

Antimicrobial Profile of Selected Snake Venoms and their associated enzymatic activities. Experimental Evaluation of Antiviral and Antibacterial Potentials of Selected Snake Venoms against Viral and Bacterial Models

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ABSTRACT

Aims: To investigate the antiviral and antibacterial profile of several crude snake venoms and to assess some of their enzymatic activities.

Methodology: The antiviral activities of *Naja haje*, *Bitis arietans*, *Naja nigricollis* and *Echis carinatus* snake venoms were investigated against *Herpes simplex virus* type1, *Rift valley fever virus* and *Vesicular stomatitis virus* using the end point of cytopathic effect method. Antibacterial activities of *Bitis arietans*, *Cerastes cerastes*, *Echis carinatus*, *Vipera lebetina*, *Naja naja*, *Pseudechis australis*, *Naja nigricollis* and *Naja haje* venoms were examined against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* using disc diffusion method. Microdilution method was used to determine the venom's minimum inhibitory concentration. L-amino acid oxidase and phospholipase A2 activities of crude venoms were evaluated using enzymatic assays.

Results: *Naja nigricollis*, *Bitis arietans* and *Echis carinatus* snake venoms, but not *N. haje* venom, exhibited significant antiviral activities against all test viruses, except for *N. haje* treated cells, except for *N. haje* treated cells. The mean depletion of viral infectivity titer of venom pretreated cells was higher than its depletion post viral infection for all three venoms showing antiviral activities. *Naja nigricollis* exhibited the highest antiviral activity against test viruses and recorded a mean depletion of viral infectivity titer in venom pretreated cells of 3.8 log (10) / ml, 3.2 log (10) / ml, and 2.5 log (10) / ml for HSV-1, RVFV and VSV, respectively. *Pseudechis australis*, followed by *Naja naja* and *Naja nigricollis* venoms, showed the highest inhibitory activity against test bacteria with inhibition zones ranging from 11-17 mm, 8-14 mm and 8-13 mm, respectively. Minimum inhibitory concentrations of test venoms against different bacterial strains ranged from 156 µg / ml to 1.25 mg / ml. Maximum L- amino oxidase activity was detected in *Naja naja*, *Cerastes cerastes* and *Pseudechis australis*. The highest Phospholipase A2 activity was identified in *Bitis arietans*, *Pseudechis australis*, *Naja naja* and *Naja nigricollis*.

Conclusion:

It can be concluded that snake venoms or their bioactive derivatives can may can be promising therapeutic agents against some microbial infections. Further investigations will be carried out for purification and more characterization of the biologically active components in snake venoms.

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Keywords: [Snake venom, antibacterial activity, antiviral activity, L- amino oxidase activity, Phospholipase A2 activity]

1. INTRODUCTION

Bacterial infections are among the 10 causes of death worldwide according to the World Health Organization (Lopez et al., 2006). The presence and emergence of resistant strains make the risk of these infections a universal problem with deleterious effects. Therefore, the discovery of new alternatives is necessary for treatment of infections involving resistant microorganisms.

Recently, new antimicrobial peptides from natural sources have drawn attention as antimicrobial agents. Since antimicrobial peptides were initially identified in frogs and insects in the 1980s, many additional peptides have been found and over 1200 have been isolated to date (Park et al., 2011). Among these antimicrobial peptides were those isolated from snake venoms.

Snake venoms are complex mixtures of toxins and enzymes with different activities on many biological systems. Among other components, snake venoms include cytotoxins, antibacterial and anti-viral factors (Chaim-Matyas et al., 1991; Borkow and Ovadia., 1994; Mollmann et al., 1997; Borkow et al., 2008). It was reported that L-amino acid oxidase present in snake venoms has many important biological properties as platelet aggregation inhibitor, bactericidal activity, antiviral activity and cytotoxicity against tumor cells (Rideiro et al., 2007). Chellapandi and Jebakumar., (2008) reported that phospholipase A2 and L-amino acid oxidase present in snake venoms had toxic and non-toxic antimicrobial activities. Moreover, it was reported that snake venoms of *Crotalus adamanteus*, *Daboia russelli*, *Agkistrodon halys*, *Pseudechis australis*, *Bungarus candidus*, *Pseudechis guttata* and *Agkistrodon rhodostoma* were effective against several bacterial strains (Samy et al., 2006; Ferreira et al., 2008). In addition, to the newly isolated snake venom derivatives, such as Crotamine, Cathelicidin BF-15 and Cathelicidin BF-30, where they exhibited broad antibacterial activities that were suggested to be based on enhancing cytoplasmic membrane permeabilization (Oguiura et al., 2011; Chen et al., 2011; Zhou et al., 2011).

