CONCLUSION
Cystatins are known for their ubiquitous presence in living system. They bind non-covalently with cysteine proteases of the papain super-family in competitive and reversible manner and are accountable in regulating the activity of cysteine proteases to keep in check the catabolism of proteins and peptides. Deregulation of cystatin function can lead to various pathological states due to the uncontrolled proteolysis. Cystatins have been classified into three distinct families based on their molecular complexity. **Family I** – the Stefins, with a molecular mass in the range of 11–12 kDa. **Family II** – the Cystatins, with a molecular mass of about 13–14 kDa and **Family III** – the Kininogens, found only in plasma are more complex members with three distinct types named as high molecular weight kininogen (MW 120 kDa), low molecular weight kininogen (68-70 kDa) and T-kininogen, found only in rat plasma (68 kDa). Cystatins have been reported from a variety of mammalian sources viz spleen, brain, parotid gland, liver, kidney, lung, skin and placenta. Isolation and purification of cysteine protease inhibitors from specific tissues could provide an insight into the *in vivo* regulation of their target proteases. In the current study a cystatin variant, caprine brain cystatin (CBC) of molecular mass 44 kDa (approx) has been isolated from caprine brain. Results obtained show a good relation of CBC with other reported cystatins in terms of physical characteristics while differ in terms of molecular mass, subunit structure, sulphhydryl groups and carbohydrate content, therefore the purified inhibitor is grouped as a variant of type I cystatins. All these characteristics suggest a different biosynthetic pattern for CBC and hence different compartmentalization to play a variety of physiological functions. Owing to the myriad of vital functions performed by cystatins in the mammalian body, this study is of great significance as it brings to light various physiochemical properties of cystatins in mammalian brain which is one of the most important organ coordinating body activities. In addition the purified inhibitor can serve as a model protein to investigate various pathophysiological phenomena concerning the human brain.

Advancement of nano-biotechnology and bioengineering areas has led to an increasing interest in the formulation of competent nano-carriers for the welfare and treatment of many human disorders. Recently these nano structured materials have
emerged as potential pharmacological units that have shown application in regenerative medicine, diagnosis and drug delivery. The fundamental aspects of nanoparticle protein interactions in nano-medicine and nano-toxicity have emerged most recently due to the recognition of nanoparticle-protein corona formation. Present study reported the formation of CBC-iron oxide ($\text{Fe}_3\text{O}_4$) and CBC-silver (Ag) nanoparticle corona that led to the passage of CBC through a variety of structural and functional changes. Evidences provided by various analysis presented the changes in conformation of CBC at both secondary and tertiary structural levels. The pattern of interaction towards these two nanoparticles (NPs) led to the adsorption of CBC to the surfaces of these nanoparticles eventually leading to the mechanical strain thereby causing a loss in functional ability, a decrease in $\alpha$-helical content led to the formation of partially unfolded structures of CBC. Partially unfolded states of the proteins are reported to be toxic for the cell. Further the pattern of interactions reported from various assays presents silver nanoparticles to be more effective in causing the structural and functional variations in CBC in contrast to the corresponding concentrations of iron oxide NPs, thereby presenting silver nanoparticles to be more toxic. Current study gives a preliminary understanding of protein nanoparticle interaction, which presents the first step towards developing strategies to ameliorate the recognized deleterious effects of nanoparticles for safer biomedical applications. Additionally corroboration of the facts presented needs an extended study involving the \textit{in vitro} and \textit{in vivo} setups.

Protein aggregation is recognised as a general phenomenon observed widely in proteins. With the aid of different spectroscopic assays and employing TEM analysis, changes in the conformation and the pattern of aggregation of CBC was analysed on treating CBC with the varying concentrations of TFE. In addition the anti-amyloidogenic and anti-fibrillating potential of flavonoids, curcumin and quercetin were also analysed using the same multi-methodological approach. The study helps to gains an insight about the favourable condition for growing CBC amyloid fibrils (observed to be 28% TFE) along with the recognition of inhibitory potential of two flavonoids, curcumin and quercetin on \textit{in vitro} CBC fibrillogenesis. The inclination shown by CBC to undergo fibrillation under experimentally induced conditions leading to decrease in CBC function indicates the possible damage the fibrils can
bring about, hence indicating the role in various brain pathologies. Further the outcome of this study will not only enable one to understand the mechanism of protein self-association and hence amyloid formation but also helps in devising strategies for the prevention or retardation of amyloid formation implicated in various amyloidogenic diseases with regard to human welfare.

Protein glycation is known to be the unwarranted post-translational modification which alters the three dimensional conformation of proteins and hence triggers its abnormal function. Products released from the Maillard reaction modifies the protein function and are also known to be involved in the advancement of diabetic complications and other forms of degenerative pathologies. In the final chapter, glycation of CBC has been studied on addition of glucose (17 and 100 mM) and ribose (17 and 100 mM) by using various techniques like UV absorption, fluorescence emission, circular dichroism (CD) spectroscopy and transmission electron microscopy (TEM). The advanced glycation end products (AGEs) produced as a result of prolonged glycation have been monitored and characterized by employing different AGE’s specific fluorescence studies. Reducing sugars have been reported for their role in protein aggregation via glycation and AGEs formation either directly or by speeding up the process of fibrillation of amyloidogenic proteins. Present study gives an insight about the glycation induced aggregation via formation of molten globular state in cystatin like thiol proteinase inhibitor purified from caprine brain, with the alterations produced showing dependency on type of sugar, concentration of sugar and the time of incubation. Ribose was found to rapidly glycate and induces aggregation in CBC in a concentration and time dependent manner. Similar trend was seen for glucose incubation as well, with 17 mM glucose concentration not showing any CBC aggregation. Thus any condition which leads to an increase in concentration of either glucose or ribose may lead to glycation of cystatin, altering its structure, rendering it unfit to bind to cathepsins, hence disrupting the protease antiprotease balance leading to various pathological states. The study gains its importance from the fact that cystatin C, a member of cystatin superfamily, found to bind and inhibit amyloid-β deposition in both in vivo and in vitro conditions, shows its protective mechanism in neurodegenerative conditions thus signifying the important role of cystatins which otherwise due to glycation and/or aggregation may not be able to perform its normal function.