Potential renoprotective effect of Silymarin against Amikacin-Induced acute nephrotoxicity in rats

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Amikacin is an aminoglycoside antibiotic which widely used in neonatal intensive care units for the treatment of severe, life-threatening gram-negative bacterial infections. Its clinical use is limited by its side effects especially high nephrotoxicity. The present study designed to evaluate the renoprotective effect of silymarin (herbal medicine) against amikacin nephrotoxicity in male Albino rats. Rats were randomly allocated into 4 groups, Group (1); served control group and orally received 1ml isotonic saline solution. Group (2); orally received 1ml of freshly prepared silymarin solution (200 mg/kg/day). Group (3); intramuscularly injected with amikacin (100 mg/kg/day). Group (4); orally received 1ml of freshly prepared silymarin for 5 successive days then intramuscularly injected with amikacin. The experiment continued for 15 days and the samples were taken at the 3rd and 10th days of treatment. Results cleared amikacin causes nephrotoxicity through increasing levels of creatinine, blood urea nitrogen, uric acid, potassium, phosphorus and total oxidant status together with decreasing levels of sodium, calcium, and total antioxidant capacity. Urine protein/creatinine ratio and microalbuminuria concentration were also increased. Amikacin nephrotoxicity was confirmed by renal cytological, histopathological and immunohistochemical findings. Results of this study cleared the restorative and renoprotective effects of silymarin through improving the renal function following its administration which mediated via the attenuation of oxidative stress and the modulation of the damaged renal tissue particularly the glomerular filtration.

Keywords: Amikacin, Silymarin, Nephrotoxicity, Oxidative Stress Biomarkers, Renal Cytology, Renal Immuno histochemistry.

INTRODUCTION

The experiential use of broad-spectrum antibiotic as aminoglycoside in 2000 has been recommended due to rapid emergence of gram negative resistant bacterial strains (Chaudhary et al. 2011). Aminoglycoside antibiotics especially amikacin, are widely used in neonatal intensive care units for the treatment of severe, life-threatening gram-negative bacterial infections. Amikacin (AK), is a group of antibacterial drugs derived from species of Streptomyces which interfere with the function of bacterial ribosome. AK, is a common antibiotic of choice for the treatment of many infections like urinary tract infection, respiratory tract infection, gynecological infection, some mycobacterial infections, burns and endocarditis (Chambers et al. 2007). AK, is recurrently used in these treatments because it has high antibacterial efficacy, rapid onset of action, synergistic effect with β-lactam antibiotics, low resistance and low costs (Roy et al. 2016). Despite all AK benefits, its clinical use is limited by its side effects especially its high nephrotoxicity (Vasquez-Mendoza et al. 2007). The nephrotoxic side effects of AK have been documented in numerous studies. These deleterious effects have
been attributed to the development of cluster of alterations in proximal tubular epithelium followed by its destruction resulting in kidney dysfunction (Cipullo and Burdmann, 2006). Aminoglycoside (including AK) administration is also reported to induce apoptosis (Klemens et al. 2003), oxidative stress and free radical generation (Parlakpinar et al. 2006). These free radicals play an important role in the pathogenesis of nephrotoxicity which might be prevented by using several antioxidants (Parlakpinar et al. 2003). Silymarin is a mixture of three flavonolignans isomers (silybin, silydianin, and silychristin) and two flavonoids (taxifolin and quercetin), extracted from milk thistle plant (Silybum marianum) (Abenavoli et al. 2010) which considered as potent antioxidant compound, it able to scavenge both free radicals and reactive oxygen species (ROS), thus increases the antioxidant potentiality of the cells by ameliorating the adverse effects of free radical reactions (Mahabady and Varzi, 2011). Additionally, silymarin has anti-inflammatory and anti-apoptotic effects as well as antioxidant effect making it an interesting herbal medicine, and these properties have classified silymarin as a potent renoprotective agent (Soto et al. 2010; Mahabady and Varzi, 2011). The protective role of silymarin against nephropathic processes was related to its silybin and silychristin isomers which shown the ability to increase proliferation rate, protein and DNA biosynthesis in renal cells that have been damaged in vitro(Kren and Walterova, 2005; Shahbazi et al. 2012). Furthermore, silymarin has been shown to be safe in animal models and no significant adverse effects are reported in human studies (Hogan et al. 2007). The purpose of present study was to evaluate the renoprotective effect of silymarin against the acute nephrotoxicity induced by amikacin in male Albino rats through measurement of renal function tests (RFT). Renal function was evaluated based on the results of different tests including the concentrations of creatinine, blood urea nitrogen (BUN) and uric acid, mineral analysis including estimation of calcium and inorganic phosphorus, electrolyte analysis including estimation of sodium and potassium together with oxidative stress biomarkers including total antioxidant capacity (TAC) and total oxidant status (TOS) in association with urine examination including measurement of urine creatinine, urine protein/creatinine ratio (U-P/C) and microalbuminuria. All these results were in correlation with hematological, renal cyto pathological, histopathological and immunohistochemical examinations.

