PROTECTIVE EFFECT OF PISTACIA KHINJUK ON GENTAMICIN-INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

Introduction and Aim: Gentamicin (GM) is used for treatment of urinary tract infections which induce nephrotoxicity. This study designed to investigate the possible protective effects of Pistacia khinjuk against GM-induced renal damage in rats. Materials and Methods: The ethanol extract of Pistacia khinjuk fruit was administered orally once for 14 days. Male wistar rats were divided into five groups of six rats in each one; first group served as control and received normal saline, second group was received GM (80 mg/kg/d, intraperitoneally) for 14 days, third, fourth and sixth groups were received simultaneous GM plus extract 150, 300 and 600 mg/ml respectively. The rats were sacrificed on the 15th day and serum was collected. Nephroprotective potential was estimated by renal functional tests such as blood urea nitrogen (BUN), creatinine(CR), total protein(TP), sodium (Na), potassium(K) levels and kidneys were dissected out for determination of reduced glutathione (GSH) and malondialdehyde (MDA) levels in renal tissues homogenate. Results: The animals which received only GM causes a significant increase in levels of CR and BUN, MDA, and significantly decrease in TP and GSH concentrations when compared with the control group. Treatment with Pistacia khinjuk produced amelioration in biochemical indices in plasma and renal tissue when compared with the GM treated group. Conclusion: simultaneous treatment of Pistacia khinjuk might have produced amelioration in biochemical indices against GM-induced nephrotoxicity. It was concluded that Pistacia khinjuk is an effective nephroprotective agent as a novel natural antioxidant.
Keywords: Gentamicin, *Pistacia khinjuk*; nephroprotective, BUN, MDA,

**INTRODUCTION**

Acute renal damage (ARD) is a kidney disease occurs when body is exposed to a drug or toxin. It is comprised 20-30% in the intensive care unit and increased mortality, hospitalized duration. Aminoglycoside antibiotics such as gentamicin (GM) are non–protein bound drugs commonly use for the treatment of severe Gram-negative infections especially in urinary tract infections. They are not metabolized and mainly excreted by glomerular filtration therefore, induce renal disease and nephrotoxicity. Ten to twenty percent of all cases of acute renal failure are related with gentamicin nephrotoxicity. GM operates as an iron chelator therefore, can induce free radical in human body [1]. Intracellular increase of aminoglycoside in lysosomes is may be to inhibit with normal biological activity, such as synthesis of protein and mitochondrial function finally cause cell death [2].

When renal injure occurs, body unable to excrete of excess urine and wastes material from the blood body and electrolytes including potassium and magnesium will all concentrated in blood. Nephroprotective compounds are the substances can protective property against nephrotoxicity. Medicinal plants have employed in traditional medicine due to the presence of various phytochemical compounds. Co-administration of different medicinal plants with nephro - bacterial protective potential along with different nephrotoxic compounds may reduce its toxicity. Free radical formation is very important in GM-induced toxicity process, therefore administration of antioxidant agents maybe valuable in reducing GM toxicity [3, 4].

Literature taught us that Plants have always been a part of medicinal practice from the start of human civilization to the present modern world. Medicinal plants mostly developed from nature that are potent, safe and inexpensive remedies in the treatment of most disease.

The study for medicinal plants revealed many plants having noticeable biological and medicinal potentials, among which one is *Pistacia khinjuk* is widely used for treat of antibacterial, antiflammatory, antiviral, antipyretic, gastrointestinal disorders in Iran. *Pistachios* are rich in phenolic compounds with antioxidant activity [5, 6]. *In vitro* antioxidant activity of methanol and ethyl acetate extracts of *P. khinjuk* was reported previously [6].
This study was carried out in order to evaluate the possible protective effects of GM-induced nephrotoxicity of *P. khinjuk* in male rats based on antioxidant activity of the *P. khinjuk* from the region of Yasuj Iran by focusing on the change of structures and functions of kidneys. This study was designed also to determine of renal tissue malondialdehyde (MDA), glutathione (GSH) content and blood urea nitrogen (BUN) serum creatinine (Cr), total protein.

**MATERIALS AND METHODS**

*Experimental animals:* 30 healthy adult male rats in-breed Wistar rats weighting between (150-200 g) of were used under standard laboratory conditions (23 ± 2°C) humidity (50 ± 5%) and 12-hour light–dark cycle, were used for the experiment during the experimental period. They had free access to standard commercial rat chow and tap water *ad libitum*. All procedures were carried out in accordance with the protocol of the care and use of laboratory animals.

GM-induced nephrotoxicity in rats follows as [7]: Thirty male Wistar albino rats were randomly divided in to five groups containing six rats each one (n= 6).

GM was administrated to animals intraperitoneal at the dose of 80 mg kg⁻¹, for 14 consecutive days.

