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The impact of omega-3 and saccharomyces cerevisiae on Amikacin-induced nephrotoxicity in rats

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ABSTRACT

Aminoglycosides as Amikacin (AMK) are widely used antibiotics however they are reported to cause considerable nephrotoxicity mediated via increased oxidative stress. The present study aimed to assess the impact of omega-3 and Saccharomyces cerevisiae (Sc) on AMK-induced nephrotoxicity in rats. Sixty Sprague Dawley rats of both sexes were assigned to ten equal groups.Group1received saline (normal control), groups 2-4 received Sc (10^9 CFU ml^{-1}; p.o.), omega-3 (200 or 400 mg/kg; p.o.), respectively, group 5 received AMK (35mg/kg/day; i.p.), groups 6-8received AMK with Sc, omega-3 (200or 400 mg/kg), respectively and groups 9-10 received AMK and Sc combined withomega-3 (200or 400 mg/kg), respectively for 4 weeks. At the end of experiments, blood samples were collected and kidneys were isolated and used for biochemical and histological studies. AMK-induced nephrotoxicity was shown by elevations in serum urea, creatinine and blood urea nitrogen parallel to decrease in serum total protein. AMK induced oxidative stress manifested by increases in kidney malondialdehyde and nitric oxide contents parallel to decreases in reduced glutathione content and superoxide dismutase activity. Besides, AMK increased kidney hydroxyproline and tumor necrosis factor-alpha contents as well as caspase-3 immunostaining. Sc, omega-3 and their combinations attenuated AMK-induced changes in kidney function tests, oxidative stress, inflammatory, apoptotic and fibrotic biomarkers. The tested agents even improved markers of kidney damage in normal animals. Histological examinations of kidney tissues confirmed the biochemical findings. Sc and omega -3 could be of therapeutic value against nephrotoxicity induced by AMK.

Key words: Amikacin, saccharomyces cerevisiae, omega-3, nephrotoxicity, oxidative stress, tumor necrosis factor-alpha.

INTRODUCTION

Drug-induced nephrotoxicity is an extremely common condition and is responsible for a variety of pathological effects on the kidneys. Drug-induced acute renal failure (ARF) accounted for 20% of all ARF cases [1].

Aminoglycosides as amikacin (AMK) and tobramycin are most important antibiotic drugs in clinical use and are essential for the treatment of severe infections caused by gram negative bacteria. These antibiotics are reported to cause nephrotoxicity, hepatotoxicity, ototoxicity and neuromuscular blockade [2]. AMK is characterized by its broader antimicrobial spectrum and its resistance to destruction by enzymes inactivating other aminoglycosides. Thus AMK is of a value in treatment of severe hospital acquired infections with multidrug resistant gram negative bacteria such as pseudomonas aeruginosa, Acinetobacter, and Enterobacter [3]. Long-term use of AMK is, however, prohibited due to its nephrotoxicity [4].

Renal cell injury may culminate in the cell death, which may occur through necrosis, apoptosis or other pathways. Chemicals in general can initiate toxicity because of their intrinsic reactivity with cellular macromolecules; they may also initiate injury indirectly by inducing oxidative stress [5].

Increased production of oxygen free radicals is involved in the induction of nephrotoxicity which is the main limiting factor in aminoglycosides clinical use [6&7]. Additionally it has been demonstrated that aminoglycosides form a complex with mitochondrial ion to catalyze the formation of free radicals [8].

The goal of reducing or protecting against aminoglycoside induced nephrotoxicity has attracted much effort over the last decade. As oxidative stress is the main underlying cause of AMK-induced nephrotoxicity, using antioxidants especially from natural origin appears motivating.

Scientific evidence reveals that a diet rich in long chain omega-3 fatty acids helps in the development of healthy brain, heart, and immune system. It has a role in joint movement, balanced mood, a sense of well-being, strength, stamina, and helps to maintain cholesterol levels within the normal range [9]. Omega -3 fatty acids contains about 60% of long-chain omega-3 fatty acids docosahexanoic acid (DHA) and eicosapentanoic acid (EPA). The most widely available source of EPA and DHA is cold water oily fish such as salmon, herring, mackerel, anchovies and sardines [10]. EPA, DHA, and α-linoleic acid (ALA) are omega-3 essential fatty acids (EFA) that are important for structural and biochemical integrity of all cells [11].

Sacchamomyces cerevisiae (Sc; yeast) is traditionally used a source of vitamin B, selenium and chromium. Clinical trials have evaluated role of yeast for in immuno-modulation, respiratory and post-surgical infections and as a source of dietary fibers to improve the lipid profile [12]. Besides, antioxidant activity of Sc was reported [13].

The present study was conducted to investigate the possible protective potentials of Sc and omega-3 in nephrotoxicity induced in rats by long-term administration of Sc.

MATERIALS AND METHODS

2.1. Animals:

Sprague Dawley rats of both sexes weighing 180–200 g were used throughout the experiments. Animals were purchased from animal house of Cairo university (Egypt), housed under standard environmental conditions (23 ± 1 ^{o}C, 55 ± 5% humidity and a 12-h light: 12-h dark cycle) and maintained with free access to water and a standard laboratory diet *ad libitum*. Experimental procedures were approved by the National Research Centre Animal Care and Use Committee and the Ethics Committee of Faculty of Pharmacy, Cairo University.

2.2. Drugs and chemicals:

Amikacin (Amikin®) and omega-3 oil were purchased from Bristol-Myers Squibb Laboratories Co. (Egypt) and PharmAssure, Incphoenx, AZ (USA), respectively. Saccharomyces cerevisiae was freeze-dried powder (baker's yeast strain) obtained from (Egypt). It was freshly prepared prior to administration.

