

## EXPERIMENTAL RESEARCHES REGARDING THE MONTELUKAST INFLUENCE IN GENTAMICIN INDUCED ACUTE NEPHROTOXICITY

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### Abstract

We investigated the effects of montelukast (Mk) in acute kidney injury (AKI) experimentally induced by gentamicin (Ge). We used Wistar rats, distributed in 4 groups: group I (control): distilled water 0.5 mL/100 gbw p.o. 10 days; group II (Ge): Ge 80 mg/kgbw i.p. 7 days; group III (Ge + Mk): Ge 80 mg/kgbw i.p. 7 days and Mk 10 mg/kgbw p.o. 10 days; group IV (Mk): Mk 10 mg/kgbw p.o. 10 days. The following parameters: serum concentration of urea, creatinine, superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant status (TAS) and urinary proteins levels were determined before the experiment ( $I_0$ ) and after 3, 7 and 10 days, and the kidneys were anatomo-pathologically evaluated. After 7 days, urea and creatinine levels significantly ( $p < 0.001$ ) increased, while SOD, GPx and TAS significantly ( $p < 0.001$ ) decreased in Ge group, compared with  $I_0$  and with Ge + Mk, Mk, respectively with the control groups. Mk reduced the severity of renal lesions induced by Ge. The dose of 10 mg/kgbw of Mk revealed a protective renal effect on the experimental AKI induced by Ge.

### Rezumat

S-au investigat efectele montelukastului (Mk) în insuficiența renală acută (IRA) indusă experimental cu gentamicină (Ge). S-au folosit șobolani Wistar împărțiți în 4 grupuri: grup I (martor): apă distilată 0,5 mL/100 g corp/zi p.o. 10 zile; grup II (Ge): Ge 80 mg/kgc/zi i.p. 7 zile; grup III (Ge + Mk): Ge 80 mg/kgc/zi i.p. 7 zile și Mk 10 mg/kgc/zi p.o. 10 zile; grup IV (Mk): Mk 10 mg/kgc/zi p.o. 10 zile. S-au urmărit concentrațiile serice de uree, creatinină, superoxid dismutaza (SOD), glutation peroxidaza (GPx), capacitatea totală antioxidantă (TAS) și proteinele urinare înainte de experiment ( $I_0$ ) și după 3, 7 și 10 zile. Pentru evaluarea anatomopatologică s-au prelevat rinichii la 3, 7 și 10 zile. La lotul Ge, după 7 zile, ureea și creatinina au crescut semnificativ ( $p < 0,001$ ), iar SOD, GPx și TAS au scăzut semnificativ ( $p < 0,001$ ) comparativ cu  $I_0$  și loturile Ge + Mk, Mk, respectiv cu lotul martor. Mk a redus severitatea leziunilor morfologice renale induse de Ge. Mk în doză de 10 mg/kgc a avut efect protector la nivel renal pe modelul experimental de IRA indus de Ge.

**Keywords:** acute kidney injury (AKI), gentamicin, motelukast, nephrotoxicity

### Introduction

Acute kidney injury (AKI) represents a major clinical problem, affecting more than 5% of the hospitalized patients, with a very high mortality rate [2, 5, 26]. Literature data show that AKI can lead to long term complications, such as: exaggerated hypertensive response to angiotensin II, proteinuria, interstitial renal fibrosis [3, 16, 25]. For this reason the development of new therapies for the management of AKI remains a target of great interest.

Gentamicin (Ge) is an antibiotic from the class of aminoglycosides, widely used for its effects on gram-negative bacteria. The clinical use of Ge is limited by nephrotoxicity, an important adverse effect representing 10-20% of all cases of AKI [14].

The toxic mechanism of gentamicin on kidney is mainly due to the induction of reactive oxygen species (ROS), which stimulate the activation of proapoptotic and proinflammatory mediators [13, 15, 20]. Montelukast (Mk), a selective CysLT1 leukotriene receptor inhibitor, is being known for its anti-inflammatory and antioxidant properties. Recent literature data show that Mk plays an essential role in removing free radicals, balancing the antioxidant status and regulating the generation of inflammatory mediators [22, 23, 24].

