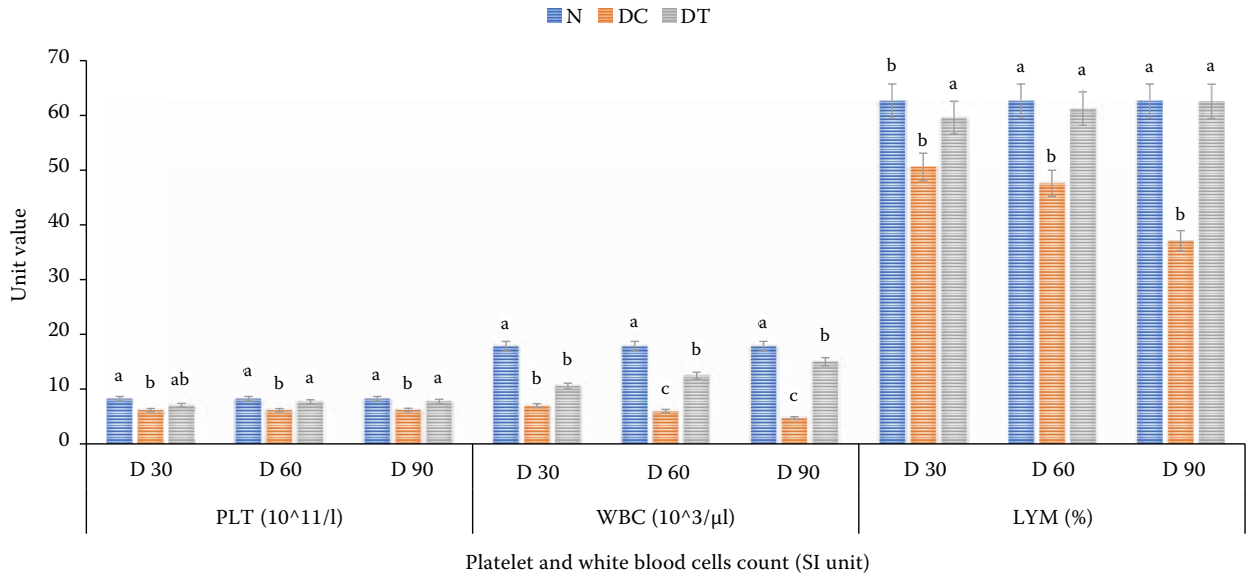

Figure 3. The graphical representation of the various thrombocyte and leukocyte indices in the different groups at varied time intervals

DC = the diabetic control without the camel milk treatment (Positive control); DT = the diabetic control treated with camel milk; N = the control without any treatment (Negative control, Placebo)

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Table 4. The histological measurements of the kidneys in the neonates delivered from dams of the experimental groups at the end of the trial

Groups	Weight of the foetus in grams (g)	Cortex (μm)	Medulla (μm)	Cortico-medullary ratio	Diameter of the renal corpuscles (μm)
N*	6.3 ± 0.9[a]	156.62 ± 12.86[a]	195.62 ± 12.86[a]	0.8060 ± 0.09[a]	87.54 ± 5.28[a]
DC**	4.1 ± 1.2[b]	80.56 ± 9.45[c]	201.69 ± 9.45[a]	0.4150 ± 0.07[c]	64.37 ± 4.81[c]
DT***	5.2 ± 0.6[ab]	96.38 ± 15.58[b]	170.57 ± 15.58[b]	0.7700 ± 0.19[b]	74.82 ± 7.51[b]

*The control without any treatment (Negative control). **The diabetic control without the camel milk treatment (Positive control). ***The diabetic control treated with the camel milk

Means having different alphabets are significantly different at $P < 0.05$

in width as compared to the normal dam's foetus and the same pattern was observed in the cortico-medullary ratio and the diameter of the renal corpuscles. However, the medullary region was seen to be unaffected by the diabetes (Table 4 and Figure 4). The diabetic dams treated with camel milk during pregnancy gave birth to foetuses with significantly ($P < 0.05$) improved diabetes-induced renal characteristics except for the medullary region.

