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RESEARCH ARTICLE

Insulin from Human, Camel and Farm Animals: Comparative Bioinformatics and Molecular Dynamics Studies

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The insulin self-association and dissociation is an important trend in drug discovery studies and insulin structural-activity optimizations. Currently, little is known about the structural basis of insulin activity in animals in general and specifically camels for their unique glycemic status. To fill a knowledge gap in insulin molecular aspects in animals and their future application in human medicine, this research used a structural analysis method that included protein sequence panel comparison followed by molecular dynamics studies. The identity% with human insulin was in the following order swine=rabbit>bovine=ovine>camel with amino acid differences range from 1-4 residues. The residue scanning and mutation of the B chain residue T30 in human insulin to the alanine residue in bovine, camel, and ovine insulin resulted in decreased insulin dimer affinity and stability with Δ stability equals 3.94, 8.43 and 8.43, respectively. The camel insulin showed the highest conformational changes, retarded arrival to the steady state of residues deviation, the most prominent asymmetric monomer fluctuations, the shortest distance between the insulin-stabilizing cysteines disulfide bonds and less conformationally stable insulin monomer 2. The camel insulin dimer is expected to be more dissociable to yield active monomers and to lesser extent swine, bovine and ovine dimers. In addition, the camel monomer is stabilized by the stronger inter-chains cysteines recognition. The merit of residue mutation at dimer interface to mimic the B-chain T30 from camel, swine, and bovine insulin might help in improving the human insulin dimer dissociation and hence the shorter duration to release the active insulin monomer.

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INTRODUCTION

The recently published camel genome sequences (Jirimutu *et al*., 2012) had to lead to the revolution in the identification of camel metabolic pathways and potential application in drug discovery against its pathogens. The differences between the host and parasite genes have been used by our group to identify structural and metabolic differences between the camel and its parasite (Kandeel and Al-Taher, 2020a, b; Kandeel *et al*., 2020a). These differences are important in the discovery of hot spots in the parasite life.

Insulin regulates glycemic balance in both ruminants and monogastric animals (SASAKI, 2002). There is no glucose-derived directly from ruminants' gastrointestinal tract due to rumen microbial fermentation. In the fed state, acetate replaces glucose as the primary substrate for energy storage and oxidation in ruminants, and it is almost entirely reliant on gluconeogenic pathways for glucose supply. Glucose hemostasis is controlled in ruminants and monogastric animals by insulin.

Insulin is a 51-amino acid protein that is mostly composed of α-helical content. The mature insulin monomer is composed of two chains, A and B chains composed of 21 and 30 amino acids, respectively. Insulin is initially contained in a zinc-bound hexameric form in the pancreas, and after secretion into the bloodstream, it is diluted to the nanomolar range, resulting in the

dissociation of monomeric form, which is the active receptor-binding form (Kadima *et al*., 1993). Previous studies showed that insulin dimers can be easily dissociated upon dilution in solution with an estimated Kd of 12 µM (Lovatt *et al*., 1996). The insulin self-association and factors affecting its dissociation have interesting applications in pharmacology and drug discovery by optimizing the insulin molecular dynamics for therapeutic purposes.

The glucose levels in camels differ greatly and are higher than in other ruminants (Nazifi *et al*., 1998). The elevated blood glucose levels in camels are thought to be due to 1) a higher glucagon level in camels than in humans and other ruminants (Abdel-Fattah *et al*., 1999); or 2) camels bearing a natural insulin resistance, which is unique to camels. Insulin resistance is described as a condition in which the target cell's sensitivity to ordinary insulin levels is reduced (Boura-Halfon and Zick, 2009); resulting in the failure of ordinary insulin levels to show its expected metabolic action. Camel adaptations to dry weather include maintaining a high blood glucose level of up to 1300 mg/dL and losing water via the urine. The camel can retain plasma water in this way, allowing it to withstand water deprivation (Yagil, 1985). Camel tissues have a low insulin sensitivity and a slow insulin response (Kaske *et al*., 2001).

There is a knowledge gap about the camel insulin and its function in camels' higher blood glucose levels and apparent insulin resistance. The structure and function of the camel insulin, as well as its similarities and differences from human and other well-characterized insulins, are unknown. Furthermore, a comparison of camel insulin's sequence and domain content with that of other well-studied s may be a starting point for determining camel insulin's biological functions. As a result, we will fill this void in this study by looking at the camel insulin sequence, structure model, and biological aspects for the first time. Camel insulin will be compared to human and farm animals insulin in this analysis. Since insulin monomer is the essential form to bind with insulin receptors, it will interesting to investigate the impact of amino acid mutations in the insulin sequence in different animals on the insulin dimer affinity and stability. The potential relation of MD simulation findings and residue scanning and mutation in human medicine is discussed.

MATERIALS AND METHODS

Retrieval of insulin sequences: The sequences used in this analysis were obtained from the GenBank and protein databases accessible online at (https://www.ncbi.nlm.nih.gov/). The sequences that were retrieved, as well as their database accession numbers, were Human AAA59172, *Camelus dromedarius* XP_031303797, *Camelus ferus* XP_032346050, Caprine XP_017898974, Ovine AAB60625, Swine 4ins PDB ID sequence, Bovine ACD35246 and Rabbit AAA19033. In addition to the old world camelids, new world camelid insulin was also retrieved. The new world camelids insulin sequence was retrieved for Alpaca (*Vicugna pacos*) XP_031546921.

Multiple sequence alignment: The sequence alignment method in the CLC genomic program (Qiagen software, Denmark) was used to match the sequences of domestic animals and human insulin.

Retrieval of crystal structures and construction of molecular models: The crystal structures were retrieved for human, bovine and pigs insulin from the protein data bank. The camel and ovine structure models were built based on the bovine's structure, while the rabbit model was built on the human structures. After model building, all structures were 3D optimized and energy minimized by the protein preparation wizard in Maestro software (Schrodinger LLC, NY, USA). All structures were modeled in dimers. Using a range of tools, including ICM Molsoft, Molegro virtual docker, and the CLC drug discovery workbench, the structure models were energy minimized and compared to human insulin.