MATERIALS AND METHODS

Drugs and Chemicals

Amikacin sulphate vial (100 mg/2ml) was obtained from GlaxoSmithKline Company, Egypt. Silymarin capsule (140 mg) was obtained from Sedico Company, Egypt.

Animal protocol

Thirty-two Sprague-Dawely male Albino rats (weighing about 180±20 g) were used in the experiment and were obtained from the Animal House, Faculty of Veterinary Medicine, Cairo University, Egypt. After the rats were allocated into the cages, they were kept in our laboratory for 7 days prior to begin the experiment for adaptation to the new environment. Rats were fed with standard laboratory diet and allowed to drink water ad libitum.

Experimental protocol

A preliminary experiment was performed to determine the appropriate dosage to make nephrotoxicity to rats. After determining dose, rats were randomly allocated into 4 groups, comprising 8 rats each as follow: Group (1); served control group and orally received 1ml isotonic saline solution. Group (2); silymarin treated group which orally received 1ml of freshly prepared silymarin solution (dissolved in distilled water) at dose of 200 mg/kg/day (Soto et al. 2010). Group (3); amikacin treated group which intramuscularly (IM) received amikacin sulphate at dose of 100 mg/kg/day (Naseer et al. 2014). Group (4); orally received 1ml of freshly prepared silymarin solution (same dose as that in silymarin treated group) for 5 successive days then rats were IM injected with amikacin (same dose as that in amikacin treated group). The experiment continued for 15 days and the samples were taken at the 3rd and 10th days of treatments.

Sample Collection and Methods

Blood samples from each group were collected at the 3rd and 10th days of treatments. The obtained blood sample from each rat (retro-orbital venous plexus) was divided into two parts. First part was anticoagulated by heparin and used for hemogram evaluation, minerals and electrolytes analysis. Second part was collected in a clean centrifuge tube and allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum
separation. Clear non hemolysed supernatant serum was harvested for biochemical studies. At the 3rd and 10th days of treatments, all rats were kept inside especially designed metal containers. Owing to the especial design of these metal containers, urine from the rats was collected in a reservoir located below, urine protein/creatinine ratio (U-P/C) (Gentilini et al. 2005) and microalbuminuria (Ng WY et al. 2000) were measured for the samples collected from this reservoir.

At the end of the experiment (10th day of treatments) and after blood sampling, rats of all groups were anaesthetized with diethyl ether and decapitated. Kidneys of each rat were removed. Impression smears from kidney were taken and stained with field stain for cytological examination (Cowell, 2008). For histopathological examination, small pieces of fresh kidney tissue were fixed in 10% buffered formalin and then placed in fresh fixative solution and embedded in paraffin, sectioned at 5μm thick and stained with Hematoxylin and Eosin (H& E), and Periodic Acid Schiff (PAS) stains for routine light microscope examination (Bancfort and Marilyn, 2008). For immunohistochemistry, selected paraffin sections were stained for inducible Nitric Oxide Synthase (iNOS) expression (Volkan et al. 2006). Cytological, histopathological and immunohistochemical changes were evaluated in several sections from each group.

**Hematological and Biochemical Studies**

**Hematological Studies**

Red blood cells (RBCs) count, packed cell volume (PCV %), hemoglobin (Hb) concentration, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), total leukocytic count (TLC) and differential leukocytic count (DLC) on Giemsa stained blood smears were performed (Feldman et al. 2000).

