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Camel Milk α-lactalbumin As A Potential Anticancer Molecule: A Bioinformatics Analysis.

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ABSTRACT

α-lactalbumin-Oleic acid complex derived from milk is well known to have anti-cancer properties in many diverse mammalian species including humans, goats and cows. The objective of the current study is to see if α-lactalbumin present in camel's milk too can serve as a potential anti-cancer molecule in its complex form. A comparative evaluation of α-lactalbumin structure among four mammalian species, viz, *Camelusferus*, *Bostaurus*, *Homo sapiens*, and *Capra hircus* has been made between the nucleotide sequences and structures of protein. Furthermore, the physico-chemical properties, amino acid composition, position of disulfide bridge formation in these species has been compared. The outcome of the present study suggests the presence of an anti-cancer property in the α-lactalbumin of camel, as has been experimentally observed in the other three species.

Keyword: Camel milk; Anti-cancer; HAMLET; XAMLET, α-Lactalbumin; protein model. Computational analysis, Protein, DNA, Physicochemical properties, amini acid

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INTRODUCTION

Over millions of years, diverse species have evolved to ensure their survival against adverse climatic and pathological environments. In case of mammals, maternal milk has paramount preventive and therapeutic benefits against vast variety of diseases, including cancer (Küçükçongaret al., 2015*)*.Cancer is the second largest killer in the world and cancer deaths have accounted for 8.2 million deaths in 2012, and by 2030, the global cancer burden is expected to reach ~20 million deaths/year (Globocan, 2012). Cancer therapeutics remains a very challenging area, not only due to complex molecular mechanisms involved in its pathogenesis but also due to the severity of side effects caused by the present treatment modalities.

Evolutionarily conserved biomolecules have played a highly significant role in carrying out vital functions across diverse species, and it is well-known that structurally similar biomolecules have similar functions. Immunologically important biomolecules play a vital role in protecting us from various infections and cancers (Corthay, 2014).The anti-cancer ability of α-lactalbumin-oleic acid complex (HAMLET) is well established (Rath et al., 2015), and it has been discovered by Hakinsson in the human milk (Hakkanson,1999).Interestingly, a variety of HAMLET-like substances from milk of other mammals including bovine, equine, porcine, ovine, and caprine species have also been discovered. For the sake of generalization, these molecules are known as XAMLET (X referring to any other mammalian species) (Kakamura et al., 2013). HAMLET specifically lyses cancer cells without having any adverse effect on the normal cells, and it has been shown to have a significantly positive response against glioblastomas, skin papillomas and bladder cancers (Mossberg et al., 2010).It shows a promising antitumor response and has no side effects.

Camel milk is well known for its medicinal properties (Agrawal et al., 2013; Dubey et al., 2016).It has been used for the treatment of various diseases like diarrhea, diabetes, food allergies and liver diseases (Ehlayel et al., 2011; Korish 2014; El Miniawy et al., 2017). Traditionally, camel milk has been used as a prophylactic and therapeutic nutraceutical against cancer in the middle-east countries. Camel is well known for its ability to withstand thermal and aquatic stress, and its milk proteins are unusually stable against high temperature and acid hydrolysis (Atri et al., 2011).Camel has a very special immune system and it exhibits the presence of camelid antibodies which contain only the heavy chains in their structure (Konning et al., 2016).
α-lactalbumin, also known as LALBA, is a predominant whey protein present in milk and colostrum. Its LALBA gene is expressed only in the mammary gland. α-lactalbumin plays a fundamental physiological role during the biosynthesis of lactose in milk. It forms the regulatory subunit of lactose synthase complex which catalyzes the final step of lactose biosynthesis. It is known to have cytotoxic, bactericidal and anti-tumor properties. Additionally, camel α-lactalbumin is relatively heat-stable when bound to calcium (Atri et al., 2010). In comparison to other milk proteins, it encodes many essential amino acids, namely, tryptophan, lysine, and cysteine. Since it is the vital component of HAMLET, it can serve as a suitable model to study anti-cancer property of HAMLET and related molecules (XAMLET) present in other mammalian species.

To understand its functional implications, Redington*et al.*has recently compared the structure of α-lactalbumin in camels and cows, and found camel protein to be more stable towards thermal and pH mediated denaturation and less stable towards guanidine hydrochloride-mediated unfolding (Redington et al., 2016). Atri et al. has conducted a comparative structural and stability analysis of cytotoxic complex of camel α-lactalbumin with oleic acid and with linoleic produced at higher temperatures (Atri et al., 2011). Recently, Shariaatikia et al. has studied the anti-cancer activity of cow, sheep, goat, mare, donkey and camel milks, their caseins, whey proteins and *in-silico* comparison of the caseins has indicated that mare, camel and donkey milks might be good candidates against the breast cancer cells (Shariaatikia et al., 2017). Comparison between the vital sequence parameters of these proteins can yield important information, and the derived physicochemical properties like instability index, pI, aliphatic index, number of charged residues have strong functional implications. Moreover, the conservation across this protein has a high degree of structural similarity, and it forms the basis of their functional relationship. Comparative sequence and structural homology analysis of this molecule with the other referred mammalian species, shown to exhibit an anti-cancer property for this molecule, forms the basis of this study. We have done a comparative analysis of the protein α-lactalbumin and its domain(s) among the four mammalian species, viz, *Camelusferus*(Camel), *Bostaurus* (Cattle), *Homo sapiens* (Human) and *Capra hircus* (Goat) at the primary, secondary and tertiary level. Furthermore, comparative analysis of physicochemical properties has also been done. Lastly, the functionally important residues are identified and the conserved α-lactalbumin structure is computed through the selected sequences and through the HMMER-profile of their non-redundant sequence homologs.