Concerning antiviral activity of snake venoms, it was demonstrated that cobra venoms of *Naja atra* and *Naja nigricollis* had antiviral activities against Sendai virus (Borkow and Ovadia., 1992). In another study, it was reported that *Crotalus durissus terrificus* snake venom was found to have an antiviral activity against Measles virus (Petricevich and Mendonca, 2003). Moreover, Crotoxin and phospholipases A2, isolated from this venom, showed antiviral activity against Dengue and Yellow fever viruses (Muller et al., 2012). Snake venom's phospholipases A2 were also a new class of HIV inhibitors that blocks the virus entry into the host cells (Fenard et al., 1999). Recently, it was reported suggested that the antiviral properties components of snake venoms appeared as new promising therapeutic alternatives against the defense mechanisms developed by viruses (Rivero et al., 2012).

The present work aimed at evaluation-evaluatingof the antimicrobial activity-activities of different crude snake venoms by testing their potency against several selected viruses and bacteria, against viral and bacterial models.

2. MATERIAL AND METHODS

2.1 Materials

Snake venoms: *Bitis arietans* (*B. arietans*), *Cerastes cerastes* (*C. cerastes*), *Echis carinatus* (*E. carinatus*), *Vipera lebetina* (*V. lebetina*), *Naja naja* (*N. naja*), *Pseudechis australis* (*P. australis*), *Naja nigricollis* (*N. nigricollis*) and *Naja haje* (*N. haje*) were kindly supplied from VACSERA Sera Plant- Egypt.

Viral models: *Herpes simplex* virus type.1 (*HSV-1*), *Rift valley fever* virus (*RVFV*) and *Vesicular stomatitis* virus (*VSV*) were kindly supplied from Applied Research Sector VACSERA – Egypt.

Bacterial strains: *Staphylococcus aureus* ATCC 6538 (*S. aureus*), *Escherichia coli*, ATCC 8739 (*E. coli*), *Salmonella typhimurium* ATCC 35664 (*S. typhimurium*) and *Pseudomonas aeruginosa* ATCC 9027 (*P. aeruginosa*) were kindly supplied from American Type Culture Collection (ATCC) – USA.

2.2 Methods

2.2.1 Cytotoxicity

Cytotoxic effect of test venoms (*N. nigricollis*, *N. haje*, *E. carinatus* and *B. arietans*) to Vero cells (Clone CCL-81- ATCC) was carried out using MTT assay (Cory et al., 1991) where test venoms were saline dissolved (Adwic-Egypt) to contain 2560 µg/ml and sterile filtered using 0.22 µm syringe filter (Millipore-USA). Precultured 96-well Vero cells plates (Nunc- USA) were treated with descending double fold serially diluted venoms at 37 °C for 24 h. Negative cell control of untreated cells was included. Residual living cells were treated with 20 µl of MTT (5 mg/ mL) (Sigma-Aldrich-USA) at 37°C for 4 h. MTT was discarded. Plates were PBS washed three times. DMSO (BDH-England) was added as 50 µl/well. Plates were shaken on plate shaker (Staurt-England) for 30 min to dissolve the produced intracellular blue formazan complex. Optical densities (O.D) were read at 570 nm using an ELISA plate reader (Dynatech -England). Data were reported for three independent experiments. Viability percentage was calculated according to Chen et al., (2009) as follows: Cell viability percentage = (O.D of treated cells / O.D of untreated cells) X 100. Results were expressed as means of three independent tests ± standard deviation.

