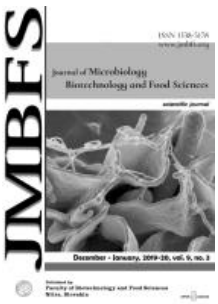

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ASSESSMENT OF CAMEL MILK YOGURT AS A COGENT APPROACH ON STREPTOZOTOCIN (STZ) INDUCED DIABETES MELLITUS IN SPRAGUE - DAWLEY RATS

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ABSTRACT

According to Global report of World Health Organization (WHO) on diabetes, a predictable frequency of diabetes in the grown-up populace has nearly elevated 2-fold since 1980 from 4.7% to 8.5%. Altogether, mainstream of individuals is suffering from Type 1 diabetes mellitus of all diabetes cases which is a serious metabolic disorder and frightening state of cases booming annually. Camel milk, fresh/ fermented has the properties to be acknowledging for its potential effect for the cure of diabetes mellitus type 1. The higher concentration of insulin/insulin mimics particles in camel milk, like half cysteine, small sized immunoglobulins and their optimistic impact on beta cells and resistance to coagulation in acidic environment of stomach contributes to hypoglycemic effect in type 1 diabetes. The persistence of this study was the evaluation of camel milk yogurt on experimental Sprague-Dawley rats for type 1 diabetes. Two types of studies were conducted with four groups depending upon the diet. The analysis of serum glucose, insulin levels, liver and kidney functioning tests, and hematological analysis were performed to access the therapeutic potential of camel milk yogurt during efficacy trials. Serum glucose and insulin results showed that camel milk yogurt exhibited 3.69 % reduction of serum glucose level in study-I (fed on cow milk yogurt) whereas, 13.88% reduction was reported in study-II (fed on camel milk yogurt). Insulin concentration was increased 1.84 % in study-I whereas, 15.22 % was increased in study-II. Categorically, insulin percentage increment enlightened that induction of camel milk yogurt to subjects revealed better results in comparison to cow milk yogurt. The protective perspectives of camel milk yogurt supplementation to rats did not impart any deleterious effect on biochemical markers of the rats. Overall research showed that camel milk yogurt administration improved the clinical symptoms of diabatic rats along with minimal determental effects on health stratum of rats group.

Keywords: Diabetes, camel milk, Insulin increment, improved clinical symptoms, minimal determental effects

INTRODUCTION

According to Global report of World Health Organization (WHO) on diabetes, a predictable frequency of diabetes in the grown-up populace has nearly elevated 2-fold since 1980 from 4.7% to 8.5%. Altogether, mainstream of individuals is suffering from Type 1 diabetes mellitus of all diabetes cases which is a serious metabolic disorder and frightening state of cases booming annually. Diabetes mellitus is a metabolic disorder in which blood glucose level is increased as compared to the normal concentration resulting in low insulin production and increased insulin resistance (**Gader and Alhaider, 2016**). In Type-1 diabetes, the beta cells of pancreas are destroyed and unable to produce required amount of insulin for proper body functioning and results in higher level of blood and urine sugar. Oral insulin therapy either hypoglycemic drugs, insulin exogenous administration many other medicines are addressed thought world to combat this prevailing ailment. Nevertheless, these approaches are not entirely suitable in a huge element of diabetic patients and essentially needs to look for novel medications with minimum other associated complications of antidiabetic drugs. Prior to insulin discovery, different natural plant extracted medication and several nutraceutical foods are claimed for the treatment of diabetes (**Lee *et al.*, 2003**). However, very less are acknowledged scientifically after remedial examination and clinical validations (**Torronen *et al.*, 2010**). Recently, the exploration for natural suitable antidiabetic agents has been engrossed as traditional medicine having hypoglycemic effects. Different experimental trails exhibited that camel milk has ability to repair damaged beta cells and possess regulatory and immune modulatory effect on the cells (**Agrawal *et al.*, 2013**). It owns 150 U/mL insulin concentrations which is highest amount of insulin as compared to other milk species. Furthermore, insulin present in camel milk has peculiar property of resistance to degradation in the acidic environment of stomach unlike human, cow and goat milk insulin (**Mullaicharam, 2014**). This insulin is encapsulated in fat globules in the form of nanoparticles which make its absorption easier and facilitate passing to the blood stream without proteolysis. This equity ranked camel milk as therapeutic and medical milk for alleviating metabolic syndrome like hyperglycemia, hyperlipidemia and insulin resistance condition (**Gader and Alhaider 2016**). On the basis of aforesaid scientific knowledge, the antidiabetic potential camel milk yogurt on Streptozotocin induced diabatic rats was explored to sensitize this indigenous and natural remedial source to lessen curative cost of local community.