Methods

Comparative sequence analysis

The α-lactalbumin protein sequences are retrieved from NCBI for the four species, viz, *Camelusferus* (camel), *Homo sapiens* (human), *Capra hircus*(goat), *Bostaurus*(cow). The sequence as well as structural similarity is computed between the four sequences, as explained subsequently.

Primary and secondary sequence comparison

Pairwise alignment of α-lactalbumin protein of all the selected species is constructed and assessed through the PRALINE multiple sequence alignment server (Bawono and Heringa, 2014). PRALINE is a fully customizable multiple and pairwise alignment application. Apart from several other currently available alignment strategies, it can integrate information from database homology searches to generate a homology-extended multiple alignment. The scoring scheme works within the range of 0 and 10, which respectively mark the least and the most conserved alignment position. The scoring is color-coded and it adds all the individual scores to obtain the final alignment score. The higher the final score, the more is the sequence identity.

The HHpred online server is used to screen the most closely related structural templates for all the selected protein sequences from the PDB database (Soding, 2005), and the structures are retrieved. As the camel sequence shows a 70% sequence identity against the other three structures, it is modelled on basis of each of these templates on basis of their HHPred alignment through MODELLER (Sali and Blundell, 1993), to further do a detailed assessment and select the best model. The constructed models are topologically compared through the TM-align server via the optimal structural superposition of their secondary structures, viz. calcium binding domains, α-helix and β-pleated sheets (Zhang and Skolnick, 2005).

TM-align deploys the TM-score rotation matrix (Zhang and Skolnick, 2005). The alignment score and sequence similarity of the evaluated sequence pair, along with their conserved domain availability are retrieved from the same source. It quantitatively estimates the mutual structural similarity of the proteins against the camel and human sequences. TM-score is usually deployed for measuring the topological similarity of two protein structures, and it is less sensitive to the local conformational variations (Runthala, 2012; Runthala and Choudhury, 2019). The magnitude of TM-score for random structure pairs is independent of their sequence lengths and its score varies from 0 to 1, where the latter defines the maximum similarity score attained by the two identical structures.Following the strict statistics of structures in the protein data bank (PDB), a score below 0.17 corresponds to the randomly chosen unrelated proteins whereas with a score more than 0.5 belongs to the same structural fold.

Comparative analysis of amino acid composition and location

The ProtParam tool is used to analyze the relative composition of all the amino acids in the considered protein within all the considered species. The difference of amino acids in primary structure is compared and analyzed using the online tool GOR (version IV). Secondary structure analysis with GORIV is performed alongside to compare the changes resulting from an amino acid shift at a particular position. Structural analysis and prediction related to the α-helix, random coil and extended strands is performed through this tool.

Evaluation of disulfide-bridge forming residues

The disulfide bridge formation and its position in the protein is compared among the four species using the same online tool GOR (version IV) through the DISULFIND tool available at PredictProteinserver. It is deployed to decode their sequence conservation. The conservation of location of these disulfide bonds is an indication of the similarity within the domain size.

Comparative Conformational Analysis

The HHpred online server is used to screen the closest set of functionally related protein templates. The protein structures of the human, cow and goat α-lactalbumin are thus obtained from the PDB. To estimate

the topological similarity of camel sequence with all these structures, its model is constructed by deploying each of these structures as a template. By using the HHPred alignment, the camel protein is modeled with each of the three templates through MODELLER.The constructed models are topologically compared through TM-Align via the optimal structural superposition of their secondary structures.

Physico-chemical properties of α-lactalbumin

The ProtParam tool at ExPASy is used to analyze the various physico-chemical properties of α-lactalbumin across the four species under consideration. The vital biological properties of a protein, including the instability index, Absorbance, Molar extinction, pI, GRAVY, Aliphatic Index and the proportion of positively and negatively charged residues are estimated for making this study fruitful for the further experimental studies.

Structural conservation

For the camel sequence, HMMER is deployed against the UNIREF90 database to build a sequence profile of evolutionarily close homologues by HMMER with the default E-value profile extension threshold of 0.0001 through consurf(Ashkenazy et al., 2016). The default strategy is used to maximally sample the resultant homologs within a mutual sequence identity range of 35-95%, and 150 sequences, sampling the resultant list, are selected for further analysis. MAFFT-L-INS-I algorithm is subsequently used to align these sequences and with the Bayesian statistical scoring, the best solution is selected as the evolutionary substitution model. The strategy is expected to map the conservation scores across the camel sequence chain and its predicted tertiary structure. In addition, the entire strategy is also repeated for only the four considered sequences, and the statistically computed evolutionary scores are compared.

RESULTS

Comparison of protein sequence of α-lactalbumin in the Camel, Humans, Cow and Goat

Amino acids in α-lactalbumin: The α-lactalbumin protein sequences for all the four species are detailed in the following Table 1. It is interesting to observe that these sequences encode the same number of 142 amino acids.

Comparison of the primary protein sequences

The primary sequence of α-lactalbumin of human, cow, and goat are compared with camel, as shown in figure 1 through PRALINE. To denote the residue conservation across these pairwise alignments, the residues are scored within a range of 0 to 10 to denote the least and most conserved residue loci within those species.

