(RESEARCH ARTICLE)

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Protective effect of *Anodonta cygnea* hemolymph against acute kidney injury induced by gentamicin in rats

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Abstract

Background: *Anodonta cygnea* hemolymph has several pharmacological activities against inflammation, bacteria and tumor. The current study aimed to explore the efficacy of *A. cygnea* hemolymph against the renal toxicity induced by Gentamicin (GM) in rats.

Methods: The animals were divided randomly into three groups (six per group): control, GM and *A. cygnea*. Tissues toxicity established after injection of GM daily for eight days at dose 100 mg/kg. Kidney functions, liver functions, oxidative stress markers and histopathology of tissues investigated in the study.

Results: *A. cygnea* treated rats showed a significant decrease in urea, creatinine, uric acid, ALT, AST, GGT and MDA levels while GSH and CAT levels increased. The histopathological of liver and kidney investigation showed partial restoration of renal architecture.

Conclusion: This study showed the *A. cygnea* potency in improving the biochemical and histopathological changes in the kidney of the rats following experimental induction of toxicity using Gentamycin.

Keywords: *Anodonta Cygnea*; Hemolymph; Gentamicin; Nephrotoxicity; Mollusca

1. Introduction

The first aminoglycoside, streptomycin, was introduced into clinical practice in 1944, and has since been followed by many drugs of this class [1]. Among the classes of aminoglycoside antibiotics, gentamicin (GEN) is widely used in clinical practice for the treatment of life-threatening Gram-negative bacterial infections [2]. However, therapeutic doses of gentamicin can produce nephrotoxicity and the use of this class of antibiotics is known as one of the most common causes of acute renal failure, which is seen in 10–20% of patients receiving the drug [3].Due to relatively large blood flow (20 % of stroke volume) and the ability to extract and concentrate hydrosoluble toxic molecules, the kidney is prone to drug induced damage. The experimental data point to the fact that medicate induced nephrotoxicity includes multiple mechanisms that can be classified as vascular, tubular and glomerular [4]. There are two main factors for kidney damage induced by GM, its accumulation in proximal tubular cells and interaction with cell membranes and organelles [5]. About 5 % of the administered dose accumulates inside these cells after glomerular filtration [6].

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Nephrotoxicity is a side effect in the use of gentamicin and is believed to be related to the generation of reactive oxygen species (ROS) in the kidney [7,8] . ROS induce cellular damage and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage [9,10,11,12]. In vivo, ROS are labeled as proximal tubule necrosis and acute kidney injury mediators [7] .However, Gentamicin clinical use has been limited because of gentamicin-induced acute kidney injury (AKI) occurs in approximately 20% of patients [13]. In addition, AKI may also lead to the failure of other organs like the lung, brain, and liver [14].

Products from freshwater and marine sources have recently become attractive as nutraceutical and functional foods and as a source material for the development of drugs and particular wellbeing nourishments [15] . The Egyptian freshwater mussel (*Anodonta cygnea*) is a Molluscan bivalve that belongs to Unionoidae common in the Egypt along the River Nile. [16] . Many in vitro and in vivo studies have found that freshwater clams possess many medical and biological effects, including antitumorigenic properties [17], hepatoprotective [18] , and cholesterol-lowering [19] . However, there is a rareness in data regarding its anti-nephrotoxic and anti-hepatoxic activities. The current study aimed to explore the efficacy of *Anodonta cygnea* hemolymph against the renal toxicity induced by Gentamicin (GM) in rats.

2. Material and methods

2.1. Materials

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

2.2. Freshwater mussel (Anodonta cygnea) hemolymph extraction

Hemolymph was collected from the posterior adductor muscle sinus of *Anodonta*, using a sterile syringe with a 25-gauge needle (Lowe & Pipe, 1994) .The haemolymph was centrifuged at 3000 rpm for 15 minutes at 4°C to remove haemocytes. The supernatant was collected by aspiration and stored at 4 °C until use.

