Introduction
A. KIDNEY: STRUCTURE AND FUNCTION

Kidney is a complex organ that regulates the amount of water lost from the body and aids in removal of waste products of protein catabolism especially urea and creatinine as well as ketone bodies from fat catabolism. They control and regulate the acid-base balance of body fluids including the blood [1]. Thus, it plays an important role in health, disease, growth and overall development of the mammalian system. Kidneys are the two bean shaped organs, one on each side of vertebral column in the lumbar region of the body. Human kidney represent about 0.5% of total body weight. Each kidney is 10 cm long, 5 cm broad and 4 cm thick. The outer surface of kidney is convex and the inner side is concave. The inner face of it has a deep notch called hilus, from where renal artery, renal vein and ureter enters the kidney. Kidney receives 20-25% of total arterial blood. About 180 liters of water is filtered through the kidney everyday but only 1.5 liter finally leaves the body as urine. Thus, kidney helps to maintain total body fluid volume and its composition.

Mammalian kidney which apparently looks homogenous, has many segments. Each kidney has two portions, the peripheral dark reddish brown region known as cortex and the central light red portion known as medulla (Figure 1a). Each kidney contains about 2 million nephrons, which is the basic functional unit. Each nephron is a tube like structure, closed at one end and open at the other. The basic anatomy of nephron is described as follows (Figure 1b): Blood enters the glomerulus through the afferent arteriole and then leaves through the efferent arteriole [2] The glomerulus is a network of upto 50 parallel branching and anatomizing capillaries covered by epithelial cells and encased in bowman’s capsule, pressure of blood in the glomerulus causes fluid to filter into bowman’s capsule and form it, the fluid flows into the proximal tubule, that lies in the cortex of the kidney along with the glomerulus. The proximal convoluted tubule then lead to a “U” shaped part, the loop of Henle [3]. The Henle’s loop has a descending limb, thin middle limb and a thick ascending limb. The ascending limb then leads to distal tubule, that lies in renal cortex.
**Figure 1a:** Cross sectional view of kidney

**Figure 1b:** Structure of a nephron
About eight of distal tubules coalesce to form the collecting duct. The largest collecting ducts empty into the renal pelvis. In each kidney, there are about 250 large collecting ducts, each of which transmits the urine from about 4000 nephrons. As the glomerular filtrate flows through the tubules, about 99% of its water and varying amounts of the solutes are normally reabsorbed into the vascular system and the remaining tubular water and dissolved substances becomes urine (Fig.2). Reabsorption through the tubular membrane is both active as well as passive. The transport of ions and solutes is mostly driven by metabolic energy yielding reactions. Amino acids, sugars, inorganic phosphate (iP) and ions like sodium (Na⁺), potassium (K⁺), chloride (Cl⁻) and bicarbonate (HCO₃⁻) are selectively reabsorbed by brush border membrane (BBM) of proximal tubule [4]. There are numerous extensions in luminal membrane of the proximal tubule, the function of which is to increase the total surface available for contact with tubular fluid, from which many components ion and solutes are reabsorbed at high rates [5]. The transport of sodium ions (Na⁺) is the major function in mammalian kidney, as in the absence of Na⁺ transport, the transport of other solutes such as amino acids and hexoses approaches zero [6]. Fromter et al. [7] suggested that one third of the net transepithelial Na⁺ flux is transported actively.

To discuss the functions of the kidney, it is necessary to know the function of a single nephron. Nephrons have various subsegments which show distinct structural and functional differences. The nephron heterogeneity adds to the variation in kidney function as a whole. Depending on the origin and location of the nephrons in cortical regional of kidney, both inter and intranephronal heterogeneity exists in mammalian kidney [8]. Those nephrons whose glomerule lie close to the surface of the kidney (i.e. approximately 0.5 – 1 mm below the capsular surface) are called cortical nephrons or superficial nephrons. Nephrons with glomeruli situated in the mid cortex deep to the superficial nephrons are referred to as midcortical nephrons. Generally, the most superficial nephrons have short loops of Henle and the loops penetrae
only a very short distance into the outer portion of the medulla. Between one fifth and one third of the nephrons have glomeruli that lie deep in the renal cortex near the medulla; these are called juxtamedullary nephrons. These nephrons have very long loop of Henle that penetrate deeply into the inner zone of the medulla [9]. All these populations of nephrons have been found to be structurally and functionally distinct [8]. Glomerular diameter, filtration rate, proximal tubular length, epithelial permeability, transport characteristics, transepithelial voltage differences and distribution of various enzyme activities contribute to distinguish different nephron populations [10]. The proximal convoluted tubules of superficial nephrons always touch the surface of the kidney, while convolutions from midcortical nephrons do so infrequently. Whereas proximal convolutions from juxtamedullary nephron run perpendicular to and interwine with medullary rays.

In intra-nephron or axial heterogeneity, the proximal tubules have been divided into three distinct morphological subsegments namely, S1, S2 and S3. Proximal convoluted tubules (PCT) both in superficial and juxtamedullary nephrons is defined as S1 subsegment and can be identified by its attachment with glomeruli on one side [11]. S2 is defined as the late superficial PCT and includes the rest of convolutions and entire pars recta (straight portion) in cortical portion of the kidney [11]. S3 is located principally in the outer stripe of outer medulla and terminal superficial PCT. S3 is identified by its medullary location and by its connection with thin limbs on distal part. All S2 subsegments as they descend from cortex into the outer stripe of outer medulla, change from the S2 to S3 cell type.

