The effect of dietary bovine colostrum supplementation on serum malondialdehyde levels and antioxidant activity in alloxan-induced diabetic rats

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Abstract

Due to the range of its constituents, colostrum has been considered as a supplement for various diverse purposes. This study was conducted to examine the effect of supplementary bovine colostrum on serum malondialdehyde (MDA), antioxidant activity (AOA) and glucose in a diabetic rodent model. Sixty male Wistar rats were divided into 10 groups of six rats each for 40 days as follows: non-diabetic; diabetic; diabetic with 10%, 20% or 30% colostrum intake; non-diabetic with 10%, 20% or 30% colostrum intake; diabetic treated with insulin; and diabetic treated with glibenclamide. Although serum MDA levels showed a significant decrease in response to insulin (2.56 ± 0.31 µmol/L) and 10%, 20% or 30% colostrum intake (0.46 ± 0.04, 0.29 ± 0.06, 0.37 ± 0.09 µmol/L, respectively), the decrease was greater in the diabetic rats (3.92 ± 0.29 µmol/L) ($p < 0.01$). Significant changes were seen in the AOA of both insulin (0.78 ± 0.11 mmol/L) and glibenclamide (0.7 ± 0.08 mmol/L) treated rats compared to the diabetic rats (0.69 ± 0.1 mmol/L); however, AOA showed a significant increase in response to 10% (1.78 ± 0.11 mmol/L), 20% (1.57 ± 0.02 mmol/L) and 30% (1.75 ± 0.02 mmol/L) colostrum ($p < 0.001$). All treated groups showed a significant decrease in serum glucose levels compared to the diabetic group (391 ± 39.79 mg/dL) ($p < 0.01$). It seems that colostrum might be a beneficial dietary supplement for reducing serum MDA and glucose levels while increasing serum AOA in type1 diabetes mellitus.

Introduction

Various disorders are now attributed to oxidative stress (Jennings *et al*., 1991; de Diego-Otero *et al*., 2009). Oxidative stress occurs in an organism when the generation of oxygen-derived free radicals or non-radicals is high and antioxidant potential low (Bagis *et al*., 2005). This situation can lead to damage to cell components such as proteins, lipids and nucleic acids, with varying consequences. Oxidants can also attack double bonds in unsaturated fatty acids and apolipoproteins, other plasma proteins and DNA (Karpfenbauer *et al*., 1998; West, 2000).

Diabetes is a widespread chronic disease that is one of the most important disorders in both industrial and non-industrial societies. Since research showed an imbalance between oxygen-derived free radicals and non-radicals and antioxidant potential in diabetic patients, it has been postulated that oxidative stress might be involved in the development of diabetes mellitus and its consequences (Lipinski, 2001; Rahimi *et al*., 2005). It has been shown that there is an increase in oxidative stress as a result of free radical generation during autoxidation of glucose in both insulin dependent (type 1) and insulin independent (type 2) diabetes. It has also been suggested that individuals with higher levels of serum antioxidants have a lower risk of type 2 diabetes (LeRoith *et al*., 2004).

Both radical and non-radical oxidants can induce lipid peroxidation, particularly of lipoproteins that contain unsaturated fatty acids (Ferns and Lamb, 2004). There is evidence of lipoprotein independent oxidative modification of macromolecules in patients with diabetes (Karpfenbauer *et al*., 1998). In addition, experimental diabetes can be induced in rats by feeding them alloxan or streptozotocin (Grankrist *et al*., 1981; Asplund *et al*., 1984). It has been shown that alloxan works through generating reactive oxygen species that kill the islet cells.

Studies have shown beneficial effects of various antioxidant therapies in patients with diabetes, including vitamin E, water soluble derivatives of vitamin E, vitamin C, and combinations of vitamin E and N-acetyl cysteine, beta-carotene and selenium (Naziroglu and

Butterworth, 2005; Jain *et al*., 2009). Other types of antioxidant have also shown to be therapeutically effective in diabetes mellitus (Lipinski, 2001).

