

# **Chapter 8**

## **Conclusion and future scope** **of study**

## Chapter 8

### Conclusion and future scope of study

In this thesis the anti-cancer property of camel milk has been studied at multiple dimensions. Further the milk was partially purified and gel filtration chromatography was done to obtain the bioactive component. Finally a comparative structural analysis of camel  $\alpha$ -lactalbumin has been done with three other species with proven anti-cancer properties attributed to an oleic acid complex of this molecule. The conclusion and the future scope of this study have been given ahead.

### 8.1 Conclusion

- Camel milk showed cytotoxicity against HeLa cells at concentrations above 10mg/ml at 24h and above 5mg/ml at 48h. Camel milk whey was cytotoxic at all the concentrations studied whereas camel milk casein was not found to be cytotoxic. The IC<sub>50</sub> of camel milk whey was 7.5mg/ml.
- Camel milk whey depicted the potential to inhibit migration of transformed cells, almost similar to the cisplatin.
- Possible induction of apoptosis by camel milk whey proteins is likely through caspase- 3 activation.
- The cells showed nuclear condensation and cell shrinkage upon staining with DAPI.
- The SDS-PAGE showed the presence of the following proteins: lactoferrin (80kDa), CSA 66KDa, Immunoglobulin heavy chain (45-55KDa) Beta Casein, (24 KDa), Alpha S1 Casein 23.5KDa, TRAIL (20kDa) KDa & alpha-lactoglobulin (14KDa)
- The active components had a protein at a molecular weight of 20kDa. This may possibly correspond to TRAIL a known immunomodulatory molecule with anti-cancer properties.

- According to LCMS/MS analysis of camel milk whey the following proteins are found to be abundantly present: K-casein (Fragment) , Alpha-s1-casein, EGF factor 8 (Fragment), Beta-casein, Lactoferrin, A-lactalbumin, Alpha-s1-casein. A-lactalbumin had a high score and coverage of 24.36 and 42.31 respectively.
- Camel  $\alpha$ -lactalbumin protein sequence shows 70% sequence identity and the alignment score is found to be ~0.86 with the other three species viz. human, cow and goat.
- Secondary structure of protein is quite similar (0.86) in spite of differences at the primary level (70% sequence identity)
- In camel  $\alpha$ - lactalbumin, the  $\alpha$ -helix is lesser and its extended strand and random coil is more than the other species studied. , There is a striking structural similarity in the Calcium binding domain, across the species studied. Structure overlapping studies indicate that overall  $\alpha$ -lactalbumin is quite similar in camel, human, cow and goat.
- The structural similarity of camel alpha- lactalbumin-oleic acid complex with the other three species is indicative of its similar therapeutic potential against cancer whereas the differences could be the possible explanation for an increased thermostability of this molecule.

**8.2 Limitation of study:** There are 2 major limitations in this study. Firstly, although a bioactive 20KDa protein with anticancer property was isolated upon purification but its identity as TRAIL is yet to be experimentally validated. Secondly, the anticancer activity of camel alpha-lactalbumin-oleic acid complex is theoretically suggested in this study but its experimental proof is still lacking.

### **8.3 Future scope of the thesis**

In this study it has been shown that the cytotoxicity is associated with camel milk whey and not the casein fraction however the exact identity of the molecule(s) need to be validated by further studies. Moreover, the mechanism of action of camel milk component (s) of various cell signaling mechanisms should be further studied. Important camel milk components should be produced by gene cloning so that they are utilized in purified form. The outcome of this work can be used for further commercialization of camel milk and its whey components as it is a nutraceutical compound without any side effects. However, animal studies related to the effect of camel milk whey and its potential in preventing and obviating are very important before its further utilization as an important nutraceutical that may be given to cancer patients.