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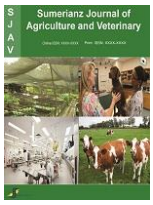

Original Article Open Access

Protective Effect of *Procambarus Clarkia* Hemolymph against Acute Kidney Injury Induced by Gentamicin in Rats

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Abstract

Background: *P.clarkii* hemolymph has several pharmacological activities against inflammation, bacteria and tumor. The current study aimed to explore the efficacy of *P.clarkii* 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 *P.clarkii*. Tissues toxicity was established after injection of GM daily for eight days at a dose 100 mg/kg. Kidney functions, liver functions, oxidative stress markers and histopathology of tissues were investigated in the study. Results: *P.clarkii* treated rats showed a significant decrease in urea, creatinine, uric acid, ALT, AST, and MDA levels while GSH and CAT levels increased. The histology of kidney investigation showed partial restoration of renal architecture. Conclusion: The study results revealed the protective role of *p.clarkii* hemolymph against gentamicin-induced acute kidney injury in rats.
Keywords: *Procambarus clarkii*; Hemolymph; Gentamicin; Nephrotoxicity, Arthropods.

1. Introduction

The kidney is a vital organ that plays an essential role in the body's healthy. Kidney diseases became dangerous issues globally with 13–15% expansion rates, matching with an expanded commonness of diabetes and hypertension [1]. Acute kidney injury (AKI) is a comparatively common condition in the intensive care unit and occurs in 20% to 30% of critically ill patients, with about 6% eventually requiring renal replacement therapy [2]. One of the most important risk factors for AKI is medication [3]. Drug-induced nephrotoxicity has been reported to contribute to up to 26% of cases of hospital-acquired AKI and 18% of cases of community-acquired AKI globally [4-6].

Drugs that may cause renal injury include many classes as antiviral drugs and aminoglycoside [7]. Gentamicin is an antibiotic called aminoglycoside that has been in use for over 40 years. That has been widely used in the treatment of infection caused by Gram-negative bacteria and is effective against Staphylococcus and Enterococcus [8, 9]. The drug is accumulated in renal epithelial cells, and induces the loss of the brush border, apoptosis, massive proteolysis, and overt necrosis of renal tubules [10]. Nephrotoxicity is a serious side effect in the use of gentamicin and is believed to be related to the generation of reactive oxygen species (ROS) in the kidney [11, 12]. ROS induce cellular damage and necrosis via lipid peroxidation and protein change [13-15]. However, Gentamicin clinical use has been limited because of gentamicin-induced acute kidney injury (AKI) occurs in approximately 20% of patients [16]. Besides, AKI may also lead to the failure of other organs like the lung, brain, and liver [17].

Natural products supply to treat various types of diseases such as cancer, inflammation and diseases of the liver. Freshwater crayfish, *Procambarus clarkii*, has been widely spread all over most of the River Nile [18]. *P.clarkii* has a bioactive compound with antimicrobial activity [19] and antitumor activity [20]. However, there is a rareness in data regarding its *anti-nephrotoxic and anti-hepatoxic* activities. The current study aimed to explore the efficacy of *P.clarkii* hemolymph against the renal toxicity induced by Gentamicin (GM) in rats.

2. Materials 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, EGY).

2.2. Procambarus Clarkii Hemolymph Extraction

The hemolymph (250–500 µl) was withdrawn from the arthrodial membrane between the coxa and the base of cheliped using a 1-ml syringe with a 23-gauge needle [21]. The hemocyte degranulation and coagulation were avoided, the hemolymph was collected in the presence of anticoagulant citrate_EDTA solution for the collection of marine invertebrate hemolymph (100 mM glucose, 30 mM trispodium citrate, 26 mM citric acid, 510mM NaCl and

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10mM EDTA.Na,; pH = 4.6) (2:1 v/v) was prepared according to Soderhlll and Smith [22]. The hemolymph was centrifuged at 3000 rpm for 15 minutes at 4°C to remove hemocytes. The supernatant was collected by aspiration and stored at 4°C until use.

2.3. Acute Toxicity Study (LD50)

LD_{50} of *P.clarkii* hemolymph was determined according to the method described by Chinedu, *et al.* [23]. The rats were fasted overnight then separated into four groups (2rats/group). Different doses of the *P.clarkii* 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:

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

Where M0: the highest dose of *P.clarkii* hemolymph that gave no mortality.

M1: the lowest dose of *P.clarkii* 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. *Ethical* Consideration and Field Study

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

2.6. 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.) [24].

Group III (GM+ *P.clarkii*): Rats in this group were treated for 7 consecutive days with *P.clarkii* (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.

2.7. 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^{o}C and the resultant supernatant was used for the biochemical analyses.

2.8. Histopathological Examination

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. The qualitative score was applied to the detected histopathological alterations; (-) no lesion, (+) mild, (++) moderate and (+++) severe lesion.

2.9. Biochemical Markers

The collected sera were used for determining creatinine, urea, uric acid, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and according to the manufacturer's instructions using Bio-diagnostic kits (Giza, Egypt).

