CHAPTER 7

Comparative computational analysis of protein structure of camel α-lactalbumin with other mammalian species

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7.1 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 (Küçükçongar et al., 2015). Cancer is the second largest killer in the world and cancer deaths have accounted for 10 million cancer-related deaths occurred in 2020. By 2030, the global cancer burden is expected to mount by about 75%, increasing to 21.4 million new cases and culminating in 13.2 million deaths/year (Sung et al., 2021). 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 and Gasser, 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 (Svensson et al., 2000). 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) (Nakamura 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 anti-tumor 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 (M. S. Ehlayel et al., 2011; El Miniawy et al., 2017; Korish, 2014). 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 (Könning et al., 2017). 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.* 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. have conducted a comparative structural and stability analysis of cytotoxic complex of camel α-lactalbumin with oleic acid and with linoleic acid produced at higher temperatures (Atri et al., 2011) . Recently, Shariaatikia et al. have 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 (Shariatikia 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, *Camelus ferus* (Camel), *Bos taurus* (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.

7.2 Outline of work

In this chapter the protein sequence of camel α-lactalbumin was compared with Human, Cow and Goat α-lactalbumin at a primary, secondary and tertiary level. In the primary sequence related studies the protein sequence of the three species was compared with camel, th**e s**equence identity

and alignment score of camel α-lactalbumin protein with other three species was done. In the secondary structural analysis secondary structures of α-lactalbumin namely, α-helix, extended strand and random coil are compared between the four species was studied. Finally to understand the overall similarity at a tertiary level the structure of α-lactalbumin was overlapped with the other three species.

7.3 Results

7.3.1 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 9. It is interesting to observe that these sequences encode the same number of 142 amino acids.

Table 9: Protein Sequence of α-lactalbumin in the four mammalian species

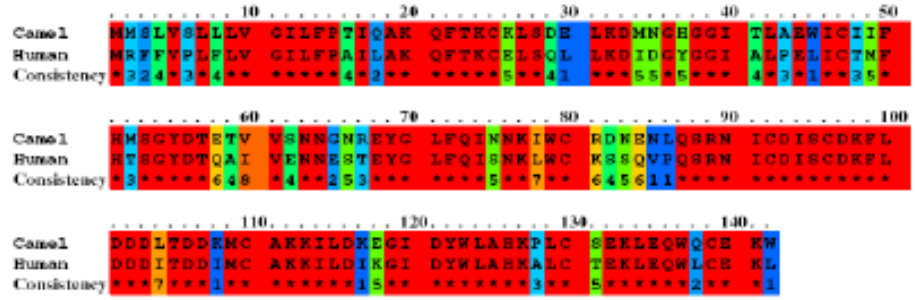
Species Name	Amino acid sequence of α-lactalbumin protein
Human	>gi|22093539|dbj|BAC06860.1| alpha-lactalbumin [Homo sapiens] MRFFVPLFLVGILFPAILAKQFTKCELSQLLKDIDGYGGIALPELICTM FHTSGYDTQAIVENNESTEYGLFQISNKLWCKSSQVPQSRNICDISCD KFLDDDITDDIMCAKKILDIKGIDYWLAHKALCTEKLEQWLCEKL
Camel	>gi|744616270|ref|XP_010995788.1| PREDICTED: alpha-lactalbumin [Camelus dromedarius] MMSLVSLLLVGILFPTIQAKQFTKCKLSDELKDMNGHGGITLAEWICI IFHMSGYDTETVVSNNGNREYGLFQINNKIWCRDNENLQSRNICDIS CDKFLDDDLTDDKMCAKKILDKEGIDYWLAHKPLCSEKLEQWQCE KW
Cow	>gi|980|emb|CAA28797.1| alpha-lactalbumin [Capra hircus] MMSFVSLLLVGILFHATQAEQLTKCEVFQKLKDLKDYGGVSLPEWV CTAFHTSGYDTQAIVQNNDSTEYGLFQINNKIWCKDDQNPHSRNICN ISCDKFLDDDLTDDIVCAKKILDKVGINYWLAHKALCSEKLDQWLC EKL