Sequence identity and alignment score of camel α-lactalbumin protein with other 3 species

Camel α-lactalbumin protein sequence shows 70% sequence identity with each of the other three considered sequences (Table 2). Furthermore, the alignment score is found to be ~0.86 for all the three species. It could be observed that the secondary structure of protein is quite similar in spite of differences at the primary level.

Comparative analysis of amino acid composition

The residue percentages encoded in the α-lactalbuminin sequences for all the selected species are computed (Table 3), and numerous observations are drawn. While the leucine is found to be the most common amino acid encoded in all these species, cysteine is equally present in the sequences. Moreover, proline content is similar in all three species (1.4%), except in Human's, where it is found to be double (2.8%); Serine content in α-lactalbumin is same for all (5.6%), except for cow where it is slightly higher (6.3%); Tyrosine content in camel α-lactalbumin is found to be the lowest (2.1%) among the other three species (2.8%), though the difference is not very significant and six amino acids (Glutamic acid, Glycine, Methionine, Asparagine, Arginine and Tryptophan) are much more frequent in camel than the other species. Furthermore, six residues

(Alanine, Phenylalanine, Glycine, Glutamine, Tyrosine and Threonine) are less frequent in camel than the other species. Lastly, besides a few residues, rest others shows a substantial variation in their percentages than their average value, which is expected for these species.

Comparison of location of amino acid residues in camel α-lactalbumin with human, cow and goat

Alterations of the vital residues recognized from the alignment of camel α-lactalbumin and the other three species are summarized in table 4. To better investigate the structural role of these mutations, the proteins are structurally evaluated. Quite interestingly, only 9/10 residue alterations are observed between the camel and the other sequences. The position of amino acid transition and the corresponding secondary structure change is almost identical when camel α-lactalbumin is compared to bovine and goat, with only single amino acid variation missing at position 89th in the analysis of camel and goat sequences.

Comparison of the secondary structure of α-lactalbumin in the four species and their disulphide bonds

Secondary structure analysis: The comparative predominance of features of secondary structures of α-lactalbumin namely, α-helix, extended strand and random coil are compared between the four species. It can be noted from table 5 that in camel, the relative proportion of α-helix is lesser than all the other three species i.e. 10.56% as compared to 25.35%, 24.65 % and 26.06% in human, cow and goat respectively. Also its extended strand is more than the other species with cow and goat being very similar i.e. 31.69 as compared to 21.13%, 25.35% and 24.65% in human cow and goat respectively. Furthermore, camel has maximum random coil too (57.75 % vs 53.52 %, 50.00% and 49.30% in human cow and goat respectively. This differentiating feature may be useful in making the camel proteins more thermo-resistant and its milk proteins resistant to acid hydrolysis.

Location of disulphide bonds in α-lactalbumin

It may be noted from figure 2 that the number of disulphide bond in α-lactalbumin is the same in all the four species, namely, 4. Moreover, the location of these disulphide bonds within α-lactalbumin is also the same in all these species. This is an indication of similarity in the size of domains in the four species under consideration**.** Furthermore, similar disulphide bonds likely lead to a similar conformation of the molecule across the four species. This similarity in structure is the basis of a predicted similar function.

Comparative structural analysis of α-lactalbumin amongst the four species

Tertiary structure of α-lactalbumin the four species

Given in figure 3 is the structure of α-lactalbumin in four different mammalian species. This protein has three main structural components is all the four species studied. These domains are the helices, the extended domain with beta-pleated sheets and the Ca^{2+} binding domain. Shown in these figures are the structures of this molecule in these four species. It may be noted that the structure of α-lactalbumin is quite similar in camel, human, cow and goat.

Tertiary structure of the α-helix, extended sheet and Calcium binding domain of α-lactalbumin in the four species

The structural similarity in the three domains (namely the Ca^{2+} binding domain, α-helices and the beta pleated sheets) of camel α-lactalbumin with the other three species are compared in this section. Aremarkable degree of similarity in the confirmation is observed between camel and the other three species when comparedindependently and as shown in figure 4 (A, B and C) below.

Structural comparison of calcium binding domain of camel α-lactalbumin with all the other three species

The Ca^{2+} binding domain is very important region determining the anti-cancer property ofα-lactalbumin-oleic acid complex. A striking similarity is observed in this domain across the considered species and is shown in the following figure 5. This similarity in structure further confirms the functional similarity of camel with these proteins.

Comparative analysis of the physico-chemical properties of α-lactalbumin

The important physico-chemical properties of α-lactalbumin, viz. molecular weight (KDa), instability index (Stable/ Unstable), molar extinction coefficient at 280nm ($cm^{-1}M^{-1}$), theoritical isoelectric point, GRAVY index (Measure of peptide solubility) and total positively and negatively charged residues, are estimated for all the four species (Table 6). It can be noted that the molecular weight of the evaluated protein does not vary significantly across the four species. Further, unlike the other three species, the human α-lactalbumin is found to be the most unstable structure, and its instability index is also higher than the defined threshold of 40.0. Assuming all pairs of Cys residues as cystienes, the absorbance is estimated and it is respectively found to be highest and lowest for the camel and human α-lactalbumin molecules, and equivalent for the cow and goat protein, as also well expected from the respectively higher and lower proportion of tryptophan in camel and human.

