International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.397.406

Research Article

Antidiabetic and Immunoprophylactic Effects of Camel Milk Filtrate and Bitter Gourd (*Momordica charantia*) Juice Against Alloxan-induced Oxidative Stress and Diabetes in Rats

[1]Ahmed M. Abdel-Salam and [2]Mona A. Al-Damegh

[1]Department of Dairy Science, National Research Centre, Dokki, Cairo, Egypt
[2]Department of Biology, College of Science and Art, Qassim University, Unaizah, Saudi Arabia

Abstract

Background and Objective: The increasing of environmental pollution and changing of the dietary habits and other lifestyle are associated with increasing the risk factors and oxidative stress. Dairy manipulation and traditional plant therapies are commonly prescribed in various countries. Therefore, the aim of the present study was to investigate the antidiabetic and immunoprophylactic effect of a combination of camel milk filtrate with bitter gourd (*Momordica charantia*). **Materials and Methods:** Fresh camel milk was subjected to membrane filtration system and milk filtrate was used. Bitter gourd was cut into small size cubes and put in a high speed electric blender to make juice. The biological evaluation of camel milk filtrate and bitter gourd juice against alloxan-induced diabetic rats were studied. The immunological analyses and some blood biochemical markers were carried out using official methods. **Results:** Administration of camel milk filtrate and bitter gourd juice showed a hypoglycemic and immunoprophylactic effect. Blood glucose levels were decreased in alloxan-induced diabetic rats fed on camel milk filtrate and bitter gourd juice groups compared with alloxan-induced diabetic group fed on basal diet (positive control). In addition, administration of camel milk filtrate and bitter gourd juice was associated with a reduction in the serum levels of Alanine aminotransaminase (ALT) and Aspartate aminotransaminase (AST), when compared with positive control. **Conclusion:** Administration of camel milk filtrate and bitter gourd juice could have hypoglycemic effect in alloxan-induced diabetic rats. This immunoprophylactic and protective effect probably due to micronutrients found in the combination and synergistic effect of camel milk filtrate and bitter gourd against oxidative stress and diabetes.

Key words: Membrane technology, camel milk filtrate, bitter gourd, oxidative stress, diabetes

Received: September 15, 2017 Accepted: December 17, 2017 Published: March 15, 2018

Citation: Ahmed M. Abdel-Salam and Mona A. Al-Damegh, 2018. Antidiabetic and immunoprophylactic effects of camel milk filtrate and bitter gourd (*Momordica charantia*) juice against alloxan-induced oxidative stress and diabetes in rats. Int. J. Pharmacol., 14: 397-406.

Corresponding Author: Ahmed M. Abdel-Salam, Department of Dairy Science, National Research Centre, Dokki, Cairo, Egypt Tel: 00201112291291

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The development of industrial technology and excessive use of the agricultural techniques, food additives, pesticides, pharmaceutical chemicals and heavy metals pollution increased the risk factors for oxidative stress. Diabetes mellitus (DM) is a major metabolic disorder characterized by chronic hyperglycemia as a result of a relative or absolute lack of insulin or its action[1].

Oxidative stress plays an important role in chronic complications of diabetes and is associated with increased lipid peroxidation[2]. An increasing in oxidative stress indices (lipid peroxidation and protein oxidation) was observed in the plasma of diabetic patients[3]. Besides, glucose autoxidation was found to be the cause of protein damage in the experimental model of DM and aging[4].

Camel milk is an important source of proteins for people who are usually living in the desert of the world. It was claimed to have protective health effects and therapeutic values. Moreover, camel milk is well known by its medical properties that have been widely exploited for human health[5] in a lot of developing countries[6]. The beneficial effects of camel milk, as can be depicted from various studies, can be attributed to many factors. Camel milk is considered to have anti-cancer[7], hypo-allergic[8] and anti-diabetic properties[9,10]. High contents of unsaturated fatty acids in camel milk contribute to its overall dietary quality[11,12]. Other components such as protective milk proteins (i.e., lactoferrin, immunoglobulins and lysozyme) were reported to play a central role in the determination of this properties[13,14].

The membrane filtration systems used worldwide in dairy industry since the 1970's. Ultrafiltration (UF) and reverse osmosis membrane systems implemented in the 1980's. Microfiltration (MF) is a low pressure-driven membrane filtration process, which is based on a membrane with an open structure allowing dissolved components to pass while most non-dissolved components are rejected by the membrane. In the dairy industry, microfiltration is widely used for bacteria reduction and fat removal in milk and whey as well as for protein and casein standardization. Ultra-filtration is characterized as having a molecular weight cut-off range from about 3000-75,000. In dairy factories most common cut-off is the dairy standard of 10,000 MW that is commonly used for fractionating whey proteins from lactose. In the dairy industry, microfiltration and ultrafiltration is used for a wide range of applications such as protein standardization of cheese milk, processing of milk powders, production of fresh cheese, concentration of milk proteins and decalcification of permeates as well as reduction of lactose in milk[15].