Goat	>gi|295774|emb|CAA29664.1| alpha-lactalbumin [Bos taurus] MMSFVSLLLVGILFHATQAEQLTKCEVFRELKDLKGYGGVSLPEWV CTAFHTSGYDTQAIVQNNDSTEYGLFQINNKIWCKDDQNPHSSNICNI SCDKFLDDDLTDDIMCVKKILDKVGINYWLAHKALCSEKLDQWLCE KL

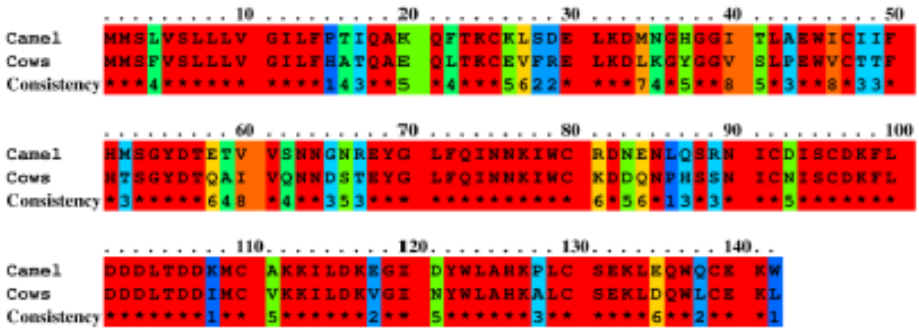
7.3.1.1 Comparison of the primary protein sequences

The primary sequence of α-lactalbumin of human, cow, and goat are compared with camel, as shown in Figure 17 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.

Camel vs Human



Camel vs Cow



Camel vs Goat

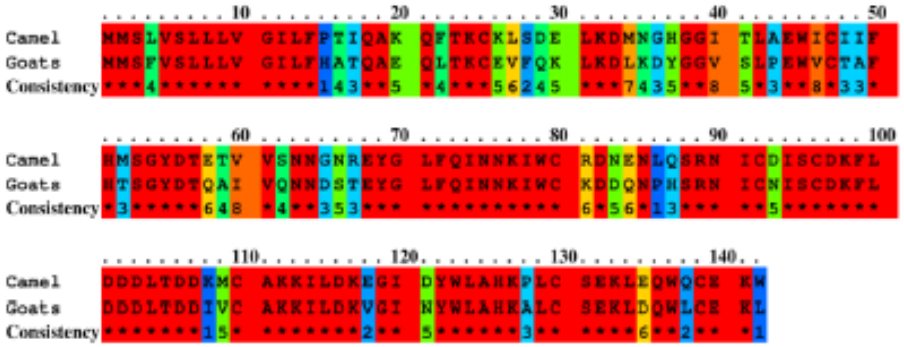


Figure 17: Sequence-comparison of camel α-lactalbumin sequence with human, cow and goat sequences

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

Camel α-lactalbumin protein sequence shows 70% sequence identity with each of the other three considered sequences (Table 10). 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.

Table 10: Alignment score of α-lactalbumin protein of camel with respect to other three species

Reference	Compared Sequence / Structure	Server Used	Parameter studied	Human	Cow	Goat
Camel	Primary sequence	Praline	Alignment score	2236	2268	2262
			Sequence Identity	70%	72%	71%
	Tertiary Structure	TM Align Score	Alignment score	0.86117	0.86324	0.86327

7.3.2 Comparison of the secondary structure of α-lactalbumin in the four species

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 11 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 comparison to 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 (Atri et al., 2010).

Table 11: 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%

7.3.3 Comparative tertiary structural analysis of α-lactalbumin amongst the four species

7.3.3.1 Tertiary structure of α-lactalbumin the four species

Given in Figure 18 is the structure of α-lactalbumin in four different mammalian species. This protein has three main structural domains in 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.