MATERIAL AND METHODS

For experiment, 3 to 4 weeks old twenty Sprague Dawley rats were obtained from NIH (National Institute of Health), Islamabad. The rats were acclimatized for two weeks in air-conditioned experimental animal room at National Institute of Food Science and Technology, university of Agriculture, Faisalabad. At the commencement of the efficacy trails some rats were sacrificed to attain baseline values of the study whereas, rest of the subjects were sacrificed at the end of the

study (60 days). Bio-evaluation of camel yogurt was done to investigate the life style related disorders with particular reference to diabetes. The feeding trails were done on rats to systematized observation, meticulous diet delivery and sophisticated and controlled environment. The study was segmented into two groups (Normal rats) and (diabetic rats) to make the comparison of treated one. Furthermore, each of two studies were divided into further two groups depending of the diet *i.e.* Y_1 (cow milk yogurt) and Y_2 (camel milk yogurt) subjected to the rats. At the end of trial, the overnight starved rats in each study were slaughtered to evaluate the treatments own effect for the designated parameters including glucose and insulin levels was assessed the methods stated by Kim *et al*. (2011) and Ahn *et al*. (2011) respectively. The protective likelihood of yogurt made from camel milk for serum aspartate aminotransferase (AST), alkaline phosphate (ALP), alanine aminotransferase (ALT) and bilirubin constituents was evaluated by the method of Akinloye *et al*. (2014). For the safety assessment of camel milk creatinine and urea was deliberated as pronounced by Hussein (2012). Red blood cells (RBC), white blood cells (WBC) and platelets were calculated by the protocols as stated by Al Haj *et al*. (2011) and Kamatani *et al*. (2010).

Statistical analysis

The results of the present study were subjected to two factorial design under CRD to check the level of significance for statistical analysis (Montgomery, 2008).

RESULTS AND DISCUSSION

Gross nutritional composition of camel milk yogurt

Camel milk is highly nutritious and unique than other ruminants' milk in terms of composition and functionality, rich source of minerals and bioactive components that offers numerous inimitable nutritional attributes. The yogurt made from camel milk was studied to know nutritional composition including protein, fat, lactose, ash, insulin, minerals and different organic acid as given in Table 2.

Feed and drink

Feed and drink intakes were recorded on daily basis, whilst body weight was measured on weekly basis. The results pertaining to efficacy are statistically elucidated to get a convincing understanding. Statistical analysis of feed intake elucidated highly significant (P<0.01) effect on diet and drink intake while significant (P<0.05) effect on body weight and their week intervals. However, interaction between (yogurt samples X weeks) was found to be non-significant (P>0.05) in both segment of studies. The feed consumption was gradually increased as a function of time (weeks) due to more body requirements of the subjects under study probably due to more physiological requirements. The feed intake in normal rats was increased from (29.65±1.23 g/rat/day) to (38.75±1.65 g/rat/day) from 1st week to the 9th week in cow milk yogurt feeding group whereas the feed consumption of camel milk yogurt group was gradually increased from (26.91±2.11 g/rat/day) to (37.55±2.18 g/rat/day) up to 9th weeks of study. Likewise, in hyperglycemic rats feed intake augmented from (28.16±1.38 g/rat/day) to (35.61±2.29 g/rat/day) in cow milk feeding group whilst feed ingestion in camel milk feeding group showed increment from (29.89±1.21 g/rat/day) to (40.79±1.70 g/rat/day) as shown in Figure 1. The more body requirement of feed in both studies could be attributed to increasing body requirements for feed of the rats with the passage of time of study. The results of drink intake showed increasing trend as a function of time from 1st to 9th week. In normal rats drink intake was observed as (19.06±1.20 mL/rat/day) and (29.83±2.88 mL/rat/day) at the initiation of the study and that increased to (25.81±1.60 mL/rat/day) and (39.88±1.44 mL/rat/day) at the termination day. Similar increase was recorded in hyperglycemic rats where drink intake of both groups increased from (19.78±1.01 mL/rat/day) to (26.30±1.43 mL/rat/day) in cow milk yogurt group on the other hand in camel milk feeding group showed increment from (31.65±1.55 mL/rat/day) to (41.55±1.99 mL/rat/day) as shown in Figure 2.