Molecular dynamics simulation (MD): Molecular dynamics simulations were done as previously described (Kandeel and Alzahrani, 2020; Kandeel *et al*., 2020b). NPT ensemble was adopted and output comprised route mean square deviation (RMSD), route mean square fluctuation (RMSF), number of hydrogen bonds, secondary structure elements during the simulation and the distance between the cysteine residues forming bonds to stabiles the insulin molecule.

Residue scanning and mutations: The human insulin structure was engineered by the introduction of several mutations based on the corresponding residues in bovine, camel, swine, ovine or rabbit insulin. The calculations included both stability and affinity options. The side chains were mutated followed by backbone minimization. The results are presented as Δ Affinity and Δ stability, indicating changes in dimer affinity and stability as a result of the mutation.

RESULTS

Comparison of camel and human insulin: We aligned the human and camel insulin sequences and obtained the alignment statistics. Fig. 1 shows a comparison of human and camel sequences. Camel and humans had a high degree of homology, with a 92.16 identity rate and four distinct amino acid residues.

Insulin in new and old world camelids: In the old world camel species, *C. dromedarius* and *C. ferus*, the sequence of insulin were compared (Fig. 2). In the old world camel species, there were no variations in the insulin protein sequence, and there was a 100 percent identity rate.

A sequence alignment was used to compare the variations between insulin in the old world and new world camelids, and the results of the alignment summary are shown in Fig. 2. Between the *Vacugna pacos* insulin and the old world camels, there was a strong identity percent of 99.7%. In the insulin sequences, there was just one amino acid variation of alanine to threonine replacement (red star).

Insulin composition in humans, camelids and other farm animals: For the insulin sequences from humans, camelids, and several farm animal species, multiple sequence alignment was created (Fig. 3).

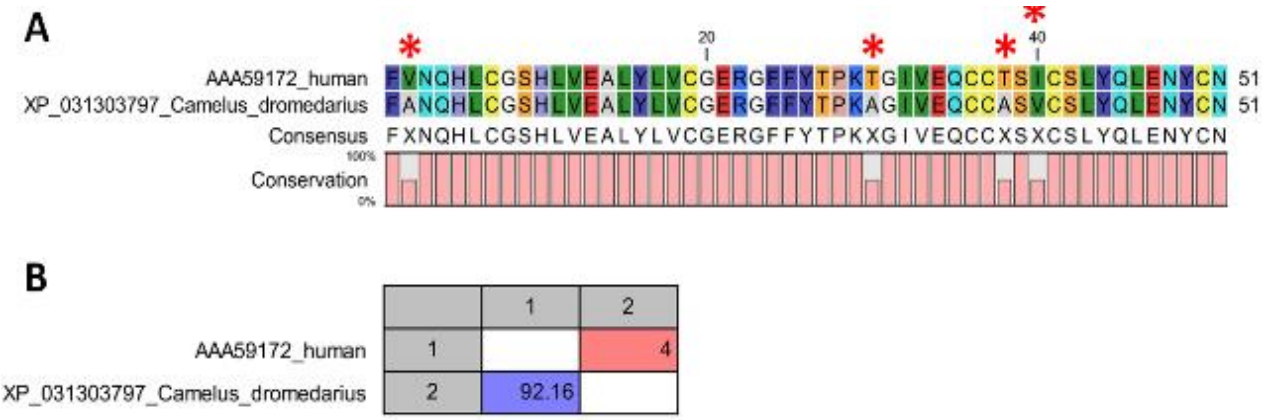

Fig. 1: Alignment of human and camel insulin. A) Pairwise alignment of human and camel insulin. B) Alignment statistics. The number of residue differences is highlighted in red and the identity% is highlighted in blue.

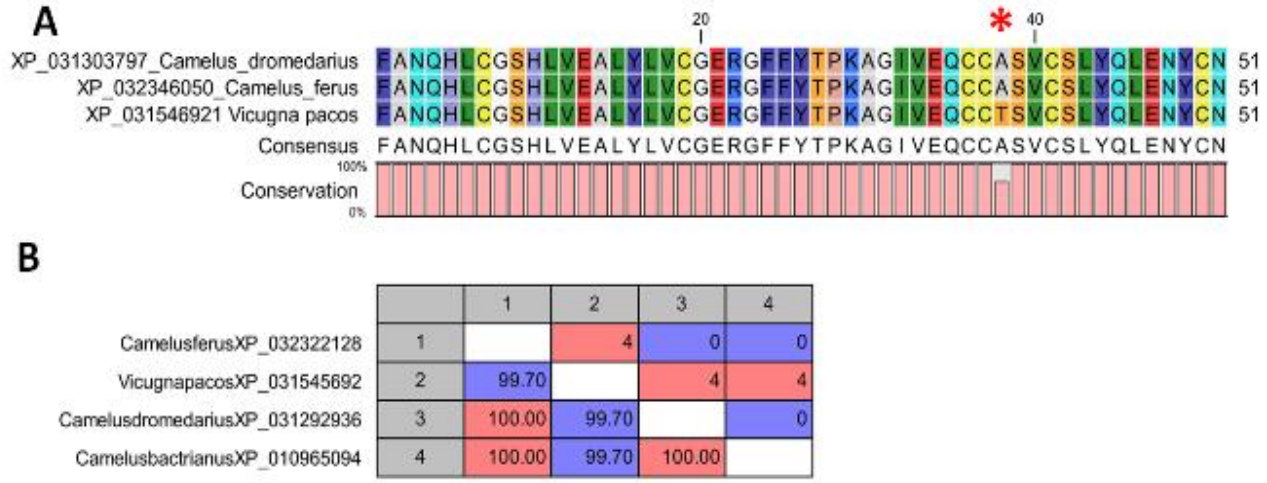

Fig. 2: Alignment of old world and new world camelids insulin. A) Pairwise alignment of old world and new world camelids insulin. B) Alignment statistics. The number of residue differences is in the upper-left panel and the identity% is in the lower-left panel.