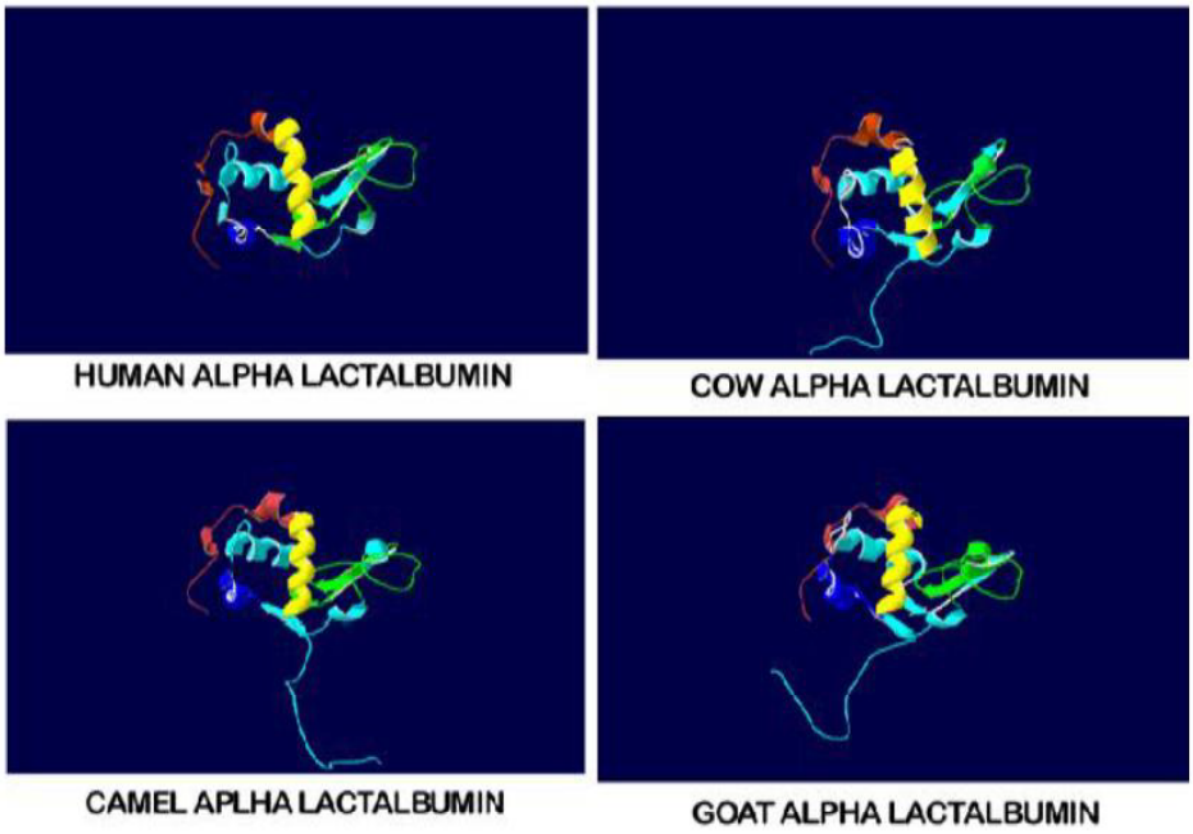


Figure 18: Tertiary structure of the α-helix, extended sheet and Calcium binding domain of α-lactalbumin in the four species

The structural similarity in the three domains (namely the Ca^{2+} binding domain, α-helices and the β-pleated sheets) of camel α-lactalbumin with the other three species are compared in this section. A remarkable degree of similarity in the conformation is observed between camel and the other three species when compared independently and as shown in Figure 19 (A, B and C). 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 human, red is for cow and black is for goat.

