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Evaluation of the Kidney Extract on Gentamicin Induced-Nephrotoxicity in Rat

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ABSTRACT

Gentamicin (GM) is an aminoglycoside antibiotic that induces tubular necrosis, increases Blood Urea Nitrogen (BUN), Creatinine (Cr) and kidney atrophy. It has been proved that the kidney extract reduces the blood pressure. Perhaps, this extract may be having healing effects on kidney injury. The aim of this study was to evaluate the effects of kidney extract on GM-induced nephrotoxicity. For this purpose, thirty rats were divided into three groups. The gentamicin (GM) group received 100 mg kg⁻¹ intraperitoneal injection whereas, gentamicin plus extract (GME) group received 100 mg kg⁻¹ plus 30 mg kg⁻¹ extract and control group received saline. After taking blood sample, the right kidneys were processed and stained with Hematoxylin-Eosin. Our findings showed that BUN and Cr increased in GM and GME groups in comparison with CON group. The length of Proximal Tubule (PT) decreased in the GM as compared to the control groups. The volumes of the kidney, cortex and medulla did not change in the GM and GME groups as compared to the CON group. It seems that GM decreases the length of PT; however, it has no effect on the volumes of the cortex and medulla. The kidney extract did not have healing effect on the structure of kidney in GM-induced nephrotoxicity.

Key words: Gentamicin, tubular necrosis, kidney volume, tubular length

INTRODUCTION

Gentamicin (GM) is an aminoglycoside antibiotic which is commonly used for the treatment of gram negative bacteria infection in human and animals (Nourani *et al.*, 2006). However, a major complication of GM treatment is nephrotoxicity. GM-induced nephrotoxicity is characterized by tubular necrosis which is localized mainly to the Proximal Tubule (PT) (Atessahin *et al.*, 2003), an increase in Blood Urea Nitrogen (BUN) and serum Creatinine (Cr) concentration (Atessahin *et al.*, 2003; Parlakpınar *et al.*, 2005; Polat *et al.*, 2006). Aminoglycosides do so by impairing mitochondrial function, interfering with tubular transport, increasing oxidative stress and forming free radicals (Markowitz and Perazella, 2005). In many cases, these changes lead to kidney atrophy (Wu *et al.*, 2002; Rymgayllo-Katska, 1989). Although, the pathogenic changes are often reversible, long time use of this drug induces irreversible renal injury. Therefore, successful prevention requires information about quantitative pathogenic changes and estimation of renal

atrophy. Stereological principles are methods that give us adequate information about tri-dimensional structure of various tissues or organs and are used for estimation of renal atrophy. One of the important indexes for measurement of atrophy is volume estimation. Studies have shown that various agents can prevent GM induced renal damage such as melatonin (Ozbek *et al.*, 2000), Vitamin E (Pedraza-Chaverri *et al.*, 2000), Zinc (Kumar *et al.*, 2000) and *Zingiber officinale* (Lakshmi and Sudhakar, 2010). Some studies have demonstrated that the extract of kidney has several chemical materials capable of reducing blood pressure (Page *et al.*, 1941; Grollman *et al.*, 1942, 1940; Hamilton and Grollman, 1958). Perhaps, this extract has properties that induce healing effect on the function and structure of the kidney.

Therefore, the aim of this study was to evaluate the possible protective effects of kidney extract on GM-induced nephrotoxicity in a rat model by stereological method.

MATERIALS AND METHODS

Experimental conditions: Thirty adult male Sprague-dawley rats weighting 190-230 g were provided from Research Animal House, Shiraz, Iran. The animals were housed under standard conditions (12:12 light: dark cycle, temperature 22±23°C and free access to tap water and basal diet). Gentamicin was purchased from Alborz darou Company, Iran. The animals were randomly divided into three groups:

- The control group (CON group) received intraperitoneal (i.p.) injection of 0.5 mL saline for 8 day
- Gentamicin group (GM group) was injected (i.p.) with gentamicin sulfate 100 mg kg⁻¹ in 0.5 mL of saline solution for 8 days
- Gentamicin+kidney extract group (GME group) received (i.p.) GM sulfate 100 mg kg⁻¹ in 0.5 mL of saline solution for 8 day plus kidney extract 30 mg kg⁻¹ in 0.5 saline for 8 days by feeding needle

At the end of the experiment, blood samples were taken from the heart of each animal for determination of Creatinine (Cr) and Urea (BUN).

