Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org

doi: 10.4103/2221-1691.290132

Impact Factor: 1.90

Allolobophora caliginosa coelomic fluid ameliorates gentamicin-induced hepatorenal toxicity in rats

Saad Bin Dajem[1], Sara Bayoumi Ali[2], Omar Ghanem Abdelrady[2], Nouraldin Mahmoud Salahaldin[2], Ahmed Muhammed Soliman[2], Yasmin Mohamed Kamal[2], Ammar Yasser Abdelazim[2], Aya Fadi Mohamed[2], Kareem Morsy[1,2], Ayman Saber Mohamed[2]✉, Sohair Ramadan Fahmy[2]

[1]*Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia*

[2]*Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt*

ABSTRACT

Objective: To explore the efficacy of earthworm's coelomic fluid against gentamicin-induced hepatic and renal toxicity in rats.

Methods: The animals were divided randomly into three groups (n = 6 per group): control, gentamicin, and *Allolobophora caliginosa* coelomic fluid-treated groups. Toxicity was established after injection of gentamicin daily for 8 days at a dose of 100 mg/kg. Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total proteins, albumin, creatinine, urea, uric acid, malondialdehyde, glutathione, catalase and histopathology of tissues were investigated in the study.

Results: *Allolobophora caliginosa* coelomic fluid significantly decreased urea, creatinine, uric acid, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and malondialdehyde levels while significantly increasing levels of total proteins, albumin, glutathione and catalase. The histopathological investigation showed partial restoration of renal and hepatic architecture.

Conclusions: This study shows the potency of *Allolobophora caliginosa* coelomic fluid in improving the biochemical and histopathological changes induced by gentamicin in the liver and kidney of the rats.

KEYWORDS: *Allolobophora caliginosa*; Coelomic fluid; Gentamicin; Hepatorenal toxicity; Earthworm

1. Introduction

Kidney diseases became dangerous issues globally with expansion rate of about 13%–15%, in line with the prevalence of diabetes and hypertension[1]. Acute kidney injury (AKI) has a major impact on health in developing countries like Egypt[2]. The kidney plays an important role in the elimination of many xenobiotics including drugs, environmental chemicals, and metals. One of the most important risk factors for AKI is medication[3]. Renal toxicity related to drug use accounts for 20% of the causes that may lead to AKI[4]. Drug-induced AKI is a significant clinical problem and accounts for the cessation of the development of many promising drug candidates[5].

Drugs that may cause renal injury include many classes such as antiviral drugs and aminoglycoside[6]. Antibiotics are among the most widely used drugs in hospitalized patients[7]. AKI is one of the most common complications of the widespread use of antibiotics[8]. Gentamicin is a common antibiotic from aminoglycosides that is used widely for the Gram-negative bacterial infection[9]. However, using gentamicin for a long time will result in undesirable side effects and one of its main side effects is hepatic and renal toxicity[10]. Gentamicin may induce vascular, glomerular, and tubular damages because of its accumulation in the proximal renal tubules in the cortex[11]. Recent studies showed that the gentamicin-induced nephrotoxicity model in the rodent is regarded as the "golden standard" for AKI-related studies[5]. This model of nephrotoxicity produces proximal tubular necrosis that would consistently translate the real condition as it is the most

✉To whom correspondence may be addressed. E-mail: ayman81125@hotmail.com

How to cite this article: Dajem SB, Ali SB, Abdelrady OG, Salahaldin NM, Soliman AM, Kamal YM, et al. *Allolobophora caliginosa* coelomic fluid ameliorates gentamicin-induced hepatorenal toxicity in rats. Asian Pac J Trop Biomed 2020; 10(9): 411-416.

Article history: Received 8 December 2019; Revision 10 January 2020; Accepted 13 May 2020; Available online 30 July 2020

closely representative of the clinical situation encountered in human beings[12]. Several studies have also shown that AKI may also lead to the failure of other organs like the lung, brain, and liver[13].

