doi.org/10.36721/PJPS.2022.35.4.REG.1067-1076.1

Glycemic control and antioxidant potential of distinct fractions of camelus dromedaries fresh milk in streptozocin induced diabetic rats

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Abstract: To analyze the antiglycemic effects and to evaluate the anti-oxidant levels in pre and post-camel milk fractions treated albino diabetic rats, sixty male Swiss albino rats weighing 30-40gm aged 2 to 3 months were randomly divided into six groups, A, B, C, D, E & F, each comprised of 10 animals. as Group A: Normal control, Group B: Streptozocin induced Diabetic group while Groups C, D, E & F were diabetic groups treated with various fractions of camel milk. Noteworthy alteration in blood glucose and antioxidant activity was observed between disease control (group B) and all the treated groups with a percentage decrease of about 25%, 12.98%, 11.57% and 10.17% in blood sugar in groups C, D, E and F respectively. Changes in total antioxidant capacity were significant with the rise of 92.30%, 30.76%, 46.15% and 38.46% respectively in groups C, D, E and F. Percentage difference in superoxide dismutase between group B and Group C of 85% was highest as compared to 45.90 %, 52.45% and 39.34% for groups D, E and F. Group C also showed a significantly higher increase in serum copper, zinc and Vitamin C. It is inferred that Camel milk has significant antiglycemic and antioxidant potential and may prove good complementary therapeutics for diabetes.

Keywords: Type 2 diabetes mellitus, hyperglycemia, antioxidants, camel milk.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is an unceasing metabolic disease due to fault in the release of insulin, action of insulin, or a combination of both, present clinically as high blood sugar levels (American Diabetes Association, 2020). The causes of type 2 diabetes are multi-factorial and colossal research has been carried out to comprehend its credible etiology. Eccentricity in insulin emission or action frequently coexists and it is obscure to discriminate the principal core explanation of hyperglycemia. The pathophysiologic aberration varies from prime insulin resistance accompanying placid or stern β-cell debilitation with ensuing insulin paucity. Insulin resistance also contributes to related metabolic tribulations, including dyslipidemia and hypertension (Galicia-Garcia *et al*., 2020).

The pervasiveness of type 2 diabetes mellitus is escalating globally and is nearly attaining epidemic magnitude (Unnikrishnan *et al*., 2017). The number of patients afflicted with diabetes mellitus was estimated to be about 382 million in 2013 which is projected to increase to 592 million adults by 2035 (Tao *et al*., 2015). It is predicted that 75% of the developing nation's population will be afflicted by diabetes by the year 2025 (Animaw & Seyoum 2017).

Pakistan is amongst one of the developing South Asian countries with a projected population of approximately 207.77 million people, ranked fifth highest-populated country in the world. Like in other developing countries; the pervasiveness of diabetes mellitus is on the rise in Pakistan. The mean frequency of type 2 diabetes mellitus in Pakistan is reported to be about 11.77% and 26.3% respectively (Meo *et al*., 2016, Basit *et al*., 2018).

Keeping in view the escalating worldwide pervasiveness, the gravity of complications and financial encumber of diabetes, its management and prevention of associated complications ought to be considered as a key objective of the country's healthcare system (Forouhi & Wareham, 2019). A collective endeavor to manage known cardiovascular risk factors including diabetes, hypertension and hyperlipidemia is needed to reduce complications and economic costs associated with type 2 diabetes mellitus (Zheng *et al*., 2018).

Management of type 2 diabetes mellitus entails a multidisciplinary approach to attain a reduction in high blood glucose and reduce its micro-vascular and macro-vascular complications (Tan *et al*., 2019).

In type 2 diabetes, conservative pharmacological management approach has largely been replaced by a range of oral antiglycemic agents because of the progressive character of the illness and frequent occurrence of concomitant diseases; patients frequently

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require the addition of one or more agents (Marín-Peñalver *et al*., 2016; Olokoba *et al*., 2012).

