Research Article

Physiological and histopathological analysis of nephroprotective effects of lactoferrin in diabetic rats

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ABSTRACT

Background:
Lactoferrin (Lf) has several actions that are interceded through different cell receptors. It was proven to have antimicrobial, antiparasitic, anti-inflammatory, antioxidant, anticarcinogenic and immunomodulating properties. Therefore, this current study aims to explain the protective effect of bovine lactoferrin (bLf) on kidney function and histopathology and the role of nitric oxide.

Methods:
In this investigation, 60 adult male rats were used, and fifty of them were used to induce diabetes by intraperitoneal injection of streptozotocin. Forty animals whose blood glucose levels exceeded 300 mg/dL were classified as diabetic. Rats (10 normal + 40 diabetics) were left for about a month without treatment. Experimental rats were arranged into 5 exploratory groups; Control, diabetes, (diabetes + insulin), (diabetes + lactoferrin), and (diabetes + insulin + lactoferrin). After 45 days of treatment, blood was collected for the determination of glucose, urea, creatinine, and uric acid. A urine sample was also collected to estimate creatinine clearance, microalbumin, nitric oxide, sodium, and potassium levels. kidney tissue samples were taken from all rats in each experiment and used to estimate nitric oxide levels and histopathological examination.

Results:
The results revealed the protective effect of lactoferrin against diabetic nephropathy in rats through biochemical and morphological confirmations, including lowered rates of blood and urine indices, and an increase in renal nitric oxide levels as compared with untreated diabetic rats or those treated with insulin alone.

Conclusion:
There was a clear improvement in the kidney function as well as in the renal histology of rats treated with (insulin + lactoferrin) compared to those treated with insulin alone. This suggests a potential protective role of Lf in diabetic kidneys, which could operate through its biological activities, such as its anti-hyperglycemic, anti-inflammatory and antioxidative actions.

1. INTRODUCTION

Diabetes mellitus (DM) is the most widely recognized cause of renal disorders leading to end-stage renal failure. The late development of diabetic kidney disease is identified with a couple of elements, for instance, genetic factors, and hemodynamic and biochemical changes (Zemin and Mark, 2011). All arteries can be negatively impacted and their sizes were changed in the diabetes mellitus (Postma, et al.,). Thus, both smaller and huge-scope angiopathy has been reported in diabetic patients. Diabetic nephropathy pathogenesis is connected with the term and capability of hyperglycemia treatment and heartbeats in diabetic patients. Most clinical assessments have shown that the soonest perceivable changes all through diabetic nephropathy in

diabetic patients will be reported within ten years since the initiation of diabetes mellitus. In any case, morphometric analysis shows that the signs can appear eighteen months after diabetes begins (Radica, et al., 2017).

Diabetic nephropathy is a reformist infection and prior findings can help in making a superior treatment plan to diminish its turn of events. For instance, utilizing sulodexide manages framework protein amassing in diabetic nephropathy (Abdulrahman, 2017). A wide extent of changes in the renal tissue has been shown in diabetes. Renal tubular functions have been altered and also glucose and sodium transportation were affected (Sandeep, et al., 2018).

Bovine Lactoferrin (bLf) is a glycoprotein containing iron that is discovered in body fluids and milk (Bartoskova, et al., 2009). Lactoferrin has several actions that are interceded through different cell receptors (Berlutti, et al., 2011). It was proven to have antimicrobial and antiparasitic activities (Velusamy, et al., 2014) anti-inflammatory (Vesce, et al., 2014), and antioxidant properties (Tsubota, et al., 2008). Also, It has been discovered that Lf improves apoptosis (Hessin, et al., 2015), and it shows anticarcinogenic (Fang, et al., 2014) and immunomodulating properties (Zimecki, et al., 2007). It was shown that Lf is expressed in different tissues and high expression of mRNA and protein is detected in the kidneys. This indicates that the kidneys express Lf which suggests that Lf may play significant roles in either inborn immunity of this organ or antioxidant-securing kidneys against some other non-microbial lesions, for example, ischemia-reperfusion and inflammations (Abrin, et al., 20114).

In the current study, we investigated the protective roles of Lf on kidney function and structure by using physiological and histopathological analysis in diabetic rats.

2. MATERIALS AND METHODS

2.1. Medications
Insulin (Mixtard, Novo Nordisk) and Lactoferrin (Radiance organic process Company - New Zealand) were utilized.

