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Lactoferrin Ameliorates the Oxidative Stress Condition Associated with *Diabetes mellitus* in Rats

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Abstract: The present study was planned to investigate the ameliorating action of lactoferrin administration on the oxidative stress condition associating diabetes mellitus in rats. Diabetes was induced by intraperitoneal injection (IP)of 60 mg/kg body weight of streptozotocin.After 2 weeks of diabetes induction, experimental rats were grouped into 4 groups; control (non-diabetic), (control + lactoferrin), (diabetic) and (diabetic + lactoferrin).Lactoferrin was administrated orally (300 mg/Kg/day) for 30 days. At the end of the treatment period (30 days), fasting blood samples were collected from rats of all experimental groups and sera were separated. Measurements included serum glucose, catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx) and malondialdehyde (MDA). Results indicated significant elevation in blood glucose of diabetic rats allover the experimental time. Anon-significant decrease in blood glucose level of control or diabetic rats treated with lactoferrin was noticed. Significant elevation in serum MDA level and significant decrease in activities of oxidative enzymes (SOD, CAT and GP_x) and GSH level were observed in diabetic rats. Oral administration of lactoferrin for 30 days induced significant reduction in serum MDA and significantly reversed the activities of oxidative enzymes to near normal values. Results declared that the treatment of diabetic rats with lactoferrin (300 mg/ kg, orally) markedly counteracts the associated oxidative stress condition evidenced by restoration of oxidative enzyme activities as well as prevention of MDA accumulation and maintenance of normal GSH content.

Key words: Lactoferrin · Diabetes Mellitus · Oxidative Stress · MDA

INTRODUCTION

The hyperglycaemia throughout DM is the important mechanism that results in oxidative stress and enlarged production of reactive oxygen species (ROS) activate several pathways including glucose autoxidation, protein kinase-C activation, hexosamine metabolism, sorbitol formation and oxidative phosphorylation [1, 2]. These pathways contribute on to a rise in oxidative stress, that square measure related to a series of injury within the tissue throughout diabetic state.

The oxidative stress in DM might arise from excessive oxygen radical production from auto-oxidation of glucose [3] glycated proteins and glycation of antioxidative enzymes that limit their capability to detoxify oxygen radicals [4]. Additionally, hyperglycaemia might stimulate cytochrome P450-like activity by excessive nicotinamide A dinucleotide phosphate-oxidase (NADPH) created by glucose metabolism [5]. Moreover, ketosis might increase oxygen radical production in diabetic patients [6].

The balance between the speed of free radicals generation and elimination is very important. Excess cellular radical generation will be harmful [7] but, if there's a big increase in radical generation, or a decrease in radical elimination from the cell, oxidative cellular stress ensues [8]. There's a clinical proof that the generation of reactive oxygen species (ROS) will increase in each kind of DM which the onset of diabetes is closely related to oxidative stress [9].

Lactoferrin (Lf) is a conjugated protein found in numerous class body fluids, together with blood, tears, saliva and bile and is particularly exuberant in milk [10]. The potential of LF has been proved by its

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physiologically pleiotropic properties, as depicted by biophylactic responses like anti-inflammatory and anti-cancer effects [11].

In recent years, interests have conjointly been developed within the involvement of LF in metabolic reactions. LF supplementation improves lipid metabolism related to reductions within the contents of cholesterol and triacylglycerol, in the course of a restrictive impact on fat accumulation within the mesentery and liver in mice [12].

Recent studies have additionally shown an in depth relationship between LF and stress; LF exerts its anxiolytic and analgesic effects in the midst of a rise in nitric oxide production or activation of the -opioid system [13, 14]. These facts might counsel the advantage of LF for the correction of glucose metabolism and diabetic care. Much attention has been centered on suppression of oxidative stress by dietary natural antioxidants [15]. This study was planned to research the ameliorative action of lactoferrin administration on the oxidative stress condition associating DM in rats.