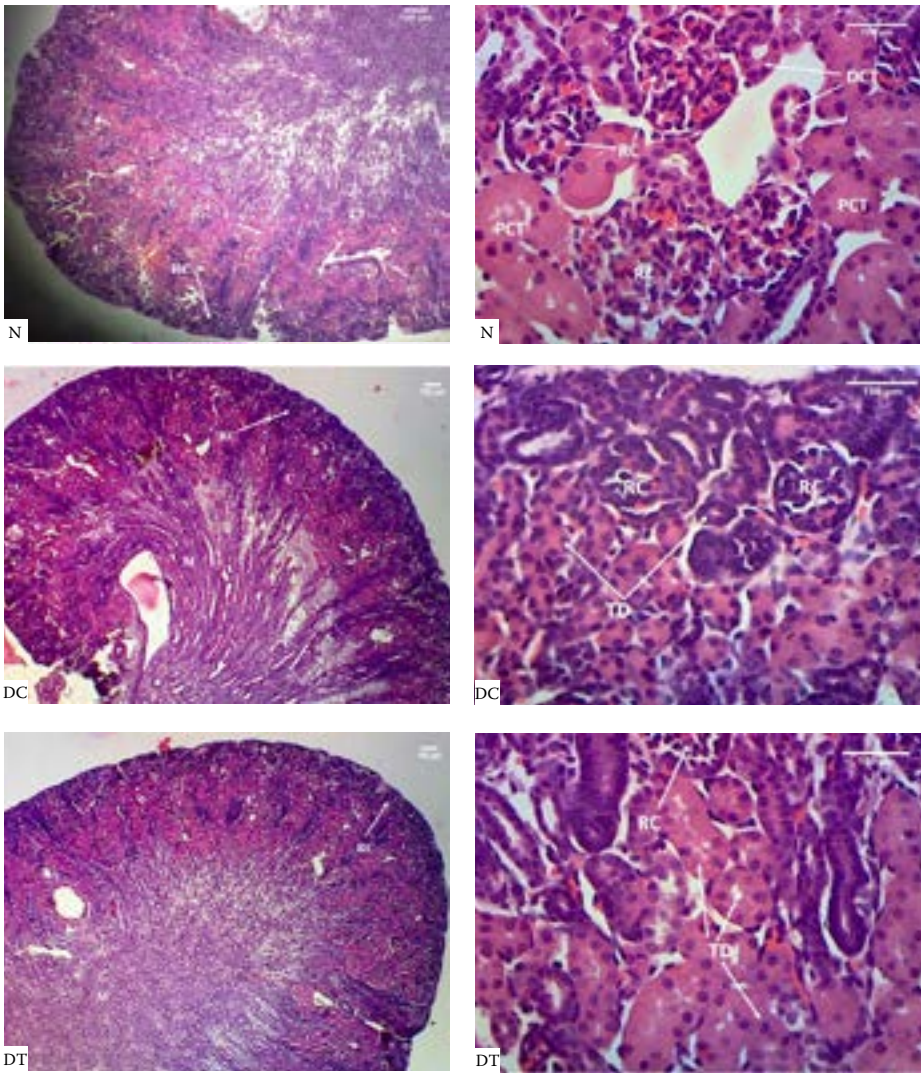

Figure 4. The histological representation of the neonates' kidneys collected from the normal (N), diabetic control (DC) and diabetic treated (DT) dams at the end of the trial (90 days). The normal foetal kidney shows the normal cellular structure of the renal corpuscles (RC) along with the proximal convoluted tubules (PCT), the distal convoluted tubules (DCT). The chronic diabetes in the gestation caused shrinkage of the renal corpuscles, the tubular degeneration (TD) and the cortical region (C) while the medulla (M) remained unaltered during the renal histogenesis. The camel milk therapy in the diabetic pregnancy protected these diabetic induced changes in the renal histogenesis (H&E, the left column histographs are at × 40 and the right column ones are at × 400 magnification)

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DISCUSSION

The endometrium and myometrium are the most dynamic compartments showing extensive remodelling during the course of the heat cycle (Salgado et al. 2009) and pregnancy allowing the appropriate development of the placenta and accounting for a successful labour (Favaro et al. 2010).

This rat model was developed for the appropriate understanding of the time-dependent induced diabetes impact on pregnant and non-pregnant animals as well as on the offspring delivered by diabetic dams. Moreover, the antidiabetic effects of camel milk were also evaluated in the long-term therapy for the management of the chronic pre-gestational diabetes.

One of the distinctive features of this project is to understand the progression of the gestational anomalies allied with the long-term diabetes in animals, thus mimicking human conditions in which the pregnancy develops into the onset of type-1 diabetes many years later.