The results illustrate visible increase in body weight as a function of time. Data recorded in normal rats at the first week explicit that body weight was increased from 226.33±3.56 g/rat to 360.54±1.56 g/rat whereas, increment of 236.02±3.83 g/rat to 403.11±3.63 g/rat was observed in cow milk yogurt and camel milk yogurt groups respectively. Likewise, in hyperglycemic group body weight was increased significantly from 1st week to 9th week of the study. The increment exhibited from (227.56±4.95 g/rat) to (313.89±6.34 g/rat) in rats consuming cow milk yogurt whilst amplification of (231.89±3.62 g/rat) to (344.14±8.70 g/rat) was observed in camel milk yogurt consuming group from initiation to termination of the study as mentioned in Figure 3.

Blood Glucose level

The statistical data indicated that camel milk yogurt exhibited significant (P<0.05) effect in hyperglycemic rats however non-significant (P>0.05) result was observed in normal rats. The serum glucose level in normal rats were recorded (86.64±4.17mg/dL) and (83.44±3.11mg/dL) in respective group cow milk yogurt and camel milk yogurt. The glucose level in hyperglycemic rats were 237.97±3.68 mg/dL in cow milk feeding group while it was lower (204.92±3.02 mg/dL) in camel milk feed group (Table 3). Overall, normal rats showed 3.69% reduction of serum glucose level whereas, 13.88% reduction was reported in hyperglycemic rats. The results of glucose reveal that camel milk yogurt administration achieved superior results as compared to the cow milk yogurt against the hyperglycemic rats. The reduction of serum glucose level was due to exceptional curative properties of camel milk proteins that mimic the insulin function and also owing the higher concentration of insulin 52 IU/L of the camel milk. Various researchers around the globe emphasized on the medicinal and nutraceutical potential of the camel milk utilization against metabolic disorders particularly diabetes. The anti-diabetic property of camel milk is authenticated by several other scientists who observed the serum glucose reduction in animal models as well as human subjects. Agrawal et al. (2005) carried a comparative study on streptozotocin (STZ) induced rat model. They documented well reduction of blood glucose level from (135.45±20.91 mg/dL) to (113.08±29.09 mg/dL) was attributed to the pasteurized camel milk induction during 4-week trial period. Similarly, Sboui et al. (2010) conducted a study on variations of blood glucose, in alloxian-induced diabetic dogs. They reported the significant reduction in blood glucose level from (10.88±0.55 mmol/L) to (5.77±0.44 mmol/L) before and after five weeks' duration. Badar. (2013). also reported the declining trend in blood glucose while examining un-denatured camel milk whey proteins as antihyperglycemic agent and acknowledged the significant reduction in blood glucose from (411±37 mg/dL) to (261±25.5 mg/dL) while performing two weeks' study on rat model.

