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Research Article

ANTIOXIDANT AND ANTI STRESS BIOMARKERS OF SOME NUTRACEUTICALS IN ALLOXAN - INDUCED DIABETIC RATS

Alofi M.T., *Zaki A.A., Abdel-Rahman H.A and El Tigani EA.
Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, University of Qassim, Qassim, P.O.Box 6622 Buraidah 51452, Saudi Arabia
Author for Correspondence

ABSTRACT
Amelioration of oxidant and stress biomarkers of diabetes mellitus (DM) is the keyto better generate new therapy. The aim of the current study was to investigate the efficacy of aqueous garlic and onion extracts and camel milk in alloxan (ALX)-induced diabetic rats with respect to body gain, glucose, antioxidant defenses, anti-inflammatory cytokines (IL-4 and IL-5), liver function tests, lipid profile, insulin and C-peptide and pancreatic histopathology. For this purpose, 60 rats were divided into five groups: normal control, diabetic control (ALX), and ALX plus treatment groups (ALX + garlic, ALX + onion and ALX + camel milk). Serum levels of glucose, ALT, AST, ALP, TGs and cholesterol were significantly increased in ALX –induced diabetic group compared with normal group. This was accompanied with reduction of Hb, C-peptide, insulin, SOD, GSH-PX, CAT, IL-4 and IL-5. The recorded drop in blood glucose levels for camel milk, garlic and onion groups were 25.75%, 19.82% and 7.93% respectively when compared to the initial level after diabetes induction. ALX- plus treatments were significantly decreased serum liver enzymes, oxidative markers and lipid profile versus each one alone. In the same time, ALX-induced inflammation was also mitigated via elevation of IL-4 and IL-5. The findings showed that garlic, onion, and camel milk treatments demonstrate a protective effect in ALX diabetic model of by modulation of oxidative and stress biomarkers. Whereas the pancreatic sections from ALX-plus treatment groups indicated the protective response. Further detailed studies are required for the evaluation of the exact protective mechanism of each treatment against diabetic complications in animal models.

Keywords: *Diabetes, Antioxidant, Anti Stress, Nutraceuticals*

INTRODUCTION
For several years, allium species such as onions and garlic and camel's milk have enjoyed special reputation as therapeutic and prophylactic agents around the world. However, there is a little information about the effect of them on the oxidant and stress diabetic biomarkers.
DM is one of the endocrine glands diseases in human and animal. About 6.3% of world populations live with diabetes. Diabetic number is predicted to reach 300 million by 2025 (Ali and Agha, 2009). Two strategies of medication all over the world to handle the oxidant and stress biomarkers in vivo aretraditional medicines and pharmaceutical drugs.
The pharmaceutical drugs used in diabetic therapy are either too expensive or have undesirable side-effects or contraindications (Pari and Amarnath, 2004).
Therefore, the search for more effective and safer agents has continued to be an area of active research to ameliorate and/or halt the progression of complications. Traditional medicine is known for its usefulness for treatment (Sboui *et al.*, 2010).
Type 1 DM is characterized by destruction of pancreatic islet β-cells and loss of insulin secretion. This subsequently leads to the liberation of pro-inflammatory cytokines and reactive oxygen species (Nicole *et al.*, 2010; Delmastro and Piganelli, 2011). DM in domestic animals has been most commonly reported in dogs (Kimmel *et al.*, 2000), horses (Philips *et al.*, 2012) and llamas and alpacas (Middleton *et al.*, 2013). Treatment is a combination of art and science, due in part to the many factors that affect the diabetic state and the animal's response (Renee *et al.*, 2010).
Hyperglycemia induces the overproduction of oxygen free radicals and consequently increases the protein and lipid oxidation (El Faramawy and Rizk, 2011; Samanthi *et al.*, 2011). Islet β-cells are highly

susceptible to oxidative stress because of their reduced levels of endogenous antioxidants (Saleh *et al.*, 2011). Increasing evidence has implicated a role for oxidative stress biomarkers in mediating diabetes-associated complications.

Oxidative stress biomarkers have been reported in the form of activities of enzymatic antioxidants, such as Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px) (Salar- Amoli *et al.*, 2009) and Catalase (CAT) (Saleh *et al.*, 2011).

The ant diabetic- properties of camel milk have been demonstrated in several studies. Malik *et al.*, (2012) have reported a unique camel milk health benefit in diabetic patients. These researchers have demonstrated that using camel milk has improved the long-term glycemic control and led to reduction in doses of insulin in patient with type-1 diabetes.

Thus, the challenge for ameliorating oxidation and stress biomarkers developing an effective antioxidant therapy of diabetes-associated complications would be the goals of this research.

