GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

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Naja nubiae venom ameliorate hepatorenal toxicity induced by Gentamicin in rats

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GSC Advanced Research and Reviews, 2021, 09(03), 030–038

Publication history: Received on 30 October 2021; revised on 03 December 2021; accepted on 05 December 2021

Article DOI: https://doi.org/10.30574/gscarr.2021.9.3.0290

Abstract

Background: In developing countries, there is no doubt that acute kidney injury and chronic liver diseases have a major impact on health. Different venom components are gaining renewed interest as potential sources of new pharmacological compounds relevant for human therapeutics.

Aim: The present study was designed to evaluate the therapeutic efficacy of the crude venom extract of the Egyptian spitting cobra (*Naja nubiae*) venom extract against gentamycin–induced nephrotoxicity and hepatotoxicity in rats. Methods: Eighteen male Wistar rats were divided into three groups, control, gentamycin, and venom extract. The hepatorenal toxicity model was induced by gentamycin (80 mg/kg, intraperitoneal) for 8 days.

Results: LD50 of venom extract in rats was 0.2mg/kg. The venom extract group showed a significant decrease in the liver enzymes, urea, uric acid, creatinine, malondialdehyde meanwhile glutathione reduced and catalase levels increased. Histological examination of liver and kidney mild protective efficacy with less extensive degenerative changes in the tissues of venom extract grou

Conclusion: The results of the present investigation showed that administration of venom extract proved therapeutic efficacy against gentamycin -induced hepatorenal dysfunction by maintain the normal functional status of the liver and kidney and normalized the antioxidant system.

Keywords: *Naja nubiae*; Spitting cobra; Hepatorenal toxicity; Gentamycin; Venom

1. Introduction

In developing countries like Egypt, there is no doubt that acute kidney injury (AKI) and chronic liver diseases significantly impact health [1]. Drug-induced hepatotoxicity and AKI are widespread conditions and are responsible for various pathological effects on the liver and kidney [2,1]. Indeed, AKI and hepatotoxicity are the most common two complications of the widespread use of antibiotics [3,4]. Aminoglycoside antibiotics have long been used for the treatment of severe Gram-negative bacterial infections. Gentamicin (GM) is the most widely used anti- Gram-negative bacterial therapy [5,6]. Despite its beneficial effects, GM has considerable nephrotoxic and hepatotoxic side effects [7]. Bin Dajem et al. reported that renotoxicity and hepatotoxicity induced by GM are mediated through the overproduction of the reactive oxygen species (ROS) that suppresses the non-enzymatic and enzymatic antioxidants [8].

Unfortunately, the recent appearance of the undesirable side effects of traditional medications and the emergence of uncommon drawbacks has forced scientists to search for new nutraceutical drugs. Thereby, developing new drugs from

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natural products may reduce the risk of toxicity and maintain a therapeutic effect when the drug is used clinically. Animal venoms are complex cocktails of complex chemical mixtures of pharmacologically active components including mixtures of enzymatic and non-enzymatic components with specific pathophysiological functions [9]. Due to the interesting properties of snake venoms in biomedical research and as tools in diagnosis and/or therapies, they might hold the next miracle for treating different diseases. In recent years, the attention of researchers has turned to snake venoms, which contain many potentially bioactive compounds not found in other venomous lineages products, representing excellent pharmacological resources that can be used in medicine.