### Materials and Methods

The experiment was carried out on Wistar male rats (210 - 320 g weight). During the experiment the animals were housed in metabolic cages, at a

temperature of 22 - 24°C, with free access to water and standard granulated food. The rats were distributed in 4 groups of 12 animals each, as following: group I (control) received distilled water p.o. 0.5 mL/100 gbw daily for 10 days, group II (Ge) received Ge 80 mg/kgbw/day i.p. daily for 7 days, group III (Ge + Mk) received simultaneously Ge 80 mg/kgbw/day i.p. daily for 7 days and Mk 10 mg/kgbw/day p.o. daily for 10 days and group IV (Mk) received Mk 10 mg/kgbw/day p.o. daily for 10 days.

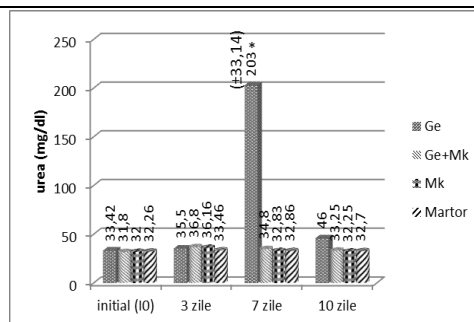
Before the beginning of the experiment ( $I_0$ ) and at 3, 7 and 10 days after the first dose of gentamicin, blood samples were collected from retro-orbital plexus, after the rats were anesthetized with halothane. Urine samples were also taken at the same time moments in the experiment. Using diagnostic kits RANDOX and Rx Daytona analyser, the following biochemical parameters: blood concentration of urea, creatinine, erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status (TAS) and urinary proteins levels were determined.

For the histopathological evaluation, at 3, 7 and 10 days in the experiment, 3 rats of each group were anesthetized and both kidneys were removed. The tissue samples were processed using haematoxylin-eosin staining and were examined by optic microscopy using Nikon Eclipse 50i device. The following changes were quantified: degeneration and necrosis of renal tubules, interstitial inflammation, accumulation of calcium and proteins in renal tubules, regeneration. It was used a lesion severity scale from 1 to 4: (1) – mild damage (< 25%), (2) – medium damage (25 - 50%), (3) – moderate damage (50 - 75%), (4) – severe damage (> 75%).

The obtained data was statistically analysed using one-way ANOVA test and t-student test. The values  $p < 0.05$  were considered statistically significant. The study protocols were approved by the Ethics Committee of Research of "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, in accordance with the Law of Research no. 206/2004, as well with the actual legislation of the European Union.

## Results and Discussion

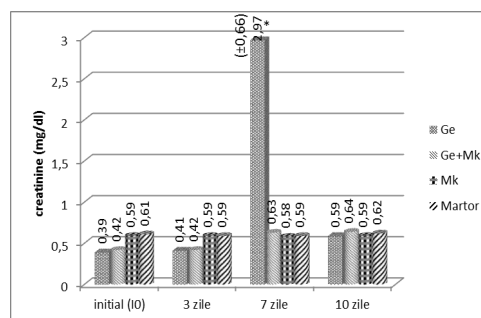
The biochemical analysis revealed that the serum concentrations of urea and creatinine increased significantly 7 days after the Ge administration ( $p < 0.001$ ). At the same time, there were not registered any significant variations of urea and creatinine levels in the group treated simultaneously with Mk and Ge, compared to initial values (Figure 1 and Figure 2).



**Figure 1.**

Variation of serum urea (mg/dL) in Ge, Ge + Mk, Mk groups compared to control group

\* $p < 0.001$  vs.  $I_0$

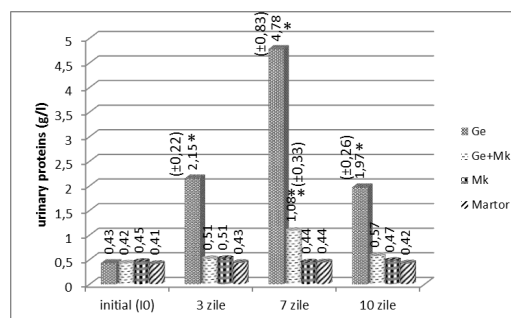


**Figure 2.**

Variation of serum creatinine (mg/dL) in Ge, Ge + Mk, Mk groups compared to control group

\* $p < 0.001$  vs.  $I_0$

The urinary proteins levels increased significantly in Ge group at 3, 7 and 10 days, compared with  $I_0$ . In Ge + Mk group, the level of urinary proteins was also increased vs.  $I_0$ , but the elevation was significantly lower than in the Ge group (Figure 3).