MATERIALS AND METHODS

Animal Models: Male albino Wistar rats, weighing between 180-200 g were housed at the Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia under hygienic conditions. The animal rooms were supplied by clean plastic cages and the animals were allowed to acclimatize to the laboratory environment for two weeks under laboratory conditions of photoperiod (12-h light&12-h dark cycle), minimum relative humidity of 40-45 % and temperature of 23 ± 2 ◦C. The rats were provided *ad libitium* with tap water.

All rats were received a commercial diet obtained from General Company of Feed Silo and Powder Mint two weeks before starting the experiment. The diet formulated to furnish all the nutrient requirements recommended by (NRC, 1985) for rats (Soybean meal 18.0%, Ground yellow corn 21.5%, Barley 10.0%, Wheat bran 14.0%, Hay 29.5%, Protein supplement 5.0 %, Vitamins & minerals mixture 0.1%, Lime stone 1.4%, Common salt 0.3%, Ca 0.1%, Lysine, meth. & cyst 0.1%). The experimental protocol was duly approved by the Animal Ethics Committee of the Institute and met the Guidelines for Care and Use of Animals in Scientific Research.

Materials: Camel milk samples were collected from a camel farm. All lactating camels were consumed the same type of food. The milk was collected in the morning in sterile screw bottles and kept on ice during transportation to the laboratory where milk bottles will be stored at 4°C. It was administered in a dose of 33 ml/kg body weight for each rat daily by oral cannula. Aqueous garlic and onion extracts were prepared from locally available bulbs according to Mahesar *et al.*, (2010).

The bulbs were peeled on crushed ice and 50 g of peeled bulbs were cut into small pieces and homogenized in 70 ml of cold, sterile normal saline in the presence of some crushed ice. The homogenization was carried out in a blender at high speed using 30-second bursts for 10 minutes. The homogenized mixture was filtered 3 times then centrifuged at 2000 rpm for 10 minutes and the clear supernatant was diluted to 100 ml with normal saline.

The concentration of this preparation was considered to be 500 mg/ml on the basis of the weight of the starting material (50g/100 ml). The aqueous extract was stored in small aliquots at 4 °C until use (Mahesar *et al.*, 2010).

Experimental Design: Diabetes was induced in fasting rats 12 h by a single intraperitoneal injection of freshly prepared ALX (120 mg/kg body weight, dissolved in 0.9% saline, Sigma Chemicals, USA). All rats were given 5% glucose during the following 24 h in drinking water.

Blood glucose was measured daily with standard glucometer (Accu-CHEK, Roche, Germany) at the next seven days. After 48 h of ALX treatment, rats with marked hyperglycemia (fasting blood glucose over 200 mg/ dl) will be selected for the study and considered as diabetic.

Rats were then divided into five groups (n=12), as follow: (G1) normal control rats not receive any treatment. (G2): control diabetic rats, receive ALX only. (G3): diabetic rats receive onion solution daily for 28 days. (G4): diabetic rats receive garlic solution daily for 28 days. (G5): diabetic rats receive camel milk daily for 28 days.

Sampling: Body weights of all groups were separately measured and recorded throughout the experimental period. Two blood samples were collected from each overnight-fasted rats from the inner can thus, of the eye using capillary tubes under mild ether anesthesia every week for four successive weeks for estimations. One sample was collected in dry tubes and left for 30 minutes at room temperature to clot and then centrifuged at 3,000rpm for 10 min. Sera harvested, labeled and stored deep-frozen (-20ºC) until used.

The another sample was collected in EDTA-tubes for hematological analysis in Beckman Coulter Clinical Chemistry AU analyzer. At the end of the experiment, grouped rats were anesthetized by diethyl ether, euthanized and sacrificed. For routine paraffin wax histopathological examination pancreatic specimens were taken fixed in 10% formal saline, processed and finally stained with Hematoxylin and Eosin stain (H&E).

Blood Biochemical Estimation: Serum was used for the determination of glucose, total protein and albumin levels using SPECTRUM kits. Globulin level was obtained by subtracting albumin from total protein of the same samples. The activity of the liver enzymes AST, ALT and ALP was determined by using Linear Chemicals. S.L. Kits. Triglyceride and cholesterol of serum were measured by Kits from LINEAR chemicals and HUMAN respectively. Interleukin-4 (IL-4) and Interleukin-5 (IL-5) were assayed by kit that pre-coated with an antibody specific to IL-4 and IL-5 respectively (CUSABIO BIOTECH CO., LTD. Lot: 004152648 and 004152649).