**Biochemical Studies**

Serum samples were prepared to assay the following kidney function tests: concentrations of creatinine, BUN and uric acid were carried out according to the methods described by Spences (1986), Tabacco et al. (1979), and Fossasati et al. (1980), respectively. Heparinized plasma was used for estimation of calcium, phosphorus, sodium and potassium concentrations according to Biggs and Moorhead (1974), Goodwin (1970), and Niels et al. (1984), respectively. Determination of serum oxidative stress biomarkers including TAC and TOS were measured according to Erel (2004) and Erel (2005), respectively. The before mentioned biochemical parameters were assayed using reagent kits of good quality and analytical grade.

**STATISTICAL ANALYSIS**

Values were expressed as mean ± SD. Statistical comparisons among the means of different experimental groups were made with completely randomized one way ANOVA by COSTAT program version 6.4. A probability "P" value of <0.05 was assumed for statistical significance.

**RESULTS**

**Hematological Findings**

Silymarin treated group revealed insignificant changes in all hematological parameters compared to control group. Amikacin treated group revealed significant decrease in PCV %, Hb concentration and RBCs count together with normal MCV and MCHC resulting in normocytic normochromic anemia in association with stress leukocytosis. Silymarin protected group showed significant improvement in all hematological parameters compared to amikacin treated group (Table, 1).

**Renal Function Tests (RFT)**

Results of silymarin treated group showed insignificant changes in all parameters of RFT compared to control group. Results of amikacin treated group when compared to the corresponding results of control group cleared the nephrotoxic effect of amikacin through the recorded significant increase in levels of creatinine, BUN, uric acid, potassium, phosphorus and TOS together with significant decrease in levels of sodium, calcium and TAC. Results of silymarin protected group revealed the positive effects of silymarin. These effects were documented through the significant decrease in levels of creatinine, BUN, uric acid, potassium, phosphorus and TOS together with significant increase in levels of sodium, calcium and TAC toward the control group levels (Table, 2).

**Urine Examination**

Insignificant changes in U-P/C ratio and microalbuminuria concentration in silymarin treated group were recorded. Significant increases in U-P/C ratio and microalbuminuria concentration were recorded in amikacin treated
group. An improvement in urine examination in the form of significant decreases in U-P/C ratio and microalbuminuria concentration toward control group were recorded in silymarin protected group (Table, 3).

**Table (1): Hematological parameters of different experimental groups at the 3rd and 10th days of treatment.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>3rd Day</th>
<th>10th Day</th>
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<tbody>
<tr>
<td></td>
<td>Group(1)</td>
<td>Group(2)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>34.20 ±1.14a</td>
<td>33.25 ±1.26a</td>
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<tr>
<td>Hb (g/dl)</td>
<td>11.04 ±0.59a</td>
<td>10.52 ±0.68a</td>
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<tr>
<td>RBCs (x10^6/µl)</td>
<td>8.19 ±1.40a</td>
<td>7.54 ±1.40a</td>
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<tr>
<td>MCV (fl)</td>
<td>43.21 ±3.01a</td>
<td>44.23 ±2.52a</td>
</tr>
<tr>
<td>MCHC (g%)</td>
<td>32.16 ±1.21a</td>
<td>30.53 ±1.28a</td>
</tr>
<tr>
<td>TLC (x10^5/µl)</td>
<td>6.24 ±1.20a</td>
<td>6.41 ±2.30a</td>
</tr>
<tr>
<td>Neutrophil (x10^3/µl)</td>
<td>5.64 ±1.22a</td>
<td>5.84 ±1.50a</td>
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<tr>
<td>Lymphocyte (x10^3/µl)</td>
<td>1.78 ±0.30a</td>
<td>1.65 ±0.06a</td>
</tr>
<tr>
<td>Monocyte (x10^3/µl)</td>
<td>0.31 ±0.06a</td>
<td>0.36 ±0.04a</td>
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<tr>
<td>Eosinophil (x10^3/µl)</td>
<td>0.42 ±0.03a</td>
<td>0.22 ±0.19a</td>
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</table>

Group (1): represents control group. Group (2): represents silymarin treated group. Group (3): represents amikacin treated group. Group (4): represents silymarin protected group. Means with different superscripts (a, b, c) within a raw are significantly different at P< 0.05.