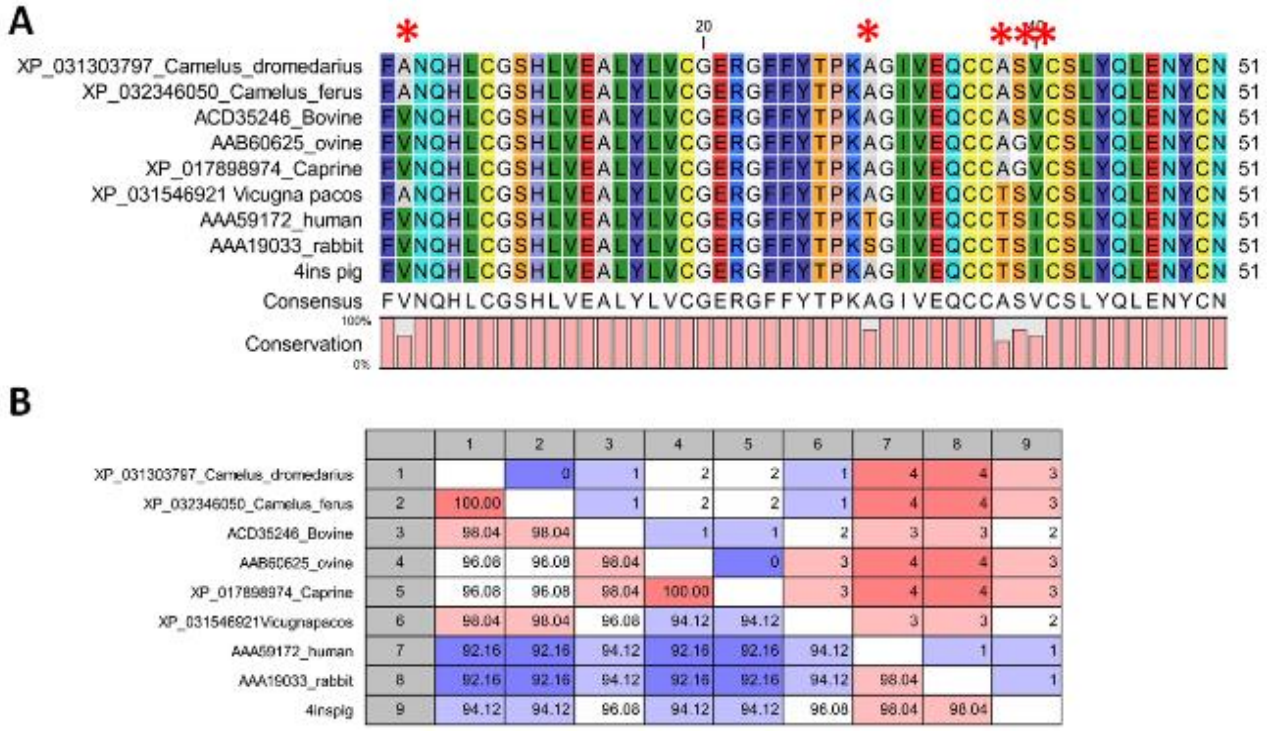

Fig. 3: Alignment of insulin from Humans, camelids and farm animals. A) Multiple alignments of insulin from Humans, camelids and farm animals. The sites of differences are marked by a red star. B) Alignment statistics. The number of residue differences is in the upper-left panel and the identity % is in the lower-left panel.

The identity% was from 92.16% to 100%. For camel insulin, the greatest number of differences was with human insulin (four amino acids). The number of amino acid differences between camel insulin and bovine, ovine, caprine, old-world camelids, rabbits and pigs was 2, 2, 2, 1, 4 and 3 amino acids, respectively (Fig. 3).

Motif and domain content: Although the insulin sequences in the selected dataset differed by around 1-4 amino acids, these discrepancies did not result in any new change in the domain and motif content. A conserved PF00049, Insulin/IGF/Relaxin family motif was found in all insulin sequences.

Conformational stability of humans, camelids and other farm animals insulins: The conformational stability of insulins was monitored through the estimation of RMSD during the MD simulation (Fig. 4). The bovine, ovine, human and rabbit insulin were stable and rapidly equilibrated within the first 100 frames and showed almost stable and fixed RMSD throughout the simulation time. The camel and swine insulins, on the other hand, spent about 300 frames in equilibration before being sable to the end of the simulation time (Fig. 4B and E).

To gain more insights into the conformational stability of insulins, the average RMSD values during the simulation time were estimated (Table 1). The camel insulin showed the greatest conformational changes with an average RMSD value of 3.04 Å. In contrast, rabbit, human and bovine insulin showed stable and low RMSD of 2.2-2.4 Å.