Despite the availability of conventional therapies for the management of diabetes mellitus, the curiosity for use of complementary and alternative medicines in the management of diabetes mellitus is growing worldwide (Kaskous, 2016, Chang *et al*., 2013). Conventional health care practices and beliefs are often recognized for this curiosity. The utilization of natural remedies for the treatment of diabetes mellitus is still rather common across the globe (Willcox *et al*., 2021). However scientific evidence is lacking regarding their efficacy and safety.

Although Camel milk (CM) has been reported to have antidiabetic activity (Mansour *et al*., 2017; Ejtahed *et al*., 2014), there is no general agreement on its use for antidiabetic purposes, primarily because of conflicting data from animal studies and a lack of scientific proof from clinical studies (Kebir *et al*., 2017, Agrawal *et al*., 2003).

Keeping in mind widespread belief and rising interest worldwide regarding the use of complementary medicines and natural remedies for prevention and cure of diabetes mellitus and lack of scientific evidence behind the potential hypoglycemic effects regarding the benefits of CM in diabetic patients based on reports regarding the presence of lactoferrin, immunoglobulins, lysozyme, insulin-like factor, antioxidant trace elements (Cu, Zn and Mg and vitamins (Vitamin C, E) in camel milk and arguments about their function in reverting the complications of diabetes mellitus, this contemporary study was carried out to measure antiglycemic efficacy, total antioxidant activity and effects on levels of zinc, copper, vitamin C using various camel milk extracts.

MATERIALS AND METHODS

Collection of camel milk

Camel milk was collected from a local camel milk vendor in Lahore in clean 500ml bottles and transported to the laboratory while on ice and stored at 40C. The milk obtained so was lyophilized and kept frozen at -800c for further experimentation.

Extraction of milk

Extraction of camel milk was carried out by adopting and modifying the method illustrated by Aschaffenburg (2009) (Aschaffenburg, 1963). Extraction with alcohol 250 ml ethyl alcohol was added to the lyophilized camel milk and stirred overnight. This alcoholic mixture was centrifuged. The supernatant was decanted as alcoholic extract and residues were collected and air-dried for successive ether and chloroform extraction. Alcoholic extract was concentrated at low temperatures on a rotary evaporator. Concentrated material marked as alcoholic extract was kept for further experiments.

Extraction with chloroform and Ether

Two hundred and fifty milliliters (250ml) chloroform was added to the leftover residues of alcoholic extraction and stirred overnight. The mixture was transferred to the separating funnel and let stand for 1 hour. Clearly, two phases have emerged. Chloroform portion was separated, transferred and air-dried in Petri dish. This was marked as chloroform extract. The residues were also air-dried and immersed in 250 ml ether and let stirred overnight. After stirring, the mixture was poured into the separating funnel. After 1 hour ether phase was separated and air-dried and marked as ether extract. The leftover residues were air-dried and marked as Residues of Extracted Camel Milk (RECM) for further experiments.

Ethical Approval

The study protocol and all of the animal handling procedures were followed according to the guidelines of the ERC of University College of Medicine & Dentistry, the University of Lahore after taking approval from ERC under Reg No: ERC/22/20/11.

Experimental animals

Sixty (60) male Swiss albino rats weighing 30-40gm with ages in a range from 2 to 3 months were taken from Animal House of IMBB, University of Lahore and were kept in well ventilated polyplastic cages in an air-conditioned room at standard temperature and humidity (700 F & 50-60% relative humidity) and exposed to 12hrs light/dark cycle for 15 days. All rats were provided a customary laboratory diet and tap water *ad libitum*. The experimental animals have received humane care. After acclimatization, animals were indiscriminately alienated into six groups namely, A, B, C, D, E & F, each encompassing 10 animals as follows.

Group A (n=10): Normal control was given normal diet and water

Group B (n=10): Diabetic group not received any treatment.

Group C (n=10): Diabetic group treated with residues of extracted camel milk (RECM)

Group D (n=10): Diabetic group treated with alcoholic extract of camel milk.

