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The Protective Effect of Omega3 Against Amikacin-Induced Nephrotoxicity in Rats

Afrah Thiab Hlail [1] Hadeel Rashid Faraj [2] Wafa S. Abdulredha [3]

[1]Department of pharmacology and toxicology, Faculty of Pharmacy, University of Thi-Qar, Iraq.
[2]Department of chemistry, college of science, University of Thi-Qar, Iraq.
[3]Department of pharmacology and toxicology, Faculty of Pharmacy, University of Thi-Qar, Iraq.
Corresponding author: Afrah Thiab Hlail
Email: afrah.thiab@utq.edu.iq

ABSTRACT

Renal system is vital for total body homeostasis because the kidney plays a principle role in the excretion of metabolic waste products and in the regulation of extracellular fluid volume, electrolyte composition, and acid-base balance. Amikacin induced renal toxicity including a variety of pathophysiological effects i.e. inflammation, specific transporters inhibition ,induction of oxidative stress and vascular alterations.Omega-3 fatty acids are unsaturated fatty acids that have a roles in human physiology including antioxidant and anti-inflammatory effects,. This study was designed to examine the impact of co administration of omega 3 with therapeutic dose of amikacin for 14 days in rats on amikacin nephrotoxic effect. The animals utilized in this study were allocated into 3 groups (eight rats each) as negative control group ,amikacin only group and omega 3 in concomitant with amikacin group .serum creatinine, serum urea ,serum malondialdehyde(MDA) and serum glutathione(GSH) levels were determined. The results showed significant increase ($P<0.05$) in serum creatinine , urea and malondialdehyde levels and significant decrease ($P<0.05$) in serum GSH level in amikacin treated group compared to the corresponding level in negative control group. Moreover, significant decrease in serum creatinine, urea, MDA levels and significant increase in GSH level in group receiving omega3 with amikacin in comparison with amikacin treated group. this study demonstrated that co-administration of omega 3 with amikacin for 14 days significantly alleviate the injurious effects of the intended antibiotic on rats' kidneys.

Keywords: Amikacin, Oxidative stress, Malondialdehyde. Glutathione,Creatinine , Urea ,Renal system and Omega 3

Correspondence:
Afrah Thiab Hlail
[1]Department of pharmacology and toxicology, Faculty of Pharmacy, University of Thi-Qar, Iraq.
Email: afrah.thiab@utq.edu.iq

INTRODUCTION

Kidney is vital organ for body homeostasis because the kidney plays a significant role in the excretion of metabolic wastes and in extracellular fluid volume, electrolyte composition, and acid-base balance regulation. In addition, renin and erythropoietin are synthesized and released in the kidney ,it also plays vital role in metabolizing vitamin D3 to the active 1, 25-dihydroxy vitamin D3 form[1,2].Intrinsic reactivity of the chemicals with cellular macromolecules can initiate toxicity , may require bioactivation either renal or extrarenal to reactive intermediate, or may indirectly initiate injury by generation of reactive species and free radicals and ultimately inducing oxidative stress[3].Renal cortex has high cytochrome P450 and glutathione-S-transferase activities , while renal medulla has high prostaglandin synthetase activity , which can catalyze co-oxidation of xenobiotics[4].Metabolism of renal xenobiotics can result in the electrophiles or free radicals production that may covalently bind to macromolecules or causing lipid peroxidation.[5]

Nephrotoxicity can define as increase in baseline serum creatinine by a 50% or decrease in creatinine clearance by 50% also elevated in blood urea nitrogen. 20% of nephrotoxicity induced by drugs such as , aminoglycosides ,chemotherapeutic agents, angiotensin converting enzyme inhibitors ,non-steroidal anti-inflammatory drugs, , angiotensin receptor blockers, vancomycin, amphotericin B as well as chemicals (heavy metals like cadmium and cobalt) and radio contrast.[6]