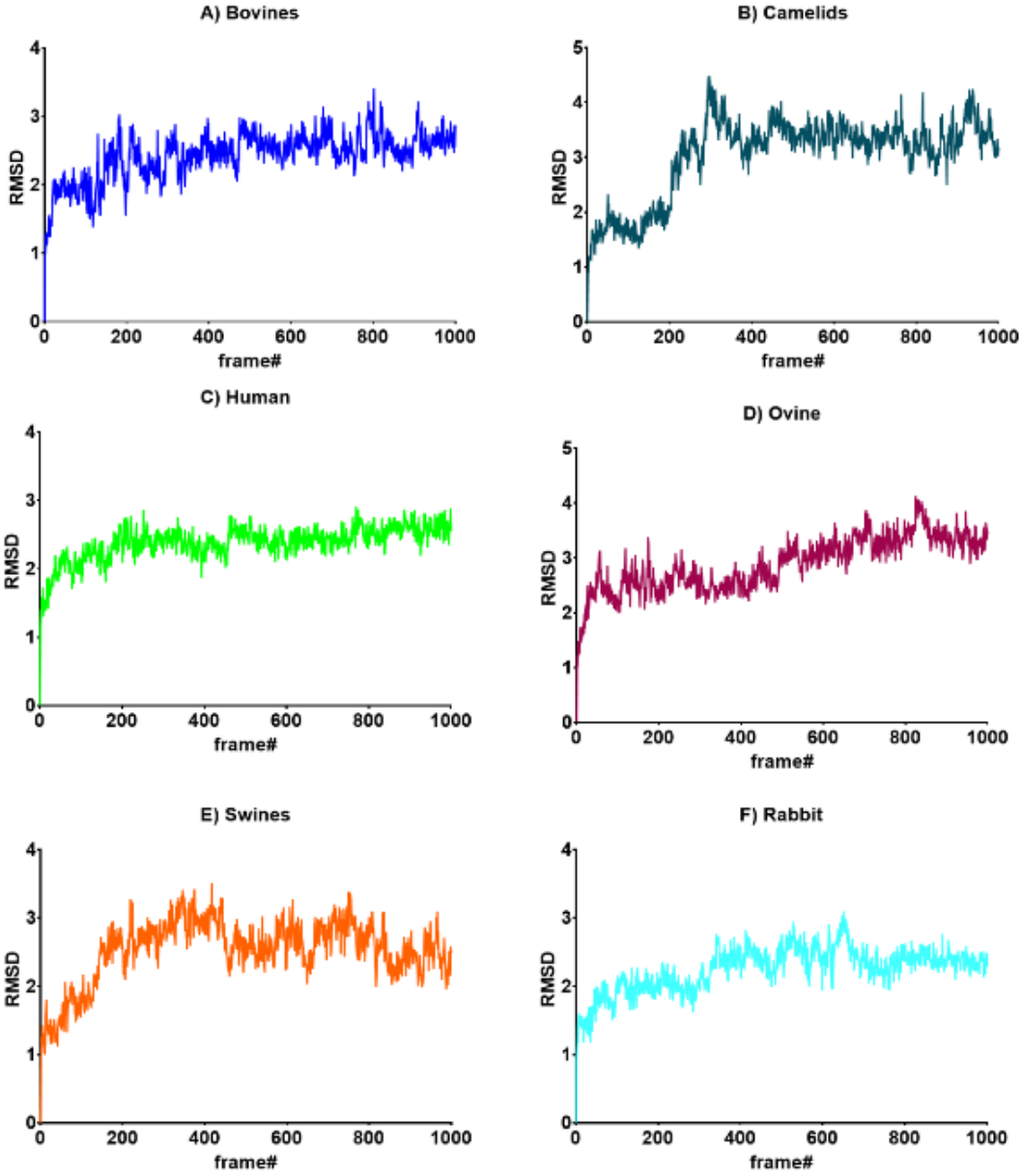

Fig. 4: The RMSD (Å) during 50 ns MD simulation of insulin from human, camel, ovine, swine and rabbits. About 1000 frames were collected during 50 ns simulation time.

Table 1: The average RMSD (Å) after 50 ns MD simulation of insulin from human, camel, ovine, swine and rabbits.

	Bovine	Camelids	Human	Ovine	Swine	Rabbit
RMSD (Å)	2.4	3.04	2.38	2.89	2.52	2.2

Insulins per-residue fluctuations: The RMSF was used to examine local changes in the insulin structure and compare the impact of observed amino acid changes in insulin sequences on the subsequent structure fluctuations (Fig. 5). The initial observation is that each monomer in an insulin dimer has different behavior. A red dashed line was added to compare the RMSF changes in each monomer (Fig. 5). This includes either a larger number of fluctuating residues as in camel insulin (Fig. 5B) or a higher magnitude of RMSF of certain residues as in bovine or rabbit insulin.

The stability of insulin cysteine bonds: The insulin molecule is stabilized by three SH bonds between the insulin molecule cysteines. These comprise A:C6-A:C11, A:C20-B:C19 and A:C7-B:C7. The length of these three bonds was monitored during the simulation as an indicator of insulin molecule stability in the examined set of insulins (Fig. 6). There is one bond within the A chain, A:C6-A:C11, and two bonds between the A and B chains, A:C20-B:C19 and AC7-BC7. The A:C6-A:C11 bond length was the shortest in camel and swine insulins (<4.6 Å). Compared with the higher bond length >5.38 in human, bovines, ovine and rabbit insulins (Table 2). A similar finding was observed with the interchains A:C7-B:C7 bond.

While most of the recordings indicated stable bond length over time, observable large drifts were observed with AC7-BC7 in humans, swine and rabbit insulin (Fig. 6).

Table 2: The average length (Å) between the three insulin disulfide bonds during 50 ns MD simulation of insulin from human, camel, ovine, swine and rabbits

	Bond length (Å)					
Bond	Bovine	Camelids	Human	Ovine	Swine	Rabbit
A:C6-A:C11	5.41	4.41	5.57	5.57	4.54	5.38
A:C20-B:C19	5.97	5.98	6.28	5.99	5.95	6.03
A:C7-B:C7	5.23	4.66	5.04	5.87	4.87	4.52

Table 3: The secondary structure elements (SSE) during 50 ns MD simulation of insulin from human, camel, ovine, swine and rabbits

SSE	Bovine	Camelids	Human	Ovine	Swine	Rabbit
α-helix	31.31	34.78	38.97	24.36	38.7	35.05
β-sheet	0.00	0.00	0.00	0.00	0.00	0.00
Total SSE	31.31	34.78	38.97	24.36	38.7	35.05

