Protective role of Pomegranate juice in inhibit nephrotoxicity induced by amikacin in Rabbits
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ABSTRACT
The current investigation aims to evaluate the role of Punica granatum juice extract to reduce nephrotoxic formation by amikacin in male and female albino rabbits by measuring some criteria for biochemical functions, such as creatinine, urea, albumin, total protein. where given amikacin (80mg / kg) daily by intramuscular injection for the period of 15 days caused a significant increase at the level of (p ≤ 0.05) in concentration of creatinine, and urea in the serum compared with a group control, while decreased concentration of albumin and total protein significantly compared with the control group. The rabbits treated with pomegranate juice 100ml daily, the concentration of 40% for a period of 15 days have recorded a significant decrease in the concentrations of creatinine and urea compared with a group rabbits treated with amikacin only, and increased significantly in albumin and total protein compared with the control group treated with amikacin only.

Keywords: nephropathy, amikacin-induced nephropathy, Pomegranate juice, rabbits

1. Introduction

Many human diseases have been recognized as being a consequence of free radicals damage (Aruoma, 2003). Interest in the role of antioxidants in human health has prompted research in the fields of food science to assess fruit and vegetable antioxidants (Kim, 2002; Hofmann et al. 2006). The majority of the antioxidant capacity of a fruit or vegetable may be from phenolics compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechins and isocatechins rather than from vitamins C, E or β-carotene (Aruoma, 2003; Mehri et al. 2005; Gil et al. 2000). These phytochemicals may help to protect cells against the oxidative damage caused by free radicals (Seeram et al. 2008, Tipoe et al. 2007). The antioxidant activity of phenolics compounds is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Azadzoi et al. 2005; Enver et al. 2003). Renal failure is accompanied by oxidative stress, which is
thought to be caused by enhanced production of reactive oxygen species and impaired antioxidant defense. 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 (Rusinol et al. 2000). They may initiate injury indirectly by inducing oxidative stress, which is caused by excessive production of reactive oxygen species and it may produce a major alteration of protein and nucleic acid structure, damage to DNA, and destruction of the cells by lipid peroxidation. An imbalance between free radicals and defense mechanism leads to cell damage and several diseases. For this reason, the role of nutrition in health has captured the interest of researchers in antioxidants and their capacity to protect the body from damage induced by oxidative stress (Mehri et al. 2005). Many plants such as Punica granatum L, Citrus lemon possess antioxidant properties. Pomegranate, (Al-Ruemman), has long been attracted a lot of attention for its medical importance(Wilfred, 1998; Lidianne, 2011). Pomegranate juice was indeed shown recently to possess impressive antioxidative properties due to its polyphenolics, tannins and anthocyanins. Also, in healthy humans. Pomegranate juice consumption was shown to possess potent antioxidative capabilities against lipoprotein oxidation, and increased serum total antioxidant status (Seeram et al. 2005; Aviram, 2002). Aminoglycosides are potent bactericidal antibiotics; they act particularly against aerobic, Gram-negative bacteria. Amikacin is one of the aminoglycoside, mostly used for treatment of severe, hospital-acquired infections with multidrug resistant Gram negative bacteria such as Pseudomonas aeruginosa, Acinetobacter, and Enterobacter (Enver et al. 2003; Hakan et al. 2003). Aminoglycoside induced nephro and oto-toxicity, which are the limiting factors for their clinical use, in which the oxygen free radicals have been involved. Aminoglycosides, exert their adverse renal effect by generation of reactive oxygen species. Additionally, it has been demonstrated that aminoglycoside form a complex with mitochondrial Fe+² to catalyze the formation of free radicals (Poormoosavl et al. 2010). Increased nephrotoxicity has been reported following concomitant parenteral administration of aminoglycoside antibiotics and cephalosporins. Concomitant cephalosporins may spuriously elevate creatinine determinations (Poormoosavl et al. 2010).

2. Methodology

2.1. Experimental Animals: In continuation of our investigation of Punica granatum fruit extract (Hazim et al. 2008), male and female rabbit, age between 8-10 months, weight: 900-1750 gram, bought from local market used in the experiment. These rabbits kept in closed wooden boxes covered thin metal of Aluminum, its distances 40x90x60cm. Floored with wood straws with complete cleaning and used antiseptic every two days, light exposist 12 hour, dark exposist 12 hour, temperature was 25±2 oC. The animals kept for two weeks to adopt them its new medium and with no disease contact. Feeding of the rabbits depend mixture of (35% wheat, 34% corn, 20% Soya bean, 10% animal protein, 1% dried milk). Added to it 50 conservative and antifungal with regulate feeding and water continuously along that period from December 2012 to April 2013.

2.2. Preparation of Plant Extract: Pomegranate’s juice (250 ml) was obtained from cold pressed fruits (about 2kg fresh fruit weight) collected from local market of Iraq during
September 2012, which was then concentrated by simple distillation. The methodologies of Harbone (1983) and Wagner et al. (1984) were adopted to prepare the ethanolic extract of concentrated juice (1:1; v/v). The mixture was shaken for 12h on a shaker with reciprocal mechanism. After ethanol evaporation and concentration of the remaining aqueous solution by freeze drying, the residue was weighed and further dried in desiccators.