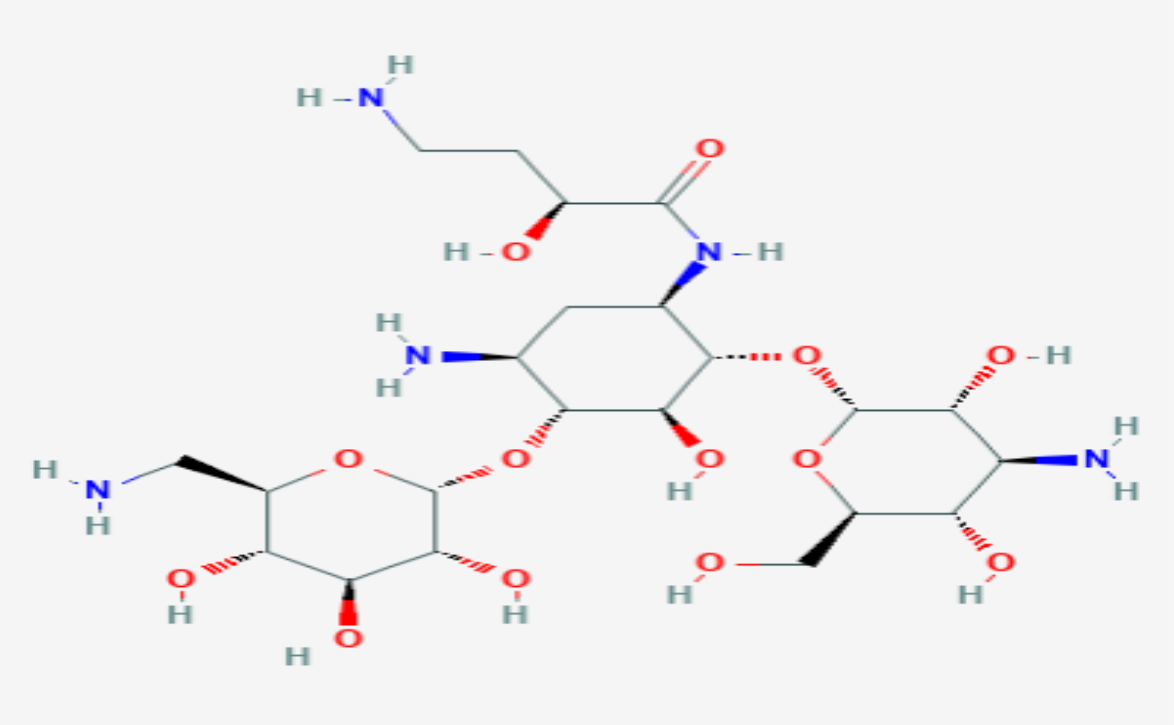
Aminoglycosides is antibactrial agents act by binding to the bacterial 30S ribosomal subunit , inhibiting the transfering of the peptidyl-tRNA from the A-site to the P-site and also causing misreading of mRNA, making the bacteria unable to synthesize proteins essential to its growth[7].They undergo glomerular filtration , and once concentrated in the urine in these segments occurs, they are known to bind to phospholipids, followed by internalization within the cell via megalin.Once inside the proximal tubular cell, they are concentrated within lysosomes and cause a stereotypical of the lysosomes disorganization termed myeloid bodies.The reduction in glomerular filtration rate (GFR) thought to be an indirect effect on the glomerulus.[8] With aminoglycosides causing myeloid bodies accumulation within lysosomes and there by inhibition of lysosomal phospholipases with a resultant decrease in sphingomyelinase activity, or an extralysosomal mechanism, with inhibition of Na, K - ATPase and phospholipase C.[9]

One of another causes of aminoglycoside- induced nephrotoxicity is related to the lipid peroxidation, when high dose of aminoglycoside antibiotics had been used or for a long time, causing renal tissue injury [10]

Key subcellular target of aminoglycoside are lysosomes, are believed to promote cellular injury through rupture and lysosomal enzymes release into the cytoplasm after excessive accumulation of reabsorbed toxicant and lysosomal overload.[11, 12].Wojckch Lesniak (2005) found that aminoglycoside exerts its adverse renal effect by inducing of oxidative stress, [13]also found that aminoglycosides form complex with mitochondrial Fe^{+2} to catalyze the free radicals formation [13,14]. previous study showed that hydrogen peroxide generated by aminoglycoside in vitro by renal cortical mitochondria is

derived from superoxide anion; both of them may generate the hydroxyl radical. [15,16]. Serum creatinine and blood urea nitrogen characteristically increase 7 to 10 days after aminoglycoside therapy initiation. In more than half of the cases with nephrotoxicity, the decline in renal function occurs only after the therapy has been completed. [17,18]

Amikacin is abroad –spectrum semi synthetic aminoglycoside antibiotics derived from kanamycin A. It was discovered firstly in 1971 although amikacin has high antibacterial efficacy, rapid onset of action, low cost, has synergism with beta-lactam antibiotics and low resistance but its clinical use is limited because of nephrotoxicity and ototoxicity. [19, 20 and 21]



