INTRODUCTION
1.1 INTRODUCTION

Health is a state of complete harmony of the body, mind and spirit. When one is free from physical disabilities and mental distractions, the gates of the soul open. - B.K.S. Iyengar

Healthy human life is always cardinal for human being starting from his birth to the end of life. The number of diseases, minor to major play a key role in disturbing the healthy human life. Along with the modernization as well as sophistication in the life, human health directly or indirectly faces challenge from several diseases resulting in survival and sometimes in surrender to diseases. Kidneys are paired organs, each sharing equally the work of removing wastes and excess water from the blood. Remarkably, a single kidney can do the job of both if one kidney is lost through injury or disease. It sometimes occurs, although rarely, that a person is born with only one kidney. Such people are able to lead normal lives. Diseases of the kidney range from mild infection to life-threatening kidney failure. The most common form of kidney disease is an inflammation of the kidney, called pyelonephritis. Symptoms of pyelonephritis include fever, chills, and back pain. Glomerulonephritis, another common kidney disease, is characterized by inflammation of some of the kidney's glomeruli (glomerulus is a round cluster of interconnected capillaries found in the cortex of a kidney, which remove body waste to be excreted as urine). This condition occurs, when the body immune system is impaired. Symptoms include blood in the urine, swelling of body tissues, and the presence of protein in the urine.
1.2 Renal Failure

Renal failure is a medical condition in which the kidneys fail to adequately filter waste products from the blood.

1.3 Common Causes of Kidney Failure

There are two main categories of kidney failure: acute renal failure which is often reversible with adequate treatment and chronic renal failure which is often not reversible.

1.4 Acute Renal Failure (ARF)

In this form of kidney failure, the kidneys stop functioning properly because of a sudden illness, a medication or medical condition causes one of the following:
1. Pre renal ARF is due to heart failure with reduced cardiac output and low blood pressure and conditions associated with diminished blood volume and low blood pressure, like severe hemorrhage. A severe drop in blood pressure or interruption in the normal blood flow to the kidneys occurs during major surgery, severe burns with fluid loss through burned skin, massive bleeding (hemorrhage) or a heart attack that severely affects heart function. Blood clots travel to the kidney and causes acute kidney failure. As long as renal blood flow does not fall below 20% of normal, acute renal failure can usually be reversed if the cause is corrected before renal cell damage occurs. When the blood flow to the kidneys decreases, the glomerular filtration rate (GFR) also decreases. This decreases the kidney's work load, and therefore decreases the kidney's requirement for energy and oxygen. Ischemia cannot persist for more than a few hours at below 20% blood flow, or the kidney will experience intrarenal ARF.

2. Intra renal ARF due to abnormalities of the kidney itself, including direct damage to kidney cells or to the kidneys' filtering units, (blood vessels, glomeruli or tubules) and it causes inflammation in the kidney called glomerulonephritis, toxic chemicals, medications and infections.

- Acute Nephritic Syndrome (Acute Glomerulonephritis; Post infectious Glomerulonephritis) – A common cause of acute glomerular capillary damage. 95% of patients with acute glomerulonephritis had damage occur to the glomeruli 1 -3 weeks after an infection elsewhere in the body. Antigen-antibody complexes are deposited in the glomeruli. Glomeruli become
blocked by inflammation. In the past, Post infectious Glomerulonephritis was usually caused by streptococcus; currently, glomerulonephritis is increasingly caused by staphylococcal and gram-negative bacteria.

- Tubular necrosis due to severe renal ischemia – The epithelium is destroyed due to severe ischemia (prerenal ARF causing intrarenal failure) and inadequate supply of nutrients and oxygen to the tubular epithelial cells. Tubular cells slough off and plug up the nephrons. This blocks urine outflow. Most common cause is a pre renal occurrence of ARF, such as circulatory shock.

- Tubular necrosis due to poisons, toxins or medications which destroy the tubular epithelial cells. Examples are carbon tetrachloride, heavy metals, ethylene glycol, insecticides and medications such as tetracyclines and cisplatin.

- Interstitial nephritis is due to vascular, glomerular or tubular damage, destroys individual nephrons and involves primary damage to the renal interstitium. Conditions causes primary interstitial damage is acute pyelonephritis and acute allergic interstitial nephritis. Pyelonephritis occurs due to bacterial infections from the bladder (most commonly due to Escherichia coli, from fecal contamination of the urinary tract) or via the bloodstream. Drugs or poisons also induce primary damage to the renal interstitium.
3. Post renal ARF due to bilateral obstruction of the urinary collecting system from calices to urethra. (Blocked urine flow from the kidney). The most common cause being kidney stones caused by precipitation of calcium, urate or cystine. Blockage of urine flow within the kidney causes sudden kidney failure, with major muscle injury. With moderate acute renal failure, there is retention of water, waste products of metabolism, and electrolytes. This causes edema and hypertension. Excessive retention of potassium can be fatal. Another possibly fatal problem is metabolic acidosis due to inability to excrete sufficient hydrogen ions. Acute renal failure may be treated and the person may never have another problem or it can be the instigating factor for chronic renal failure. Chronic renal failure may show up immediately or many years down the road.

1.5 Chronic Renal Failure

Chronic renal failure is due to irreversible loss of large number of functioning nephrons. A clinical symptom shows less than 30% normal functioning nephrons. In general, chronic renal failure occurs due to the same reason as acute renal failure occurs, but the progression is slower. Often the initial insult to the kidney leads to progressive deterioration of kidney function and increased loss of nephrons over time until it reaches end-stage renal failure and is placed on dialysis or receives a kidney transplant. When nephrons are lost, other nephrons take on a larger load. They adapt and excrete normal amounts of water and solutes until the kidney is reduced to 20-30% normal nephron mass. The glomeruli are injured due to increased pressure and stretch from the increased blood pressure on the glomeruli. This
causes sclerosis and further destruction of the kidneys. The only method known by conventional medicine to slow the glomerular damage down is to lower blood pressure and glomerular hydrostatic pressure by using drugs such as angiotensin-converting enzyme inhibitors to block formation of angiotensin II. In chronic kidney failure, the functioning of the kidney gradually declines, over a period of years. Most commonly, caused by illnesses such as diabetes, uncontrolled high blood pressure or chronic kidney inflammation (glomerulonephritis or pyelonephritis). Also due to long-term exposure to lead, mercury or certain drugs, especially painkillers. The most common causes of end stage renal failure are diabetes mellitus, hypertension and glomerulonephritis. Diabetic nephropathy is the most common cause of renal failure. Almost all insulin-dependent diabetics have histological evidence of glomerulosclerosis. 35% will develop clinical nephropathy, usually about 5-20 years after diagnosis. 5-15% of non-insulin dependent diabetics also develop nephropathy. Renal failure accounts for 48% of the diabetic deaths in those who acquire IDDM before age 20. Hypertension and atherosclerosis can be a primary cause of renal damage. Renal failure also induces hypertension and lead to increased renal damage. Even in “normal” people benign nephrosclerosis takes place which diminishes normal kidney function to 60% by age 80. Benign nephrosclerosis in association with severe hypertension leads to rapidly progressing malignant nephrosclerosis. Chronic glomerulonephritis is a slow progressive disease often leading to irreversible renal failure. It can be a primary disease following acute glomerulonephritis or secondary to systemic diseases, such as diabetes or lupus erythematosus. It usually begins with precipitated antigen-antibody complexes in the
glomerular membrane. There is inflammation, thickening, and eventually, fibrous tissue.

1.6 Signs & Symptoms of Renal Failure

Patients with slightly diminished renal reserve are asymptomatic and renal dysfunction can only be detected by lab tests. Mild to moderate renal failure brings about vague symptoms such as nocturia, fatigue and decreased mental acuity.

- Changes in urination - making more or less urine than usual, feeling pressure when urinating, and changes in the color of urine, foamy urine, or having to get up at night to urinate.

- Edema or even CHF, there is swelling of the feet, ankles, hands, or face (Due to fluid the kidneys can't remove taking up residence in these tissues.)

- Fatigue or weakness (a build-up of wastes or anemia can cause these symptoms when the kidneys begin to fail.)

- Muscle cramps or twitching

- Shortness of breath due to anemia as well as the buildup of fluid in the lungs.

- Ammonia breath or an ammonia or metal taste in the mouth - waste build-up in the body can cause bad breath, Changes in taste, or an aversion to protein foods like meat.
• Back or flank pain - due to kidney location on either side of the spine.

• Itching - waste build-up in the body can cause severe itching, especially of the legs.

• Loss of appetite

• Nausea and vomiting

• Hypertension

• Yellow brown skin or even uremic frost.

• Dark circles under the eyes.

• More hypoglycemic episodes, if diabetic

1.7 Treatment of Renal Failure

Treatment consists of measures to help control signs and symptoms of chronic kidney failure, reduce complications and slow the progress of the disease. Sometimes kidney diseases lead to kidney failure which requires dialysis or a kidney transplant to keep you alive, when the kidneys have products from the blood when the kidneys fail.

Treatment options for Kidney Failure

Dialysis – Removes waste and extra fluid from the blood.

Transplantation – If you start dialysis you will also be assessed for your suitability for transplantation. Health issues may prevent this option.
Conservative or supportive Care – If you decide that dialysis or transplant is not for you, then your health-care team will support you to stay as healthy as possible without dialysis. Your life-span however will be limited.

