Experimental Researches on the Effects of Some Antibiotics on Carrageenan-induced Rat Paw Inflamation

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The aim of our study was to evaluate the effects of some antibiotics on hepatic and renal integrity in rats with acute paw inflammation. Acute inflammation was induced after sub-plantar administration of carrageenan in rats. The substances: prednisone (PDN), indomethacin (IND), cefotaxime (CEF), gentamicin (GEN) and sulfamethoxazole (SMT) were injected intraperitoneally, 30 min before carrageenan administration. Saline was intra-paw administered in control group. Prednisone and indomethacin were injected in a single daily dose, and the antibiotics every 12 hours, during 3 days. Specific biochemical parameters, liver and kidney tissues were examined after 3, 24 and 72 hours. Experimental protocol was conducted according to the recommendation of our University Committee for Research and Ethical Issues. The use of GEN, SMT and CEF resulted in exacerbation of hepatic enzymes activity, increasing in serum creatinine, blood urea and uric acid levels, statistically significant comparing with control group. Histopathological examination revealed the alteration of liver and kidney architecture in groups treated with GEN and SMT. The effects of GEN were more accentuated than those of SMT and CEF. The treatment with GEN and SMT manifested hepatic and renal toxicity in rats with experimental carrageenan-induced acute paw inflammation.

Keywords: cefotaxime, gentamicin, sulfamethoxazole, hepatic, real, toxicity

The efficacy of antimicrobial therapy is determined by both pharmacokinetics and pharmacodynamics effects. The toxicity of antibacterial antibiotics was mainly associated with high doses, long-term administration, the drug associations or the person's allergic terrain [1-2] . It is known that some anti-infective agents produce a series of specific side effects, which can be particularly severe. A side effect of an antibacterial drug is defined as an unwanted reaction that occurs in addition to its desired therapeutic action and which, in most cases interferes with the patient's ability to tolerate the compound by requiring discontinuation of treatment [2-3]. The manifestation of these side effects is a concern not only because they cause host injuries, but also because interrupt or complicate therapy and can also contribute to excessive medical costs [1, 4-5].

Antibacterial and antifungal compounds are the therapeutic agents most commonly associated with hepatotoxicity [6,18], effects mainly due to the large prescription and the non-medical use of these drugs [8,10]. Hepatotoxicity induced by the antibiotics is usually asymptomatic, transient and associated only with mild hepatic impairment. In rare cases, however, significant morbidity, the need for liver transplantation and even death by acute liver failure have been reported [11-13].

Literature data highlights that hepatotoxicity induced by antibacterial drugs appears to be of an idiosyncratic nature in most cases and is due to an immunological reaction, or is a response to the presence of hepatotoxic metabolites [14,8,19].

We aimed to evaluate the effects of some antibacterial chemotherapeutic drugs (from the groups of cephalosporins, aminoglycosides, sulfonamides) on the liver and renal function in rats with carrageenan-induced paw inflammation.

For the experiments, white male Wistar rats (200-250g) from the bio-base of the Grigore T. Popa University of Medicine and Pharmacy Iasi were used. In order to acclimate to the experimental environment, the animals were housed in the laboratory the day before, being maintained under standard conditions (with constant temperature of 21 ± 2° C, relative humidity of 50-70% and alternating illumination regimen 12 /12 h of light/dark ratio)[15]. Rats received standard granulated feed and water *ad libitum*, except for the day the studies were conducted. To avoid the chronobiological influences, experimental research took place between 8-14 a.m.

The substances used: carrageenan, prednisone, indomethacin, cefotaxime, gentamicin, and sufamethoxazole were obtained from Sigma-Aldrich,

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Chemical Co, Germany. The solutions were prepared extemporaneously and heated to 36.7°C prior to injection.

The animals were randomly assigned into 7 groups of 6 rats each, treated intraperitoneally, according to the following protocol: Group 1 (Control): rats with intra-paw injection of saline - control group without inflammation. For the following groups, carrageenan-induced paw inflammation was induced. Group 2 (SS): saline solution - 0.3 mL/100g body - control group with inflammation; Group 3 (PDN): prednisone 5 mg/kbw/day; Group 4 (IND): indomethacin 150 mg/kbw/day; Group 5(CEF): cefotaxime 200 mg/kbw/day; Group 6 (GEN): gentamicin 20 mg/kbw/day; Group 7 (SMT): sulfamethoxazole 250 mg/kbw/day.

