Evaluation of the antioxidant protective effect of oxytocin and silymarin against gentamicin-induced nephrotoxicity in rat

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Gentamicin is an effective aminoglycoside antibiotic against severe infections. In spite of inducing nephrotoxicity and oxidative damage, gentamicin is used clinically due to its wide spectrum of activities against Gram negative bacterial infections. Its nephrotoxicity occurs by selective accumulation in renal proximal convoluting tubules. Its nephrotoxicity involves renal free radical generation and reduction in antioxidant defense mechanisms. A potential therapeutic approach to protect or reverse gentamicin-induced oxidative stress and nephrotoxicity would have more importance for clinical consequences. Therefore, the present study was designed to investigate the possible antioxidant protective effects of oxytocin and silymarin against gentamicin-induced renal damage in rats. A total of 40 adult male albino rats were divided into four groups. The first group is the control group that received normal saline (1ml/kg/i.p/day for 8 consecutive days), the second group was treated with gentamicin (80mg/kg/i.p/day for 8 days), the third group was treated with gentamicin (80mg/kg/i.p/day for 8 days) and oxytocin (5 I.U/kg/i.p/day for 8 days) and the fourth group was treated with gentamicin (80mg/kg/i.p/day for 8 days) and salymarin in a dose of (50 mg/kg orally/day for 8 days). Some biochemical and histopathological examinations of kidneys were performed after treatment for evaluation of the oxidative stress and renal nephrotoxicity. Gentamicin treatment significantly increased serum urea and creatinine levels and AST activities. Also gentamicin significantly decreased the total antioxidant capacity and catalase activity in renal tissues. Renal tissue malondialdehyde (MDA) has a nonsignificant increase, while renal reduced glutathione (GSH) wasn’t changed. Study of renal morphology showed degenerative changes in the form of cloudy swelling, hydropic degeneration and glomerular necrosis in gentamicin group. Administration of oxytocin and silymarin with gentamicin ameliorated to some degree the biochemical changes and oxidative stress parameters against gentamicin-induced nephrotoxicity. It was concluded that treatments with these antioxidants could have beneficial effects in treatment of gentamicin induced nephrotoxicity.

Gentamicin is an aminoglycoside antibiotic widely used in veterinary and human clinical practice for the treatment of Gram-negative bacterial infections. Treatment with gentamicin often associated with an induction of nephrotoxicity, which is seen in 10–20% of patients of acute renal failure (Ali, 1995). Despite the introduction of newer and less toxic antibiotics, gentamicin continues playing a useful role in the treatment of serious enterococcal, mycobacterial and Gram-negative infections, due to its effectiveness against resistant β-lactamic microorganisms, its low cost, and the low levels of resistance among members of Family Enterobacteriaceae (Edson and Terrell, 1999). Gentamicin accumulates in the renal cortex and induces renal morphological changes characterized by tubular necrosis, which is localized mainly in the proximal tubules and an overall syndrome very similar in humans and experimental animals. The effects of gentamicin on biological membranes appear to be important in its toxicity. Gentamicin undergoes partial re-absorption by proximal tubular cells by absorptive endocytosis that results in the fusion of endocytic vacuoles with lysosomes where the drug accumulates. This accumulation induces the process of lysosomal phospholipidosis, resulting in tubular necrosis, which is a key

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pathological mechanism contributing to renal toxicity (Pitchai et al., 2010). The other important mechanism which involved in gentamicin nephrotoxicity is the generation of reactive oxygen species (ROS) (Kadkhodaei et al., 2005). Gentamicin directly increases the production of mitochondrial ROS from the respiratory chain (Morales et al., 2010). Many strategies have been reported to ameliorate gentamicin nephrotoxicity in experimental animals which focused on the use of various antioxidants (Ali, 2003).

Oxidative stress represents a major common pathway of cellular injury (Sohal, 2002). Antioxidants in general show marked nephroprotective activities against gentamicin-induced nephrotoxicity. Therefore, many products reported to have antioxidant potentials possess nephro-protective potentials, these include for example Nigella sativa (Yaman and Balikci, 2010), grape seed extract (Safa et al., 2010), green tea extract (Sara et al., 2009), vitamin E and N-acetyl cysteine (Patel et al., 2011) and sesame oil (Periasamy et al., 2010).

Silymarin, an antioxidant flavonoid complex isolated from the seed of Silybum marinus (milk thistle) possesses a powerful free radical scavenging properties (Kren and Walterova, 2005). In addition, silymarin regulates the intracellular contents of the reduced glutathione (GSH) (Borsari et al., 2001). Silymarin is used clinically to treat chronic inflammatory liver disease and hepatic cirrhosis. Recuperative effect of silymarin on the renal tissue damage may be related to an increase in the activity and recovery of gene expression of antioxidant enzymes which in addition to the glutathione system constitute some of the most important defense mechanisms against free radicals damage (Soto et al., 2010).