Momordica charantia commonly known as bitter gourd or bitter melon is a tropical and a subtropical climber of the family Cucurbitaceae. It is a green fruit, although bitter, that has been used to fight cancer, diabetes and many infectious diseases. It is one of the most favorite vegetables in China, Malaysia, India and tropical Africa. All parts of the plant, including the fruit taste is very bitter[16]. It has been reported that oral administration of *M. charantia* could lead to the secretion of insulin from endocrine pancreatic β cells[17]. In Ayurvedic medicine, various parts of *Momordica charantia* are recommended for many of certain diseases like anemia, blood diseases, cholera, ulcer, bronchitis, diarrhea, dysentery, sexual tonic and as a cure for gonorrhea[18].

The aim of the present study was to investigate the antidiabetic and immunoprophylactic effect of a combination of camel milk filtrate with bitter gourd (*Momordica charantia*) against alloxan-induced oxidative stress and diabetes in rats.

MATERIALS AND METHODS

Camel milk samples were obtained from healthy lactating animals in Al-Qassim area and then stored at -20°C until analysis. Bitter gourd (*Momordica charantia*) was purchased from Indian fresh fruits and vegetables market, Saudi Arabia. Chemicals and pure reagents were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA) and Roche Diagnostics (Roche Professional Diagnostics , Rotkreuz, Switzerland.

Production of camel milk filtrate: Fresh camel milk was subjected to membrane filtration system using a membrane with a molecular weight cut off allowed to pass some of protein and peptides in final milk filtrate. The QuixStand™ benchtop membrane filtration system was used in the present experiment to produce camel milk filtrate (Fig. 1). The configuration of this system is designed to accommodate a full range of hollow fiber ultrafiltration (UF) and microfiltration (MF) cartridges. The pore size of the UF cartridge is ranging from 1,000-750,000 nominal molecular weight cutoff (NMWC) and the pore size for MF cartridge is ranging from 0.1-0.65 micron. In the present study, MF cartridge was used at a pore size of 0.1 micron to produce camel milk filtrate. The obtained camel milk filtrate was collected, then pasteurized and stored in a sterile dark bottles at -4°C until use in the experiment.

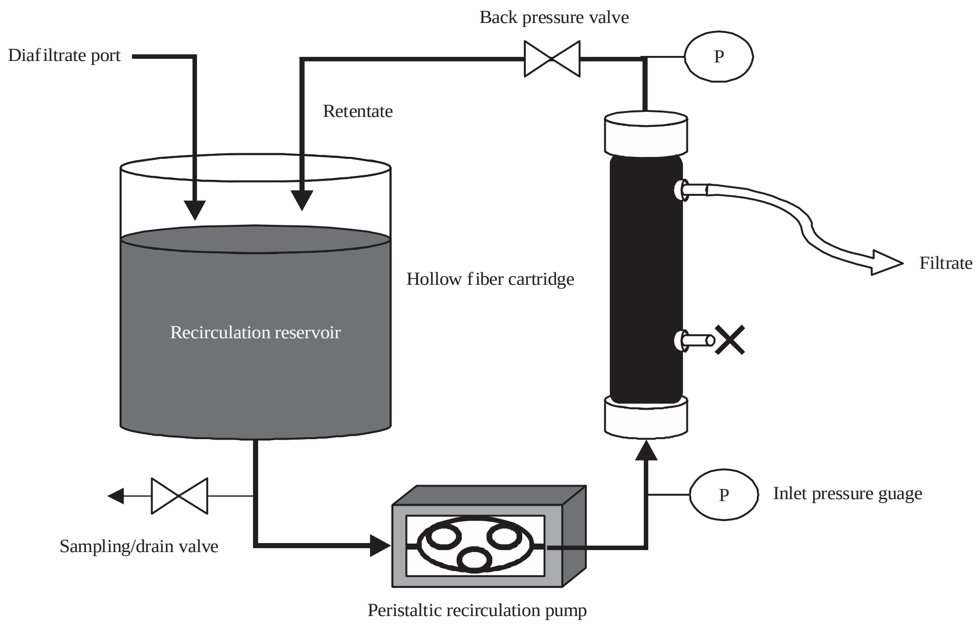

Fig. 1: QuixStand benchtop membrane filtration system was used in the experiment to form camel milk filtrate rich in protein and peptides

Preparation of aqueous extracts of bitter gourd (*Momordica charantia*) juice: Firstly, the outer green flesh of the bitter gourd was cut into small to medium-size cubes with a sharp knife. The seeds and white flesh were removed from the bitter gourd with a spoon to leave the green outer section, then put in a high speed electric blender to make juice. The final concentration of juice was 20% by adding distilled water. The extract juice was filtered twice through cheese-cloth (50% cotton/50%polyester) and was preserved in sterile dark bottles (500 mL) at -4°C for using in experiment.