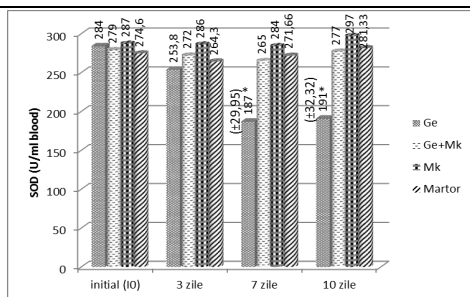


**Figure 3.**

Variation of urinary proteins (g/L) in Ge, Ge + Mk, Mk groups compared to control group

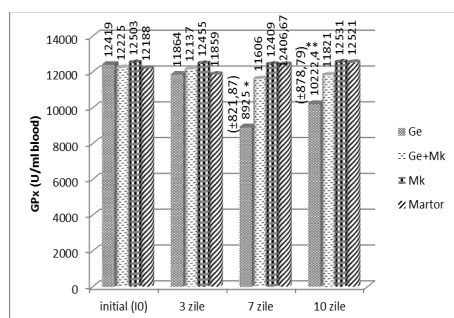
\* $p < 0.001$  vs.  $I_0$ ; \*\*  $p < 0.001$  vs. Ge at 7 days

The values of SOD and GPx decreased significantly at 7 and 10 days in the group treated with Ge, compared to the initial  $I_0$  determination. During the experiment, there were observed no significant variations in the activity of antioxidant enzymes in Ge + Mk group (Figure 4 and Figure 5).

**Figure 4.**

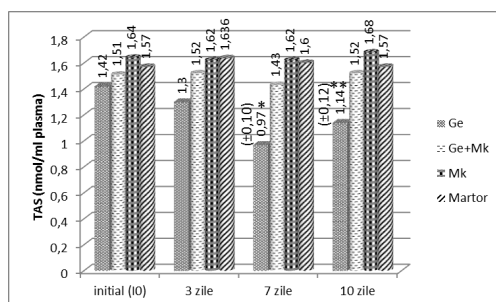
Variation of erythrocyte SOD (U/mL blood) in Ge, Ge + Mk, Mk groups compared to control group

\* $p < 0.001$  vs.  $I_0$

**Figure 5.**

Variation of GPx (U/mL blood) in Ge, Ge + Mk, Mk groups compared to control group

\* $p < 0.001$  vs.  $I_0$ ; \*\* $p < 0.01$  vs.  $I_0$

**Figure 6.**

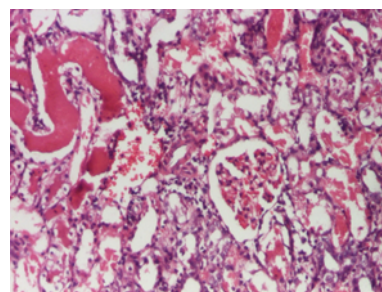
Variation of plasmatic TAS (nmol/mL plasma) in Ge, Ge + Mk, Mk groups compared to control group

\* $p < 0.001$  vs.  $I_0$ ; \*\* $p < 0.05$  vs.  $I_0$

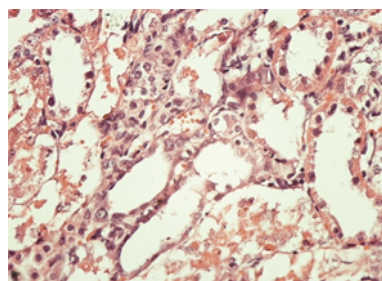
The total antioxidant status (TAS) in Ge group decreased significantly at 7 and 10 days compared to  $I_0$ . There were observed no significant variations

of TAS in Ge + Mk group, compared to  $I_0$  determination (Figure 6).

The histopathological evaluation revealed a normal kidney morphology in both Mk and control groups. After 7 days of treatment, it was noticed the degeneration and the presence of necrosis (3<sup>rd</sup> degree) zones, localized mostly in the proximal and distal renal tubules, associated with areas of inflammation (3<sup>rd</sup> degree), in the group treated with Ge (Figure 7). At the same time, in the Ge + Mk group, there were identified degeneration and necrosis areas, but with a milder degree of severity compared to those observed in the Ge group (Figure 8). Moreover, the inflammatory processes were found in smaller areas (1<sup>st</sup> degree), associated with regenerative modifications (3<sup>rd</sup> degree) in the proximal and distal tubules (Table I).