2.2.2 Antiviral activity

The non-toxic concentrations of *N. haje*, *B. arietans* (10 µg/ml), *N. nigricollis* and *E. carinatus* (20 µg/ml) venoms were used to evaluate the antiviral activities against *HSV-1*, *RVFV* and *VSV* (Petricevich and Mendonca, 2003). Test viruses were titrated on non washed 24 h venom pretreated cells and another set of cells were treated with venom 1 h post virus adsorption. Virus titer was determined according to Reed and Muench, (1938). The differences between the virus titers in venom treated and untreated cells represent the antiviral activities. Statistical differences between the virus titer in venom treated cells and its titer in untreated cells were determined using one way ANOVA. Differences at $P \leq .05$ were considered significant.

2.2.3 Antibacterial activity

2.2.3.1 Disc diffusion susceptibility test

Antibacterial activities of snake venoms were evaluated using disc diffusion method (Chellapandi and Jebakumar, 2008). Bacterial inoculums were adjusted to an absorbance of 0.132 at 600 nm (equivalent to 1×10^8 colony forming units / ml). Two hundred µl of bacterial inoculums were spread on tryptic soya agar plates (90 mm diameter) (Gibco - USA) using sterile cotton swabs (Nunc-USA). The surface of the medium was allowed to dry for about 3 min. Sterile filter paper discs (5 mm diameter) (Whatman - United Kingdom) loaded with 200 µg/disc of each snake venom sample were placed onto the surface of agar plates. Discs containing antibiotics (Bioanalyse - Netherlands) were used as positive drug controls. Negative control discs were also included. Plates were incubated at 37°C for 24 h. The diameters of inhibition zones were measured in millimeter including the 5-mm diameter of the filter disc. Results were means of three independent tests ± standard deviation.

2.2.3.2 Minimum inhibitory concentrations (MICs)

MICs of test venoms were carried out according to Andrews, (2001). Test venoms (5 mg/ ml) were descending ordered double fold serially diluted in normal saline (0.9%) in 96-well microtiter plate and inoculated with equal volume of bacterial inoculums (1×10^5 CFU / ml). Gentamicin (Memphis - Egypt) prepared as 128 µg/ml was processed in the same way. Negative control wells were included. Plates were incubated at 37°C for 24 h. O.D was measured at 600 nm using ELISA plate reader (Dynatech medical products-England) (Sachidananda et al., 2007). Percentage of inhibition was calculated as follows:

Percentage of inhibition = $[1 - (a/b) \times 100]$

a = O.D of bacteria with test material

b = O.D of control well containing saline, bacteria and media

2.2.4 Enzymatic assays

2.2.4.1 L-amino acid oxidase assay

L-amino acid oxidase (LAO) activity was determined according to Knox and Pitt., (1957); Wellner and Lichtenberg., (1971), where 11.7 ml of 200 mM sodium phosphate buffer (BDH-England), 3 ml of 10 mM L-phenylalanine solution (Sigma-Aldrich-USA) and 14 ml of 2000 mM boric acid solution (BDH-England) were mixed by stirring and adjusted to pH 6.5 with 1 M HCl or 1 M NaOH. Aliquot of 2.87 ml of the mixture was pipetted into cuvettes, mixed with 0.03 ml catalase (60 000 unit/ml) (Sigma-Aldrich-USA) and left to equilibrate at 37°C. Absorbance at 308 nm was monitored till constant reading. Test venoms (0.1 ml, 1 mg/ml) were added and immediately mixed. Increase in absorbance for approximately 10 min was recorded for both test and blank. One unit of enzyme activity was defined as the unit of enzyme that oxidatively deaminate 1 µmole of L-phenylalanine per min. Results were expressed as mean of three independent tests $\pm$ standard deviation.