Chemical structure of Amikacin [22]

Amikacin AK induced renal toxicity involve a variety of pathophysiological effects i.e. inflammation, specific transporters inhibition, induction of oxidative stress and vascular alterations. The pathogenesis of nephrotoxicity is predicted due to the ability to interact with membrane anionic phospholipids electrostatically and to interrupt structure and function of membrane[12].Administration of amikacin may also produce reactive oxygen and nitrogen species and induce apoptosis. Reactive species play a critical role in drug induced renal dysfunction and toxicity to other organs[23].In addition, it has been proposed that aminoglycosides (e.g., Amikacin) form a complex with a mitochondrial Fe2+ to promote the formation of free radicals[24].Other studies reported that AK accumulated most blatantly in renal cortex then generate free radicals and the over production of free radicals lead to destroying of unsaturated fatty acids in cell membrane which ,in turn lead to an increase in lipid peroxidation and rising of MDA level and decrease in endogenous glutathione (GSH) level in renal tissue[25].

Mechanism of amikacin induced renal impairment is attributed to oxidative stress and inflammation[19, 20 and 21].Oxidative stress caused by excessive production of reactive oxygen species and it is produce major interrelated disturbance of cellular metabolism, including protein and nucleic acid structure alteration, DNA damage, induction of apoptosis, elevation in intracellular free calcium, damage to membrane ion transport and damaging of the cells by lipid peroxidation[26,27].The antioxidants play a critical role in the defense against the deleterious effects of oxidant agents produced in the biological system .The protective antioxidant mechanisms involve both enzymatic substances (e.g. catalase, glutathione peroxidase and superoxide dismutase) and non-enzymatic substances (such as tocopherols, phenolic compounds, flavonoids, catechins, ascorbic acid , omega3 and carotenoids).[28]

Omega-3 fatty acids are a group of fatty acids that involved two or more double bonds ("polyunsaturated"), one of this double bonds is located three carbon positions from the methyl terminus ("omega-3" or "n-3"). omega-3 fatty acids that are longer than 14 carbon cannot be synthesized in sufficient amounts in the body ,so the body obtained its need from dietary sources (i.e., they are "essential" fatty acids).The parent omega-3 fatty acid, -linolenic acid (ALA), is derived from vegetable seed oils (Figure 1)[29]. ALA is converted to the longer chain omega-3 fatty acid eicosapentanoic acid in the endoplasmic reticulum via desaturase and elongase enzymes (EPA; Figure 1).