The standard curves of cytokines were constructed before measurements. The antioxidant activity of serum was determined by the measurement of activity of enzymes GSH-PX (BIODIAGNOSTIC Kits, CAT. No 2578); SOD (BIODIAGNOSTIC Kits, CAT. No. 2563) and CAT (BIODIAGNOSTIC Kits, CAT. No. 2552).

Quantitative determination of rat C-peptide levels in serum was done by solid phase direct sandwich ELISA Kits (SE120040-1KT. Lot No. CPT4779, Sigma Aldrich)**.** Quantitative determination of rat Insulin levels in serum was done by the solid phase two-site enzyme immunoassay Insulin ELISA Kits. (SE120069-1KT. Lot No. INS4565, Sigma Aldrich).

Obtained data were calculated and statistically analyzed by SPSS 19 version for Windows. All data were recorded on an individual basis. Data were expressed as means ± SD.

RESULTS AND DISCUSSION

Results

ALX treated group showed a significant decrease in the body weight gain % than control group during the four weeks of the experiment. The treatment with camel milk alleviate gain to the same values of control one. The treatment with garlic and onion extracts showed a significant decrease in the body weight gain % than control group at the end of experiment indicating non improvement (table 1).

The blood glucose levels of the ALX group increased significantly when compared to the control rats. The treated groups showed variety of drop in blood glucose levels when compared to the initial blood glucose level after 72 hrs.of diabetes induction.

The drop was obviously recorded for camel milk, garlic and onion groups were 25.75%, 19.82% and 7.93% respectively (table 2).

The total serum protein was lower in ALX group than that in control group at the 28^{Th} day. While, it increased in garlic treated group at the 7^{th} day and camel milk treated one at the 28^{Th} day in comparison with ALX group.

Decreasein serum albumin and globulin were recorded in ALX group relative to control at the 7^{th} and 28^{Th} daysfor albumin and at the 7^{th} day for globulin.

The treatment with camel milk were recorded a significant raise in the globulin level when compared to the ALX group at the same day (table 3).

ALX group showed a significant higher cholesterol and triglycerides level as compared to the control one. Treatment with garlic (at the 21^{th} and 28^{th} days) significantly diminish cholesterol level relative to ALX group (table 4).

Serum activities of ALT, AST and ALP were found to be significantly increased in ALX group as compared to control one at the 21th day; at the 21th and 28th days and at 21th and 28th days of the present study for the three enzymes respectively.

In contrast, the treatment with onion (at the 21th day), garlic (at the 14th and 21th days), and camel milk (at the 21th day) were recorded a significant decline in the ALT levels. For AST, the treatment groups with onion, garlic or camel milk (at the 7th and 14th days), were recorded a significant decline in the AST levels relative to ALX group at the same day. While, ALP levels were found to be significantly decreased with the treatment with garlic (at the 21th and 28th days), and camel milk (at the 7th, 21th and 28th days) relative to ALX group (table 5).

ALX group illustrated a significant lower hemoglobin concentration as compared to the control rats as well as to other treatments (Data not shown).

ALX group showed a significant lower GSH-PX, SOD and CAT activities as compared to the control rats throughout the experimental period.

Treatment with garlic (at the 21th day) and camel milk (28th day) were significantly increase GSH-PX activity when compared to the ALX group. Also, the overall mean of SOD was showed a significant elevation in garlic treated group and CAT in onion and camel milk treated groups in comparison with ALX group (table 6).

The analysis of IL-4 and IL-5 levels revealed a significant decrease in ALX group (at 7th and 28th days) as compared to control one. The treatment with onion (at 7th and 21th days), camel milk (at 14th and 21th days) showed a significant elevation in IL-4 concentrations whereas, IL-5 concentrations were found to be significantly increase in treatment groups with onion (at 28th day), garlic (at the 28th days), camel milk (at 28th day) in comparison with ALX rats at the same day.

The overall mean of IL-5 were showed a significant increase in camel milk treated groups in comparison with ALX group (table 7).

Insulin and C-peptide estimation revealed a significant decrease in ALX group as compared to control one. The treatment with garlic (at 21th days), and camel milk (at 21th and 28th days) showed a significant elevation in insulin concentration.

C-peptide levels were found to be significantly increase in treatment groups with onion (at 21th and 28th day), garlic (at the 21th days) and camel milk (at 21th and 28th day) in comparison with ALX group at the same day (table 8).

The histopathological features of paraffin wax pancreatic sections from control rats were noted a normal well defined encapsulated Langerhans islets normally distributed within the acinar portion (Figure 1A). While, ALX-treated group were showed sever pathological changes characterized by pyknosis, karyorrhexis, karyolysis and necrotic vaculation (Figure 1B). ALX – plus treated group sections were noted a variable slight to moderate degenerative pathological response (Figure 1 C, D, and E) in comparison to control.