2.2.4.2 Phospholipase A2 assay

Phospholipase A2 (PLA2) assay was carried out according to the acidimetric method of Tan and Tan., (1988) where an egg yolk suspension was prepared by mixing three equal parts consisting of one part chicken egg yolk, one part 18 mM calcium chloride (ICI –UK), and one part 8.1 mM sodium deoxycholate (BDH-England). The pH of the egg yolk suspension was adjusted to 8.0 using 1 M sodium hydroxide (ICI –UK), and stirred for 10 minutes to ensure homogenous mixing. One hundred µl of 1mg/ml saline dissolved test venom was added to 15 ml of egg yolk suspension to initiate the hydrolysis. The initial decrease in pH was measured by a pH meter (Denver-USA). A decrease of 1 pH unit corresponds to 133 µmoles of fatty acid released. Enzyme activity was expressed as mean of three independent tests and was expressed as µmoles of fatty acid released per min $\pm$ standard deviation.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Cytotoxicity

Data recorded revealed that 100 % cellular viability was noticed on using *N. nigricollis*, *N. haje*, *E. carinatus* and *B. arietans* as 20 µg /ml, 10 µg/ml, 20 µg/ml and 10 µg/ml respectively (Figure 1).

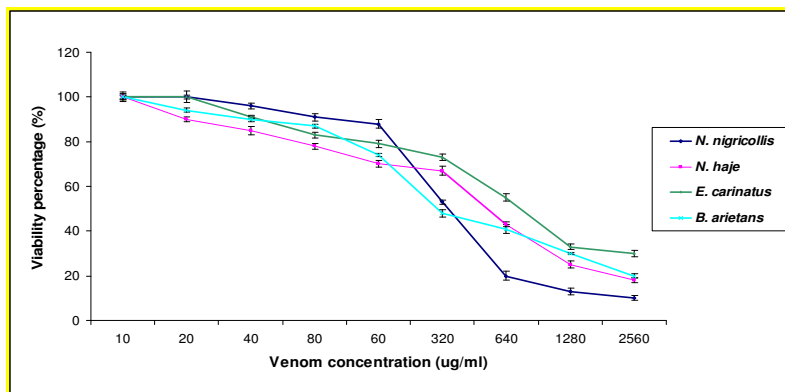


Figure 1. Evaluation of cytotoxicity of double fold serially diluted test venoms (2560 µg/ml) to Vero cells 24 h post treatment. Recorded values were the mean of three independent experiments ± S.D.

3.1.2 Antiviral activity

Data recorded revealed that *N. nigricollis*, *B. arietans* and *E. carinatus* venoms showed antiviral activities against test viruses in both venoms pretreated cells and 1 h post infection compared with the viral infectivity titer of non treated cells. The induced antiviral potential post viral infection showed a slightly depleted titer compared with its depletion in venom pretreated cells. On the other hand In contrast, *N. haje* venom didn't show significant viral depletion rate against test viruses at $P \leq 0.05$ (Table 1).

Table 1. Evaluation of antiviral activities of test venoms using cell culture assay.

Test venoms	Test viruses	Mean virus titer log (10) / ml ± SD			Mean depletion titer log (10)/ml	
		Untreated cells	Venom pretreated cells	1 h post viral infection	Venom pretreated cells	1 hr post viral infection
<i>N. nigricollis</i>	HSV-1	5.3 ± 0.07	1.5 ± 0.15	3.2 ± 0.09	3.8*	2.1**
	RVFV	6.7 ± 0.05	3.5 ± 0.08	4.9 ± 0.14	3.2*	1.8**
	VSV	8.5 ± 0.1	6 ± 0.12	6.9 ± 0.16	2.5*	1.6**
<i>B. arietans</i>	HSV-1	5.1 ± 0.08	2.6 ± 0.06	3.6 ± 0.13	2.5*	1.5**
	RVFV	6.3 ± 0.11	4.5 ± 0.12	5.2 ± 0.08	1.8*	1.1**
	VSV	8.3 ± 0.13	7 ± 0.11	7.5 ± 0.12	1.3*	0.8**
<i>E. carinatus</i>	HSV-1	5.1 ± 0.14	3.2 ± 0.09	3.9 ± 0.08	1.9*	1.2**
	RVFV	6.5 ± 0.09	4.9 ± 0.1	5.7 ± 0.15	1.6*	0.8**
	VSV	8.4 ± 0.12	7.3 ± 0.07	7.7 ± 0.11	1.1*	0.7**
<i>N. haje</i>	HSV-1	5.2 ± 0.11	5.1 ± 0.03	5 ± 0.04	0.1	0.2
	RVFV	6.3 ± 0.1	6.1 ± 0.07	6.2 ± 0.02	0.2	0.1
	VSV	8.2 ± 0.13	8.1 ± 0.1	8 ± 0.08	0.1	0.2

(*, **): The differences between the mean virus titer in venom treated cells compared with untreated cells were statistically significant at $P \leq 0.05$.