Other chemicals, reagents, and reagent kits, used in the present study, were of the highest analytical grade available.

2.3. Experimental design:

Rats were randomly allocated into 10 groups (n=6) and treated as follows: Group 1was given saline p.o. (normal control). Group 2 was given Sc (10^{9} CFU ml^{-1}; p.o.) [14]. and groups 3 and 4 were given omega 3 (200 and 400 mg/kg; p.o.), respectively [15]. Group 5 was given AMK (35mg/kg; i.p.) [16]. Groups 6-8were given AMK concurrently with Sc, omega 3 (200 or 400 mg/kg), respectively and groups 9 and 10 were given AMK and Sc together with omega 3 (200 or 400 mg/kg), respectively. All treatments continued daily for four weeks.

2.4 Methods:

2.4.1 Preparation of blood and tissue samples:

At the end of experimental period, blood samples were collected from retro-orbital venus plexus of all animals under light ether anesthesia and were used for serum separation.

Thereafter, animals were sacrificed by decapitation, and then kidneys were removed. One kidney was homogenized in phosphate buffer and the prepared homogenates (10%) were used for determination of the chosen markers in kidney. The other kidney was used for histopathologic examinations.

2.4.2. Determination of the chosen biochemical markers:
Serum samples were used for biochemical analysis of creatinine according to the method of *Bartles et al.(1972)* [17], uric acid according to the method of *Barham and Trinder (1972)*[18], blood urea nitrogen (BUN) according to the method of *Fawcett and Soctt, (1960)* [19], total protein according to the method of Gornal *et al*.(1949) [20], and tumor necrosis factor-alpha (TNF-α) according to the method of *Bonavida, (1991)* [21].

Kidney homogenates were used for determination of nitric oxide (NO) content according to the method of *Montgomery and Dymock (1961)* [22], malondialdehyde (MDA) content according to the method of *Begona Ruiz-Larrea et al.(1994*) [23], reduced glutathione (GSH) content according to the method of *Beutler et al. (1963)* [24], superoxide dismutase (SOD) activity according to the method of *Marklund and Marklund (1974)* [25], and hydroxyproline (HYP) content according to the method of *Ronis et al.(2010)* [26].

2.4.3. Histopathological studies:
Kidney specimens from all animals were dissected immediately after death, and fixed in 10% neutral-buffered formal saline for at least 72 hours. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6 µm thick were cut and stained with hematoxylin and eosin [27].

2.4.4. Immunohistochemical determination of caspase-3:
Staining for apoptosis was performed with signal-cleaved caspase-3 immunohistochemical detection kit (Cell signaling technology, USA) that utilized avidin-biotin immunoperoxidase method to detect intracellular caspase-3 protein. Staining was performed on 5 µm paraffin sections from the left kidney by a standard technique using rat anticleaved caspase-3 (clone Asp 175, 1:50) [28]. Known positive control sections for apoptosis were used. For negative control, primary antibody was replaced with normal rat serum. Images were captured and processed using Adobe Photoshop version8.0.

2.5. Statistical analysis:
All results were expressed as mean ± standard error (SE).Data were analyzed using one-way analysis of variance followed by least significant difference test. Difference among groups was considered significant when p value is < 0.05.

RESULTS

3.1. Effect on kidney functions
Administration of Sc and two doses of omega-3(200 mg/kg and 400 mg/kg) for four weeks in normal rats showed a significant decrease in serum creatinine level by 49.31%, 18.75% and 46.528%, respectively. Significant increase in uric acid by 20.22% was shown in Sc group. BUN levels was not changed in Sc group and were decreased in omega-3(200 mg/kg and 400 mg/kg) groups by 18.65% and 38.59%, respectively and serum total protein level was significantly increased by Sc and omega-3(200 mg/kg and 400 mg/kg) by 37.798%, 15.68% and 22.949% respectively as compared to normal control group (Table 1).

AMK injection for four weeks resulted in a significant increase in serum creatinine, uric acid and BUN levels by 91.66 %, 69.32 % and 117.67%, respectively and a significant decrease in serum total protein level by 45.06 % as compared to normal control group (Table 1). Meanwhile administration of Sc, the two doses of omega-3 and the combination of Sc with either doses of omega-3 with AMK showed a significant decrease in serum creatinine by 35.50%, 27.17% and 28.98%, 31.52% and 42.75%, respectively. Uric acid levels showed no changes in groups receiving Sc or omega (200 mg/kg) but were significantly decreased in omega (400 mg/kg) group and the groups receiving Sc with any of the doses of omega-3by 14.76% 18.65% and 38.59%, respectively. In the same context, BUN levels showed significant decreases in groups treated with Sc, both doses of omega-3 and the combination of Sc with any of the doses of omega-3by 18.40%, 48.98% 34.30% 53.51% and 53.65%, respectively. Regarding serum total protein, treatment with Sc, both doses of omega-3 and their combinations caused a significant increase in its level by 52.93%, 25.70% 26.08% 70.51% and 83.74%, respectively as compared to normal control group (Table 1).

3.2. Effect on serum TNF-α content
Administration of Sc and omega-3(400 mg/kg) for four weeks in normal rats showed a significant decrease in TNF–α level by 26.69%and 28.21%, respectively as compared to normal control group (Figure 1).

AMK injection for four weeks showed a significant increase in serum TNF – α level by 334.11 % as compared to normal control group (Figure 6).While Administration of Sc, two doses of omega-3 and their combinations for four

weeks with AMK injection showed a significant decrease in serum TNF – α level by 52.42%, 41.13% 48.99%, 58.65% and 59.93%, respectively as compared to AMK control group (Figure 1).