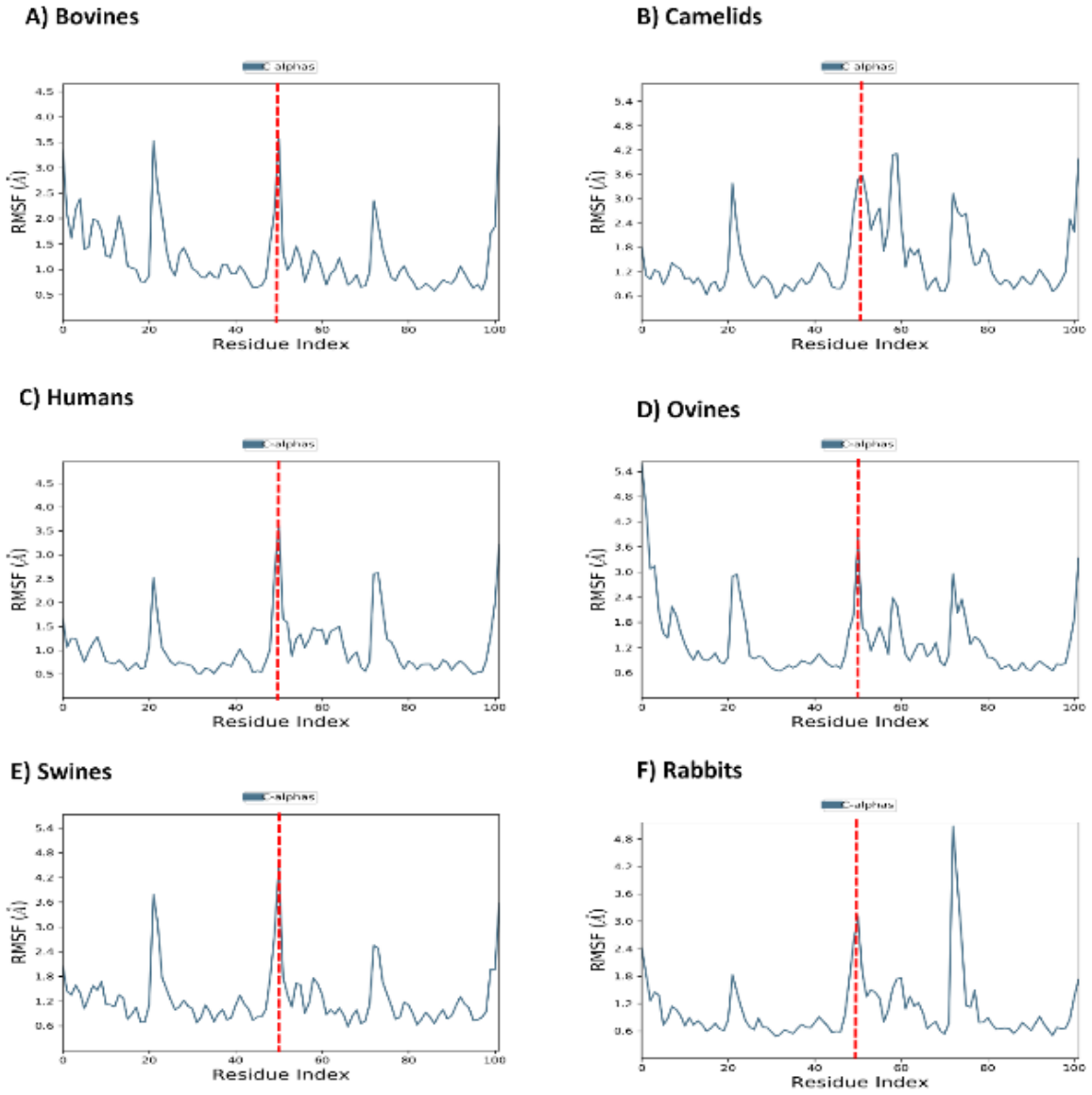

Fig. 5: The RMSF (Å) during 50 ns MD simulation of insulin from human, camel, ovine, swine and rabbits. Insulin dimers were used in MD simulation. The red dashed line was added to mark the differences between each monomer.

Table 4: Residue scanning and mutation. The human insulin was taken as a template. Point mutations were induced in the human insulin corresponding to its equivalent residue in camel, bovine, ovine and rabbit insulin. The effect of mutation was evaluated on the insulin dimer affinity and stability. *Δ Affinity indicates the change in insulin dimer affinity in response to the mutation. Δ Stability indicates the change in insulin dimer stability in response to the mutation*

Insulin type	Mutation	Δ Affinity	Δ Stability
Bovine	A: T8A	0.01	0.99
	A: I10V	0.00	0.65
	B: T30A	8.4	7.9
	C: T8A	0.02	3.94
	C: I10V	0.00	0.04
	D: T30A	0.48	4.98
Camelids	A: T8A	0.01	0.99
	A: I10V	0.00	0.65
	B: F1V	0.00	10.05
	B: T30A	0.06	8.43
	C: T8A	0.02	3.94
	C: I10V	0.00	0.04
	D: F1V	0.00	0.72
	D: T30A	0.48	4.99
Ovine	A: T8A	0.01	0.99
	A: S9G	0.00	-0.4
	A: I10V	0.00	0.65
	B: T30A	0.06	8.43
	C: T8A	0.02	3.94
	C: S9G	0.00	3.75
	C: I10V	0.00	0.04
	D: T30A	0.48	4.98
Rabbit	B: T30S	-0.07	-0.69
	D: T30S	-0.07	-0.69

The insulins secondary structure elements (SSE): The changes in SSE during simulation were analyzed (Table 3). All contributions to SSE were related to α-helix only. The human insulin had the highest -helix content, while the ovine insulin had the lowest. Similar to RMSF, there was a difference in the SSE in each monomer of insulin dimer (Fig. 7).

Residue scanning and mutations: Taking the human insulin structure as a template, several point mutations were added and their effect on insulin dimers affinity and stability were studied (Table 4). For instance, three point mutations were adopted in human insulin with the three different residues in bovine insulin comprising A:T8A, A:I10V and T30A. Four-point mutations were induced in human insulin to mimic the camel insulin residues. T30S was the only different residue between rabbit and human insulin. All mutations had negative effects on human insulin dimer affinity and stability, except for rabbit T30S and ovine S9G.

DISCUSSION

The aim of this study was to analyze the potential differences between camel, human and other farm animals' insulin to better understand the potential contributing factors for camel resistance to high glucose contents as well as shed insights into the insulin dimer stability among farm animals insulin.