**Table (2): Renal function tests of different experimental groups at the 3rd and 10th days of treatment.**

<table>
<thead>
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<th>Parameters</th>
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<th>10th Day</th>
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<tbody>
<tr>
<td></td>
<td>Group (1)</td>
<td>Group (2)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.78 ±0.64a</td>
<td>1.82 ±0.49a</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>24.53 ±1.56a</td>
<td>25.61 ±1.41a</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.68±1.16a</td>
<td>3.12±1.02a</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.02±1.06a</td>
<td>7.82±0.66a</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>5.62±1.02a</td>
<td>4.95±1.21a</td>
</tr>
<tr>
<td>Sodium (meq/L)</td>
<td>134.51±12.06a</td>
<td>144.53±19.06a</td>
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<tr>
<td>Potassium (meq/L)</td>
<td>3.98±0.52a</td>
<td>4.12±1.12a</td>
</tr>
<tr>
<td>TAC (mmol/Trolox Equiv/L)</td>
<td>4.18±0.42a</td>
<td>4.23±1.22a</td>
</tr>
<tr>
<td>TOS (µmol H2O2 Equiv/L)</td>
<td>13.18±3.01a</td>
<td>11.12±2.08a</td>
</tr>
</tbody>
</table>

TAC: represents total antioxidant capacity. TOS: represents total oxidant status.
Group (1): represents control group. Group (2): represents silymarin treated group. Group (3): represents amikacin treated group. Group (4): represents silymarin protected group. Means with different superscripts (a, b, c) within a raw are significantly different at P< 0.05.

Table (3): Urine protein/creatinine ratio (U-P/C) and microalbuminuria concentration of different experimental groups at the 3rd and 10th days of treatments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>3rd Day 3rd</th>
<th>3rd Day 10th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td></td>
<td></td>
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<tr>
<td>U-P/C</td>
<td>0.71±0.12a</td>
<td>0.69±0.16a</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>25±1.15a</td>
<td>23±1.18a</td>
</tr>
<tr>
<td>Group (2)</td>
<td>0.73±0.05a</td>
<td>0.84±0.15a</td>
</tr>
<tr>
<td>Group (3)</td>
<td>0.84±0.03a</td>
<td>1.74±0.08b</td>
</tr>
<tr>
<td>Group (4)</td>
<td>0.82±0.14a</td>
<td>0.84±0.15a</td>
</tr>
<tr>
<td>Group (1)</td>
<td>0.71±0.12a</td>
<td>0.69±0.16a</td>
</tr>
<tr>
<td>Group (2)</td>
<td>0.73±0.05a</td>
<td>0.84±0.15a</td>
</tr>
<tr>
<td>Group (3)</td>
<td>0.84±0.15a</td>
<td>0.84±0.15a</td>
</tr>
<tr>
<td>Group (4)</td>
<td>0.82±0.14a</td>
<td>0.84±0.15a</td>
</tr>
</tbody>
</table>

Cytological Findings
Renal cytological findings of silymarin treated group were similar to that of control group. Both groups showed normal renal tubular epithelial cells which appeared round to polygonal cells with round, centrally placed nucleus and abundant light blue cytoplasm (Fig. 1, a). Renal tubular cells often remain together as recognizable tubule fragments of various sizes or occur as single cells. Amikacin treated group revealed hyperplasia of renal cells manifested by presence of binucleated renal cells with various degrees of vacuolation and degeneration of renal tubular epithelium (Fig. 1, b-d). Free nucleus with histocytic and lymphocytic infiltration were also seen (Fig. 1, e). Silymarin protected group exhibited all the cytological findings manifested in amikacin treated group but with lesser degrees to be resemble the findings of normal renal tubular epithelial cell (Fig. 1, f).