The theoretical pI score of these proteins are found to be quite similar and lesser than 7.0. The GRAVY index score, a measure of the average values of hydropathy for the protein solubility, is found to be negative for all the species besides camel. The aliphatic index, which is positively correlated with thermo-stability, is dependent on the relative proportion of Ala, Val, Ile and Leu. While the human and camel α-lactalbumin respectively shows the highest and lowest relative proportions, the cow and goat shows an almost similar trend.

Structural conservation

ConSurf is used to estimate the degree of conservation for the camel sequence against the set of the considered bovine sequences. It estimates the statistical conservation score for every residue for the constructed alignment, and is a widely used strategy to decipher the functionally vital segments. On basis of the considered sequences and their alignment, the residue-conservation is plotted over the tertiary structure, predicted by Consurf, for the camel sequence. Through the per-residue score, the evolutionarily retained sites are mapped for the camel sequence. The rate of evolution is estimated using the Bayesian method and the conservation pattern is plotted on the protein sequence (Figure6).

The conservation for the variable regions, average and highly conserved positions is respectively mapped over the predicted camel structure as turquoise, white and maroon. This analysis shows the average residue substitution score of 0.293343 for the camel sequence, within the upper and lower bounds of 0.381128 and $1.01758e^{-07}$ respectively. However, repeating the analysis for the camel sequence by HMMER through Consurf, it yields 1708 unique sequences from a total of 2043 statistically top-scoring hits, and the 150 sequences, maximally sampling the non-redundant dataset, are finally considered to estimate the evolutionarily significant conservation across the camel sequence. In contrast to the earlier analysis, it yields an average residue substitution score of 1.22186, along with the upper and lower bounds of 2.39966 and $1.01758e^{-07}$ respectively. Comparing the scores for the consurf evaluation of the considered bovine set and the top-ranked homologues of camel sequence, the scores are found to be sufficiently lower for the former case. With an increased dataset in the latter case, it could be well expected due to sequence diversification.

DISCUSSIONS

In this study, a comprehensive comparative analysis of the structure of camel α-lactalbumin is done with three other mammalian species, namely, human, cow and goat. Proteins are compared at primary, secondary and tertiary levels. The residue composition and physiochemical properties are also compared for α-lactalbumin within the four considered species. The primary sequence analysis of α-lactalbumin protein suggests a very high degree of similarity amongst the considered species**.** For the reason that protein structures are robust over the sequence alterations, there exists an even higher degree of similarity at the secondary structure level.

Shariatikia et al. has already investigated the anticancer activity of cow, goat, sheep, mare, donkey and camel milks, and their casein and whey proteins (Shariatikia et al., 2017). Experimentally as well as *in silico* analysis highlight a strong correlation between the anticancer activity of milk caseins, its physicochemical properties and secondary structure. Comparison of amino acid content of α-lactalbumin across the four

species shows that leucine is the most common amino acid present in all of them, and cysteine is equivalently encoded in all the four species. Not only an equal number of each residue is present in α-lactalbuminin in all the four species, the same amino acids (aspartic acid, leucine and isoleucine) are predominantly present. In addition, the number of disulphide bonds (4), and their localization, is also same in all these species, and is an indication of their equivalent domain-size. Further, the position of residue transition and the corresponding secondary structure change is almost identical with only single amino acid variation when camel is compared to goat. Moreover, four positions are also similar where the same amino acid and secondary structure changes are observed. By analyzing the relative proportion of α-helix, **β**-sheets and extended structure, it is observed that the proportion of α-helix is lesser and extended strand is more in camel's α-lactalbumin in contrast to the other species. Camel encodes the maximum proportion of random coil segments. The structure of Camel α-lactalbumin is quite similar to that of other species, and the topology of calcium binding domain is found to be substantially similar than the α-helices/**β**-sheets. The domain is vital to interact with the α-lactalbumin and oleic acid to form the complex, responsible for the anti-cancer activity. It is also a strong indication that camel milk could possibly show the best anti-cancer activity among all these protein molecules.

Studies on the physiochemical properties of α-lactalbumin across the species indicate that the molecular weight of this protein does not vary significantly among the four species. Camel α-lactalbumin has the maximum absorbance whereas its human homologue shows the minimum absorbance. The absorbance of cow and goat's α-lactalbumin stands equal, and a similar pattern is also reflected in the molar extinction coefficient, as expected.

Protein GRAVY (grand average of hydropathy) value is calculated by adding the hydropathy value for each residue and dividing by the length of the sequence. The hydropathy index of an amino acid is a number representing the hydrophobic or hydrophilic properties of its side chain. The larger the number is, the more hydrophobic the amino acid. The measure of GRAVY index is negative for camel, bovine and goat's α-lactalbumin, which is indicative of their hydrophilic nature. Only human α-lactalbumin has a positive value, indicating its hydro phobic nature. Hence comparing α-lactalbumin molecule of camel with that of human, cow and goat, a significant similarity is observed for the sequence, physiochemical similarity and conserved surface. This similarity in structure or sequence conservation is the basis of a predicted functional similarity. α-lactalbumin is a very important component of HAMLET and XAMLET, that have been experimentally proven to have anti-cancer properties without any side effect (Spolaore et al., 2010; Zhang et al., 2009).In light of the fact that the other three species namely human, cow and goat are known to have an anti-tumor property associated with the α-lactalbumin-oleic acid complex (Brinkmann et al., 2011), it seems highly likely that α-lactalbumin-oleic acid complex present in camel milk should also have an anti-cancer activity. In view of the unique camel immune response, stability of camel milk proteins to higher temperature and acid hydrolysis imparts a special significance to the camel milk (Faye, 2014). Hence, a similar molecule with anti-cancer property and encoded in camel should be known as CAMLET, synonymous with HAMLET for Humans, BAMLET for Bovines, GAMLET for goats and XAMLET as a generalization for all mammalian species, as has earlier been proposed by our group [9].