Table 1: Effects of saccharomyces cerevisiae and/or omega-3 on serum creatinine, uric acid, BUN, total protein in normal and amikacin (AMK) - treated rats.

Parameters / Groups	Creatinine (mg/ dL)	Uric acid (µmol/L)	Blood urea nitrogen (g/dl)	Total protein (g/dL)
Control (saline)	1.44 ± 0.054^{A}	308.9 ± 28.4^{A}	6.79 ± 0.25^{AC}	9.63 ± 0.56^{A}
Saccharomyces cerevisiae(10^9 CFU/ml)	0.73 ± 0.044^{C}	246.48 ± 8.39^{C}	5.99 ± 0.27^{C}	13.27 ± 0.43^{C}
Omega- 3 (200 mg/kg)	1.17 ± 0.120^{D}	270 ± 10.73^{AC}	4.23 ± 0.12^{D}	11.14 ± 0.23^{D}
Omega- 3 (400 mg/kg)	0.77 ± 0.056^{C}	259.82 ± 11.80^{AC}	4.77 ± 0.24^{D}	11.84 ± 0.36^{D}
Control	2.76 ± 0.078^{B}	523.15 ± 18.55^{B}	14.78 ± 0.42^{B}	5.29 ± 0.57^{B}
Saccharomyces cerevisiae (10^9 CFU/ml)	1.78 ± 0.065^{E}	474 ± 30.82^{BD}	12.06 ± 0.4^{E}	8.09 ± 0.58^{E}
Omega- 3 (200 mg/kg)	2.01 ± 0.073^{F}	464.52 ± 11.30^{BD}	7.54 ± 0.31^{A}	6.65 ± 0.35^{F}
Omega- 3 (400 mg/kg)	1.96 ± 0.049^{EF}	445.91 ± 32.68^{D}	9.71 ± 0.24^{F}	6.67 ± 0.32^{F}
Saccharomyces cerevisiae+ omega- 3 (200 mg/kg)	1.89 ± 0.089^{EF}	425.54 ± 2.51^{D}	6.87 ± 0.17^{A}	9.02 ± 0.46^{AE}
Saccharomyces cerevisiae+ omega- 3 (400 mg/kg)	1.58 ± 0.093^{AE}	321.26 ± 21.44^{AC}	6.85 ± 0.24^{A}	9.72 ± 0.35^{A}
AM (35 mg/kg)				

Data were expressed as mean ± S.E (n= 6).
Groups with different superscripts are significantly different at p< 0.05.

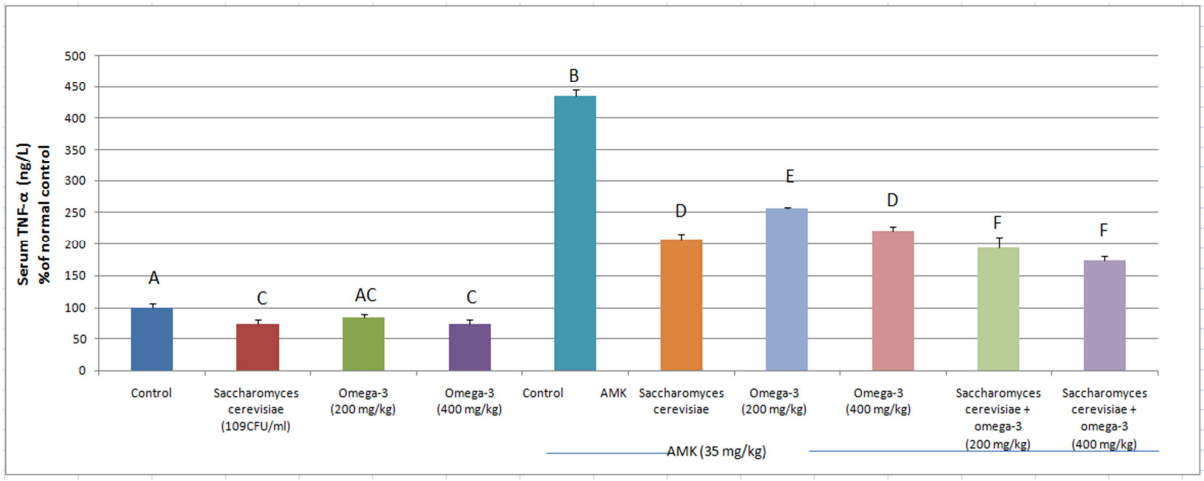

Figure (1): Effects of saccharomyces cerevisiae and/or omega-3 on serum tumor necrosis factor (TNF-α) level in normal and AMK - treated rats

Data were expressed as mean ± S.E (n= 6).
Groups with different superscripts are significantly different at p< 0.05.

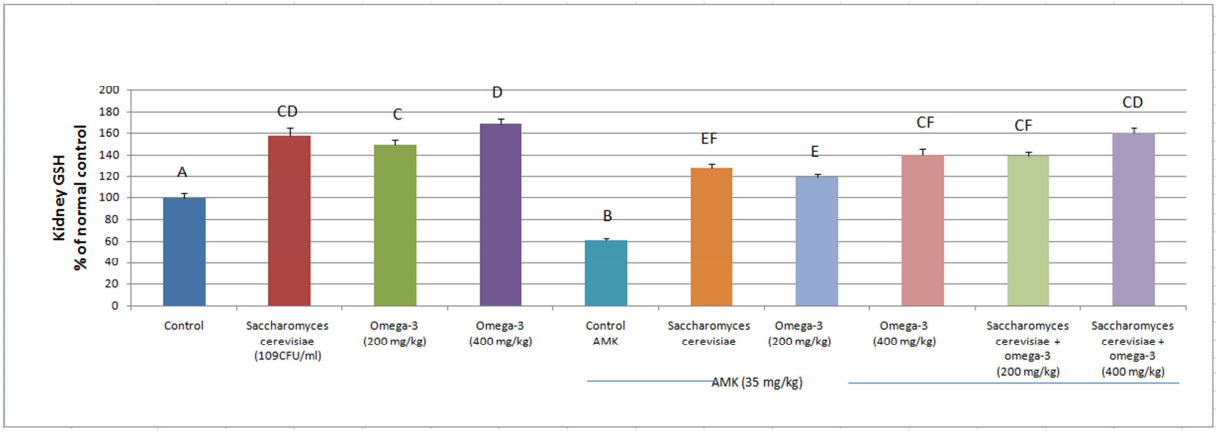

Figure (2): Effects of saccharomyces cerevisiae and/or omega-3 on kidney reduced glutathione (GSH) content in normal and AMK - treated rats