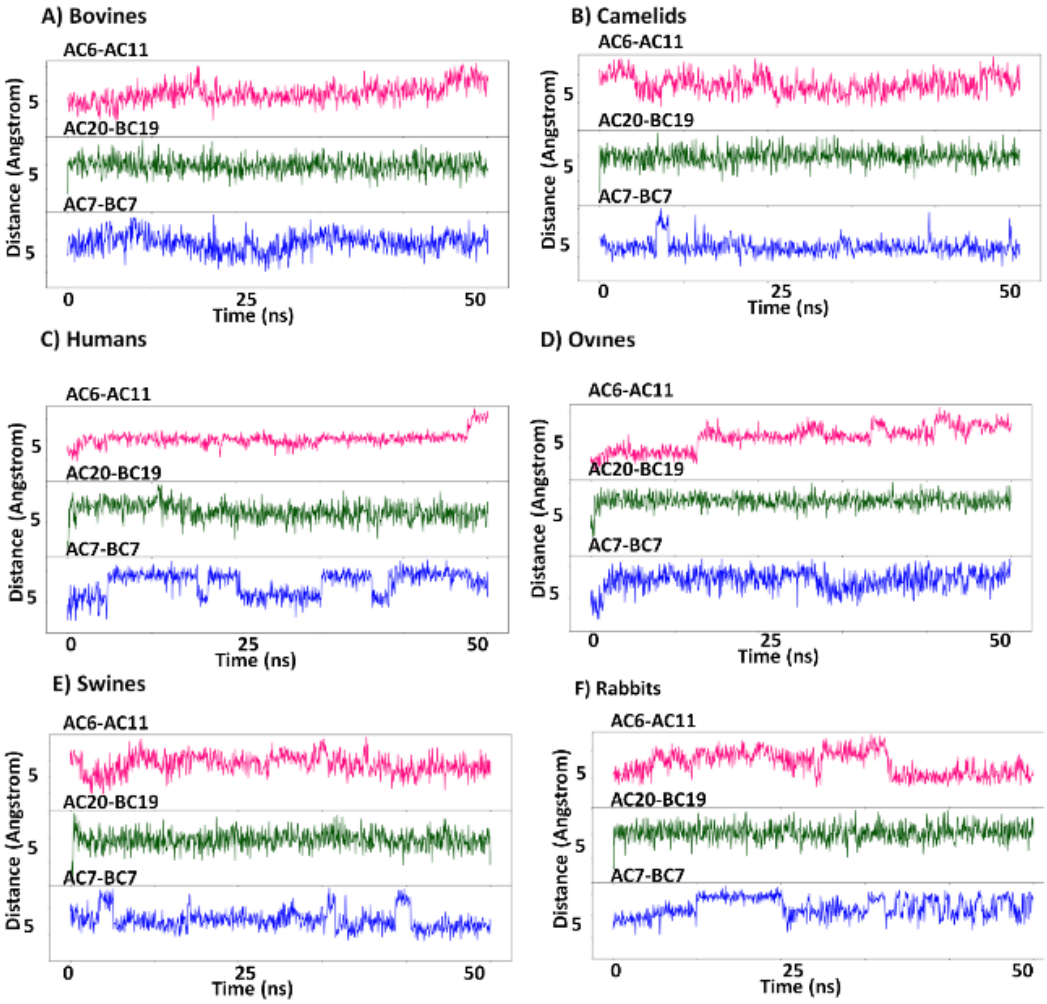

Fig. 6: The length of the three cysteine-cysteine bonds AC6-AC11, AC20-BC19 and AC7-BC7 during 50 ns MD simulation of insulin from human, camel, ovine, swine and rabbits.

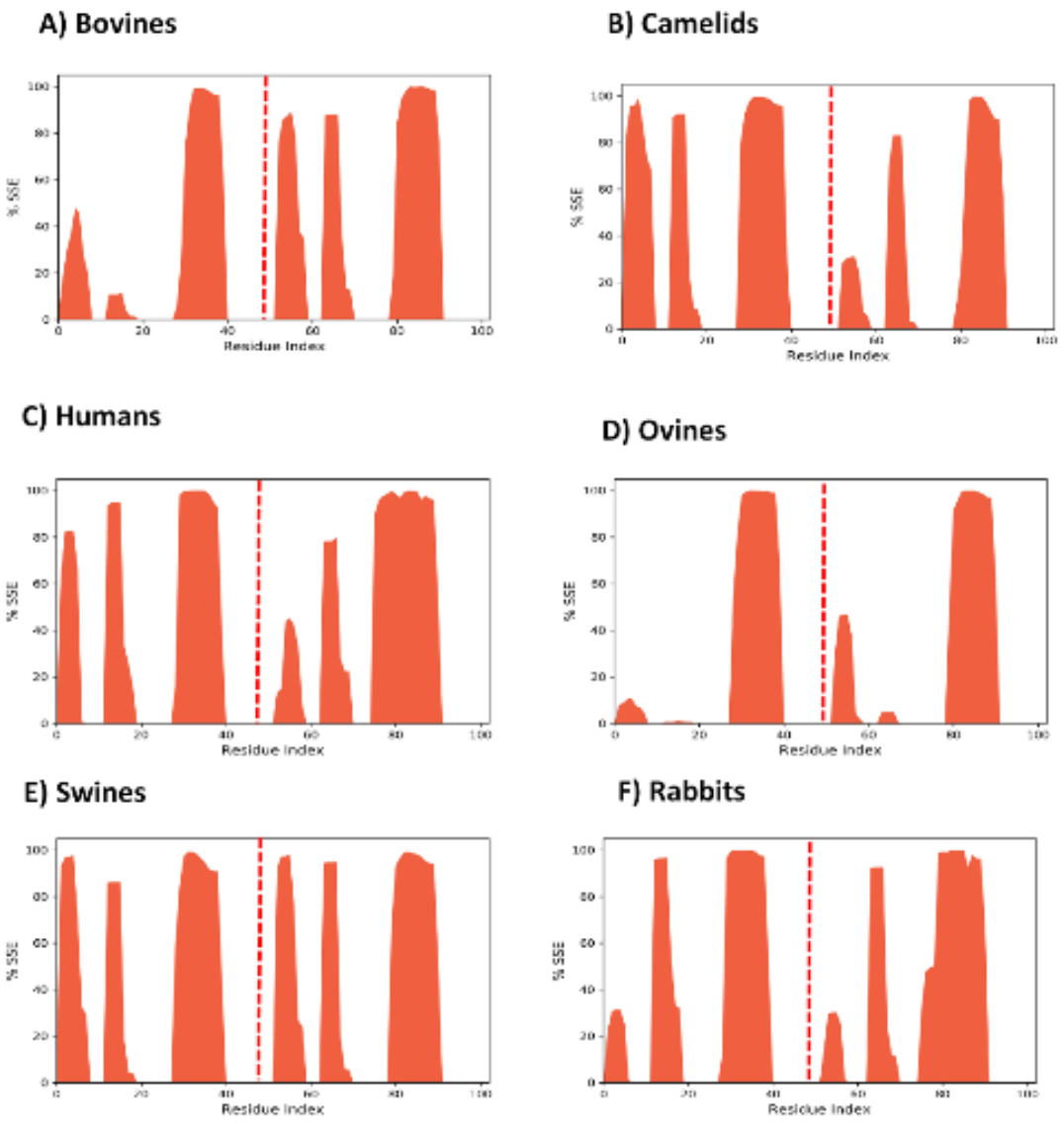

Fig 7: The secondary structure elements (SSE) during 50 ns MD simulation of insulin from human, camel, ovine, swine and rabbits. The red dashed line was added to mark the differences between each monomer.