Discussion

ALX - induced diabetes was associated with the characteristic loss of body weight, which was due to increased muscle wasting from loss of proteins (AI Abayomi *et al.*, 2011). We observed that the weight loss was attenuated by camel milk treatment, which might be a reflection of the improved health as previously reported by Thomson *et al.*, (2007).

The blood glucose levels of the ALX group continued to increase significantly throughout the experimental period.

ALX is selectively toxic to insulin producing pancreatic β cells because it preferentially accumulates in it through uptake via the GLUT2 glucose transporter (Etuk, 2010). The treatment groups of the current study showed variety of drop in blood glucose levels when compared to the initial blood glucose level after 72 hrs. diabetes induction.

Camel milk group recorded the highest drop while the lowest drop obtained after onion treatment. The hypoglycemic potential of camel milk was previously evaluated in patients with DM (Agrawal *et al.*, 2011). This was attributed to the low degree of phosphorylation of the caseins in camel milk (Shori,

2012). Insulin-like protein in milk protein could be protected in the stomach and absorbed efficiently into blood stream to reach the target (Malik *et al.,* 2012).

The total serum protein, albumin and globulin were lower in ALX group than that in control group. While, total serum protein increased in garlic treated group at the 7th day and camel milk treated one at the 28Th day in comparison with ALX group.

The treatment with camel milk were recorded a significant raise in the globulin level when compared to the ALX group at the same day.

This data are in agreement with Hasan and Abdulsattar (2015) who observed a reduction in protein concentration in sera of diabetic patients. Such reduction was reported to occur in inflammatory process and chronic inflammatory diseases.

ALX - induced diabetic rats showed a significant higher cholesterol and triglycerides levels as compared to the control one. Treatment with aqueous garlic extracts significantly diminish cholesterol level relative to ALX group.

This data agree with the previous works of Chidiebere and James (2011) who reported that the hypolipidaemic and hypocholestrolemic activities of garlic on experimental animal models and humans could be attributed to allicin and its derivative compounds.

Serum ALT, AST and ALP activities were determined to evaluate the hepatic functions. These enzymes activities were found to be significantly increased in ALX group as compared to control one. The time of increase in the activities of the liver enzymes was differ. The result give an indication on the hepatotoxic effect of ALX (Najla *et al.,* 2012).

The results of Lucchesi *et al.,* (2013) & Lucchesi *et al.,* (2015) revealed that changes in blood liver enzymes and the morphological and ultra structural lesions found in the livers of animals were closely correlated to DM-induced stress in liver cells. In contrast, the treatment with onion, garlic and camel milk (at the 21th day) were ameliorated the ALT and AST activities. While, ALP was alleviated with the treatment with garlic and camel milk. The beneficial health effects of camel milk were extended to the liver function as reported by Hamad *et al.,* (2011).

El-Din *et al.,* (2014) showed that combined administration of garlic and onion produced a better and significant decrease in liver serum liver enzymes in non-alcoholic fatty liver disease rats. Recently, Moodley *et al.,* (2015) suggested that garlic might ameliorate STZ-induced hepatocyte injury in diabetic rats.

Diabetic rats showed a significant lower GSH-PX, SOD and CAT activities as compared to the non-diabetic control rats throughout the experimental period. Treatment with garlic and camel milk were significantly increase GSH-PX activity when compared to the ALX group. In addition, the overall mean of SOD was showed a significant elevation in garlic treated group and CAT in onion and camel milk treated groups in comparison with ALX group.

This date are in agreement with Chiu *et al.,* (2005) who reported that the activity of the GSH -PX was decreased in plasma of chemically induced diabetic animals and rats. Diabetes was associated with a decrease in SOD (Khan *et al.,* 2015) in animal studies.

The protective effects of camel milk might be attributed to its antioxidant activity (Shori and Baba, 2012 & Shori, 2013). It has been reported that camel milk possesses high levels of vitamins content (Al-Humaid *et al.,* 2010).

These vitamins are antioxidants that are useful in preventing tissue injury associated with toxic agents such as ALX (Shori, 2015) and thereby remove free radicals.

The analysis of IL-4 and IL-5 levels revealed a significant decrease in ALX group as compared to control one. The treatment with onion and camel milk showed a significant elevation in IL-4 levels whereas, IL-5 levels were found to be significantly increase in onion, garlic and camel milk treated groups. The 2 cells (including IL-4, and IL-5) was protective against diabetes progression in rodents. Hence, numerous studies have revealed that treatment of mice model of type 1diabetes with IL-4 delays the onset of spontaneous diabetes and reduces its incidence (Kretowski *et al.,* 2000). Pancreatic expression of IL-4, moreover, completely prevents diabetes in mice (Jeker *et al.,* 2012).