The Nubian spitting cobra (*Naja nubiae*) belonging to the genus *Naja* (cobras) is a species of spitting cobra native to Africa [10]. The Egyptian Spitting cobra is characterized by a cytotoxic pattern of envenomation [11]. Moreover, the use of the Nubian spitting cobra confers certain beneficial advantages, including analgesic, antipyretic, and anti-inflammatory activities. [12]. Different venom components are gaining renewed interest as potential sources of new pharmacological compounds relevant for human therapeutics. Phospholipase A2 from bee venom has been found to have an ideal protective effect against acetaminophen-induced hepatotoxicity [13]. cathelicidin peptide derived from the venom of the sea snake, *Hydrophis cyanocyntus*, exerts a protective effect in mouse models of lung inflammation and infection [14]. In addition, a study of Ebaid et al. [15] proved the potential capability of Samsum ant to restore oxidative stability and improve kidney functions after CCL4 acute injury. However, it was reported that the snake venoms usually contain two categories of components that act antagonistically through activation or inhibition of coagulation factors [16]. Thereby, the present study was designed to evaluate the therapeutic efficacy of the crude venom extract of the Egyptian spitting cobra (*Naja nubiae*) venom extract (NnvE) against GM –induced nephrotoxicity and hepatotoxicity in rats.

2. Material and methods

2.1. Chemicals

All chemicals and Kits were purchased from the Biodiagnistic Company (El Moror St, Dokki, EGY).

2.2. Collection of snake venom, preparation and storage

The Nubian spitting cobra was identified by Department of Wild Life Management and Zoo Medicine, Faculty of Veterinary Medicine, Suez Canal University. Ten specimens of *Naja nubiae* snakes were captured from the Nubian area (Aswan, Egypt) in April 2017. The milking process of the cobra collected the crude venom according to the method described as the ‘‘Mirtschin technique’’ [17]. The snake was forced to bite through a para-film membrane, stretched over a 70 ml vial. The venom was collected in the vial below the membrane. It was mixed with sterile distilled water in 1:10 ratio, lyophilized using a lyophilizer (LABCONCO lyophilizer, shell freeze system, USA) and then kept at -20 C^{o}.

2.3. Ethical Consideration

This study's experimental protocols and procedures were approved by the Cairo University, Faculty of Science, Institutional Animal Care and Use Committee (IACUC) (Egypt). All the experimental procedures were carried out in accordance.

2.4. Animals

Adult male Wistar rats (*Rattus norvegicus*) with an average body weight of 150 - 170 gm were purchased 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) with 12:12 h day/night cycle. They were nourished standard chow pellets and drinking water *ad libitum*.

2.5. Determination of LD_{50}

The LD50 of the venom was calculated using the arithmetic method of Karber as modified by Aliu and Nwude [18]. Twenty female rats were used for the determination of the LD50. The rats were divided into four groups (A-D) of five animals each. The groups were given different doses of the extracted venom by intraperitoneal route as follows: Group A: saline serves as a control, Group B: 0.2 mg/kg, Group C: 0.4 mg/kg, and Group D: 0.6 mg/kg. The animals were then observed for 24 h for signs and symptoms of toxicity and death. The LD50 was calculated using the formula:

$$LD_{50} = LDy - \Sigma (Dd \times md)/N$$

LDy =Highest dose (LD_{100}), N =Number of animals per group, Dd =Dose difference., Md =Mean dead.

2.6. Experimental design

Eighteen rats were divided into three groups;

Group, I was the control group of rats and they received dist. water orally. Group II was the model gentamicin group and they received 80 mg/kg of gentamicin i. based on the protocol set by Jabbari et al. for 8 days [19].

Group III was the venom treatment group, where the rats received firstly gentamycin for 8 days then administrated 1/10 venom crude extract for another 7 days.

2.7. Animal handling and specimen collection

After the end of the experiment, the mice were fully anesthetized with 3% sodium pentobarbital, and the chest was opened. A needle was inserted through the diaphragm and into the heart. Negative pressure was gently applied once the heart had been punctured, and the needle was repositioned as required until blood flowed into the syringe.

2.8. Samples preparations

Blood was collected from all animal groups in tubes containing EDTA for hematological assays and biochemical analysis. The blood samples were centrifuged at 3000 rpm for 20 minutes. The collected serum stored at -20 Co until used for biochemical assays. Liver and kidney tissues were homogenized (10% w/v) in ice-cold 0.1 M Tris-HCl buffers (pH7.4). The homogenate was centrifuged at 3000 rpm for 15 min. at 4oC and the resultant supernatant was used for the biochemical analyses.