At day 30, the uterine morphometry of the diabetic control (DC) rats was similar to that of the placebo ones (N) except the epithelial height which was significantly ($P < 0.05$) reduced. However, the diabetic rats treated with camel milk (DT) showed a non-significant effect on the endometrium and myometrium. However, the thickness of the endo- and myometrium remained unchanged in the diabetic rats treated with the camel milk (DT). These findings are in agreement with those of Ali et al. (2017) in which the short duration of time (< 6 to 7 weeks) did not provoke any alterations in these layers of the uterus. Whilst, at day 60, there was a declining trend ($P < 0.05$) in all the studied micro-anatomical layers of the endometrium and myometrium in the DC group (Table 2 and Figure 1). These results are supported by the data of Usman et al. (2018). They showed that hyperglycaemia promotes noticeable uterine alterations in a time-based manner. These changes are directly related to the duration and intensity of the hyperglycaemia, but the mechanism behind these time-dependent diabetes-induced changes in the uterus is not explained as yet. Though, Tatewaki et al. (1989) ascribed the atrophy of the myometrium and a decrease in the number of smooth muscle fibres to the progress of the disease which led to the loss of contractibility.

The administration of camel milk restored these values towards normal. Its antidiabetic effect on these altered indicators may be attributed to the high concentration of the insulin-like proteins present in the camel milk. These cysteine containing proteins are resistant to gastric milieu, therefore, can easily bypass it and be absorbed directly in the circulation system from the stomach, which may consequently improve the glycaemic control (Mirmiran et al. 2017).

Moreover, higher concentrations of antioxidant vitamins (A, B, C, and E) constrain the diabetes encounter by activating the antioxidant system in the body (Mullaicharam 2014).

These time-dependent changes in the diabetic pregnant and non-pregnant uterus were observed for the first time. These findings suggest that only a long-term course of diabetes has the potential to alter the uterine histometry (Table 2 and Figure 1). However, it was also reported that a short-term effect of diabetes can result in anomalies of the uterine blood flow by increasing the vascular permeability (Brownlee 2001). The gestational growth and remodelling of the uterus are the basic events that are directly influenced by the extracellular matrix (ECM). Long-standing diabetes hampers the synthesis of the ECM in the pregnancy by the production of reactive oxygen species during the cellular stress (Favaro et al. 2010). Hence, these observed changes in the uterine morphometric indicators may be linked to the oxidative stress that subsequently hinders the production of ECM which leads to the thinning of the endometrial tissue.

Camel milk has many anti-hyperglycaemic constituents like zinc, antioxidant vitamins and insulin. The latter also acts as an anti-inflammatory agent. These agents limit the diabetic impact by suppressing the hyperglycaemic stress and oxidative load on the cells through promoting glutathione activity, an anti-oxidant system (Mullaicharam 2014). Therefore, the group treated with camel milk showed a profound recovery in the DT rats at day 90 also.

The haematological profile depicted diabetes-associated anaemia in the form of a declining trend in the RBC counts and its indices at every sampling interval (day 30, 60 and 90) in the DC rats. This pattern was highly significant ($P < 0.01$) in the pregnant diabetic rats which is in accordance with the results of Usman et al. (2018). In a normal pregnancy, a progressive increase in the blood plasma volume is reported to cope with the large blood flow towards the foetus. In contrary to blood plasma, no such

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increment happens in the case of the RBCs that resulted in the diluted RBCs and its indices like haemoglobin and haematocrit (Soma-Pillay et al. 2016).

Hyperglycaemia induces lipid oxidation that caused the non-enzymatic glycosylation of the RBCs (Oyedemi et al. 2011), which results in their poor oxygen-carrying capacity. The statistical analysis of haematological parameters presented a non-significant ($P > 0.05$) interaction among the groups as well as over time. Following the camel milk treatment of the diabetic rats, these altered parameters were commendably improved (Figure 2). This restoration towards normal values indicates that the camel milk constituents, like antioxidants (vitamin C and zinc) and insulin (anti-inflammatory agent), reduce the diabetes-induced haemolysis and lipid oxidation through the activation of the antioxidant system of the body (Rahimi et al. 2011; Mullaicharam 2014), increases the concentration of iron in the form of lactoferrin (ten times more than other ruminant's milk) and vitamin B that enhance the production of erythropoietin, a protein that stimulate haematopoiesis (Rukkayya et al. 2018).