Several studies have been conducted on the relationship between levels of dietary antioxidant intake and the occurrence of cardiovascular diseases and related disorders in patients with diabetes (Lee *et al*., 2006; Al-Azzawie and Alhamdani, 2006). Both epidemiologic and experimental studies have shown an inverse relationship between dietary antioxidant intake and diabetes side effects (Montonen *et al*., 2004; Psaltopoulou *et al*., 2009). However, it has been suggested that monotherapy with a single antioxidant may be insufficient to counterbalance severe oxidative stress such as that seen in diabetes (Stevens *et al*., 2000).

Colostrum is a pre-milk substance produced immediately after birth. The useful properties of colostrum have been recognized since ancient times. More recently, it has been used as, for example, an immunomodulator, an antibacterial agent (Waaga-Gasser, 2007), an anti-inflammatory in rheumatoid arthritis and a vaccine carrier (Uruakpa *et al*., 2002). Since colostrum is a naturally balanced compound containing vitamins E, C and A, various minerals, amino acids with sulfide residues, and polypeptides, it has been suggested that it could serve as an antioxidant in the body (Butler, 1995). Indeed, the high antioxidant capacity of colostrum can protect newborns from an environment rich in oxygen after birth (Thapa, 2005).

To our knowledge, there is no published literature addressing the effect of dietary colostrum supplementation on the oxidative stress-related parameters in patients with diabetes. This study was undertaken with the objective of examining the effect of dietary intake of colostrum on serum malondialdehyde (MDA), antioxidant activity (AOA) and glucose in rats with alloxan-induced diabetes.

Materials and Methods

Animals were handled following the recommendations of the Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Iran concerning animal care. Bovine colostrum was freshly prepared by Talise Nemone Inc. (Tehran, Iran) from Holstein cows within a few hours of calving. The cows were in their second or third parturition. Colostrum was kept on ice while transferred to the Department of Biochemistry, University of Tehran, where it was aliquoted and kept at -80ºC until use. An aliquot was kept in liquid nitrogen for further analysis.

Sixty male Wistar rats (277 ± 48 g) were purchased from Razi Vaccine and Serum Institute (Tehran, Iran) and transported to the Department of Biochemistry, Faculty of Veterinary Medicine, University of Tehran. Rats were acclimatized with a dark–light cycle (12/12

h) at 22ºC for 10 days and fed normal chow diet prepared by Pars Dan Company (Tehran, Iran). Both diet and water were available ad libitum. The rats were then divided into 10 groups of three rats each. Each experiment was done in duplicate. Diabetes was induced by intraperitoneal injection of alloxan (150 mg/kg body weight) (Ahmed *et al*., 2005) and the development of diabetes was checked by measuring blood glucose concentration. Rats with blood glucose levels of > 150 mg/dL were considered to be diabetic (Para *et al*., 2005). Blood glucose levels were estimated by glucometer (Cleverchek TD-24209, Taiwan). All experimental groups received normal chow diet throughout the experiment while being treated for 40 days as follows:

1) diabetic group: diabetic rats received 0.9% saline solution through gavage
2) non-diabetic (healthy) group: healthy rats received 0.9% saline solution through gavage
3) non-diabetic with 10% colostrum intake group: healthy rats received bovine colostrum based on 10% of their body weight
4) diabetic with 10% colostrum intake group: diabetic rats received bovine colostrum based on 10% of their body weight
5) non-diabetic with 20% colostrum intake group: healthy rats received bovine colostrum based on 20% of their body weight
6) diabetic with 20% colostrum intake group: diabetic rats received bovine colostrum based on 20% of their body weight
7) non-diabetic with 30% colostrum intake group: healthy rats received bovine colostrum based on 30% of their body weight
8) diabetic with 30% colostrum intake group: diabetic rats received bovine colostrum based on 30% of their body weight
9) diabetic with insulin intake group: diabetic rats received insulin subcutaneously (9 IU/kg body weight/day) (Para *et al*., 2005)
10) diabetic with glibenclamide intake group: diabetic rats received oral glibenclamide (0.5 mg/kg body weight) (Marathe *et al*., 2006).