The insulin dimer is a stable input for structural investigations since the monomeric form is almost contains disordered structural components (Ganim *et al*. 2010). Therefore, insulin dimer was selected for MD simulations in this study.

Insulin is composed of two chains, A and B chains. The A chain is composed of two anti-parallel α-helices α1 and α2 covering the residues ranges A2-A8 and A13-A20, respectively. The B-chain has a single broad helix (α3), which is formed by residues B23-B43 and ends in an unstructured C-terminus loop (residues B44-B51). The helical structure of the insulin molecule is maintained by a single salt bridge (between GluB38 and ArgB43) and three disulfide bonds (Sundaram *et al*. 2021). The insulin structure is maintained by a hydrophobic core composed of A2A, A3V, B29G, B32L, B33V, B36L and B45F. This hydrophobic core is conserved in humans, bovine, ovine, swine, camelids and rabbit insulin (Fig. 3).

In the insulin monomer, the last four residues of the B chain C-terminal residues are exposed to solvent and its mutation affects the insulin dimer affinity. For instance, insulin aspartate has rapid action due to the reduced dimer affinity after mutation of the B-chain proline (BP28) to aspartate (Owens and Vora 2006). In this study, insulin of bovine, camel, swine, ovine and rabbits showed mutation in the B chain at the last residue position BT30 in human insulin. The BT30 residue is replaced by alanine in bovine, camel, swine and ovine insulin. While it is replaced by serine in rabbits (Fig. 3). Residue scanning and mutation studies revealed that mutation at this site affected the dimer affinity and stability (Table 4). The BT30A mutation resulted in decreased dimer affinity and stability. This may indicate that dimer affinity is lower in bovine, camel, swine, and ovine insulin than in human insulin. As a result, these insulins might be easily dissociated and activated than human insulin. In contrast, BT30S in rabbits improved the dimer affinity and stability.

Analysis of RMSD reveals that camel insulin undergoes more structural transitions than other animals and human insulin (Fig. 4). This might be due to the largest number of different (four) residues compared with human insulin. The late arrival to the steady-state in camel insulin implies more time required for the system water to penetrate the camel insulin molecule. In contrast to other insulins, which reached RMSD value of 2-3 Å within the first ten nanoseconds.

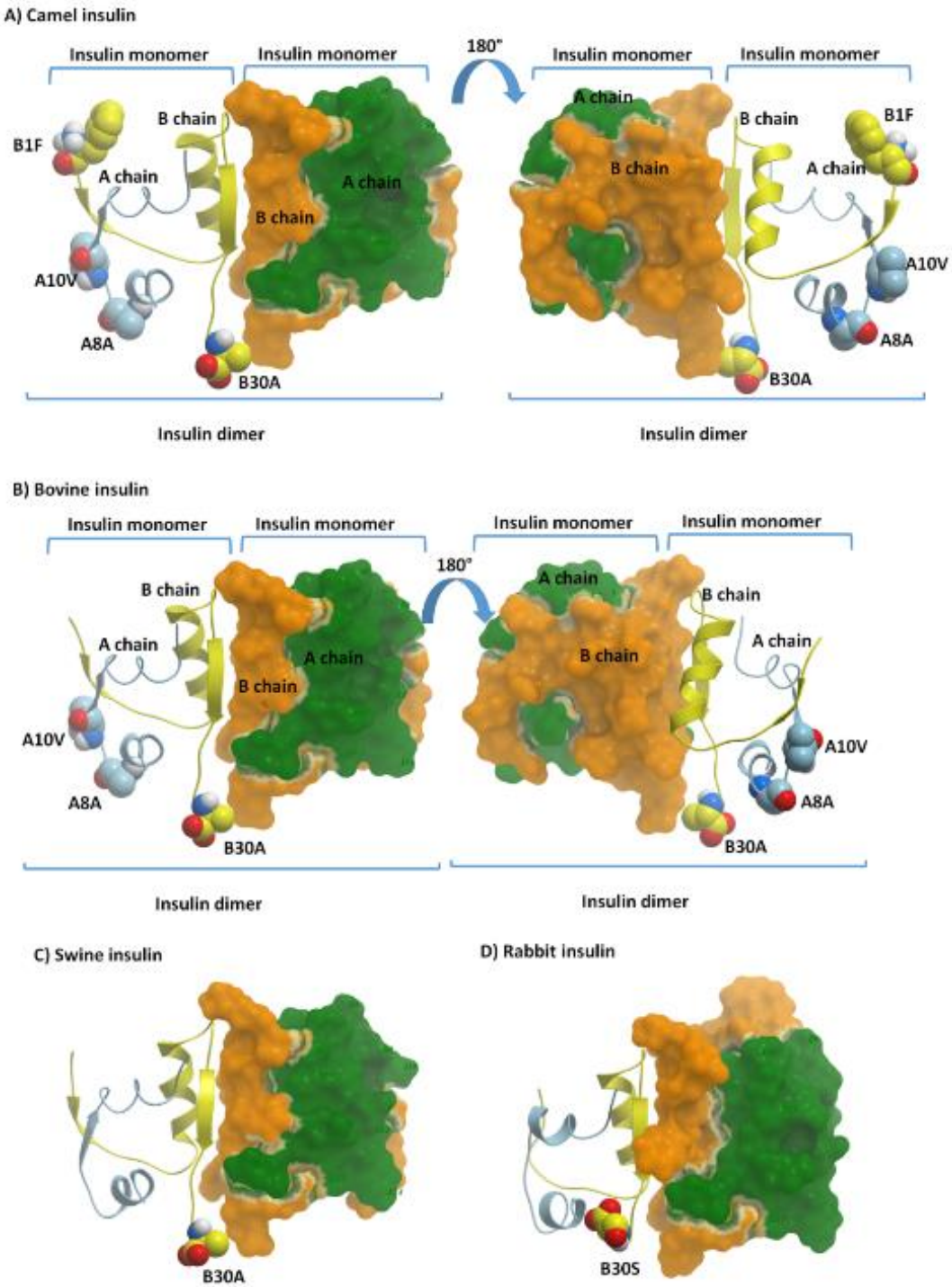

Fig. 8: Graphic representation of insulin dimer from camel, bovine, swine and rabbit. The insulin monomers are displayed either in cartoon (left) or surface representation (right). The residues differences between the selected insulins and the human insulin are represented in balls coloured by the atom type.