Group E (n=10): Diabetic group treated with chloroform extract of camel milk

Group F (n=10): Diabetic group treated with ether extract of camel milk

Each extract was dissolved in 5 ml of dimethylsulfoxide (DMSO) and 1 ml of each was given orally to each rat in their respective groups corresponding to 50ml fresh milk/rat/24 hours (as per dosage used by Meena, S *et al*., 2016) for 30 days and at the end of the study all rats were euthanized and the blood sample was collected by cardiac puncture for biochemical estimations.

Induction of diabetes

Diabetes in rats was produced by three days' successive intraperitoneal injection of 45mg/kg body weight Streptozocin (STZ) in 0.1M cold citrate pH 4.5 as per protocol followed by Nacer *et al.* (2020). The level of blood glucose was checked by drawing blood from the tail of the rats with Accu-Check Performa blood glucose meter (Roche, Germany) before the STZ injection and after three days of the last injection. Diabetes was considered induced if the blood glucose levels were found above 300 mg/dl.

Biochemical estimations

The animals have fasted for 6 hours and blood was obtained for estimation of blood glucose from the tip of the tail of all rats in groups A, B and C on days, 0, 15 and 30. On the culmination of the study day i.e. day 30 all the rats were anesthetized and blood was collected by cardiac puncture (Williams *et al.,* 2020). Blood was processed to estimate total antioxidant capacity, copper, zinc and vitamin C levels.

Estimation of serum Zinc (Zn) and Copper (Cu)

The levels of plasma Zn and Cu were calculated by spectrophotometer (932 plus, GBC, Australia) using an air-acetylene flame without backdrop correction at 213.9 and 324.71 nm, respectively. Samples were digested in an H2O2/HNO3 mixture in a start D microwave-assisted digestion system (Milestone Microwave Laboratory station ETHOSD) and subsequently brought up the volume with double deionized water (Guo *et al.,* 2011)

Estimation of Superoxide dismutase (SOD)

The estimation of SOD was carried out by using superoxide dismutase activity colorimetric assay kit (Bio Vision Inc.) as per manufacturer instructions and method followed by Ga Yoon *et al*. (2017). Blood was collected in citrated tubes and centrifuged at 1,000xg for 10 minutes at 4^{o}C. The plasma layer was removed without disturbing the buffy layer and stored at -80^{o}C. Absorbance was read at 450 nm utilizing an EZ Read 400 ELISA Reader (Biochrom, Holliston, USA).

Estimation of Glutathione peroxidase

Oxidative capacity was identified by assessing the glutathione peroxidase activity colorimetric assay kit (Bio Vision Inc) according to the manufacturer's instructions and as followed by Zhou *et al.* (2019). Samples were pooled with 5,5′-dithio-bis(2-nitrobenzoic acid) and the quantity of total GSH present in each sample was estimated by determining levels of yellow-colored derivative 5-thio-2-nitrobenzoic acid at 340 nm using an EZ Read 400 ELISA Reader (Biochrom, Holliston, USA).

Estimation of total antioxidant capacity (TAC)

TAC was calculated by FRAP test-taking after Rubio *et al.* (2016) utilizing Bio vision kit CAT: K515. Ferric diminishing capacity of plasma (FRAP) measure is based on the rule of decrease of ferric-tripyridyltriazine (Fe3+-TPTZ) complex to ferrous tripyridyltriazine (Fe2+-TPTZ) by the antioxidants in a sample at low pH. Fe2+-TPTZ exhibits blue color with assimilation greatest at 593 nm. The alteration in absorbance is related to the antioxidant capacity (Rubio *et al*., 2016).

Estimation of vitamin C

For estimation of plasma vitamin C, samples were instantly treated with 4% metaphosphoric Corrosive/ dithiothreitol as a stabilizer. The item was at that point coupled to o-phenylenediamine to create a chromophore and absorbance was measured at 340 nm concurring to the strategy utilized by Guo & Wang (2012).

STATISTICAL ANALYSIS

Data was entered and analyzed in SPSS V25. Total antioxidant capacity, copper (µg/dl), zinc (µg/dl) and vitamin C were presented as mean ± SD in Group A, B, C, D, E and F. Comparison of blood glucose levels between Groups A, B, C, D, E & F and Changes in serum SOD, Catalase & Glutathione peroxidase levels between Groups A, B, C, D, E & F were presented as Bar charts. Statistical analysis was done by using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test. Paired-t test was used to compare the groups. P-values less than 0.01 were considered significant and less than 0.001 were highly significant respectively.