Unfortunately, the traditional medications used to treat nephrotoxicity and hepatotoxicity are not enough and can often cause serious side effects, so developing new drugs from natural products may reduce the risk of toxicity and maintain a therapeutic effect when the drug is used clinically. Using natural products against tissue toxicity induced by gentamicin has proven to be highly efficient[10]. Emerging evidence suggests that earthworms can be an alternative medication because of its abundance, easy access and minor side effects[14]. Coelomic fluid of earthworm has much useful biological activity against inflammation[15], cytotoxic[16], fungi[17] and cancer[18]. However, there is limited data regarding its anti-nephrotoxic and anti-hepatoxic activities. Therefore, the current study aimed to explore the efficacy of earthworm's coelomic fluid against the hepatic and renal toxicity induced by gentamicin in rats.

2. Materials and methods

2.1. Materials

Gentamicin was purchased from a local pharmacy, Cairo, Egypt. All chemicals and kits were purchased from the Biodiagnistic Company (El Moror St, Dokki, EGY).

2.2. Coelomic fluid extraction

Allolobophora caliginosa (*A. caliginosa*) coelomic fluid was extracted by the thermal shock method according to Patil & Biradar[19]. The earthworms (15 g) were subjected to hot water bags (55-60 ℃) and the fluid was collected in a clean dry test tube.

2.3. Acute toxicity study (LD_{50})

LD_{50} of *A. caliginosa* coelomic fluid was determined according to the method described by Chinedu *et al*[20]. The male Wistar rats were fasted overnight and then separated into four groups (2 rats/group). Different doses of the *A. caliginosa* coelomic fluid (10, 100, 300 and 600 mg/kg) were administered. The animals were observed for 1 h post-administration and then 10 min every 2 h interval for 24 h. The animals were monitored for any change in behaviors such as paw licking, fatigue, semi-solid stool, salivation, writhing and loss of appetite in addition to mortality. LD_{50} was calculated from the following formula:

$LD_{50} = (M_0+M_1)/2 = (300+600)/2 = 450$ mg/kg

where M_0 is the highest dose of *A. caliginosa* coelomic fluid that caused no mortality; M_1 is the lowest dose of *A. caliginosa* coelomic fluid that caused mortality.

2.4. Animals and drug treatment

Adult male Wistar rats (*Rattus norvegicus*) with an average body weight of 150-170 g were bought from the National Research Center, Egypt, grouped and housed in polypropylene cages (six animals/cage) in a well-ventilated animal house at a temperature of (23 ± 2) ℃ under 12:12 h day/night cycles. They were allowed to adapt to the environment one week before starting the experiment and fed standard chow pellets and water *ad libitum*.

2.5. Ethical statement

Experimental protocols and procedures used in this study were endorsed by the Institutional Animal Care and Use Committee (IACUC) (Egypt) (CUIF2518).

2.6. Experimental design and grouping

The rats were randomly separated into 3 groups (n=6 per group) as follows: Group Ⅰ received intraperitoneal injection of 0.9% saline daily for 15 consecutive days; Group Ⅱ was treated for 8 consecutive days with gentamicin (100 mg/kg body weight *i.p.*)[21], and then injected intraperitoneally with saline for another 7 d; Group Ⅲ was treated for 8 d with gentamicin (100 mg/kg body weight *i.p.*) and then received *A. caliginosa* coelomic fluid (45 mg/kg body weight) for another 7 d.

On day 16, the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The chest was opened and the blood was collected by the cardiac puncture. The blood collected from the rats was separated by centrifugation at 3 000 rpm for 15 min to get sera, which were stored at –80 ℃ for the biochemical measurement. The liver and kidney were removed and immediately dried using filter paper to remove traces of blood; the left and the right kidneys were weighted.

2.7. Liver and kidney homogenate

Liver and kidney tissues were weighted and homogenized (10% w/v) in ice-cold 0.1 M Tris-HCl buffers (pH 7.4). The homogenate was centrifuged at 860 ×g for 15 min at 4 ℃ and the resultant supernatant was used for the biochemical analyses.

Table 1. Effect of *Allolobophora caliginosa* coelomic fluid on kidney functions in gentamicin treated rats.