3.1.3 Antibacterial activity

It was found that *S. aureus* was the most susceptible strain to test venoms. *N. naja*, *P. australis* and *N. nigricollis* venoms showed the highest antibacterial activity. *E. carinatus* showed the least antibacterial activity against *S. aureus* and *E. coli*, while other bacterial strains were not affected. In contrast, *N. haje* venom showed no activity against test strains. Only *N. nigricollis* and *N. naja* showed inhibitory activity against *P. aeruginosa*, whereas *S. typhimurium* was susceptible to *C. cerastes*, *P. australis* and *N. naja* (Table 2). It was found that the minimum inhibitory concentrations (MICs) of test venoms against test microorganisms ranged from 156 µg / ml to 1.25 mg / ml, while MICs of gentamicin were much lower and ranged from 0.25 to 0.5 µg / ml (Table 3).

Table 2. Antibacterial activity of snake venoms against selected bacterial strains using disc diffusion method.

Snake venom	Mean bacterial inhibition zone diameter (mm) ± SD			
	Micro-organism			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Pseudomonas aeruginosa</i>
<i>Bitis arietans</i>	13 ± 0.1	–	–	–
<i>Pseudechis australis</i>	17 ± 0.85	12 ± 0.71	11 ± 0.86	–
<i>Cerastes cerastes</i>	12 ± 0.88	10 ± 0.55	10 ± 0.57	–
<i>Naja nigricollis</i>	13 ± 0.71	11 ± 0.82	–	8 ± 0.81
<i>Naja naja naja</i>	14 ± 0.91	12 ± 0.45	11 ± 0.62	8 ± 0.61
<i>Vipera lebetina</i>	12 ± 0.73	11 ± 0.68	–	–
<i>Echis carinatus</i>	12 ± 0.65	9 ± 0.51	–	–
<i>Naja haje</i>	–	–	–	–
Gentamicin	11 ± 0.83	18 ± 0.73	20 ± 0.50	12 ± 0.56
Penicillin	27 ± 0.12	7 ± 0.98	10 ± 0.77	8 ± 0.66

(-) = No inhibition

Table 3. Assessment of the Minimum inhibitory concentrations (MICs) of test venoms using microdilution method.

Snake venom	Minimum inhibitory concentration (MIC) (µg / ml)			
	Microorganism			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Pseudomonas aeruginosa</i>
<i>Bitis arietans</i>	312.5	NA	NA	NA
<i>Pseudechis australis</i>	156	312.5	625	NA
<i>Cerastes cerastes</i>	625	1250	1250	NA
<i>Naja nigricollis</i>	312.5	625	NA	1250
<i>Naja naja naja</i>	156	312.5	625	1250
<i>Vipera lebetina</i>	625	625	NA	NA
<i>Echis carinatus</i>	625	1250	NA	NA
Gentamicin (µg / ml)	0.5	0.25	0.25	0.5

(NA) = Non applicable

3.1.4 Enzymatic assays of L-amino acid oxidase and phospholipase A2

Enzymatic activity revealed the existence of LAAO and PLA2 in tested venoms. Maximum LAAO activity was detected in *N. naja*, *C. cerastes* and *P. australis*. Moderate activity was recorded in *V. lebetina* and *N. nigricollis*. The highest PLA2 activity was identified in *B. arietans*, *P. australis*, *N. naja* and *N. nigricollis*. Moderate activity was noticed in *V. lebetina* and *C. cerastes*. *N. haje* showed the least LAAO and PLA2 activity (Figure. 2) and (Figure. 3).