Histopathological Findings
Histopathological findings of silymarin treated group were similar to that of control group. Both groups showed normal histological structure of renal parenchyma. Amikacin treated group revealed glomerular, tubular sand vascular histopathological alterations and the nature of these alterations include degenerative and inflammatory reactions. Renal glomeruli showed exudation of eosinophilic proteinous material in Bowman’s space with focal necrosis of parietal lining epithelium (Fig. 2, a), renal tubules showed degenerative and necrotic changes that were obvious in proximal convoluted tubules. Renal blood arterioles showed hyalinization of smooth muscle layer with perivascular edema. Interstitial tissue showed edema with mononuclear cell infiltration, this lesion was severe in perivascular area (Fig. 2, b). Appearance of hyaline droplets in tubular epithelium was evident (Fig. 2, c).

Histopathological renal alterations were reduced in silymarin protected group; degenerative tubular changes were confined to cortical area with individual epithelial necrosis, interstitial edema became mild and confined to perivascular area with few mononuclear cell aggregation (Fig. 2, d-e). Appearance of regenerative renal tubules was evident in this group which manifested by slightly basophilic cytoplasm with active vesicular nuclei (Fig. 2, f).

Immunohistochemical Findings
Findings of silymarin treated group were similar to that of control group. Amikacin treated group PAS positive histological section showed massive disruption of the apical brush borders of tubular epithelium with reduced staining affinity for PAS stain in the apical cellular part indicating cellular disruption (Fig. 3, a), while focal disruption was evident in individual tubular epithelium with normal to slightly narrowed tubular lumina (Fig. 3, b). Expression of iNOS was relatively dense and affect multifocal tubular epithelial cells (Fig. 3, c). Silymarin protected group showed slight reduction in brown color intensity as well as the distribution of iNOS expression in the tubular epithelial cytoplasm (Fig. 3, d).
Figure (1): Cytological findings of renal smear in different experimental groups

a) Silymarin treated group as well as control group showed normal renal tubular epithelial cell (arrow). (Field stain, x1000)
b) Amikacin treated group showed hyperplasia of renal cell manifested by presence of binucleated renal cell (long arrow) with free nucleus (short arrows). (Field stain, x1000)
c) Amikacin treated group showed vacuolation and degeneration of renal tubule (arrow). (Field stain, x1000)
d) Amikacin treated group showed vacuolation and degeneration renal epithelium (long arrow) with free nucleus (short arrow). (Field stain, x1000)
e) Amikacin treated group showed histocytic (short arrows) and lymphocytic (L-arrow) infiltration. (Field stain, x1000) Silymarin protected group showed nearly normal renal tubular epithelial cell (arrow). (Field stain, x1000)
f) Silymarin protected group showed nearly normal renal tubular epithelial cell (arrow). (Field stain, x1000)
Figure (2): Renal histological findings of different experimental groups

a) Amikacin treated group showed distension of Bowman’s space by eosinophilic proteinous material associated with perivascular edema, hyalinization of renal arteriole with tubular epithelium vacuolization and necrosis (H &E, ×400).

b) Amikacin treated group showed perivascular mononuclear cell aggregation (H &E, ×400).

c) Amikacin treated group showed tubular epithelial swelling with intracytoplasmic hyaline droplets (H &E, ×400).

d) Silymarin protected group showed vacuolization of tubular epithelium with focal interstitial edema (H &E, ×400).

e) Silymarin protected group showed few mononuclear cell infiltrations in renal interstitium (H &E, ×400).

f) Silymarin protected group showed regenerative renal tubules (H &E, ×400)
Figure (3): Immunohistochemical analysis of different experimental groups

a) PAS stained renal section of amikacin treated group showed massive disruption of apical brush borders of proximal tubular epithelium (×600).

b) PAS stained renal section of silymarin protected group showed moderate disruption of apical brush borders of proximal tubular epithelium (×600).

c) Renal histological section of amikacin treated group stained for iNOS showed intense and diffuse iNOS in tubular epithelium in amikacin treated group (×400).

d) Renal histological section of silymarin protected group stained for iNOS showed moderate brownish staining in tubular epithelium (×400).