The platelet count and immune parameters of the blood followed the same pattern as that of the RBCs and its indices in the DC rats. During a normal pregnancy in women, the platelet counts tend to fall progressively (Soma-Pillay et al. 2016). Thrombocytopenia may contribute towards excessive internal bleeding and bruising which is sometimes fatal due to blood loss. The platelet and leukocyte production are controlled by the liver through the release of thrombopoietin, a platelet synthesis hormone, and erythropoietin (Jelkmann 2001).

The platelet and leukocyte production are controlled by the liver through the release of thrombopoietin, a platelet synthesis hormone, and erythropoietin (Jelkmann 2001). In chronic diabetes, deteriorating changes have been reported in the hepatic tissue (Ali et al. 2017; Muzaffar et al. 2018; Usman et al. 2018) in terms of its histology and physiology. These hepatic cellular changes in the hepatic tissue lead to the decreased synthesis of haemostatic hormones and the excessive peripheral consumption of plasma fibrinogen (Korish 2014). However, after the camel milk treatment, in the DT rats, there was a signification ($P < 0.05$) improvement in the leukocyte and platelet counts of the diabetic rats (Figure 3). The higher contents of the insulin present in the camel milk which also acts as anti-inflammatory agent on the hepatic tissue may revive the hepatic structure that boosts the platelet count through thrombopoietin synthesis. The immune-boosting effect of the camel milk may be associated with the higher contents of trace elements like iron and zinc (Hafez and Gad 2018). Thus, the reversal of similar haemostatic activation processes by the CM in chromic diabetes can be explained by the effect of the bioactive antithrombotic component present in the ingested CM (Korish 2014).

The intrauterine weight of the foetus was severely affected in the DC rats, which is comparable to the findings of Sinzato et al. (2012). This abridged prenatal growth of foetus might be due to the diabetes-induced degenerative changes in the uterus of their respective dams due to the abnormal synthesis of the ECM. A hyperglycaemic surge disturbs the progesterone concentration by deteriorating the hypothalamic-hypophyseal gonadal axis, the cellular architecture of the *corpora lutea* and the placenta by the formation of syncytial fibrotic knots. Albeit the progesterone concentration was not determined in this study, it should be related with the altered uterine histomorphometry and decreased foetal weight. Moreover, the foetal weight is also affected by the diabetes induced hyperketonaemia and the syncytial fibrosis of the placenta (Amaral et al. 2008; Ateeq et al. 2019).

However, the offspring exposed to gestational diabetes are more prone to be overweight and have other related conditions due to the failure of the glucose homeostasis (Yessoufou and Moutairou 2011; Garcia-Vargas et al. 2012). These variations in the results of the foetal weight may be associated with the duration of diabetes as chronic pre-gestational diabetes impairs the uterine architecture discussed earlier, which may have a possible role in the stunted foetal growth.

Camel milk-treated hyperglycaemic dams gave birth to foetuses with weights comparable to the placebo dams' foetuses because of the improved maternal glycaemic control. This positive change may be linked to the presence of small molecules of cysteine containing the insulin-like protein and lactoferrin that regulate the host immune system and inhibit the hyperglycaemic stress through protecting the hypothalamic-hypophyseal gonadal axis (Malik et al. 2012).

Nephrogenesis in hyperglycaemic dam's foetus has not been properly described earlier. Chronic hyperglycaemic intrauterine conditions lead to the shrinkage of the different renal regions in foe-

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tuses except for the medullary region (Table 3 and Figure 4). Although there are no contemporary studies present to compare these data, though Martins et al. (2014) recorded defective renal functions in three-month-old pups delivered from diabetic dams. This renal dysfunction may be linked to the defective nephrogenesis due to subnormal uterine environment during the prenatal period in the chronic diabetes. Camel milk was used as an antihyperglycaemic agent containing some elements which facilitate the foetus to accommodate in the diabetic uterus.

These findings broaden our understanding of the uterine anomalies associated with the long-term effect of diabetes that severely affects the intrauterine growth of a foetus. Moreover, these diabetes-induced changes can be reversed by the use of camel milk, therefore, it can be helpful in the management of chronic diabetes especially during the gestation period. For future studies, it is suggested to evaluate the effect of the chronic pre-gestational diabetes in dams on their offspring during the latter stages of life and the role of camel milk in the gestational diabetes.

Conflict of interest

The authors declare no conflict of interest.

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