Oxytocin (OT) is a neurohypophysial nonapeptide synthesized in the paraventricular and supra optical nuclei of the hypothalamus (Olson et al., 1992). Its receptors are widely distributed in the central nervous system and have been identified in the kidney heart, thymus and pancreas (Gimple and Fahrenholz, 2001). Oxytocin has antioxidant properties and modulates the immune and anti-inflammatory response in wound healing and sepsis induced animal models after subcutaneous injection. (Išeri et al., 2005).

The present study examined the protective antioxidant effects of silymarin and oxytocin on gentamicin-induced nephrotoxicity. Some biochemical parameters for renal assessment and histopathological examination of kidney also performed. It is a new trial. There protective antioxidant properties were recorded previously against experimental gentamicin nephrotoxicity in dogs and cisplatin nephrotoxicity in rat respectively.

**Material and methods**

**Drugs.**

Gentamicin-sulphate (GARAMYCIN®) was obtained commercially as AN injectable solution produced by Schering-Plough (U.S.A). Oxytocin (Syntocinon®) was obtained commercially as injectable solution produced by (Novartis Pharam).

Silymarin (Legalon®) a commercially product produced by MAAdAUS GmbH. (Germany).

**Experimental animals.** Forty adult male albino rats weighed (170±30 g) were supplied by the Egyptian Organization for Biological Products and Vaccines. They had free access to water Ad libitum and a normal rat chow diet. The animals were housed 5 rats per cage.

**Experimental design.** One week after acclimatization, the rats were randomly divided into four experimental groups (10 rats in each); the first was considered as the (C) control group, they injected intraperitoneally (i.p) with 0.9% NaCl (1ml/kg/day/for 8 days). Whereas, the second one (gentamicin group, G) received gentamicin alone (80 mg/kg/i.p/day for 8 consecutive days), (Patel, et al., 2011). The third one (GO group) received the same previous dose of gentamicin in addition to oxytocin (5 I.U/kg/i.p for 8 consecutive days), (British pharmacopoeia, 1985). The fourth one (GS group) received the same previous dose of gentamicin in addition to silymarin orally using gavage (50 mg/kg/day for 8 consecutive days, (Gholamreza et al., 2005). Twenty four hours after the last doses of treatments, blood samples were collected via retro-orbital bleeding for serum separation and storage at -80 °C for further biochemical analysis. Rats were then sacrificed and kidneys were removed for histological and biochemical studies.

**Renal tissue homogenate preparation.** Animals were sacrificed and kidneys were immediately removed and washed using chilled saline solution (0.9%). One gram of kidney was, homogenized in 5 ml phosphate buffer saline (pH 7.4), and then centrifuged at 3000xg for 15 min at 4°C. The supernatant was collected and stored at -80°C for further analysis.
Biochemical parameters for renal function assessment. Serum urea level and serum creatinine level were determined according to Yong (2001) by using commercially available kits (Diamond, Cairo, Egypt). Activity of aspartate amino transferase (AST; EC 2.6.1.1) was determined according to Bergmeyer et al. (1977) using commercially available kits (BioSystems S.A, Barcelona, Spain).

Determination of some antioxidant parameters and lipid peroxidation in kidney tissue homogenate. Antioxidant parameters including total antioxidant capacity (TAC), reduced glutathione (GSH) and catalase activity were measured in kidney tissue homogenate using commercially available kits (Biodiagnostic, Cairo, Egypt), according to Koracevic et al. (2001); Beutler, et al. (1963) and Aebi (1984) respectively. Also lipid peroxidation (malondialdehyde (MDA) was measured according to Ohkawa et al. (1979) by using of the commercially available kits (Biodiagnostic, Cairo, Egypt).

Histopathological examination. Kidneys were sectioned and fixed in 10% formalin and then embedded in paraffin. Sections were cut at 7 µm and stained with haematoxylin and eosin and then examined using light microscope, according to Bancroft and Steven (1983).

Statistical analysis. Statistical analysis was carried out using GraphPad Instat software (version 3., ISS-Rome, Italy). Unless differently specified, groups of data were compared with un-paired t-test and one-way analysis of variance (ANOVA) followed by Tukey-kramer (TK) multiple comparisons post-test. Values of P<0.05 were regarded as significant. The data, as clearly indicated are reported in tables as mean ± standard error (S.E).