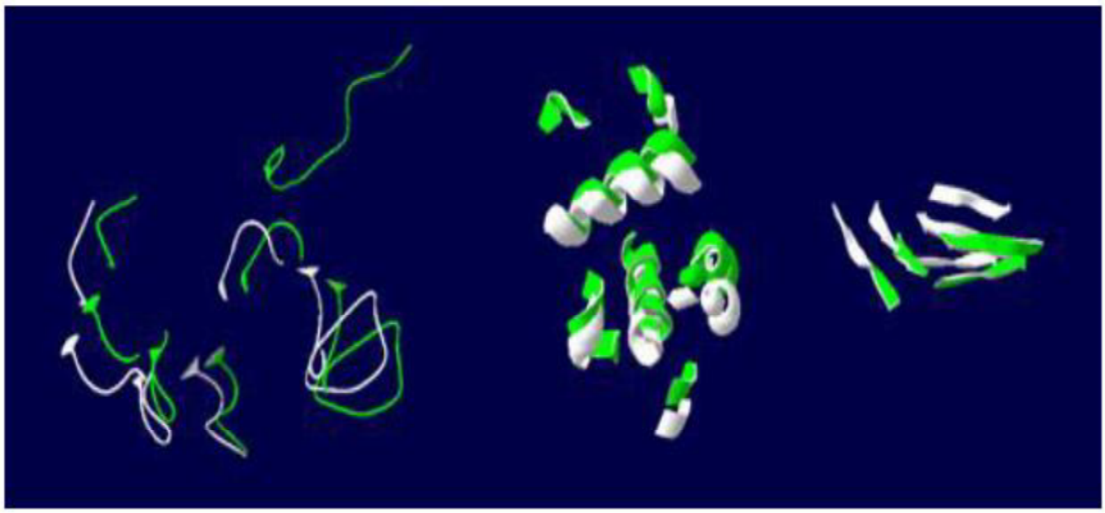


Figure 19A: Structure of Ca^{2+} binding domain (left), α-helices (middle), beta-pleated sheets (right) of α-lactalbumin of camel compared with human

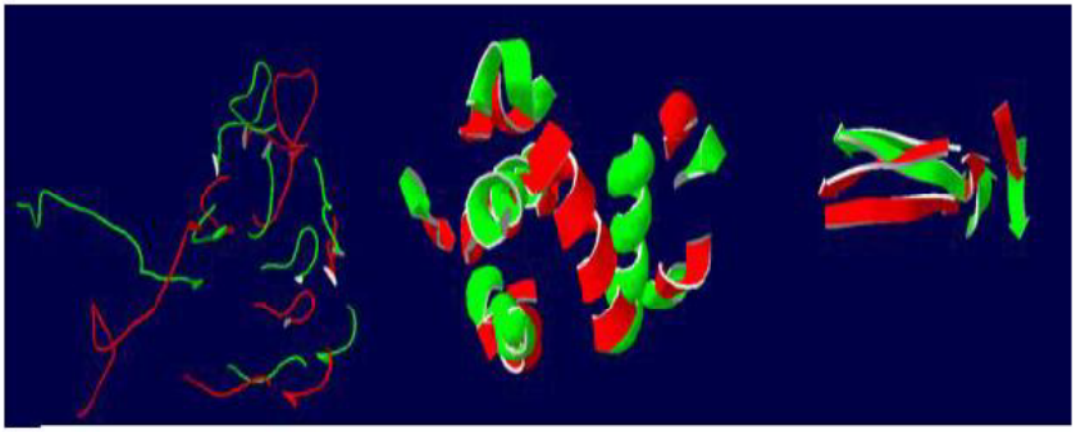


Figure 19B: Structure of Ca^{2+} binding domain (left), α-helices (middle) , beta pleated sheets (right) of α-lactalbumin of camel compared with cow