RESULTS

Glucose level

The amendment in blood glucose levels between diverse groups has appeared in fig. 1. Discernible drop off in blood glucose was observed when compared between streptozocin induced diabetes in group B and treated groups C, D and E & F with a decrease in blood glucose from 285 mg/dl in group B to 211 mg/dl, 248 mg/dl, 252 mg/dl and 256 mg/dl in groups C, D, E and F respectively. Among the treated groups C, D, E & F, insignificant variation was found among the three groups, however statistically significant ($P<0.001$) decrease in blood glucose (25%) in group C was observed in contrast to group B (fig. 1).

Antioxidant activity

The results showed comparable efficacy of all extracts of camel milk in raising antioxidant activity among all the four treated groups C, D, E & F, however group C which was treated with RECM showed better antioxidant activity as compared to alcohol (Group D), chloroform (Group E) and ether (Group F) extract treated groups. Superoxide dismutase (SOD) levels were found to be elevated among all the treated groups C, D, E and F as compared to the diabetic untreated group (group B).

However, the percentage variation between groups B and C of 85% was found to be highest as compared to 45.90%, 52.45% and 39.34% for groups D, E and F respectively (figs. 2 & 3).

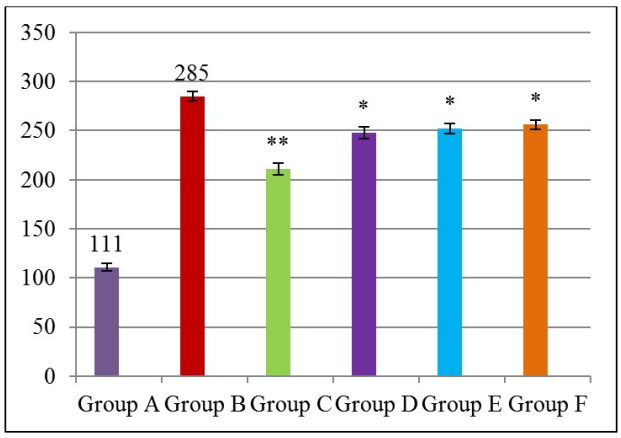

Fig. 1: Comparison of blood glucose levels between Groups A, B, C, D, E & F

Group A: Control, Group B: Diabetic (Untreated), Group C: Diabetic+RECM, Group D: Diabetic + Alcoholic extract, Group E: Diabetic+Chloroform extract, Group F: Diabetic +Ether extract. * P< 0.01, ** P <0.001

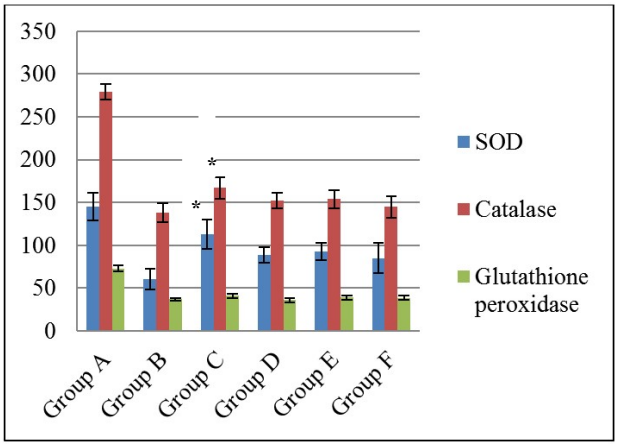

Fig. 2: Comparison of serum SOD, Catalase & Glutathione peroxidase levels between Groups A, B, C, D, E & F

Group A: Control, Group B: Diabetic (Untreated), Group C: Diabetic+RECM, Group D: Diabetic + Alcoholic extract, Group E: Diabetic+Chloroform extract, Group F: Diabetic +Ether extract