Data were expressed as mean ± S.E (n= 6).
Groups with different superscripts are significantly different at p< 0.05.

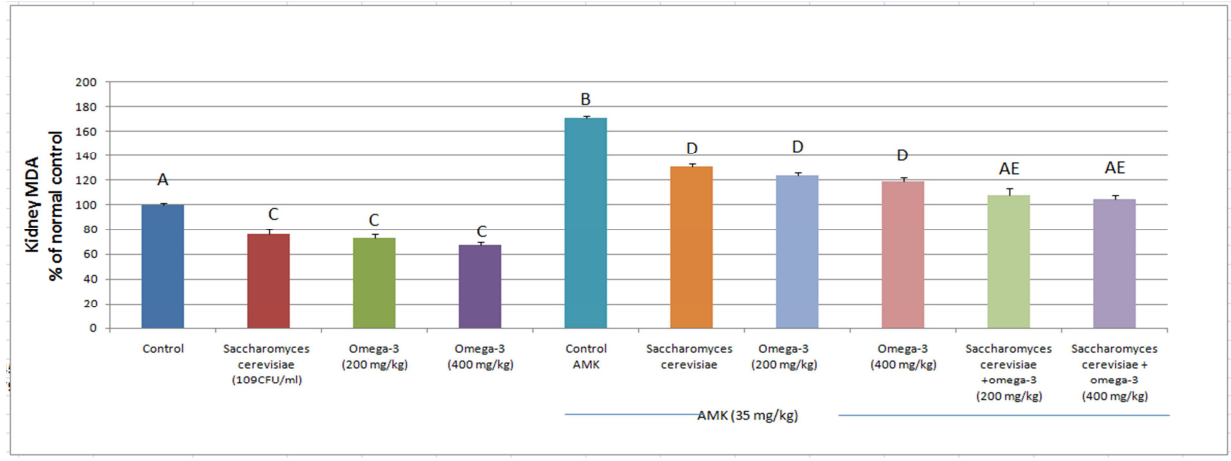

Figure (3): Effects of saccharomyces cerevisiae and/or omega-3 on kidney malondialdehyde (MDA)content in normal and AMK - treated rats

Data were expressed as mean ± S.E (n= 6).
Groups with different superscripts are significantly different at p< 0.05.

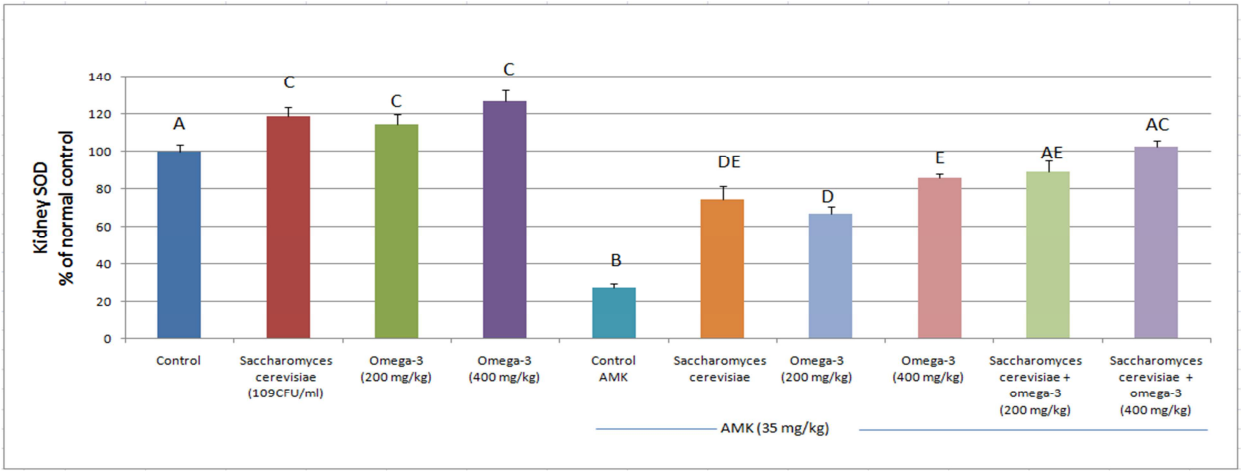

Figure (4): Effects of saccharomyces cerevisiae and/or omega-3 on kidney super oxide dismutase (SOD) activity in normal and AMK - treated rats.

Data were expressed as mean ± S.E (n= 6).
Groups with different superscripts are significantly different at p< 0.05.

