SUMMARY AND CONCLUSION

1. Treatment of goat red blood cells with phenylhydrazine (1mM) caused alterations in the biomarkers of oxidative stress indicating peroxidative damage to the red blood cells. Co-incubation of RBCs with PHZ and aqueous bark extract of TA protected the RBCs from being damaged due to oxidative stress as is evident from the biomarkers of oxidative stress, protein carboxylation, status of GSH, GSSG and GSSG:GSH ratio and the activities of antioxidant enzymes. However, aqueous bark extract of TA alone had no effect on the parameters studied.

2. Phenylhydrazine treatment of goat RBCs induce a significant translocation of PS and PE from the inner to the outer leaflet and PC from the outer to the inner leaflet of the red cell membrane. Co-incubation of RBCs with aqueous bark extract of TA and PHZ tend to protect the cells from such traslocation of phospholipids. Aqueous bark extract of TA alone had no effect on the translocation of phospholipids.

3. Red blood cells treated with PHZ causes alterations in a number of important cytoskeletal membrane proteins when compared to normal membrane proteins from control RBCs. SDS-PAGE analysis of membrane proteins of RBCs co-incubated with aqueous bark extract of TA and PHZ shows a remarkable improvement in the intensity of RBC cytoskeletal membrane protein bands. In addition, the densitometric scan of the SDS-PAGE further confirms the protective role of the aqueous bark extract of TA. However, this aqueous bark extract alone exhibited no effect on the cytoskeletal membrane proteins.
4. The activities of the metabolic enzymes G6PDH, hexokinase, aldolase and LDH are altered by treatment of RBCs with PHZ. However, co-treatment of the RBCs with aqueous bark extract of TA and PHZ protected the changes in the activities of these enzymes consequently protecting the RBCs metabolic status. Aqueous bark extract of TA alone had no effect on the metabolic status of red blood cells.

5. Membrane-bound enzymes are important in maintaining the normal physiology of erythrocytes. A decrease in activities of membrane bound enzymes- Na\(^+\)/K\(^+\)-ATPase, Mg\(^{2+}\)-ATPase and AchE have been observed with PHZ treatment. Co-treatment of RBCs with aqueous bark extract of TA and PHZ restored the activities of these enzymes significantly. However, aqueous bark extract of TA alone had no effect on the activities of membrane-bound enzyme studied.

6. There was a significant increase in the level of ROS production in the RBCs following treatment with PHZ. Co-treatment of goat RBCs with PHZ and aqueous bark extract of TA prevented the enhancement of ROS production of goat RBCs.

7. Flow cytometry study revealed remarkable changes in the granularity of RBCs following PHZ treatment. No marked change in granularity was observed following co-treatment of RBCs with PHZ and aqueous bark extract of TA.

8. Protective effect of aqueous TA bark extract against PHZ treatment on the structural integrity and morphology of RBCs was evident from SEM and AFM studies. However, Aqueous bark extract of TA alone had no effect on the morphology of red blood cells.
Thus, the present findings suggest that the aqueous TA bark extract provides protection against oxidative stress mediated changes induced by PHZ \textit{in vitro}, in goat RBCs as is evident from the effects on the bio-markers of oxidative stress, the activities of antioxidant enzymes, the cytoskeletal architecture of RBC membrane, its metabolic status and functions and the cellular morphology. The aqueous TA bark extract has been reported to contain various bioactive compounds such as phenolics, flavonoids, proanthocyanidins and many others which are known to possess antioxidant activity. It can be assumed that these bioactive compounds may be responsible for the protective effects of the aqueous TA bark extract against PHZ induced oxidative stress in goat RBCs \textit{in vitro}. Moreover, our studies revealed that this aqueous TA bark extract do possess a strong antioxidant potential and it is unique in specifically scavenging \textquoteleft OH and O$_2^\cdot$ . Thus, the present study indicate that the aqueous TA bark extract may have future therapeutic relevance in situations of $\beta$-thalassemia, in particular, hemolytic anemia and other diseases involving oxidative stress, in general.