SUMMARY AND CONCLUSION
6. SUMMARY

The field of solid organ transplantation has made great progress over the past decade. Many new agents have joined the armamentarium of immunosuppressive drugs for use in various combinations of immunosuppressive regimens. Also, a few drugs with novel mechanisms of action are currently being evaluated in early clinical trials. The use of these new immunosuppressive drugs has significantly decreased the rates of acute rejection (AR) over the past 10 years. Cyclosporine A (CsA) has revolutionized the field of organ transplantation and is widely used in the treatment of many autoimmune diseases. Despite these advances, CsA is associated with major renal complications including acute nephrotoxicity, chronic tubulointerstitial fibrosis, and rarely hemolytic uremic syndrome. The pathogenesis of CsA nephropathy is multifactorial and includes several factors like inflammation, apoptosis, fibrosis and transformation of renal proximal tubular cells by the epithelial mesenchymal transition (EMT) process. Among the possible mechanisms, there is evidence for a direct tubular toxicity of CsA. An increased rate of tubular cell apoptosis was observed in human renal biopsy specimens obtained from patients with CsA nephrotoxicity. In addition, several independent groups have shown that CsA induces apoptosis in tubular cells in a dose- and time-dependent manner.

CsA promoted a Bax-dependent, caspase-dependent pathway of mitochondrial injury that leads to apoptosis. The unraveling of the apoptotic pathways activated in the course of tubular cell death induced by different stimuli may provide the basis for the therapeutic targeting of apoptosis in the
course of acute or chronic renal injury. In addition, the mechanisms by which some lethal pathways are initially activated but does not progress to the point of inducing cell death. Apoptotic is an integral part of the normal functioning of the kidney and other organs. Dearrangements in its regulation, as a hypothesis, may result in renal disease. The apoptotic rate of renal cells might be abnormally increased in nephropathies characterized by cell death or cell depletion like acute tubular necrosis, acute rejection, necrotizing glomerulonephritis or renal atrophy, and during proliferative glomerulonephritis, polycystic renal disease, renal fibrosis and neoplastic.

Understanding the mechanisms of physiologic cell death (apoptotic or programmed cell death), provides new diagnostic opportunities and therapeutic approaches to the clinical management of renal disease. The availability of advanced techniques in cellular and molecular biology to study the genes that regulate apoptosis has resulted in an exponential growth of information, especially in the fields of immunology, oncology, neurology and development. Apoptosis has an important role not only in the physiological processes of kidney growth and remodeling but also in various renal diseases and drug-induced nephrotoxicity. Apoptosis in the kidney is a double-edged sword because not only it leads to tissue loss and dysfunction but also contributes to eliminate intoxicated cells and to control proliferative responses. Investigation into the cellular and molecular mechanisms of cyclosporine action has yielded evidence that several cellular processes are altered by this compound. We have reviewed the concept of apoptosis, the genes involved in its regulation, its role in physiology and its possible
participation in renal disease based on the international Literature and our laboratory experiences.

Herbal medicine is increasingly gaining greater acceptance from the public and medical profession due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life. In current years, substantial attention has been directed towards credentials of plants with antioxidant ability that may be used for human expenditure. The task of free radicals in many disease conditions has been well customary. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging critical biomolecules. Medicinal plants possess important contributions to the pharmaceutical, agriculture and food industries. With the onset of the synthetic era, pharmaceutical industries producing lot of synthetic drugs, help to alleviate chronic diseases. Recently there is an emerging trend in research to support the biological activities of medicinal plants. Many scientific researchers have reported about the efficacy of the role of medicinal plants in the treatment of diverse diseases. The plant which shows most promising and distinct results among, is Terminalia arjuna, (Family-Combretaceae) popularly known as arjuna. The bark stem powder found to be useful for “hritshool” (angina) and cardiac ailments by the ancient physicians. Recently there has been renewed interest in this plant because of its multimode cardioprotective activity. In the traditional system of medicine, it is used in the treatment of diseases like cardiac failure, cirrhosis of liver, asthma, bronchitis, cough, tumors, inflammations, diabetes, piles & leukorrhea. Its constituents include tannins, triterpenoids, flavonoids, gallic
acid, mannitol and phytosterols. The arjunolic acid is the major triterpenoid constituents present in TA bark extract. Pharmacological studies showed cardioprotective, antifertility, antioxidant, antibacterial, antifungal, anti HIV and anti neoplastic activities. The possible mechanism by which Arjunolic acid influences the inflammatory process is by the maintenance of intracellular antioxidative status, by attenuating the activation of Nfkb and controlling the mitochondrial dependent and independent apoptotic signaling pathways.