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Control	0.40±0.20	25.69±2.87	1.53±0.22
Gentamicin	0.82±0.05[a]	34.45±2.03[a]	2.63±1.10[a]
A. caliginosa coelomic fluid	0.68±0.24[b]	26.27±10.46[b]	1.66±0.64[b]

Values are expressed as mean ± SD (n = 6 per group). a: Indicates statistical significance as compared to the control group; b: Indicates statistical significance as compared with the gentamicin group.

2.8. Biochemical markers

The collected sera were used for determining aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, creatinine, urea, and uric acid according to the manufacturer's instructions using Biodiagnostic kits (Giza, Egypt).

2.9. Oxidative stress markers

The supernatant of the homogenate of the liver and the kidney was used for biochemical analysis according to the manufacturer's instructions using Biodiagnostic kits (Giza, Egypt). Malondialdehyde (MDA), reduced glutathione (GSH) and catalase (CAT) were determined.

2.10. Histopathological examination

Liver and kidney tissues were fixed in 10% formal saline, embedded in paraffin and sectioned. Then, the sections were stained with hematoxylin and eosin (H&E) for histological examination using a light microscope (OPTRCH, Germany).

2.11. Statistical analysis

All data were expressed as mean ± standard diviation (SD). The comparisons within groups were tested using one-way analysis of variance (ANOVA) with Duncan *post hoc* test and $P < 0.05$ was considered statistically significant. SPSS, for Windows (version 15.0) was used for the statistical analysis.

3. Results

3.1. Kidney function biomarkers

As shown in Table 1, creatinine, urea, and uric acid concentrations were increased significantly ($P<0.05$) in the gentamicin group compared to the control group, which were decreased ($P<0.05$) after treatment of *A. caliginosa* coelomic fluid.

3.2. Liver function biomarkers

A significant increase ($P<0.05$) in the levels of AST, ALT, and ALP was observed in the gentamicin treated-group compared to the control group. On the other hand, gentamicin induced a significant decrease ($P<0.05$) in the levels of total proteins and albumin. Administration of *A. caliginosa* coelomic fluid alleviated hepatotoxic effect induced by gentamicin by increasing the levels of total proteins and albumin and decreasing the levels of AST, ALT, and ALP ($P<0.05$) (Table 2).

3.3. Oxidative stress biomarkers

MDA levels in the liver and the kidney were elevated while GSH and CAT levels decreased after gentamicin administration compared with the control group. Treatment with *A. caliginosa* coelomic fluid reversed the changes induced by gentamicin (Table 3).

3.4. Histopathology of the liver

The liver tissue of rats in the control group showed normal liver architecture. The liver section of gentamicin showed severe destructive changes in hepatic architecture. Normal hepatocyte was observed in the *A. caliginosa* coelomic fluid-treated group with moderately improved liver architecture (Figure 1).

3.5. Histopathology of the kidney

The renal tissue of the control group demonstrated the normal appearance. Gentamicin-treated rats showed severe shrinking and degeneration of glomeruli and cytoplasm of some cells of the renal tubules. Treatment with *A. caliginosa* coelomic fluid improved the architecture of renal tissue (Figure 2).

Table 2. Effect of *Allolobophora caliginosa* coelomic fluid on liver functions in gentamicin treated rats.

Groups	AST (U/mL)	ALT (U/mL)	ALP (U/L)	Protein (g/dL)	Albumin (g/dL)
Control	17.83±1.32	23.46±2.01	177.65±5.41	6.41±0.15	4.33±0.17
Gentamicin	24.56±2.47[a]	37.64±9.72[a]	256.29±99.23[a]	5.44±0.15[a]	3.62±0.17[a]
A. caliginosa coelomic fluid	19.29±4.29[b]	29.94±15.38[b]	184.17±13.20[b]	5.93±0.15[b]	3.95±0.07[b]

Values are expressed as mean ± SD (n = 6 per group). Letter a indicates statistical significance as compared with the control group; letter b indicates statistical significance as compared to the gentamicin group. AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase.

Table 3. Effect of *Allolobophora caliginosa* coelomic fluid on oxidative stress parameters in gentamicin treated rats.