Chemical composition of camel milk filtrate and bitter gourd juice: Chemical composition of camel milk filtrate and bitter gourd juice were carried out according the official methods of analysis[19].

Experimental animals: Forty male Swiss albino rats, weighing about 150±40 g were obtained from the Experimental Animal Unit, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Animals were housed in cages and assigned to be given a basal diet pellets (Protein 20.0%, fat 4.0%, fiber 4%, vitamin mixtures 1% and salt mixtures 4%) for 2 weeks for adaptation. Clean drinking water was allowed according to AIN-93 guidelines[20]. Animal procedures were performed in accordance with the ethics committee of Qassim University and according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. Animals were kept under standard conditions of temperature and humidity along the experimental period (42 days). Rats were randomly divided into five-test groups (n = 8 rats/group).

- Group 1 was kept as untreated, control group (negative control) fed on the basal diet only and normal saline
- Group 2-5 were treated with alloxan as inducer of diabetes
- Group 2 was fed on the basal diet only and used as a positive control
- Group 3 was fed on basal diet+camel milk filtrate replacing drinking water
- Group 4 was fed on basal diet+bitter gourd juice replacing drinking water
- Group 5 was fed on basal diet+camel milk filtrate+bitter gourd juice (1:1) replacing drinking water

The feeding experiment have been continued for 6 weeks. At the end of the experimental period and after an overnight fast, rats were anesthetized by exposure to an atmosphere of a ratio 1:2:3 of ethanol: chloroform: diethyl ether, respectively[21]. After anesthetizing rats were sacrificed and a blood sample from each rat was collected using blood capillary tubes. Blood samples were then centrifuged at 3500 rpm for 15 min. Sera were harvested in labeled tubes and deep frozen (-20°C) for biochemical analysis. This experiment have been conducted during 2014-2015.

Agarose gel electrophoresis of serum proteins: Changes in serum proteins of rats fed on camel milk filtrate and bitter gourd juice were monitored using agarose gel electrophoresis method reported by Werner and Reavill[22] using a kit and an automated SAS electrophoresis unit (Helena, UK). Electrophoresis was conducted at a voltage of 100 V for 12 min on a high resolution agarose gel. After electrophoresis, gels were transferred to SAS 4 unit (Helena, UK) for staining, de-staining and drying. Gels were then photo scanned and interpretation of the revealed peaks was carried out using a densitometer software (Platinum, Helena, UK).

Biochemical analysis: Blood glucose concentration were determined by the Haemo-Glukotest 20-800-R and measurements read with a Reflolux test (Boehringer-Mannheim)[23]. The serum levels of alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) were determined according to the method described by Reitman and Frankel[24]. Urea was determined according to the method of Tietz[25]. Creatinine was determined according to the method of Bousnes and Taussky[26]. Triglycerides were determined according to the method of Stein and Myers[27].

Statistical analysis: Mean, standard deviation and coefficient of variation of the obtained data were calculated and conducted according the method described by Miller and Miller[28].

RESULTS

The chemical composition of camel milk, bitter gourd juice and camel milk filtrate is shown in Table 1. Table 2 shows the glucose level in serum of alloxan-induced diabetes rats fed on camel milk filtrate and bitter gourd juice.

The obtained results showed that the glucose levels were decreased in serum of alloxan-induced diabetic rats fed on camel milk filtrate and bitter gourd juice groups compared with alloxan-induced diabetic group fed on basal diet (positive control). The mean values of glucose level (mg dL^{-1}) were 116.5±2.64, 121.25±5.188 and 114.5±8.88 in serum group fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with negative (114.75±3.77) and positive (399.25±68.67) control groups.

Table 3 and 4 show alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) levels in serum of alloxan-induced diabetes rats fed on camel milk filtrate, bitter gourd juice and their combination.

The mean values of ALT levels (U L^{-1}) in serum were 32±4.53, 30±3.57 and 31±4.83 when rats were fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with positive control (56±6.97). Also, the AST levels (U L^{-1}) in serum were141±12.47, 130±9.67 and 136±9.73 when rats were fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with positive control (192±11.43).

Table 5 shows the triglycerides levels (mg dL^{-1}) in serum of alloxan-induced diabetes rats fed on camel milk filtrate and bitter gourd juice. It can be observed that no significant effect of all treatments on the serum levels of triglycerides when compared with control.

Table 6 and 7 show the urea and creatinine levels in serum of alloxan-induced diabetes rats fed on camel milk filtrate and bitter gourd juice.