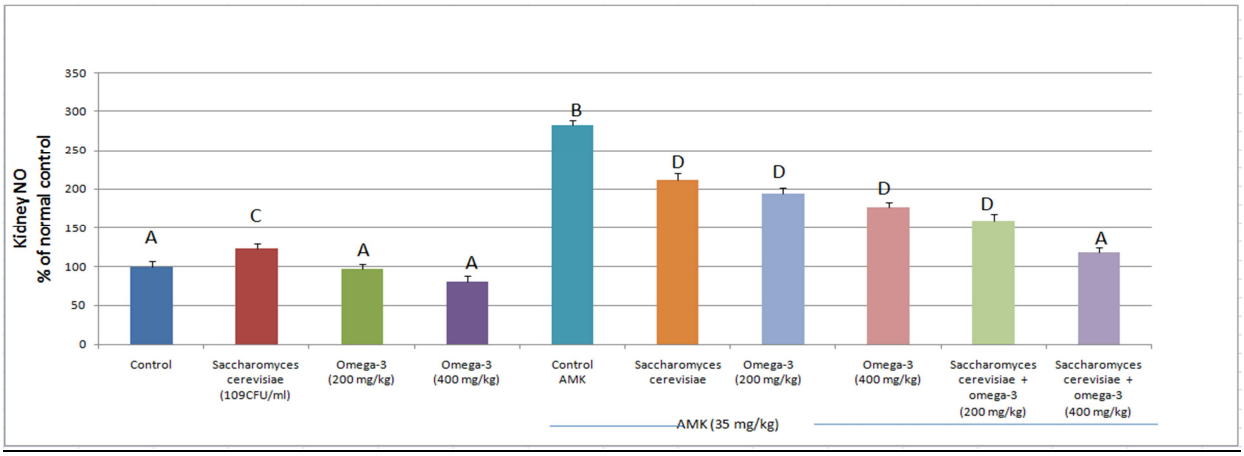

Figure (5): Effects of saccharomyces cerevisiae and/or omega-3 on kidney nitric oxide (NO)content in normal and AMK - treated rats

Data were expressed as mean ± S.E (n= 6).
Groups with different superscripts are significantly different at p< 0.05.

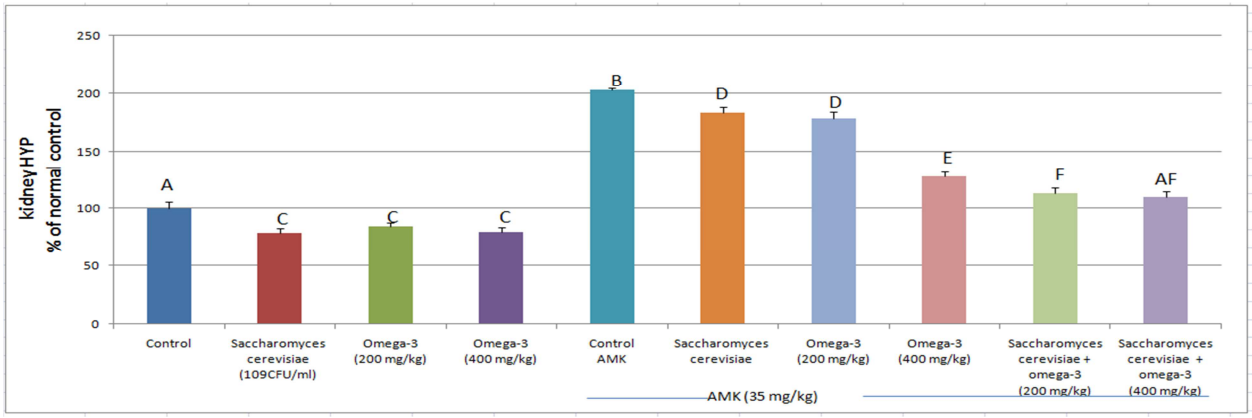

Figure (6): Effects of saccharomyces cerevisiae and/or omega-3 on kidney hydroxyproline (HYP)content in normal and AMK - treated rats

Data were expressed as mean ±S.E (n= 6).
Groups with different superscripts are significantly different at p< 0.05.

3.3 Effect on kidney GSH, MDA, NO, HYP contents as well as SOD activity

Administration of Sc and the two doses of omega-3 for four weeks in normal rats showed a significant increase in kidney GSH content by 58.20%, 49.85% and 68.95%, respectively (Figure 2). The same regimens decreased MDA kidney content significantly by 23.90%, 27.373% and 32.482%, respectively (Figure 3) and increased SODactivity by 18.587%, 14.428% and 26.73%, respectively, as compared to normal control group(Figure 4).In the same context, NO kidney content was elevated by 23.4% in Sc-treated group and showed no change in other groups (Figure 5); Meanwhile administration of Sc and the two doses of omega-3reduced kidney HYP content by 21.51%, 15.55% and 20.47%, respectively as compared to normal control group(Figure 6). AMK injection for four weeks showed a significant decrease in kidney GSH content and SOD activity by 39.40% and 70.49%, respectively as compared to the normal group (Figures 2&4).In the same context, AMK increased kidney MDA, NO and HYP contents 70.80%, 181.8%, 102.63%, respectively as compared to normal control group (Figures 3,5,and 6).While administration of Sc, two doses of omega-3 and their combinations for four weeks concomitant with AMK showed a significant increase in GSH kidney content by 112.31%, 96.55% 132.51%, 130.54% and 165.02%, respectively parallel to decrease in MDA kidney content by 23.29%, 27.24%,30.34%, 36.64% and 38.56%, respectively as compared to AMK control group (Figures2 &3).The same regimens reduced NO kidney content by 25.06%, 31.15% 37.28%,43.42% and 57.84%, respectively parallel to increased SOD activity by 151.6% , 127.31% 191.04%,201.7% and 247.69%, respectively as compared to AMK control group(Figures 4&5). Finally administration of Sc, the two doses of omega-3 and their combinations with AMK reduced kidney HYP content by 9.46%, 12.15% 36.57%, 43.87% and 45.53%, respectively as compared to AMK control group (Figure 6).