Insulin and C-Peptide levels revealed a significant decrease in ALX group as compared to control one throughout experimental period. The data enforces the previous study of Kanchana *et al.,* (2011) who reported that STZ-induced diabetic rats showed significant reduction in the levels of insulin and C-Peptide. This might be due to the destruction of the pancreatic -cells and thereby induces hyperglycemia. Lebedev *et al.,* (2007); Nagy and Mohamed (2014) indicated that a single dose of ALX to adult male albino rats was suitable to induce hypoinsulinemia state. A significant increase in the levels of plasma insulin and C-peptide observed in milk administered diabetic rats in the present study might be due to the increased pancreatic secretion of insulin from the existing remnant -cells (Nagy and Mohamed, 2014). In addition, the results agree with El- Said *et al.,* (2010) who found that the mean serum insulin level was significantly higher for diabetic rabbits treated with camel milk for 4 weeks than for untreated diabetic rabbits and insulin-treated diabetic rabbits.

The histopathological features of diabetic rat sections were showed sever pathological changes, a results agree with Lebedev *et al.,* (2007) & Nagy and Mohamed (2014) who indicated that a single dose of ALX to adult male albino rats was suitable to induce histological changes of the islets of Langerhans characterized appearance. While treated diabetic rats sections were noted a variable slight to moderate pathological response in compare to the features of pancreatic sections from sound control rats which were noted normal well defined encapsulated Langerhans islets distributed within the acinar portion without any degenerative or necrotic changes.

In summary, the findings in this study show that garlic, onion, and camel milk treatments demonstrate a protective effect in the ALX model of diabetes by modulation of oxidative and stress biomarkers. Detailed studies are required for the evaluation of the exact protective mechanism of each treatment against diabetic complications in animal models.

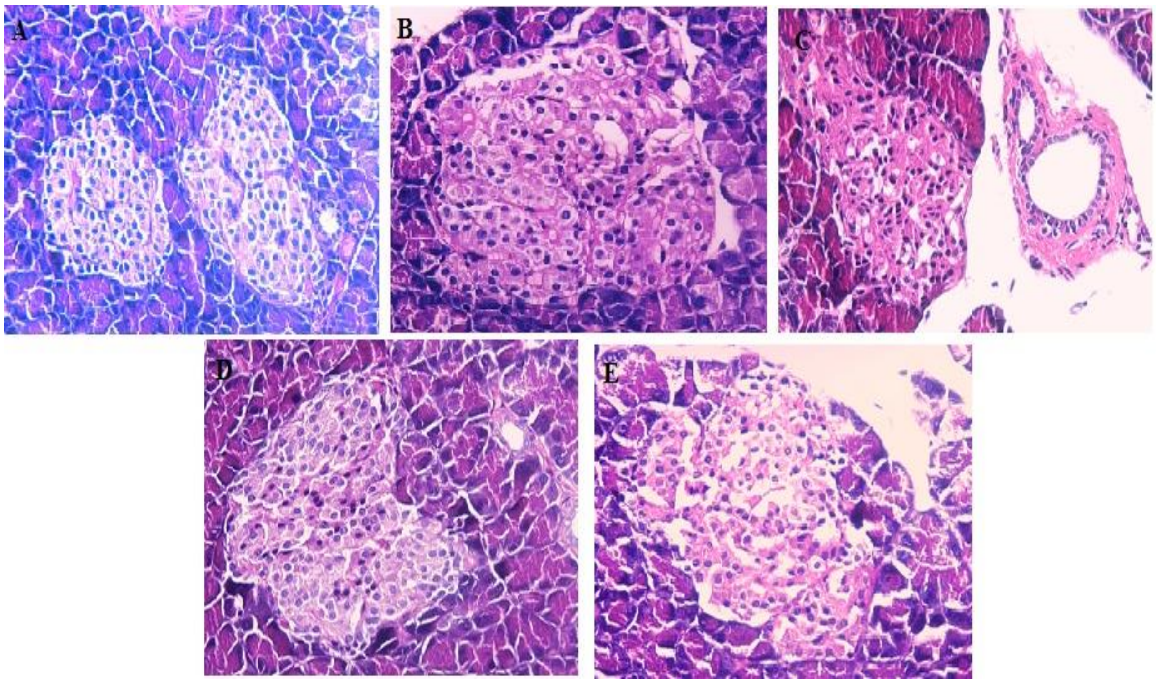

Figure (1): Histopathology of Pancreatic Sections (H&E. 400X Magn)
A: Control Rats Noted Normal Well Defined Encapsulated Langerhans Islets Distributed within the Acinar Portion
B: ALX- Diabetic Rat Section Showed Sever Pathological Changes (Pyknosis and Vaculation)
C-D-E: ALX-Plus Treated Diabetic Rat Section (Garlic, Onion Extract and Camel Milk) were Noted a Variable Slight to Moderate Pathological Response