Groups	MDA (nmol/g tissue)		GSH (mg/g tissue)		CAT (U/g tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	2.39±0.20	2.90±0.12	27.29±1.52	21.81±1.03	60.18±1.25	23.05±2.01
Gentamicin	4.17±0.61[a]	5.33±0.91[a]	18.41±1.45[a]	17.50±0.98[a]	28.87±17.46[a]	12.66±1.54[a]
A. caliginosa coelomic fluid	3.41±0.44[b]	4.14±0.44[b]	22.53±1.89[b]	19.28±1.00[b]	47.03±4.97[b]	19.91±7.72[b]

Values are expressed as mean ± SD (n = 6 per group). Letter a indicates statistical significance as compared to the control group; letter b indicates statistical significance as compared to the gentamicin group. MDA: malondialdehyde; GSH: reduced glutathione; CAT: catalase.

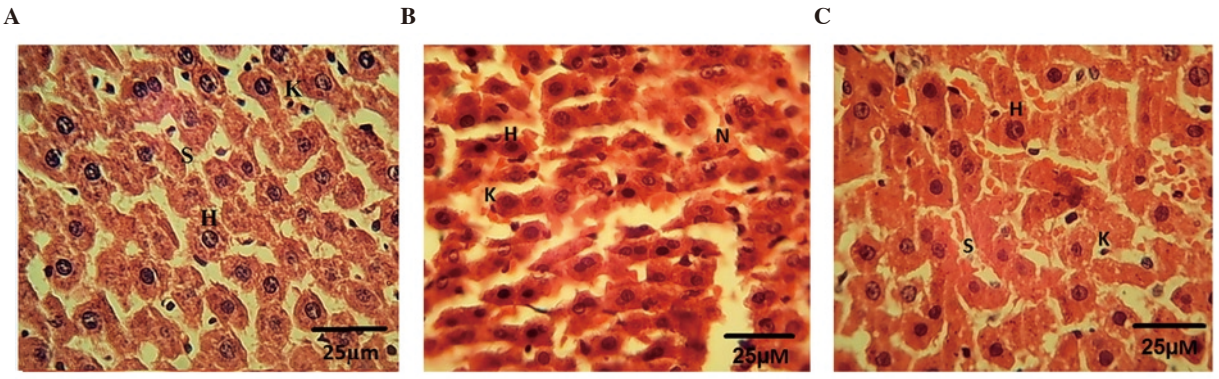

Figure 1. Histopathological examination of the liver (H&E ×400). (A) The control group shows a normal liver architecture, including hepatocyte (H), sinusoids (S) and Von Kupffer cells (K). (B) The gentamicin group shows necrosis (N) and sever destructive changes in hepatic architecture. (C) The group treated with *Allolobophora caliginosa* coelomic fluid shows normal hepatocyte and moderately improves the liver architecture.

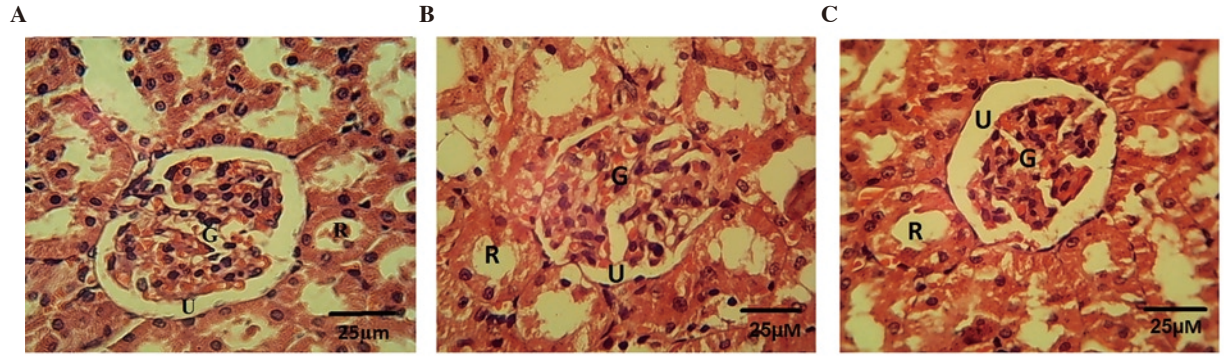

Figure 2. Histopathological examination of the kidney (H&E ×400). (A) The control group shows the normal appearance of the tissue where glomeruli (G) appears as dense tufts of capillaries enclosed in the outer layer of Bowman capsules, urinary space (U), and renal tubules (R). (B) The gentamicin group shows severe shrinking and degeneration of glomeruli and degenerated cytoplasm of some cells of the renal tubules. (C) The group treated with *Allolobophora caliginosa* coelomic fluid shows improvement in architecture of renal tissue.