Table 1: Protein Sequence of α-lactalbumin in the 4 mammalian species

Species Name	Protein sequence of α-lactalbumin
Human	MRFFVPLFLVGILFPAILAKQFTKCELSQLLKDIDGYGGIALPELICTMFHTSGYDTQAIVENNESTEYGLFQISN KLWCKSSQVPQSRNICDISCDKFLDDDITDDIMCAKKILDIKGIDYWLAHKALCTEKLEQWLCEKL
Camel	MMSLVSLLLVGILFPTIQAKQFTKCKLSDELKDMNGHGGITLAEWICIIFHMSGYDTETVVSNNGNREYGLF QINNKIWCRDNENLQSRNICDISCDKFLDDDLTDDKMCAKKILDKEGIDYWLAHKPLCSEKLEQWQCEKW
Cow	MMSFVSLLLVGILFHATQAEQLTKCEVFRELKDLKGYGGVSLPEWVCTTFHTSGYDTQAIVQNNDSTEYGLF QINNKIWCKDDQNPHSSNICNISCDKFLDDDLTDDIMCVKKILDKVGINYWLAHKALCSEKLDQWLCEKL
Goat	MMSFVSLLLVGILFHATQAEQLTKCEVFQKLKDLKDYGGVSLPEWVCTAFHTSGYDTQAIVQNNDSTEYGLF QINNKIWCKDDQNPHSRNICNISCDKFLDDDLTDDIVCAKKILDKVGINYWLAHKALCSEKLDQWLCEKL

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Table 2: Alignment score of α-lactalbumin protein of camel with respect to other 3 species

Reference	Compared sequence/ structure	Server Used	Parameter studied	Human	Cow	Goat
Camel	Primary sequence	Praline	Alignment score	2236	2268	2262
			Sequence	70%	70%	70%
	Tertiary structure	TM-Score	Alignment score	0.86	0.863	0.86

Table 3: Comparison of average residue percentages encoded in the α-lactalbumin protein of the four selected species, in correlation with their average score.

Amino Acid	Camel	Human	Cow	Goat	Average
ALANINE	2.8%	4.9%	3.5%	4.9%	4.03%
CYSTEINE	5.6%	5.6%	5.6%	5.6%	5.60%
ASPARTIC ACID	9.2%	8.5%	9.2%	9.9%	9.20%
GLUTAMIC ACID	6.3%	5.6%	4.9%	4.2%	5.25%
PHENYLALANINE	3.5%	5.6%	4.2%	4.2%	4.38%
GLYCINE	5.6%	4.9%	4.9%	4.2%	4.90%
HISTIDINE	2.1%	1.4%	2.8%	2.8%	2.28%
ISOLEUCINE	8.5%	9.9%	6.3%	6.3%	7.75%
LYSINE	9.2%	8.5%	8.5%	9.2%	8.85%
LEUCINE	11.3%	12.7%	12%	12%	12.00%
METHIONINE	3.5%	2.1%	2.1%	1.4%	2.28%
ASPARAGINE	6.3%	2.8%	5.6%	5.6%	5.08%
PROLINE	1.4%	2.8%	1.4%	1.4%	1.75%
GLUTAMINE	4.2%	4.9%	4.9%	5.6%	4.90%
ARGININE	2.1%	1.4%	0.7%	0.7%	1.23%
SERINE	5.6%	5.6%	6.3%	5.6%	5.78%
THREONINE	4.2%	4.9%	5.6%	4.9%	4.90%
VALINE	2.8%	2.8%	5.6%	5.6%	4.20%
TRYPTOPHAN	3.5%	2.1%	2.8%	2.8%	2.80%
TYROSINE	2.1%	2.8%	2.8%	2.8%	2.63%

Table 4: Residue variation within the α-lactalbumin sequence of camel in contrast to the other three species

Against human

Position	Altered Residue		Altered secondary structure	
	Camel	Human	Camel	Human
6	S	P	E	C
16	T	A	C	H
18	Q	L	C	H
26	K	E	C	H
41	T	A	E	C
43	A	P	E	C
75	N	S	C	E
78	I	L	E	C

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81	R	K	E	C
117	K	I	C	E

Against cow

Position	Altered Residue		Altered secondary structure	
	Camel	Cow	Camel	Cow
16	T	A	C	H
17	I	T	C	H
20	K	E	C	H
22	F	L	C	H
26	K	E	C	H
43	A	P	E	C
81	R	K	E	C
89	R	S	E	C
93	D	N	C	E
121	D	N	E	H

Against goat

Position	Alteredresidue		Altered secondary structure	
	Camel	Goat	Camel	Goat
16	T	A	C	H
17	I	T	C	H
20	K	E	C	H
22	F	L	C	H
26	K	E	C	H
43	A	P	E	C
81	R	K	E	C
93	D	N	C	E
121	D	N	E	H

Table 5: Representation of secondary structure of α-lactalbumin in the four species

	α-helix	Extended Strand	Random-coil
Human	25.35%	21.13%	53.52%
Camel	10.56%	31.69%	57.75%
Cow	24.65%	25.35%	50.00%
Goat	26.06%	24.65%	49.30%