Table 1: Body Weight Changes (g) and Body Weight Gain (g and %) in ALX Rats Treated with Aqueous Garlic and Onion Extracts and Camel Milk

Sampling/ Grouping	Initial body weight	Day 7	Day 14	Day 21	Day 28	Overall mean	Loss or gain (g)	Loss or gain %
Control	181.33±2.69	190.12±2.02	198.35±4.37	208.87±3.26	215.37±4.38	198.81±3.34	45.69±3.28	12.70±1.34
ALX	185.625±4.41	200.87±3.31	198.12±6.08	202.75±1.74	198.125±2.09	197.108±3.53	15.81 c ±3.23	6.48 a±1.05
ALX+Onion	194.75±3.52	198.37±3.50	209.125±4.47	215.25±3.543	224.25±4.61	208.35±3.93	30.554**±3.18	4.129*±0.61
ALX+Garlic	195.12±3.34	197.62±2.06	203.62±2.95	210.25±4.750	218.87±3.28	205.13±3.27	24.75*±4.62	2.94*±0.63
ALX+Milk	188.13±3.66	191.62 ±2.99	212.37±11.67	220.55±2.21	228.125±8.29	208.161 ±5.76	41.014**±4.71	11.176*±2.05

Table 2: Glucose Changes (mg/dl and %) in ALX Rats Treated with Aqueous Garlic and Onion Extracts, Camel Milk

Sampling/ Grouping	Day 7	Day 14	Day 21	Day 28	Overall mean	After 72 hrs.	Changes in level	Changes in level %
Control	123.47 ±12.82	112.12 ±6.44	127.38±9.08	111.13±8.39	118.52 ±9.18	118.47 ±7.82	-11.35±2.0	10.002±1.04
ALX	354.921c±12.35	387.540c±17.130	418.432c±11.29	365.643c±10.654	371.543±13.761	358.289±10.285	+19.62±2.24	5.05±0.54
ALX+ Onion	306.472±13.22	311.219±17.31	306.929±14.69	311.189±12.00	306.532±14.35	328.743±13.61	-18.29±2.7	7.93±1.01
ALX+Garlic	291.784±16.083	276.200±14.36	285.160±15.90	301.191*±17.11	282.083±14.36	378.542±16.61	-75.59±5.53	19.82±1.05
ALX+Milk	242.469**±14.028	271.041±12.34	284.388±19.77	250.023**±9.13	256.980**±12.82	341.329±14.76	-89.44±4.73	25.75±2.5

Table 3: Total Protein (g/dl), Albumin (g/dl), and Globulin (g/dl) Changes in ALX Rats Treated with Aqueous Garlic and Onion Extracts and Camel Milk

Sampling/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
Total Protein	Control	7.216±0.32	6.043±1.60	7.842±0.97	7.748±0.60	7.212±0.87
	ALX	7.397±0.76	7.830±0.91	7.738±0.80	6.116a±0.68	7.170±0.79
	ALX +Onion	7.785±0.53	7.467±0.93	7.124±0.30	6.250±0.11	7.156±0.46
	ALX +Garlic	8.790*±0.32	7.701±0.56	7.389±1.91	7.191±0.17	7.767±0.74
	ALX +Milk	6.318±0.06	7.132±0.39	7.398±0.64	7.945**±0.66	7.198±0.44
Albumin	Control	3.205±0.95	3.605±0.04	3.704±0.11	4.585±0.16	3.774±0.32
	ALX	4.563a±0.75	3.515±0.52	3.001±0.18	3.727a±0.03	3.501±0.27
	ALX +Onion	4.604±0.69	4.049±0.25	3.201±0.44	3.298±0.11	3.788±0.37
	ALX +Garlic	3.494±0.67	3.051±0.15	4.078±0.33	3.008±0.96	3.407±0.23
	ALX +Milk	4.456±0.63	3.038±0.07	3.018±0.25	3.789±0.85	3.575±0.35
Globulin	Control	4.583±0.26	3.316±1.15	4.223±0.62	3.313±0.73	3.858±0.69
	ALX	3.422a±0.53	3.037±0.82	3.894±0.42	3.199±1.28	3.388±0.76
	ALX +Onion	3.698±0.65	3.925±1.10	3.274±0.44	2.446±1.06	3.335±0.81
	ALX +Garlic	3.605±0.63	3.000±1.00	3.182±0.42	4.200±0.99	3.496±0.76
	ALX +Milk	4.568*±0.59	3.985±0.93	4.129±0.44	3.007±0.96	4.322*±0.52