3.4. Effect on kidney caspase-3

The histopathological investigations revealed that AMK treated group stained immunohistochemically for caspase -3 showed intense positive results in many tubular cells denoting apoptosis of these cells (Figure 7e). These results were reduced in groups receiving Sc or omega-3 (200 or 400 mg/kg) or both together (Figures7 f-j).

3.5. Results of histopathologic studies

The histopathological investigations revealed that AMK treated group stained with hematoxylin and eosin stain showed marked cellular infiltration with atrophy of the capillary tuft and noticeable vacuolar degeneration in many tubular cells (Figure 8e).These results were reduced in groups receiving Sc or omega-3 (200 or 400 mg/kg) or both together where reduction of vacuolar degeneration of tubular cells was noted when compared with the AMK group (Figures 8 f-j).

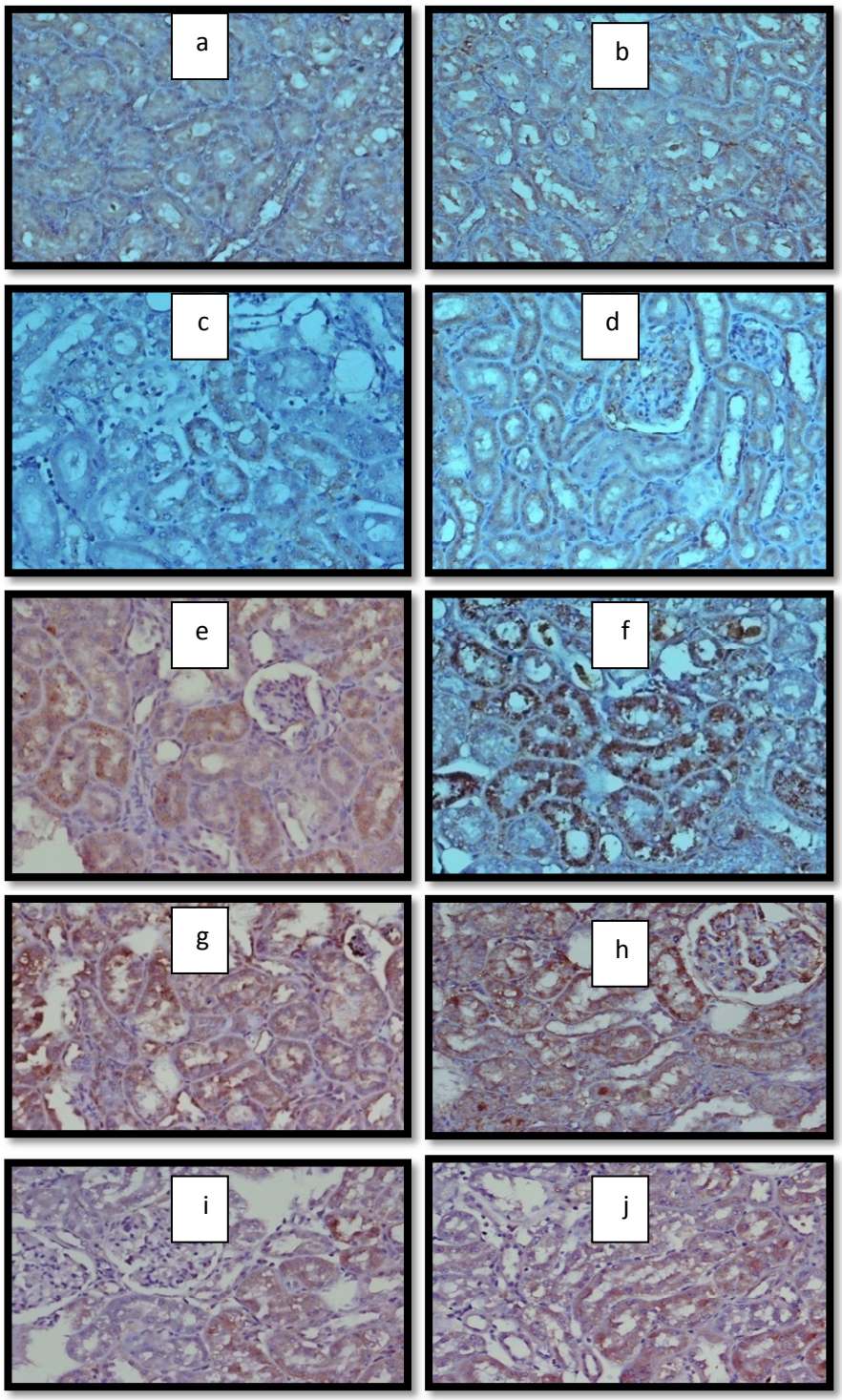

Figure 7: Photomicrograph of a sections of renal tissue stained immunohistochemically for caspase-3: (a) normal control rat (b): normal rat receiving Sc(C):normal rat receiving omega-3 (dose) (d): normal rat receiving omega-3(dose) (e): control AMK (f): rat receiving Scand AMK (g): rat receiving omega-3 in low dose and AMK(h): rat receiving omega-3 in high dose and AMK(i): rat receiving Sc, omega-3 in low dose and AMK (j): rat receiving Sc and omega-3 in high dose and AMK