Then the animals were sacrificed and their right kidneys were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm and stained with Hematoxylin-Eosin. The slides were observed by light microscopy to evaluate the tubular necrosis.

Extraction of kidney: Boiling extract method was used: the fresh sheep kidney was cut in small pieces and boiled for 90 min in normal saline. The mixture was then filtered. The filtrate was evaporated to dryness. Amount 3 g of the dry extract was dissolved in 100 mL of normal saline and 0.5 mL of this solution was fed to each rat (Joseph, 1907). Our university Ethical Committee approved this research.

Stereological study: The rats were anaesthetized and their right kidneys were removed. The pelvis of the kidney was then dissected out and the kidney was weighed and the primary volume, "V primary" was measured using the immersion method (Silva and Merzel, 2001). Briefly, the weight of a container filled with distilled water was measured and then the kidney was suspended by a thin thread in the container. The new weight in grams, minus the weight of the container and water divided by ~1.0, (the specific gravity of distilled water) was the volume of the kidney in cubic centimeters. After fixation in neutral buffered formaldehyde for 1 week, the kidney was processed and stained. The shrinkage was estimated. Estimation of tissue shrinkage and some stereological

parameters requires isotropic uniform random sections (Nyengaard, 1999; Mandarim-de-Lacerda, 2003). These sections were prepared by the orientator method. Briefly, each kidney was placed on a circle with equal division and sectioned into half randomly according to the divisions. The cut surface of the one half of the kidney was then placed on the second circle with non-equal sinus-weighted divisions and the second cut done by selecting another random number. Two halves of the kidney was sectioned into slabs in the direction of the second cut with an interval of ~0.5 mm. Then 9-11 slabs were collected. By a trocar, a circle was punched from a kidney slab. The diameters of the circular piece were measured and the area of the circle was estimated. The cut surfaces of the slabs and circular piece were embedded in paraffin. After sectioning (5 µm thicknesses) and staining with Hematoxylin-Eosin, the area of the circular piece was measured again and volume shrinkage was calculated as:

$$\text{Volume shrinkage} = 1 - (\text{AA}/\text{AB})^{1.5}$$

where, AA and AB were the area of the circular piece after and before processing, sectioning and staining, respectively. The final volume of the kidney was corrected using:

$$V_{\text{final}} = V_{\text{primary}} \times (1 - \text{volume shrinkage})$$

The sections were analyzed using a video-microscopy system made up of a microscope (E-200, Nikon, Japan) connected to a video camera (Sony, Japan, SSC Dc 18P), a P4 PC computer and a LG monitor (Flatron 1752 s). 10-12 microscopic fields were examined in each kidney. By means of a stereology software designed at our laboratory (Stereological Research Laboratory, Shiraz University of Medical Sciences, Shiraz, Iran), the stereological probe (composed of 25 points) was superimposed upon the images of the tissue sections viewed on the monitor and volume density (V_v) or the fraction of the unit volume of the kidney which have been occupied by each parameter, including the renal cortex, medulla, Proximal Tubule (PT), Distal Tubule (DT), Collecting duct (CT), loop of Henle (HT) were obtained using a point-counting method and the following formula:

$$V_v = P_{\text{structure}} / P_{\text{reference}}$$

where, ' $P_{\text{structure}}$ ' and ' $P_{\text{reference}}$ ' were the number of test points falling on the histological parameter and on all the area of the section, respectively. Volume fraction of the cortex, medulla was estimated at magnification of 375 and the other parameters at 1500 on the monitor. The fractional volume was multiplied by the final volume of the kidney to obtain the absolute volume of the parameters to prevent the 'reference trap' (Nyengaard, 1999; Mandarim-de-Lacerda, 2003). The length density of the tubular structures of the kidney including the PT, DT, CT and HT were estimated by randomly overlaying an unbiased counting frame (Nyengaard, 1999; Mandarim-de-Lacerda, 2003) with an area of 2800 µm² on the monitor live images. The tubules which lied completely inside the counting frame or partly inside the counting frame but only touching the top and right lines were counted. The tubules in contacted with the bottom and left lines were ignored. The Length density (L_v) of the each tubular structure was calculated as:

$$L_v = 2\Sigma Q / \Sigma P \times (a/\text{frame})$$

where, ΣQ denotes the total number of the tubules counted per kidney, (a/frame) equals the area associated with a frame, and " a/frames " is the total number of frames counted. Finally, the total

length of each tubule in the kidney, L , was estimated by multiplying the Length density (L_v) by the final volume of the kidney.