The positive control drugs in the experiment: prednisone (corticosteroid with anti-inflammatory and immunosuppressive effects), and indomethacin (non-steroidal anti-inflammatory with strong anti-inflammatory effects) were administered in a single daily intraperitoneal dose of 5 mg/kg body weight, respectively of 150 mg/kg body weight for 3 days[16-17]. The chemotherapeutic drugs were administered every 12 h intervals for 3 days. Saline solution was used as control drug in the experiment.

The standard model of acute rat paw inflammation induced after the local injection of an irritant compound was used. This test is a classic recognized method, a reproducible and useful experimental model, for evaluating the anti-inflammatory action of various substances, assessing the effects of these agents on the basis of their ability to decrease the evolution, or even prevent the occurrence of acute inflammation of the laboratory animals paw's tissues [20-21].

30 min after the intraperitoneal injection of the investigated substances, 0.1 mL of 1% carrageenan in saline was administered subcutaneously into the plantar face of the left hind paw. Injection of this irritant agent induced after about an hour, an evident inflammation, local edema, which increased the paw volume. The administration of carrageenan into the animal's paw causes a biphasic response: a rapid inflammation, lasting 6 h, and a tardive reaction phase, with a maximum intensity of 72 h, and then decreasing in time to 96 hours [22-24].

The influence of the investigated substances on the liver and kidney function, was evaluated by the assessing of some specific biochemical parameters, and by histopathological examination.

Before the administration of the substances (M0 - moment zero), at 3 , 24 h and 72 days in the experiment, the animals were anesthetized with diethyl ether and venous blood (2 mL) was collected from the retroorbital plexus to determine: the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), respectively the serum levels of creatinine, urea and uric acid. At the end of the experiment the animals were euthanized under general anesthesia with enflurane, liver and kidney fragments were taken for histopathological investigation. The tissue fragments were fixed in 10% formaldehyde, embedded in *paraffin wax* and sectioned into 5μm *slices* using a laser microtome. The samples stained with Masson's trichrome were examined using a Nikon E600 Ti Eclipse inverted optical microscope, equipped with a digital camera to take the images.

The experimental studies were approved by the Ethic Committee on Research and Ethical Issues of our University, in agreement with the international ethical regulations, regarding the handling of laboratory animals [25-26].

The results were expressed as the mean of values ± the standard deviation (SD) of mean and statistically analyzed using SPSS program for Windows 10, version 17.0 and ANOVA method.

Results and discussions

The intra-plantar injection of 1% carrageenan induced a local inflammation revealed by increased paw volume, redness (observable in direct examination) and local pain (manifested by the licking animal's behavior) in saline solution group. Inflammation developed progressively, being maximally 6 h after the chemical irritant administration, thereafter it started to slowly diminish, but persisted for 72 h in the experiment.

The biochemical investigations revealed a statistically significant increase in ALT activity compared to the control group, for the groups treated with: PDN, IND, GEN, SMT at 3 h; PDN at 24 hours; PDN, CEF, GEN, SMT at 72 hours (fig. 1.A.). Substantial increases in serum AST activity, statistically significant compared to the group treated with saline, was also observed, for the groups that received: PDN, IND, GEN, SMT at 3 h; PDN at 24 h; GEN, SMT at 72 hours (fig. 1.B.).

Laboratory investigations have shown an increase in serum creatinine values, statistically significant comparing with control group, for PDN and GEN 3 and 24 h; and for PDN, IND and SMT 72 h after the acute inflammation occurred. No important changes in serum creatinine levels, between the IND, CEF, and the group treated with saline, were noted, throughout the experiment (fig. 2.).