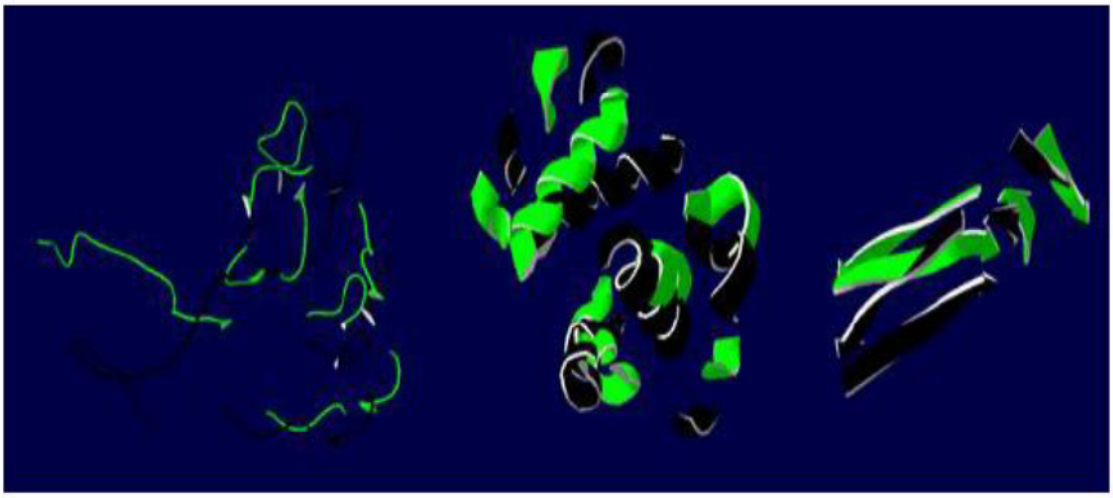


Figure 19C: Structure of Ca^{2+} binding domain(left), α-helices (middle), beta pleated sheets (right) of α-lactalbumin of camel compared with goat

7.3.3.2 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 of α-lactalbumin-oleic acid complex. A striking similarity is observed in this domain across the considered species and is shown in the following Figure 20. This similarity in structure further confirms the functional similarity of camel protein with these proteins.

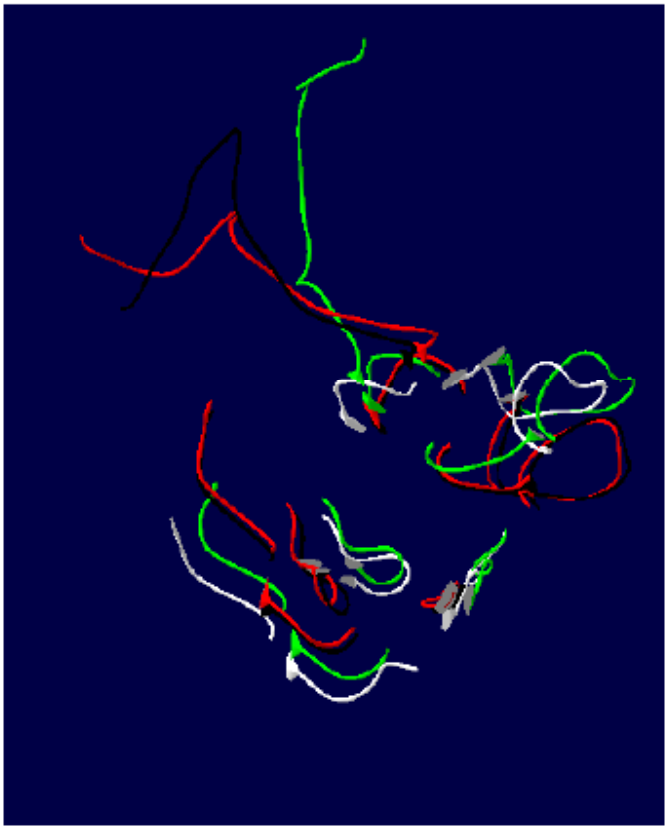


Figure 20: Comparison of calcium binding domain of α-lactalbumin among the four species. In this figure, green indicates the structural component for camel, white is for humans, red is for cow, and black is for goats.

7.4 Discussion

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 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., (2017) have already investigated the

anti-cancer activity of cow, goat, sheep, mare, donkey and camel milks, and their casein and whey proteins. Experimentally as well as *in silico* analysis highlight a strong correlation between the anti-cancer activity of milk caseins, its physicochemical properties and secondary structure. 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. 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.

7.5 Conclusion

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. Studies have experimentally validated that HAMLET induces cytotoxicity in cell lines without causing any damage to normal cells. A similar observation has also been made for BAMLET and GAMLET (Zhang et al., 2010). 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 Camel α-lactalbumin – Oliec acid complex (CAMLET) with the other three species is indicative of its similar therapeutic potential against cancer whereas the differences could possibly be the explanation for an increased thermostability of this molecule as observed by Atri.,et al in 2010.