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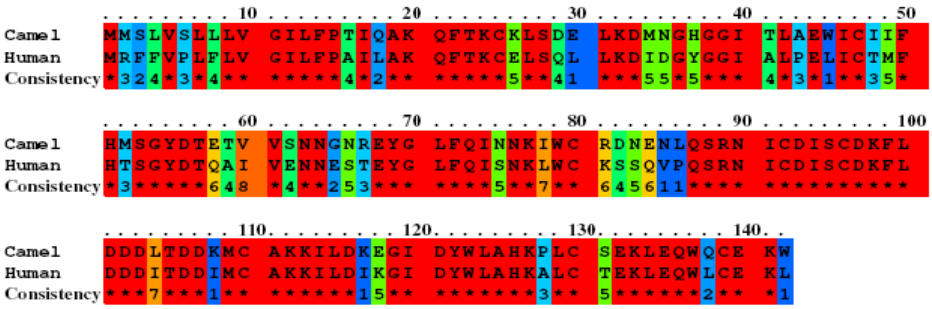
Table 6: Comparison of the physico-chemical properties of α-lactalbumin in the four species.

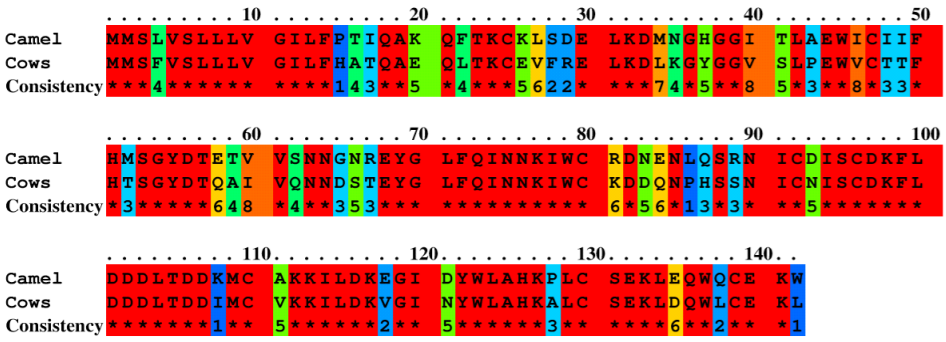
Organism	Mol. weight (KDa)	Instability index (Stable/ Unstable)	Absor bance	Molar extinction coefficient	Isoele-ctric point	GRAVY index	Total positively charged residues	Total negatively charged residues
Camel	16.4	33.62/ Stable	1.94	32,470	5.0	-0.327	16	22
Human	16.2	46.61/ Unstable	1.41	22,960	4.83	0.069	15	20
Cow	16.2	27.85/ Stable	1.75	28,460	4.92	-0.169	13	20
Goat	16.2	28.31/ Stable	1.75	28,360	5.06	-0.196	14	20

Figure 1: Sequence-comparison of camelα-lactalbuminsequence with human, cow and goat sequences.

Camel vs Human



Camel vs Cow



Camel vs Goat

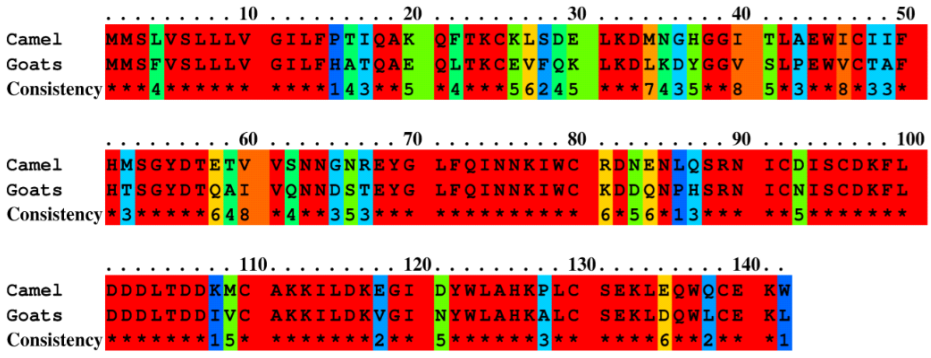


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Figure 2: Depiction of location of α-lactalbumindisulphide bonds in of the four species.