1.8 Transplantation

The first real move towards clinical transplantation took place in 1962, when Professor Tom Starzl began transplanting organs with the help of immunosuppressive agents using a method similar to that which we use today. In 1966, the HLA (Human Leukocyte Antigen) system was discovered, assisting in the determination of organ compatibility. In 1980 a new breakthrough was achieved by Borel, a Swiss researcher from the Sandoz Company, developed Cyclosporine A, a new immunosuppressive substance which is still widely used.Regularly transplanted organs can be divided into two types. The first type consists of organs which have no mechanical replacement, such as the liver or the heart. If transplant fails, a repeat transplant is immediately required. This possesses a very serious problem as there is no “dialysis” for a liver or heart. Transplantation of organs which do have mechanical replacement, for example kidneys, is easier for if the new kidney is rejected, the possibility exists of returning to dialysis. A second problem in organ transplantation is the risk of serious infections which attack patients receiving immunosuppressive treatment. The most important issue is the selection of compatible organs which will not be damaged as a result of being removed from the donor’s body. There are cases of serious infection following transplant, occurs principally in patients receiving immunosuppressive therapy. Such infection is likely to attack patients
receiving damaged organs with strong immunosuppressive treatment to suppress rejection. Clinical transplants of the following have been carried out for many years: kidneys (since 1962), liver (since 1963), heart and lungs (1968), and pancreas and intestines in recent years.

1.9 Liver Transplantation

In 1963 Prof. Starzl carried out the first liver transplant in Denver, Colorado. Thereafter, few others (one surgeon in South Africa, and Prof. Kilna in Cambridge, for instance), continued transplanting livers. Since then, progress in surgical procedure has solved a number of problems which were encountered. In a liver transplant several blood vessels have to be connected as well as the bile ducts. There are also problems associated with the size-compatibility of the organ, particularly when an adult’s liver is transplanted into a child. In the event of definitive rejection of the organ which cannot be suppressed by immunosuppressive treatment, a new organ must immediately be transplanted, and any available source is approached for this purpose since without a liver the patient will die. Owing to the shortage of organs from dead donors, attempts are being made to use live donors – a relative of the patient, most often parents to children and to remove just a portion of the liver. In the case of dead donors, the liver is divided into two in order that one organ can be used for two patients, even though this is a much more complicated procedure and involves more danger to the recipients. Liver transplants are carried out only in large medical centers, unlike kidney transplants which are much simpler and are routinely performed in most hospitals.
1.10  Heart Transplantation

Until 1980 transplants, especially of kidneys, were mostly taken for granted despite the fact that the success rate for transplantation of the heart, liver, pancreas and other organs was not high. The turning point occurred with the discovery of Cyclosporine A, a more potent immunosuppressive substance, which was put into use in 1980. Since then transplant results have dramatically improved. Heart transplants began in 1968, carried out in South Africa by Christiaan Barnard, followed by Dr. Kantrowitz in California. In any event, many others subsequently attempted heart transplants, but owing to the high rate of failure, enthusiasm waned considerably. For a time few centers made further attempts at heart transplants until Cyclosporine came into use in the 1980’s. Since then heart transplants have become routine and are carried out in many medical centers. As mentioned, the problem with heart transplantation is that if we fail to control rejection using immunosuppressive, a repeat transplant needs to be immediately carried out. Today life expectancy of a year after a heart transplant is about 50%-60%.

1.11  Lung Transplantation

A lung transplant is required in the case of chronic lung failure. The number of patients requiring such a procedure is constantly rising. The transplant is complicated since the lung is very sensitive, and hence the failure rate is high. There are only a few medical centers in the world where lung transplants are carried out. At first the practice was to transplant the heart and
lungs as one unit, but today sufficient experience exists for the transplantation of lungs alone to be performed.

### 1.12 Pancreas Transplantation

Pancreas transplants too are becoming increasingly common. The indication for a pancreas transplant is severe diabetes, especially juvenile diabetes, which affects the kidneys (some patients undergo transplant of both kidney and pancreas). The organ can come from a dead donor or from a live relative. Experiments have been carried out at the University of Minnesota, a good procedure has been developed for transplantation of a portion of the pancreas from a living relative. During 1980’s the revolution of Cyclosporine affected pancreas transplants, as well as other organs, and the number of transplants started rising exponentially as did the life expectancy figures for the organs transplanted. This improvement was due to not only to the introduction of Cyclosporine, but also to the increased skill of the surgeons. With a growing number of such operations, their experience in pancreas transplants increased, and the results improved accordingly.

### 1.13 Intestine Transplantation

The transplant of intestines is also an important development. There are many patients whose intestines cease to function due to arterial or other damage. Such patients live permanently on intravenous nutrition, and therefore looking for ways to transplant intestines. This operation is still very complicated, the results are not yet satisfactory, and there are only a small
number of centers which perform the procedure. Intestine transplantation is said to be still in its experimental stages.

1.14 Kidney Transplantation

Kidney transplants are routinely carried out almost worldwide. The kidney comes from a living relative, a live donor who is not related, or from a dead donor. A review of the results of kidney transplants shows 90% success during the first year for kidneys from live relatives, and 75% success during the first year from dead donors. Currently there are approximately one hundred kidney transplants carried out each year, with the demand reaching around two hundred and fifty. The organ-demand curve in recent years has been rising exponentially while the supply of organs has remained constant, which are entirely unethical.

The field of solid organ transplantation made great progress over the past decade. Many new agents have joined the armamentarium of immunosuppressive drugs for use in various combinations of immunosuppressant regimens. Also, a few drugs with novel mechanism 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. In kidney transplantation, AR rates were high in the 1960s with allograft survival at 1 year being 50%. In the Cyclosporine (CsA) era, AR rates decreased significantly with a corresponding increase in allograft survival rates to more than 80%. The use of more potent immunosuppressive therapy over the past 10 years has further
decreased AR rates and improved allograft survival rates in kidney transplant recipients to about 15% and 90%-95%, respectively, at 1 year post-transplantation.

Calcineurin inhibitors have been an integral part of immunosuppressive therapy for kidney transplantation since 1980s. They are the backbone of maintenance immunosuppressive regimens post-liver and post-heart transplant. Cyclosporine is a lipophilic cyclic polypeptide CNI manufactured in an oil-based formulation (Sandimmune) in the early 1980s. Its use was hindered by poor and erratic absorption, despite its relative success in preventing AR in recipients of solid organ transplantation. The availability of a microemulsion formulation of CsA (Neoral) in the mid 1990s, significantly improved bioavailability and minimized the variability in pharmacokinetic characteristics seen with the original oil based formulation. Acute rejection rates and allograft survival were comparable up to 2 years post transplant between the two CsA formulations in de novo kidney recipients and in recipients of a second kidney transplant. A trend toward lower AR rates and a lower requirement of monoclonal antibody treatment for AR was observed in patients receiving CsA administration. The dose limiting effect of CsA is paradoxically nephrotoxicity, occurs as a result of direct vasoconstriction on the kidney vasculature. Cyclosporine induced nephrotoxicity can present as a reversible decline in glomerular filtration rate in up to 30% of patients and can progress to irreversible dysfunction in up to 15%. The latter is significantly associated with CAN (chronic allograft nephropathy) in kidney transplant recipients as well as non renal transplant receipients and can limit long term allograft survival.
1.15 Autoimmune disorders

Previous reports demonstrate that direct and specific effects of CsA on renal cells, suggesting immunosuppressive activities like autoimmune disorders or graft rejection, as well as nephrotoxic phenomena, due to the interference of the drug with intrinsic regulatory mechanisms within the kidney. Kidney transplantation remains the treatment of choice for end-stage renal disease (ESRD) even in patients with autoimmune disease (Haubitz et al., 1997). Kidney abnormalities are severe, with potential risk of renal failure despite intensive therapy including high dosage of corticosteroids and immunosuppressant therapy. Currently, transplantation has been recognized as a good therapeutic option for ESRD to autoimmune disease. Recurrence of histologic lesions related to autoimmune disease in the grafted kidney noted in 16% to 40% of patients, undergo transplantation because of Wegener granulomatosis (Wrenger et al., 1996; Almkuist et al., 1981). In contrast to other nephropathies, recurrence of autoimmune disease is severe, leading to rapid graft loss despite appropriate therapy (Lionaki et al., 2008). It was reported that graft survival in kidney recipients with autoimmune disease is similar to that in other kidney recipients (Haubitz et al., 1997; Schmidt et al., 2002; Lionaki et al., 2008; Wrenger et al., 1996). These findings have been confirmed from US Renal Data System registry.

1.16 Immunosuppressive Drug after Renal Transplant

The introduction of cyclosporine into clinical practice in 1980s has dramatically improved one year kidney transplant survival and reduced
rejection episodes. Improvement of long-term outcomes not changed and five year graft survival rates are far below 80%; data from the European Renal Associations – European Dialysis and Transplant Association (ERA-EDTA) registry showed only modest improvement in actual kidney graft half lives. Several studies indicate chronic allograft nephropathy (CAN) and death with a functional graft (Morris, 2004; Pascual et al., 2002; Kreis & Ponticeli, 2001) as principal causes of late kidney graft loss. The calcineurin inhibitors (CNIs) cyclosporine remains the cornerstones of immunosuppressive therapy characterized by chronic nephrotoxicity and contribute to chronic allograft nephropathy. These considerations have prompted the development of new immunosuppressive agents and protocols, the goal of which has shifted from acute rejection prophylaxis to management of chronic allograft nephropathy and minimisation of total immunosuppression. The aim of strategies focused on minimizing total immune suppression would be the reduction of unwanted side effects and the preservation of graft function without any increase in rejection rate.