2.3. Acute toxicity study (LD50)

LD_{50} of *A. cygnea* hemolymph was determined according to the method described by Chinedu et al.. [20]. The rats were fasted overnight then separated into four groups (2 rats/group). Different doses of the *A. cygnea* hemolymph (10, 100, 300 and 600 mg/kgm) are administered to the rats. The animals were observed for o'clock post-administration and then 10 minutes every 2 hours interval for 24 hours. The animals were monitor 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} calculated from the following formula:

$$LD_{50} = \frac{M0+M1}{2} \ (300+600)/2 = 450 \ mg/kg$$

Where, M0: the highest dose of *A. cygnea* hemolymph that gave no mortality.
M1: the lowest dose of *A. cygnea* hemolymph that gave mortality.

2.4. Animals and drug treatment

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

2.5. Experimental design and grouping

The rats had been randomly separated into three groups (n=six per group) as follows:

- Group I (Control): Rats of this group injected i.p. with 0.9% Saline daily for 15 consecutive days.
- Group II (GM): Rats in this group were injected i.p. with saline for another 7 consecutive days and then treated for 8 consecutive days with GM (100 mg/kg body weight i.p.) [21].
- Group III (GM+ *A. cygnea*): Rats in this group were treated for 7 consecutive days with *A. cygnea* (45 mg/kg body weight) and then injected GM (100 mg/kg body weight i.p.) for another 8 consecutive days.

On day 16, the rats were anesthetized by intraperitoneal injection 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 3000 rpm for 15 minutes to get sera, which were stored at -80°C for the biochemical measurements. The liver and kidney were removed and immediately blotted using filter paper to remove traces of blood and for each group.

2.6. 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°C and the resultant supernatant was used for the biochemical analyses.

2.7. Histopathological examination

Kidney tissues were fixed in 10% formal saline, embedded in paraffin and sectioned. Then, the sections stained with hematoxylin and eosin (H&E) for histological examination using a light microscope. The qualitative score was applied to the detected histopathological alterations; (-) no lesion, (+) mild, (++) moderate and (+++) severe lesion.

2.8. Biochemical markers

The collected sera were used for determining creatinine, urea, uric acid, aspartate aminotransferase (AST) , alanine aminotransferase (ALT) and Gamma-glutamyl transferase (GGT) , and according to the manufacturer's instructions using Bio-diagnostic 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), glutathione reduced (GSH) and catalase (CAT) were determined.

2.10. Statistical analysis

All data were expressed as means ± standard error of the mean (SEM). The comparisons within groups were evaluated utilizing one-way analysis of variance (ANOVA) with Duncan post hoc test was used to compare the group means and $p < 0.05$ was considered statistically significant. SPSS statistical software package for Windows (version 21.0) was used for the statistical analysis.

3. Results

3.1. Kidney functions biomarkers

According to the data represented in Table 1, creatinine, urea, and uric acid concentrations increased significantly ($p<0.05$) in the GM group, as compared to the control group. While a significant decrease ($p<0.05$) was recorded in the creatinine, urea and uric acid concentrations in the *A. cygnea* hemolymph group as compared to the GM group.

Table 1 Effect of *A. cygnea hemolymph* on kidney functions in GM treated rats

Variable	Control	GM	
		Vehicle	*A. cygnea*
Creatinine (mg/dl)	0.77±0.05[a]	1.62±0.20[b]	0.94±0.10[a]
Urea (mg/dl)	26.35±1.47[a]	55.62±9.86[b]	36.98±4.29[a]
Uric acid (mg/dl)	1.44±0.13[a]	2.30±0.33[b]	1.94±0.23[a]

Values are mean ± SEM (n= 6). Values with different superscript letters are significantly different (P < 0.05).

3.2. Liver functions biomarkers

Regarding the hepatotoxic effect of GM, data recorded in Table 2 showed a significant increase ($p < 0.05$) in the levels of AST, ALT and GGT while total proteins decreased in the GM treated-group as compared to the control group. However,

A. cygnea hemolymph administration decreased the studied liver enzyme activities significantly (p < 0.05) and increased total proteins concentrations as compared to the GM group.

Table 2 Effect of *A. cygnea* hemolymph on liver functions in GM treated rats

Variable	Control	GM	
		Vehicle	***A. cygnea***
AST (U/ml)	6.71±0.21[a]	12.50±0.67[c]	8.16±0.29[b]
ALT(U/ml)	41.56±1.59[a]	76.92±3.04[c]	60.20±2.35[b]
GGT(U/ml)	16.50±0.97[a]	34.80±1.58[b]	19.02±1.11[a]
Protein (g/dl)	5.76±0.53[c]	1.93±0.09[a]	3.83±0.25[b]

Values are mean ± SEM (n= 6). Values with different superscript letters are significantly different (P < 0.05).