**Figure 7.**

Group Ge 7 days, col. HE, x100. Necrosis and degeneration (3<sup>rd</sup> degree), inflammation (3<sup>rd</sup> degree), hyaline casts.

**Figure 8.**

Group Ge + Mk 7 days, col. HE, x40. Necrosis and degeneration (2<sup>nd</sup> degree), inflammation (1<sup>st</sup> degree), regeneration (3<sup>rd</sup> degree).

**Table I**

Quantitative analysis of histopathological changes in Ge, Ge + Mk, Mk groups compared to control group after 7 days

| Group    | n | tubular necrosis |   |   |   |   | tubular degeneration |   |   |   |   | inflammation |   |   |   |   | regeneration |   |   |   |   |
|----------|---|------------------|---|---|---|---|----------------------|---|---|---|---|--------------|---|---|---|---|--------------|---|---|---|---|
|          |   | 0                | 1 | 2 | 3 | 4 | 0                    | 1 | 2 | 3 | 4 | 0            | 1 | 2 | 3 | 4 | 0            | 1 | 2 | 3 | 4 |
| Control  | 6 | 6                | 0 | 0 | 0 | 0 | 6                    | 0 | 0 | 0 | 0 | 6            | 0 | 0 | 0 | 0 | 6            | 0 | 0 | 0 | 0 |
| Ge       | 6 | 0                | 2 | 0 | 4 | 0 | 0                    | 0 | 0 | 6 | 0 | 0            | 0 | 2 | 4 | 0 | 0            | 0 | 0 | 0 | 0 |
| Ge + Mk* | 6 | 0                | 1 | 2 | 3 | 0 | 0                    | 0 | 3 | 3 | 0 | 3            | 3 | 0 | 0 | 0 | 3            | 0 | 0 | 3 | 0 |
| Mk       | 6 | 6                | 0 | 0 | 0 | 0 | 6                    | 0 | 0 | 0 | 0 | 6            | 0 | 0 | 0 | 0 | 6            | 0 | 0 | 0 | 0 |

n – number of examined sections

(1) – mild damage (< 25%), (2) – medium damage (25 - 50%), (3) – moderate damage (50 - 75%), (4) – severe damage (> 75%)

\* $p < 0.05$  vs. Ge

The understanding of aminoglycoside nephrotoxicity is clinically important and the investigation based on the use of experimental animal models may lead to a better knowledge of cellular mechanisms of renal injury. Gentamicin-induced oxidative stress may induce renal vasoconstriction and the decreasing of glomerular filtration rate [13, 17]. In our study, the i.p. administration of 80 mg/kgbw Ge, for 7 days was associated with an increase of serum urea and creatinine, compared to control group ( $203 \pm 33.14$  mg/dL vs.  $32.86 \pm 4.62$  mg/dL for urea, respectively  $2.97 \pm 0.66$  mg/dL vs.  $0.59 \pm 0.05$  mg/dL for creatinine), after 7 days of treatment. Simultaneous oral administration of 10 mg/kgbw Mk for 10 days resulted in a decrease of renal impairment induced by Ge, evidenced by the reduction of serum urea and creatinine levels ( $34.8 \pm 6.49$  mg/dL vs.  $203 \pm 33.14$  mg/dL for urea, respectively  $0.63 \pm 0.06$  mg/dL vs.  $2.97 \pm 0.67$  mg/dL for creatinine) at the same time of determination. Similar results were reported by other authors [11, 22]. Mk, a selective reversible cys-leukotriene-1 (CysLT1) receptor antagonist, possesses antioxidant properties [9], and it is a well-known anti-asthmatic drug, reducing airway eosinophilic inflammation. Literature data also show that the CysLT1 antagonist has an anti-ulcer activity, being beneficial in various inflammatory bowel diseases [6, 28], as well as a protective effect in burn-induced oxidative injury of the skin [21]. Mk has also partially protective activity in the experimental intoxication with carbon tetrachloride [7]. Some recent studies highlight that Mk is a potent free radical scavenger, balancing oxidant-antioxidant status and regulating the generation of inflammatory mediators [13, 24]. Mk treatment antagonizes the CysLT1 receptors and could decrease cytokine production and renal lesions in nephrotoxicity induced by Ge.