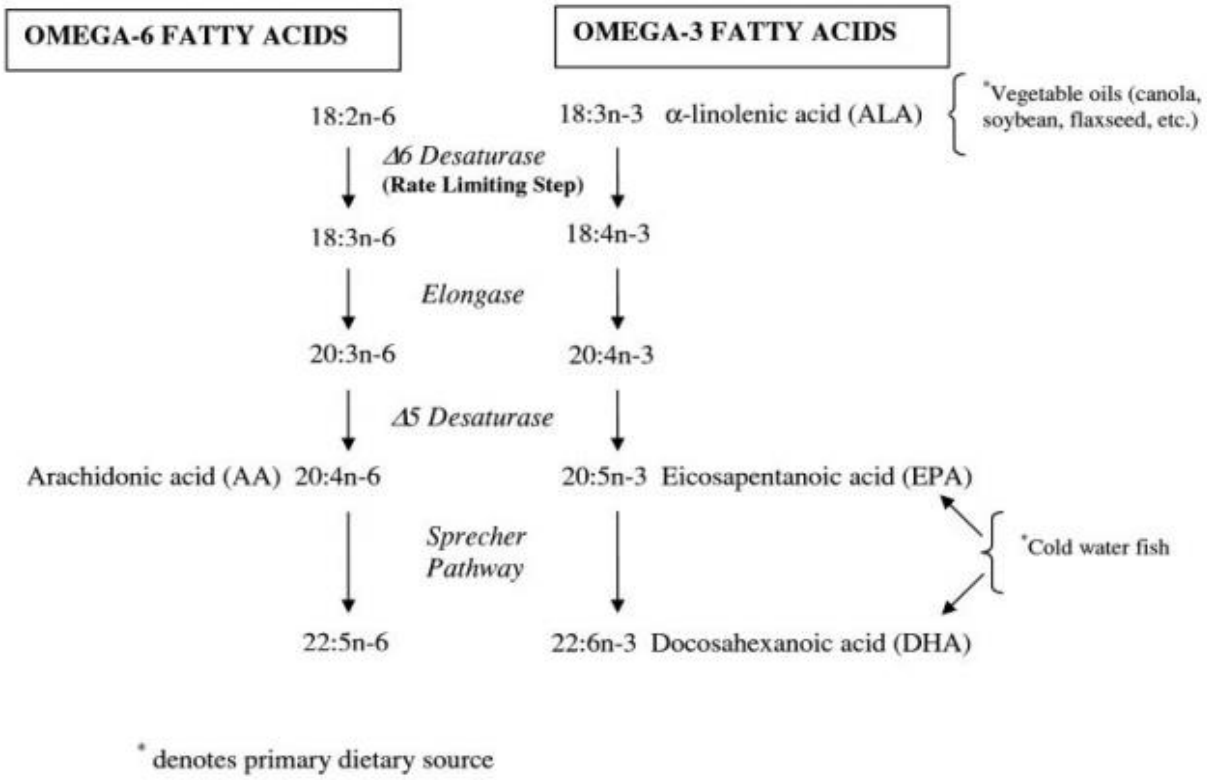


Figure 1. Elongation of omega-3 and omega-6 fatty acids [29]

Cyclooxygenase, lipoxygenase, or cytochrome P450 AA monooxygenase convert omega-6 fatty acid arachidonic acid (AA) to a host of bioactive eicosanoid products intracellulary, they involved prostaglandins, leukotrienes, thromboxanes, lipoxins, epoxyeicosatrienoates, and hydroxyeicosatetraenoates. Some of these AA-derived eicosanoids induce prothrombotic, proinflammatory, or proarteriosclerotic effects, including platelet aggregation , vasoconstriction, chemotaxis, increased vascular permeability, and cytokine releas.(Figure 2)[30,31].