The level of serum catalase also showed comparable results between groups C, D, E & F with an increase in serum catalase levels of 167, 152, 154, 145 (U/ml) respectively as compared to untreated from 138 (U/ml) in diabetic group B. The percentage difference between groups B with other treated groups was found to be high with RECM treated group C with a percentage rise of 21.01% in serum catalase levels 138 (U/ml) in group C as compared to groups D, E, F which showed the percentage changes of 10%, 11.59% & 5.07 % respectively. The percentage difference in serum glutathione peroxidase levels when compared between group B with group C, D, E and F was found to be mild, however, group C showed a rise of about 10.81% as compared to groups D, E and F where group D showed a mild decrease in levels of glutathione as compared to other treated groups with the percentage change of about 2.70% whereas group E & F showed a rise of about 5.40% & 4.86% in serum glutathione levels respectively (figs. 2 & 3).

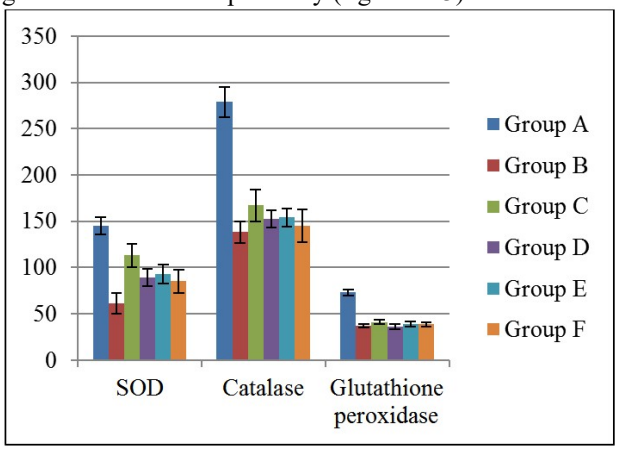

Fig. 3: Changes in serum SOD, Catalase & Glutathione peroxidase levels between Groups A, B, C, D, E & F

Group A: Control, Group B: Diabetic (Untreated), Group C: Diabetic+RECM, Group D: Diabetic + Alcoholic extract, Group E: Diabetic+Chloroform extract, Group F: Diabetic +Ether extract

Total antioxidant capacity was found to be significantly increased when diabetic rats were treated with RECM (group C) as compared to group D, E & F. which showed improvement in total antioxidant capacity but not that much as observed in group C rats. The percentage change in total antioxidant capacity was found to be high in RECM treated group C with a percentage rise of 92.30% as compared to groups D, E, F which showed percentage changes of 30%, 46.15% & 38.46 % respectively. When groups C, D, E and F were compared with group B, a statistically remarkable distinction was witnessed between groups B & C with a p-value <0.01 as compared to groups D, E and F (table 1).

Group C also showed a significantly higher increase in serum copper, zinc and Vitamin C as compared to alcohol, ether and chloroform extracts of camel milk with a rise in serum copper levels showing a percentage increase of 12.5 % as compared to 46.9 µg/dl (6.59%), 45.3 µg/dl (2.9%) and 46.1 µg/dl (4.77%) in groups D, E and F respectively (table 1 & fig. 4).

Serum levels of Zinc were also found to be raised with a statistically significant percentage difference of 34.69% (P<0.01) as compared to 4.08%, 13.63%, 8.16% for groups D, E and F respectively (table 1).

Table 1: Effect of camel milk extracts on serum total antioxidant capacity, copper, zinc & vitamin C levels

	Group A	Group B	Group C	Group D	Group E	Group F
Total antioxidant capacity(µm/L)	3.1±0.02	1.3±0.03	2.5±0.029*	1.7±0.035	1.9±0.029*	1.8±0.38
Copper(µg/dl)	66±13.5	44±7.6	49.5±10.2**	46.9±8.4	45.3±9.4	46.1±6.8
Zinc(µg/ml)	0.9±0.01	0.49±0.012	0.66±0.013*	0.51±.015	0.55±0.02	0.53±0.03
Vitamin C	6.2±1.1	3.8±0.98	4.4±1.3**	4.1±1.2*	4.0±1.4	4.3±1.5*