2.9. Biochemical parameters

The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed by the technique for Reitman and Frankel [20], serum alkaline phosphatase (ALP) [21] serum creatinine [22], urea [23] and uric acid [24] according to the manufacturer's instructions using Bio-diagnostic kits (Giza, Egypt). MDA level is a record of lipid peroxidation and it was assessed by Ohkawa *et al* [25], glutathione reduced (GSH) [26] and catalase [27] were determined in the liver and kidney homogenate supernatant as indicated by the produces directions utilizing Biodiagnostic (Giza, Egypt).

2.10. Histopathological examination

Liver and kidney tissues were fixed in 10% formal saline, embedded in paraffin, and sectioned. At that point, the sections were stained with hematoxylin and eosin for histological assessment utilizing a light magnifying lens.

2.11. Statistical analysis

Values were expressed as means ±SE. 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, for Windows (version 15.0) was used for the statistical analysis.

3. Results

3.1. LD50 Results

Table 1 Determination of LD50 of the crude venom of snake (*Naja nubiae*) in rats

Dose	No of Death	Mean Death	Dose Diff	Mean Death x Dose Diff.
Control	0	0	0	0
0.2	0	0	0.2	0
0.4	3	1.5	0.2	0.3
0.6	4	3.5	0.2	0.7
Sum (Σ)				**1**

By applying the formula LD50 =LDy – Σ (Dd x md)/N the LD50 could be calculated as follows; LD50=0.6 – 1/5= 0.2 mg/kg

None of the animals in Group A showed any clinical or behavioral changes throughout the observation period. However, depression, weakness, and loss of appetite in the first 5 hr were observed in Groups C, D animals treated with the higher doses of the extracted venom (Table 1).

3.2. Liver function markers

Significant (P<0.05) increase was noticed in AST, ALT, and ALP activities of GM groups as compared with the control grou In the venom-administered group rats, there was a significant (P<0.05) reduction to the liver parameters as compared with the GM group (Table 2).

Table 2 Effect of crude venom on liver function parameters of gentamicin induced hepatorenal toxicity in rats

Groups	AST (U/ml)	ALT (U/ml)	ALP (IU/L)
Control	64.04 ±1.27^{a}	36.44 ± 0.55^{a}	123.56±1.61^{a}
GM	86.09 ± 0.90^{c}	50.36 ±0.91^{c}	167.30±1.92^{c}
Venom	74.18 ±0.96^{b}	40.62 ± 0.79^{b}	147.65±2.11^{b}

Data are expressed as mean ± SD (n=5). Values with different superscripts within one column differ significantly (*P*<0.05).

3.3. Renal function markers

The levels of urea, uric, and creatinine in the GM group were increased significantly (P<0.05) in comparison to those of the control group, which indicates damage to the kidney and the occurrence of dysfunction. Meanwhile, levels of urea, uric, and creatinine in the venom-administered rats were significantly (P<0.05) decreased in comparison to those of the GM group (Table 3).

Table 3 Effect of crude venom on kidney function parameters of gentamicin induced hepatorenal toxicity in rats

Groups	Urea (g/dl)	Uric (mg/dl)	Creatinine (mg/dl)
Control	33.55 ±1.18 ^{a}	1.83 ± 0.09^{a}	1.24 ± 0.05 ^{a}
GM	50.47 ± 1.25 ^{c}	6.93 ±0.34^{b}	2.67 ± 0.05 ^{c}
Venom	38.89 ± 0.86 ^{b}	2.31 ±0.04^{a}	1.46 ±0.05 ^{b}

Data are expressed as mean ± SD (n=5). Values with different superscripts within one column differ significantly (*P*<0.05).