In the present study the possible beneficial effect of Arjunolic acid in ameliorating CsA induced nephrotoxicity without affecting its immunosuppressive activity was elucidated.

♦ Assessment of renal function revealed increase in urea, uric acid and creatinine levels in CsA induced rats. Arjunolic acid treatment was able to significantly reduce their levels and maintain the urea and creatinine clearance.

♦ Administration of Arjunolic acid to CsA induced rats significantly increased the antioxidant status, showing its potent antioxidant activity.

♦ A remarkable elevation in the activity of the antioxidant enzymes and significant improvement of non enzymic antioxidants was observed on Arjunolic acid treated rats when compared with the CsA induced group of rats.
♦ Alterations in mitochondrial enzymes of TCA cycle and Electron transport chain complexes were restored back to near normal levels after AA administration to CsA induced animals.

♦ Arjunolic acid has a good role in maintaining lysosomal enzyme levels in experimental Animals.

♦ Antihypercholesterolemic effect of Arjunolic acid was proved in this model of toxicity.

♦ Histopathological analysis revealed dilatation of proximal tubules, vacuolization, tubular cell desquamation and intraluminal cast formation with massive infiltration of inflammatory cells in CsA induced animals. This was significantly reduced in Arjunolic acid treated rats.

♦ Transmission electron microscopic analysis revealed autophagosome with disrupted mitochondrial structure, lipid droplets accumulation, mitochondrial membrane damage with loss of cristae in CsA induced animals. This was significantly reduced in Arjunolic acid treated rats.

♦ The inflammatory markers of Nfkb expression studied by western blot analysis were strongly inhibited by Arjunolic acid in renal tissues of CsA challenged rats.
♦ Bax expression promotes the susceptibility of the cell to growth factor deprivation induced apoptosis, upregulated by cyclosporine was reduced by Arjunolic acid administration.

♦ Bcl2, a key factor of suppressing reactive oxygen species were strongly inhibited by cyclosporine in renal tissues of CsA induced rats was confirmed by western blot analysis.

♦ Cytochrome C, a key factor in apoptotic cells, upregulated by cyclosporine was reduced by Arjunolic acid administration.

♦ Caspase-3, an important effector enzyme in apoptosis common to both death receptor and mitochondrial dependent apoptotic mechanism, were strongly inhibited by Arjunolic acid in renal tissues of CsA induced rats as studied by western blot analysis.

♦ IL-6, an intrinsic element in the cell mediated inflammatory response, upregulated by cyclosporine was reduced by Arjunolic acid.

♦ The proinflammatory cytokines such as IL1-α and IL1-β expression were strongly inhibited by Arjunolic acid in renal tissues of CsA challenged rats was studied by western blot analysis.
Summary of Pathway

Figure 54:

- CsA
- Bax translocation
- Cytochrome c release
- Caspase-9 activation
- Caspase-3 activation
- Apoptosis
CONCLUSION

The clinical and pathological outcome during heart, liver, lung and kidney transplantation as well as autoimmune disease treated with CsA, is the chronic nephrotoxicity. The pathogenesis of CsA nephrotoxicity is multifunctional, including inflammation, apoptosis, fibrosis and transformation of renal proximal tubular cells by the epithelial mesenchymal cells.

Hence the search for a multitargeted drug to mitigate the nephrotoxic effect of CsA is the need of the hour. Terminalia arjuna commonly known as abana and used as cardiotonic for decades in ayurvedha, unani, homeopathic and allopathic, serve as the source for isolation of triterpenoid arjunic acid. The results of the present study reveal that, AA could be a novel therapeutic agent in targeting the down regulation of oxidative stress, inflammatory and apoptotic pathways.

CsA induced increase in pro apoptotic markers and decrease in antiapoptotic protein were attenuated by arjunolic acid. Hence arjunolic acid has significant potential as a therapeutic intervention for chronic CsA induced nephrotoxicity.
MECHANISTIC PATHWAY ABOUT THE ACTION OF ARJUNOLIC ACID ON CYCLOSPORINE (A) INDUCED NEPHROTOXICITY

- Apoptosis
- Tissue fibrosis
- Inflammation
- Oxidative stress
- Immunosuppression
- Cell death
- Nephrotoxicity

CsA

+ Cas-3
+ IL-6
+Bax, -Bcl2
+Cyt c.
+Nfkβ

AA inhibition
FUTURE PROSPECTUS

Future research should focus on defining the cellular and molecular targets, the optimal time frame and the specific strategies for therapeutic intervention of arjunolic acid on chronic cyclosporine induced nephrotoxicity. The special consideration should be given to optimizing modes of local delivery and target of action of AA, that moderate apoptosis so as to target only specific cell such as renal tubular cells for limited period and limit interference with the process of beneficial apoptosis.