Blood Insulin level

The statistical analysis elucidated that camel milk yogurt showed highly significant (P<0.01) results in hyperglycemic rats conversely non-significant (P>0.05) affect was perceived in normal rats. The result elucidated insulin level 8.67±0.54 mg/dL in Y_1 and was higher as 8.83±0.58mg/dL in Y_2 among normal rats. However, mean values pertaining to insulin in hyperglycemic rats were 6.57±0.17 mg/L in cow milk yogurt and 7.57±0.13mg/L in camel milk yogurt groups (Table 3). The Insulin concentration was increased 1.84% in normal rats whereas, 15.22% was increased in hyperglycemic rats. The insulin concentration was found to be lower in hyperglycemic rats due to diabetic rats in this group and their body was unable to produce sufficient amount of insulin as compared to in normal rats. Therefore, hyperglycemic rats were fed with camel milk yogurt to fulfill their insulin requirements of the rats. Categorically, insulin percent increment enlightened that induction of camel milk yogurt to subjects reveals better results in comparison to the cow milk yogurt. The results revealing the substantial increment in serum insulin in the normal rats that may be attributed to the higher concentration of insulin in raw camel milk as well as the exceptionality of camel insulin as resistance to proteolysis during the acidic environment in the stomach due to nano encapsulation in fat globules. Camel milk also enriched with insulin mimic particles like 5, 8-diacetyloxy-2, 3-dichloro-1, 4-naphthoquinone that works same like insulin for the activation of glucose receptor on the surface of the cell (**He *et al*., 2011**). The current study of insulin concentration in hyperglycemic rats are in harmony of Diab et al. (2012) who elucidated the effects of camel milk on glycemic regulation of STZ induced diabetes mellittus in rats. They compared different diabetic rat groups treated with or without camel milk supplementation and conclusively documented significant increase in insulin concentration (14.1±1.2 µU/mL) compared to diabetic rats without the camel milk induction (2.62± 0.28 µU/mL). In another study, El-Said et al. (2010) also reported the elevation in insulin concentration from (2.4 ±0.1) to (7.90±0.9 µU/mL) in rabbits during the four-week efficacy trails. Similarly, Al-Numair et al. (2011) documented handsome increment from 5.53±0.41 µU/mL to 9.97±0.8 µU/mL in insulin concentration as results of camel milk supplementation while executing the 45 days' efficacy trails.

Liver Functioning Tests

Liver functioning tests were performed to know the side effects of camel milk yogurt administration. The results for the serum aspartate transaminase (AST), Alkaline transaminase (ALT) and Alkaline phosphate (ALP) revealed highly significant (P<0.05) results in hyperglycemic rats while non-significant (P>0.05) effects were noticed in normal rats. The serum AST, ALT and ALP concentration exhibited un-substantial decline with the administration of cow milk yogurt and camel milk yogurt in normal rats and in hyperglycemic rats. However, effect of camel milk was more obvious and higher in comparison to cow milk yogurt as shown in Table 4. The current results of serum aspartate transaminase are in harmony of Hamad et al. (2011) who evaluated the safety status of liver biomarkers in different diabetic groups treated with camel, buffalo and cow milk and highly significant results (38%) were documented in AST, 41% in ALT level for camel milk treated group. Likewise, Khan et al. (2013) reported the affected decay in elevated level of liver enzymes AST during one-month supplementation of camel milk in STZ induced rats. Likewise, Al-Fartosi et al. (2011) observed the higher concentration of ALT due to liver injury in paracetamol hepatotoxicity

rats initially but after the oral administration of camel milk the level of ALT liver biomarker reduced significantly. The findings of ALP level in the current study are in harmony of Diab et al. (2012), who concluded that the reduction was found in ALP level in hyperglycemic rats with the provision of camel milk. Khan et al. (2013) measured hepatotoxicity in STZ induced rat model study and well documented the reduction level in ALP. The observed values for the ALP were (110±6.9 U/L) and (103± 4.5 U/L) in camel milk and insulin treated groups respectively as compared to the controlled group (149±7.9U/L).

Kidney functioning test

In order to determine the any harmful effect of camel milk yogurt, different kidney functioning test like serum creatinine, urea and bilirubin contents was evaluated. The statistical data (F value) exhibited ominous variations (P<0.05) on serum urea and creatinine level in hyperglycemic rats but non-significant (P>0.05) result was found in normal rats. The reduction in urea, creatinine and bilirubin level in serum by cow milk yogurt and camel milk yogurt has given in Table 4. The results exhibiting significant effect of camel milk yogurt in decreasing serum urea, creatinine and bilirubin as compared to cow milk yogurt. The results of serum urea and creatinine are in line with the findings of Afifi, (2010) and Amjad et al. (2012) who reported the reduction in serum urea contents while accomplishing the study on anti-diabetic effect of camel's milk. The current outcomes of serum creatinine are in agreement with the findings of Diab et al. (2012) they have reported significant variation in serum creatinine level. The current results are in harmony of Zeweil et al. (2016), who assessed the camel milk effects on liver cirrhosis rats and conclusively documented that camel milk provision maintained the bilirubin contents while carrying the efficacy trails. Al-Hashem (2009) revealed the camel milk potential to maintain the bilirubin contents while executing the study of camel milk effects on aluminum chloride induced rats and reported the observed value for bilirubin content was (0.73± 0.06 mg/dL).