Discovered in the lab of Sandoz in Switzerland in 1972, cyclosporine A (CsA) is the revolutionized transplant medicine. Initially discovered for novel antifungal agents, found to have many immunologic properties and attractive agent for immunosuppression following renal and other solid organ transplants. Cell-mediated immunity is involved in autoimmune and chronic inflammatory conditions. A review from Hariharan et al., published in 2000 looking at graft survival in more than 93,000 transplants from 1988 to 1996 revealed one-year graft survival rates of 94 and 88% in living related and
decreased donor allografts, respectively (Harihan et al., 2000). The most recent data from United Network for Organ Sharing (UNOS) from 1998 to 2007 reveals current one-year adjusted survival rates to be 96.6 and 91.6% in living related and deceased donor renal allografts, respectively. Despite the marked improvement in the rates of acute rejection and one-year graft survival, long term data has been somewhat disappointing, with current age-adjusted graft survival at five years only 81.4% in living related donors and 71.6% in those with decreased donor transplants. This difference has been attributed to the nephrotoxic effects of cyclosporine. In the first attempt of using cyclosporine for immunosuppression following transplant, Calne et al., using a dose of 25mg/kg found a significant but unexpected nephrotoxicity. CsA nephrotoxicity may be dose dependent and reversible upon dose reductions or discontinuation of the drug. Since that time, one of the major reasons given for the lack of long-term improvement in graft survival has been chronic calcineurin (CNI) nephrotoxicity.

1.17 Cyclosporine

Cyclosporine is a potent immunosuppressive agent prevents or delays rejection of solid organ allografts or xenografts in various animal models including skin, heart, kidney, pancreas, small intestine and lung. It delays graft-versus-host disease after bone marrow transplantation in rodents. Successful kidney, pancreas, liver, heart, bone marrow and heart-lung allogeneic transplants have been performed in man using cyclosporine. Cyclosporine may be used alone or with low-dose corticosteroids in the prophylaxis of organ rejection following solid organ transplants. It is also
used in the treatment of transplant rejection in patients previously receiving other immunosuppressive agents. Studies in animals suggest that cyclosporine inhibits the development of cell-mediated reactions, including allograft immunity, delayed cutaneous hypersensitivity, experimental allergic encephalomyelitis, Freund's adjuvant arthritis, graft-versus-host disease and also T-cell dependent antibody production. It also inhibits lymphokine production and release, including interleukin 2 or T-cell growth factor (TCGF). Cyclosporine appears to block the resting lymphocytes in the G0 or G1 phase of the cell cycle, and inhibits the antigen-triggered release of lymphokines by activated T-cells.

**Figure 2:**

1.18 Cyclosporine structure

1.18.1 Description

It is a cyclic polypeptide consisting of 11 amino acids. It is produced as a metabolite by the fungus species *Beauveria nivea* (formerly *Tolypocladium inflatum Gams*).
Chemical Name


Chemical formula: C62H111N11O12

Figure 3:

Molecular Weight: 1202.635

Cyclosporine is rich in hydrophobic amino acids, neutral, insoluble in water (0.004% w/w) and n-hexane, but is very soluble in all other organic solvents and in lipids (olive oil) (Petcher et al., 1976).
1.18.2 Distribution

Cyclosporine is distributed largely outside the blood volume with a volume of distribution of 3.5 L/kg (average). Within the blood, distribution is concentration-dependent, with 33-47% present in plasma, 4-9% in lymphocytes, 5-12% in granulocytes and 41-58% in erythrocytes. At higher concentrations the leucocytes and erythrocytes become saturated. In plasma, approximately 90% is bound to proteins, mostly lipoproteins.

1.18.3 Metabolism

Cyclosporine is extensively bio transformed to approximately 15 metabolites. There is no single major metabolic pathway. All metabolites identified, contains intact cyclic peptide structure of the parent compound. Major pathways consist of mono- and dihydroxylation and N-demethylation at various positions. Hepatic dysfunction, as measured by a rise in serum bilirubin, associated with proportional rise in cyclosporine blood concentrations.

1.18.4 Excretion

There is a high variability in the data reported on the terminal half-life of cyclosporine, depending on the assay applied and target of population. The terminal half-life ranged from 6.3 hours in healthy volunteers to 20.4 hours in patients with severe liver disease. Elimination is primarily biliary with only 6% of the oral dose excreted in the urine and only 0.1% is unchanged drug.
1.19 Mechanism of Action

Cyclosporine A has potent immunosuppressive properties due to its ability to block the transcription of cytokine genes in activated T cells (Rovira et al., 2000). CsA forms a complex with cyclophylin D, inhibiting the peptidyl-prolyl cis-trans isomerase activity of this protein. Additionally, the CsA-cyclophilin complex inhibits the activity of calcineurin (Rusnak & Mertz, 2000). Calcineurin is a Ca\(^{2+}\) and calmodulin-dependent serine/threonine phosphatase (protein phosphatase 2B), regulates nuclear translocation and subsequent activation of the nuclear factor of activated T cells known as NFAT transcription factor. Prevention of NFAT dephosphorylation is an important step for its translocation to the nucleus, blocks cytokine production. In addition to the calcineurin/NFAT pathway, recent studies indicate that CsA also blocks the activation of stress-activated protein kinase JNK (c-jun NH2-terminal kinase) and the mitogen-activated protein kinase p38 signaling pathway triggered by antigen recognition (Matsuda & Koyasu, 2000). Despite this beneficial role of CsA in organ transplantation, CsA has significant side effects such as hypertension, renal and muscular toxicity. Probably, these effects of CsA are related to ROS generation (Buetler et al., 2000)
1.20 Cyclosporine Nephrotoxicity

CsA possesses severe toxic effects, most notable is acute and chronic nephrotoxicity, but also include hypertension, hyperlipidemia, gingival hyperplasia, hyperkalemia, neurotoxicity, hypomagnesaemia, hyperuricemia, and thrombotic microangiopathy (Kahan, 1989). These effects are due to calcineurin inhibition in non lymphatic tissues (Williams & Haragsim, 2006). The electrolyte disturbances are due to alterations in tubular function and ion homeostasis (Naesens et al., 2009; Heering & Grabensee, 1991). The nephrotoxic effects have garnered over the years and have two components, acute nephrotoxicity caused by vascular dysfunction and chronic fibrotic form. Cyclosporine A-induced nephropathy model helps to understand the pathological mechanism involved in progressive glomerulosclerosis, tubulointerstitial fibrosis and tubular necrosis.
1.20.1 Acute Nephrotoxicity

The findings of nephrotoxicity in early studies using CsA as an immunosuppressant leads to research in the pathophysiology of this process. Vasoconstriction of the afferent arterioles was suggested by Murray et al., (1985), in which rats were administered CsA infusions (20mg/kg) resulting in significant reduction in renal blood flow and rise in renal vascular resistance. Barros et al., (1987) demonstrated increase in vascular resistance in afferent and efferent arterioles with a reduction in renal plasma flow and GFR, attenuated by pretreatment with the angiotensin-converting enzyme inhibitor captopril and the calcium channel blocker, verapamil. This vascular mediated effect occurs due to an imbalance in vasoconstrictor and vasodilator factors.

Cyclosporine increases the vasoconstrictor factors endothelin as well as thromboxane in addition to its activation of the rennin angiotensin system (RAS). Activation of the RAS by CsA is by two mechanisms, a direct effect on juxtaglomerular cells (JG) and indirectly through arterial vasoconstriction and reduced renal plasma flow. Inhibition of calcineurin by CsA leads to a reduction in NFAT-mediated COX-2 expression and downstream production of arachidonic acid metabolites thereby favoring vasoconstriction (Ocherl et al., 2002). CsA promotes alterations in glomerular permeability, endothelin dysfunction, production of oxygen-free radicals and superoxide, and interference with normal tubular function (Diederich et al., 1994). The role of innate immune system has been implicated in the nephrotoxicity of CsA. Recent reports suggest that upregulation of toll-like receptors (TLR) and TNF-α, responsible for dendritic cell maturation, stimulated by endogenous,
noninfectious ligands (i.e., injured tubular epithelial cells) and stimulates secretion of chemokines that initiate phagocytic influx and immune activation. CsA provides a link between innate immunity and direct toxic effects of CsA on renal tubular cells (Lim et al., 2005).

1.20.2 Chronic Nephrotoxicity

CsA-treated patients revealed tubulo interstitial injury, significant reductions in GFR, renal plasma flow, and renal blood flow and focal glomerular sclerosis, which appear to correlate with degree of renal impairment (Myers et al., 1984). Further evidence for chronic nephrotoxicity related to long-term cyclosporine use is the clinical and pathologic findings of impaired renal function in heart, liver, and lung transplants as well as patients with autoimmune disease treated with cyclosporine (Myers et al., 1988; Falkenhain et al., 1996; Zaltzman et al., 1992; Bagnis et al., 2002). Another commonly described finding is the interstitial or striped fibrosis. This is secondary to the aforementioned vasoconstrictive effects of CsA with subsequent arteriolar luminal narrowing. The subsequent tissue ischemia/hypoxia leads to a reperfusion injury with the formation of reactive oxygen species and free radicals leading to cellular injury and apoptosis (Naesens et al., 2009; Diederich et al., 1994). Cyclosporine has also been shown to upregulate TGF-β expression in juxta glomerular cells. TGF-β promotes fibrosis by increasing the production of extracellular matrix proteins and induction of epithelial mesenchymal transition (Naesens et al., 2009; Shehata et al., 1995). Findings demonstrated less interstitial fibrosis, striped fibrosis, glomerulosclerosis, and mesangial matrix accumulation, lesions
previously attributed to long-term use of CNI (Calcineurin inhibitor). Overall, this suggests factors other than cyclosporine alone contribute to chronic allograft dysfunction. The toxic side effect of acute nephrotoxicity as a result of cyclosporine administration has been well documented and widely accepted, the concept of chronic nephrotoxicity seems a matter still up for debate.