DISCUSSION
Nephrotoxicity is a major clinical complication of aminoglycoside antibiotics. Aminoglycosides appear to induce their nephrotoxic side effects by three general mechanisms: renal tubular toxicity, reduced glomerular filtration, and reduced renal blood flow (Wargo and Edwards, 2014). AK is an aminoglycoside antibiotic which used in clinical practice to treat severe gram negative infectious diseases. However, its nephrotoxicity has limited the expand use of it. Proximal renal tubular cells are the primary site of damage in patients treated with AK. Nephrotoxic side effects of aminoglycoside antibiotics have been documented in numerous species of experimental animals (El Mouedden, 2000). One mechanism of this nephrotoxicity is believed to involve ROS generation. Some studies demonstrated that, antioxidant administration has ameliorated AK induced nephrotoxicity (Parlakpinar et al. 2006). The present study aimed to evaluate the renoprotective effect of silymarin on AK induced nephrotoxicity in rats through hematological findings, measurement of RFT and oxidative stress biomarkers, estimation of U-P/C ratio and microalbuminuria, all these measures were in close contact with renal cytological, histopathological and immunohistochemical examinations.

Hematological parameters usually reflect the physiological responsiveness of animal to its external and internal surroundings and this is serving as an actual tool for monitoring animal health. Hematological profile in animals is an important indicator of physiological or pathophysiological status of the body.

Results of the present study showed that amikacin exposure provoked deleterious effects on the renal tissue with loss of the functional integrity of the kidney as was evidenced by the observed normocytic normochromic anemia in amikacin treated group. As it is known, Erythropoietin (Epo) is the major physiological regulator of erythropoiesis and the renal peritubular cells are the primary site of its production. Also, it has been reported that, regulation of Epo production is related to the proximal tubular function (Latimer, 2011). Therefore, one of the major causes of anemia induced by amikacin treatment is Epo deficiency following the kidney injury which was documented.
Acute nephrotoxicity or acute renal failure (ARF) is a clinical syndrome characterized by the sudden onset of hemodynamic, filtration and excretory failure of the kidney with subsequent accumulation of metabolic toxins and dysregulation of fluid, electrolyte and acid–balance (Abeer et al. 2012). Biochemical analysis results of amikacin treated group compared to control group revealed marked elevation in renal biomarkers parameters including serum creatinine, BUN and uric acid, which giving an indication for reduction in the glomerular filtration rate. Since serum creatinine and urea are waste products of protein metabolism that need to be excreted by the kidney; therefore, such increase of serum creatinine and urea as reported in this study confirm the occurrence of nephrotoxicity resulting in kidney dysfunction (Enver et al. 2003). In amikacin treated group, elevated levels of serum creatinine, BUN, uric acid and TOS were seen in association with hyperkalemia and hyperphosphatemia. Significant hyponatremia, hypocalcemia with decreased level of TAC was also recorded. In general, these biochemical findings indicated that, amikacin induced acute kidney injury (Soria et al. 2005; Michael et al. 2010). Aminoglycoside antibiotics including amikacin are known to be transported and accumulated within lysosomes of renal proximal tubular cells causing proximal tubular cell injury and necrosis. The pathogenesis of amikacin nephrotoxicity is postulated to its capacity to disrupt membrane structure and function (Kaloyanides, 1992). It was found that, amikacin cause ATP depletion from either mitochondrial damage or direct inhibition of mitochondrial oxidative phosphorylation causing an oxidative injury (Joel et al. 2002), this injury was confirmed in the present study with the significant increase of TOS and significant decrease of TAC. Also, renal tubular cells undergo necrosis when their cellular ATP stores are severely depleted to a level incompatible with maintenance of basal metabolism and activity of membrane transport pumps (Wilfred et al. 1998). This nephrotoxicity was documented by the observed various degrees of vacuolization and degeneration of renal tubular epithelium during cytological and histopathological examinations.

It was demonstrated that, between silymarin treated group and control group, no significant differences in all hematological and biochemical parameters were observed. On the other hand, it was recorded that, in silymarin protected group, silymarin conspicuously restored the hematological and RFT results toward the levels of control group, also silymarin conferred a protective effect to kidney against oxidative damage by decreasing the level of TOS and increasing the level of TAC. Two major mechanisms have been suggested for this renoprotective effect of silymarin. First mechanism is due to its potential antioxidant effect which mediated by scavenging of free radicals, decreasing formation of ROS and inhibiting fatty acid peroxidation. Second mechanism involves anti-inflammatory and anti-apoptotic actions through interference with nuclear factor kappa-B (NF-κB) and modulation of iNOS expression (Vaid and Katiya, 2010; Shahbazi et al. 2012).