Table 1: Chemical composition of camel milk, bitter gourd juice and camel milk filtrate

Parameters	Camel milk	Bitter gourd juice 20%	Camel milk filtrate
Dry matter (D.M.) (%)	12.67	1.22	6.17
Moisture (%)	87.28	98.76	93.52
Fat (%)	3.30	0.06	0.02
Protein (%)	3.32	0.36	1.55
Carbohydrates (%)	5.10	0.51	3.72
Ash (%)	0.92	0.67	0.88
Freezing point	-0.580	0.140	-0.436
Density (g cm^{-3})	1.03	1.01	1.02
Fat/D.M. (%)	25.83	0.19	0.32
Protein/D.M. (%)	26.36	29.16	25.16
Carbohydrates/D.M. (%)	40.26	41.76	60.31
Ash/D.M. (%)	7.54	28.89	14.20

Table 2: Levels of blood glucose between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice

	Blood glucose (mg dL^{-1})				
Parameters	Min	Max	Mean	SD	CV
Negative control	112.0	120	114.70	3.77	3.28
Positive control	332.5	475	399.25	68.67	17.20
Camel milk filtrate group	114.0	120	116.50	2.64	2.27
Bitter gourd juice group	115.0	126	121.25	5.19	4.27
Camel milk filtrate+bitter gourd group	102.0	123	114.50	8.88	7.76

Min: Minimum values, Max: Maximum values SD: Standard deviation, CV: Coefficient of variation

Table 3: Levels of alanine aminotransaminase (ALT) activity between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice (U L^{-1})

	ALT(U L^{-1})				
Parameters	Min	Max	Mean	SD	CV
Negative control	18	27	22	4.34	19.80
Positive control	48	65	56	6.97	12.48
Camel milk filtrate group	26	37	32	4.53	14.47
Bitter gourd juice group	25	33	30	3.57	11.95
Camel milk filtrate+bitter gourd mixture	25	36	31	4.83	14.95

Min: Minimum values, Max: Maximum values, SD: Standard deviation, CV: Coefficient of variation

Table 4: Levels of aspartate aminotransaminase (AST) activity between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice (U L^{-1})

	AST (U L^{-1})				
Parameters	Min	Max	Mean	SD	CV
Negative control	103	145	125	17.40	13.87
Positive control	180	206	192	11.43	5.95
Camel milk filtrate group	127	155	141	12.47	8.86
Bitter gourd juice group	122	144	130	9.67	7.43
Camel milk filtrate+bitter gourd mixture	130	151	136	9.73	7.12

Min: Minimum values, Max: Maximum values SD: Standard deviation, CV: Coefficient of variation

Table 5: Levels of triglycerides between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice (mg dL^{-1})

	Triglycerides (mg dL^{-1})				
Parameters	Min	Max	Mean	SD	CV
Negative control	50.00	52.00	51.25	0.88	1.71
Positive control	48.67	54.67	50.96	2.83	5.55
Camel milk filtrate group	50.33	52.00	51.11	0.68	1.34
Bitter gourd juice group	47.67	57.67	51.50	4.82	9.36
Camel milk filtrate+bitter gourd mixture	48.33	53.33	50.42	2.11	4.19

Min: Minimum values, Max: Maximum values, SD: Standard deviation, CV: Coefficient of variation

Table 6: Levels of urea between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice (mg dL^{-1})

	Urea (mg dL^{-1})				
Parameters	Min	Max	Mean	SD	CV
Negative control	24	33	28	3.86	13.93
Positive control	15	23	19	3.32	17.66
Camel milk filtrate group	26	34	30	3.11	10.42
Bitter gourd juice group	24	28	26	1.50	5.92
Camel milk filtrate+bitter gourd mixture	25	31	27	2.84	10.43

Min: Minimum values, Max: Maximum values, SD: Standard deviation, CV: Coefficient of variation

Table 7: Levels of creatinine between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice (mg dL^{-1})

	Creatinine (mg dL^{-1})				
Parameters	Min	Max	Mean	SD	CV
Negative control	0.357	0.52	0.42	0.074	17.66
Positive control	0.371	0.53	0.45	0.092	20.38
Camel milk filtrate group	0.571	0.60	0.48	0.013	2.22
Bitter gourd juice group	0.36	0.40	0.38	0.015	4.07
Camel milk filtrate+bitter gourd mixture	0.56	0.60	0.47	0.015	2.66

Min: Minimum values, Max: Maximum values, SD: Standard deviation, CV: Coefficient of variation

The urea levels (mg dL^{-1}) in serum were 30±3.11, 26±1.50 and 27±2.84 in rats' groups fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with positive control (19±3.11). The highest levels of urea were observed when rats were given camel milk filtrate.