2.3. Experimental Method

**Group 1**-for rabbits were treated with intravenous (I.V) of normal saline for 14 days, this group served as control.

**Group 2**-four rabbits were treated by intramuscular injection of (80) mg/Kg/day of amikacin for 14 days. This group served as positive control for nephrotoxicity induced by amikacin.

**Group 3**-four rabbits treated with oral total dose of 100ml several time daily, the concentration of 40% pomegranate juice concomitantly treated by intramuscular injection dose of amikacin (80)mg/kg/day) for 14 days. This group utilized to investigate the possible protective effect of pomegranate against nephrotoxicity induced by amikacin. All animals were anesthetized by ether and sacrificed after 15 days of treatment

3. Results

3.1. The histology of the kidney of the controlled group: Kidneys of group 1(control) showed that the cortex of the kidney was containing glomeruli of normal size and structure surrounded by Bowman's capsule (fig. 1). The cortex also occupied by the proximal convoluted tubules which were formed by epithelial cells of pyramidal form, also there was many distal convoluted tubules of normal form which lined by cuboidal cells, so the lumen of these tubules appeared wider than the proximal convoluted tubules. The medulla of kidney was formed by the tubules and collecting ducts which were lined by cuboidal epithelial cells (fig. 2). the tubules were very thin surrounded by squamous cells which were the thin segments of Henle loop.
Section of the kidney show normal structure appearance of the glomerulai and renal tubules of control group (H&E X20).

The medulla of kidney formed by collecting ducts, tubules and Henle loops (H&E X20).

3.2. Nephrotoxic effects of amikacin:

3.2.1. Effect of amikacin on the histology of the kidney: The cortex was containing the glomeruli which were appeared mostly atrophied inside the Bowman's capsule, so the capsular space of bowman was wide (fig. 3a), some of the glomeruli were segmented and broken into many segments. The proximal convoluted tubules were containing a few of desquamated epithelial cells from its wall, whereas the lumen containing a glomerular filterate and some of degenerated cells (fig. 3b). The distal convoluted tubules revealed normal. The renal medulla was containing degenerated cells in its tubules and collecting ducts, and there was another tubules and collecting ducts of normal structure.

Figure 3a, 3b: Sections of kidney shows degenerative changes and necrosis of renal tubules in amikacin treated group (H&E X 40).
3.2.2. Effects of combination of pomegranate with amikacin on the histology of the kidney: There were some of the glomeruli which appeared hypertrophied (fig. 4a), while others showed atrophied pattern (fig. 4b).

**Fig. 4a (H&E X20)**
**Fig. 4b (H&E X 40)**

Figure 4a, 4b: Sections of kidney show slightly degenerative changes with regeneration of renal tubule in which the appearance look like the normal in amikacin with pomegranate treated group.

The proximal convoluted tubules were of normal structure; the distal convoluted tubules appeared of normal shape and structure (fig. 5) while some of the tubules were containing a glomerular filtrate. The renal medulla appeared of normal structure and shape, the renal tubules, collecting ducts and the interstitial connective tissue were intensively infiltrated with different types of connective tissue cells (fig. 6).

**Fig. 5: Sections of kidney showing the proximal and distal convoluted tubules of normal shape and structure (H&E X 20).**

**Fig. 6: Sections of Kidney showing** renal medulla normal structure and shape of collecting ducts and interstitial connective tissue infiltration (H&E X 20).

The effect of amikacin on the renal function showed significant increase (p<0.05) in the serum levels of both creatinine and urea of rabbits treated with 80 mg/kg/ day of amikacin
(group 2) compared to the corresponding levels in the control animals of group 1, while there are a significant decrease (p<0.05) in the serum levels of both creatinine and urea of rabbits treated with 80 mg/kg/ day of amikacin + 100 mg/kg/day of pomegranate juice (group 3) compared to the corresponding levels of rabbits treated with 80 mg/kg/ day of amikacin (group 2). Serum levels for creatinine were (2.9±0.070 mg/dL and 1.70±0.157 mg/dL) and that for urea were (25.4±0.917 mg/dL and 19.220±0.431 mg/dL) in group 2 and 3, respectively. (Table 1, fig. 1 and 8).