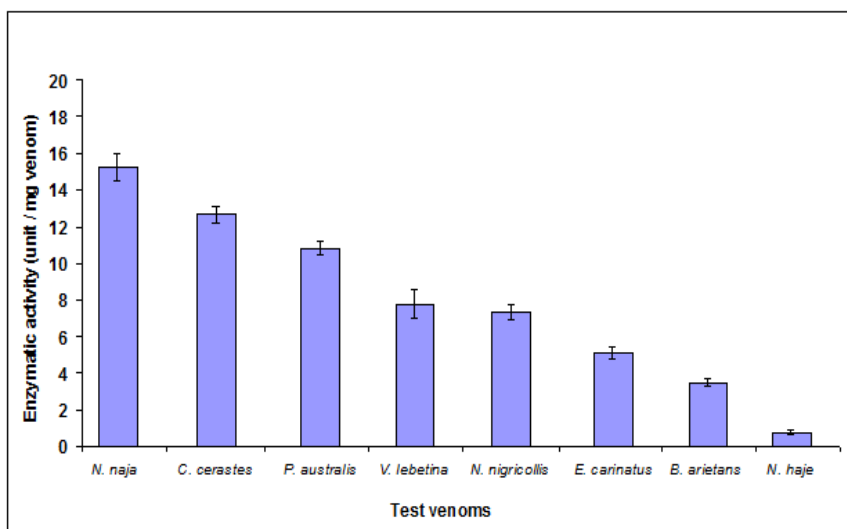
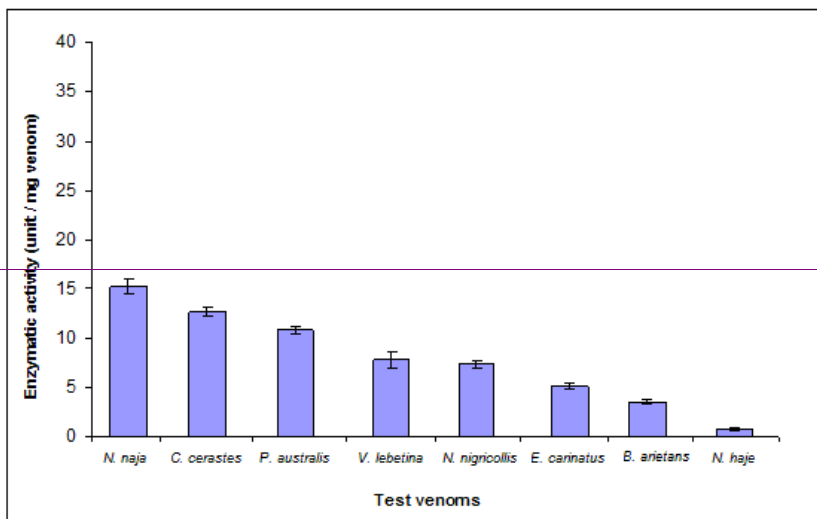


Fig. 2. Enzymatic activity of L-amino acid oxidase based on the oxidative deamination of L-phenylalanine per minute. Results were expressed as mean of three independent tests $\pm$ standard deviation.

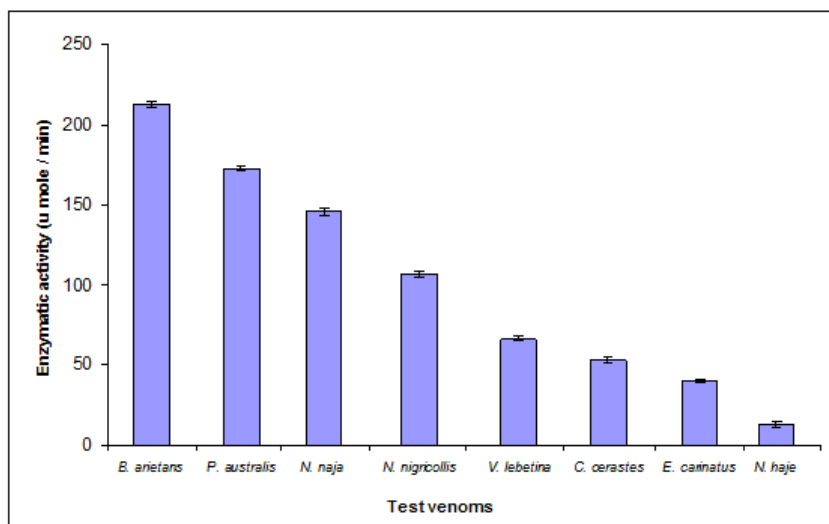


Fig.3. Enzymatic activity of phospholipase A2 expressed as the mean μmoles of fatty acid released per minute. Values were means of three independent tests ± standard deviation.