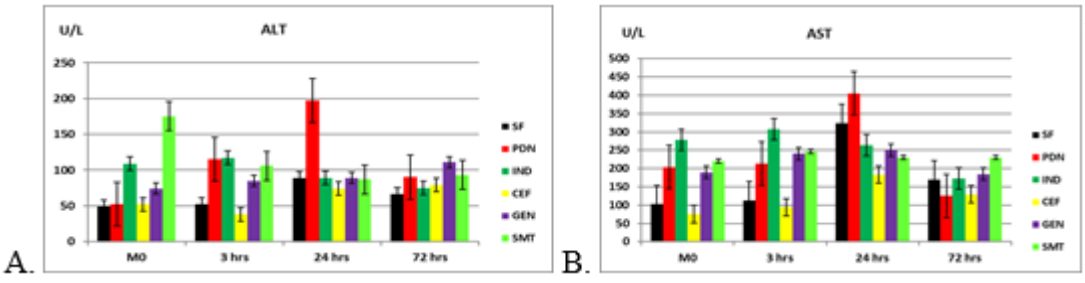

Fig. 1. The effects of CEF, GEN, SMT on the ALT (U/L) (A.) and AST (U/L) activity (B.) (data were expressed as arithmetic mean ± SD of mean values for 6 animals in a group)

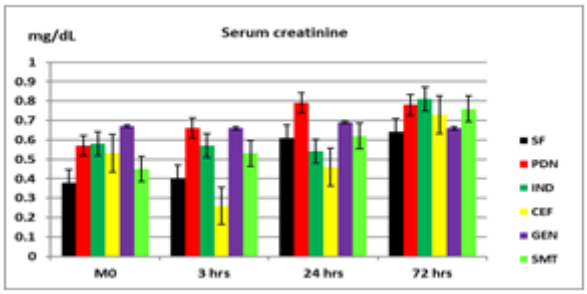

Fig. 2. The effects of CEF, GEN, SMT on the serum creatinine (mg/dL) levels (data were expressed as arithmetic mean ± SD of mean values for 6 animals in a group)

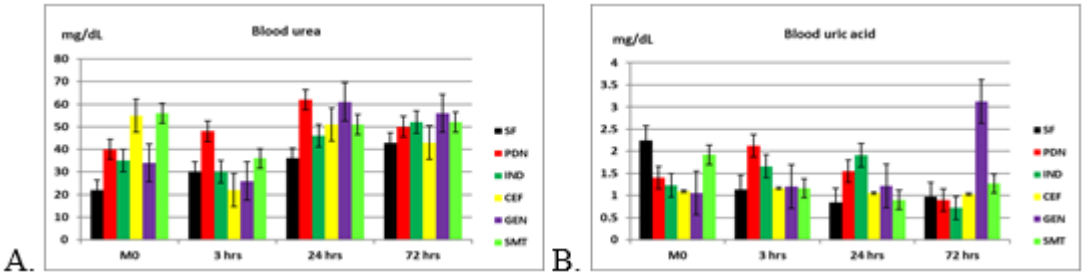

Fig. 3. The effects of CEF, GEN, SMT on the blood urea (mg/dL) and uric acid (mg/dL) levels (data were expressed as arithmetic mean ± SD of mean values for 6 animals in a group)

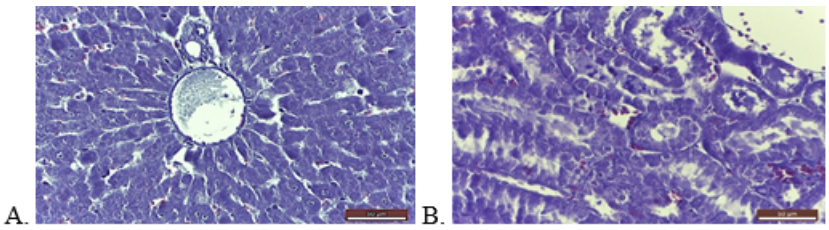

Fig. 4. The effects of the PDN on rat's liver (A) and renal (B) architecture (Masson's trichrome stain)

Substantial increases in blood urea levels, statistically significant compared to control, were detected for the groups treated with: PDN at 3 h; PDN, CEF, GEN, SMT at 24 hours; GEN at 72 h in this experimental-induced paw inflammation test in rats (fig. 3.A.). Important increases in plasma uric acid values, statistically significant compared to the group treated with saline solution, were also evidenced, for PDN and IND at 3 h; for PDN, IND, CEF and GEN at 24 h; GEN and SMT at 72 h in the experiment (fig. 3.B.).

No pathological aspects were noted in any of the examined organs (liver, kidneys) that were taken from animals in the control group.