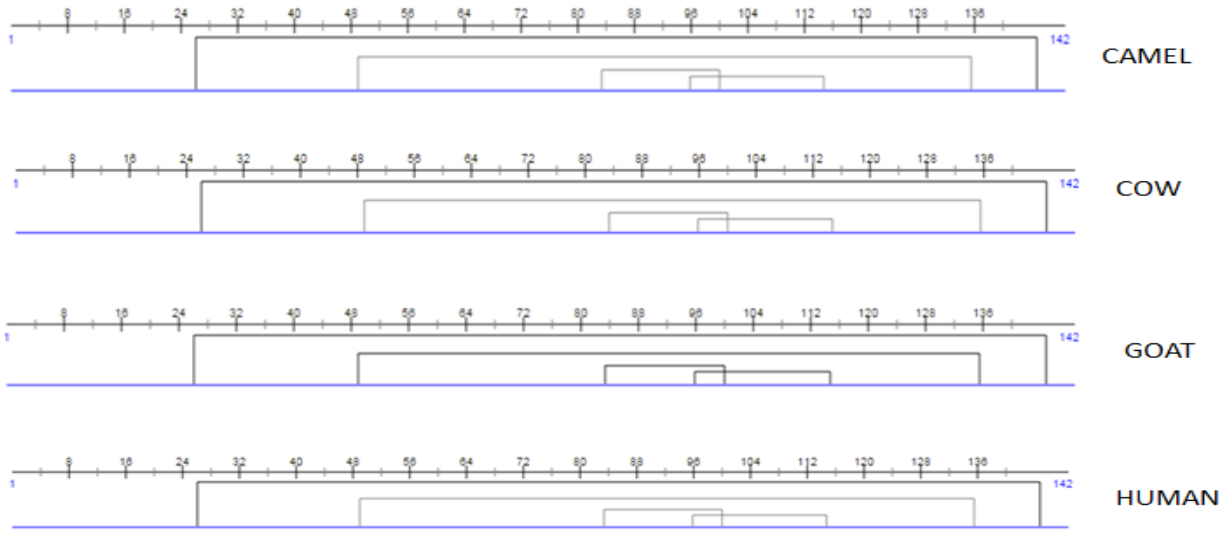

Figure 3: Structure of α-lactalbumin in human, camel, cow and goat

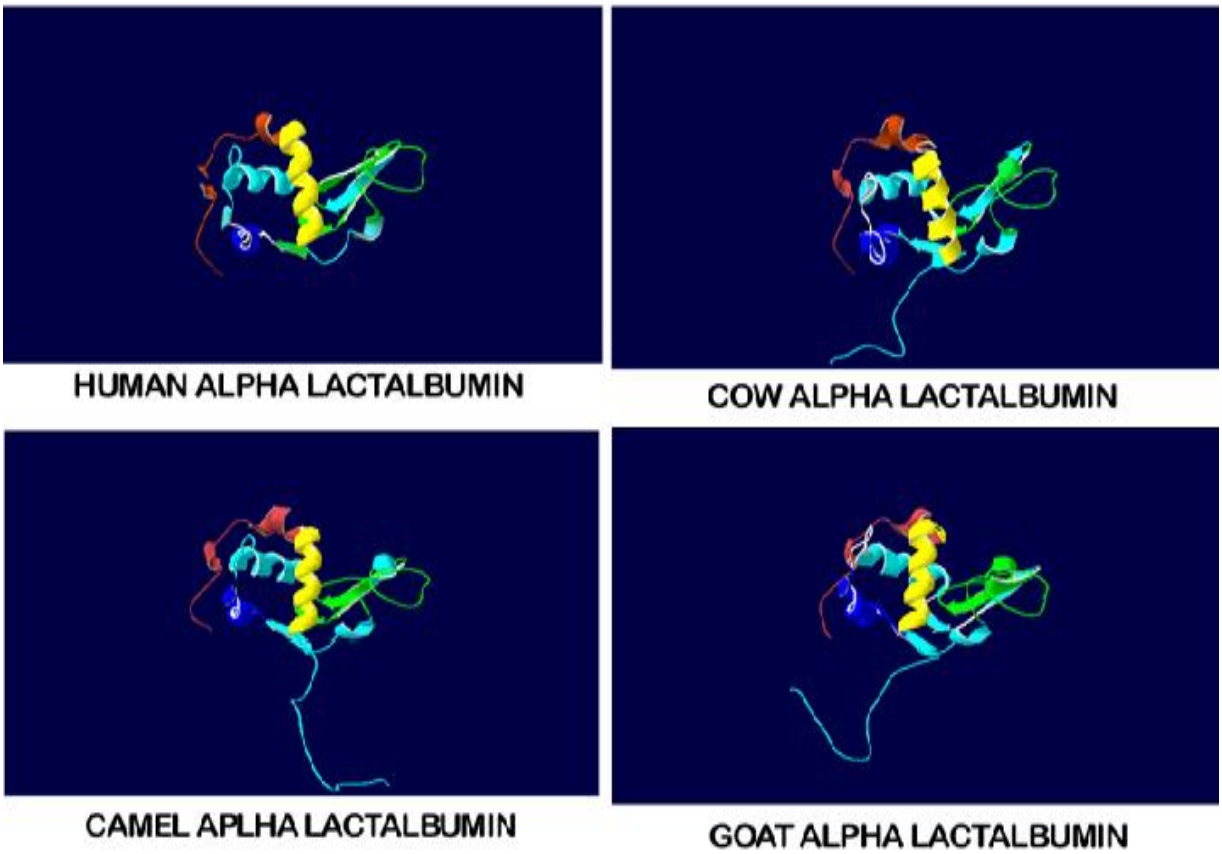

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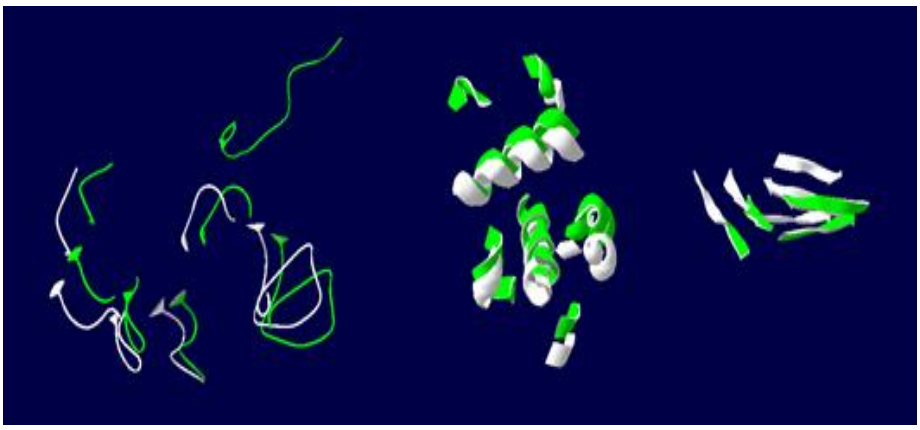
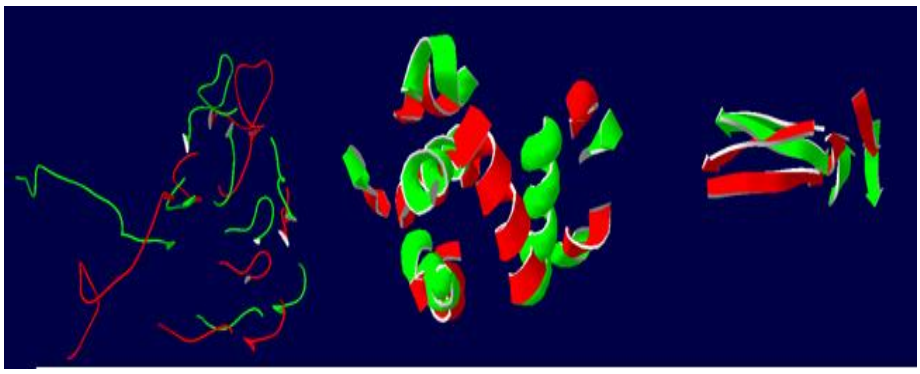
Figure 4: Comparison of Ca^{2+} binding domain (left), α-helices (middle), beta-pleated sheets (right) of camel's α-lactalbumin with the other three species. A, B and C depict the comparison of camel α-lactalbumin with that of human, cow and goat respectively. In the below figures, green indicates the structural component for camel, white is for humans, red is for cow, and black is for goat.

Structure of Ca^{2+}binding domain (left), α-helices (middle), beta-pleated sheets (right) of α-lactalbuminof camel compared with human:



Structure of Ca^{2+}binding domain (left), α-helices (middle) , beta pleated sheets (right) of α-lactalbuminof camel compared with cow:



Structure of Ca^{2+}binding domain(left), α-helices (middle), beta pleated sheets (right) of α-lactalbumin of camel compared with goat:

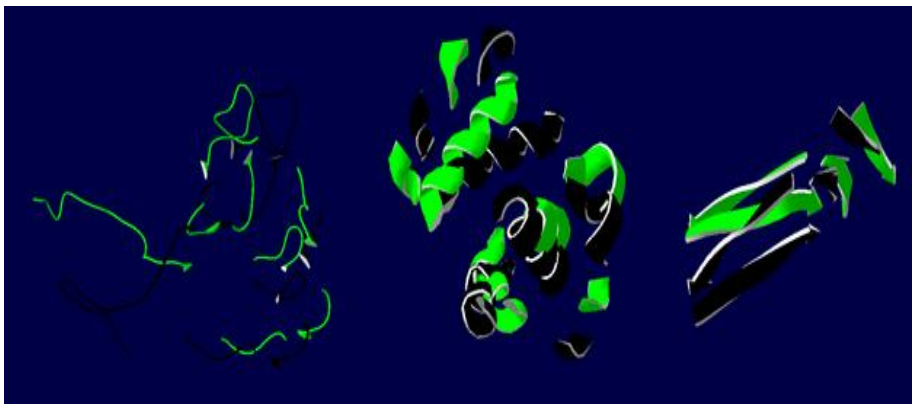