Table 4: Cholesterol (mg/dl) and Triglyceride (TG) (mg/dl) Changes in ALX Rats Treated with Aqueous Garlic and Onion Extracts and Camel Milk

Sampling/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
Cholesterol	Control	116.822±7.07	120.908±4.30	126.816±8.16	111.997±5.23	119.135±6.19
	ALX	118.955±11.21	128.462±4.53	113.197±9.46	142.618c±7.12	125.808±8.08
	ALX +Onion	114.043±7.68	88.187±9.94	92.512±9.84	79.162*±17.5	93.476±11.24
	ALX +Garlic	88.683±6.91	87.862±7.64	71.032*±6.18	80.712*±9.55	82.072**±7.57
	ALX +Milk	97.695±9.47	81.348*±6.19	92.452±6.38	91.856±6.80	90.837±7.21
TGs	Control	67.118±7.10	64.582±3.52	60.797±4.28	69.315±4.86	65.453±4.94
	ALX	87.206±9.12	71.782±4.45	74.953±3.83	86.216a±5.47	80.039a±4.121
	ALX +Onion	60.651±5.43	62.440±5.21	71.576±4.52	82.625±3.02	69.323±4.54
	ALX +Garlic	74.231±4.54	64.905±8.15	68.335±4.39	97.672±4.84	76.285±5.484
	ALX +Milk	84.827±5.75	83.533±8.30	75.454±8.95	79.097±5.29	80.727±7.07

Table 5: Alanine Aminotransferase ALT (U/dl), Aspartate Aminotransferase AST (U/dl) and Alkaline Phosphatase ALP (U/dl) Activity Changes in ALX Rats Treated with Aqueous Garlic and Onion Extracts and Camel Milk

Sampling		Day 7	Day 14	Day 21	Day 28	Overall mean
ALT	Control	37.458±6.672	35.676±6.265	31.333±5.901	39.936±4.340	36.101±5.794
	ALX	39.525±4.116	47.182±4.410	51.810b±5.781	41.749±2.329	45.066±4.159
	ALX +Onion	38.644±3.100	37.805±4.719	32.774*±4.697	40.735±5.254	37.489±4.4425
	ALX +Garlic	30.054±2.514	32.076*±4.998	32.891**±3.144	41.213±4.766	34.058*±3.855
	ALX +Milk	33.610±3.958	34.440±2.608	33.577*±4.925	40.074±4.102	35.425*±3.898
AST	Control	26.752±2.713	23.891±2.142	26.496±1.203	21.252±2.040	24.597±2.024
	ALX	32.018±4.700	24.036±2.351	38.895b±3.184	36.396c±3.808	32.836a±3.110
	ALX +Onion	27.110*±2.849	33.043*±1.627	34.813±13.205	31.755±24.521	31.680±10.550
	ALX +Garlic	24.136*±2.153	39.560±12.271	35.279±11.566	31.371*±1.764	32.586±6.938
	ALX +Milk	24.535**±3.02	28.697±2.063	36.356±1.464	25.419*±1.926	28.751*±1.120
ALP	Control	54.978±8.613	60.303±8.671	64.998±8.330	61.895±7.324	60.543±8.234
	ALX	64.234±6.311	58.689±9.893	81.176a±4.082	79.613a±6.031	70.928±6.579
	ALX +Onion	56.731±9.663	58.366±10.292	60.155±10.499	60.597±6.748	58.962±9.301
	ALX +Garlic	53.606±6.642	58.219±8.295	59.622**±6.406	61.063*±7.880	58.127*±5.305
	ALX +Milk	44.441*±6.600	66.113±10.324	63.369*±10.389	61.160*±5.906	58.770±8.304

Table 6: Glutathione Peroxidase (GSH -PX -U/ml), Superoxide Dismutase (SOD- U/ml) and Catalase (CAT - U/ml) Activity Changes in ALX Rats Treated with Aqueous Garlic and Onion Extracts and Camel Milk