Table 1: Effect of amikacin and the combination of P.G with amikacin on the serum Creatinine and Urea,

<table>
<thead>
<tr>
<th>Group No</th>
<th>Parameter</th>
<th>Serum Creatinine mg/dL</th>
<th>Serum Urea mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1  X±S</td>
<td>Control</td>
<td>0.660±0.121</td>
<td>17.060±0.359</td>
</tr>
<tr>
<td>Group 2  X±S</td>
<td>Amikacin</td>
<td>2.900±0.070</td>
<td>25.400±0.917</td>
</tr>
<tr>
<td>Group 3  X±S</td>
<td>Amikacin With pomegranate juice</td>
<td>1.700±0.157</td>
<td>19.220±0.431</td>
</tr>
</tbody>
</table>

X= mean, SD= standard deviation, * =significant (p<0.05)

Fig 7: Effect of Amikacin on the serum creatinine.
3.2.3. Effect of the combination of pomegranate with amikacin on the serum Albumin and Protein levels: There were significant increase (p<0.05) in the serum levels of Albumin of rabbits treated with 80 mg/kg/day of amikacin+100 mg/kg/day of pomegranate (group 3) compared to the corresponding levels of rabbits treated with 80 mg/kg/ day of amikacin(group 2), the serum levels of albumin were (1.598±0.566 dL and 3.683±0.887 dL) in group 2 and 3, respectively; while there were significant decrease(p<0.05) in the serum levels of protein in rabbits treated with 80 mg/kg/ day of amikacin+100 mg/kg/day of pomegranate (group3) compared to the corresponding levels of the rabbits treated with 80 mg/kg/ day of amikacin respectively(group2). (Table 2, figure 9 and figure 10)

Table 2: Effect of amikacin and the combination of P.G with amikacin on the serum Albumin and Protein

<table>
<thead>
<tr>
<th>Group No</th>
<th>Parameter</th>
<th>Serum Albumin mg/dL</th>
<th>Total Serum Protein mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Control</td>
<td>3.64±0.186</td>
<td>6.72±0.404</td>
</tr>
<tr>
<td>Group 2</td>
<td>Amikacin</td>
<td>2.06±0.098</td>
<td>4.12±0.647*</td>
</tr>
<tr>
<td>Group 3</td>
<td>Amikacin</td>
<td>3.66±0.067</td>
<td>5.5±0.408*</td>
</tr>
<tr>
<td></td>
<td>With pomegranate juice</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig 9: Effect of Amikacin on the serum protein

Fig 10: Effect of Amikacin on the serum albumin

X= mean, SD= standard deviation, * =significant (p<0.05)
4. Discussion

Aminoglycoside antibiotics have long been used as antibacterial therapy. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic side effects (Enver et al. 2003). It has been reported that amikacin may induce free radical production which implicates a variety of pathological processes (Hakan et al. 2003, Arvind et al. 2008). In this study the marked elevation of the levels of both serum creatinine and urea in group 2 compared with group 1 were observed and give an indication to 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 increase of serum creatinine and urea as reported in this study confirm an indication of functional damage of the kidney and these results were in consistent with other studies (Enver et al. 2003; Hakan et al. 2003). Also it was found that aminoglycosides cause renal tubular cells undergo necrosis when their cellular Adenosine Tri Phosphate (ATP) stores are severely depleted to a level incompatible with maintenance of basal metabolism and activity of membrane transport pumps (Wilfred et al. 1998). Results of this study showed an improvement in the serum creatinine and urea levels of rabbits treated with combination of pomegranate juice with amikacin (group 3) compared with group 2, and these levels are near the levels in group1. These results are in agreement with results of other study which showed that combination of cimetidine (an inhibitor of cytochrome P450) with gentamicin showed decrease in serum urea and creatinine levels (Poormoosavl et al. 2010). The elevation of the levels of both serum creatinine and urea in group 2 compared with group1 attributed to the free-radical scavenging properties of the Pomegranate juice, where it help in maintaining the levels of reduce of both serum creatinine and urea. The antioxidant effects of Pomegranate juice was attributed to its constituents like antioxidant trace elements and flavonoids compounds; therefore Pomegranate juice has been suggested to be able to decrease lipid peroxidation (Rosenblat et al. 2006). Also the antioxidant activity of Pomegranate juice is due to phenolic compounds and enzymes (glucose oxidase, catalase and peroxidase) (Seeram et al. 2004; Gil et al. 2000). Results of this study are in agreement with results of (Aviram et al. 2000), which found that natural Pomegranate juice has protective effect against the damage in liver and kidney cells from oxidative stress induced by toxic level of lead in rats (Aviram et al. 2002). Pomegranate juice in the present study was used as the antioxidant of choice, as it is very rich in polyphenol and demonstrates high capability to scavenge free radicals and to inhibit Low density lipoprotein oxidation in vitro and in vivo (Enver et al. 2003, Hakan et al. 2003; Poormoosavl et al. 2010; Hazim et al. 2008). It have been shown to increase serum antioxidant capacity or decrease oxidative damage of biomolecules (Harbone et al. 1983; Arvind et al. 2008). Pomegranate juice has shown significant anti atherosclerotic, anti-hypertensive, antioxidant, and anti-inflammatary effects in human subjects and rabbits models. Pomegranate juice has also been shown to prevent oxidative destruction of nitric oxide and enhance its antioxidant and anti-inflammatory functions (Ignarro et al. 2006).
5. Conclusion

It is concluded that pomegranate juice could be useful for reducing the nephrotoxic effects of amikacin.

6. Acknowledgments

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References