The Ge induced nephrotoxicity was associated with a low activity of antioxidant enzymes [22, 27]. The results of the present study showed that in rats treated with Ge, an acceleration of lipid peroxidation and a reduction of antioxidant enzymes activity (with a decreasing of SOD, GPx and TAS levels, compared with initial values  $I_0$  and with control group) occur. It is known that Mk inhibits neutrophil infiltration and lipid peroxidation [19]. Our study demonstrated that Mk administration prevented the decrease of the antioxidant enzymes (SOD, GPx) activity and TAS in Ge + Mk group, compared to Ge group. After 7 days of treatment, the level of SOD in Ge + Mk group compared to Ge group was  $265 \pm 27.72$  U/mL vs.  $187 \pm 29.95$  U/mL, suggesting a protective role of Mk. The obtained data is consistent with other existing studies in the literature [4].

According to biochemical changes that sustain the antioxidant effect of Mk, the morphological

evaluation revealed that Mk also was efficient in protecting the kidney against injury induced by Ge. Our data are consistent with other results on Mk effects in experimental acute renal failure [5, 12]. In Ge group there were produced important structural changes in the kidney, with cortical damage, tubular epithelium alteration, degeneration (3<sup>rd</sup> degree of severity), necrosis (3<sup>rd</sup> degree), tubular dilations and protein accumulation at the tubules level. Similar modifications have been reported in other studies that investigated the histopathological alterations of renal tissue in animals treated with Ge [8, 18]. These morphological disturbances may be due to the formation of reactive radicals, as a consequence of the oxidative stress induced by Ge.

In our study the histopathological lesions observed in Ge + Mk group had a milder severity compared with those in Ge group (2<sup>nd</sup> degree for degeneration and necrosis, 1<sup>st</sup> degree for inflammation). Our obtained data are consistent with the information existing in other studies [12, 24].

## Conclusions

The administration of Mk 10 mg/kgbw p.o. for 10 days manifested a protective effect against the acute kidney failure induced by Ge. Mk diminished renal function impairment, limited the lipid peroxidation process and improved the severity of histopathological lesions induced by Ge.

## References

1. Balgradean M., Cinteza E., Filipoiu F., Jinga V., Gentamicin, infections and acute tubular necrosis in children. *Farmacia*, 2013; 61(4): 772-780.
2. Barrantes F., Tian J., Vasquez R., Prognosis of acute kidney injury. *Crit. Care Med.*, 2008; 36: 1397-1403.
3. Basile D., Donohoe D., Roethe K., Osborn J., Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. *Am. J. Physiol.*, 2001; 281: F887-F889.
4. Bathia Lovkesh, Bathia Vivek, Grover Manav, Nephroprotective effect of fresh leaves extracts of *Sida Cordifolia* Linn in gentamicin induced nephrotoxicity in rats. *Intern. J. Res. in Pharmacy and Science*, 2012; 2(2): 151-158.
5. Buzoianu E., Moiceanu M., Cora F., Plesca D.A., The influence of controller therapy on correlation between feno and asthma control in children. *Farmacia*, 2015; 63(2): 196-200.
6. Cuciureanu M., Caruntu I.D., Kuchar M., Nechifor M., The influence leukotriene receptors antagonist on experimentally induced ulcer in rats. *Rev. Med. Chir.*, 2008; 112: 750-756.
7. Cuciureanu M., Caruntu I.D., Paduraru O., Stoica B.A., Jerca L., Crauciuc E., The protective effect of Montelukast sodium on carbon tetrachloride