Group A: Control, Group B: Diabetic (Untreated), Group C: Diabetic+RECM, Group D: Diabetic + Alcoholic extract, Group E: Diabetic+Chloroform extract, Group F: Diabetic+Ether extract. * P< 0.01, ** P <0.001

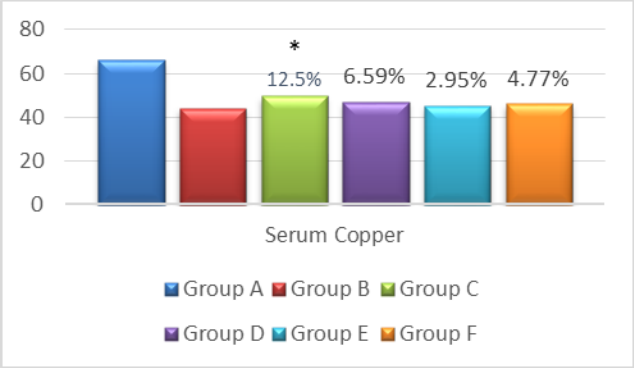

Fig. 4: Changes in Serum Copper levels between Groups C, D, E & F in comparison with Group B
Group A: Control, Group B: Diabetic (Untreated), Group C: Diabetic+RECM, Group E: Diabetic+Chloroform extract, Group F: Diabetic +Ether extract

RECM treated group C showed a highly significant increase in serum vitamin C levels statistically (P<0.001) when compared with vitamin C levels in group B. The rise in vitamin C concentrations in groups D and F was mild but statistically significant (P<0.01) as compared to group B (table 1).

DISCUSSION

The present study has observed the promising antiglycemic effects of alcoholic, ether, chloroform extracts of came milk as well as residues of extracted camel milk (RECM) on serum catalase, superoxide dismutase, glutathione peroxidase, total antioxidant capacity, serum zinc, copper and vitamin C levels in streptozocin induced diabetic rats after treatment with different extracts of camel milk for 30 days. Among the treated groups C, D, E & F, Group C showed a marked and significant increase in all the parameters as compared to group D, E and F. To the best of our knowledge, the present study is unique from other studies measuring the effects of camel milk using the extracts of camel milk to investigate the anti-diabetic effects.

Information accessible from experimental and human studies has pointed out that camel milk has assenting impacts on glycemic control, by plummeting fasting blood glucose, diminishing insulin resistance and improving diabetic dyslipidemia. It has been shown to reduce the risk of diabetes in communities who frequently drink camel milk, in contrast to the population not consuming camel milk (Ejtahed *et al*., 2014; Agrawal *et al*., 2003). This potential positive role of CM is ascribed to the presence of high concentrations of insulin & insulin-like proteins with resultant augmentation in insulin receptors activity (Khalesi *et al*., 2017). It has been reported in the literature that CM reduces βeta cell injuries due to the presence of some specific forms of protective proteins e.g. lactoferrin and lactoperoxidase, as well as antioxidant agents including Zinc, Vitamin C and B3 (Ayoub *et al*., 2018; Mostafidi *et al*., 2016; Kula, 2016).

The improvement in blood glucose observed in our study was in accordance with other studies (Fallah *et al*., 2020; Mirmiran *et al*., 2017; Shori, 2015; Ejtahed *et al*., 2014), however, the outcome of most of these studies was only to observe the efficacy of raw camel milk on blood glucose in comparison to our study in which we evaluated different extracts of camel milk in relation to their effects on blood glucose as well as their antioxidant potential in streptozocin induced diabetic rats. Our findings are in accordance with Sboui *et al*. (2010) who compared the efficacy of camel milk in comparison to that of cow milk in diabetic dogs and reported a considerable drop off in blood glucose and cholesterol concentrations in camel milk treated group in contrast to a group treated with cow milk, which showed an increase in the blood glucose and cholesterol levels after 4 weeks (Sboui *et al*., 2010). The other studies (Korish *et al*., 2020; Mansour *et al*., 2017; Meena *et al*., 2016; Khan *et al*., 2013) conducted on diabetic rats with a few dissimilarities in the study protocols, documented the efficiency and ascendancy of camel milk in correcting hyperglycemia and plummeting insulin resistance.