Patients treated with CsA are high risk of developing renal injury (Burdmann, 2003). CsA nephrotoxicity can manifest as acute azotemia and is reversible after reducing the dose, or irreversible chronic progressive renal disease (Kahan, 1989). Other renal effects of CsA include tubular dysfunction, and hemolytic uremic syndrome lead to acute graft loss (Kahan, 1989). Chronic CsA nephrotoxicity manifests as renal insufficiency, tubular function dysfunction, and increases blood pressure (Kahan, 1989). A study of more than 11000 non-renal transplant recipients revealed that 17% of patients using CsA developed chronic renal failure (defined as an estimated GFR 29 mL/min per 1.73 m2) at a median follow-up of 36 months (Ojo et al., 2003). The risk continued to increase over time up to 5 years. These patients had 4.6-fold increase in the risk of death compared with those without chronic renal failure. The mechanisms responsible for chronic CsA nephrotoxicity are not well understood. Renal failure progressed and reached end stage renal disease in some of the patients. The understanding of the CsA renal effects is not only a research interest, but also clinically important in developing a strategy in the prevention and treatment of the disease.
Cyclosporine A is one example of a toxicant that acts at several sites within the kidney. It can injure both endothelial and tubular cells. Endothelial injury results in increased vascular permeability and hypovolemia, which activates the sympathetic nervous system. Injury to the endothelium also results in increases in endothelin and thromboxane A2 and decreases in nitric oxide and vasodilatory prostaglandins. Finally, cyclosporine A may increase the sensitivity of the vasculature to vasoconstrictors, activates the renin-angiotensin system, and increase angiotensin II levels. All of these changes lead to vasoconstriction and hypertension. Vasoconstriction in the kidney contributes to the decrease in glomerular filtration rate (GFR), and the
histologic changes in the kidney are the result of local ischemia and hypertension).

1.21 Role of Free Radicals in CsA induced Nephrotoxicity

In chronic nephrotoxicity, CsA induced increase in $O_2^-$ was demonstrated in renal tubular and endothelial cells (Dehornedo et al., 2007; Raymond et al., 2003; Vetter, 2003). Mitochondria possesses complex antioxidant system that detoxify these ROS, among them, there are enzymes (superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase, NADP transhydrogenase) and other molecules (like glutathione) that combined, constitute an effective antioxidant system. $O_2^-$ released in the respiratory chain is metabolized by the mitochondrial superoxide dismutase to hydrogen peroxide and is then detoxified by intra and/or extra mitochondrial glutathione peroxidase. Nishida, (2003) demonstrated increase in superoxide anion radical ($O_2^-$) and hydrogen peroxide ($H_2O_2$) level following CsA administration. CsA increases mitochondrial $O_2^-$ and oxidation of NADPH, which is essential for the maintenance of reduced state of complex I of the mitochondrial respiratory chain (Kim et al., 2003; Kowaltowski et al., 2001; Raymond, 2003; Vetter, 2003).

ROS are highly reactive and react with intracellular molecules, mainly unsaturated fattyacids and transmembrane proteins triggering lipid peroxidation and protein damage. Oxidation of these molecules promotes increased cellular membrane permeability, consequent alteration in ionic gradients, disruption of several membrane functions and metabolic processes.
CsA dependent lipid peroxidation increases malondialdehyde (MDA) concentration in endothelial, mesangial (or) tubular epithelial cells is observed (Amudha et al., 2006; Parra Cid, 2003; Wongmekiat et al., 2008). Lipid peroxidation induced by CsA was dose dependent and paralleled the renal functional alterations (Parra Cid, 2003) as well as structural damage (Wongmekiat et al., 2008). Important antioxidants are catalase, superoxide dismutase, reduced glutathione, glutathione peroxidase and GSH, found in relatively high concentrations in the kidney (Atessahin et al., 2007). It plays a pivotal role in the protection of cells against oxidative stress and detoxification of xenobiotics, including CsA (Tariq et al., 1999). Together with glutathione peroxidase, converts H$_2$O$_2$ to non toxic products, thereby maintaining the integrity of the mitochondria and cell membranes (Hager et al., 2006), Tariq et al., 1999). A significant decrease in the level of renal reduced glutathione (GSH) after CsA administration was observed in many studies (Amudha et al., 2006; Anjaneyulu et al., 2003; Hager et al., 2006; Wongmekiat et al., 2008).

Reactive oxygen species (ROS) release and consequently oxidative stress proposed as an alternate source of CsA dependent kidney damage (Dehornedo et al., 2007; Hager et al., 2006; Hakan et al., 2007; Jern, 2005). CsA causes imbalance in the cellular oxidative status as a result of excessive ROS formation. The production of ROS by CsA is postulated, due to action of CsA as an uncoupler and inhibitor of the mitochondrial electron transport system, drug’s action on NADPH oxidase or xanthine oxidase, decreased cellular antioxidant system or CsA metabolism by cytochrome p450 (Jern,
Ros attack unsaturated bonds of membrane lipids by autocatalytic process, result in lipid peroxidation. The oxidative breakdown of membrane polyunsaturated fatty acid causes increased cellular membrane permeability, with subsequent alterations of ionic gradients and disruption of several membrane functions and metabolic processes (Parra Cid, 2003). The increased levels of ROS and lipid peroxidation products following CsA have been reported in many experimental studies invitro and invivo (Amudha et al., 2006; Anjaneyul et al., 2003; Inselmann et al., 1990; Tariq et al., 1999; Zhong, 2001).

ROS are predominantly produced in mitochondria and plays important role in apoptosis. Mitochondria are the important cell organelles in research and regulate energy balance (Wallace, 1999). Various NAD/NADP-linked enzymes are intricately involved in the maintenance of the reduced redox state in mitochondria in and provide the reducing power to generate ATP via oxidative phosphorylation (Lee et al., 1965; Maechler & Wollheim, 2001). There is a huge understanding between cytosolic and mitochondrial enzymes to maintain the regulation of several biological functions. Mitochondrial antioxidant defense mechanisms counteract these reactive species, and are exhausted due to inordinate production of free radicals, leads to disruption of mitochondrial membrane (Trushina & Murray, 2007). The mitochondria are involved in diverse processes; modulate cell operation like cell cycle regulation and apoptosis.

Antioxidant defenses fall into two categories; enzymatic and nonenzymatic. Superoxide dismutases are metalloproteins that dismutate the
Superoxide radical (O2●) to hydrogen peroxide (H2O2) and molecular oxygen. Three types of Superoxide dismutases are found in eukaryotic cells; CU/Zn Superoxide dismutase, predominantly located in the mitochondria and EC superoxide dismutase, which are found in the extracellular space. Catalase, a heme protein located predominantly in peroxisomes and the inner mitochondrial membrane, catalyzes the conversion of H2O2 to H2O is also accomplished by the reaction with glutathione catalyzed by glutathione peroxidases, a family of cytosolic seleno enzymes. Non-enzymatic defenses include small molecules such as membrane associated α-tocopherol, ascorbate and glutathione.

Figure 6:

1.22 Role of nitric oxide in CsA induced renal apoptosis

NO is a molecule of both anti-oxidant and pro-oxidant properties, depending on the availability and concentration of potential reaction partners such as superoxide and hydrogen peroxide or other reactive oxygen species (ROS). In mammals, the synthesis of NO is catalysed by nitric oxide synthase (NOS), exists in three distinct isoforms (Nathan & Xie, 1994). NOS catalyses
oxidation of amino acid L-arginine, give rise to citrulline and NO. Molecular cloning and sequence analysis has revealed the existence of three distinct NOS isotypes and is expressed in endothelial cells (eNOS) and neurones (nNOS) or are induced (iNOS) by endotoxin and by inflammatory cytokines such as interleukin 1 (IL-1) or tumour necrosis factor α (TNF-α) in macrophages and many other cell types (Moncada et al., 1991; Nathan & Xie, 1994). iNOS requires a delay of 6–8 h before the onset of NO production but, once induced, this enzyme is active for hours to days and produces NO in 1000-fold larger quantities than the constitutive enzymes eNOS and nNOS. NO stimulates guanylate cyclase activity and triggers cyclic GMP (cGMP), an important messenger mediating physiological functions of NO as vascular homeostasis. Higher concentrations of NO produced by iNOS interact with thiol groups or transition-metal containing proteins and alters protein function or initiate gene expression to protect cells. There is a continuous shift at even higher concentrations of NO towards cell damage or apoptosis, with other factors in the microenvironment of a cell critically influencing the final outcome (Brune et al., 1998).