At the end of the experiment, the rats were deeply anesthetized with chloroform and bled through a cardiac puncture. Sera separated by centrifugation at $3,000 \times g$ were transferred immediately into liquid nitrogen while awaiting analysis. In all sera, MDA levels and total AOA were measured using spectrophotometric methods as described in our colleagues' previous work (Mokhber-Dezfouli *et al*., 2008). MDA levels were estimated by the thiobarbituric acid reaction according to the method of Ledwożyw *et al*. (Ledwozyw *et al*., 1986). Briefly, 1 mL of plasma was mixed with 2 mL of freshly prepared TCA-TBA-HCl reagent (30 g trichloroacetic acid, 0.75 g thiobarbituric acid and 4.2 mL concentrated HCl were

mixed and diluted to 200 mL with distilled water) and 1.5 µL butylhydroxytoluene (0.05%). This mixture was boiled for 30 min in a boiling water bath, cooled to room temperature and the *n*-butanol extractable layer centrifuged at $3000 \times$ g for 10 min. The supernatant was removed and its absorbance was read at 535 nm. An MDA standard curve was obtained using MDA bis (S4258497 537, Merck Company, Tehran, Iran). AOA was measured according to the method of Koracevic *et al.* (Koracevic *et al.*, 2001). A standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction, leading to the formation of hydroxyl radicals. These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS). Antioxidants from the added sample suppress the production of MDA or TBARS. The reaction was measured spectrophotometrically (UV-120-12, Shimadzu) at 532 nm and the inhibition of color development defined as the AOA.

These parameters were compared between the control and each treatment group using Student's t- test with Sigma Stat version 2.03 (Systat Software Inc., Richmond, CA, USA); α in all cases was 5% ($p < 0.05$).

Results

Values for MDA, AOA and glucose in diabetic and non-diabetic rats under the different treatment conditions are shown in Table 1. They show that alloxan induced a significant increase ($p < 0.001$) in the MDA levels of the treated rats compared to the non-diabetic rats.

Dietary supplements of colostrum decreased serum MDA in both diabetic and non-diabetic groups. Although 10% supplementation with colostrum caused no significant change to MDA, higher colostrum consumption (20% and 30%) caused a marked change ($p < 0.001$) In diabetic rats, however, all levels of colostrum supplementation led to a significant decrease ($p < 0.001$) in MDA. Although 20% colostrum led to a greater decrease in MDA than 10% colostrum, there was no significant difference between 20% and 30% intake. Insulin treatment caused a significant decrease in MDA levels ($p = 0.027$), but glibenclamide did not have such an effect.

AOA showed a significant decrease in the diabetic rats compared to the non-diabetic rats ($p < 0.001$). However, dietary intake of colostrum caused a significant increase in both diabetic and non-diabetic rats. We did not find any significant difference in the AOA among the diabetic and non-diabetic colostrum intake groups, and neither insulin nor glibenclamide produced any significant increase in AOA.

Alloxan caused a significant increase in glucose levels in diabetic rats (about 334%) compared to the non-diabetic rats. In the diabetic groups receiving 10%, 20% and 30% colostrums, there were approximately 39%, 79% and 73% decreases in glucose, respectively. The higher colostrum intake groups (20% and 30%) showed higher serum glucose concentrations than the lower intake group (10%). Although colostrum decreased the serum glucose of both diabetic and non-diabetic rats, its effect in the former. Both insulin and glibenclamide decreased serum glucose compared to the diabetic control group.

Discussion

As shown in Table 1, by the end of experiment MDA, AOA and glucose had all been restored to levels comparable to those of the normal controls. It is well documented that alloxan selectively destroys pancreatic ß-cells through hydroxyl radical toxicity. Interestingly, in the present study, we found an improvement in the glucose level of diabetic rats treated with colostrums, which returned to the baseline level. Renewal of the β-cells could be a causal factor in this improvement. Indeed, there is a balance between ß-cell generation and loss. Renewal of ß-cells in diabetes has been shown in several animal models. Gorray *et al.* (Gorray *et al.*, 1986) demonstrated the generation of ß-cells following alloxan injection in guinea pigs. Potentiation of glucose-induced insulin release and also peripheral uptake of glucose have been postulated as possible mechanisms for hypoglycemia in diabetic animals treated with certain plant derived therapeutic agents (Gonzalez *et al.*, 1992; Trivedi *et al.*, 2004). In the present study, we have shown significant decreases in MDA in rats with alloxan-induced diabetes treated with colostrum. Twenty percent and 30% of body weight colostrum intake per day prevented lipid

Table 1: Serum malondialdehyde (MDA) and antioxidant activity (AOA). Values (mean ± SEM) were compared between diabetic and treatment groups using Student's t-test. Insulin and glibenclamide treated groups were compared with the diabetic group.