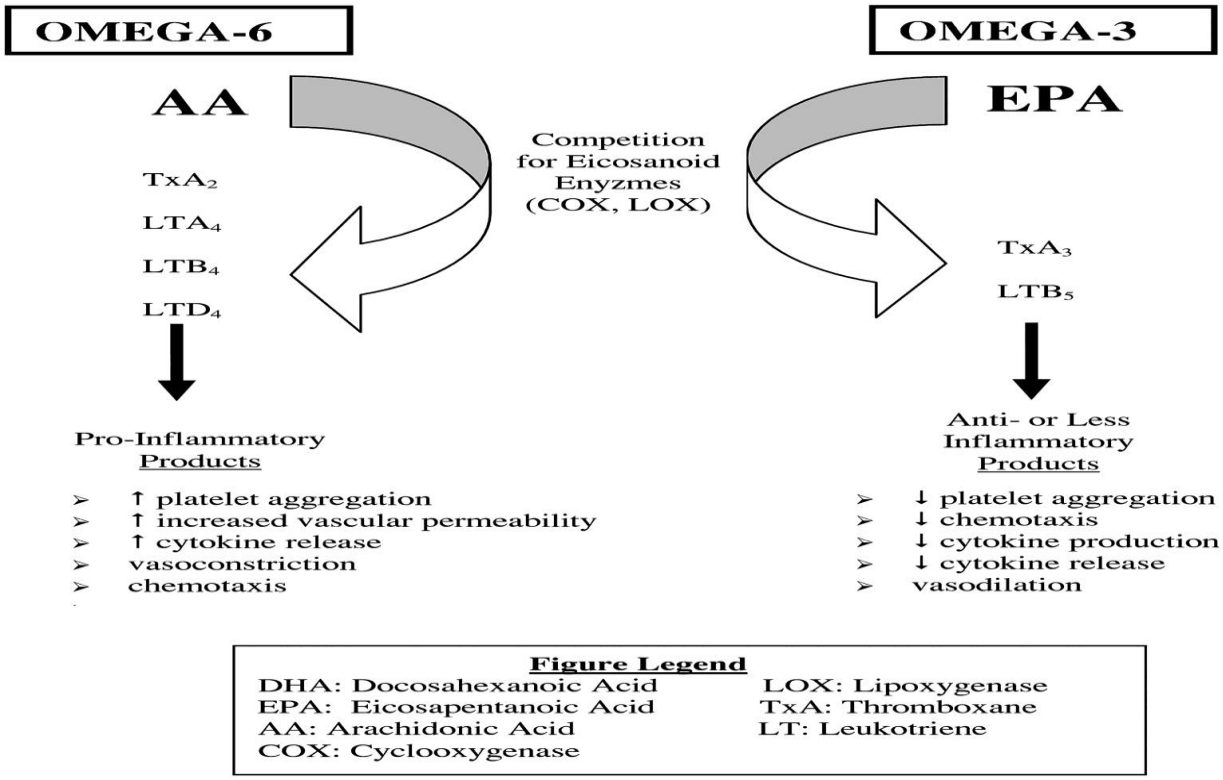


Figure 2. Synthesis of pro- and anti-inflammatory eicosanoids. [31]

By competing with AA both for incorporation into the lipid pool (in particular, cell membrane phospholipids) and therefore reducing the supply of AA, in addition to the enzymes involved in synthesis of eicosanoid , EPA shunts production toward eicosanoids with anti-inflammatory and antiarteriosclerotic effects (Figure 2)[31].These involve down regulation of pro inflammatory cytokines[33–35], and cell surface molecules involved in cell adhesion and activation[30,32]. The mechanism may include molecules such as resolvin E1, a newly discovered lipid byproduct of EPA that suppresses activation of NF-κB[36].Omega-3 fatty acids have clinical benefits for such many inflammation-mediated diseases as rheumatoid arthritis, inflammatory bowel disease ,and certain diseases of skin and nephropathies.[37-40]

Animals and Method

24 Male albino strain rats with an average weight of (150-200g) in each group there is 8 rats. Obtained from collage of sciences Thi-Qar University and maintained at my house under conditions of controlled temperature. The animals were fed commercial pellets.

Experimental protocol

Group1: Eight rats were injected intraperitoneally normal saline for fourteen days. This group served as negative control.

Group2: Eight rats were injected intrapritoneally 120mg/kg/day of amikacin [19] for 14 consecutive days. This group served as positive control of nephrotoxicity induced by amikacin.

Group 3: Eight rats received oral omega3 (100 mg /day) given once daily [41] concomitantly with intraperitonial dose of amikacin (120 g/kg/day) for 14 days. This group utilized to find the protective effect of omega 3 against amikacin induced nephrotoxicity.

All animals were euthanized by anesthetic ether on day 15 and sacrificed.

Material and method

Preparation of serum samples

After euthanization of rats by anesthetic ether, blood was collected from the neck and put in plain tube; the clot was dispersed with glass rod and then centrifuged at 3000 (rpm) for 15 minutes.Serum was stored at -20ºC until used for the determination of creatinine, urea, glutathione and malondialdehyde.

Biochemical analysis

Determination of serum creatinine

Serum creatinine concentrations were determined according to Jaff reaction[42] using ready-made kit for this purpose.The principle depends on the reaction of creatinine with picric acid under alkaline conditions to form a red-colored product that can be measured at 500nm. Using UNICO 1100 spectrophotometer. The red color intensity is directly proportional to creatinine concentration, which was expressed in mmol/L.

Determination of serum urea

Serum urea levels were determined using urease-modified Barthelot reaction by a ready-made kit for this purpose.[43]In alkaline medium, the ammonium ion reacts with the salicylate and hydrochlorites to form a green-colored indophenol (2,2 dicarboxyl indophenols), which can be measured spectrophotometrically at 580 nm using UNICO 1100 spectrophotometer. Levels of blood urea nitrogen were expressed in mmol/L.

Determination of serum glutathione (GSH) concentration

Total thiol groups contents, which can be used as indicator for reduced glutathione (GSH), was determined [44] by enzyme linked immunoassay method by ELISA Reader supplied by, BDH chemical, Ltd., Poole, England.

Determination of serum malondialdehyde concentration

Malondialdehyde (MDA), the end product of lipid peroxidation, was measured as thiobarbituric acid (TBA) activity by using the colorimetric method recommended, MDA level of the plasma was measured according to a modified method of Fong et al. [45,46]

Statistical Analysis

Statistical analysis was performing using unpaired Student´s t-test. P-values
less than 0.05 were considered significant for all data showed in the results of this study

Results

Groups of rats intraperitoneally administered 120mg /kg/ day of amikacin for 14 days showed significant increase in serum creatinine ,blood urea, MDA and significant decrease in GSH activity ((P<0.05)) compared to negative control group as shown in table 1.