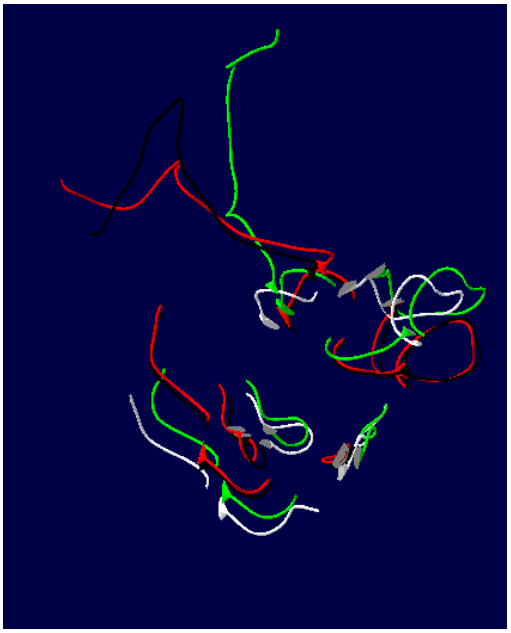


Figure 5: Comparison of calcium binding domain of α-lactalbumin among the four species. In this figure, green indicates the structural component for camel, white is for humans, red is for cow, and black is for goats.



CONCLUSIONS

A high degree of structural similarly at almost all levels is indicated when camel α-lactalbumin is compared with human, cow and goat α-lactalbumin. It is well known that structural similarity is the key to establish the functional similarity. It has been experimentally validated that HAMLET induces cytotoxicity in cell lines without causing any damage to normal cells. A similar observation has also been made for BAMLE and GAMLET. In contrast, the camel protein is hereby predicted to be more stable, as has also been experimentally validated (Atri et al., 2010). The structural similarity of CAMLET with the other three species is indicative of its similar therapeutic potential against cancer whereas the differences could be the explanation for an increased thermostability of this molecule.

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