Group	Non-diabetic	Diabetic	Non-diabetic + 10% colostrum	Diabetic + 10% colostrum	Non-diabetic + 20% colostrum	Diabetic + 20% colostrum	Non-diabetic + 30% colostrum	Diabetic + 30% colostrum	Diabetic + insulin	Diabetic + glibenclamide
MDA (µmol/L)	0.88 ± 0.12*	3.92 ± 0.29	0.77 ± 0.04*	0.46 ± 0.04*	0.38 ± 0.03*	0.29 ± 0.06*	0.36 ± 0.02*	0.37 ± 0.09*	2.56 ± 0.31*	3.94 ± 0.44
AOA (mmol/L)	1.23 ± 0.04*	0.69 ± 0.1	1.56 ± 0.11*	1.78 ± 0.11*	1.74 ± 0.11*	1.57 ± 0.02*	1.80 ± 0.07*	1.75 ± 0.12*	0.78 ± 0.11	0.7 ± 0.08
Glucose (mg/dL)	85.99 ± 7.77*	391 ± 39.79	94.52 ± 5.67*	239.35 ± 29.13**	126.74 ± 12.92*	83.42 ± 8.29*	115.11 ± 13.80*	105.56 ± 8.45*	83.91 ± 4.02*	125 ± 1*

*Significant difference at $p < 0.001$, **Significant difference at $p < 0.01$

peroxidation in diabetic rats. This potent effect of colostrum on serum MDA levels in diabetic rats could be due to synergism among different antioxidants to increase the AOA. This is in agreement with suggestions that dietary supplementation with a single agent is not sufficient to counterbalance all of the adverse effects of diabetes mellitus (Stevens *et al.*, 2000).

Colostrum contains several enzymatic (e.g. lactoperoxidase, catalase, superoxide dismutase) and non-enzymatic (e.g. vitamins A, E and C, lactoferrin) antioxidants (Przybylska *et al.*, 2007).The effects of some of these non-enzymatic antioxidants have been shown in the prevention of diabetes mellitus or the alleviation of its complications. Giannini *et al.* (Giannini *et al.*, 2007) showed that high dose vitamin E supplementation reduced plasma levels of markers of oxidative stress and improved antioxidant defense in young patients with type 1 diabetes mellitus. They showed that while, short term (2 months) dietary vitamin E intake did not have any beneficial effect, a longer duration of intake had a preventive effect on lipid peroxidation, based on plasma MDA measurements. However, different experiments have reported conflicting results for vitamin E therapy in patients with diabetes (Hamblin *et al.*, 2007). These controversies could be due to the type of vitamin E used, its dose and the duration of treatment. In the present study, we used natural animal products and were able to ignore considerations such as dose, type and duration that occur with vitamin E monotherapy. In line with the decrease in serum MDA levels, we found a significant increase in the serum AOA of diabetic rats treated with dietary colostrum. It has previously been shown that the antioxidant pool is drastically aggravated in hyperglycemia due to the persistent challenge by reactive oxidants and free radicals. Inouye *et al.* (Inouye *et al.*, 1999) suggested that an elevation in glucose concentration may depress natural antioxidant defense agents such as vitamin C and glutathione. Zarban *et al.* (Zarban *et al.*, 2009) have shown that colostrum has a greater antioxidant capacity than transitional and mature milk. Therefore, colostrum especially that taken immediately on calving, could be considered as a safe dietary supplement beneficial for people with diabetes mellitus. No adverse effects have been reported from colostrum added to rats' diets (Davis *et al.*, 2007).

In conclusion, this study demonstrated beneficial effects of dietary colostrum as a hypoglycemic and antioxidant agent in rats with alloxan- induced diabetes. Use of colostrum may be of prophylactic value in reducing complications resulting from oxidative stress in diabetes mellitus; however, further studies monitoring different oxidative stress related markers are needed.

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