4. Discussion

Aminoglycoside antibiotics like gentamicin and amikacin usually associated with nephrotoxicity and hepatotoxicity[22]. Antioxidant supplement is important for antibiotic treatment to protect the liver and kidney against the side effects. Earthworm's coleomic fluid has strong antioxidant activity[23]. This study sheds light on the therapeutic effect of *A. caliginosa* coelomic fluid on gentamicin-induced hepatorenal toxicity in rats.

Damaged organ function and accumulation of a series of compounds are indicative of the progression of kidney disease[24]. Increasing concentration of serum urea or creatinine is the first diagnosis of the hepatorenal syndrome[25]. The current investigation showed that consuming gentamicin for 8 d produced a marked increase in creatinine levels in the blood and urea nitrogen compared to the normal control. The reduction of glomerular filtration rate following treatment with gentamicin may be attributed to a tubular blockage induced after apoptosis and cellular necrosis[26]. The present findings demonstrated that the administration of *A. caliginosa* coelomic fluid at 45 mg/kg for 7 d significantly decreased the level of creatinine, urea and uric acid in the gentamicin treated group. Phenolic compounds present in *A. caliginosa* coelomic fluid were responsible for the antioxidant activity that protected the kidney from oxidative stress-induced renal injury[23].

The main functions of the liver are protein synthesis, and the handling of enzymes, such as ALP and release of AST and ALT. Liver enzymes leaked into the blood during liver injury[27]. The present study illustrated that gentamicin administration elevated AST, ALT, and ALP enzyme activities in the serum of rats as compared to the normal control group. The elevation of liver enzymes was related to the increase in hepatic cell membrane fluidity that led to enzyme release into the circulation[28,15].

Current results indicated that the administration of *A. caliginosa* coelomic fluid at 45 mg/kg for 7 d significantly decreased levels of serum AST, ALT and ALP activities when compared to the gentamicin treated group. Consistent with the current study, Balamurugan *et al.*[29] reported that the earthworm extract prevents the formation of the reactive oxygen groups, preventing the damage on the hepatic cells and modulating the genes responsible for the synthesis of antioxidant enzymes.

Protein concentration in the blood was decreased significantly after gentamicin injection. This decrease may due to the damage occurred in the liver by necrosis induced by gentamicin[30]. Regarding the effect of *A. caliginosa* coelomic fluid on gentamicin-treated rats, the results indicated a significant rise in the total protein level and albumin. Moreover, the increase in serum albumin may be due to

enhanced synthesis of proteins and albumin[31].

Oxidative stress resulted from the imbalance between the formation of reactive oxygen species (ROS) and the body's antioxidant defense capacity[32]. ROS has the potential to oxidize biomolecules including proteins, lipids, and DNA[33]. Accumulated evidence indicates that gentamicin stimulates the intracellular formation of ROS such as superoxide anion and hydrogen peroxide that cause kidney damage[34]. Increase ROS production leads to damage in the liver and kidney cells, consequently impairing the functional capacity of these tissues[5]. In addition, lipid peroxidation was considered as an important marker for the structural and functional alternation in cellular membranes[35]. MDA is polyunsaturated fatty acid peroxidation and hence an important sign of lipid peroxidation[36]. The current study revealed that gentamicin administration significantly caused an elevation in the MDA level in the liver and kidney, indicating enhanced lipid peroxidation which leads to tissue damage and the failure of the antioxidant defense system to prevent excessive free radical formation[37]. Moreover, an elevation in MDA indicated a decrease in the polyunsaturated fatty acid content, which serves as a substrate for the free radical attack[38]. It was recorded that gentamicin induced oxidative stress occurs in renal cells[39]. However, treatment with *A. caliginosa* coelomic fluid in the present study significantly decreased the MDA level compared to the gentamicin treated group, suggesting that the protective mechanism of *A. caliginosa* coelomic fluid in the kidney may be attributed to its antioxidant activity.