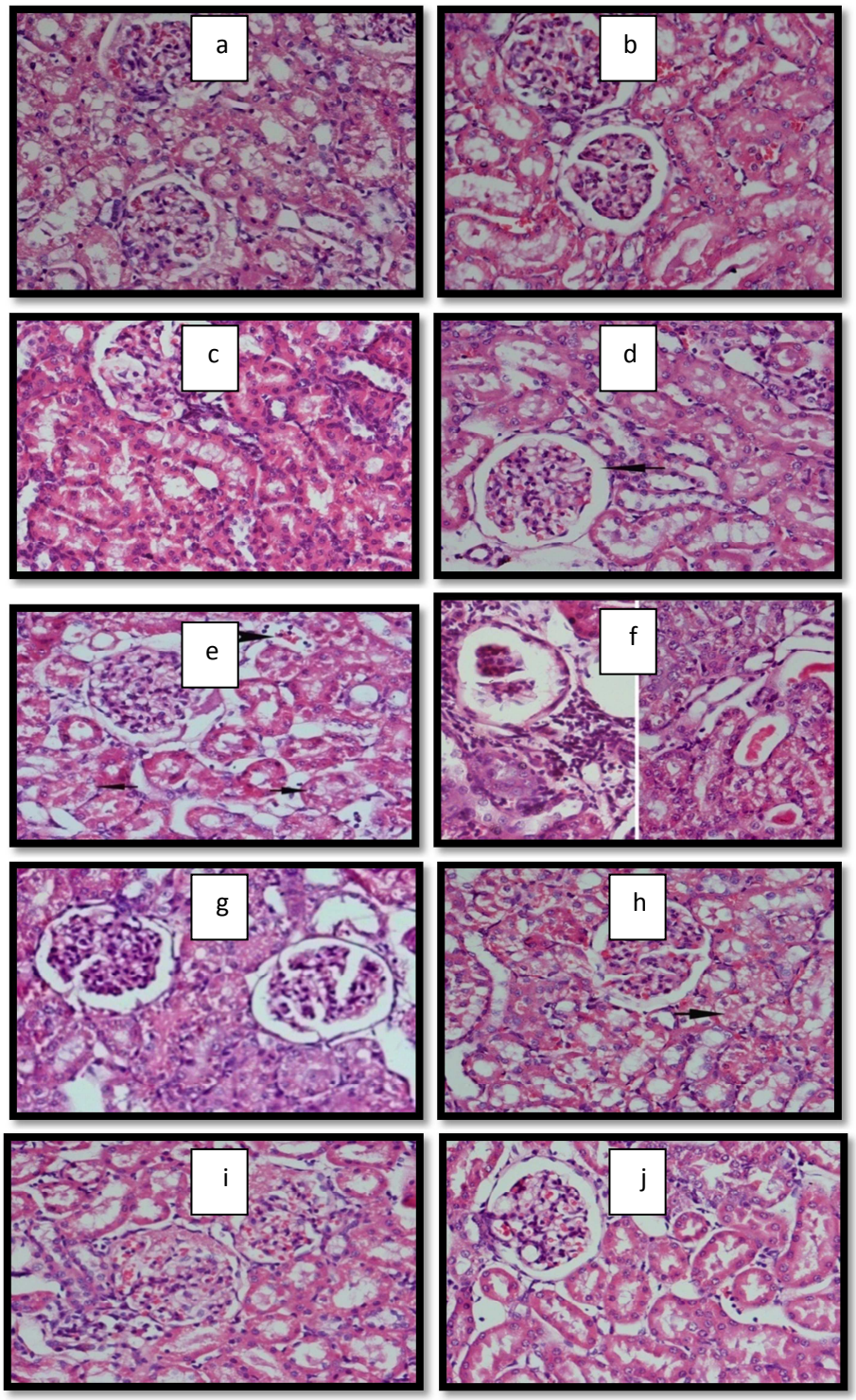

Figure 8: Photomicrograph of a section of renal tissue stained (Hx& EX 200): (a) normal control rat (b): normal rat receiving Sc (C):normal rat receiving omega-3 (dose) (d): normal rat receiving omega-3(dose) (e): control AMK (f): rat receiving Scand AMK (g): rat receiving omega-3 in low dose and AMK(h): rat receiving omega-3 in high dose and AMK(i): rat receiving Sc, omega-3 in low dose and AMK (j): rat receiving Sc and omega-3 in high dose and AMK

DISCUSSION

In the current study the marked elevation of the levels of both serum creatinine and urea observed by AMK give an indication of the reduction in the glomerular filtration. Since serum creatinine and urea are waste products of protein metabolism that need to be excreted by the kidney; therefore such increases of serum creatinine, uric acid and urea as reported in this study confirm functional damage of the kidney and are consistent with other studies [29&30].

The reduction in total protein (TP) in AMK treated group in our study may reflect the damage to kidneys and liver, where protein is synthesized. In another study, activities of aspartate amino transferase (AST) and alanine amino transferase (ALT) in serum were significantly higher in AMK intoxicated rats [31], also, renal damage by aminoglycosides can in turn leads to liver injury [32&33].

In the present study, serum TNF-α level was elevated in AMK control rats, when compared to normal control group. These results are in agreement with *Morsy et al (2013)* [34] who proved that TNF- α level was elevated by other nephrotoxicants as methotrexate in rats. TNF-α is a key element in a network of pro-inflammatory chemokines and cytokines activated in the kidney by nephrotoxic agent [35].

Moreover, the results of this study showed a significant increase in the contents of the end product of lipid peroxidation, MDA and decrease in GSH in AMK intoxicated group. Previous studies showed significant increase in the serum level of MDA in AMK-treated rats, suggesting the involvement of oxidative stress in its nephrotoxicity [36&29]. MDA is one of the well-known secondary products generated after exposure to reactive oxygen species and free radicals, and it may be used to evaluate oxidative damage by measuring serum levels of thiobarbituric acid reactive substance [37].

HYP is a sensitive marker for renal fibrosis and toxicity [38]. The elevation in HYP in our results in AMK group may be due to increased kidney epithelial cell extracellular matrix degradation and more specifically collagen degradation by AMK-mediated activation of kidney metalloprotease [39&40].