2.2. Animals
Sixty Sprague-Dawley male rats of an average weight of 150 g were utilized. Their fasting glucose levels were around 100 ± 5.0 mg/dl. Ten rats were taken as control normal rats. Fifty rats were utilized for induction of diabetes by intraperitoneal infusion (IP) of 60 mg/kg body weight of streptozotocin and were checked by testing blood tests for hyperglycemia (> 300 mg/dl), 48 h post-injection. Forty rats with glucose levels surpassing 300 mg/dl were sorted as diabetic rats. All (10 normal +40 diabetic) were left for 4 weeks without treatment, at that point characterized into the accompanying exploratory groups. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Committee of Animal Care and Use Umm AlQura University, Makkah, KSA with approval number (HAPO-02-K-012-2021-08-420).

2.3. Experimental groups
1. Group I/Control rats (10 rats). They were given saline only through a stomach tube and every day infused subcutaneously with 0.1 ml saline solution for 6 weeks.
2. Group 2/diabetic/non-treated rats (10 rats). They were given saline only and every day infused subcutaneously with 0.1 ml saline solution for 6 weeks.
3. Group 3/diabetic/Insulin treated rats (10 rats). They were every day infused subcutaneously with 2U insulin (Mixtard, Novo Nordisk) and orally administered with 1 ml saline for 6 weeks.
4. Group 4/diabetic/Lactoferrin treated rats (10 rats) were given Lf (200 mg/kg/day, p.o.) [11] and every day infused SC with 0.1 ml saline for 6 weeks.
5. Group 5/diabetic/insulin + lactoferrin treated rats (10 rats) were given Lf (200 mg/kg/day, p.o.) [11] and day-by-day infused SC with insulin (Mixtard, Novo Nordisk) for 6 weeks.

2.4. Sampling and measurements
At the end of the test period, blood samples were withdrawn in the morning from all experimental rats by orbital sinus technique under ether sedation. Fasting plasma glucose levels were surveyed colorimetrically. Serum samples were obtained and kept at - 20°C till the next biochemical analysis. Serum levels of urea, uric acid, and creatinine were estimated colorimetrically (Young, 1990; Rock, 1987). Levels of sodium and potassium were determined (] Wood, 1976) by utilizing Spectrophotometer-FP 20 (SEAC - Radim company, Italy, S/N: 701111). Twenty-four-hour urine samples were collected from rats of all groups by utilizing metabolic cages. Urine was utilized to estimate creatinine clearance (Young, 1990), microalbumin (Cambiaso, 1988), nitric oxide (Gallinelli, et al., 2009), and sodium and potassium concentration s (Rock, 1987). The rats were sacrificed by cervical dislocation under ether sedation. Immediately, dissection for the two kidneys of each rat was done and flushed with phosphate buffer saline (PBS) to dispose of blood. Specific parts from the two kidneys were homogenized (MPW-120 homogenizer, Med instruments, Poland) in PBS to get 20% homogenate that was kept for 12 hours at – 20°C. Two freezing-defrosting cycles were done to break the cell boundaries, the homogenates were centrifuged under cooling at 5000 x g for 5 minutes (Sigma and laborzentrifugen, 2k15, Germany). The supernatant was eliminated promptly and measured for Nitric oxide (Gallinelli, et al., 2009). Samples of the two kidneys from all experimental rats were treated with 10% buffered formalin (72 hrs) and then, washed, dehydrated, and embedded in paraffin. A stained section of 5μm with Eosin and Hematoxylin (Drury , 1967) was prepared for histological analysis.

2.5. Statistical analysis
ANOVA test was used to analyze the differences among experimental groups using the general linear model procedure (SAS) at the significant level of ($p < 0.05$).

3. RESULTS

3.1. Physiological analysis showed positive effects of lactoferrin treatment in the regulation of blood glucose level and kidney functions:

Here, we studied the effects of lactoferrin treatment in diabetic rats using physiological analysis. Data from Fig. (1) shows that glucose levels significantly increased all over the trial periods in diabetic rats and diabetic rats treated with lactoferrin only compared with the control rats (P≤0.05). Treatment with Lf alone was associated with lower blood glucose levels, although it was non-significant. Treatment with insulin + lactoferrin produced a marked lowering in blood glucose levels compared with diabetic rats or diabetic rats treated with lactoferrin alone.
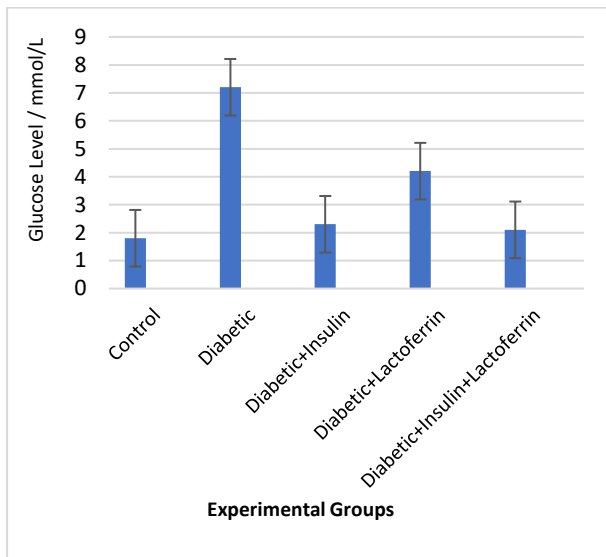