Many inflammatory diseases are accompanied by an increase in NO production and, in appropriate animal models; a beneficial action of iNOS inhibitors has been demonstrated. Currently studying physiological or synthetic agents inhibit cytokine-induced iNOS expression at the transcriptional level (Pfeilschifter et al., 1996). Cytokine -induced iNOS expression is inhibited by dithiocarbamates (Eberhardt et al., 1994), dexamethasone (Pfeilschifter & Schwarzenbach, 1990; Kunz et al., 1996) and
Cyclosporine A (Muhl et al., 1993; Kunz et al., 1995) that diminish activation or binding of NF-kB. NF-kB regulates the expression of almost 400 different genes, which include enzymes (e.g., COX-2, 5-LOX, and iNOS), cytokines (such as TNF, IL-1, IL-6, IL-8, and chemokines), adhesion molecules, cell cycle regulatory molecules, viral proteins, and angiogenic factors. The molecular mechanisms by which NO affects gene expression are poorly understood, and NO responsive promoter element has been identified. Evidence shows that NO preferentially alter transcription factors and is sensitive to changes in the cellular oxidation–reduction (redox) status. At least two well-defined transcription factors, NF-kB and activator protein 1 (AP-1) were regulated by the intracellular redox state (Sen & Packer, 1996). NO is established as a potent inducer of apoptosis in certain cell types, contradictory effects have been reported, with NO displaying anti-apoptotic effects in lymphocytes, Bells, eosinophils, hepatocytes and endothelial cells (Dimmeler & Zeiher, 1997). Anti-apoptotic activities of NO were observed at concentrations of only 2–10 % (Haendeler et al., 1997). Several groups reported and favoured a direct inhibition of caspases by reversible S-nitrosylation and therefore an interaction between NO and NO/O₂⁻ derived congenitors and the executioners of apoptotic signal transduction (Li et al., 1997; Haendeler et al., 1997; Dimmeler & Zeiher, 1997).

A number of pathological conditions are associated with the simultaneous generation of nitric oxide (NO) and O₂⁻. NO sources are restricted to the activity of the various NO synthases, whereas O₂⁻ arises from multiple sources, including electron leak from the mitochondria,
NADPH oxidase, xanthine oxidase, and uncoupling of NO synthases. Once a flux of NO and O$_2$ •$^-$ is produced simultaneously in close proximity, the generation of peroxynitrite is considerably enhanced. Peroxynitrite-dependent cytotoxicity is then mediated by a myriad of effects including lipid peroxidation, protein nitration and oxidation, DNA oxidative damage, activation of matrix metalloproteinases (MMP), and inactivation of a series of enzymes. Mitochondrial enzymes are particularly vulnerable to attacks by peroxynitrite, leading to reduced ATP formation and induction of mitochondrial permeability transition by opening of the permeability transition pore, which dissipates the mitochondrial membrane potential. These events result in cessation of electron transport and ATP formation, mitochondrial swelling, and permeabilization of the outer mitochondrial membrane, allowing the efflux of several proapoptotic molecules, including cytochrome c and apoptosis-inducing factor (AIF). In turn, cytochrome c and AIF activate a series of downstream effectors that eventually lead to the fragmentation of nuclear DNA.

In addition to its damaging effects on mitochondria, peroxynitrite inflicts more or less severe oxidative injury to DNA, resulting in DNA strand breakage which in turn activates the nuclear enzyme poly (ADP-ribose) polymerase (PARP). Activated PARP consumes NAD to build-up poly (ADP-ribose) polymers (PAR), which are themselves rapidly metabolized by the activity of poly (ADP-ribose) glycohydrolase (PARG). Some free PAR exit the nucleus, travel to the mitochondria and amplify the mitochondrial efflux of AIF (nuclear to mitochondria cross-talk). Mild damage of DNA activates
the DNA repair machinery. On the contrary, excessive oxidative and nitrosative stress-induced DNA damage occurs various forms of reperfusion injury and other pathophysiological conditions, the cell may be executed by apoptosis in case of moderate PTP opening and PARP activation with preservation of cellular ATP, or by necrosis in the case of widespread PTP opening and PARP overactivation, leading to massive NAD consumption and collapse of cellular ATP.

**Figure 7:**

**Molecular mechanisms of peroxynitrite-mediated cell death.**

The mechanism of CsA-induced nephrotoxicity is not fully understood, but seems to be associated with renal hypoxia and increases synthesis of free radicals (Zhong et al., 1999; Kumar et al., 1999). In addition, nitric oxide (NO) participates in CsA-dependent nephrotoxicity (Paller et al.,...
1998; Yu et al., 1994). Although the mechanism of NO-induced apoptosis is well established (Markewitz et al., 1993; Bobadilla et al., 1998; De Nicola et al., 1993), the interaction between immunosuppressors and NO in terms of potentiation of apoptosis still remains elusive. Expression of endothelial NO synthase (NOS-3) found in epithelial cells of the kidney, including proximal tubule, thick ascending limb, and inner medullar collecting duct and in interstitial cells (Haynes et al., 1997). Proinflammatory cytokines, such as interleukin-1 (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α), is the important mediator in progression of acute renal failure, and exerts cytotoxic effects by the expression of high-output NO synthase NOS-2 (Glynne & Evans, 1999). NO and its derived metabolite peroxynitrite (ONOO⁻) participate in renal tubular cell injury, by regulating renal hemodynamics and sodium tubular transport, and cytotoxic mechanisms responsible for acute renal allograft rejection, where macrophages express high levels of NOS-2 (Romagnani et al., 1999). Apoptosis caused by NO in peritoneal macrophages in inhibited by treating the cells with CsA. Immunosuppressors and NO act on apoptotic signaling in PTEC and induce apoptotic death. Immunosuppressors and NO exert a synergistic action, induces apoptosis on PTEC, probably by increasing the release of mitochondrial apoptotic mediators as reflected by the enhancement in the activity of caspases 3 and 7.
1.23 Role of Bax in CsA induced renal apoptosis

Bax is a member of Bcl-2-like family of proteins (Oltvai et al., 1993), forms heterodimers with Bel-2. In cells with both Bcl-2 and bax, the Bcl-2/Bax ratio appears as important in determining the susceptibility of the cell to growth factor deprivation-induced apoptosis. Excessive bax predisposes cells to apoptosis under these conditions, but not in the presence of growth factors. The original report suggested that murine kidney expresses two bax transcripts of 1.5 and 1 kb abundantly. Over expression of Bax, the endogenous antagonist of Bcl-2, result in the development of renal cysts. In cultured tubular cells, decreased Bcl-2, and increased levels of Bax, is associated with the development of cell death and inter nucleosomal DNA fragmentation in experimental acute renal failure (Ortiz et al., 1993; Ortiz & Neilson, 1993). Apoptosis induced by Cyclosporine A on tubular epithelial cells is related to the translocation of Bax to mitochondria as Bax antisense oligodeoxynucleotides prevented cyclosporine A induced apoptosis (Justo et al., 2003).

ER stress-induced apoptosis is mediated by the activation of Bax and Bak at the mitochondria through upregulation of the upstream BH3-only proteins (i.e. BIM and PUMA) leading to mitochondrial outer membrane permeabilization, cytochrome c release, and subsequent apoptosome assembling. Under certain conditions such as mitochondrial calcium overload and oxidative stress, cytochrome c release can occur independently of Bax and Bak through opening of the mitochondrial permeability transition pore
(PTP) and forms several components including CypD and VDAC. Opening of the PTP leads to the expansion of the mitochondrial matrix, resulting in sufficient swelling to rupture the outer mitochondrial membrane and cytochrome c release. Stimulation of ER stress in conjunction with mild serum withdrawal triggers apoptosis in a Bax/Bak and CypD-independent manner. This alternative death pathway is mediated by the activation of caspase-9. Under normal conditions growth factor signaling inhibits this alternative cell death pathway at the level of (i) mitochondria, or by (ii) blocking the activation of a specific ER-dependent event.

Figure 8:

A BAX/BAK and CypD-independent intrinsic apoptosis
1.24 Role of Bcl-2 in CsA induced renal apoptosis

Bcl-2 itself is a suppressor of apoptosis that homodimerizes or heterodimerizes with homologous protein Bax (Oltvai et al., 1993). Bcl-2 comprises both death antagonist and death agonist; differ in structural features and their expression. Members of Bcl-2 family possess a carboxy terminal transmembrane region (Yang et al., 1995) and influences subcellular distribution. Bcl-2 is found to be associated with mitochondria, nuclear membrane and smooth endoplasmic reticulum (Krajewski et al., 1993; Halder et al., 1994). Bcl-2 is important in membrane lipid integrity by suppressing the generation of reactive oxygen species (Kane et al., 1993). Experimental evidence suggesting regulation of intracellular Ca^{2+} homeostasis by Bcl-2 (Baffy et al., 1993; Lam et al., 1994). Bcl-2 is implicated in protein transport across biological membranes (Liu et al., 1996; Kluck et al., 1997; Yang et al., 1997). Bcl-2 proteins forms ion channels on synthetic lipid membranes (Minn et al., 1997; Schendel et al., 1997) and promotes susceptibility to apoptosis.