The histopathological examination of tissue samples (liver, kidney) taken from animals receiving the PDN, revealed a slight change in liver structure and a moderate alteration of renal architecture (fig. 4.A., fig. 4.B.);

The treatment with IND has been associated with severe hepatic degeneration, alteration of the hepatocytes structure, activation of Kupffer cells and moderate congestion of sinusoidal capillary (fig. 5.A.). The renal structure was harshly modified, manifested by disruption of the normal renal histology, with total or partial desiccation of urinary tubular epithelium. Devitalized neutrocytes with the hypochromic cytoplasm are detached from the tubular epithelium (fig. 5.B.).

Intraperitoneal injection of CEF did not induce noticeable changes in the liver architecture (fig. 6.A.). Histologic kidney examination revealed the presence of low to moderate toxic nephritis, as evidenced by minor nephrocytes alterations (fig. 6.B.).

The administration of GEN resulted in severe hepatitis, proved by important hepatocyte degeneration. The liver cells have fragmented membranes, disorganized and vacuolized cytoplasm, and hyperhydrated nuclei (Fig. 7.A). The microscopic kidney evaluation showed the congestion of glomerular capillaries and severe necrosis of the epithelium of the urinary tubes. In the proximal segment, the integrity of the tubular epithelium is seriously altered. The hypochromic cytoplasm and the cellular residues in the tubular lumen are also observed. Some nephrocytes still adherent to the basal lamina are swollen, hyperhydrated, with intracytoplasmic protein filaments and centrally located hypercalcemic nuclei. The lesions fall into a severe toxic tubulo-nephritis (fig. 7.B.).

The use of SMT determined extensive liver damage, characterized by the detachment of hepatocytes from the Remak's horns. The disconnected cells are isolated, polygonal, with dark cytoplasm and picnic nuclei (fig. 8.A.). Moderate degeneration of proximal tubular epithelium was noted, manifested by partial nephrocyte desquamations

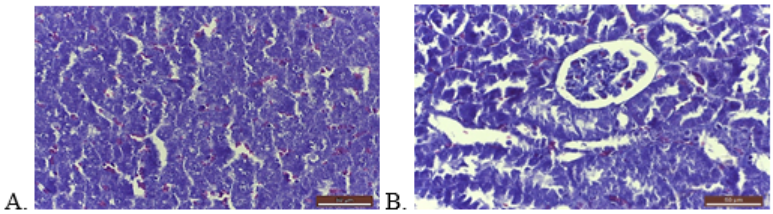

Fig. 5. The effects of the IND on rat's liver (A) and renal (B) architecture (Masson's trichrome stain)

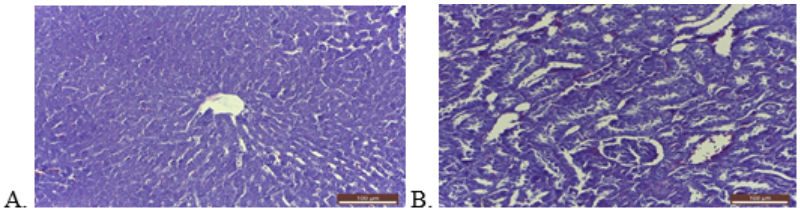

Fig. 6. The effects of CEF on rat's liver (A) and renal (B) architecture (Masson's trichrome stain)

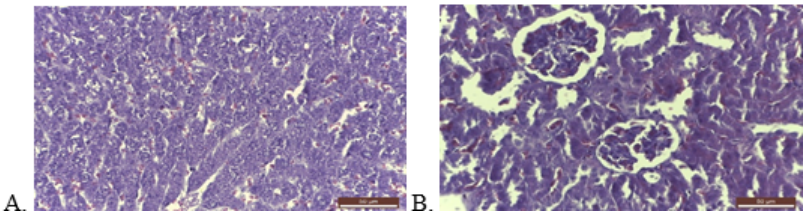

Fig. 7. The effects of GEN on rat's liver (A) and renal (B) architecture (Masson's trichrome stain)

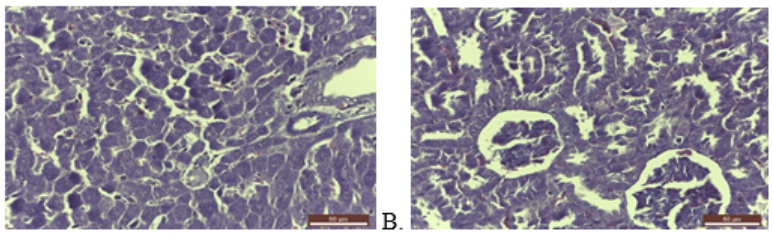

Fig. 8. The effects of SMT on rat's liver (A) and renal (B) architecture (Masson's trichrome stain)

and slightly retracted glomeruli with a tendency to segregate glomerular capillaries.