Besides morphological differences, the functional inter and intra-nephronal differences have also been observed in proximal tubule. The reabsorption of solutes i.e. aminoacids, sugars, inorganic phosphate (iP) and sodium ion (Na+) occurs maximally at proximal tubules of the cortical region, by brush border membrane [12]. In S1 subsegment of early PCT, Na+−K+ ATPase activity, oxidative metabolism and active transport is relatively high, resulting in very
efficient Na\(^+\) coupled net reabsorption of glucose, amino acids, phosphate and net secretion of hydrogen ions \[8\]. Transport capacities of glucose, iP and H\(^+\) by S2 decrease progressively along the proximal convolutions. Finally, in S3 segment transport of organic acids and bases mainly takes place; Na\(^+-K\)^\(^+\) ATPase activity and Na\(^+\) transport are found to be relatively low. The retention of Na\(^+\) ions and iP in body by kidney is required for proper growth and development.

**B. RENAL FAILURE**

It refers to any condition that seriously interferes with kidney function. Nephrons are susceptible to damage due to many factors such as poisons, drugs, aging, infection, trauma, drugs, cancer, autoimmune diseases and genetic predisposition. If any of these occurs, the entire nephron stops functioning. When the number of functioning nephrons drops below 25 percent or when damage occurs too suddenly for the remaining nephrons to compensate, kidney failure occurs. There are two types of renal failure, which are as:

1. Chronic renal failure, which is an irreversible loss of function that occurs, progressively over months or years.
2. Acute renal failure, in which there is sudden loss of function that is sometimes but not always reversible.

**ACUTE RENAL FAILURE**

It is a clinical situation where kidney function is seriously interfered. It occurs so suddenly that surviving nephrons do not have time to recompense. Failing kidneys cannot adequately clear the blood from certain toxins. These include urea (a nitrogen containing by product of protein metabolism) and creatinine (a chemical byproduct of muscle exertion). As a result when the kidney fails, there is an abnormally high level of these wastes products in plasma \[13\]. Other components such as phosphorus, calcium, sodium, potassium and chloride, may also rise or fall abnormally. Failing kidney may also produce extremely dilute urine or urine that contains too much protein (proteinuria). ARF is
caused either by ischemia or toxic insult to the kidney which interferes with energy production, thereby leading to loss of active absorption and secretion functions of the kidney [14]. In ARF, both kidneys stop their function in a short period of time. It begins with tubular necrosis and continues until renal function and structure have essentially recovered due to reperfusion of blood or by administration of various drugs, hormones or antioxidants [15-18]. However, the time course of injury and recovery overlap remain variable due to the extent of damage.

**Pathophysiology of ARF**

ARF can be grossly divided into three phases:

1) Pathogenic phase, 2) Manifestation phase and 3) Recovery phase. In the pathogenic phase, a progressive disintegration and necrosis of tubular epithelial cells occur, leading to the functional loss of the kidney which is manifested by the reduction in creatinine clearance [19]. In the second phase, long lasting effects are observed that severely effect the clearance of creatinine and which can continue for several days depending on the degree of renal damage. In the recovery phase, there is an increase in the concentrating ability of the kidney with eventual normalization of kidney function.

Acute renal failure may occur due to certain factors [20-23]. The classical events that lead to the loss of renal function in ARF include tubular leakage across the damaged epithelium, tubular obstruction or interstitial compression, a decrease in renal blood flow and glomerular membrane permeability. Physiologic and morphologic studies suggest that there are four major possible pathogenic mechanisms of ARF, which include renal vasoconstriction, glomerular permeability, tubular obstruction and tubular leakage [24]. However, the extensive research of the past 40 years using morphology, clearance and micropuncture techniques were unable to provide a universal view regarding the mechanism for the pathogenesis of ARF. A decrease in glomerular permeability explains the reduction in filtration rate but not loss of
tubular function. The reason for not reaching to a conclusion, could be due to the fact that most of the evidences are based on structural alterations rather than on functional parameters.

C. ANTIBIOTIC NEPHROTOXICITY

The clinical use of antibiotics as antibacterial agents have been found to be associated with renal dysfunction. The clinical and morphological expression of antibiotic nephrotoxicity represents a spectrum of alterations ranging from acute renal failure and tubulointerstitial nephrotoxicity to selected renal tubular disorders.

General features of antibiotics

Aminoglycosides form a class of antibiotics that are highly polar cations composed of various sugar molecules in glycosidic linkage with amino group containing side chains. Aminoglycosides have low lipid solubility and a low capacity for penetrating membranes. They are poorly absorbed from gut, therefore are given intramuscularly or intravenously. They have a broad antimicrobial spectrum, extending from gram positive aerobic cocci to gram negative bacilli. However, their exact mechanisms of action is unknown [25]. Aminoglycosides act by inhibiting the synthesis of bacterial proteins via interference with the activity of ribosomes. However, serious toxicity is a major limitation to usefulness of these drugs. The nephrotoxic potential varies among individual aminoglycosides.