3.2 DISCUSSION

Animal venoms are complex mixtures of proteins and non proteins components with several biological activities. Snake venoms represent an essentially unexplored source of bioactive compounds that may cure disease which do not respond to currently available therapies (Jorge et al., 2011). In the present study, the non toxic concentrations of test venoms were first assessed to be examined in the evaluation of the antiviral activities of test venoms against the selected viral models. Data recorded revealed that *N. nigricollis*, *B. arietans* and *E. carinatus* venoms showed higher antiviral activities against HSV-1, RVFV and VSV on venom pretreated cells compared with the antiviral activities post cellular infection. These results suggested that these venoms exhibited their antiviral activity due to partial blocking or interfering with Vero cell receptors probably inducing inhibition of virus entry to target host cells. In addition, they might possess a viricidal effect on the adsorbed-virus. These results are in agreement with other studies despite the use of different virus models; *Crotalus durissus terrificus* (Cdt) venom had anti-measles virus activity that may be of potential clinical interest, where the inhibition of viral replication in Vero cells was observed when Cdt venom was added either before or during measles virus adsorption. It was also reported that little inhibition was observed when Cdt venom was added 1 h post infection, suggesting that the viral infection was inhibited at the time of the initial stages of infection during the period of adsorption and viral invasion (Petricevich and Mendonca, 2003). Moreover, it was reported that, herpoxin isolated from *Naja Kaouthia* snake venom, when inoculated onto Vero cells pretreated with HSV-2 didn't inhibit the appearance of CPE by HSV-2. However, pretreatment of Vero cells with herpoxin for 24 h followed by HSV-2 cell infection, inhibited CPE. This observation revealed that the receptors required for HSV-2 infection on Vero cells appeared to be blocked by the herpoxin inhibitor (Meier, 1997).

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Several reports had attempted to elucidate the mechanisms controlling the antiviral activity of snake venoms. In a study of Meenakshisundaram et al., (2009) it was found that the homology between a short segment in *Human immunodeficiency virus* and the highly conserved amino acid residues of snake venom neurotoxins resulted in competition between both the virus and venom on cell receptors, preventing virus entry into the host cell.

Test venoms showed variable antibacterial activity against *S. aureus*, *P. aeruginosa* and *E. coli*. Among the examined bacteria, *S. aureus* (Gram positive bacteria) showed the highest susceptibility to the test venoms, followed by the Gram negative bacteria; *E. coli* then *P. aeruginosa*. While, *S. typhimurium*, Gram negative bacteria, exhibited the least susceptibility to test venoms. These results are in agreement with San et al., (2010) who reported that Gram negative bacteria were more resistant to test crude venoms. In addition, resistance of the Gram negative bacteria could possibly be due to outer membrane charged lipopolysaccharides, as charges could affect the uptake of antimicrobial peptide resulting in bacterial resistance to these peptides (Devine and Hancock, 2002). The antibacterial profile of venoms against tested Gram negative rods was variable; this finding might reflect the presence of different components that are involved in antibacterial activity or might reflect differences between Gram negative rods. Moreover, broad antibacterial activities had previously been reported to *N. naja*, *C. cerastes* and *V. lebetina* venoms (Zolfagharian et al., 2002; Sachidananda et al., 2007; Hafshejani, et al., 2009). Venoms were further studied for their minimum inhibitory concentrations (MICs). The MIC values obtained were much higher than that of the conventional antibiotics; this could be attributed to the use of crude venoms rather than purified components.