MATERIALS AND METHODS

Animals: Seventy Sprague-Dawley male rats of average weight 200 g were used. The animals were maintained at a controlled temperature of 24 ± 1°C with a 12-12 h light-dark cycle (light cycle, 07:00–19:00). They were allowed free access to water and standard chow ad libitum. The standard chow contains 24.9% protein, 4.6% fat, 4.5% fiber, 6.6% ash, 51.0% nitrogen free extract and consisted of 3.45 kcal/g. Forty rats were subjected for diabetes induction. Diabetes was induced by intraperitoneal injection (IP) of 60 mg/kg body weight of streptozotocin. Diabetes was verified by testing blood samples for hyperglycemia (>300 mg/dl), 48h post-induction. Thirty rats having blood glucose level exceeding 300 mg/dl were considered as diabetic rats. All rats (30 normal +30 diabetic) were left for 14 days without treatment, then classified into the following experimental groups. The animals were treated according to the national and international ethics guidelines stated by the ethics committee of Umm-Alqura university and all procedures and experiments were performed according to the protocol approved by it and the earliest scientifically justified endpoint was used in this study to prevent pain or distress in the experimental animals.

Experimental Groups: Experimental rats were divided into the following groups:

- Group I /control/saline: (n=15) they were orally administrated with 1 ml isotonic saline daily for 30 days.
- Group II /control / lactoferrin: (n=15) they were orally administrated with lactoferrin (Radiance Nutritional Company -New Zealand) / (300 mg/Kg/day) daily for 30 days.
- Group III /diabetic/saline: (n=15) they were orally administrated with 1 ml isotonic saline daily for 30 days.
- Group IV /diabetic / lactoferrin: (n=15) they were orally administrated with lactoferrin (300 mg/Kg/day) for 30 days.

Sampling and Techniques: At the end of the treatment period, individual fasting blood samples were collected at nine am from rats of all experimental groups under ether anesthesia by orbital sinus puncture. Measure of fasting plasma glucose level was performed by Accu-Check meter, Roche Diagnostics, GmbH, Germany, for monitoring of diabetic condition.

Blood was collected and centrifuged at 4000 revolutions per minute for five minutes. The serum collected was assayed for biochemical parameters, including catalase (CAT), superoxide dismutase (SOD), glutathione(GSH), glutathione peroxidase (GPx) and malondialdehyde (MDA). Serum catalase (CAT) activity was assayed by the method of Aeibi [16]. The reduced GSH level was measured by adopting the method described by Weckbecker and Cory [17]. The SOD assay measured all 3 types of SOD (Cu/Zn-, Mn- and Fe-SOD) and its activity was estimated according the spectrophotometric method, as described by Masnini [18]. The GPx activity was determined using Randox Laboratories kits (Antrim, UK). Lipid peroxidation end product MDA was measured by the method outlined by Esterbauer *et al.* [19].

Statistical Analysis: Data were analyzed using "ANOVA" test to analyze the differences among groups using general linear model procedure (SAS). Level of significance used in all results was ($p \leq 0.05$).

RESULTS

Table (1) shows that the blood glucose level was significantly elevated allover the experimental time in diabetic groups as compared to control groups (p= .05). Treatment with lactoferrin was associated with a decrease in its level, although it was insignificant.

Table 1: Blood glucose level of control and lactoferrin-treated rats

Blood glucose level	Control+saline	Control+lactoferrin	Diabetic+saline	Diabetic+lactoferrin
	90.20±4.05[a]	82.30±3.88[a]	325±11.45[b]	311±10.20[b]

Values are means ± SE, values having different letters are significantly different at $p \leq 0.05$.