Table 8: Changes in albumin levels (%) between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice

	Albumin levels (%)				
Parameters	Min	Max	Mean	SD	CV
Negative control	41.40	42.40	41.96	0.38	0.91
Positive control	39.48	41.20	40.52 ↓	0.70	1.73
Camel milk filtrate group	44.40	45.91	45.12 ↑	0.61	1.35
Bitter gourd juice group	43.11	48.39	45.93 ↑	2.32	5.05
Camel milk filtrate+bitter gourd group	43.65	46.19	44.60 ↑	0.95	2.13

Min: Minimum values, Max: Maximum values, SD: Standard deviation, CV: Coefficient of variation, ↓: Decrease ↑: Increase

Table 9: Changes in α_1-globulin (alpha1-globulin) levels (%) between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice

	α_1-globulin (alpha1-globulin) levels (%)				
Parameters	Min	Max	Mean	SD	CV
Negative control	13.12	16.65	15.12	1.30	8.64
Positive control	12.79	17.81	16.02 ↑	1.96	12.25
Camel milk filtrate group	13.30	15.54	14.52 ↓	0.98	6.81
Bitter gourd juice group	10.78	14.99	13.57 ↓	1.65	12.17
Camel milk filtrate+bitter gourd group	12.09	14.43	13.49 ↓	0.86	6.38

Min: Minimum values, Max: Maximum values, SD: Standard deviation, CV: Coefficient of variation, ↓: Decrease, ↑: Increase

Table 10: Changes in α_2-globulin (alpha$_2$-globulin) levels (%) between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice

	α_2-globulin (alpha$_2$-globulin) levels (%)				
Parameters	Min	Max	Mean	SD	CV
Negative control	5.91	8.79	7.25	1.05	14.50
Positive control	7.76	8.96	8.44 ↑	0.44	5.24
Camel milk filtrate group	5.82	6.47	6.12 ↓	0.28	4.61
Bitter gourd juice group	6.57	9.01	7.56 ↓	0.89	11.90
Camel milk filtrate+bitter gourd group	5.97	7.44	6.63 ↓	0.55	8.38

Min: Minimum values, Max: Maximum values, SD: Standard deviation, CV: Coefficient of variation, ↓: Decrease, ↑: Increase

Table 11: Changes in β-globulin (beta-globulin) levels (%) between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice

	β-globulin((beta-globulin levels (%)				
Parameters	Min	Max	Mean	SD	CV
Negative control	18.95	21.17	20.32	0.85	4.20
Positive control	19.24	20.26	19.70 ↓	0.37	1.91
Camel milk filtrate group	18.59	19.80	19.02 ↓	0.46	2.42
Bitter gourd juice group	16.94	21.13	18.95 ↓	2.01	10.56
Camel milk filtrate+bitter gourd group	17.70	20.51	19.39 ↓	1.03	5.34

Min: Minimum values, Max: Maximum values, SD: Standard deviation, CV: Coefficient of variation, ↓: Decrease, ↑: Increase

Table12: Changes in γ-globulin (gamma-globulin) levels (%) between alloxan-induced diabetes rats groups fed on camel milk filtrate and bitter gourd juice

	γ-globulin (gamma-globulin) levels (%)				
Parameters	Min	Max	Mean	SD	CV
Negative control	10.32	13.15	11.72	1.24	10.62
Positive control	10.44	16.72	12.98 ↓	2.34	18.08
Camel milk filtrate group	15.26	23.68	18.92 ↑	3.09	16.36
Bitter gourd juice group	12.72	15.17	13.58 ↑	0.96	7.14
Camel milk filtrate+bitter gourd group	16.35	19.61	17.53 ↑	1.23	7.05

Min: Minimum values, Max: Maximum values, SD: Standard deviation, CV: Coefficient of variation, ↓: Decrease, ↑: Increase

The creatinine levels (mg dL^{-1}) in serum were 0.48±0.013, 0.38±0.015 and 0.47±0.015 in the rats' groups that were fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with both negative (0.42±0.074) and positive control groups (0.45±0.092).

Table 8-12 show change in serum proteins (%) of alloxan-induced diabetic rats that were fed on camel milk filtrate, bitter gourd juice and their mixture. The obtained results showed that the levels of albumin were 45.12±0.70, 45.93±2.32 and 44.6±0.95 when rats fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with both negative (41.96±0.91) and positive (40.52±0.70) control groups (Table 8).

The serum levels of α_1-globulin were 14.52±0.98, 13.57±1.65 and 13.49±0.86 in rats' groups fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with negative (15.12±1.30) and positive (16.02±1.96) control groups (Table 9). The serum levels of α_2-globulin were 6.12±0.28, 7.56±0.89 and 6.63±0.55 in rats' groups fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with negative (7.25±1.05) and positive (8.44±0.44) control groups (Table 10). The serum levels of β-globulin were 19.02±0.46, 18.95±2.01 and 19.39±1.03 in rats' groups fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with negative (20.32±0.85) and positive (19.70±0.37) control groups (Table 11). The serum levels of γ-globulin were 18.92±3.09, 13.58±0.96 and 17.53±1.23 in rats' groups fed on camel milk filtrate, bitter gourd juice and their mixture, respectively, compared with negative (11.72±2.34) and positive (12.98±2.34) control groups (Table 12).