Hematological analysis

The effect of camel milk was also evaluated for hematological indicators including red blood cells (RBC), white blood cells (WBC) and platelets count (PLC). The statistical results showed that the effect of (camel milk yogurt) administration on the red blood cells, white blood cells and platelets count was found to be non-significant (P>0.05) in both studies as illustrated in Table 5. The current results pertaining to RBC's showed an increase in RBC with administration of camel milk are in harmony with the findings of Dallak, (2009) who authenticated that camel's milk administration significantly increased in total erythrocytes count while exploiting the camel milk potential to assess the red blood cells count in anemic rats. The current results of WBC's are in normal range as reported by Sarfraz, (2014) they studied the effects of camel milk supplementation on blood profile of hepatitis patients. In another study, Elattar et al. (2010) reported that the leucocytes contents were also in line with the present who elucidated the potential of whey protein isolates (WPI) for the hepatitis C virus management. The group of researchers Ali *et al*. (2017) reported that camel milk treatment significantly (P<0.05) improved hyperglycemic altered erythrocytes and its indices, leucocytes and lymphocytes but not the platelets count. A significant (P<0.05) increase in platelet count was observed in case of individual treatments. The substantial increase in platelets count was observed by Sarfraz. (2014). They conducted the study on camel milk supplementation in hepatitis C virus patients. Likewise, the results pertaining to PLC of the current study are in harmony with the findings of Elattar et al. (2009) they studied the curative potential of whey protein isolates in hepatitis C patients.

Table 1 Different studies carried out in efficacy trials

Studies	Normal rats		Hyperglycemic rats	
Groups	1	2	1	2
Diets	Y_1	Y_2	Y_1	Y_2

Y_1 = yogurt made by cow milk
Y_2 = yogurt made by best selected camel milk

Table 2 Gross nutritional composition of camel milk yogurt

Parameters	Camel milk Yogurt	Minerals	(g/100g)	Organic acids	(mg/L)
Protein (%)	4.29±0.14	Ca	134.33±3.05	Acetic acid	275.25±37.87
Fat (%)	3.28±0.35	Mg	10.68±0.19	Butyric acid	1769.00±10.61
Lactose (%)	5.39±0.11	Zn	0.75±0.07	citric acid	5320.25±3.59
Ash (%)	1.09±0.381	Na	53.24±3.14	Lactic acid	7411.75±37.09
Insulin (IU/L)	24.91±1.03	K	143.71±3.64	Pyruvic acid	35.37±3.68

The values are stated as mean± SD (n=3)
Mean values containing different letters are significantly from others ($P \leq 0.05$)

Table 3 Effect of yogurt on serum glucose and insulin level (mg/dL) during the studies

	Glucose level (mg/dL)		Insulin level (mg/dL)	
Studies	Y_1	Y_2	Y_1	Y_2
Normal rats	86.64±4.17	83.44±3.11	8.67±0.54	8.83±0.58
Hyperglycemic rats	237.97±3.68^a	204.92±3.02^b	6.57±0.17^b	7.57±0.13^a

^{ab}Means in rows with similar superscripts do not differ (P>0.05), Results are expressed as mean ± standard deviation of means; n=5