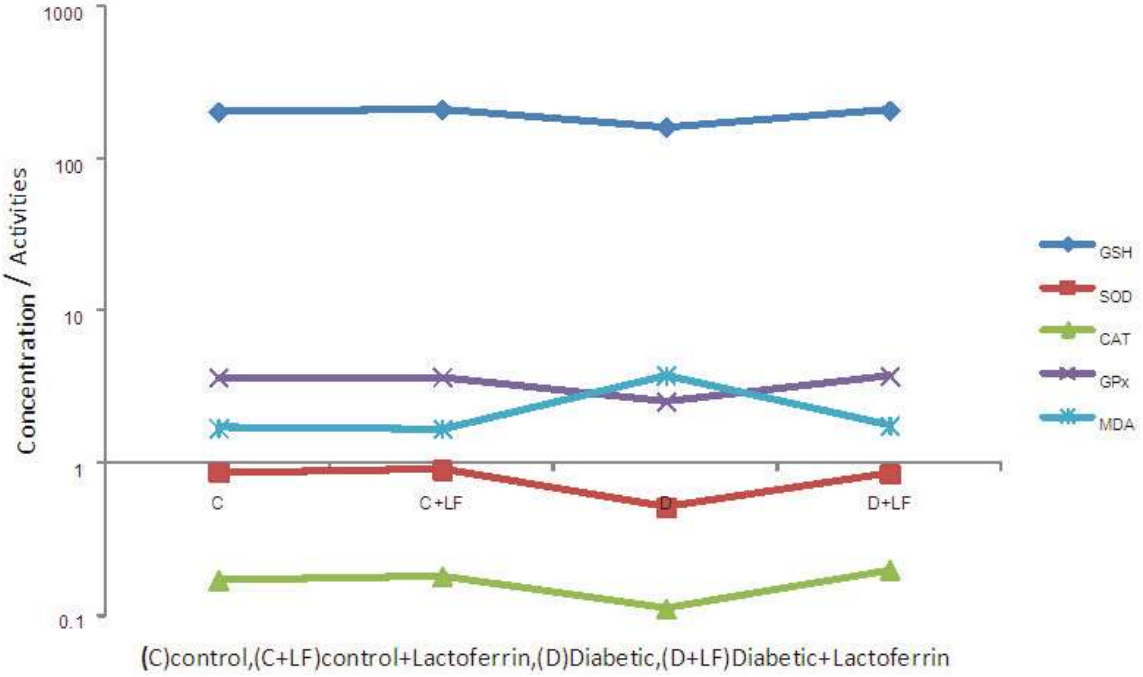

Fig. 1: Antioxidant parameters in serum of normal and diabetic rats after 30 days of Lactoferrin treatment

Fig. (1) represents the levels of GSH, SOD, CAT, GP_x and MDA in serum of control and experimental animals. A significant elevation in serum MDA was observed in diabetic rats when compared with control rats. Activities of oxidative enzymes (SOD, CAT and GP_x) and GSH level were significantly decreased in diabetic animals when compared with control rats. Oral administration of lactoferrin (300mg/kg body weight) for 30 days showed significant reduction in serum MDA and significantly reversed these enzymes to near normal values ($p \leq 0.05$).

DISCUSSION

Lipid peroxidation is one of the characteristic features of chronic diabetes. Physiologically, low concentrations of lipid peroxides are found in tissues. Karpen *et al.* [20] observed an elevated level of lipid peroxides in the plasma of diabetic rats. Lipid peroxide mediated tissue damage has been observed in the development of both types 1 and 2 diabetes. Nakakimura and Mizuno [21] have reported that the increased level of MDA is an index of lipid peroxidation. The obtained results showed that in diabetic animals the level of MDA was high in plasma, due to increased lipid peroxidation.

In lactoferrin treated diabetic rats, the MDA levels was low in plasma, which may be due to the free radical scavenging action of active ingredients in lactoferrin.

SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O2•-), which damages the membrane and biological structures [22]. CAT has been shown to be responsible for the detoxification of significant amounts of H_2O_2 [23]. SOD and CAT are the two major scavenging enzymes that remove the toxic free radicals in vivo. Reduced activities of SOD and CAT in plasma have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals (O2•-) and hydrogen peroxide [24]. Lactoferrin treated rats showed decreased lipid peroxidation that is associated with increased activity of SOD and CAT.

Gpx catalyzes the reduction of H_2O_2 to H_2O and O_2 at the expense of GSH. GPx activity is also reduced in diabetic condition. This may be due to inactivation of the enzyme involved in disposal of oxygen species and also insufficient availability of GSH [25]. The present study also observed the depleted levels of GSH in STZ diabetic rats and elevation of GPx after treatment with lactoferrin.

Treatment of diabetic rats with Lf markedly counteracts the oxidative stress status, as evidenced by restoration of plasma levels of SOD, CAT and PGx activities as well as prevention of MDA accumulation and maintenance of normal GSH plasma contents. In addition, many studies reported the antioxidant effect of Lf [26-28] and mentioned that the binding of Lf to the cells limited the process of membrane lipid peroxidation, because Lf is not entirely saturated and is capable to scavenge free iron radicals that are cytotoxic activators of the lipid peroxidation and oxidative stress and subsequently suppress free radical-mediated damage [29].

CONCLUSIONS

In conclusion, the results of the current study declared that the treatment of diabetic rats with Lf (300 mg/ kg, orally) markedly counteracted the associated oxidative stress condition evidenced by restoration of oxidative enzyme activities as well as prevention of MDA accumulation and maintenance of normal GSH serum content. These findings revealed that this protective potential of Lf is possibly through its antioxidant properties.

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