Most of the clinical trials have shown the valuable effects of camel milk through a drop in FBS, HbA1c, insulin dosage and increased insulin levels (Mirmiran *et al*., 2017; Mihic *et al*., 2016; Ejtahed *et al*., 2014). Clinical trials in humans by Agrawal *et al*. (2003) scrutinize the corollary of a combination of camel milk and insulin in

type1 diabetic patients reported a significant reduction in fasting blood sugar from (P-value = 0.002), HbA1c (P-value = 0.002) similar to the changes in blood glucose levels observed in our study (Agrawal *et al*., 2003).

The possible mechanisms underlying the above-mentioned benefits of camel milk observed in experimental and clinical trials justifying its antiglycemic effects are based on the fact that it contains a substantial amount of small insulin-like molecules (Ayoub *et al*., 2018; Abdulrahman *et al*., 2016).

The hypothesis that camel milk residues stimulate beta cells to release insulin and reduce the blood sugar level seems appropriate after observing the results of the present study in which a decrease in blood glucose levels was observed in all treated groups as well as previously reported data in the literature (Agrawal *et al*., 2020). RECM was found to have greater antiglycemic activity as compared to alcohol, chloroform and ether extracts, the possible reason for which is not known.

Unlike that of other trials (Yadav *et al*., 2017; Ranasinghe *et al*., 2015) on camel milk which observed the efficiency of CM on blood sugar and insulin levels along with beneficial effects on lipid profile, our study also revealed the antioxidant effects of CM extracts with a significant increase in serum glutathione, catalase, superoxide dismutase as well as total antioxidant capacity in all the treated groups of rats which relates the reported affirmative function of CM in diabetes mellitus associated with the high echelon of antioxidants claiming to boast anti-inflammatory role on the pancreatic β-Cells (Prasad & Bao, 2019).

Escalating data advocate, a pivotal part of oxidative stress in the pathogenesis of diabetes mellitus and its associated complications. Hyperglycemia independently has a central role in the development of diabetic complications through glycation process, called as Maillard or browning reaction that engross irretrievable binding of glucose to proteins; these glycated proteins then go through supplementary reactions forming advanced glycation end products (AGEPs) (Nowotny *et al*., 2015) which then bind to their respective receptors called RAGE. The binding of AGEPs with RAGE provokes intracellular oxidative stress and spur of inflammatory mediators with amplified vascular permeability and vascular complications of diabetes (Singh *et al*., 2014).

Although hyperglycemia is a sovereign risk factor for oxidative stress that leads to impairment of both insulin secretion and its actions. However, high levels of glucose are not the only facet accountable for the generation of ROS. Some studies imply an important role of transition metals such as Cu and Zn as catalysts of oxidative stress (Lowe *et al*., 2017). Homeostasis of trace elements like zinc and copper can be altered in patients suffering from type 2 diabetes mellitus which may append insulin resistance and diabetic complications (Basaki *et al*., 2012). Camel milk was found to contain lofty levels of antioxidants like zinc, selenium and other trace elements known to thwart lipid peroxidation, by invigorating the anti-oxidant system.

Camel milk has been claimed to have antioxidant effects by altering antioxidant enzymes and nonenzymatic antioxidant molecules (Khan *et al*., 2021). The rise in Glutathione peroxidase, superoxide dismutase and myeloperoxidase significantly after CM utilization has been reported in the literature (Abdel-Salam & Al-Damegh, 2018). These antioxidant enzymes provide primary defense against the production of free radicals. Copper deficiency reduces the capacity of superoxide dismutase to metabolize free radicals which may lead to the development of diabetic complications (Sanjeevi *et al*., 2018; Qiu *et al*., 2017).