DISCUSSION

In arid and semi-arid regions, camel milk represents an important dietary component with high nutritional values for humans[29]. Ultrafiltration process of milk by using 100,000 molecular weight cut off membrane produces a milk rich in α-lactalbumin, β-lactoglobulin and peptides. Milk and whey contains many proteins with excellent properties which are also of a high nutritional value. Now-a-days, a large amount of whey protein concentrate with high nutritional value are available due to popularity of membrane filtration technology in dairy industries. Therefore, whey protein concentrate has become increasingly important in the field of functional food, dietetic and pharmaceutical industries[30]. Surprisingly, It has been found that the ratio of α-lactalbumin to β-lactoglobulin in the filtrate is substantially greater than that in the starting material when a 10,000 molecular weight cut off membrane were used. In addition, the ratio of α-lactalbumin/β-lactoglobulin in the filtrate using a 100,000 molecular weight cut off membrane was not significantly lower than that obtained from a 50,000 molecular weight cut off membrane. It was expected that the rate of permeation of α-lactalbumin through the 100,000 molecular weight cut off membrane is significantly higher than that through a 50,000 molecular weight cut off membrane[30].

The obtained results indicated that feeding alloxan-induced diabetic rats on camel milk filtrate and bitter gourd juice might showing hypoglycemic, immunoprophylactic and decreased blood glucose level. This positive effect probably due to many potentially bioactive compounds found in both camel milk filtrate and bitter ground juice. There are elevation in blood glucose levels in positive control group. Administration of camel milk filtrate and bitter gourd juice in alloxan-induced diabetic rats caused a high reduction in the blood glucose levels compared to the diabetic control rats.

The increased blood urea in male rats fed on camel milk filtrate compared with control groups could be due to higher micronutrients and protein content in camel milk filtrate as resulted from membrane filtration system. Also, elevated blood urea is known to be a function of or related to increased protein catabolism in mammals and/or the conversion of ammonia to urea as a result of increased synthesis of arginase enzyme involved in urea production. Serum urea levels are known to be elevated by high-protein diets. Although daily production of creatinine is relatively constant and serum levels are unaffected by diet, but its levels are generally used as an indicator of kidney function[31].

Table 8-12 show change in serum proteins (%) of rats fed on camel milk filtrate and bitter gourd juice and treated with alloxan for inducing diabetes. Induction of diabetes in rats by alloxan resulted in a decrease in the concentration of albumin, beta and gamma-globulins and slight increase in alpha globulin as can be noticed in positive control group. This reduction in albumin and gamma-globulins levels were shifted after giving rats camel milk filtrate and bitter gourd. In addition, the levels of alpha and beta globulins in the same group were similar with that found in the negative control group.

The data revealed that administration of camel milk filtrate and bitter gourd juice augmented the immune system by elevating albumin and gamma-globulins which might overcame negative effects of alloxan. Previously, patients with certain autoimmune and allergic diseases, such as systemic lupus, multiple sclerosis, autoimmune thyroiditis or atopic eczema, often show an increased lymphocyte stimulation by oxidative stress agents *in vitro*[32]. A hallmark of such autoimmune induction is the accompaniment of an immune shift, in which there is usually an initial skewing toward a Th2-like immune environment[33].

Research studies on camel milk and its relation with diabetes indicated that drinking camel milk daily decreases the blood glucose level and reduces insulin requirement by 30%[34]. These studies revealed that camel milk may provide an insulin-like protein in a different form than in other mammals and/or delivers some other therapeutic compounds that boosts the health of diabetic patients. However, the mechanism is not yet fully understood[34].

Clinical studies reported that the administration of insulin is incapable of overcoming mucosal barriers and is degraded by digestive enzymes before it enters the bloodstream[35]. On other hand, the insulin-like protein of camel milk might resist degradation in the stomach by nanoparticles and might be absorbed efficiently into blood stream to reach the target[36]. This might be due to the fact that camel milk does not coagulate in an acidic environment and it has a higher buffering capacity than the milk of other ruminants[9]. Also, it was reported that no differences were noted in the sequence of camel milk insulin-like protein and its digestion pattern in comparison with the other sources of milk[36]. Other studies found that amino acid sequence of some camel milk protein is rich in half cysteine which has a superficial similarity with the insulin family of peptides[37]. Hamers-Casterman *et al.*[38] and Agrawal *et al.*[34] reported that the small size and weight of camel milk immunoglobulin may offer enormous potential through interaction with the host cell protein and cause an induction of regulatory cells and finally result in a downward regulation of the immune system and β-cell salvage. Bottomley[30] reported that some of whey proteins (α-lactalbumin and β-lactoglobulin) and milk peptides were passed into the permeate using ultrafiltration membrane system with a 30,000-50,000 molecular weight cut-off. In addition, the concentration of these proteins and peptides were increased when a 100,000 molecular weight cut off membrane was used.