3.4. Oxidative stress markers in liver and kidney

Administration of GM compared to the control group significantly reduced the levels of GSH and CAT; meanwhile, it increased MDA concentration (Table 4). Treatment of rats with snake venom significantly reversed the changes made by gentamicin and resembled more closely to that of the control grou

Table 4 Effect of crude venom oxidative stress parameters of gentamicin induced hepatorenal toxicity in rats

Groups	MDA (nmol/gm)		GSH (mg/g)		CAT (U/gm.tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	0.78 ± 0.04^{a}	1.04 ±0.08^{a}	2.60± 0.14^{b}	5.38 ±0.21^{c}	21.04 ±0.59^{c}	17.34 ± 0.44^{c}
GM	1.30 ±0.03^{c}	1.59 ± 0.03^{c}	1.52±0.21^{a}	2.91±0.13^{a}	12.47 ±0.49^{a}	9.36±0.53^{a}
Venom	1.00 ±0.02^{b}	1.29 ±0.03^{b}	2.35 ±0.10^{b}	4.72±0.19^{b}	17.31±1.42^{b}	13.85 ±0.44^{b}

Data are expressed as mean ± SD (n=5). Values with different superscripts within one column differ significantly (*P*<0.05).

3.5. Histopathology of liver

The Control group showed normal morphological features of hepatic parenchyma with almost intact radiating hepatocytes and intact vasculatures without abnormal tissue alterations (Figure 1). GM group showed alternated areas of degenerative changes of hepatocytes in pericentral and periposrtal zones (arrow) with a focal aggregation of inflammatory cells (star). Moderate congestion of portal blood vessels accompanied with hyperplasia of bile ducts

(arrowhead) as well as inflammatory cell infiltrates was recorded; Venom group sample showed wide areas of almost intact hepatocytes with few scattered degenerated cells (arrow). Intact vasculature with many activated kupffer cells (dashed arrow)

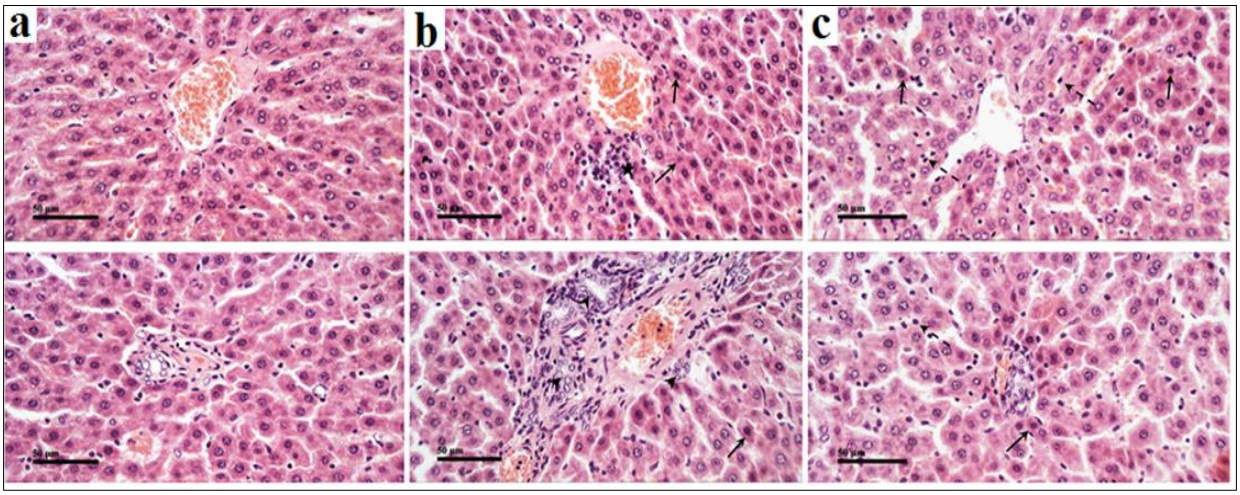

Figure 1 Comparison between the histology of the liver tissues between control group (a), gentamicin group (b) and venom group (c)