- induced hepatopathy in rat. *Prostaglandins Other Lipid. Mediat.*, 2009; 88: 82-88.
8. Erdem A., Gundogan N.U., Usubutun A., The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. *Nephrol. Dial. Transplant*, 2000; 15: 1175-1182.
9. Ersahin M., Cevik O., Akakin D., Sener A., Ozbay L., Yegen B.C., Sener G., Montelukast inhibits caspase-3 activity and ameliorates oxidative damage in the spinal cord and urinary bladder of rats with spinal cord injury. *Prostaglandins Other Lipid. Mediat.*, 2012; 99(3-4): 131-139.
10. Funk J.A., Schnellmann R.G., Persistent disruption of mitochondrial homeostasis after acute kidney injury. *Am. J. Physiol. Renal Physiol.*, 2012; 302(7): F853-864.
11. Iliescu R., Hancianu M., Reckelhoff J.F., Testosterone supplements accelerate progression of kidney injury in a rat model of reduced renal mass. *Farmacia*, 2014; 62(1): 119-128.
12. Kose E., Beytur A., Dogan Z., Ekincioglu Z., Vardi N., Cinar K., Turkoz Y., Soysal H., Ekinci N., The effects of montelukast against amikacin-induced acute renal damage. *Eur. Rev. Med. Pharmacol. Sci.*, 2012; 16(4): 503-511.
13. Lopez-Novoa J.M., Quiros Y., Vicente L., New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney International*, 2011; 79: 33-45.
14. Mathew T.H., Drug-induced renal disease. *Med. J. Aust.*, 1992; 156:724-728.
15. McWilliam S.J., Antoine D.J., Sabbisetti V., Turner M.A., Farragher T., Bonventre J.V., Park K.B., Smyth R.L., Pirmohamed M., Mechanism-based urinary biomarkers to identify the potential for aminoglycoside-induced nephrotoxicity in premature neonates: a proof-of-concept study. *PLOS ONE*, 2012; 7(8): e43809.
16. Metha R.L., Pascual M.T., Soroko S., Spectrum of acute renal failure in the intensive care unit: The PICARD experience. *Kidney Int.*, 2004; 66: 1613-1621.
17. Mingeot-Leclercq M.P., Tulkens P.M., Aminoglycosides: nephrotoxicity. *Antimicrob. Agents Chemother.*, 1999; 43: 1003-1012.
18. Muthuraman A., Singla S.K., Rana A., Singh A., Sood S., Renoprotective role of flunarizine (mitochondrial permeability transition pore inactivator) against gentamicin induced nephrotoxicity in rats. *Yakugaku Zasshi*, 2011; 131: 437-443.
19. Padmini M.P., Kumar J.V., A histological study on gentamycin induced nephrotoxicity in experimental Albino rats. *IOSR J Dental&Medical Sciences*, 2012; 1(1): 14-17.
20. Quiros Y., Vicente-Vicente L., Morales A.I., An integrative overview on the mechanisms underlying the renal tubular cytotoxicity of gentamicin. *Toxicol. Sciences*, 2011; 119(2): 245-256.
21. Sener G., Kabasakal L., Cetinel S., Contuk G., Gedik N., Yegen B., Leukotriene receptor blocker montelukast protects against burn-induced oxidative injury of the skin and remote organs. *Burns*, 2005; 31: 587-596.
22. Sener G., Sehirli O., Velioglu-Ogunc A., Montelukast protects against renal ischemia/reperfusion injury in rats. *Pharmacol. Res.*, 2006; 54(1): 65-71.
23. Tintinger G.R., Feldman C., Theron A.J., Anderson R., Montelukast: more than a cysteinyl leukotriene receptor antagonist? *Scientific World Journal*, 2010; 10: 2403-2413.
24. Tugtepe H., Sener G., Cetinel S., Velioglu-Ogunc A., Yegen B.C., Oxidative renal damage in pyelonephritic rats is ameliorated by montelukast, a selective leukotriene CysLT1 receptor antagonist. *Eur. J. Pharmacol.*, 2007; 557(1): 69-75.
25. Uchino S., Kellum J.A., Bellomo R., Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA*, 2005; 294: 813-818.
26. Waikar S.S., Curhan Gc., Wald R., Declining mortality in patients with acute renal failure. *J. Am. Soc. Nephrol.*, 2006; 17: 1143-1150.
27. Walker P.D., Barri Y., Shah S.V., Oxidant mechanism in gentamicin nephrotoxicity. *Ren. Fail*, 1999; 21: 433-442.
28. Wallace J., McKnight G.W., Keenan C.M., Byles N.I., MacNaughton W.K., Effects of leukotrienes on susceptibility of the rat stomach to damage and investigation of the mechanism of action. *Gastroenterology*, 1990; 98: 1178-1186.