Statistical analysis: The results were analyzed using Mann-Whitney U test. p-value less than <0.05 was considered as statistically significant.

RESULTS

Histopathological study: Histological examination of the kidney in the CON group, as expected, was showed an entirely normal histological feature (Fig. 1). In the GM-group in marked swelling, vacuolization and necrosis of the epithelial cells of the PT, DT, HT and CT. However, these changes were more than those in PCT. The lumen of the tubules was filled with hyaline casts and desquamation epithelial cells. Glomeruli were hypertrophied (Fig. 2). Treatment with the extract of kidney in the GME group was showed no changes in the structure of the kidney tubules as compared with the GM group (Fig. 3).

Biochemical assay: Figure 4 shows that the BUN and Cr of the serum in the GM group was significantly higher than in the CON group ($p < 0.05$) while BUN and Cr in the GME group had no significant changes compared as GM group. It is possible that the extract does not decrease quantities of BUN and Cr.

Stereological examination: The length of PT, DT, HT and CT in different groups is shown in Fig. 5. According to these results the length of PCT was decreased in the GM group as compared to the CON group; however, the length of DT, HT and CT was the same between the GM and CON

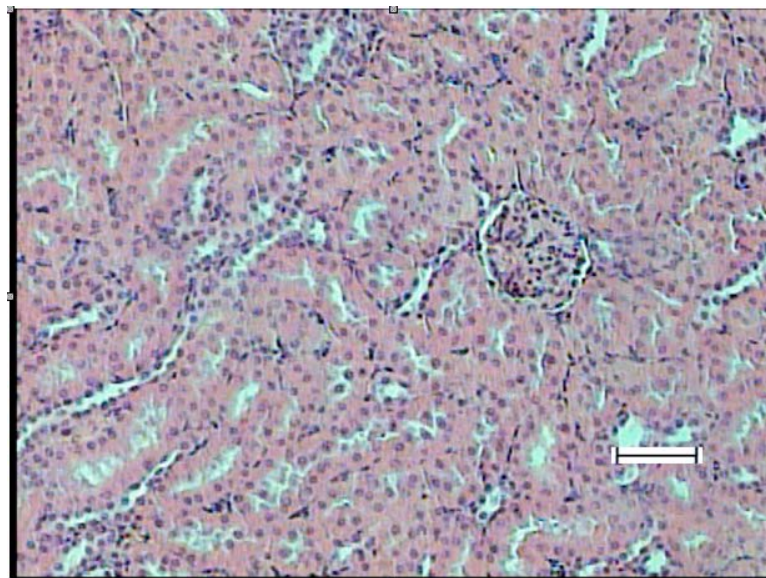


Fig. 1: Histological structure of kidney in control group. Tubules of kidney are normal. Scale bar = 50 μm

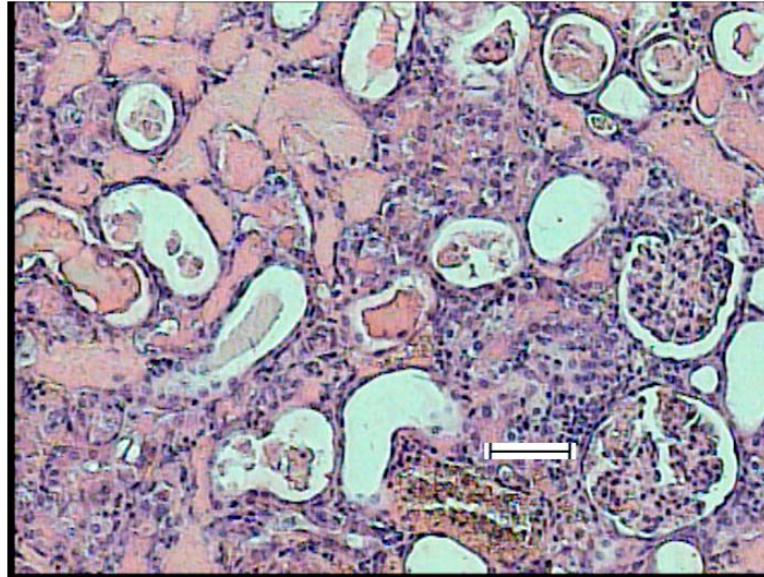


Fig. 2: Structure of kidney cortex in gentamicin group. Epithelial cells necrosis was marked in proximal tubules. Tubules of kidney were filled with hyaline casts. Scale bar = 50 μ m