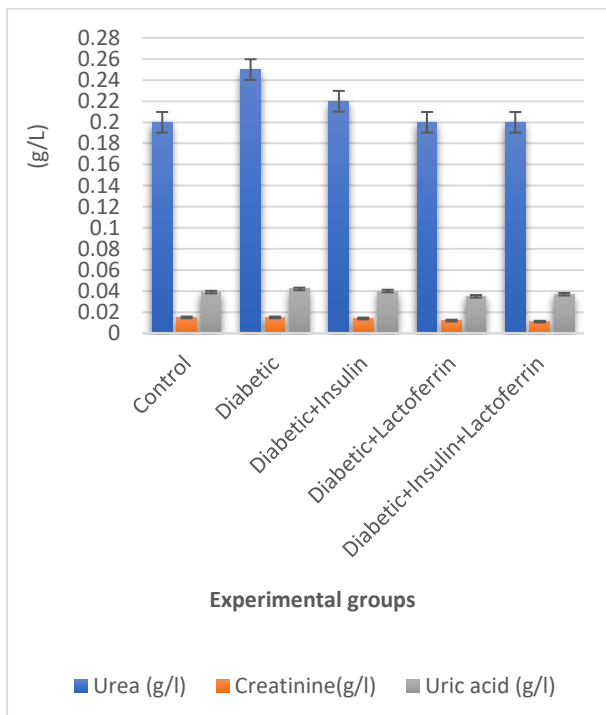**Fig. 1. Blood glucose levels in control and experimental rat groups.**

Fig. 2. Renal blood parameters in control and experimental rat groups.

Fig. (2) shows that concentrations of blood kidney function parameters, including urea, creatinine, and uric acid, were significantly increased in untreated diabetic animals versus control ones. However, their levels were shown to be significantly lowered in rats of all treated groups versus those of untreated animals (P ≤ 0.05). Moreover, a significant decrease was detected in rats treated with lactoferrin alone or combined with insulin as compared with those of untreated rats.

Fig. (3) shows that creatinine clearance was altogether diminished in untreated animals practically identical to the control (P≤0.05). A significant increase in its level, approaching the control value, was recorded in rats treated with either Lactoferrin alone or combined with insulin as compared with untreated rats or rats treated with insulin alone (P≤0.05). Levels of urinary microalbumin, sodium, and potassium were significantly increased in untreated animals versus control values (P≤0.05). A significant decrease in their levels was recorded in rats treated with either lactoferrin alone or combined with insulin as compared with untreated rats or rats treated with insulin alone (P≤0.05).
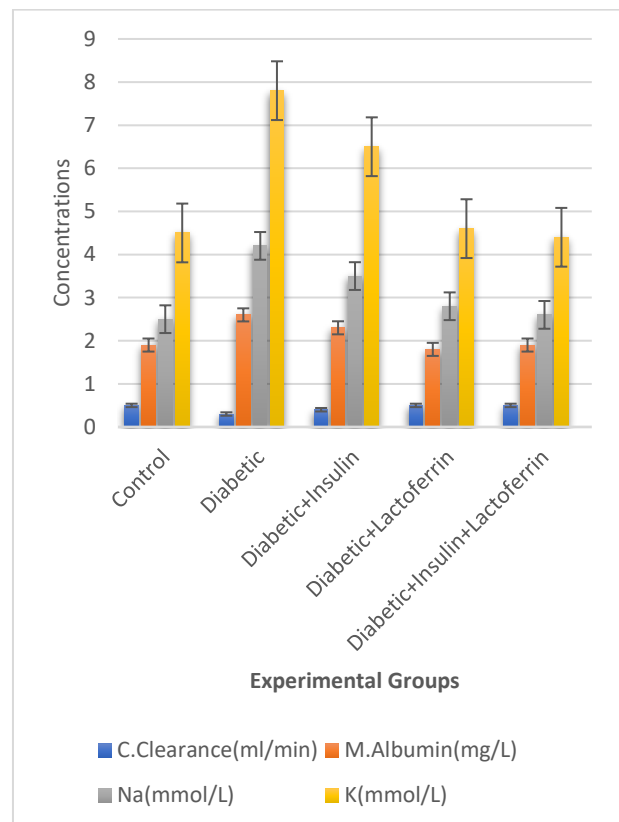

Fig. 3. Urine parameters of control and diabetic rat groups.