Table 4 Effect of camel milk yogurt on liver and renal functioning

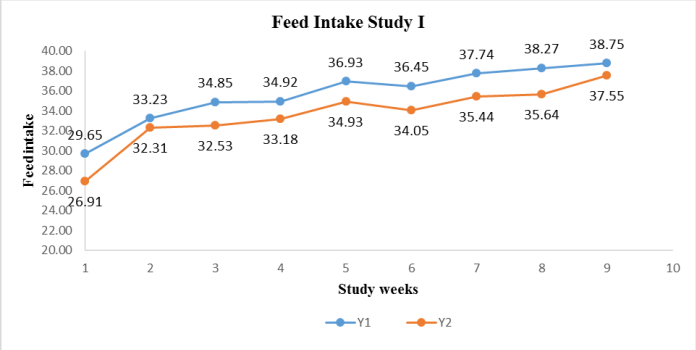
Studies	Liver Functioning Tests						Renal Functioning Tests					
	Aspartate Aminotransferase (AST) level (IU/L)		Alanine Transaminase (ALT) level (IU/L)		Alanine Phosphatase (ALP) level (IU/L)		Biological urea nitrogen (BUN) level (mg/dL)		Creatinine level (mg/dL)		Bilirubin contents (mg/dL)	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Normal Rats	107.62±3.76	104.43±4.34	46.54±1.52	44.39±1.47	151.93±3.54	146.12±2.73	21.53±0.66	20.93±0.71	0.88±0.01	0.86±0.02	0.60±0.040	0.57±0.032
Hyperglycemic Rats	143.38±3.13^a	129.12±4.15^b	55.66±1.88^a	49.87±1.69^b	226.48±2.15^a	209.82±3.31^b	28.17±0.65^a	26.33±0.22^b	0.97±0.02^a	0.90±0.03^b	0.55±0.031	0.58±0.038

^{ab}Means in rows with similar superscripts do not differ (P>0.05), Results are expressed as mean ± standard deviation of means; n=5

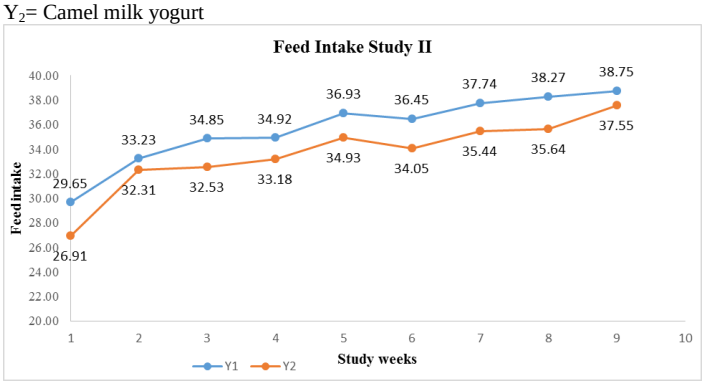
Table 5 Effect of camel milk yogurt on hematological parameters

Studies	Red Blood Cell (RBC) (cell/nL)		White Blood Cells (WBCs) (cells/pL)		Platelets Count (PLC) x 10^9/L	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Normal Rats	7.75±0.46	7.96±0.51	15.51±0.61	15.18±0.46	7.65±0.51	7.86±0.43
Hyperglycemic rats	7.02±0.26	7.43±0.27	16.01±0.31^a	15.13±0.42^b	7.05±0.42	7.46±0.48

^{ab}Means in rows with similar superscripts do not differ (P>0.05), Results are expressed as mean ± standard deviation of means; n=5