Sampling/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
GSH -PX	Control	6.011±0.38	8.93±1.15	7.07±2.32	9.88±1.23	7.97±0.67
	ALX	5.560±0.91	5.00±1.21	4.31a±1.71	5.07a±2.41	4.98a±0.96
	ALX +Onion	5.46±0.73	6.12±0.06	7.40±0.20	6.82±0.42	6.46±0.41
	ALX +Garlic	5.66±0.97	6.16±1.06	8.50*±1.28	5.76±1.56	6.52±1.21
	ALX +Milk	5.59±0.98	7.07±1.054	6.45±1.25	8.58*±1.49	6.92*±0.19
SOD	Control	127.54±15.27	126.39±13.26	126.41±15.97	122.32±14.44	125.66±14.73
	ALX	75.72a±12.48	88.72a±14.81	101.04±14.82	83.37a±8.45	87.21a±7.64
	ALX +Onion	127.61±14.17	111.90±9.31	120.28±14.49	113.94±12.43	118.43±12.60
	ALX +Garlic	124.36±11.32	120.35±12.42	122.08±14.20	111.08±14.72	125.66*±11.73
	ALX +Milk	102.22±14.24	80.27±12.63	88.43±10.28	81.93±14.90	89.21±12.64
CAT	Control	117.40±10.10	126.11±9.23	122.71±8.48	127.46±8.83	123.42±7.16
	ALX	91.94±13.92	95.17±13.11	92.41±11.24	104.71±12.23	96.06a±6.62
	ALX +Onion	123.48±6.06	118.24±7.79	108.32±6.812	117.74±11.10	116.9*±7.9437
	ALX +Garlic	97.68±6.06	96.88±8.78	85.35±7.83	103.68±5.64	95.90±5.08
	ALX +Milk	127.74±6.51	117.54±10.73	116.53±5.54	119.12±6.04	120.23*±7.21

Table 7: Interleukin-4 (IL-4 pg/ml) and Interleukin-5 (IL-5 pg/ml) Changes in ALX Rats Treated with Aqueous Garlic and Onion Extracts and Camel Milk

Sampling/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
IL-4	Control	16.73±0.53	15.27±0.32	15.36±0.33	16.81±0.48	16.04±0.42
	ALX	13.15b±0.61	14.17±0.55	14.37±0.46	14.17a±0.54	13.96a±0.54
	ALX +Onion	16.89*±0.44	15.22±0.59	16.31*±0.58	16.16±0.43	16.14*±0.51
	ALX +Garlic	13.37±0.62	16.01±0.39	15.94±0.55	14.21±0.67	14.88±0.56
	ALX +Milk	14.01±0.58	16.21*±0.41	16.79*±0.48	16.11±0.58	15.78±0.515
IL-5	Control	16.73±0.53	14.92±0.34	14.48±0.23	14.456±0.23	15.148±0.33
	ALX	13.12a±0.62	13.45±0.61	14.47±0.29	12.37a±0.58	13.16a±0.32
	ALX +Onion	14.54±0.23	14.25±0.33	14.00±0.24	14.87±0.35	14.41±0.29
	ALX +Garlic	14.72±0.25	14.76±0.33	14.47±0.31	14.62±0.39	14.64±0.32
	ALX +Milk	14.97±0.29	14.73±0.19	14.83±0.27	15.09**±0.36	14.91*±0.28

Table 8: Insulin (uU/ml) and C-Peptide (ng/ml) Changes in ALX Rats Treated with Aqueous Garlic and Onion Extracts and Camel Milk

Sampling/ Grouping		Day 7	Day 14	Day 21	Day 28	Overall mean
Insulin	Control	16.34±1.32	13.67±0.84	16.47±1.044	14.25±1.04	14.57±2.01
	ALX	11.46a±1.72	9.05a±1.254	8.87c±0.97	8.33b±1.01	9.83a±1.16
	ALX +Onion	9.45±1.322	10.57±1.865	11.64±2.433	11.65±2.520	11.48±1.511
	ALX +Garlic	11.32±1.11	9.65±2.05	13.54*±1.52	11.44±1.23	11.34±1.05
	ALX +Milk	10.32±1.02	10.46±1.06	13.35*±1.48	13.44*±1.28	12.75±1.12
C-peptide	Control	6.15±0.43	7.26±0.60	6.24±0.45	6.31±0.71	6.43±0.63
	ALX	2.15c±0.27	2.46c±0.19	2.24c±0. 03	2.35c±0.37	2.34c±0.24
	ALX +Onion	3.75±0.51	3.61±0.96	4.36*±0.35	4.034*±0.65	4.15*±0.44
	ALX +Garlic	3.76±0.35	3.46±0.51	4.36*±0.98	3.15±0.47	3.64±0.65
	ALX +Milk	2.38±0.03	3.15±0.56	4.54*±0.64	4.33*±0.63	4.26*±0.41

Mean ± Standard deviation (SD) and standard error (SE)
(a, b,c) Values of the diabetic groups were differs significantly from the value of control group within the same day at P<0.05,P<0.01 and P<0.001 respectively.
(*) (**)(***)Values of the treated groups were differs significantly from the value of diabetic group within the same day at P- < 0.05 , P < 0.01 and P < 0.001 respectively.

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