The conformational stability as evidenced by RMSF (Fig. 5) indicated maximal fluctuations at the loops of C-terminal of A-chain, N-terminal of B-chain and C-terminal of B-chain. This is a characteristic feature for all protein systems, in which loops at terminals are more flexible than other parts or the helical components. This finding agrees with the previous RMSF studies of insulins (Papaioannou *et al*., 2015). However, the residues fluctuation features were not completely conserved or asymmetrically displayed in the insulin dimers. The asymmetric behavior of insulin monomers agrees with the previous findings of MD simulation of insulin dimer (Falconi *et al*., 2001). This was attributed to the different number of chain-to-chain hydrogen bonds and the higher number of residues forming a random coil in the first monomer (Falconi *et al*., 2001). This indicates that the second monomer is more rigid and structured during simulation. However, in this study second monomer in camel insulin was less rigid and structured by showing a lower percentage of SSE (Fig. 7B).

The insulin structure is stabilized by three disulfide bonds between A:C6-A:C11, A:C20-B:C19 and A:C7-B:C7. The former bond has been associated with the prevention of amyloid body formation and insulin

denaturation (Dec *et al*., 2019). The camel structure showed the shortest A:C6-A:C11 length, which might modulate the denaturation of camel insulin.

Conclusions: Overall, the several mutations in the human insulin could lead to lowered dimer affinity and potentially easier dimer dissociation. These mutations were derived from their corresponding residues in the bovine, camel, swine and ovine insulin. These mutations comprise A: T8A, A: I10V, B: T30A and B: F1V. The observed adverse changes in residues affinity and stability of insulin dimers after mutation of human insulin to camel, bovine or swine insulin residues suggests its application in improving the dissociation of human insulin dimers dissociation. Of special interest, the T30A mutation can be used to improve the dissociation of human insulin dimers.

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Authors contribution: MK designed and performed this study. MK, KA, SA, IA and Sal analyzed data, and MK wrote the manuscript. MG, MB, SA and SI revised the manuscript. All authors approved the final version of the manuscript.

REFERENCES

Abdel-Fattah M, Amer H, Ghoneim M, *et al*., 1999. Response of one-humped camel (camelus dromedarius) to intravenous glucagon injection and to infusion of glucose and volatile fatty acids, and the kinetics of glucagon disappearance from the blood. J Vet Med Ser A 46:473-81.

Boura-Halfon S and Zick Y, 2009. Serine kinases of insulin receptor substrate proteins. Vit Horm 80:313-49.

Dec R, Koliński M and Dzwolak W, 2019. Beyond amino acid sequence: disulfide bonds and the origins of the extreme amyloidogenic properties of insulin's H-fragment. The FEBS J 286:3194-205.

Falconi M, Cambria MT, Cambria A, *et al*., 2001. Structure and stability of the insulin dimer investigated by molecular dynamics simulation. J Biomol Str Dynam 18:761-72.

Ganim Z, Jones KC and Tokmakoff A, 2010. Insulin dimer dissociation and unfolding revealed by amide I two-dimensional infrared spectroscopy. Phys Chem Chem Phys 12:3579-88.

Jirimutu, Wang Z, Ding G, *et al*., 2012. Genome sequences of wild and domestic bactrian camels. Nat Comm 3:1202.

Kadima W, Øgendal L, Bauer R, *et al*., 1993. The influence of ionic strength and pH on the aggregation properties of zinc-free insulin studied by static and dynamic laser light scattering. Biopolymers: Orig Res Biomol 33:1643-57.

Kandeel M and Al-Taher A, 2020a. Metabolic drug targets of the cytosine metabolism pathways in the dromedary camel (Camelus dromedarius) and blood parasite Trypanosoma evansi. Trop Anim Health Prod 52:3337-58.

Kandeel M and Al-Taher A, 2020b. Uridine 5'-monophosphate (UMP) metabolising enzymes uracil phosphoribosyltransferase and orotidine-5'-phosphate decarboxylase/ump synthase in camels and Trypanosoma evansi. J Camel Prac Res 27:81-8.

Kandeel M and Alzahrani A, 2020. Molecular dynamics simulation of carbonyl reductase I clarifies the structural switch in drug metabolism. J Taibah Univ Sci 14:1326-34.

Kandeel M, Dalab A, Al-Shabebi A, *et al*., 2020a. Bioinformatics of pyrimidine metabolism in camels and Trypanosoma evansi: uridine 5'-diphosphate (UDP) metabolic pathways and targeting ATP diphosphatase. J Camel Prac Res:1.

Kandeel M, Kitade Y and Almubarak A, 2020b. Repurposing FDA-approved phytomedicines, natural products, antivirals and cell protectives against SARS-CoV-2 (COVID-19) RNA-dependent RNA polymerase. Peer J 8:e10480.

Kaske M, Elmahdi B, Engelhardt Wv, *et al*., 2001. Insulin responsiveness of sheep, ponies, miniature pigs and camels: results of hyperinsulinemic clamps using porcine insulin. J Comp Physiol B 171:549-56.

Lovatt M, Cooper A and Camilleri P, 1996. Energetics of cyclodextrin-induced dissociation of insulin. Eur Biophys J 24:354-57.

Nazifi S, Rezakhani A and Gheisari H, 1998. Physical, biochemical and cytologic properties of blood and synovial fluid in clinically normal adult camel (Camelus dromedarius). J Vet Med Ser A 45:155-60.

Owens D and Vora J, 2006. Insulin aspart: a review. Expert Opinion on Drug Metabol Toxicol 2:793-804.

Papaioannou A, Kuyucak S and Kuncic Z, 2015. Molecular dynamics simulations of insulin: elucidating the conformational changes that enable its binding. PloS One 10:e0144058.

SASAKI Si, 2002. Mechanism of insulin action on glucose metabolism in ruminants. Anim Sci J 73:423-33.

Sundaram V, Ramanan RN, Selvaraj M, *et al*., 2021. Structural stability of insulin aspart in aqueous cholinium aminoate ionic liquids based on molecular dynamics simulation studies. J Mol Liq 322:114501.

Yagil R, 1985. The desert camel. Comparative Physiol Adap Karger.