The action of the antibiotic in the body is not limited to the destruction of the etiologic agent, but unfortunately it is accompanied by various important side effects, with a much higher risk versus benefit, becoming an important aspect in clinical trials [9]. The use of some anti-infective agents has been associated with a number of particular adverse effects, ranging from mild to very severe, depending on the drug used, the targeted bacteria and the patient [10]. The safety profiles of newer anti-infective agents, are often not as well established compared to those with long-term use.

In the occurrence of adverse events attributable to anti-infective agents are, particularly involved, three main mechanisms: the exaggerated response to the known pharmacological effects of drugs, immunological reactions to the compound or its metabolites, and the toxic effects of them [8]. The most of the adverse reactions to antibiotics are precipitated by an exaggeration of the known pharmacodynamic effects of drugs and are often avoided by the appropriate dose adjustment [27]. In addition to the direct influence of anti-infective agents, however, many host factors (genetic constitution, integrity of drug removal mechanisms, associated pathologies), may affect the frequency and severity of adverse events related to their administration [28].

Antibiotics act preferentially, are transformed and removed from the body, but also have important side effects (nephrotoxicity, hepatoxicity, cardiotoxicity). Despite their particular toxicity, antibiotics can cause serious side effects, mainly due to poor metabolism of the drug, or excessive dosing regimen [29-31].

We investigated the influence of different chemotherapeutic antibacterial drugs on liver and kidney function and structure, in rats with acute experimental-induced paw inflammation. The local administration of 1% carrageenan caused a characteristic acute inflammation (manifested by the presence of inflammatory exudates rich in neutrophils infiltration, the presence of increased concentrations of nitrite / nitrate ions: NO2- / NO3- and a high level of prostaglandin E2), after approximately one hour, with edema, which produced swelling of the paw, erythema and hyperalgesia [8]. These specific clinical manifestations of acute inflammation, produced shortly after the intravenous administration of the irritant agent, are induced by the action of the pro-inflammatory compounds: reactive species of oxygen, histamine, bradykinin, tachykinins, complement, hydrogen ions and nitrogen species [32-33].

The administration of GEN and SMT was accompanied by liver and kidney toxicity, proved by the increase in serum creatinine values, respectively in blood urea and uric acid levels, and also, by typical histopathological alteration of the normal liver and renal aspect. GEN induced the most accentuated liver and kidney damages, and CEF only a slight functional and structural modifications.

Our results are consistent with the communicated data, revealing a diminution of the glomerular filtration rate, the increase in glomerular sizes with alteration in morphology and density, the increase of cell apoptosis and necrosis, after treatment with high doses of gentamicin [34]. The aminoglycoside-induced lesions and their precise mechanism at the proximal renal tubular cells, are not fully understood, being suggested: the destruction of epithelial tubular cells, especially in the proximal segment, in associated with an important inflammatory process, and the functional alteration of cellular components, involved in the transport of water and electrolytes [35-36].

Conclusions

In our experimental condition the treatment with gentamicin and sulphamethoxazole induced liver and kidney injury proved by the specific blood parameters disturbances and by characteristic disturbances in hepatic and renal architecture.

References

1.GRIGORYAN L, BURGERHOF JG, DEGENER JE, DESCHEPPER R, et al, Pharmacoepidemiol Drug Saf, 2007, 16(11): 1234-1243.

2.LIU M, HINZ ERM, MATHENY ME, et al, J Am Med Inform Assoc, 2013, 20(3): 420-426.

3.KOURKOUTA L, KOTSIFTOPOULOS CH, PAPAGEORGIOU M, ILIADIS CH, MONIOS A, The Rational Use of Antibiotics Medicine, J Health Commun, 2017, 2: 36.

4.SARBU, I., VASSU, T., CHIFIRIUC, M.C., et al, Rev Chim (Bucharest), **68**, no.12, 2017, p. 3015

5.BARRIERE SL, Expert Opin Pharmacother, 2015, 16(2): 151-3.