Snake venoms are composed of a mixture of proteins and peptides (90-95%), but also include free amino acids, nucleotides, lipids, carbohydrates and metalloproteinase enzymes (5%) (Rivero et al., 2012). The ability of some snake venom toxins to cause toxicity is associated with their high specificity and affinity for functional organized cells and tissues. In spite of their toxicological effects, several isolated snake venom proteins (such as, phospholipases A2, metalloproteinases, serineproteases, L-amino acid oxidases, lectins and others) and peptides (bradykinin potentiators, analgesic peptides and others) have found practical application as pharmaceutical agents with significant therapeutic values (Soares et al., 2012). In an attempt to investigate the bioactivity of venoms components; L- amino oxidase and phospholipase A2, enzymatic assay for LAAO and PLA2 in test venoms revealed variable contents and the highest enzymatic activities were related to the antibacterial one. On the contrary, *N. haje* venom that showed minor enzymatic activities, compared to the other test venoms, also showed absence of antibacterial activity. These results suggested an association between antibacterial properties of test venoms to their LAAO and PLA2 contents. In that context, other studies also reported that the antibacterial activity of snake venoms might be attributed to LAAO and PLA2 components (Chioato and Ward., 2003; Samy et al., 2006 & 2007; San et al., 2010; Paulchamy., 2010; Torres et al., 2010; Okubo et al., 2012; Vargas et al., 2012). However, these results could not be conclusive since *B. arietans* venom showing the highest PLA2 activity, didn't show antibacterial activity especially against Gram negative organisms. These discrepancies might be attributed to that *B. arietans* venom showed very low LAAO activity and might indicate the significance of the presence of both enzymes for effective antibacterial activity. Another explanation for these discrepancies might be due to that, the snake venom antimicrobial mechanism is complex and is affected by many factors such as amino acid sequence, net charge of protein, three dimensional structure, bacterial membrane composition and salinity of the environment (Nair et al., 2007; San et al., 2010). In addition, the presence of enzymes in snake venoms does not guarantee the antibacterial activity as the bacterial cell wall may avoid or affect the actions of these proteins against them (Ferreira et al., 2008).

Concerning the mechanism of action of LAAO and PLA2, it was reported that LAAO catalyzes the oxidative deamination of L-amino acids converting them into α -keto acids, ammonia and hydrogen peroxide (Du and Clemetson., 2002) & (Chellapandi and Jebakumar., 2008). This hydrogen peroxide is involved in the antibacterial effect of these venoms (Zhang et al., 2003). In the mean time, it was reported that the structural determinant of the antibacterial effect of PLA2 is the cationic (positively

charged) and hydrophobic residues located at the C-terminal region of these proteins that bind with high affinity to negatively charged bacterial lipopolysaccharide (LPS). This binding reduces its specificity to physiological tissue targets in eukaryotic cells thus contributing to the antibacterial activity of these proteins (Lomonte et al., 2009). Antibacterial activity of PLA2 was explained in a different way where, it was reported that the electrostatic interactions between PLA2 molecules and bacterial membrane promoted the formation of negatively charged peptidoglycan pores in the bacterial cell membrane thus inhibiting bacteria by disturbing the outer membrane of the bacterial cell wall, resulting in damaging of critical intercellular targets after internalization of these peptides (Yeaman and Yount, 2003; Barbosa et al., 2005; Samy et al., 2007).

4. CONCLUSION

The present study illustrated the antimicrobial potential of different snake venoms where, three venoms, *N. nigricollis*, *B. arietans* and *E. carinatus*, proved antiviral activity, as well as three venoms showed antibacterial activity, *P. australis*, *N. nigricollis* and *N. naja*. The most promising venom was *N. nigricollis* exhibiting broad antimicrobial profile against test viruses and bacteria. Further studies are required to determine if there is indeed a correlation between the venoms biocidal potentials and their enzymatic content. The active antimicrobial components should be purified, investigated for determination of their mechanism of action and examined over a wide range of microbial models. It is also recommended to sequence these antimicrobial proteins and use them as a prototype for drug design to present a new strategy for antimicrobial therapy depending on snake venoms and their derivatives.

AUTHORS' CONTRIBUTIONS

'Shebl, R. I', performed the practical work, wrote the first draft of the manuscript.
'Mohamed, A. F', wrote the protocol, managed the literature searches.
'Ali A. E', managed the analysis of the study, reviewing and editing the manuscript.
'Amin, M. A', designed the study and managed the analysis of the study.
All authors read and approved the final manuscript.

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