3.3. Oxidative stress biomarkers

Data recorded in Table 3 displayed a significant increase (P< 0.05) in the levels of liver and kidney MDA and significant decreases in both GSH and CAT levels after GM administration compared with the control group. However, treatment with *A. cygnea* hemolymph caused significant (*P* < 0.05) decrease in the liver and kidney MDA level and an increase in the liver and kidney GSH level and CAT levels, as compared to the corresponding GM intoxicated groups (Table 3).

Table 3 Effect of *A. cygnea* hemolymph on on oxidative stress biomarkers in GM treated rats

Variable	Organ	Control	GM	
			Vehicle	***A. cygnea***
MDA (nmol/g.tissue)	Liver	17.22±1.98[a]	41.86±2.89[c]	28.32±4.75[b]
	Kidney	2.13±0.18[a]	4.49±0.19[b]	3.92±0.39[b]
GSH (mg/g.tissue)	Liver	18.99±1.91[b]	11.55±0.94[a]	15.55±0.89[a]
	Kidney	5.28±0.75[c]	3.51±0.11[a]	4.11±0.21[bc]
CAT (U/g.tissue)	Liver	24.46±1.18[c]	4.13±1.18[a]	11.11±0.44[b]
	Kidney	6.86±0.99[b]	3.73±0.29[a]	4.94±0.41[a]

Values are mean ± SEM (n= 6). Values with different superscript letters are significantly different (P < 0.05).

3.4. Histopathology of kidney

Table 4 Qualitative score of histopathological lesions in kidneys

	Control	GM	*A. cygnea*
Congestion	-	+++	++
Perivascular edema and inflammation	-	+++	-
Nephrosis	-	+++	++
Interstitial nephritis	-	+++	+

(-) absent, (+) mild, (++) moderate and (+++) severe.

Control group showed normal histology of renal cortex and medulla; in which the cortex appeared containing numerous glomeruli and both types of renal tubules. The renal medulla was formed of renal tubules and collecting ducts (Fig. 1A&B). Kidneys of GM group showed various histological alterations, cortical blood vessels were severely congested with perivascular edema, mononuclear inflammatory cells infiltration, interstitial nephritis represented by mononuclear cells infiltration, necrosis and cystically dilated tubules (Fig. 1C&D). Using treatment 1 had a mild ameliorative action against gentamycin induced renal toxicity, some renal tubules exhibited degenerative changes as vacuolation of the epithelial lining with few necrotic tubules, minute focal aggregations of mononuclear inflammatory