The commonly used, clinically available aminoglycoside antibiotics are gentamicin, tobramycin, streptomycin, amikacin and kanamycin.

TOBRAMYCIN, belongs to the family of nebramycins and is derived from the actinomycetes Streptomyces tenebrius. It is active against Pseudomonas aeroginosa but less active against proteus. Chemically, it is a six-membered aminocyclitol ring with amino group side chain (Fig.2). It is used to treat
bacterial conjunctivitis, cystic fibrosis and abdominal infections. However, about 30% of patients treated with it show signs of nephrotoxicity [26].

**GENTAMICIN**, is produced by Microspora purpura and is effective against Pseudomonas, E. coli, Proteus and Mycobacterium tuberculosis. Chemically, it is a sugar in glycoside linkage with side chain amino groups (Fig.2). Nephrotoxicity, is the most unavoidable effect associated with its use [27,28]. Therefore, proper dosage schedule of the drug becomes important.

**STREPTOMYCIN**, is obtained from Streptomyces griseus. It is an organic base which forms water soluble salts (Fig.2). The organisms sensitive to streptomycin are Mycobacterium tuberculosis, Shigella species, E. coli, Proteus and Pseudomonas aeruginosa. The drug is more effective at alkaline pH. therefore its activity is reduced in blood and serum. Renal irritation, however, occurs in patients receiving streptomycin [26].

**AMIKACIN**, is a semisynthetic derivative of kanamycin A with pharmacokinetic properties similar to the kanamycin (Fig.2). It is active against Klebsiella, E. coli and Proteus. However, acute renal deterioration occurs with its usage which manifests as enzymuria and polyuria [29].

**KANAMYCIN**, is a water soluble antibiotic derived from Streptomyces kanamyceticus. It is effective against many gram-negative organism like E. coli. Klebsiella, Aerobacter, Salmonella, Shigella, Vibrio and Brucella. The chemical structure of kanamycin is given in Fig.2. Acute tubular necrosis however, develops characterized with polyuria and proteinuria [30]. Therefore, utmost care is taken during its administration.

Studies indicate that gentamicin and tobramycin are equally nephrotoxic and are slightly more nephrotoxic than amikacin [31]. In animal studies, streptomycin is the least nephrotoxic. The nephrotoxicity of kanamycin
Figure 2: The chemical structures of various commonly used antibiotics.

Aminoglycosides are sugars in glycosidic linkage with side chain containing amino groups.
Streptomycin

Kanamycin
lies between that of streptomycin and neomycin [32]. Concentration of drug found in renal cortex in experimental animals has been used to measure the relative nephrotoxicity produced by an antibiotic. However, some controlled clinical trials have given different estimates of their relative nephrotoxicity [33-36].

Antibiotics are rapidly distributed throughout the extracellular fluid. Because of their polar nature, these are largely excluded from most cell. The potential for aminoglycoside nephrotoxicity seems to depend on the number of ionizable amino groups (NH$_2^+$) contained on their molecule and on the derived cationicity. The cationic charge of these aminoglycosides is correlated with the degree of their interaction with the membrane and interference with mitochondrial function. Although a good correlation exists between the cationicity and specific (renal, ototoxicity or neurotoxicity) or whole animal toxicity but aminoglycosides with similar cationic charges may exhibit different clinical or experimental toxicities. Studies show that aminoglycosides containing equal number of aminogroups (five NH$_2^+$ groups) exhibit different nephrotoxicities [37]. Therefore, factors other than the molecular cationic charge are of importance. These may include charge orientation, position and an inherent propensity of the aminoglycoside molecular structure for causing toxic injury to intracellular organelles.

**Molecular and pharmacologic aspects**

Antibiotics are nephrotoxic because a small concentration 5% is retained in the epithelial cells lining in the S1 and S2 segments of the proximal tubules after glomerular filtration. The drugs accumulated by these cells are mainly localized with endosomal and lysosomal vacuoles and with golgi complex [36-38]. They elicit an array of morphological and functional alterations of increasing severity (Fig.3). In humans, only after few days of administration of clinical dose, aminoglycosides induces conspicuous changes in lysosomes of proximal tubular cell [38]. These changes are accompanied by signs of tubular dysfunctions i.e. release of BBM and lysosomal enzymes, decreased
reabsorption of filtered proteins, wasting of ions and glucose and phosphlipiduria. High doses of aminoglycosides (more than 40 mg/kg body weight) in animals rapidly induce cortical necrosis and renal dysfunction [30,39]. At this stage a number of structural, metabolic and functional alterations occur in tubular cells that lead to cell dysfunction or death. Many changes occur due to the direct effect of drug on apical membrane during its initial stages of uptake in proximal tubules [40, 41], whereas effects like inhibition of protein synthesis mitochondrial alterations, modulation of gene expression must involve uptake and intracellular distribution of the drug to corresponding targets (Fig. 3).