GSH is one of the essential compounds needed to maintain cell integrity, participate in cell metabolism and has an important role in catalysis. GSH protects tissues against free radicals, peroxides and many toxic agents[40]. Indeed, the enhancement of lipid peroxidation is a consequence of the reduction of GSH to certain critical levels[41]. The present study showed a reduction in GSH content after the gentamicin administration. Increase ROS production induced by gentamicin leads to inhibition of glutathione-associated enzymes[42]. Treatment with *A. caliginosa* coelomic fluid restored GSH content, which might be attributed to the presence of high level of glutathione, polyphenol chemicals, and amino acids.

CAT enzyme protects the cell from the harmful effects of hydrogen peroxide by converting it into free oxygen and water[43]. CAT activity in gentamicin treated rats was inhibited due to excess production of ROS[44]. Administration of *A. caliginosa* coelomic fluid enhanced the activity of CAT and the increase may be due to the presence of polyphenolic compound especially chlorogenic acid that can scavenge free radicals[45]. The protective effect of *A. caliginosa* coelomic fluid against gentamicin-induced toxicity in the liver and the kidney may be through modulation of the balance between the formation of ROS and internal antioxidant system.

In summary, this study demonstrated the potency of *A. caliginosa* coelomic fluid in ameliorating the biochemical and histopathological changes in the liver and the kidney of rats induced by gentamicin.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at Faculty of Science, Cairo University, Egypt for supporting the current work.

Funding

This study was supported by the Deanship of Scientific Research at King Khalid University through Research Group Project under grant number (R.G.P.1–56 –40).

Authors' contributions

SRF developed the theoretical formalism. AMS, YMK and AYA performed the analytic calculations. SBA, OGA and NMS contributed to data collection and interpretation. AFM and KM drafted the article. ASM done critical revision of the article. SBD supervised the project.

References

[1] Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of chronic kidney disease: A position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2005; **67**(6): 2089-2100.

[2] Abdelsalam M, Elnagar SS, Mohamed AH, Tawfik M, Ahmed N. Community acquired acute kidney injury in Mansoura Nephrology Dialysis Unit: One year prospective observational study. *Nephron* 2018; **140**(3): 185-193.

[3] Perazella MA. Drug-induced acute kidney injury: Diverse mechanisms of tubular injury. *Curr Opin Crit Care* 2019; **25**(6): 550-557.

[4] Al-Naimi MS, Rasheed HA, Hussien NR, Al-Kuraish HM, Al-Gareeb AL. Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. *J Adv Pharm Technol Res* 2019; **10**(3): 95-99.

[5] Mohamed D, Khairy E, Saad S, Habib E, Hamouda M. Potential protective effects of Dapagliflozin in gentamicin induced nephrotoxicity rat model *via* modulation of apoptosis associated miRNAs. *Gene* 2019; **707**: 198-204.

[6] Vormann MK, Gijzen L, Hutter S, Boot L, Nicolas A, van den Heuvel A. Nephrotoxicity and kidney transport assessment on 3D perfused proximal tubules. *AAPS J* 2018; **20**(5): 90.

[7] Hong S, Valderrama E, Mattana J, Shah HH, Wagner JD. Vancomycin-induced acute granulomatous interstitial nephritis: Therapeutic options. *Am J Med Sci* 2007; **334**(4): 296-300.

[8] Khalili H, Bairami S, Kargar M. Antibiotics induced acute kidney injury: Incidence, risk factors, onset time and outcome. *Acta Med Iran* 2013; **51**(12): 871-878.

[9] Martin J, Barras M, Yui N, Kirkpatrick C, Kubler P, Norris R. Gentamicin monitoring practices in teaching hospitals – time to undertake the necessary randomised controlled trial. *J Clinic Toxicol* 2012; **2**: 146.