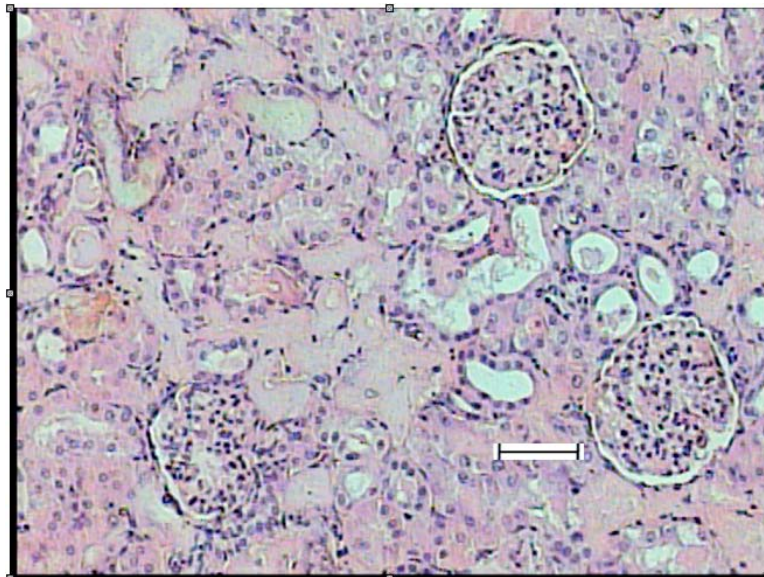


Fig. 3: Structures of kidney cortex were the same in gentamicin plus extract group with gentamicin group. Scale bar = 50 μ m

groups. The length of these tubules in the GME group compared as GM group had no significant changes. It seems that this extract has no effect on the renal tubular length.

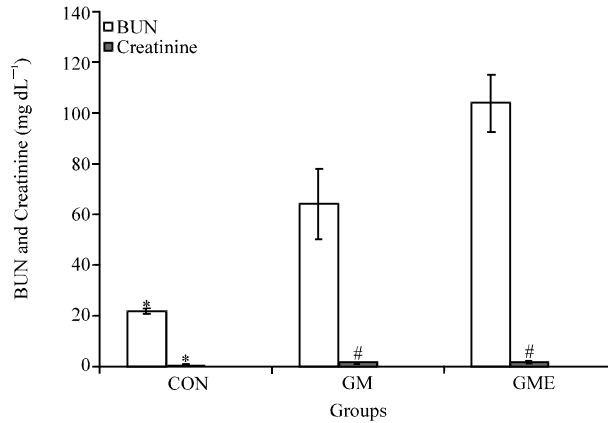


Fig. 4: Rate of BUN and cratinin in different experimental groups. *Significantly compared as control group ($p \leq 0.05$)

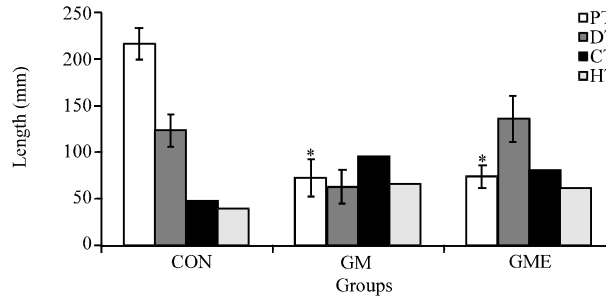


Fig. 5: Length measurement of PT, DT, CT, HT in different experimental groups. *Significantly compared as control group ($p \leq 0.05$)