**Tubular necrosis**

Although histopathological studies strongly support the concept that tubular necrosis is the primary causes of functional toxicity. However, the mechanism of this necrosis remains unsettled. Different hypothesis have been given to explain the unambiguity. First hypothesis assumes that antibiotics exert their toxicity in direct relation to their local concentration. This would therefore designate lysosomes as key site and lysosomal alterations as a main target. However, so far, links between lysosomal alterations and cell necrosis have not been found. A second hypothesis is that aminoglycosides become toxic once they are released from lysosomes. This means if triggered abruptly, the release of large quantities of aminoglycosides from lysosomes could indeed cause the simultaneous development of a number of otherwise unrelated metabolic changes, many of which are capable of causing cell death. The question, then is to distinguish between real toxic events from trivial or secondary effects. A typical example is inhibition of mitochondrial respiration and Ca\(^{2+}\) transport or lipid peroxidation, both of which were claimed to cause irreversible cell damage but detailed studies showed that they occur after cell death [42]. Finally a third hypothesis is that drug stored in lysosomes is in parallel to endocytic uptake and a small amount of aminoglycosides reaches a critical, nonlysosomal target and causes toxicity.
Figure 3: Ultrastructural alterations induced in proximal tubular cells during antibiotic treatment.

(A) Control

(B) Low dose of aminoglycoside showing enlargement of lysosomes and deposition of polar lipids as myeloid bodies.

(C) High doses giving apparent rupture of lysosomes, extensive mitochondrial swelling and damage, dilation of the endoplasmic reticulum cisternae, shedding of the apical brush-border villi, pericellular membrane discontinuities, and the occurrence of apoptotic nuclei.
While the determinants of cell damage still remain undefined, more knowledge concerning the mechanisms causing the impairment of the renal function is to be available. Activation of the renin-angiotensin system and the ensuring local vasoconstriction appear to be primarily responsible for the decrease in glomerular filtration [40]. This explains very well the aggravating effect of nonsteroidal antiinflammatory drugs on aminoglycoside nephrotoxicity, since these drugs inhibit the production of vasodilatatory prostaglandin PGE$_2$ [42,43].

**Interaction of Aminoglycosides with Phospholipids:**

The kidney has a large capacity to compensate for tubular insults but it has been found that tubular cells undergo a marked proliferative response, even after low-dose treatment with antibiotics [44]. While the molecular mechanisms of toxicity themselves are still unclear, it remains that the drugs must physically interact with one or several cellular constituents to initiate the cascade of events leading to toxicity. Two phenomena appear to be essential in this context, namely the uptake of antibiotics into proximal tubular cells and their interactions with phospholipids in cell [45].

Aminoglycosides binds to the BBM in cationic form. The initial points of attachment are probably the acidic phospholipids mainly phosphatidylserine [46]. Thereafter, they are transferred to the transmembrane protein megalin, with which they become internalized in endosome. N-Acetyl neuraminic acid, the cortical content of which is increased in the kidneys during gentamicin treatment [47] could also be involved. Perhaps the most interesting aspect of this uptake process, from toxicological point of view is its saturable character at concentrations which are clinically relevant (apparent Km in rats, 15µg/ml) [48].

Once transferred from endosomes to lysosomes through the physiological process of endosome lysosome fusion, aminoglycosides will be exposed to a fairly acidic pH, at which they will be fully protonated and therefore expected to bind tightly to negatively charged structures. In vitro studies show that
Figure 4: Skeleton view of the mode of assembly of Gentamicin (green) with phosphatidylinositol (hydrocarbon is in grey and oxygen and phosphorus atom in red). The molecular modeling approach suggests that the N-6 amino group are oriented in opposite direction (towards the lipophilic phase). Gentamicin lies above the plane of inositol moieties and far way from the water phase (bottom).
Gentamicin
these drugs bind tightly to acidic phospholipids, primarily by electrostatic forces and cause a marked decrease in the mobility of the phosphate heads in membrane bilayers [49]. As shown in Fig. 4, gentamicin bound to phosphatidylinositol lies close to the interface, being inserted in the monolayer at the level of the phosphogroup and extending toward the hydrophobic phase up to the level of the ester linkage of the fatty acids [50]. The binding of aminoglycosides to lipid bilayers causes their aggregation as well as the inhibition of the activities of phospholipases [51]. The later is due to the neutralization of the surface negative charge, which these enzymes require to fully express their activity [52,53].

Enzyme inhibition and membrane aggregation most likely account for the accumulation of myeloid bodies in lysosomes. Myeloid bodies isolated from the renal cortex essentially contain phospholipids and proteins. The phospholipid accumulation, inhibition of phospholipase activity together with extent of phospholipidosis induced by the aminoglycosides correlates with their nephrotoxic potential [45].

D. PROTECTION AGAINST ANTIBIOTIC NEPHROTOXICITY

Protection against antibiotic nephrotoxicity has attracted much effort and attention over the last decade. These efforts can be subdivided into several types of approaches, which are as illustrated in Table 1. Some of these are discussed below.