The Bcl-2 related protein constitutes biologically relevant classes of apoptosis regulators acting at the effector stage (Kroemer, 1997; Reed, 1997) of apoptosis, functioning as suppressors of apoptosis and promoters of cell death. The vulnerability of cells to diverse apoptotic stimuli is determined by the relative ratio of various pro-apoptotic and anti-apoptotic members of the Bcl-2 family (Oltvai et al., 1993; Yang & Korsmeyer, 1996). The Bcl-2 interacting protein, Bax is a pro-apoptotic member of the Bcl-2 family and is induced by γ-radiation, chemotherapeutic drugs and other forms of genotoxic
stress (Kitada et al., 1996; Thomas et al., 1996). Bax is a death promoter neutralized by heterodimerization with Bcl-2 (Zhan et al., 1994). The ratio of Bcl-2 to Bax determines the cellular susceptibility toward apoptosis. During apoptosis, it activates cystein proteases called Caspases in a cascade like fashion (Thornberry & Lazebniky, 1998). The active form of interleukin-converting enzyme (ICE), Caspase-1, and Caspase-3 are involved in the final induction of apoptosis. Apoptosis and its modulators (Bax and Bcl-2) are associated with progression of tubular atrophy and renal fibrosis in rats, subjected to extensive renal ablation (Yang et al., 2001). CsA treatment favors Bax expression, which remains diametrically opposed to Bcl-2, raising the possibility of CsA nephrotoxicity and graft survival is improved by regulators of renal apoptosis.

In the Bcl-2-regulated pathway (mitochondrial or intrinsic pathway) of apoptosis, a range of stimuli triggers the activation of BH3-only proteins, which then bind via BH3: groove interactions to specific prosurvival proteins. Certain BH3-only proteins (eg, tBid and Bim) can directly bind and activate the effector Bcl-2 proteins Bak and Bax to expose their BH3 domain. If prosurvival proteins are occupied by BH3-only proteins, activated Bak and Bax are free to homo-oligomerize via a BH3: groove interaction. The Bak and Bax oligomers form pores and Permeabilize the mitochondrial outer membrane leading to the release of several proapoptotic proteins including cytochrome c. Once in the cytosol, cytochrome c binds to the adaptor protein Apaf-1 to activate the downstream caspases and cleaves multiple cellular
proteins during apoptosis. In the death receptor pathway (extrinsic pathway), the Fas, TNF-α, and TRAIL ligands bind to their cell surface receptors to activate caspase-8. Activated caspase-8 then directly activates the downstream caspases, which is sufficient to cause cell death. Certain cells (Type II cells) require caspase-8 to cleave Bid (tBid) and recruit the mitochondrial arsenal of proapoptotic proteins to fully activate the downstream caspases.

**Figure 9:**

*The pathways to apoptotic cell death*

1.25 **Role of Caspase in CsA induced renal apoptosis**

Caspase is discovered as a cytokine-processing enzyme designated as interleukin-1 β-converting enzyme (ICE). Due to the rapid expansion of the expressed sequence tag (EST) database and the presence of the conserved
pentapeptide sequences QACR (N/Q) G at the caspase active site, over 14 new caspases have been cloned in a short period of time (Thornberry & Lazebnik, 1998). Caspase-1 and caspase-11 have been shown to function mainly in cytokine processing (Li et al., 1995; Kuida et al., 1995; Wang et al., 1998). Whereas, caspase 2, 3, 6, 7, 8, 9, 10 are involved in the regulation and execution of apoptosis (Kuida et al., 1995; 1996; Hakem et al., 1998; Varfolomeev et al., 1998; Bergeron et al., 1998). The functions of the remaining caspases are unknown. All apoptotic caspases exist in normal cells as inactive enzymes analogous to the zymogens involved in the regulation of blood clotting. When cells undergo apoptosis, these caspases become activated through one or two sequential proteolytic events and cleave the single peptide precursor into the large and small fragments and constitutes active enzyme (Thornberry & Lazebnik, 1998). Currently two well characterized caspase-activating cascades regulate apoptosis: one is initiated from the cell surface death receptor and the other is triggered by changes in mitochondrial integrity.

CsA promotes caspase-independent release of cytochrome c and Smac/Diablo from mitochondria. CsA causes caspase-dependent loss of mitochondrial membrane potential. Caspase-2, caspase-3, and caspase-9 were activated, and specific caspase inhibitor prevented apoptosis and increased long-term survival. Bax promotes the release of cytochrome c to the cytoplasm, contributes the formation of apoptosome, which leads to the activation of caspase-9 (Sun et al., 1999). Activated caspase-9, in turn, activates caspase-3. Both caspase-3 and -9 play a vital role in CsA-induced
apoptosis. Indeed, inhibition of caspase-9 or caspase-3 activity prevented features of apoptosis and increases long-term cell survival.

Caspases have diverse functions; essential role in initial signaling events (caspase-8, caspase-9) and downstream proteolytic cleavages (caspase-3) (Rudel, 1999; Thronberry et al., 1997). Protease inhibitors, including macromolecular and peptide-based inhibitors of caspases, are highly effective in preventing apoptotic cell death in both in vitro and in vivo models of apoptosis (Thronberry, 1997; Krajewska et al., 1997). Among identified caspases, the best functionally correlated with the phenotype of apoptosis is caspase-3 (Krajewska et al., 1997), mainly activated in death receptor and mitochondrial routes (Rudel, 1999). Fewer studies on the regulators of apoptosis in CRF models, shows data on the contribution of the caspases, in experimental cyclosporine A–induced nephrotoxicity (Shihab et al., 1999). The main role of caspase-1 is to activate inflammatory IL-1β and IL-18, whereas caspase-8 is required for compensatory liver proliferation (Launay et al., 2005; Ben Moshe et al., 2007) Specific inhibitors of caspase 2, 3, 8, or 9 decrease apoptosis and prolong cell survival in tubular cells exposed to CsA (Justo et al., 2003).
Figure 10:

Two major intracellular pathways cause apoptosis. Binding of FasL to CD95 (Fas receptor) leads to recruitment and activation of pro-caspase 8, which subsequently activates caspase-3. Activated caspase-3 is responsible for induction of several events that lead to apoptosis. A second apoptotic pathway is initiated by leakage of cytochrome c from the mitochondria and together with caspase-9 and APAF1 forms the apoptosome and activates caspase-3. The integrity of the mitochondrial membrane is regulated by proteins of the Bcl-2 family, Bax and Bak being, pro-apoptotic, Bcl-2 and Bcl-
Apoptosis resistance in ulcerative colitis results from overexpression of FLIP, leading to the impairment of caspase-mediated pathway of apoptosis. The mitochondrial pathway is intact. Fas-induced apoptosis is normal but the mitochondrial pathway is affected by an imbalance of pro- and antiapoptotic Bcl-2 family members. Activation-induced apoptosis of T cells is impaired.

1.26 Role of Cytochrome C in CsA-induced renal apoptosis

The apoptosis-inducing activity of cytochrome c seems to be independent of its redox status (Liu et al., 1996, Yang et al., 1997, Kluck et al., 1997; Bossy-Wetzel et al., 1998). Cytochrome c is released from mitochondria in cells, undergoing apoptosis induced by a variety of stimuli, includes DNA-damaging agents, kinase inhibitors, and activation of cell surface death receptors (Yang et al., 1997; Scaffidi et al., 1998). Once released from the mitochondria, cytochrome c works together with the other two cytosolic protein factors, Apaf-1 and procaspase-9, to activate caspase-3 (Li et al., 1997). In CsA-treated tubular epithelial cells, Bax translocation and cytochrome c release were caspase-independent phenomena, placing them upstream of or parallel to caspase activation. Bax promotes the release of cytochrome c to the cytoplasm, where it contributes to the formation of the apoptosome, which leads to the activation of caspase-9 (Sun et al., 1999).

In healthy cells, a major fraction of cytochrome c (Cyt c) is associated with the mitochondrial IM lipid cardiolipin (CL). This pool of Cyt.
c is mobilized by the disruption of electrostatic interactions with CL or by the oxidation of CL mediated by reactive oxygen species (ROS) and by the CL-oxygenase activity exhibited by Cyt c itself. A limited release of Cyt c enhances the generation of ROS at the levels of the oxidative phosphorylation complexes (OXPHOS) I and III, thus favoring CL peroxidation and further Cyt c release. Once in the cytosol, a low amount of Cyt c released following a limited mitochondrial membrane permeabilization (MMP) and activates caspase-3 (Casp-3) pool. Activated Casp-3, enters IMS through the partially permeabilized mitochondrial OM and cleave a 75-kDa component of the respiratory complex I. This provokes the disruption of the respiratory chain followed by an intense generation of ROS and favors MMP by interacting with the permeability transition pore complex (PTPC) and supports further Cyt c release by oxidizing. At the ER, the second messenger IP3 binds to its receptor (IP3R) to modulate Ca\textsuperscript{2+} release. While physiological concentrations of Ca\textsuperscript{2+} enhance the IP3R channel activity, higher concentrations inhibit the receptor and establishing a negative-feedback regulatory loop. Low amounts of cytosolic Cyt c are able to bind type I IP3R and remove such Ca\textsuperscript{2+} dependent inhibition, thus promoting unrestrained release of Ca\textsuperscript{2+} from the ER. Ca\textsuperscript{2+} favors MMP by direct effects on the PTPC. The mobilization of ICS proteins occurring along with cristae remodeling represents an additional mechanism to account for the biphasic release of Cyt c during apoptosis.
1.27 Role of IL-6 in CsA induced renal apoptosis

IL-6 is a pleiotropic cytokine, described as pro- and anti-inflammatory properties (Gadient & Patterson, 1999; Jones et al., 2001). IL-6 is produced in copious amounts by endothelial cells in response to proinflammatory signals including TNF-β (Shalaby et al., 1989) and hypoxia, (Yan et al., 1995) and shows response to tissue injury and organ failure (Sekiyama et al., 1994; Macgowan et al., 1997). On target cells, IL-6 acts by binding to a specific cognate receptor (IL-6R), triggers gp130 and activates Jak/STAT signaling pathway. The cellular distribution of IL-6R is limited to a few cell types, including hepatocytes, and some leukocyte subpopulations, including monocytes, neutrophils, and some T cells and B cells (Jones et al., 2001). IL-6R exists as soluble form (sIL-6R) binding to IL-6 stimulates cells
via direct interaction with gp130 (Jones et al., 2001; Rose-John, 1994). IL-6 possesses pleiotropic effects, having both inflammatory processes and tissue protection (Mizuhara et al., 1994; Camargo et al., 1997; Yoshizawa et al., 1996; Galun et al., 2000; Hecht et al., 2001). Administration of cyclosporine A (25 mg/kg/day i.p.) for five weeks developed nephropathy in mice by increasing serum level of IL-6 and activating NAD(P)H oxidase (LaSpina et al., 2008).