[10]Rizwan F, Yesmine S, Banu SG, Chowdhury IA, Hasan R, Chatterjee TK. Renoprotective effects of stevia (*Stevia rebaudiana* Bertoni), amlodipine, valsartan, and losartan in gentamycin-induced nephrotoxicity in the rat model: Biochemical, hematological and histological approaches. *Toxicol Rep* 2019; **6**: 683-691.

[11]Sassen MC, Kim SW, Kwon TH, Knepper MA, Miller RT, Frøkiaer J, et al. Dysregulation of renal sodium transporters in gentamicin-treated rats. *Kidney Int* 2006; **70**: 1026-1037.

[12]Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: An integrative point of view. *Kidney Int* 2011; **79**: 33-45.

[13]Kao CC, Yang WS, Fang JT, Liu KD, Wu VC. Remote organ failure in acute kidney injury. *J Formos Med Assoc* 2019; **18**(5): 859-866.

[14]Deng Z, Gao S, Xiao X, Yin N, Ma S, Li W, et al. The effect of earthworm extract on mice S180 tumor growth and apoptosis. *Biomed Pharmacother* 2019; **115**: 108979.

[15]Li C, Chen M, Li X, Yang M, Wang Y, Yang X. Purification and function of two analgesic and anti-inflammatory peptides from coelomic fluid of the earthworm, *Eisenia foetida*. *Peptides* 2017; **89**: 71-78.

[16]Augustine D, RaoR S, Anbu J, Chidambara MK. *In vitro* cytotoxic and apoptotic induction effect of earthworm coelomic fluid of *Eudrilus eugeniae*, *Eisenia foetida*, and *Perionyx excavatus* on human oral squamous cell carcinoma-9 cell line. *Toxicol Rep* 2019; **6**: 347-357.

[17]Fiołka MJ, Czaplewska P, Macur K, Buchwald T, Kutkowska J, Paduch R, et al. Anti-*Candida albicans* effect of the protein-carbohydrate fraction obtained from the coelomic fluid of earthworm *Dendrobaena veneta*. *PLoS One* 2019; **14**(3): e0212869.

[18]Fiołka M, Rzymowska J, Bilska S, Lewtak K, Dmoszyńska-Graniczka M, Grzywnowicz K, et al. Antitumor activity and apoptotic action of coelomic fluid from the earthworm *Dendrobaena veneta* against A549 human lung cancer cells. *APMIS* 2019; **127**(6): 435-448.

[19]Patil SR, Biradar PM. Earthworm's coelomic fluid: Extraction and importance. *Int J Adv Scie Res* 2017; **2**(2): 01-04.

[20]Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. *Toxicol Int* 2013; **20**(3): 224-226.

[21]Jabbari M, Rostami Z, Jenabi A, Zahedi-Shoolami L, Mooraki A. Simvastatin ameliorates gentamicin-induced renal injury in rats. *Saudi J Kidney Dis Transpl* 2011; **22**(6): 1181-1186.

[22]Khaksari M, Esmaili S, Abedloo R, Khastar H. Palmatine ameliorates nephrotoxicity and hepatotoxicity induced by gentamicin in rats. *Arch Physiol Biochem* 2019; **26**: 1-6.

[23]Grdisa M, Popovic M, Hrzenjak T. Glycolipoprotein extract (G-90) from earthworm *Eisenia foetida* exerts some antioxidative activity. *Comp Biochem Phys A* 2001; **128**(4): 821-825.

[24]Vanholder R, Baurmeister U, Brunet P, Cohen G, Glorieux G. A bench to beside view of uremic toxins. *J Am Soc Nephrol* 2008; **19**: 863-870.

[25]Gine P, Guevara M, Arroyo V, Rode J. Hepatorenal syndrome. *Lancet* 2003; **362**(9398): 1819-1827.

[26]Randjelovic P, Veljkovic S, Stojiljkovic N, Sokolovic D, Ilic I. Gentamicin nephrotoxicity in animals: Current knowledge and future perspectives. *EXCLI J* 2017; **16**: 388-399.

[27]Sheila S, Dooley J. *Diseases of the liver and biliary system*. 9th ed. USA: Backwell Scientific Publications; 1993.

[28]Galaly SR, Ahmed OM, Mahmoud AM. Thymoquinone and curcumin prevent gentamicin-induced liver injury by attenuating oxidative stress, inflammation and apoptosis. *J Physiol Pharmacol* 2014; **65**(6): 823-832.