Momordica charantia (bitter gourd or bitter melon) is a popular plant and used for the treating diabetes related condition in Asia, South America, the Caribbean, India and east Africa. Ayurvedic studies showed that fruits part of *Momordica charantia* contains an array of biologically active plant chemicals including proteins, flavonoids, triterpens, saponins, steroids, balkaloids and organic acids. This could explain the anti-bacterial, anti-fungal, anti-parasitic, anti-viral, anti-fertility, anti-tumorous, hypoglycemic and anti-carcinogenic properties of this plant[39-40]. Also, it is a potent hypoglycemic agent due to alkaloids, insulin like peptides and a mixture of steroidal sapogenins known as charantin[10].

CONCLUSION

It is concluded that used camel milk filtrate produced from the microfiltration cartridges with pore sizes 0.1 micron and was a rich in low molecular weight protein, peptides and micronutrients. Administration of a combination of camel milk filtrate produced by membrane technology and bitter gourd juice decreased blood glucose level in rats and showed a hypoglycemic and immunoprophylactic characteristics. This effect may be due to the bioactive components found in camel milk filtrate and bitter gourd juice. Further studies are needed to clarify and identify the mode of action of each of these bioactive components.

SIGNIFICANCE STATEMENT

This study discovers the antidiabetic and immunoprophylactic effect of a combination of camel milk filtrate with bitter gourd (*Momordica charantia*) against alloxan-induced oxidative stress and diabetes in rats. The combination of camel milk filtrate with bitter gourd decreased the blood glucose levels and improves the immunoprophylactic and protective properties against oxidative stress and diabetes.

ACKNOWLEDGMENTS

The authors would like to thank Professor El-Agamy E. I professor of Dairy and immunology in Applied Medical Sciences Department, Community College, Qassim University, Saudi Arabia and Professor Abd El-Salam M. H. Professor of Dairy Science, National Research Centre, Egypt and Editors of Journal of Egyptian Dairy Science for their ongoing cooperation and suggestions. Also, authors would like to thank Dr. Hamad, E. M. for revising and editing language of this manuscript. This study did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

REFERENCES

1. Kumar, P.J. and M. Clark, 2002. Textbook of Clinical Medicine. Saunders Publ., London, UK., pp: 1099-1121.
2. Elangovan, V., E. Shohami, I. Gati and R. Kohen, 2000. Increased hepatic lipid soluble antioxidant capacity as compared to other organs of streptozotocin-induced diabetic rats: A cyclic voltammetry study. Free Radical Res., 32: 125-134.
3. Sato, Y., N. Hotta, N. Sakamoto, S. Matsuoks, N. Ohishi and K. Yagi, 1979. Lipid peroxide level in plasma of diabetic patients. Biochem. Med., 21: 104-107.
4. Hunt, J.V., C.C. Smith and S.P. Wolff, 1990. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes, 39: 1420-1424.
5. Kenzhebulat, S., B. Ermuhan and A. Tleuov, 2000. Composition of camel milk and its use in the treatment of infectious diseases in human. Proceedings of the 2nd Camelid Conference on Agroeconomics of Camelid Farming, September 8-12, 2000, Sheffield, England, pp: 101.