**Figure 12:**

**Interleukin 6 signaling via the cognate IL-6R and sIL-6R**

[Diagram]

Cellular activation by IL-6 requires binding to its cognate receptor and the resulting dimerization of gp130. This mediates phosphorylation of gp130-associated JAKs and facilitates docking of STAT-1/STAT-3 factors to gp130, and their phosphorylation. Monomeric STAT subunits form homo- and heterodimers and translocate into the nucleus and initiates gene expression. Activation of the Ras-Raf pathway leads to the recruitment of the
transcription factors AP-1 and NF-IL-6. Signaling through gp130 via the [sIL-6R/IL-6] complex activates a similar series of cellular events. Although there is no direct evidence for sIL-6R facilitating Ras-Raf signaling, since activation of this pathway is associated with IL-6-mediated proliferation it is possible that the [sIL-6R/IL-6] complex may stimulate this cascade.

1.28 Role of IL-1α in CsA induced renal apoptosis

Interleukin 1alpha (IL-1alpha) is an important regulatory cytokine, the release of which after an injury induces activation of transcription factors nuclear factor (NF) kappa B and activator protein (AP-1), and promote expression of genes involved in cell survival, proliferation, and angiogenesis. IL-1alpha serve as an autocrine factor and stimulates prosurvival transcription factors and target genes in cancer (Wolf et al., 2001). IL-1 stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. IL-1 proteins are involved in the inflammatory response, identified as endogenous pyrogens, and stimulate the release of prostaglandin and collagenase from synovial cells. Interleukins are a group of proteins present in immune system cells. They are involved in cell-to-cell communication and possess many functions within the immune system. Interleukin-1 alpha is described as "pro-inflammatory" because it stimulates the activity of genes involved in inflammation and immunity. This protein plays a critical role in protecting the body from foreign invaders such as bacteria and viruses. It is also involved in bone resorption, the breakdown and removal of bone tissue. Interleukin-1 alpha is
produced as a long protein and is trapped within cells. Studies suggest that the effects of IL1A variations are probably related to the role of interleukin-1 alpha in promoting inflammation. In 1991, Hasegawa et al., 1991 reported that glomerular basement membranes from diabetic rats induced significantly increased amounts of TNF-α and IL-1 in cultured peritoneal macrophages (Hasegawa et al., 1991). In experimental models of diabetic nephropathy, renal expression of IL-1 increases (Sassy-Prigrnt et al., 2000; Navarro et al., 2006), with subsequent increase in the expression of chemotactic factors and adhesion molecules. IL-1 enhances the synthesis of ICAM-1 and vascular cellular adhesion molecule-1 by glomerular endothelial cells and induces de novo synthesis and expression of ICAM-1 by glomerular mesengial cells and renal tubular epithelia. In addition, this cytokine induce transient expression of E-selectin by endothelial cells (Brady, 1994; Park et al., 2000). Interleukin-1 alpha was effective in stimulating hypercalcemia and bone resorption both in vivo and in vitro but Cyclosporine A was effective in inhibiting IL-1 alpha-mediated bone resorption only in vitro.

IL-1 is involved in the development of abnormalities in intraglomerular hemodynamics related to prostaglandin synthesis by mesengial cell. Invitro studies demonstrate IL-1 directly increases vascular endothelial cell permeability (Royall et al., 1989). IL-1 dysregulates the generation of hyaluronan content in glomeruli by renal proximal tubular epithelial cells. Primary cultures of human proximal tubular epithelial cells stimulated with IL-1 and increases significantly hyaluronan concentrations in
the culture supernatant (Jones et al., 2001). Increased production of glomerular hyaluronan initiates glomerular hypercellularity in experimental model of diabetes (Mahadevan et al., 1995). The IL-6 response to IL-1α was insensitive to CsA. IL-1α play a role in the induction of trauma associated CsA insensitive IL-6 secretion (Hedges et al., 2006). CsA specifically suppressed IL-1 induced increase of COX2 mRNA and protein levels, but CsA had no effect on basal COX1 expression.

**Figure 13:**

IL-1 family members are the released mediators. IL-1α and β, the two best characterized IL-1 family agonists, are translated in the cytoplasm as 31 kD pro-forms. Pro-IL-1α and β are then proteolytically cleaved by calpain and caspase-1, respectively, to produce the mature proteins. Caspase-1 is activated by recruitment to multimeric inflammasomes, enhancing caspase-1 autoproteolysis. Pro-IL-1α, mature IL-1α and mature IL-1β can be released...
from cells and bind to transmembrane IL-1RI (RI) on IL-1-responsive cells. This leads to the recruitment of IL-1RAcP (AcP) to IL-1RI. A multi-protein complex is recruited to the cytoplasmic domain of the receptor dimer, leading to the activation of NFkβ and mitogen-activated protein kinases, and to changes in gene expression and RNA stability. IL-1RA, the best developed anti-IL-1 therapeutic agent, acts as a competitive antagonist, binding IL-1RI but failing to recruit IL-1RAcP and activate signal transduction. IL-1, interleukin-1; IL-1RA, IL-1 receptor antagonist; IL-1RI, type I IL-1 receptor; IL-1RAcP, IL-1 receptor accessory protein; NFkβ, nuclear factor kB.

1.29 Role of IL-1 β in CsA induced renal apoptosis

IL-1 (β-converting enzyme (ICE), a cysteine protease, activates pro-interleukin-1 β (pro-IL-1β), is a mammalian homologue of CED-3 (Thornberry et al., 1992; Yuan et al., 1993; Miura et al., 1993). Previous studies showed that increased ICE induces programmed cell death and releases IL-1β during apoptosis mediated stimuli (Miura et al., 1993; Hogquist et al., 1991; Miura et al., 1995; Zychlinsky et al., 1994). ICE is the major (if not the only) protease responsible for processing pro-IL-1β (Li et al., 1995; Kuida et al., 1995). IL-1β can either stimulate or inhibit cell death. A number of signal transduction mechanisms mediate the biological effect of IL-1β. IL-1β also induces apoptosis in pancreatic RIm5F cells via a pathway that is dependent on its ability to induce nitric oxide production (Ankarcrona et al., 1994). Nitric oxides are the strong and direct mediators of apoptosis (Ankarcrona et al., 1994; Haimovitz-Friedman et al., 1994). IL-1β activates
JNK-p38 signaling pathway and cell death (Raingeaud et al., 1995). IL-1 β plays a pivotal role in cellular homeostasis by modulating the apoptotic cascade and activating the immune system. CsA reduces IL-1β in mesengial cells and reduces cytokines in mRNA level. CsA specifically impaired the IL-1β triggered degradation of inhibitory NF-kB. Interleukin -1-β-converting enzyme (ICE), also known as caspase-1, a specific intracellular cysteine protease required for the processing of some cytokines lacking a signal peptide to allow for release of the mature proteins from the intracellular compartment (Gu et al., 1997). The precursors of IL-β and IL-18 identified as substrates for ICE (Gu et al., 1997; Bazan et al., 1996; Ghayur et al., 1997). The pro IL-β and pro IL-18 are inactive until cleavage by ICE occurs (Cerretti et al., 1992; Puren et al., 1998; Thornberry et al., 1992). The role of IL-β in intestinal inflammation depends both on the up-regulation of IL-β production as well as the level of its naturally occurring inhibitor, the IL-1 receptor antagonist (IL-1Ra).

Is IL-1 just the tip of the ICEberg? Caspase-1 has a broad range of substrate specificity and extends far beyond inflammation. Depicted here is an iceberg of caspase-1 substrates in which IL-1- and inflammation-related protein substrates are situated at the tip of the iceberg. The uppercase ICE in ICEberg relates to the former name for caspase-1, ICE and contributes to the phenotype of a pyroptotic cell death. In addition to inflammation, substrates related to the ontologies of cell death, cytoskeleton and metabolism are shown.
1.30 Role of CsA nephrotoxicity in renal Apoptosis

Apoptosis is a programmed cell death, possesses characteristic morphological and biochemical changes (Nagata, 1997; Schwartzman et al., 1993; Ellis et al., 1991). Apoptosis plays important role in regulating renal cell number in health and disease (Savill, 1994; Savill et al., 1996). Apoptosis is an important pathogenic mechanism in renal diseases, during development and injury (Harris, 1988; Baker et al., 1994; Shimizu et al., 1995; Tang et al., 1996; Koseki et al., 1992). Lethally injured cells may die by either necrosis or apoptosis, depending on the severity of the injury (Lieberthal et al., 1996; Lieberthal et al., 1996). Severe renal ischemia results in acute tubular necrosis, chronic low grade ischemia leads to apoptosis in renal tubular cells (Gobe et al., 1990; Schumer et al., 1992; Gobe & Axeleson, 1987; Woo,
The pathogenesis of chronic CsA nephrotoxicity shows low grade hypoxic injury to renal tubular cells (Andoh et al., 1997). The mechanism of extracellular matrix accumulation in chronic CsA nephrotoxicity have been well explored (Shihab et al., 1996; Shihab et al., 1997) whereas, the pathogenesis of tubular atrophy and cell loss is poorly understood. Recently showed that apoptotic cell death occurs in CsA associated fibrosis with the evidence of acute tubular necrosis (Thomas et al., 1998).