6.CHIRIAC, P.C., POROCH, V., et al, Rev Chim (Bucharest), **69**, no.4, 2018, p.915

7.KYRIAKIDIS I, TRAGIANNIDIS A, et al, Expert Opin Drug Saf, 2017, 16(2): 149-165.

8.ROBLES M, TOSCANO E, et al, Curr Drug Saf, 2010, 5(3): 212-22.

9.AMINOV RI, Front Microbiol, 2010, 1: 134.

10.BJORNSSON ES, Scand J Gastroenterol, 2017, 52(6-7): 617-623.

11.STINE JG, LEWIS JH, 2013, 17(4): 609-42.

12.PANDIT A, SACHDEVA T, et al, J Appl Pharm Sci, 2012, 2(5): 233-243.

13.POLSON JE, Clin Liver Dis, 2007, 11(3): 549-561.

14.NJOKU DB, Int J Mol Sci, 2014, 15: 6990-7003.

15.PINZARIU, A.C., PASCA, S.A., SINDILAR, A.,DROCHIOI, C., BARAN, M., OBOROCEANU, T., NICULESCU, S., CRAUCIUC, D.V., CRAUCIUC, E.G., LUCA, A., BUTCOVAN, D., SINDILAR, E.V., MOCANU, V., Rev Chim (Bucharest), **68**, no.9, 2017, p.2139

16.CONG HH, KHAZIAKHMETOVA VN, ZIGASHINA LE, Int J Risk Saf Med, 2015, 27 Suppl 1: S76-7.

17.SMIT HF, KROES BH, JVAN DEN BERG AJ, et al, J Ethnopharmacol, 2000, 73(1-2): 101-109.

18.BUTCOVAN, D., LUPUSORU, C.E., BARAN, D., et al, Rev Chim (Bucharest), **67**,no.10, 2016, p. 2012-2014.

19.TEUSAN, A., LUPUSORU, R.V., JELIHOVSCHI,I., et al. Rev Chim (Bucharest), **67**,no.3,2016, p. 476

20.FEHRENBACHER JC, VASKO MR, DUARTE DB, Curr Protoc Pharmacol, 2012, 0 5: Unit 5.4., 1-11.

21.POSADAS I, BUCCI M, ROVIEZZO F, et al, Br J Pharmacol, 2004, 142(2): 331-338.

22.NECAS J, BARTOSIKOVA L, Vet Med (Praha), 2013, 58(4): 187-205

23.KOKSAL, M., OZKAN, I., YARIM, M., BILGE, S.S., BOZKURT, A., EROL, D.D., Rev Chim.(Bucharest), **62**,no.11, 2011, p.1069

24.CHOPADE AR, SAYYAD FJ, NAIKWADE NS, Pharmacol Rep, 2014, 66: 353-362.
25.ANDERSEN MI, WINTER LMF, An Acad Bras Cienc, 2017, 1-14.
26.FESTING S, WILKINSON R, EMBO Rep, 2007, 8(6): 526-530.
27.TAMMA PD, AVDIC E, LI DX, DZINTARS K, COSGROVE SE, JAMA Intern Med, 2017, 177(9): 1308-1315.
28.TESCHKE R, DANAN G, Int J Mol Sci, 2018, 19, 216: 1-5.
29.KILLE JW, 2nd ed., 2017, p. 501.
30.JELIHOVSCHI, I., DROCHIOI, C., BADESCU, A.C.,et al.Rev Chim(Bucharest), **68**,no.12, 2017, p.2853
31.YÝLMAZ C, OZCENGIZ G, Biochem Pharmacol, 2017, 133: 43-62.
32.VAZQUEZ E, NAVARRO M, SALAZAR Y, et al, Inflamm Res, 2015, 64(5): 333-42.
33.MICHAELIDOU AS, HADJIPAVLOU-LITINA D, Chem Rev, 2005, 105(9): 3235-71.
34.DENAMUR S, BOLAND L, BEYAERT M, et al, Toxicol Appl Pharmacol, 2016, 309: 24-36.
35.QUIROS Y, VICENTE-VICENTE L, et al, Toxicol Sci, 2011, 119(2): 245-256.
36.LOPEZ-NOVOA JM, QUIROS Y, et al, Kidney Int, 2011, 79(1): 33-45.

Manuscript received: 21.01.2018