In our study, the photomicrograph section of renal tissue of AMK control group stained immunohistochemically for caspase-3 showed intense positive result in many tubular cells denoting apoptosis of these cells.

Apoptosis is a controlled type of cell death that is energy- dependent and characterized by cell shrinkage, chromatin condensation, membrane budding, phosphatidylserine externalization, and activation of a family of cysteine proteases called caspases [41].

Caspase activation is thought to be a key step in the genesis of apoptosis, and numerous stimuli activate caspases, including those that activate plasma membrane death receptors (caspase- 8) and cause mitochondrial dysfunction (caspase- 9) [42].

Results of the current study showed that the treatment with Sc reduced creatinine and uric acid. These results were in harmony with a previous study which showed that pretreatment with Sc against hepatic and renal injuries caused by the mycotoxin [43]. Also, mice treated with the yeast strains *of Rhodotorulaglutinis* significantly decreased creatinine and uric acid levels of aflatoxins -treated animals [44].

The treatment with Sc during intoxication with the AMK ameliorated the GSH levels and SOD activity compared to control, where MDA level decreased and SOD activity increased, along with an increase in GSH contents. Other studies showed that dietary yeast stimulates both immune and antioxidant responses after exposure to pathogens [45]. Moreover, Tovar-*Ramírez et al. (2010)* [46] reported that live marine yeast positively enhanced antioxidant status of sea bass larvae by means of preventing oxidative stress, and by maintaining a stable activity and gene expression of SOD and glutathione peroxidase (GPx).

The lowered levels of uric acid and BUN in AMK in combination with Omega-3 in both dose levels could be explained by the antioxidant effect of omega-3 fatty acids. The antioxidant and/ or anti-inflammatory effects of omega- through scavenging of free radicals and inhibiting lipid peroxidation have been reported previously [47]. This oxidant/antioxidant theory may explain the protection role of omega-3 FAs against AMK-induced nephrotoxicity. The anti-inflammatory property of omega-3 fatty acids is due to the action of eicosapentaenoic acid, which is one of the components of omega-3 through suppression of the production of AA metabolites [48].

Our result suggests an immune-modulatory role for omega-3-fatty acids based on the fact that it reduces the release of TNF–α and also its supplementation increases antioxidant levels. These results are in agreement with *and Mansara et al., 2015* [49] and *Salama et al., 2015* [50] who proved reduction of TNF–α release using omega-3 & 6 - fatty acids.

Our immunohistochemically results for caspase-3 showed reduction of positive cells in groups receiving omega-3 and/or Sc, if compared with the AMK control group

Human body has different antioxidant system to counteract oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants metabolic antioxidants), or externally supplied through dietary supplements (exogenous antioxidants/nutrient antioxidants). This explains why omega-3 and Sc (as antioxidants) reduced apoptosis. The role of antioxidants is to neutralize the augmented ROS to protect the cell against their adverse effects thus to contribute in the diseases prevention. The antioxidant system could work in two ways whether they can break the chain of death mechanisms or they could prevent the initiation of such cell death mechanisms. If the antioxidant interfere with the chain of ROS mediated death mechanism then they steals an electron from the free radical and forms a second radical. Second radical exerts same effect on another molecule and continues until the formation of non-reactive product by a chain breaking antioxidants like vitamin C, E or it simply convert into non-oxidative stable molecule. For the use of antioxidant in preventive mode it should neutralize the ROS at initiation phase thus stabilize the transition metal radical. Reports have showed that deficiency of nutrient antioxidant is also one of the causes of number of chronic age related neurodegenerative diseases [51]. *Singh 2015* [52] showed similar results, reporting that antioxidants reduce ROS.

The current result showed that kidney HYP content was reduced in AMK treated groups when treated with omega- 3 or Sc or their combination. The present results find support in those of *Yan-yan et al (2011)* [38] which confirmed prevention of mercuric chloride-induced elevation in HYP content by antioxidants as vitamin E, by stopping of ROS production, oxidative stress and lipid peroxidation.

In the present study, kidney NO content in rats treated with AMK alone was found to be higher than in control and omega-3 groups. The results obtained in this study suggest that omega-3fatty acids can react with free radicals acting as a free radical scavenger, which may lead to reduce NO concentration [53]. Omega 3- EFA prevents cellular oxidative stress by diminishing NO generation and reducing lipid peroxidation in cells. Thus administration of omega-3 EFA may have ameliorating effects on cellular damage by two mechanisms: ω-3 EFA results in enhanced defense against free oxygen radicals. Second, omega-3 EFA may replace fatty acid components of the cell membranes that had been attacked by superoxide anions, hydrogen peroxide, and hydroxyl radicals [54&55].

Barbosa et al.(2013) [56] proved that ω-3 EFA supplementation may have free radical scavenger activity. Administration of ω-3 EFA may stimulate vitamin E incorporation into membranes to avoid lipid peroxidation resulting from increased membrane omega-3 EFA content [57].Treatment with omega-3 EFA has been reported to decrease lipid peroxidation in the corpus striatum and to increase antioxidant enzyme activities in the hippocampus and corpus striatum of rats [58].

This was proved by our histological results which showed reduction in the cellular infiltration with atrophy of the capillary tuft together with vacuolar degeneration in many tubular cells in groups receiving omega-3 and/or Sc when compared to AMK treated group.

Omega- 3 fatty acids and Sc could serve as antioxidants and cytoprotective that make the cells less susceptible to the damaging action of toxic agents [14&15].

CONCLUSION

Sc and omega-3 proved ~~a~~ therapeutic effectiveness against AMK induced renal toxicity and this effectiveness is further improved when omega-3 and Sc are combined together, and thus, they should be considered when over consuming this antibiotic.

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