**Figure 15:**

Multiple intersections between apoptosis and bioenergetics

Mitochondria are the cell’s powerhouse, the site where the vast majority of ATP is synthesized. ATP synthesis is driven by the electrochemical gradient built across the inner mitochondrial membrane (IM) by the oxidative phosphorylation complexes (OXPHOS). To generate this electrochemical gradient, OXPHOS pump protons from the matrix to the intermembrane space (IMS), leading to the formation of a transmembrane...
potential ($\Delta \Psi_m$) as well as to the generation of reactive oxygen species (ROS). In healthy cells, ROS are kept at harmless levels by the activity of both nonenzymatic and enzymatic antioxidant systems. Among the former, a prominent role is played by nonoxidized glutathione (GSH), thioredoxin (Trx), and NAD(P)H and glutathione S-transferase (GST), glutathione peroxidase (GPx), and the manganese-dependent superoxide dismutase (MnSOD) represent redox-active enzymes. This delicate equilibrium breaks down when apoptosis is induced, following distinct but sometimes overlapping mechanisms. $\Delta \Psi_m$ dissipation is promoted by proapoptotic stimuli as diverse as members of the Bcl-2 family of proteins (Bax, Bak, tBid), $\text{Ca}^{2+}$ and cytosolic metabolites (all of which promote the opening of the PTPC, i.e., the permeability transition pore complex), and the activation of caspases (that may degrade OXPHOS subunits). The progressive loss of $\Delta \Psi_m$ is often accompanied by an increased generation of ROS and saturates antioxidant systems and induce the functional impairment of mitochondria, by arresting oxidative phosphorylation and feed-forward mechanisms on the PTPC. ROS accumulates and increases $\Delta \Psi_m$, as induced by inhibitors of the ATP synthase (complex V) like oligomycin or acidification of the mitochondrial matrix. Finally, decreased ATP production, protein thiol oxidation, lipid peroxidation, and the activation of stress response genes intervene, in the scenario of a bioenergetic crisis that progressively leads the cell to death.

Apoptosis is the mechanism of cell clearance and progression of kidney injury in several animal models, including ischemia-reperfusion injury (Yang et al., 2001). Chronic kidney disease, (Schelling & Cleveland, 1999)
diabetic nephropathy, (Zhang et al., 1997) and obstructive kidney disease (Zhang et al., 2001). The major multigene families involved in the molecular controls of cell survival or death is the Bcl-2 gene family. Its members include inhibitors (eg, Bcl-2, Bcl-XL, Bcl-w, and Mcl-1) and accelerators of apoptosis (eg, Bax, Bcl-Xs, Bak, Bik, and Bad) (Ortiz et al., 2000). Heterodimerization between anti apoptotic Bcl-2 and pro apoptotic Bax may negate the function of either protein. The ratio of Bcl-2 to Bax appears to determine a cell’s fate (Hockenbery et al., 1993; Oltvai et al., 1993). Caspases is the family of cysteine proteases and are activated during programmed cell death. Among them, caspase-3 (CPP32/Yama/apopain) is an executor of apoptosis (Ortiz et al., 2000). Several recent studies have demonstrated that cyclosporine treatment directly induces apoptosis in the renal tubular cell line and human proximal tubular epithelial and endothelial cells in vitro (Amore et al., 2000, Healy et al., 1998; Hortelano et al., 2000). Cyclosporine-induced apoptotic cell death is closely associated with activation of proapoptotic genes and altered regulation of apoptosis-regulating genes (Shihab et al., 1999).

1.31 Need for safer drug – Arjunolic acid

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 (Berman, 2000). 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 (Pinn, 2000).

Medicinal plants possess important contributors 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. With the passage of time, many problems associated with frequent use of synthetic drugs become prominent like severe side effects and resistance of microbes against these drugs. On the other side synthetic drugs are expensive and large population cannot afford these drugs. In recent times, research on medicinal plants has been intensified all over the world. The natural pharmaceuticals are receiving extraordinary importance and popularity as safe, efficacious and cost effective medicines with extraordinary benefits due to combination of medicinal ingredients with vitamins and minerals (Ahmad & Husain, 2008). Recently there is an emerging trend in research to support the biological activities of medicinal plants. Many scientific researchers have been reported about the efficacious and chemotherapeutic role of medicinal plants in the treatment of diverse diseases.

The use of medicinal plant either as a single drug or in combination increases health care of human being. Medicinal plants are the important source of unknown chemical substances with potential therapeutic effect. Several medicinal plants found to be beneficial for cardiac ailments in “Atharva Veda” an ancient treatise from which Ayurveda, the Indian system
of Medicine owes its origin (Shatvaleka, 1943; Dwivedi & Chaturvedi, 2000). The plant which shows most promising and distinct results among, is Terminalia arjuna popularly known as arjuna (Dwivedi & Udupa, 1989). 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.

The world health organization estimated about 75% of the world’s population still relies on plant derived medicines, obtained from traditional healers, for its basic health care needs. The arjuna, scientific name Terminalia arjuna, is a lofty evergreen plant, indigenous to the Indian subcontinent. The bark of the arjuna possesses therapeutic properties and used for centuries to treat different conditions. Arjuna is an exceptional herb, aids in facilitating a hale, hearty heart and diminishes anxiety and apprehension. In addition, bark of the arjuna endorses effectual functioning of the cardiac muscles. Arjuna is a preferred herb in Ayurveda, the ancient Indian medical science, for treating as well as maintaining the health of the cardiovascular system. Terminalia arjuna bark is commonly known as arjuna bark or arjun and abundantly available throughout India. This plant contain 15 % tannins, tritepenoid saponins, flavonoids, calcium, aluminium and magnesium salts along with coloring matter and sugars are the other constituents of arjun. Terminalia arjuna possesses wide-ranging therapeutic properties and has the potential to treat numerous medical conditions, especially those pertaining to the heart and circulation system. Terminalia arjuna possesses hypolipidemic, anticoagulant, hypocholesteremic, antihypertensive, antiviral, antithrombotic,
antifungal and antibacterial properties. The therapeutic properties regarding cardiovascular health are attributed to triterpenoids enclosed by the arjuna. Similarly, flavonoids and tannins naturally present in the herb possess anticancer properties.

**Figure 16:**

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Terminalia arjuna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common name</td>
<td>Arjun</td>
</tr>
<tr>
<td>Family</td>
<td>Combretaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Terminalia</td>
</tr>
<tr>
<td>Species</td>
<td>T. Arjuna</td>
</tr>
<tr>
<td>Part used</td>
<td>Leaves, roots, barks</td>
</tr>
</tbody>
</table>

The effect of a compound formulation (abana) containing Terminalia arjuna 30 mg per tablet, studied in isoproterenol-induced myocardial necrosis in rats. Increase in serum CPK, SGOT, SGPT and SGOT following
myocardial necrosis were significantly reversed by abana. The drug shows 90% protection against reduction in glycogen levels in ischemic rats. The beneficial effect of abana was further evident by reduction in mitochondrial enzymes such as α-kG and succinate dehydrogenase (SDH) by 44% and 48%, respectively (Tandon et al., 1995).

Effect of arjunolic acid derived from Terminalia arjuna (15 mg/kg body weight) on antiplatelet activity, electrocardiographic changes, serum marker enzymes, antioxidant status, lipid peroxide and myeloperoxidase (MPO) were measured and compared with the acetyl salicylic acid (ASA) in rats subjected to isoproterenol challenge. The drug was given intraperitoneally before and after isoproterenol administration. Arjunolic acid treatment prevented the decrease in the levels of SOD, CAT, glutathione peroxidase, ceruloplasmin, α-tocopherol, reduced glutathione (GSH), ascorbic acid, lipid peroxide and MPO. Cardioprotection conferred by arjunolic acid could possibly be due to the protective effect against the damage caused by myocardial necrosis (Sumitra et al., 2001).
Research on AA aims to untie its multifunctional therapeutic applications. Being known for its cardio protective effect over centuries, experimental studies have proved functions such as prevention of myocardial necrosis, platelet aggregation and coagulation and lowering of blood pressure, heart rate and cholesterol levels, which lend support to the claim for its
traditional usage. Apart from its cardio protective effects, AA protects the
cells from metal induced toxicity and it also possesses anti-inflammatory,
antidiabetic, antitumor, antimicrobial activity. AA is a potent antioxidant and
a free radical scavenger (Manna et al., 2008). Sinha et al., (2008) have
reported the efficacy of AA against arsenic induced nephrotoxicity in mouse
model. AA also represents a potential therapeutic option to protect renal tissue
from the detrimental effects of acute acetaminophen overdose (Ghosh et al.,
2008).
REFERENCES (INTRO)


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