3.6. Histopathology of kidney

Figure 2 show histopathological images of the kidney of all groups. No histopathological changes were observed in the kidney in the control grou Normal kidney tissue (control group); showed normal histological structures of renal tissue in cortical and medullary zones with almost intact renal corpuscles (star), different nephronal segments and vasculatures. GM group showed wide areas of the degenerated renal tubular epithelium with pyknotic nuclei (arrow) and many congested intertubular blood vessels and glomerular capillaries (stars). The venom-treated group showed mild protective efficacy with less extensive degenerative changes of lining tubular epithelium (arrow) alternated with intact tubules. However; intraluminal eosinophilic casts (star) were recorded in may tubular segments of all groups.

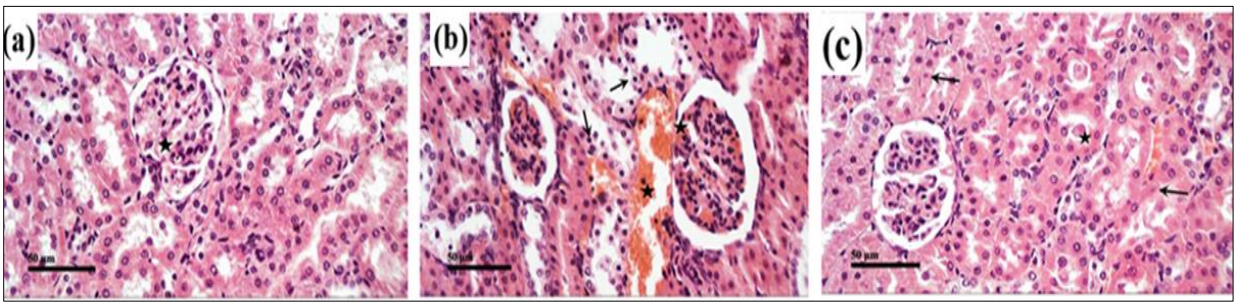

Figure 2 Comparison between the histology of the kidney tissues between control group (a), gentamicin group (b) and venom group (c)

4. Discussion

Gentamicin is one of the important broad-spectrum antibiotics that are highly potent and have satisfactory therapeutic efficacy in the treatment of life-threatening infections [28]. Its usage is severely limited by its physiological side effects, such as hepatotoxicity and nephrotoxicity [7]. For the therapeutic strategies of liver and kidney injuries and diseases, it is important to find a complementary compound that can block this injury. The pharmaceutical industry produced several venom derived-drugs such as Captopril, Aggrastat, and Eptifibatide [29]. The present study represented the first report studying the anti- nephrotoxic and anti- hepatotoxic effects of the Egyptian spitting cobra (*Naja nubiae*) crude extract in rats.

Hepatic dysfunction was the most common regimen-related toxicity reported in patients treated with GM. In conjunction with the reports of Galaly *et al*. [30], and Khaksari *et al*. [31], data from the present study showed that GM

caused hepatic damage with a significant increase in serum levels of ASAT, ALAT and AL Microsomal activation of the drugs primarily occurs in the hepatic tissues. It was reported that aminoglycosides disrupt the signal transduction pathway and increase cellular permeability by acting on membrane phospholipids [32]. The hepatic tissue damage induced by GM in the present study can be confirmed by the histological alterations of liver tissues and increased liver enzymes in the serum. The results of the present investigation showed that administration of NnvE proved therapeutic efficacy against GM-induced hepatic dysfunction through decreasing the activities of ASAT, ALAT, and ALP enzymes, showing it's potential to maintain the normal functional status of the liver. Histopathological observation of the liver tissue had directly supported this conclusion.