Results

Protective effect of oxytocin and silymarin on gentamicin induced nephrotoxicity. Gentamicin administration to rats resulted in significant increase in serum urea, creatinine levels and AST activity in all groups of rat in comparison to control. Administration of oxytocin and silymarin significantly reduced these elevations to a certain degree in comparison to gentamicin alone. It was obvious in GO group. The result was presented in table (1).

Table (1): Effect of gentamicin, oxytocin and salym arin on serum parameters of kidney function in control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Gentamicin (G)</th>
<th>Gentamicin+ Oxytocin (GO)</th>
<th>Gentamicin+ Silymarin (GS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>37.7 ± 2.59</td>
<td>77.8 ± 6.6***</td>
<td>51.46 ± 1.89* &quot;a&quot;</td>
<td>63.19 ± 4.44** &quot;b&quot;</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.49 ± 0.14</td>
<td>1.24 ± 0.13*</td>
<td>0.99 ± 0.19* &quot;a&quot;</td>
<td>1.2 ± 0.17* &quot;b&quot;</td>
</tr>
<tr>
<td>Serum AST ( U/L)</td>
<td>32.53 ± 2.87</td>
<td>45.16 ± 5.3*</td>
<td>25.14 ± 1.58 b</td>
<td>20.78 ± 3.12 a</td>
</tr>
</tbody>
</table>

*** significantly different at p<0.001 from control. ** significantly different at p<0.01 from control
* significantly different at p<0.05 from control. "a" significantly different at p<0.01 from gentamicin group and
"b" significantly different at p<0.05 from gentamicin group.

Effect of oxytocin and silymarin in combination with gentamicin on some antioxidant parameters and lipid peroxidation in renal tissue. Gentamicin administration resulted in a significant decrease in TAC and catalase enzyme in renal tissue homogenate of all groups of rat in comparison to control. Reduced glutathione not changed; while MDA was increased none significantly in the G and GO groups respectively.

Histopathological examination of renal tissue. Light microscopic examination of renal cortex of control rats showed numerous renal corpuscles and tubules. Each renal corpuscle was formed of a tuft of glomerular capillaries surrounded by Bowman’s capsule with outer parietal layer of simple squamous epithelium and inner visceral layer surrounding the glomerulus. The two layers were separated by narrow urinary space (Photo A). Light microscopic examination of the renal cortex of (G group) revealed degenerative changes in the form of cloudy swelling and hydrophobic degeneration. Glomerular necrosis was clear. Hyaline casts were present in the tubular lumen (Photo B). Light microscopic examination of the renal cortex of (GO group), Photo (C) showed the same histopathological picture as the (G group) but with less extensive degree. Renal
cortex of (GS group) showed degenerative changes but with less necrotic changes in comparison to (G and GO groups) as showed in (Photo D).

Table (2): Effect of gentamicin, oxytocin and silymarin on some antioxidant parameters and lipid peroxidation in control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Gentamicin (G)</th>
<th>Gentamicin+ Oxytocin (GO)</th>
<th>Gentamicin+ Silymarin (GS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC (nM/g tissue)</td>
<td>2 ± 0.08</td>
<td>1.65 ± 0.11*</td>
<td>1.49 ± 0.07**</td>
<td>1.69 ± 0.04*</td>
</tr>
<tr>
<td>GSH (mg/g tissue)</td>
<td>14.16 ± 1.72</td>
<td>14.09 ± 0.79</td>
<td>15.27 ± 1.93</td>
<td>14.58 ± 1.18</td>
</tr>
<tr>
<td>Catalase (U/g tissue)</td>
<td>0.69 ± 0.1</td>
<td>0.28 ± 0.06**</td>
<td>0.25 ± 0.03***</td>
<td>0.37 ± 0.04*</td>
</tr>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>22.92 ± 1.59</td>
<td>26.42 ± 2.71</td>
<td>24 ± 1.3</td>
<td>18.1 ± 0.83</td>
</tr>
</tbody>
</table>

***significantly different at p<0.001 from control, ** significantly different at p<0.01 from control and * significantly different at p<0.05 from control

Discussion

It is known that aminoglycosides (especially GM) can cause nephrotoxicity. It has been estimated that up to 30% of the patients treated with aminoglycosides for more than 7 days showed some signs of nephrotoxicity. Many antibacterial drugs with equal and sometimes better sensitivity and safety profiles than aminoglycosides are available, but the latter drugs still remain a clinically important group.
of antibiotics as they have an excellent antibacterial profile against Gram-negative life-threatening infections, and there is more experience with these than with the other newer antibacterial drugs.