2.10. 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.

2.11. 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 *P.clarkii* hemolymph group as compared to the GM group.

Table-1. Ameliorative effect of *P. clarkii* hemolymph on kidney functions in GM treated rats

Variable	Control	GM	
		Vehicle	*P.clarkii*
Creatinine (mg/dl)	0.73 ± 0.05^{a}	1.78 ± 0.22^{c}	0.86 ± 0.16^{b}
Urea (mg/dl)	25.03 ± 1.40^{a}	61.18 ± 10.85^{b}	31.24 ± 1.60^{a}
Uric acid (mg/dl)	1.75 ± 0.32^{a}	2.53 ± 0.36^{b}	1.70 ± 0.42^{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 and ALT while total proteins and albumin decreased in the GM treated-group as compared to the control group. However, *P.clarkii* hemolymph administration decreased the studied liver enzyme activities significantly (p < 0.05) and increased total proteins and albumin concentrations as compared to the GM group.

Table-2. Ameliorative effect of *P. clarkii* hemolymph on kidney functions in GM treated rats

Variable	Control	GM	
		Vehicle	*P.clarkii*
AST (U/ml)	6.19 ± 0.20^{a}	14.25 ± 0.76^{c}	8.31 ± 0.24^{b}
ALT(U/ml)	38.36 ± 1.47^{a}	87.68 ± 3.47^{c}	62.27 ± 2.88^{b}
Protein (g/dl)	5.32 ± 0.49^{c}	2.20 ± 0.10^{a}	2.82 ± 0.15^{b}
Albumin (g/dl)	2.81 ± 0.19^{c}	1.72 ± 0.06^{a}	1.94 ± 0.05^{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 *P.clarkii* hemolymph caused a 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. Ameliorative effect of *P. clarkii* hemolymph on kidney functions in GM treated rats

Variable	Organ	Control	GM	
			Vehicle	*P.clarkii*
MDA (nmol/g.tissue)	Liver	18.05 ± 2.07^{a}	40.10 ± 2.77^{c}	26.20 ± 1.33^{b}
	Kidney	1.85 ± 0.12^{a}	4.73 ± 0.20^{c}	2.73 ± 0.46^{b}
GSH (mg/g.tissue)	Liver	21.36 ± 2.15^{c}	11.40 ± 0.92^{a}	16.14 ± 0.74^{b}
	Kidney	6.18 ± 0.88^{c}	3.16 ± 0.12^{a}	3.97 ± 0.11^{b}
CAT (U/g.tissue)	Liver	27.51 ± 1.33^{c}	11.65 ± 1.36^{a}	18.67 ± 1.42^{b}
	Kidney	8.02 ± 1.16^{c}	3.57 ± 0.28^{a}	4.25 ± 0.35^{b}

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

3.4. Histopathology of Kidney

The control group showed normal histology of the 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). 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. 1B). The renal cortex of rats from P.clarkii hemolymph group appeared normal except for the presence of severely congested blood capillaries and few tubules containing eosinophilic cast (Fig. 1C). A qualitative score of histopathological lesions in the kidneys of each group is represented in Table 4.

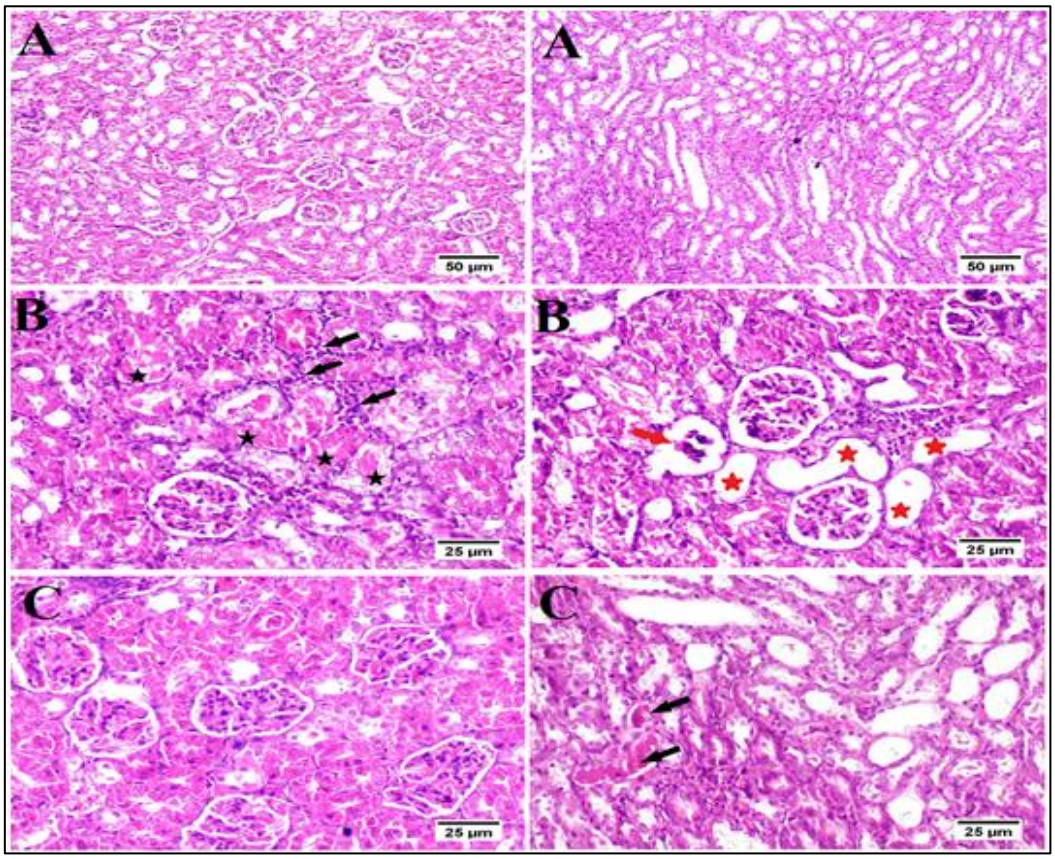
Table-4. Qualitative score of histopathological lesions in kidneys