Group I: as a control group was given normal saline, intraperitoneally; Group II as a GM-treated group were received GM (i.p) route; Group III: Rats in this group were injected with GM and administered Pistacia *khinjuk* extract by oral gavage (150 mg/ kg, p.o); Group IV and V: Rats were received GM and administered (300 and 600 mg/ kg, p.o) extracts by oral gavage for 14 consecutive days.

After dosing on day 15, animals were anaesthetized and the 5 ml blood was collected by cardiac puncture and serum for biochemical assays was rapidly separated by centrifugation at a speed of 2000/rpm for 15 minutes and stored in a freezer set at -20°C for analysis.

Rats were killed under anesthesia by cervical dislocation; kidneys were isolated from each rat, one-half of the kidneys were prepared for tissues homogenization of biochemical parameters such as MDA and GSH levels. Serum blood urea nitrogen (BUN) creatinin(CR), sodium (Na) and potassium (K) concentrations were measured by using an auto analyzer (RA...
1000, USA). The MDA level was determined based on the thiobarbituric acid [8]. GSH level was determined by dithionitrobenzoic acid method described by Ellman [9].

Statistical analysis
Results were expressed Mean ± SD from six animals in each group. Comparison between groups were made by using one way analysis of variance (ANOVA), followed by dunnett’s Multiple Comparisons test with the help of SPSS 13.0 for Windows. Results were considered significant at $p < 0.05$.

RESULTS
In GM group serum CR and BUN levels were significantly increased compared to control group ($p<0.01$). The total serum protein concentration was significantly decreased in GM treated group compared to control group ($p<0.01$). However Na+ and K+ concentration were unchanged in all groups when compared with the control group (Table 1).

Co-Administering of $PKE$ (600 mg/kg, p.o) with GM significantly ($p<0.01$) reduced the levels of serum BUN and CR compared to GM treated group and the TP concentration was significantly ($p<0.01$) increased (Table 1). GM treated group had significantly increased level of MDA ($p < 0.001$) in kidney tissue, while in extract treatment group particularly in 600 mg dose significantly decreased GSH levels when compared with the control group (Table 2). Simultaneous administration with $PKE$ normalized in kidney tissue MDA levels ($p < 0.01$) when compared to GM treated group. However, simultaneous treatment with $PKE$ provided a significant increase in kidney GSH levels ($p < 0.01$) (Table 2).

GM treated group had significantly increased weight of kidney and body weight of rats ($p < 0.05$) (Table 3). However uses of PKE normalized kidney and body weight in higher doses (Table 3).

Table 1. The effects of $Pistacia khinjuk$ extract on blood biochemical tests in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>BUN(mg /dl)</th>
<th>CR(mg /dl)</th>
<th>TP(g /dl)</th>
<th>Na(mEqL$^{-1}$)</th>
<th>K (mEqL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>19 ±1.2</td>
<td>0.7± 0.1</td>
<td>6.5 ± 0.5</td>
<td>137 ±5.5</td>
<td>4.5 ±0.15</td>
</tr>
<tr>
<td>GM 100(mg/kg)</td>
<td>50 ± 2.5$^a$</td>
<td>3.2±0.32</td>
<td>3.6 ±0.42$^a$</td>
<td>140 ±2.9</td>
<td>4.3 ±0.2</td>
</tr>
<tr>
<td>PKE150 mg / kg</td>
<td>38 ±2.1$^b$</td>
<td>2.1±0.25</td>
<td>4.9 ±0.58</td>
<td>138 ±4.3</td>
<td>4.4 ±0.22</td>
</tr>
<tr>
<td>PKE300 mg / kg</td>
<td>31 ±2.1$^b$</td>
<td>1.3±0.25$^b$</td>
<td>5.3 ±0.58$^b$</td>
<td>139 ±4.3</td>
<td>4.3 ±0.22</td>
</tr>
<tr>
<td>PKE 600 mg / kg</td>
<td>22 ±1.4$^c$</td>
<td>0.8 ±0.15$^c$</td>
<td>6.1 ±0.47$^c$</td>
<td>138 ±3.6</td>
<td>4.5 ±0.25</td>
</tr>
</tbody>
</table>

$^a$Statistically significant difference versus Negative group ($P < 0.001$). $^b$Statistically significant difference versus group GM ($P < 0.05$). $^c$Statistically significant difference versus group GM ($P < 0.01$). Values are expressed as mean ± SD (n = 6).