[29]Balamurugan M, Parthasarathi K, Ranganathan LS, Cooper EL. Hypothetical mode of action of earthworm extract with hepatoprotective and antioxidant properties. *J Zhejiang Univ Sci B* 2008; **9**(2): 141-147.

[30]Adedeji AL, Adedosu OT, Badmus JA, Adeleke GE, Afolayan IR, Olarinde IF. Aqueous extract of *Hibiscus sabdariffa* calyx modulates gentamicin activity in rats. *Asian Pac J Health Sci* 2016; **3**(3): 178-187.

[31]Fahmy SR. Anti-fibrotic effect of *Holothuria arenicola* extract against bile duct ligation in rats. *J Altern Complement Med* 2015; **15**(14): 1-12.

[32]Kang R, Zhang Q, Zeh HJ, Lotze MT, Tang D. HMGB1 in cancer: Good, bad, or both? *Clin Cancer Res* 2013; **19**(15): 4046-4057.

[33]Klaine SJ, Alvarez PJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, et al. Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. *Environ Toxicol Chem* 2008; **27**(9): 1825-1851.

[34]Yarijani ZM, Najafi H, Shackebaei D, Madani S, Modarresi M, Jassemi S. Amelioration of renal and hepatic function, oxidative stress, inflammation and histopathologic damages by *Malva sylvestris* extract in gentamicin induced renal toxicity. *Biomed Pharmacother* 2019; **112**: 108635.

[35]Halliwell B, Murcia M, Chirico S, Aruoma O. Free radicals and antioxidants in food and *in vivo*: What they do and how they work. *Crit Rev Food Sci Nutr* 1995; **35**: 7-20.

[36]Rio D, Stewart A, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *NMCD* 2005; **15**: 316-328.

[37]Kim KA, Kim JY, Lee YA, Song KJ, Min D, Shin MH. NOX1 participates in ROS-dependent cell death of colon epithelial Caco2 cells induced by *Entamoeba histolytica*. *Microbes Infect* 2011; **13**(12-13): 1052-1061.

[38]Al-Majed AA, Mostafa AM, Al-Rikabi AC, Al-Shabanah OA. Protective effects of oral arabic gum administration on gentamicin-induced nephrotoxicity in rats. *Pharmcol Res* 2002; **46**: 445-451.

[39]Aldahmash BA, El-Nagar DM, Ibrahim KE. Reno-protective effects of propolis on gentamicin-induced acute renal toxicity in swiss albino mice. *Nefrologia* 2016; **36**(6): 583-722.

[40]Hiraishi H, Terano A, Ota S, Mutoh H, Sugimoto T, Harada T, et al. Protection of cultured rat gastric cells against oxidant-induced damage by exogenous glutathione. *Gastroenterology* 1994; **106**(5): 1199-1207.

[41]Srivastava S, Das M, Seth P. Enhancement of lipid peroxidation in rat liver on acute exposure to styrene and acrylamide a consequence of glutathione depletion. *Chem Biol Interact* 1983; **45**: 373-380.

[42]Erjaee H, Azma F, Nazifi S. Effect of caraway on gentamicin-induced oxidative stress, inflammation and nephrotoxicity in rats. *Vet Sci Dev* 2015; **5**: 90-94.

[43]Liu RM, Gaston Pravia KA. Oxidative stress and glutathione in TGF-β-mediated fibrogenesis. *Free Radical Bio Med* 2010; **48**(1): 1-15.

[44]Ademiluyi AO, Oboh G, Owoloye TR, Agbebi OJ. Modulatory effects ofdietary inclusion of garlic (*Allium sativum*) on gentamycin-inducedhepatotoxicity and oxidative stress in rats. *Asian Pac J Trop Biomed* 2013; **3**: 470-475.

[45]Aldarraji QM, Halimoon N, Majid NN. Antioxidant activity and total phenolic content of earthworm paste of *Lumbricus rubellus* (red worm) and *Eudrilus eugenia* (African night crawler). *J Entomol Nematol* 2013; **5**(3): 33-37.