Kidneys are self-motivated organs and represent a major con-trol system maintaining the body's hemostasis. During the progression of the renal disease, changes in renal function are one of the most common manifestations of severe illness accompanied by failing organ function leading to the accumulation of a series of compounds [33]. The hepatorenal disorder is most commonly diagnosed by a finding of increased levels of serum creatinine, urea, and uric acid [34]. Experimental impairment of kidney function is induced through treatment by specific chemicals or drugs. Viewed in conjunction with previous studies [35,36,8], data from the present investigation reflect that GM induced marked alteration on renal functions as manifested by a significant increase in the kidney function markers, serum creatinine, urea and uric acid. The recorded increase in serum creatinine in the present study may be due to the hepatic damage which evolved into a stage with features of the hepatorenal syndrome [37]. Moreover, the marked alteration in renal functions as manifested by a significant increase in the recorded kidney function markers following administration of GM may suggest the possible upregulation in the synthesis of creatinine that needs to be excreted with urine. In addition, it was reported that GM is selectively taken by the cells lining proximal convoluted tubules [38]. When the concentration of GM reached the toxic threshold, it induced oxidative stress, which triggers lysosomal, mitochondrial, and cell membrane damage [38]. Study-ing new therapeutic agents, including nutraceu-ticals, usually need induction of kidney dysfunction in experimental animals. The present study showed a significant decrease in the serum creatinine, urea, and uric acid following treatment with NnvE that may be a contributory self-healing mechanism restoring the kidney structure and function.

Oxidative stress stimulated by GM is a systemic phenomenon. Several studies have shown that GM administration leads to the generation of oxidative stress in the liver and kidney with a resulting decrease in the activities of antioxidant enzymes and an increase in lipid peroxidation in these tissues [39,40]. In conjunction with the reports of Yarijani et al. [41] data from the present investigation reflects that oxidative stress in the liver and kidney is a common feature of GM toxicity. In conjunction with the reports of Yarijani *et al*., MDA levels increased significantly in the liver and kidney tissues of GM treated rats as a result of lipid peroxidation and hepatorenal toxicity [41]. The histological observations of hepatic and renal tissues, like moderate congestion of portal blood vessels accompanied with hyperplasia of bile ducts and many congested intertubular blood vessels and glomerular capillaries recorded in the present study, maybe due to significant oxidative injury. However, the reduction of lipid peroxidation-mediated oxidative stress may be an effective strategy for the prevention and treatment of hepatorenal injuries. Inconsistent with the report of both Abdel-rahman and AbdelMoneim [42], and Elshater *et al*. [43], the present study showed that treatment of GM pretreated rats with NnvE significantly decreased MDA levels which may be due to its antioxidant mechanism. Several reports stated that bee venom therapy is a potent antioxidant that led to a decrease in the levels of reactive oxygen species (ROS) [44,45].

Reduced glutathione (GSH) is one of the body’s most important antioxidant that plays a common role in cellular resistance to oxidative damage as a free radical scavenger. There is accumulative evidence that a decrease in GSH level and the decrease in catalase (CAT) activity referring to the enhancement of the adaptive mechanism that copes ROS [41,8]. Moreover, the concomitant decrease in GSH with MDA increase following GM administration in the present study demonstrates the role of the oxidative mechanism in hepatorenal syndrome [41]. Accordingly, antioxidant therapy represents a potential strategy to prevent hepatorenal injury. Treatment with NnvE in the present study normalized the antioxidant levels through its antioxidant effect that can scavenge free radicals.

5. Conclusion

The results of the present investigation showed that administration of NnvE proved therapeutic efficacy against GM-induced hepatorenal dysfunction by maintain the normal functional status of the liver and kidney and normalized the antioxidant system.

Compliance with ethical standards

Acknowledgments

This work was supported by Cairo University.

Disclosure of conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Declarations of interest

All Authors decelerate that no conflict of interest.

Statement of ethical approval

The experiments were carried out according to international guidelines for care and use of laboratory animals. The protocol was approved by the Faculty of Science, Cairo University, Egypt, and Institutional Animal Care and Use Committee (IACUC), (Egypt).

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