Fig. (4) represents renal and urinary nitric oxide levels. Renal nitric oxide was markedly diminished in untreated animals as compared with the control (P≤0.05). A significant increase in its level, approaching the control value, was recorded in rats treated with either Lf alone or combined with insulin as compared with untreated rats or rats treated with insulin alone (P≤0.05). No significant alterations were recorded in urinary nitric oxide levels among rat groups.
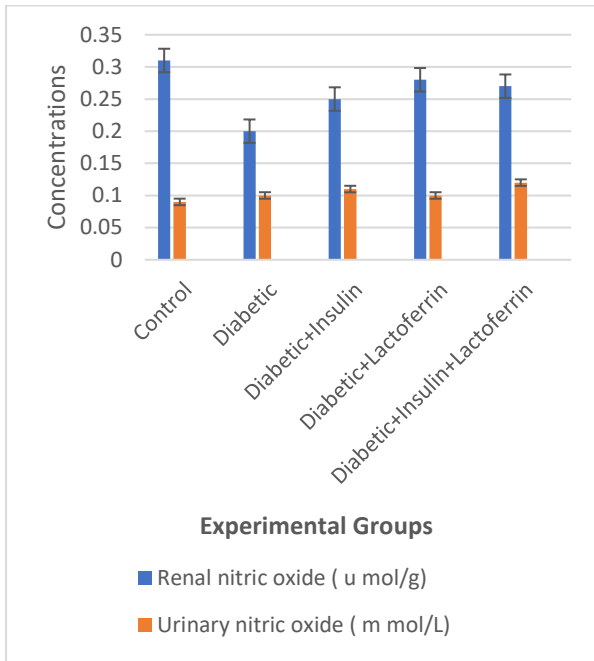
Fig. 4. Renal and urinary nitric oxide in control and diabetic rat groups.

3.2. Histopathological sections revealed a protective role for lactoferrin treatment on diabetic kidneys:

Histological analyses of the kidney showed that renal tissue of normal rats (Fig.5A) showed normal glomeruli (green arrow) and renal tubules (black arrow). diabetes strongly debilitated the renal tubule.
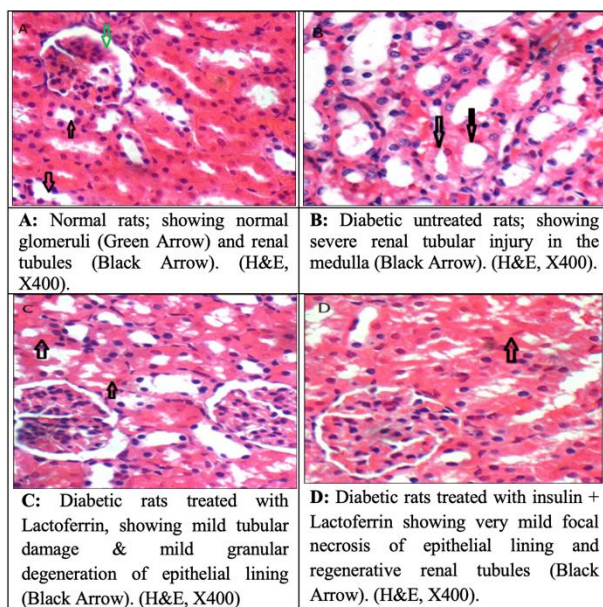

A: Normal rats; showing normal glomeruli (Green Arrow) and renal tubules (Black Arrow). (H&E, X400).

B: Diabetic untreated rats; showing severe renal tubular injury in the medulla (Black Arrow). (H&E, X400).

C: Diabetic rats treated with Lactoferrin, showing mild tubular damage & mild granular degeneration of epithelial lining (Black Arrow). (H&E, X400)

D: Diabetic rats treated with insulin + Lactoferrin showing very mild focal necrosis of epithelial lining and regenerative renal tubules (Black Arrow). (H&E, X400).

Fig. 5. Renal tissues of normal (A) and experimental rats (B, C, D).