I. Decreasing or preventing antibiotic accumulation by kidney:

Antibiotic accumulation could be reduced either by impairing their uptake or by enhancing their release. Reduction of uptake has been obtained by complexing the aminoglycosides extracellularly or by competing with / decreasing drug binding to the BBM. Various compounds have been used in this context however, all such approaches could not be translated into clinical applications [54]. Early studies with animals also revealed that administration of the daily dose of gentamicin as a single dose was considerably less toxic,
TABLE 1. Main approaches toward reduction of aminoglycoside nephrotoxicity

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Compound</th>
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<tbody>
<tr>
<td>I. Decrease or prevention of drug accumulation by kidneys</td>
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<tr>
<td>(a) Intracellular complexation of aminoglycosides with</td>
<td>Dextran sulfate</td>
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<tr>
<td>(i) Polyanionic compounds</td>
<td>Inositol hexasulfate</td>
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<td>(ii) Acidic drugs</td>
<td>Piperacillin</td>
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<td></td>
<td>Latamoxef-moxalactam</td>
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<td>Fosfomycin</td>
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<td>(b) Competition with or decrease in aminoglycoside binding to brush border membrane by</td>
<td>Pyridoxal-5'-phosphate</td>
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<tr>
<td>(i) Raising the urine pH</td>
<td>Bicarbonate</td>
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<td>(ii) competitors</td>
<td>Ca^{2+}</td>
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<td>(iii) Increase in exocytosis</td>
<td>Lysine</td>
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<td></td>
<td>Fleroxacin</td>
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<tr>
<td>II. Prevention or decrease of lysosomal phospholipase inhibition by using</td>
<td></td>
</tr>
<tr>
<td>(a) Derivatives with lesser intrinsic binding</td>
<td>Isepamicin, arbekacin, 1-N- and 6'-N-peptidic and aminoacid derivative of netilmicin</td>
</tr>
</tbody>
</table>
(ii) Other substitution

(iii) Fluorinated derivatives

(iv) Disaccharidic aminoglycosides

(b) Coadministration of agent preventing intralysosomal phospholipidosis lead to

(i) Intralysosomal sequestration of aminoglycosides

(ii) Increase of membrane negative charge

(iii) Other

III. Protection against necrosis and other gross cellular alterations using

(i) Antioxidants

(ii) Antioxidant and multifactorial factors

(iv) Disaccharidic aminoglycosides

Astromicin (fortimicin)

Dactimicin (2-N''-formidoyl-astromicin)

Polyaspartic acid

Daptomycin

Torbafylline

Deferoxamine

Methimazole

Vitamin C

Lipoic acid

Deoxycortisone and saline drinking

Ca\(^{2+}\) channel blockers

Platelet activation antagonists

Ulinastatin

Fibroblast growth factor 2

Heparin-binding epidermal growth factor
effective, on the basis of finding that gentamicin forms complexes with mitochondrial Fe\textsuperscript{2+} to catalyse the formation of free oxygen radical [61]. However, biophysical and biochemical considerations in use of antioxidants like deferroxamine, suggest that the protective effect of such compounds depend critically on the dosage of gentamicin [62]. Several other agents like epidermal growth factor, endothelin [19], atrial natriuretic factor [63], glycine [64], insulin like growth factor [65] and platelet activating factor [66] have been reported to ameliorate renal toxicity. However, in above mentioned attempts, a generalized mechanism for the damage or repair of acute renal failure in kidney particular, is not given. Very recently, protective role of melatonin, garlic extracts and gum arabic on antibiotic induced acute renal injury has been found [67-69]. Studies have also indicated the role of mixed protein diet in amelioration of gentamicin induced nephrotoxicity in rats [70]. Compounds like tempol (4-hydroxy tempo), S-allylcystein, diallyl disulfide and lipoic acids has been shown to attenuate aminoglycoside induced renal damage [71-73]. Various herbal extracts like Pongamia Pinnata and Jawarish Zaroooni Sada also possess nephroprotective effects [74, 75]. Treatment with SOD and vitamin E was found to protect against gentamicin induced nephrotoxicity in rats [76, 78]. More and more extensive research in this field is however, going on.

**F. FISH OIL PROTECTION**

Present mainly in seafood, and therefore better known as “fish oils”, exert a remarkable variety of biological effects [78]; therefore, they are currently being tested as therapeutic options in a variety of clinical situations, ranging from cardiovascular diseases [79] to hyperlipidemia [80], cancer [81], inflammatory disorder [82], respiratory diseases [83] and diabetes [84]. Fish oil is an oil rich in omega 3 fatty acids or n-3 fatty acids (PUFA) (Table 2).
Biologic properties of fish oil

Humans like all mammals cannot synthesis fatty acids (FA) with double bonds distal to the ninth carbon atom although, they are able to elongate to a certain extent and further desaturate the aliphatic chain. Between families of FA derived from linoleic acid (C18: 2n-6) and α-linolenic acid (C18:3n-3) there is no practical inter-conversion. Although humans can desaturate and elongate α-linolenic acid to eicosapentaenoic acid (EPA) and further, to docosahexaenoic acid (DHA), these processes are likely to be slow and possibly increasingly limited by age [85] and disease status like hypertension [86] or diabetes [87]. Therefore, linolenic acid, EPA and DHA are to a large extent nutritionally essential and are exclusively derived from fish and marine oils (Table 2).