Table 2. The effects of Pistacia khinjuk extract on renal MDA and GSH contents in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA(nmol/g protein)</th>
<th>GSH (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>74 ± 4.5</td>
<td>12.5 ± 0.5</td>
</tr>
<tr>
<td>GM100 (mg/kg)</td>
<td>119.2 ± 5.1</td>
<td>8.6 ± 0.55</td>
</tr>
<tr>
<td>PKE 150 mg / kg</td>
<td>95.22 ± 4.8</td>
<td>13.1 ± 1.45</td>
</tr>
<tr>
<td>PKE 300 mg / kg</td>
<td>86.9 ± 2.9</td>
<td>14.2 ± 0.8</td>
</tr>
<tr>
<td>PKE 600 mg / kg</td>
<td>69 ± 3.2</td>
<td>11 ± 0.11</td>
</tr>
</tbody>
</table>

*Statistically significant difference versus Negative group (P < 0.001).  
^Statistically significant difference versus group Gentamicin (P < 0.05).  
$Statistically significant difference versus group Gentamicin (P < 0.01).  
Values are expressed as mean ± SD (n = 6).

GM: gentamycin , PKE: Pistacia khinjuk extract, MDA: Malondialdehyde, GSH: reduced glutathione.

Table 3. The effects of Pistacia khinjuk extract on Weight of kidney and body of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight of kidney(g)</th>
<th>Body weight(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0.62 ± 0.02</td>
<td>180 ± 2.5</td>
</tr>
<tr>
<td>GM100 (mg/kg)</td>
<td>0.93 ± 0.03</td>
<td>161 ± 2.4</td>
</tr>
<tr>
<td>PKE 150 mg / kg</td>
<td>0.72 ± 0.01</td>
<td>165 ± 1.5</td>
</tr>
<tr>
<td>PKE 300 mg / kg</td>
<td>0.64 ± 0.02</td>
<td>189 ± 2.9</td>
</tr>
<tr>
<td>PKE 600 mg / kg</td>
<td>0.65 ± 0.03</td>
<td>185± 2.4</td>
</tr>
</tbody>
</table>

*Statistically significant difference versus Negative group (P < 0.01),  
^Statistically significant difference versus group Gentamicin (P < 0.05).Values are expressed as mean ± SD (n = 6). GM: gentamycin , PKE: Pistacia khinjuk extract

DISCUSSION

The natural drugs without side effects are remedies source for cure of the diseases. Drugs which obtained from herbal medicine comprise a main part and popular of therapeutics in the traditional medicine.
Medicine plant are important for modern medicine as they are utilized as sources of direct treatment, serve as raw source for semi synthetic chemical compounds, can be used as taxonomic markers for the discovery of new compounds [10]. GM is generally used for treatment of gram negative bacterial infections. Though some of the patients which receive GM, for more than seven days, associate with serious complications and nephrotoxicity. For this reason, clinical usage of GM are limiting in practice [11].

In the present study renal damage was induced by GM in 14 days and evaluated by determination of antioxidant enzyme activities and renal biochemical tests. GM nephropathy was associated with low GSH content in the renal cortex due to reduced of renal antioxidant activity and presence of oxidative damages.

The high generation of free radicals in GM-induced nephropathy could cause inactivation of antioxidant enzyme system in organisms. It is well known that use of GM in rats induce hydrogen peroxide and hydroxyl radicals in renal cortical mitochondria and result in increase in lipid peroxidation.

Therefore, the decreased of intracellular GSH and the increase of hydrogen peroxide and hydroxyl radicals are the triggering agents in GM nephrotoxicity. Results of this study confirmed that nephropathy which produced by GM which evidenced by insufficiency of the glomerular filtration rate and tubular injury because the generation of free radicals which may be directly affected filtration surface and decreased glomerular filtration rate [12].

Increase in the blood urea nitrogen, creatinine and uric acid levels in serum strongly revealed insufficiency of kidney function. In present study Protective effect of *Pistacia khinjuk* was evaluated at 150, 300 and 600 mg/kg against GM induced toxicity. In this study, consumption of hydroalcoholic extract of *Pistacia khinjuk* cause decreased in BUN, CR and uric acid concentration. Similar findings were reported by researchers in literature [13, 14].

According to much animal research there was reported a positive correlation between oxidative damages and nephrotoxicity induced by GM. This oxidative stress associated with high elevations in BUN, CR and acute tubular necrosis [15].

Therefore, screening of BUN and CR tests has been used to examine drug-induced nephropathy in experimental animal and man [16]. Administration of *Pistacia khinjuk* at the
oral doses of 300 and 600mg/kg/day significantly lowered elevations in the BUN and CR concentration in serum in treated groups when compared to the control rats.

Flavonoid compounds and other phytochemical compounds in medicinal plants due to their potent antioxidant effects can inhibit xenobiotic-induced nephrotoxicity in rat [17]. The present nephroprotective mechanism of the *Pistacia khinjuk* against cellular damage could be due to antioxidant potential and presence of flavanoids and phenolic substances.

CONCLUSION

*Pistacia khinjuk* had a protective potential against GM induced nephrotoxicity could be by antioxidant capacity.

ACKNOWLEDGEMENTS

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REFERENCES