cells were observed in some instances. The renal medulla appeared apparently normal (Fig. 1E&F). A qualitative score of histopathological lesions in the kidneys of each group represented in table 4.
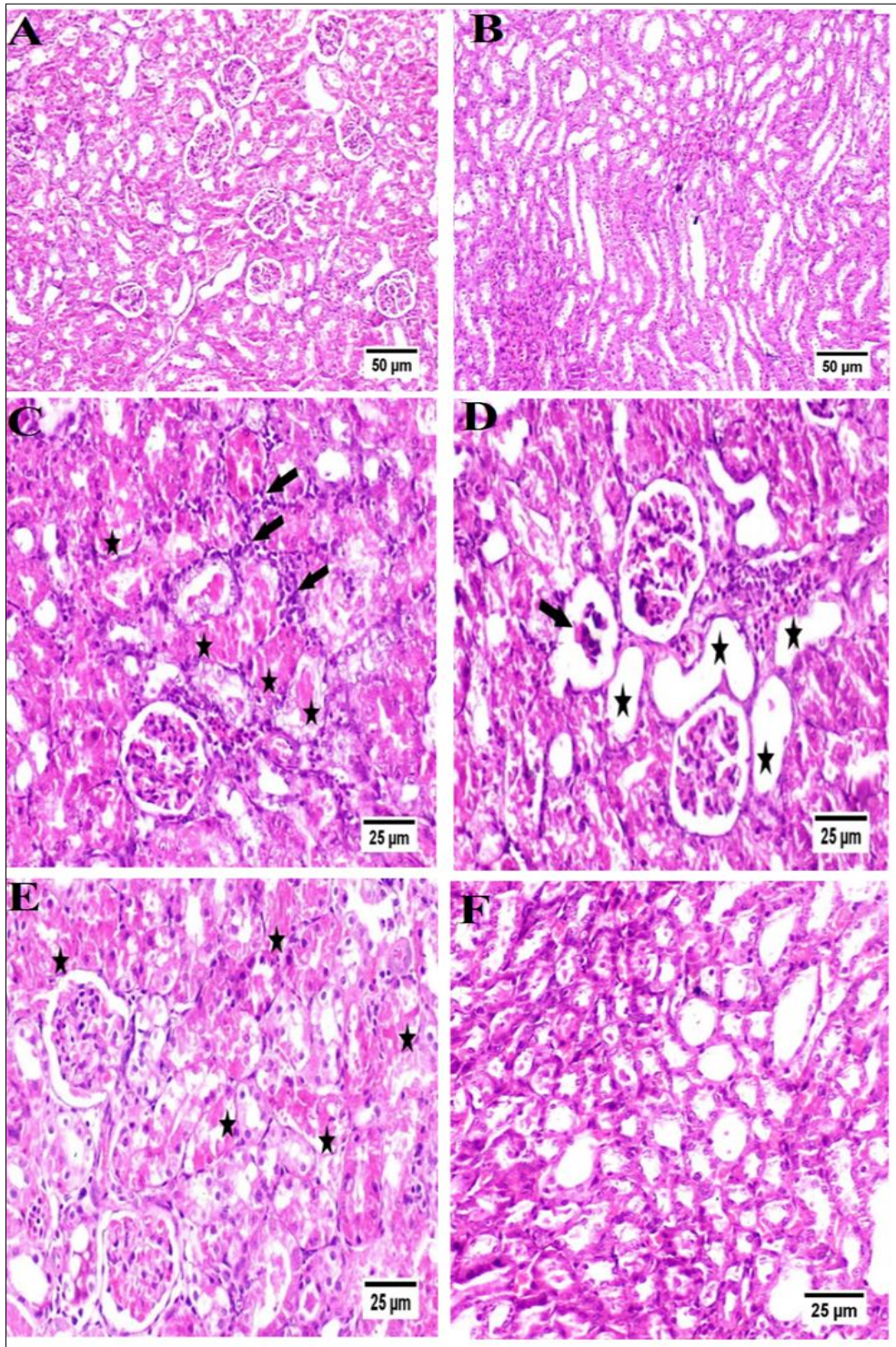

Figure 1 A&B Kidneys of rat (control group) showing normal renal cortex and renal medulla. C&D: GM group, showing degeneration and necrosis in the epithelial lining of the renal tubules (black stars) with interstitial mononuclear inflammatory cells infiltration (black arrows), cystic dilatation of the renal tubules (red stars), atrophy of the glomerular capillary tuft (red arrow) and focal mononuclear inflammatory cells aggregation. E&F: Kidneys of rat (*A. cygnea* group) showing apparently normal renal medulla with degenerating renal tubules (stars) (H&E)

4. Discussion

Gentamicin is a hydrophilic drug that is distributed to body water and excreted unchanged by the kidneys, predominantly by glomerular filtration [22]. The clinical use of gentamicin is limited due to its nephrotoxicity which is characterized by direct tubular necrosis [3]. In the present study, we evaluate the protective effect of *Anodonta cygnea* hemolymph against GM -induced nephrotoxicity in rats.

In the present study, it was shown that administration of gentamicin for one week to rats caused a reduction in glomerular filtration rate which correlated with increased creatinine, urea and uric acid in serum. The reduction in glomerular filtration is probably linked to oxidative stress (ROS) [23] . Aminoglycoside antibiotics including GM can deliver nephrotoxicity in human. Proximal tubular cells are a major site of damage in patients treated with GM [24] .