Effect of n-3 fatty acids on Eicosanoid production

n-3 FA exert an incredible variety of biologic effects (Fig. 5) many of which have implications for kidney diseases. No unifying mechanisms exists, at the moment, for all these effects. Most of the multifaceted actions of n-3 FA relates to the activities of metabolites derived from EPA as opposed to those derived from arachidonic acid (AA) [88-90]. Metabolites of EPA and AA (two fatty acids with 20 carbon atoms) are known as “eicosanoids”, which include prostaglandins (PG), thromboxanes (Tx), leukotrienes (LT), hydroxy fatty acids, and more recently lipoxins, epoxy-fatty acids and isoprostanes. Alterations in eicosanoid synthesis and metabolism occur when concentrations of EPA and DHA increase relative to AA. Thus, in platelets and in other tissues including the kidney, an inactive TXA3 is produced at the expense of TxA2, which is a potent vasoconstrictor and inducer-amplifier of platelet aggregation. On the other hand, an active prostacyclin (PGI3) with similar effects to PGI2, a vasodilator and inhibitor of platelet aggregation, is produced by the endothelium after ingestion of n-3 FA. Therefore, the balance between proaggregatory and antiaggregatory derivatives is shifted towards inhibition of platelet aggregation and a decrease in vasodialators.
Figure 5: Biological effects of n-3 FA and the rationale for their use in renal diseases. Tx—thromboxane; PG—prostaglandin, LT—leukotriene; PAF—platelet activating factor, VLDL—very low density lipoprotein, PDGF—Platelet – derived growth factor, IL—interleukin, TNF—tumor necrosis factor, RBC—red blood cells.
**Primary Effects**
- Effects on Eicosanoids and Related lipid mediators
  - decreased platelet and mesangial production of TXA2
  - unchanged or increased production of vascular PGI2
  - production of an inactive TXA3 and of an active PGI3
  - decreased leukocyte production of LTB4, C4, D4, E4
  - leukocyte production of less active LTB5, C5, D5, E5
  - decreased leukocyte production of PAF
- Eicosanoid-independent inhibition of platelet function (adhesion, aggregation)
- Decrease in plasma triglycerides and VLDL
- Decrease in endothelial production of PDGF
- Decreased monocyte production of IL-1, IL-2, TNF
- Decreased leukocyte chemotaxis
- Primary decrease in endothelial permeability
- Increase in endothelial-dependent vasodilation
- Decreased vascular responses to angiotensin II and nor ephinephrine
- Decrease in fibrinogen concentration
- Increased RBC flexibility

**Pathophysiological consequences**
- Increase in renal vasodilatory reserve
- Decreased efferent arteriolar resistance
- Decrease in platelet function
- Decreased activity of immune system
- Reduction in blood pressure
- Reduction in plasma, blood viscosity
- Reduction in vascular permeability
- Decrease in renal vasodilatory reserve

**Renal Effects**
- Retardation of progression of renal failure
- Decrease proteinuria
- Decreased activity of immune system
- Reduced proteinuria
- Special effects in selective glomerular diseases
- Amelioration of renal diseases with immune and inflammatory components
- Reduction of cyclosporine nephrotoxicity
- Reduction in vascular permeability
With respect to the kidney, renal production of \( \text{TxA}_2 \) is reduced by the treatment with n-3 FA. Thromboxane \( \text{A}_2 \) is elevated in lupus erythematosus [91], chronic glomerular disease [92], diabetic nephropathy [93], renal damage caused by cyclosporine [94] and renal transplant rejection [95] and has been linked to proteinuria in some patients [96]. In a recent study, more than 40% reduction in urinary \( \text{TxB}_2 \) excretion was detected after 6 weeks of treatment with 8 g/day of n-3 FA [97]. It has also been found that dietary n-3 FA may increase renal vasodilatory reserve, which is likely to be a compensatory mechanism during the progression of kidney disease [88-99]. It is noteworthy in this respect, that n-3 FA exert an effect on renal eicosanoids qualitatively and quantitatively different from nonsteroidal antinflammatory agents, which have been shown to drastically reduce the production of renal vasodilatory PG and to precipitate a reduction in renal function probably consequent to a reduction in renal plasma flow [91-99]. EPA and DHA enriched diets have also been shown to alter the production of lipoxygenase derivatives, decreasing the production of bioactive LT (leukotrine), which are mediators of inflammation, vasoconstriction and increased vascular permeability [100]. Decreased production of immunomediators may explain the favorable effects of n-3 FA supplementation in immune renal disease in animal as well as human studies. Fish oil also reduce endothelial cell production of platelet derived growth factor like proteins. This reduced production thereby alters proliferative responses in the kidney of some forms of glomerular disease [101].

**Hemodynamic effects of fish oil**

One of the most remarkable and consistent effects of n-3 FA supplementation in humans in their ability to decrease plasma concentration of triglycerides and very low density lipoproteins [102-105]. The effects on cholesterol are much more variable and detrimental. Renal failure is accompanied almost invariably by alterations of the lipid profile [106], which may contribute to accelerated atherosclerosis in kidney patients. The most frequent lipid abnormality in renal patients is indeed hypertriglyceridemia, particularly when glomerular
filtration rate is greatly decreased. Less consistent alterations include an increase of total cholesterol and a reduction in high density lipoprotein cholesterol. Lipid abnormalities may not only be a consequence of renal disease, but may contribute to its progression [107]. Furthermore, recent studies have suggested that the tissue cortical fatty acid profile of the kidney in various models of experimental renal injury, is abnormal and these changes which have been correlated with structural injury, may be modified by dietary fatty acids [107]. Thus, treatment with fish oils might be beneficial in ameliorating renal as well as systemic consequences of dyslipidemia; other “anti-atherogenic” effects may contribute to reducing the risk of coronary heart disease [107]. Several studies have reported a hypotensive effect of fish oils. When hypertension is a consequence of renal damage/disease, it may contribute to the rate of progression of the renal disease itself [108]. Reductions in blood pressure are generally felt to be beneficial in hypertensive patients with renal disease [109]. Fish oils prolong bleeding time and decrease platelet function [110]. These effects may be beneficial in patients with glomerulonephritis because platelet activation [111] and increased platelet consumption [112] have been demonstrated in such patients and because of earlier positive results of platelet inhibitor therapy in patients with membrane proliferative disease [113].