Table1. Effect of AK on serum creatinine,urea , MDA, and GSH in comparison with negative control group.

Group/ n.=8	Serum creatinine (mmol/L)	Blood urea (mmol/L)	Serum MDA (µmol/L)	Serum GSH (µmol/L)
Negative Control	44.2 ±5.02	8.4 ±0.2	4.55 ±0.09	3.68 ±0.83
Intrapritoneal amikacin120mg/kg/day for 14 consecutive days	57.4 * ±9.2	9.8 * ±0.6	7.16 * ±1.008	1.8 * ±0.51

- Each value represents Mean ± standard deviation (SD).
- P * < 0.05 significant difference in comparison with the negative control group
-n: number of animals.

Coadminstration of intraperitoneal amikacin with oral omega3 by gavage tube resulting in significant decrease((P<0.05)) in (S.cr,.urea,MDA) in comparision with group treated with amikacin only and significant increase((P<0.05)) in GSH compared with amikacin only treated group as shown in table 2

Table 2. Effect of coadminstration of omega3 and AK on serum (creatinine, urea, MDA and GSH) in comparison with amikacin treated group

Group/ n.=8	Serum creatinine(mmol/L)	Blood urea(mmol/L)	Serum MDA (µmol/L)	Serum GSH (µmol/L)
Intrapritoneal amikacin120mg/kg/day of for 14 consecutive days	57.4 ±9.2	9.8 ±0.6	7.16 ±1.008	1.8 ±0.51
Coadminstrationof orally omega 3(100 mg/day and intraperotonial amikacin120mg/kg/day	49.2*± 6.4	7.9*±0.7	4.3*±0.008	4.63*±0.978

-Legend as in table 1

In this study I noticed that there is significant decrease ((P<0.05)) in (Scr, B.urea and MDA) in group administered omega 3 (100 mg /day) with 120mg/ kg/ day of AK compared with negative control group that administered just intraperitonial normal saline , also there is significant increase((P<0.05)) in GSH in group administered omega 3 (100 mg /day) with AK 120mg/ kg/ day compared with negative control group that administered just intraperitonial normal saline as shown in table 3.

Table 3. Effect of coadminstration of omega3 and AK on serum (creatinine, urea, MDA and GSH) in comparison with negative control group.

Group/ n.=8	Serum creatinine(mmol/L)	Blood urea(mmol/L)	SerumMDA(µmol/L)	SerumGSH(µmol/L)
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-Legend as in table 1.

Discussion and Conclusion

Nephrotoxicity is a principle complication of treatment with aminoglycoside antibiotics, that used for treatment of severe gram-negative bacterial infections [21]. Amikacin possess serious nephrotoxic adverse effects which may result in acute renal injury; therefore, a study of their renal toxicity is important. Aminoglycosides reabsorbed and accumulated in the renal proximal tubular cells that results in renal injury. furthermore, tubuloglomerular degeneration resulting from generation of reactive species that creating state of oxidative stress [47]. According to other studies, aminoglycosides generate reactive oxygen and nitrogen species that lead to decrease glomerular filtration rate and causing renal tubular necrosis [48, 49 ,50 and 51]. Baylis and Reneke, 1977 showed that aminoglycosides have ability to damage the function of glomerulus and then, reducing the output of the afferent glomerular arteriolar causing decreasing of glomerular filtration rate .Other literatures also showed significant elevation of serum creatinine and urea levels and decreased levels of creatinine clearance in amikacin treated group as a result of toxic effect of amikacin on renal system .[50,52]

In this study it was found that elevation of the serum urea and creatinine in amikacin treated group compared with negative control group that give us indication of a decrease in the glomerular filteration rate. The waste products of protein metabolism are urea and creatinine that need to be excreted by the renal system , therefore significant elevation in blood urea nitrogen and serum creatinine which found in this study reflect the state of physiological damage to the kidney caused by amikacin , also these parameters considered as biochemical markers of nephrotoxicity which may be caused by oxidative tissue damage and consequently inflammation of renal tissue.[53] Furthermore this study showed a significant elevation in malondialdehyde (MDA) which is the end product of lipid peroxidation and decrease glutathione levels in the serum of group administered amikacin compared with negative control group , this agree with most study that showed amikacin was concentrated in the cortex and produce large amount of reactive oxygen species that destroying the phospholipids membrane that causing increasing MDA level and exhausting of endogenous GSH ,also they found that after exposure to reactive oxygen species ,free radicals and oxidant substances , there is formation of MDA which is the well-known by products of lipid peroxidation and it may be used to evaluate oxidative stress by measurement of serum levels of thiobarbituric acid reactive substance[25,54,55,56and57] .As mentioned above, amikacin lead to lipid peroxidation by significantly elevated tissue MDA level, the lipid peroxidation induced by free oxygen radicals was involved in destroying and damaging the cell membrane which contribute for development of amikacin induced severe kidney damage