Zinc has several favorable effects in both type-1 and type-2 diabetes (Pompano & Boy 2021, Ranasinghe *et al*., 2015; Jayawardena *et al*., 2012; Al-Maroof & Al-Sharbatti, 2006). It plays an important function in the antioxidant protection system by remarkably acting as a cofactor of the superoxide dismutase enzyme and by amending the glutathione metabolism (Cruz, 2015). β-cells are exceptionally rich in zinc with a significant role in the normal functioning of the islet cells of the pancreas (Ranasinghe *et al*., 2015). Zinc regulates insulin receptors, extends the action of insulin and endorses healthy lipid profiles (Bjørklund *et al*., 2019). High prevalence of zinc deficiency is found in developing countries which could be contributing factor for heavy diabetic burden among them (Ranasinghe *et al*., 2015; Black, 2003). Zinc deficiency has been reported to decrease superoxide dismutase and glutathione peroxidase levels (Duzguner & Kaya, 2007; Shaheen & Abd El-Fattah, 1995). Camel milk is known to have higher zinc content that is known to kindle the secretory bustle of islets of β cells. In addition to this, camel milk insulin is enclosed in nanoparticles that allow its swift entry into the circulatory system (Aqib *et al*., 2019). The significant increase in serum zinc levels and improved blood glucose in our study can be correlated well with the long-term benefits of camel milk extracts on glycemic control as well as in the prevention of complications of diabetes mellitus.

Decreasing oxidative stress by camel milk may be attributed to the presence of the high amount of vitamins A, C, E besides minerals like magnesium and zinc, known to have antioxidant properties which are found to be crucial for glutathione production, antioxidant enzymes activities and absorption of these antioxidant vitamins. Changes in zinc levels observed in our study are most

likely a result of the high zinc content of camel milk extracts used in our study. The antioxidant activity of different extracts observed in our study is also in accordance with reports published in the literature that camel milk reduces the action of oxygen radicals by increasing glutathione (Ibrahim *et al*., 2018; Al-Ayadhi & Elamin, 2013). Decrease in tumor necrosis factor-α and down regulation of iNOS, the key resource of NO production during inflammation has been reported by camel milk (Korish 2014), reinstating normal ß-Cell function and stifling provocative cytokines responsible for ß-Cell apoptosis, a hallmark of insulin-dependent diabetes mellitus.

Studies have revealed the implication of oxidative stress in the pathogenesis of diabetes mellitus by impaired glutathione metabolism and decreased Vitamin C levels (Asmat *et al*., 2016). Antioxidant vitamins A, C and E are found to be reduced in type 2 diabetes, probably caused by the amplified need to control the disproportionate oxidative stress related to abnormalities in glucose metabolism (Balbi *et al.,* 2018; Valdes-Ramos *et al*., 2014). In addition to other trace elements, Camel milk is also considered the richest source of vitamin C (Swelum *et al.*, 2021). The increment seen in the levels of vitamin C levels in our study is in accordance and can be justifiably related to the vitamin C concentration in camel milk extracts used in the present study. The rise in vitamin C levels in the present study in all the treated groups is an indicator for the presence of vitamin C in camel milk and its impact on increment in serum vitamin C levels which can be correlated with long-term benefits in preventing oxidative stress-induced diabetic complications.

Our findings contradict that of a study by Alabdulkarim *et al.* (2012) who compared the effects of camel and cow milk on plasma glucose and triglyceride levels in healthy female albino rats but reported no significant effect (Alabdulkarim, 2012).

CONCLUSION

Despite exquisite studies on the impact of nutraceuticals on diabetes, the effect of camel milk remains largely unexplored. Therefore, the present study was intended to decipher the underlying mechanisms about antidiabetic activities of camel milk supporting a possible beneficial role of camel milk in improving glycemic control. The present study can be considered as the first to observe the antiglycemic and antioxidant effects of different fractions of camel milk to establish the active ingredients responsible for its antiglycemic activity. Further large-scale experimental and clinical trials using extracts of camel milk are recommended to corroborate the annotations of the current study and to validate the efficacy of active ingredients in camel milk including vitamins and minerals for providing long-term benefits in diabetes mellitus.

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