**Beneficial Effects of Fish Oil in Various Kidney Diseases**

In normal rats, fish oil supplementation caused a significant increase in single nephron glomerular filtration rate and single nephron plasma flow [114]. These hemodynamic actions, recently confirmed in humans [99], may have consequences in determining the effects of n-3 FA on the course of experimental and clinical renal disease. Recent observations have shown that n-3 FA of fish oils may act to modulate cell activation by interfering with a variety of intracellular signaling mechanism or acting themselves as intercellular second messengers in cell activation [115]. In general, n-3 FA have been found to reduce the increase in intracellular calcium [116] in
particular, the enrichment of cellular phospholipids with DHA was suggested to inhibit calcium transients [116]. n-3 FA may also modulate post receptor signaling pathways and the formation of second messengers [117]. Several studies in experimental animals have supported the idea that fish oils may be beneficial in renal disease.

Dietary supplementation of fish oil was found to prevent proteinuria and prolong survival in animal model for human systemic lupus erythematosus [115-118]. Fish oils with multiple beneficial effects, including a reduction of accelerated atherosclerosis, has emerged as an important cause of decrease in human suffering from systemic lupus erythematosus [120]. The finding of beneficial effects of n-3 FA has been extended to other mouse models of immune renal disease [119-124]. In animal models of progressive glomerular sclerosis, supplementation with fish oil particularly its EPA content proved beneficial [125]. There are a few clinical studies which also indicate that n-3 FA reduce proteinuria in patients with chronic glomerular nephritis, chronic pyelonephritis, and nephrosclerosis [96,120,126,127]. With regard to other animal models of renal disease [particularly of cyclosporine nephrotoxicity], fish oil administration was found to be effective in protecting against functional and structural consequences of cyclosporine use possibly associated with a reduction in renal thromboxane production [124,128,129].

Immunoglobulin A nephropathy is one of the most common chronic glomerular diseases, often resulting in a progressive course of proteinuria, hypertension and glomerulosclerosis [130]. Recently, favorable results have been reported with use of fish oil. Researchers have concluded that fish oil supplementation is effective in slowing the progression of IgA nephropathy [131]. Besides no adverse effects of fish oil supplementation in such a disorder was observed. Proteinuria was markedly decreased and glomerular filtration rate significantly improved following supplementation in IgA nephropathic patients [132]. Kidney transplant patients and the ones requiring hemodialysis after kidney failure were also benefited with use of fish oils [133]. The efficacy of fish oil
supplementation in patients with active ulcerative colitis [134] as well as on psoriasis is well established [135].

**G. OLIVE OIL**

In the Mediterranean regions, olive oil is one of the main sources of dietary fatty acids. Olive oil, is an oil rich in monounsaturated fatty acids (MUFA) and essentially contains oleic acid and a series of polyphenols [136] (Table 2). Olive oil is also related to cardiovascular health. It is beneficial in lowering LDL-cholesterol but not HDL-cholesterol, and decreasing the susceptibility of LDL to oxidation, which in turn reduces the atherogenicity of the LDL and the development of heart disease [137]. It has also been reported that oleic acid is not necessarily the only component responsible for this effect and that other antioxidant compounds contained in the nonglyceride fraction of olive oil such as sterols, polyphenols and tocopherols may contribute to these beneficial results [138]. There are reports which suggest olive oil has hypercholesterolaemic effect in serum and liver of rats [139]. However, in human hypocholesterolaemic effect of MUFA is well established [137,140,141] although the mechanism by which it is brought about remains unclear. In comparison to fish oil, olive oil is less hypocholeslerolaemic [142,143].

Less attention has been paid to the effect of oleic acid enriched diets. However, in vitro studies indicate that olive oil inhibits platelet function and thromboxane synthesis [144] and stimulates the uptake of free radicals by leukocytes [89,144]. Olive oil also enhances the glutathione system thereby decreasing the induced tissue oxidative stress [145]. The beneficial role of olive oil on liver tissue regeneration following hepatic resection in rats has also been established [146]. In fact, olive oil has been used as placebo in most of studies involving fish oil.

More and more research is being focused on investigating the effect of olive oil on various biological processes. The higher levels of monounsaturated fatty
acids and a good content of natural antioxidants are main advantages of olive oil. This may constitute an explanation of the ability of the mediterranean diet, of which olive oil is a major component, to prevent cardiovascular disease.