In experimental animals, several strategies to ameliorate the toxicity have been attempted. These include controlling the time of administration of the antibiotics and co-administering agents to mitigate the renal toxicity. In view of their excellent safety and efficacy profiles, antioxidant drugs were found to produce the best nephroprotection as reviewed by (Koyner et al., 2008). In this study we used two types of antioxidant drugs. One is hormonal (oxytocin) and the other is a medicinal plant (silymarin) as a new trial to mitigate gentamicin nephrotoxicity.

The present study indicated that marked significant elevations of serum urea, creatinine concentrations and also AST activity were evident suggesting a significant functional impairment of kidney in gentamicin-induced group. Similar pattern of changes were also observed by (Lafayette et al., 2001; Othman et al., 2010; Yaman and Balikci 2010). Administration of oxytocin or silymarin with gentamicin significantly decreased the elevation of serum urea, creatinine concentrations and also AST activity in comparison with gentamicin alone. This was obvious in the GO group indicating somewhat protection against gentamicin nephrotoxicity, these findings similar to results obtained by (Laila et al., 2011).

Similar results suggested that silymarin protected against cisplatin-induced renal toxicity (Karimi et al. 2005) and against adriamycin induced cardiotoxicity and nephrotoxicity in rats (Nagla et al., 2008).

Several mechanisms are involved in gentamicin-nephrotoxicity. One of these mechanisms is the increase of reactive oxygen species (ROS) generation. These radicals include hydroxyl radicals and hydrogen peroxides in the renal cortex that eventually change antioxidant levels within the renal tissue and lead to structural and functional deterioration (Pedraza-Chaveri, 2000; Maldonado et al., 2003). In our study this indicated by significant decrease of total antioxidant capacity and catalase enzyme in the renal tissue homogenate in the all groups which administrated by gentamicin specially the GO group. This indicated oxidative stress due to consumption of TAC and catalase enzyme in converting the toxic radicals to non-toxic end products (Shimeda et al., 2005).

Reduced glutathione serves as a scavenger of different free radicals and is one of the major defenses against oxidative stress (Rao and Shaha, 2001), any factor that leads to oxidative stress may result in GSH depletion due to consumption of the latter in counteracting free radicals and reactive oxidant intermediates (Ursini and Bindoli 1987).

In our study there was no significant variation of the renal homogenate level of reduced glutathione between all groups of rats. Our results agreed with Chao-Hen and Jerry (1982), Soejima et al. (1998) and Karahan et al. (2005), but another studies stated that gentamicin caused a decrease in renal glutathione levels in rats (Enver, 2003). These differences may be due to the doses of gentamicin used and period of administration.

The increases in the levels of lipid peroxidation products such as MDA are indices of membrane lipid damage (Kalpana et al., 2009).

Most of previous studies reported that gentamicin caused increases in renal MDA levels as an index of lipid peroxidation. In our study administration of gentamicin alone or with oxytocin increase the level of renal MDA but this was not significant in comparison with control group (Enver et al., 2003). Some scientific papers reported that in tissue neither MDA determination nor TBA-test response can generally be regarded as a diagnostic index of the occurrence extent of lipid peroxidation, fatty hydroperoxide formation, or oxidative injury to tissue (Janero, 1990; Ayşen et al., 2000).

Another study reported that oxytocin prevented both lipid peroxidation and GSH depletion in the renal tissue invaded by bacteria, (Nes et al. 2006). Results in Table (2) showed that administration of silymarin protected to some degree the gentamicin depletion of antioxidant, this is similar to result detected by (Varzi et al., 2007) who found that Silymarin and vitamin E decreased gentamicin-induced nephrotoxicity in dogs.

In the present work the histological examination of the renal cortex revealed degenerative changes in the form of cloudy swelling, hydropic degeneration, glomerular necrosis and hyaline casts in the tubular lumen in gentamicin treated group. These changes appear to be less severe in the oxytocin and silymarin treated groups. This result was agreed
with result revealed by (Srinivasan et al. 2009) who find that gentamicin caused sever tubular necrosis, glomerular atrophy and severe extensive damage in kidney. Also the result was agreed with the result obtained by (Pitchai et al., 2010; Laila et al., 2011) who found that oxytocin decrease the degenerative changes occurred by cisplatin-induced nephrotoxicity. Also treatment with oxytocin resulted with the reduction of glomerular atrophy and inflammation, which was limited to local sites (Nes et al., 2006).

Conclusion

Our study showed that oxytocin administration with gentamicin mitigated to some degree the nephrotoxic effect of gentamicin on serum renal function parameters represented by urea, creatinine and AST. While silymarin administration with gentamicin minimize to some degree the deleterious effect of gentamicin on total antioxidant capacity and the catalase levels of renal tissue. We suggest that these antioxidants should be administrated enough time before the beginning of gentamicin treatment and then continued with it in an attempt to induce significant effects.

References