The renal tissues of untreated diabetic rats showed severe renal tubular damage in the medulla; the collecting tubules showed apoptotic epithelial linings with mildly congested peri-tubular capillaries (Fig. 5B). Kidney tissues of diabetic animals treated with Lf alone indicated mild tubular damage with mild granular degeneration of their epithelial lining (Fig5.C). Diabetic rats treated with insulin + Lf possess very mild focal necrosis of epithelial cell lining associated with consistently regenerative renal tubules (Fig5.D). Renal tissue assessment demonstrated that lactoferrin treatment in diabetic rats kept up the ordinary renal architecture. Its protective role was superimposed when joined with insulin treatment.

4. DISCUSSION

Diabetes mellitus is the most well-known reason for ongoing renal problems and end-stage kidney infection in many patients around the world. The advancement of diabetic nephropathy is related to a few factors, for example, hereditary vulnerability, and hemodynamic and biochemical changes (Keane, et al., 2015). In this study, a significant elevation in the level of blood glucose was found in diabetic animals. This may be because of the annihilation of beta cells in the pancreas by STZ, sustaining the way that STZ prompts diabetes, apparently through the production of oxidative free particles (Gupta, et al., 2004). The stature of glucose in STZ-treated animals is a result of an oxidative condition conveyed in the pancreas and a view of a single DNA break in pancreatic beta cells (Yamamoto, et al., 1981). Treatment of diabetic rats with Lf was related with brought down blood glucose levels. This outcome lies corresponds with that of Yuta et al (2017), who found that Lf can smother hyperglycemia, joined by raised plasma levels of insulin, and ascribed such effect to the fleetingly quickening glucagon-like peptide - 1(GLP-1) discharge and upgrade of glucose assimilation from the small intestine.

The present findings demonstrated that oral Lf has a defensive impact against the nephropathy induced by diabetes in rats, this defensive impact was proposed by biochemical and morphological confirmations, including lower blood and urinary kidney indices and minimal damage in the renal tubules versus the untreated diabetic rats or diabetic rats treated with insulin alone. Results also showed that the improvement in kidney parameters and morphology in rats treated with the insulin+Lf group was more pronounced than in insulin alone.

Diabetic kidney affections are reported by proteinuria, declined renal parameters, and diminishing renal clearance (Bieke, et al., 2004). A few pathways were proposed to involve the advancement of diabetic kidney sickness. progressed glycation endproduct (AGE) formation as an outcome of continued or unstable hyperglycemia has been involved. Besides, the aldose reductase/polyol pathway was also shared (Sabbatini, et al., 1992).

Likewise, different vasoactive variables were added to the advancement of diabetic microvascular complications including nitric oxide (NO) as a vasodilator (Bieke, et al., 2004). In the present study, the renal content of nitric acid was significantly decreased in diabetic untreated rats as compared with control and lactoferrin-treated rats. The increased production of

glycation endproducts may affect the NO synthase pathway. Another Possible pathway whereby high glucose diminishes NO bioavailability incorporates NO catch by glucose was proposed (Komers, et al., 2003). Furthermore, a significant improvement of urinary microalbumin after receiving Lf was observed as compared with diabetic untreated or treated with insulin alone. Furthermore, Histopathological examinations have outlined many kidney changes in the renal tissue of diabetic rats. Impaired renal function and transport disorders were accounted for (Nudler, et al., 2009). In parallel, the present study revealed tubular injury particularly in the proximal tubules of the outer medulla in diabetic untreated rats. Moreover, Onoja et al.(2018) exhibited a prior existence of irregular cells in the mass of renal tissues that could prompt cell harm in diabetic rats.

The obtained data revealed that Lf treatment minimizes to a higher extent the renal damage associated with diabetes mellitus compared with untreated diabetic rats or diabetic rats treated with insulin alone. This protective action of lactoferrin might be due to its ameliorating action to the oxidative stress condition associated with diabetes mellitus and decreased production of harmful free radicals.

5. CONCLUSION

In conclusion, Lf treatment in diabetic rats was found to be beneficiary in controlling diabetic nephropathy evidenced by maintaining normal biochemical kidney parameters and, also demonstrated by histopathological assessments. This suggests a potential protective role of Lf in diabetic kidneys and this could be through its biological activities such as anti-hyperglycemia, anti-inflammatory and antioxidative actions.

ABBREVIATION

LF- Lactoferrin
DM- Diabetes mellitus
mRNA- Messenger Ribonuclic acid
IP- Intraperitoneal
SC- Subcutaneously
PBS- Phosphate buffer saline
DNA- Deoxy ribonucleic acid
GLP-1- Glucagon-like peptide-1

AUTHOR CONTRIBUTION

All authors Equally contributed to this project.

SOURCE OF FUNDING

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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