Recent insight into use of fish oil have led to conclude that fish oils have attractive properties for use in kidney disorders. Consumption of omega-3 fatty acids found in fish oils were found to protect against cardiovascular disease in people in developed countries. However, increased consumption of omega-6 fatty acids found in vegetable oils like olive oil also has beneficial effects. Thus, keeping in view the importance of these two oils they were chosen to investigate if they have any protective role against toxic effects of commonly used antibiotics.
Table 2 Composition of fish oil and olive oil [147].

<table>
<thead>
<tr>
<th>Components</th>
<th>Fish oil</th>
<th>Olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fatty acids (g/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0 (Myristic acid)</td>
<td>3.90</td>
<td>1.02</td>
</tr>
<tr>
<td>16:0 (Palmitic acid)</td>
<td>9.38</td>
<td>5.25</td>
</tr>
<tr>
<td>16:1 n-7 (Palmitoleic acid)</td>
<td>5.10</td>
<td>0.32</td>
</tr>
<tr>
<td>18:0 (Stearic acid)</td>
<td>2.10</td>
<td>1.72</td>
</tr>
<tr>
<td>18:1 n-9 (Oleic acid)</td>
<td>6.25</td>
<td>36.25</td>
</tr>
<tr>
<td>18:1n-7 (Vaccenic acid)</td>
<td>0.55</td>
<td>3.8</td>
</tr>
<tr>
<td>18:2 n-6 (Linoleic acid)</td>
<td>0.86</td>
<td>0.55</td>
</tr>
<tr>
<td>18:3 n-3 (Linolenic acid)</td>
<td>0.92</td>
<td>-</td>
</tr>
<tr>
<td>18:4 n-3 (Stearidonic acid)</td>
<td>2.45</td>
<td>-</td>
</tr>
<tr>
<td>20:1 n-9 (Gadoleic acid)</td>
<td>0.81</td>
<td>-</td>
</tr>
<tr>
<td>20:4 n-6 (Arachidonic acid)</td>
<td>0.70</td>
<td>-</td>
</tr>
<tr>
<td>20:5 n-3 (Eicosapentaenoic acid)</td>
<td>8.73</td>
<td>-</td>
</tr>
<tr>
<td>22:5 n-6 (Docosapentaenoic acid)</td>
<td>0.27</td>
<td>-</td>
</tr>
<tr>
<td>22:6 n-3 (Docosahexaenoic acid)</td>
<td>4.27</td>
<td>-</td>
</tr>
<tr>
<td>24:0 (Lignoceric acid)</td>
<td>0.34</td>
<td>-</td>
</tr>
<tr>
<td>2. Cholesterol (mg/kg)</td>
<td>1500</td>
<td>2.54</td>
</tr>
<tr>
<td>3. Squalene (mg/kg)</td>
<td>7400</td>
<td>300</td>
</tr>
<tr>
<td>4. Tocopherols (mg/kg)</td>
<td>300</td>
<td>47</td>
</tr>
<tr>
<td>5. Polyphenols (mg/kg)</td>
<td>-</td>
<td>740</td>
</tr>
</tbody>
</table>
Scope of the work

Aminoglycosides are potent, water soluble antibiotics. Because of their soluble nature, they do not cross biological membranes readily. So for systemic therapy, they are supplied by intravenous, intraperitoneal or intramuscular injection. For the same reason, once in body, they are largely confirmed to extracellular spaces, having correspondingly small volumes of distribution and mainly eliminated unchanged in urine.

Antibiotics have been one of the commonest causes of drug-induced nephrotoxicity [148]. The pathogenesis of such drug induced nephrotoxicity is directly related to the accumulation of drug within the renal cortex. Animal studies have revealed that antibiotics like gentamicin is absorbed by pinocytosis and stored in lysosomes of proximal tubular cells. Once inside the cell, they then induce conspicuous changes. Phospholipidosis is caused by a decrease in the activity of lysosomal phospholipase and sphingomyelinase. These drugs also alter sodium and potassium permeability of inner mitochondrial membrane and cause a deterioration of certain parameters of oxidative phosphorylation [149]. Prolonged treatment with antibiotics also cause release of brush border and lysosomal enzymes, decreased reabsorption processes and increase in blood urea and creatinine [150]. Despite well documented nephrotoxicity, antibiotics are still widely used for treatment of severe bacterial infections. Although a clear recognition of the treatment-related risk factors combined with the schedule of the drug and effective monitoring procedures, have improved to some extent the situation, we are still short of having brought the safety of main wide-spectrum antibiotics. Chemical research aimed at obtaining intrinsically less toxic compounds has met with only moderate success and few of the other approaches proposed to reduce the toxicities of the available agents have reached practical clinical applications. Yet, it seems important to maintain and develop agents to improve their therapeutic indices. The present work was designed and undertaken to present in a prospective way, a strategy that may eventually lead to their safer use. A
comparative study of fish oil and olive oil on the reversal of nephrotoxicity induced by commonly used antibiotics was done. Antibiotics selected for the study included gentamicin, tobramycin, streptomycin, kanamycin and amikacin. All these are nephrotoxic but vary in their dosage selected to induce maximum nephrotoxicity. Fish oil was selected for the study because of their efficacy and potential clinical utility in number of diseases. The study was further extended to compare the effects observed in fish oil to olive oil, which has some similar constituents on lesser proportion like that of fish oil, in these types of drug induced nephrotoxicity.