Gentamicin treatment causes hepatotoxicity as clearly indicated by the significant increase in serum level of ALT, AST, GGT and decrease protein concentration as those of control rats. These result due to damage in renal brush border of epithelial cells in the proximal tubule and it is a sensitive indicator of GM toxicity [25] . Increase AST and ALT and decrease protein levels in circulation indicate necrosis and membrane damage in the liver [26,27]. The results obtained in this study are in agreement with other reports [28,29]. Histopathological lesions observed in this study correlate the serum level of liver function enzymes concentration induced with gentamicin.

Oxidative stress is a biochemical disequilibrium propitiated by excessive production of free radicals (FR) and ROS, which provoke oxidative damage to biomolecules that cannot be counteracted by antioxidative systems [30] . Involvement of reactive oxygen species (ROS) in nephropathies of human are now characterized as a key element [31] . Aminoglycoside antibiotics can stimulate the formation of ROS, which may be directly involved in gentamicin-induced acute renal failure and membrane lipid peroxidation [32]. Gentamicin exposure to rats mediates the generation of ROS that play a significant role in the progression of hepatic and renal injuries including array of biomolecules such as membrane lipids, protein and nucleic acids especially in some organelles such as mitochondria and lysosomes of renal tissues [33]. There have been reports that treatment with gentamicin produces oxidative stress in renal tubule cells, both in vivo and in vitro [34,35].

The current study revealed that GM administration significantly caused an elevation in the MDA level. The elevated level of MDA (a marker of lipid peroxidation in tissues) results in the reduction of polyunsaturated fatty acid content, which serves as substrate of free radicals particularly, hydrogen peroxide and superoxide. These aggravated free radicals affect antioxidant functions of SOD, catalase, GSH and GPx [36] . The imbalance between the generation and degradation of ROS caused oxidative stress and eventually the generation of free radicals and cellular damage [37] . Other report also shows that, cationic interaction of aminoglycosides with anionic phospholipid (kidneys) induces nephrotoxicity [24].

Glutathion reduced [GSH] has been to be an important cellular protectant against reactive oxygen metabolites in several cells by serving as a substrate for glutathion peroxidase [38,39] . The present study confirmed the findings of several studies on GM toxicity in rats, [29]; [3] [40] [28] by demonstrating significant decrease in GSH in the kidney and liver tissues of GM treated rats. A considerable decline in GSH content after the GM treatment in the present investigation may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from GM.

The nonenzymatic component of the self-defense system; renal glutathione (GSH) and enzymatic components; CAT was diminished in the rats treated with gentamicin as compared to the respective control group ,so that GM induces hydrogen peroxide, hydroxyl radical and superoxide anion production from renal mitochondria [41,42] causing oxidative stress. The Catalase (CAT) reduces hydrogen peroxide produced by dismutation reaction and prevents generation of hydroxyl radicals thereby protecting the cellular constituents from oxidative damage in peroxisomes.GM administration in the present study result in the significant decrease in CAT activity. In consonance with our results, [43] reported that GM lead to decrease of antioxidant enzymes CAT)

Antioxidant and anti-inflammatory agents play a critical role in body protection by scavenging active oxygen and free radicals and neutralizing lipid peroxides [44,45]. Therefore, there is need for a natural product that protects the body but cost-effective, safe and without side effects. So, the present study conducted to study the antioxidant properties of freshwater *Anodomta cygnea*. The present findings demonstrated the hepatorenal protective effect of freshwater mussel *A. cygnea* hemolymph at 45.6 mg/Kg for 7 days significantly. The obtained results showed significant reduction in MDA and increase in the GSH levels, CAT activity. The enhancement in antioxidant system may be due to carotenoids, steroids, phenolics and terpenoides compounds present in mollusks that can scavenge free radicals [46,47,48,49] . In addition, many studies on bioactive compounds from molluscs exhibiting antioxidant, antitumor and antibacterial

activities have been reported worldwide [50,51]. Moreover, [52] reported that clam extract such freshwater mussel *Coelatura aegyptiaca* lead to improves the antioxidant system and reduced lipid peroxidation.

5. Conclusion

This study demonstrated the freshwater mussel *A. cygnea* hemolymph potency in ameliorating the biochemical and histopathological changes in the kidney of the rats following experimental induction of renal toxicity using gentamycin. The protection effect of *A. cygnea* hemolymph my through suppressing of oxidative stress, and protecting the internal antioxidant system.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest associated with this work.

Statement of ethical approval

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

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