and these results are in agreement with studies done by Atef M. Al-Attar et al and K Kaynar et al [25,52]

Oxidative stress requires either increased reactive oxygen species formation or decreased antioxidant defence mechanism. [58,59]. A one of enzymatic metabolic antioxidant that protect cells from free radical toxins is glutathione (GSH) which is found usually in its reduced form,by action of glutathione reductase enzyme which revers it from its oxidized form (GSSG is constitutively active and inducible when there is oxidative stress)[57] ,for this reason GSH is an essential endogenous antioxidant and its level in the tissue is considered a critical determinant of the threshold for tissue injury and an explanation for decreased GSH after amikacin treatment to the increased consumption of GSH in non enzymatic and enzymatic detoxification of oxygen radicals with efflux of GSSG being the major factor in charge of maintenance of redoxe ratio[60,61] Concerning the results of this study which showed amikacin toxic effects is in harmony with similar other study in which a significant consumption of GSH in renal cells resulting to their damage due to elevationt of lipid peroxidation[62].They were found that amikacin promote renal tissue damage via excessive oxidative stress and inflammation.[63,64]. This brust of oxidative stress lead to reduction in GSH level in amikacin treated group as compared with the negative control group, amikacin induced nephrotoxicity is multifactorial depend on free radicals' formation and inflammatory responses. [63,65]

As amikacin-causig nephrotoxicity may be due to the oxidative stress; therefore, antioxidant agents could be able to attenuate amikacin –induced nephrotoxicity. according to this concept, this study is carried out to evaluate the protective effect of omega 3 because of its proven antioxidant activity.

This study show significant reduction in s.creatinene , urea, MDA,levels and significant increase in GSH level in group receiving omega3 concomitantly with amikacin in compartion with rats group administered amikacin 120 mg \kg \ day .

Omega 3 may prevents nephrotoxicity directly by scavenging ROS and RNS including hydroxyl radical (•OH), singlet oxygen (1O2), hydrogen peroxide (H2O2), superoxide radical (Ō• 2), nitric oxide (NO) and perioxynitrate anion (ONOŌ) and reduce their generation by inhibiting the activity of inducible nitric oxide synthase (iNOS) this agree with other study which state that

omega3 have anti-inflammatory and resolution-directed activities represent an indirect mechanism by which n-3 Poly Unsaturated Fatty Acids lowering endogenous ROS and changing cellular redox status [66].

Antioxidant effect of omega 3 may showed either directly by scavenging free radicals or indirectly by increasing the activity of antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GRd) and the expression of endogenous antioxidant defense (glutathione). These antioxidant enzymes have a critical role in detoxifying of free radicals to less toxic species i.e., when SOD dismutases Ō• 2 to H2O2 which in turn, decrease free radical mediated cellular damage ; CAT converts H2O2 to oxygen and water , GPX oxidizes GSH to glutathione disulfide GSSG then GRd reduce GSSG to GSH therefore, the antioxidant capability of omega3 involve reducing the ROS production which is in turn leading to improvement in renal function and GFR and correct the serum creatinine and urea levels and this agreement with other study .

Omega-3 fatty acids (Omega-3 FAs) are considered as strong antioxidants and their role as anticancer agent has been extensively confirmed in most of the human malignancies [68, 69]. Furthermore, the anti-inflammatory potential of Omega-3 FAs in many chronic diseases has been suggested [69]. In addition, another finding indicates that Omega-3 FAs act synergistically with certain chemotherapeutic agents[18]. Omega-3 FAs were found to play protective roles in the liver, cardiovascular system, and kidney and they have been widely used in clinical peroperative total parenteral nutrition [70]. Therefore, this study prove that Omega3 have nephroprotective effects by its antioxidant capacity against amikacin nephrotoxic effect.

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