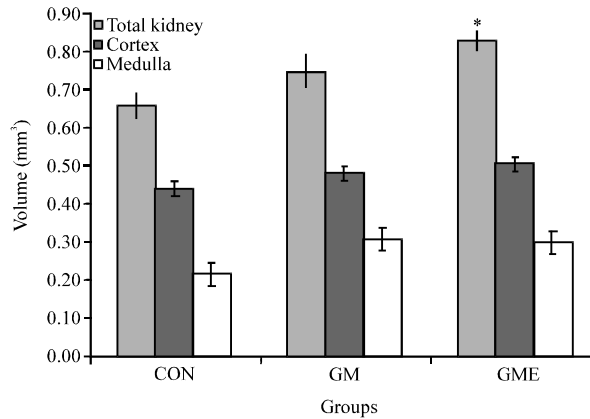


Fig. 6: Amount of volumes of total kidney, cortex and medulla in different experimental groups. *Significantly compared as control group ($p \leq 0.05$)

Figure 6 shows that the total volume of the kidney and volumes of the cortex and medulla did not change in the GM group in comparison with the CON group. These parameters were same in the GME and GM groups.

DISCUSSION

Drugs are a common source of acute kidney injury. GM is a group of aminoglycosides that increase BUN and Cr. These factors are an important index of the kidney injury. In many cases, the kidney injury is accompanied with tubular cell necrosis (Atessahin *et al.*, 2003) and renal atrophy (Wu *et al.*, 2002; Rymgayllo-Katska, 1989). One of the most important indexes for measurement of atrophy is volume estimation. Research has shown that different drugs can cause changes in the volume of kidney. Chronic and acute doses of cisplatin increase the kidney volume about 25 and 42%, respectively (Zahiri *et al.*, 2003). Acute dose of Glycerol reduced the volume of kidney about 58% (Farzaneh *et al.*, 2002). The volume of the kidney increased approximately 25% in nandrolone decanoate treated mice in comparison with the CON group (Hoseini *et al.*, 2009). The results of study revealed that GM decreased the length of PCT but had no effect on DT, HT and CT. Histological studies in this research also showed that the most effect of cell necrosis was observed in the epithelial of PT. Other researches also confirm this result (Atessahin *et al.*, 2003; Parlakpınar *et al.*, 2005; Svara *et al.*, 2010). So, perhaps GM reduced the number of epithelial cell and length shortening of PT. According to this result, it was expected that the volume of cortex decrease but in this stereological study, it was found that GM had no effect on the cortex volume. Histological study in this research also showed that the amount of cast and desquamated epithelium in the PT has increased and the glomeruli were hypertrophied. So, it may be concluded that reduction of the cortex volume in PT can be compensated by excess cast and glomerular hypertrophied. In the all of the above, information about the amount of renal volume and kidney atrophy may be useful in the choice of drug dosage.

Several agents were suggested to prevent GM induced nephrotoxicity such as Dietary fish oil (Priyamvada *et al.*, 2008) Green tea (Abdel-Raheem *et al.*, 2010) paricalcitol (Park *et al.*, 2010) but they were not found suitable for clinical practice. In the early of 19 century, researches on the treatment of organ disorders used the extract of different organs. The material of hypothalamic extract inhibits progesterone secretion (Acheson and Leavitt, 1972). The parathyroid extract enhances the tubular calcium re-absorption (Widrow and Levinsky, 1962). The extract of the kidney when administrated to animals with experimental renal hypertension cause a decline in the blood pressure (Page *et al.*, 1941; Grollman *et al.*, 1942, 1940; Hamilton and Grollman, 1958). It seems that the extract of the kidney useful for the kidney injury. Our stereological study showed no significant differences between the kidney volume and volumes of the cortex and medulla in the GM and GME groups. The histological study also showed no structural changes between the two groups. Therefore, it seems that the extract of the kidney has no effect on the tubular necrosis. So despite the fact that the extracts of the kidney could reduce the pressure but they do not have any effect on GM induced nephrotoxicity. Probably, procedure of providing the extract has been important. Seemingly, different extracts contain different material or factor that could exert different effects. In addition, the type of animal which give the extract is also important. The extract of the rabbit's kidney injected into the rabbit caused a slight increase in the blood pressure. The extract of the dog's kidney injected into dog caused a fall in pressure and the extract of cat's kidney injected into cat caused rise in the pressure (Pearce, 1909). So, the type of animal from which the extract was prepared is important.

CONCLUSION

GM decreases the length of PT but it doesn't affect the length of DT, HT and CT. Additionally, GM has no effect on the total volume and volumes of the cortex and medulla. The extract of kidney has not healing effect on the structure of kidney in GM-induced nephrotoxicity.

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