6. Mal, G., D.S. Sena, V.K. Jain and M.S. Sahani, 2006. Therapeutic value of camel milk as a nutritional supplement for Multiple Drug Resistant (MDR) tuberculosis patients. Isr. J. Vet. Med., 61: 88-91.
7. Magjeed, N.A.A., 2005. Corrective effect of milk camel on some cancer biomarkers in blood of rats intoxicated with aflatoxin B1. J. Saudi Chem. Soc., 9: 253-264.
8. Shabo, Y., R. Barzel, M. Margoulis and R. Yagil, 2005. Camel milk for food allergies in children. Isr. Med. Assoc. J., 7: 796-798.
9. Agrawal, R.P., D.K. Kochar, M.S. Sahani, F.C. Tuteja and S.K. Ghorui, 2004. Hypoglycemic activity of camel milk in streptozotocin induced diabetic rats. Int. J. Diabetes Dev. Countries, 24: 47-49.
10. Agarwal, M. and R. Kamal, 2004. *In vitro* clonal propagation of *Momordica charantia* L. Indian J. Biotechnol., 3: 426-430.
11. Karray, N., C. Lopez, M. Ollivon and H. Attia, 2005. La matiere grasse du lait de dromadaire: Composition, microstructure et polymorphisme Une revue. OCL Oleagineux Corps Gras Lipides, 12: 441-448.
12. Konuspayeva, G., E. Lemarie, B. Faye, G. Loiseau and D. Montet, 2008. Fatty acid and cholesterol composition of camel's (*Camelus bactrianus*, *Camelus dromedarius* and hybrids) milk in Kazakhstan. Dairy Sci. Technol., 88: 327-340.
13. El-Agamy, E.I., 2006. Camel Milk. In: Handbook of Milk of Non-Bovine Mammals, Park, Y.W. and G.F.W. Haenlein (Eds.). Blackwell Publishing, Oxford, UK., ISBN-13: 9780470999721, pp: 297-344.
14. Konuspayeva, G., B. Faye and G. Loiseau, 2009. The composition of camel milk: A meta-analysis of the literature data. J. Food Compos. Anal., 22: 95-101.
15. Pouliot, Y., 2008. Membrane processes in dairy technology-from a simple idea to worldwide panacea. Int. Dairy J., 18: 735-740.
16. Taylor, L., 2002. Technical Data Report for Bitter Melon (*Momordica charantia*), Herbal Secrets of the Rainforest. 2nd Edn., Sage Press Inc., Los Angeles, London.
17. Jeevathayaparan, S., K.H. Tennekoon and E.H. Karunanayake, 1995. A comparative study of the oral hypoglycaemic effect of *Momordica charantia* fruit juice and tolbutamine in streptozotocin induced graded severity diabetes in rat. Int. J. Diabetes, 3: 99-108.
18. Kirtikar, K.R. and B.D. Basu, 1987. Indian Medicinal Plants. Bishen Singh Mahendra Pal Singh, Dehra Dun, India.
19. AOAC., 1990. Official Method of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
20. Reeves, P.G., F.H. Nielsen and G.C. Fahey Jr., 1993. AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition *Ad hoc* writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.
21. Wawersik, J., 1991. History of anesthesia in Germany. J. Clin. Anesth., 3: 235-244.
22. Werner, L.L. and D.R. Reavill, 1999. The diagnostic utility of serum protein electrophoresis. Vet. Clin. North Am.: Exotic Anim. Pract., 2: 651-662.
23. Brodrick, J.M., P.S. Colloby and E.F. Legg, 1987. Comparison of two new ward-based glucose meters. Pract. Diabetes Int., 4: 170-172.
24. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
25. Tietz, N.W., 1970. Fundamentals of Clinical Chemistry. 1st Edn., W.B. Sounders, Philadelphia.
26. Bousnes, R.W. and A.A. Taussky, 1945. On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 158: 581-591.
27. Stein, E.A and G.L. Myers, 1995. National cholesterol education program recommendations for triglyceride measurement: Executive summary. The national cholesterol education program working group on lipoprotein measurement. Clin. Chem., 41: 1421-1426.
28. Miller, J.C. and J.N. Miller, 1992. Statistics for Analytical Chemistry. 2nd Edn., Ellis Horweed, New York, London, Tokyo.
29. FAOSTAT., 2013. Camel milk (whole, fresh) production (tonnes). FAO Statistics Division, Rome, Italy. http://faostat.fao.org/site/569/default.aspx#ancor
30. Bottomley, R.C., 1991. Process for obtaining concentrates having a high α-lactalbumin content from whey. U.S. Patent 5,008,376, April 16, 1991. https://www.google.com/patents/US5008376
31. Myers, G.L., W.G. Miller, J. Coresh, J. Fleming, N. Greenberg and T. Greene, 2006. Recommendations for improving serum creatinine measurement: A report from the laboratory working group of the national kidney disease education program. Clin. Chem., 52: 5-18.
32. Prochazkova, J., I. Sterzl, H. Kucerova, J. Bartova and V.D. Stejskal, 2004. The beneficial effect of amalgam replacement on health in patients with autoimmunity. Neuroendocrinol. Lett., 25: 211-218.
33. Hudson, C., L. Cao, J. Kasten-Jolly, J. Kirkwood and D. Lawrence, 2003. Susceptibility of lupus-prone NZM mouse strains to lead exacerbation of systemic lupus erythematosus symptoms. J. Toxicol. Environ. Health Part A, 66: 895-918.
34. Agrawal, R.P., S. Saran, P. Sharma, R.P. Gupta, D.K. Kochar and M.S. Sahani, 2007. Effect of camel milk on residual β-cell function in recent onset type 1 diabetes. Diabetes Res. Clin. Pract., 77: 494-495.
35. Mullaicharam, A.R., 2014. A review on medicinal properties of camel milk. World J. Pharm. Sci., 2: 237-242.
36. Malik, A., A. Al-Senaidy, E. Skrzypczak-Jankun and J. Jankun, 2012. A study of the anti-diabetic agents of camel milk. Int. J. Mol. Med., 30: 585-592.

37. Beg, O.U., H. von Bahr-Lindstrom, Z.H. Zaidi and H. Jornvall, 1986. Characterization of a camel milk protein rich in proline identifies a new β-casein fragment. Regul. Pept., 15: 55-61.
38. Hamers-Casterman, C., T. Atarhouch, S. Muyldermans, G. Robinson and C. Hammers *et al*., 1993. Naturally occurring antibodies devoid of light chains. Nature, 363: 446-448.
39. Beloin, N., M. Gbeassor, K. Akpagana, J. Hudson, K. de Soussa, K. Koumaglo and J.T. Arnason, 2005. Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. J. Ethnopharmacol., 96: 49-55.
40. Zafar, R. and Neerja, 1991. *Momordica charantia*-a review. Hamdard Medicus, 34: 49-61.