	Control	GM	*P.clarkii*
Congestion	-	+++	++
Perivascular edema and inflammation	-	+++	+

Nephrosis	-	+++	+
Interstitial nephritis	-	+++	+

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

Figure-1. A: Kidneys of rat (control group) showing normal renal cortex and renal medulla. **B**: 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. **C**: Kidneys of rat (*P.clarkii* group) showing apparently normal renal cortexand renal medulla with presence of intraluminal protein cast (arrow) (H&E)



4. Discussion

Gentamicin (GM) is a widely used aminoglycoside antibiotic, it is known to be potentially nephrotoxic despite close attention to the pharmacokinetics and dosing schedules of the drug [25, 26]. In the present study, we evaluate the protective effect of *Procambarus clarkii* hemolymph against GM -induced nephrotoxicity in rats

The current investigation showed that consuming gentamicin for one week produced a marked increase in creatinine urea and uric acid. It was established that GM is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation which leads to a reduced glomerular filtration rate [27].

AST and ALT are the most significant biomarkers used in liver disease diagnostics [28]. The present study illustrated that GM administration elevated AST and ALT, enzyme activities and decrease protein concentration in the serum of rats. Increase AST and ALT and decrease protein levels in circulation indicate necrosis and membrane damage to the liver leading to leakage of these enzymes into the circulation and loss of liver synthesis function [29, 30].

Oxidative stress occurs when the balance between antioxidants and ROS are disrupted because of either depletion of antioxidants or accumulation of ROS [31, 32] ROS, by-products of aerobic metabolism, produce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, proteins and DNA [33, 34]. Oxidative stress plays a major role in the nephrotoxicity of gentamicin [35]. There have been reports that treatment with gentamicin produces oxidative stress in renal tubule cells, both in vivo and in vitro [36, 37]. Malondialdehyde (MDA) results from lipid peroxidation of polyunsaturated fatty acids [38]. The current study revealed that GM administration significantly caused an elevation in the MDA level. The elevation in MDA suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals [39]. GSH protects cells against free radicals peroxides and other toxic compounds [40, 41]. Furthermore, GSH reduces peroxides and maintains protein thiols in the reduced state [42]. The present study showed a reduction in GSH content in GM administration. The decrease in the concentration of GSH after the GM treatment might be due to the increased consumption of GSH in the non-enzymatic removal of oxygen radicals. Catalase (CAT) is a common enzyme found in almost all living organisms exposed to oxygen, where it catalyzes hydrogen peroxide decomposition into water and oxygen [43]. The increased production of ROS in GM mediated nephrotoxicity leads to the inactivation of antioxidant enzymes [44].

The present findings demonstrated the hepatorenal protective effect of *P.clarkii* hemolymph at 45.6 mg/Kg for 7 days significantly. The enhancement in the antioxidant system may be due to phenolic and flavonoid compounds present in *P.clarkii* that can scavenge free radicals [20, 45]. Besides, Fahmy and Hamdi [46] reported that the administration P. clarkia hemolymph attenuate disrupted hepatic and erythrocytes ROS their antioxidant action. Moreover, Stimulation of protein synthesis after *P.clarkii* hemolymph administration was established as a contributory hepatoprotective mechanism that accelerates the process of regeneration and the development of hepatic cells [47].

5. Conclusion

This study demonstrated the *P.clarkii* hemolymph potency in ameliorating the biochemical and histopathological changes in the kidney of the rats following experimental induction of renal toxicity using Gentamycin (GM). The protection mechanism of *P.clarkii* hemolymph may through suppressing the formation of reactive oxygen species and enhancing the internal antioxidant system.

5.1. Compliance with Ethical Standards

- The authors declare no conflict of interest
- Test conventions and techniques utilized in this examination were endorsed by the Cairo University, Faculty of Science, Institutional Animal Care and Use Committee (IACUC) (Egypt) (CUIF2518). All the experimental procedures were completed as per universal rules for the consideration and utilization of research facility animals.

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