Assessments of microalbuminuria and U-P/C ratio are more sensitive predictor for the acute tubular injury (Menezes et al. 2010), since the changes in microalbuminuria and U-P/C ratio occur before serum urea and creatinine. In the present study, higher microalbuminuria concentration and U-P/C ratio than those of control group were observed in amikacin treated group which denote the presence of acute kidney injury, this injury was documented with the changes observed in hematological and biochemical parameters in association with renal cytological and histopathological findings. An improvement in concentrations of microalbuminuria, and U-P/C ratio toward control group level was recorded in rats treated with silymarin prior to amikacin injection (silymarin protected group), these findings confirmed the nephroprotective effect of silymarin.

Cytological examination of amikacin treated group showed various degrees of renal tubular epithelium vacuolation and degeneration together with hyperplasia of renal cells, free nucleus with pyknotic cells that reflect renal ischemia and infarction, these changes may be due to the direct irritative effect of amikacin on the renal cells after absorption and during excretion (Varzi et al. 2007). Such functional disturbance in amikacin treated group could indicate the ability of amikacin to activate oxidative stress in the mitochondria of kidney proximal convoluted tubules (PCT) and endothelial cells, followed by ROS generation and cellular injury with consequent renal damage (Kara et al. 2016). All before mentioned cytological findings were manifested in silymarin protected group but with lesser degrees to be resemble those findings of normal renal tubular
epithelial cell, which confirm the potential renoprotective effect of silymarin.

Histopathological findings of amikacin treated group showed its nephrotoxic effect in the form of moderate glomerular and tubular changes which affected mainly the cortical tissue in addition to hyalinosis of small blood vessels with perivascular edema and moderate mononuclear cell infiltration. This effect was related to the reabsorption of amikacin by proximal tubular epithelium that affect mitochondrial function and cause cell membrane disruption (Kara et al. 2016) together with disturbance of membrane integrity and enhancement of tubular epithelial swelling (Dabak and Kocaman, 2015).

Immunohistochemical findings suggested that, amikacin induced its nephrotoxicity through induction of nitric oxide production as indicated by increased expression of iNOS in tubular epithelium and increased production of nitric oxide, resulting in cytotoxic effect on the surrounding cells (Volkan et al. 2006; Ozbek et al. 2009).

Both histopathological and immunohistochemical findings of silymarin protected group confirmed its potential renoprotective effect against nephrotoxicity induced by amikacin through decreasing iNOS expression with subsequent decreasing nitric oxide production thus reduced the deleterious effect of amikacin and induced regeneration of renal tubules.

In the present study, all renal cytological, histopathological, and immunohistochemical findings were parallel with findings of hematological, biochemical and urine examinations in amikacin treated group, and these findings cleared that, free radical generation following amikacin administration may have critical role in the observed nephrotoxicity (Vaid and Katiyar, 2010). An improvement was recorded in silymarin protected group through the observed cytological and histopathological lesser degrees of renal tubular damage together with the improvement in the results of hematology, RFT, oxidative stress biomarkers and urine examination. These improvements could indicate the ability of silymarin to normalize the renal function through its potential antioxidant effect, anti-inflammatory and anti-apoptotic actions by scavenging the free radicals (Shahbazi et al. 2012).

CONCLUSION
Using parameters of hematology, renal function tests include oxidative stress biomarkers, urine examination, renal cytology, histopathology and immunohistochemistry, the present study show the positive effect of silymarin administration for reducing the nephrotoxicity induced by amikacin in rats. This study might serve as a guide for determining and, if necessary, for taking early measures against nephrotoxicity that might develop in human at normal usage doses. For this reason, it is necessary to further confirm the findings of this study within the context of human clinical studies.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest”.

AUTHOR CONTRIBUTIONS
AA designed and wrote the manuscript. AA and SI executed and followed up the experiment. AA, SI performed animal treatments, hematological parameters, renal function tests, and renal cytology. FF performed renal histopathology and immunohistochemistry. AA and SI analysed the data. All authors interpreted the data, revised and approved the final version.

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