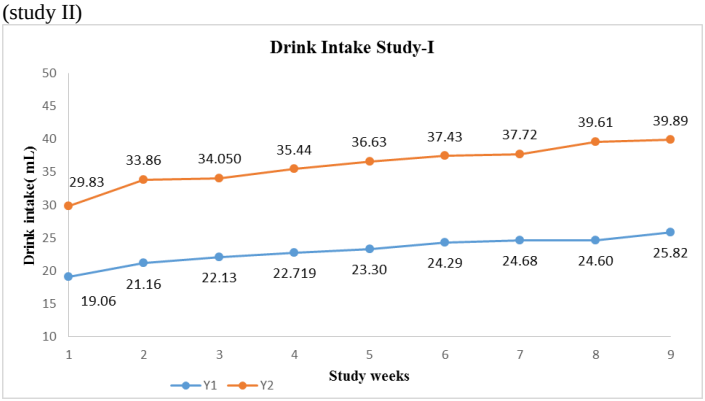


Y_1= Cow milk yogurt
Y_2= Camel milk yogurt

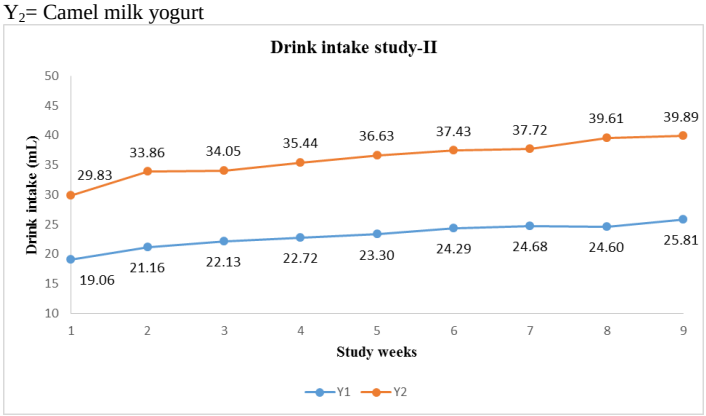


Y_1= Cow milk yogurt
Y_2= Camel milk yogurt

Figure 1 Feed intake (g)/rat/ day in normal rats (study I) and hyperglycemic rats (study II)

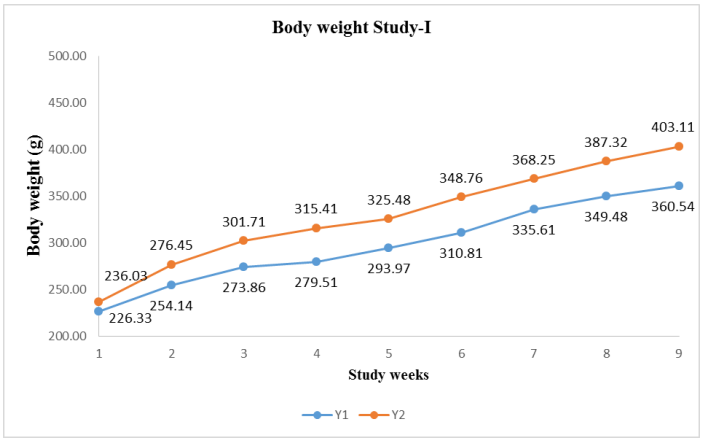


Y_1= Cow milk yogurt
Y_2= Camel milk yogurt

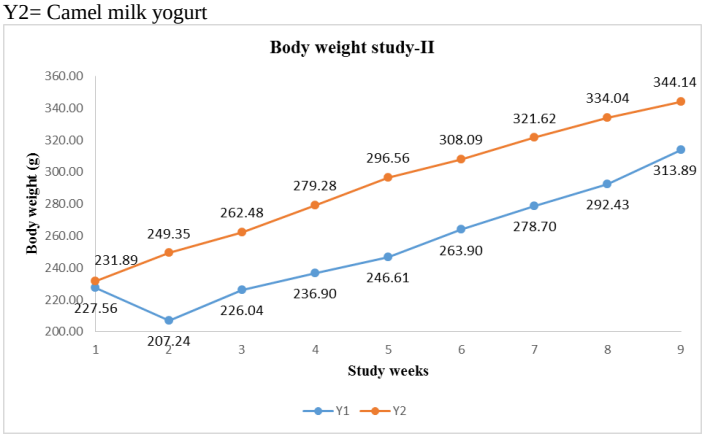


Y_1= Cow milk yogurt
Y_2= Camel milk yogurt

Figure 2 Drink intake (mL/rat/ day in normal rats (study I) and hyperglycemic rats (study II)



Y1= Cow milk yogurt
Y2= Camel milk yogurt



Y_1= Cow milk yogurt
Y_2= Camel milk yogurt

Figure 3 Drink intake (mL/rat/ day) in normal rats (study I) and hyperglycemic rats (study II)

CONCLUSION

Bio-efficacy trials showed reduction in serum glucose and increase in serum insulin of the diabetic rats during experimental trails. This established that camel milk yogurt holds higher antidiabetic potential as compared to cow milk yogurt due to the presence of significant amount of insulin and insulin mimics particles and other protective protein in camel milk yogurt. Besides probable health benefits of camel milk yogurt administration signposted that its consumption did not impart any deleterious effect on biochemical markers of the rats. Camel milk commercialization will open new era in dairy marketing of Pakistan by the provision of camel milk and its products. Importance of camel milk diet-based therapy should be introduced via mass media communication. Our farmers in rural areas produce a lot of commodities that do not capture the economic value, if we figure out new ways to capture and create low cost products with significant benefits; we can not only benefit our rural economy but also create other important spill over benefits such as low disease burden.

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