SCOPE OF THE STUDY
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All immunosuppressants and surgical techniques have obviously increased the short-term graft survival rate, but not the long-term graft survival rate. Therefore improving the long-term survival rate/life quality of graft and recipient becomes a critical issue. Current strong immunosuppressants such as CsA can effectively control acute rejection and protect the graft from injury by suppressing the immune cell activation, proliferation, differentiation and migration. However, it also has strong toxicity to various tissues that seriously decrease the long-term graft/patient survival rate and their life quality. Until now, renal transplantation is still the most common therapeutic option for patients with end-stage renal disease. Long term use of CsA could cause CCN (chronic cyclosporine nephrotoxicity) through directly injuring renal cells, activating the renin-angiotensin-aldosterone system (RAS) and up regulating the transforming growth factor-β (TGF-β) increasing free radical production. All these lead to renal cell apoptosis and finally to CRD (chronic renal disease). Therefore, renal cell apoptosis might be one of the primary causes in CRD. To reveal the mechanisms and provide new ideas for prevention, intervention and further studies of CRD, we systematically evaluated the studies of apoptotic mechanisms and interventions of CsA-induced renal cell apoptosis.

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 diseases. The task of free radicals in many disease conditions has been well customised. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging critical biomolecules. Medicinal plants are being investigated in the past to unravel the scientific principles behind their pharmacological properties. A scientific analysis of herbal armamentarium has lead to the identification of potent drug molecule AA. Hence the present study has been undertaken systematically to examine the potential of Arjunolic acid (AA) against renal dysfunction due to mitochondrial damage caused by Cyclosporine (A) and investigated the mechanism of action of AA against apoptosis caused by CsA toxicity.

The following biochemical and molecular analysis were carried out in invivo and invitro, in order to study the efficacy of Arjunolic acid and to explore the mechanism of action involved in exhibiting renoprotective effect against Cyclosporine A (CsA) induced renal apoptosis.

1. Biochemical parameters were carried out to assess the renal function. The levels of urea, uric acid, Creatinine were estimated in serum.

2. The levels of enzymic antioxidants such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione -S-transferase (GST), Glutathione reductase (GR) and Non-enzymic antioxidant such as reduced glutathione (GSH) were estimated.
3. Lipid peroxidation (LPO) were estimated by Malondialdehyde (MDA) formed as an end product of the Peroxidation of lipids, which serves as an index of the intensity of oxidative stress and Nitrite were also studied in mitochondria.

4. The damage caused to mitochondria and the enzymes of mitochondrial origin play an important role during Cyclosporine Nephrotoxicity. Therefore the prime objective of the study was to evaluate whether arjunolic acid could preserve mitochondrial function.

5. In order to determine the potential of the drug, the activity of the lysosomal enzymes were assayed.

6. The citric acid cycle and oxidative phosphorylation are central biochemical pathways in cellular energy metabolism. Therefore the prime objective of the study was to evaluate whether arjunolic acid could preserve energy potential of the cell.

7. Membrane associated signaling processes is the critical part of the complex pathways that transduce changes in cellular metabolism and hence in the present investigation the levels of mitochondrial complexes I-IV were assayed.

8. Cytosolic and mitochondrial enzymes plays crucial role in maintainance of favourable physiological conditions in the cell. Therefore the mitochondrial enzymes of NADH DH, ICDH, α-
KGDH were analyzed and the preventive role played by Arjunolic acid was assayed.

9. The permeability of cell membranes to various ions depends on lipid constituents. Lipids are an imperative group of compounds involved in cellular function, rendering substantial contribution to the surface properties of the cell. In order to determine the potential of the drug, the levels of individual lipids were assayed which maintained the cellular membrane integrity.

10. Dearrangement of lipids is considered to be of potential importance in the development of ischemic injury and hence in the present investigation, the levels of individual lipids were assayed.

11. Transmission electron microscopic analysis is the elective tool, performed to reveal oxidative renal damage in mitochondria and other structural damage due to CsA administration. Co-administration of AA was able to nullify the structural damage caused by CsA.

12. Western blot analysis of Bax was performed to study the susceptibility of the renal cell to growth factor deprivation induced and CsA promoted apoptosis.

13. The regulation and execution of apoptosis were studied by the western blot analysis of Caspase-3.
14. Generation of ROS and levels of oxidative phosphorylation complexes were studied by the western blot analysis of Cytochrome C.

15. Western blot analysis of Bcl-2 was carried out to determine the membrane lipid integrity by suppressing generation of reactive oxygen species.

16. Role of oxidative stress and the levels of oxygen free radicals were studied by the western blot analysis of Nfk-b.

17. Western blot analysis of IL-6 was assayed to determine anti-inflammatory effects against CsA induced renal apoptosis.

18. Western blot analysis of IL-1α, a proinflammatory factor stimulating inflammation and immunity was carried out to study the therapeutic potential of Arjunolic acid on inflammatory condition.

19. Western blot analysis of IL-1β was studied to determine the cellular hemostasis by modulating apoptotic cascade and immune system.

20. Cell viability was assessed using MTT dye and cytotoxic index was calculated to improve the cytoprotective potential of Arjunolic acid.
21. The cardioprotective triterpenoid arjunolic acid was soluble and the purity of the compound was assayed by using different methods as FTIR, HPLC, NMR and IR.

22. Hence the powerful phytochemical AA is taken from a well known TA, a cardioprotective. The multifunctional effect of the compound AA is studied against well known immunosuppressant CsA.