

Antimicrobial Profile of Selected Snake Venoms and their associated enzymatic activities.

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ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

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

Methodology: We tested the antiviral activity of the non-toxic concentrations of *Naja haje*, *Bitis arietans*, *Naja nigricollis* and *Echis carinatus* venoms against *Herpes simplex virus* type.1, *Rift valley fever virus* and *Vesicular stomatitis virus* using cell culture assay. Antibacterial activities of *Bitis arietans*, *Cerastes cerastes*, *Echis carinatus*, *Vipera lebetina*, *Naja naja naja*, *Pseudechis australis*, *Naja nigricollis* and *Naja haje* venoms were evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* using disc diffusion assay. We determined the minimum inhibitory concentrations of venoms by broth microdilution method. We evaluated the L-amino oxidase and phospholipase A2 components of the crude venoms using enzymatic assays.

Results: The mean depletion of viral infectivity titer of *Bitis arietans*, *Naja nigricollis* and *Echis carinatus* pretreated cells and post viral infection were significant at $P=.05$ for all test viruses, except for *N. haje* treated cells. The mean depletion of viral infectivity titer of 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, 2.5 log (10) / ml and 2.5 log (10) / ml for HSV-1, RVFV and VSV respectively. *Pseudechis australis*, followed by *Naja naja naja*, and *Naja nigricollis* venoms showed the highest inhibitory activity against test bacteria with zone of inhibition ranging from 17-19 mm, 14-8 mm, and 13-8 mm respectively. The minimum inhibitory concentrations of test venoms against test microorganisms ranged from 156 µg / ml to 1.25 mg / ml. Maximum L- amino oxidase activity was detected in *N. naja* and *P. australis* and *C. cerastes*. The highest Phospholipase A2 activity was identified in *B. arietans*, *N. naja*, *P. australis* and *N. nigricollis*.

Conclusion: We identified the antimicrobial potential of different snake venoms where, three venoms proved antiviral activity, as well as three venoms showed antibacterial activity. Enzymatic assays of tested venoms suggested that the venom's antibacterial activity was directly proportional to L- amino acid oxidase and phospholipase A2 contents. Further investigations will be carried out for more characterization and purification of different proteins in snake venoms.

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 (Ferreira et al., 2008). 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 cytotoxic, antibacterial and anti-viral factors (Chaim-Matias 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* and *Pseudechis guttata*, and *Agkistrodon rhodostoma* were effective against several bacterial genera (Samy et al., 2006; Ferreira et al., 2008).

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). Meanwhile, *Crotalus durissus terrificus* snake venom was found to have an antiviral activity against measles virus (Petricevich and Mendonca, 2003). Snake venom's phospholipases A2 were a new class of HIV inhibitors that blocks the virus entry into the host cells (Fenard et al., 1999). In addition, the antiviral activity of L-amino acid oxidase from the snake *Bothrops jararaca* against Dengue virus has also been reported (Sant'Ana et al., 2008).

The present work aimed at evaluation of the antimicrobial activity of different snake venoms against viral and bacterial models, and attempts to investigate their bioactive enzymatic components.

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 naja* (*N. naja*), *Pseudechis australis* (*P. australis*), *Naja nigricollis* (*N. nigrocallis*) and *Naja haje* (*N. haje*) were kindly supplied from VACSERA Sera Plant- Egypt.

- Virus 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.

- Bacteiral 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 512 µg/0.1 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 hrs. Negative cell control was

included. Residual living cells were treated with 20 µl of MTT (5 mg/ mL) (Sigma-Aldrich-USA) at 37°C for 4 hours. MTT was discarded. Plates were PBS washed three times. DMSO (BDH-England) was added as 50 µl/well. Plates were shaken on plate shaker (Stuart-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 (Berridge et al., 2005). 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.

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 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. Difference at ($P = .05$) was 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 expressed as means of three independent tests each performed in triplicate $\pm$ 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 double fold serially diluted 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 (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 buffer, bacteria and media

2.2.4 Enzymatic assays

2.2.4.1 L-amino acid oxidase assay

L-amino acid oxidase (LAAO) activity was determined according to Knox and Pitt., (1957); Lee et al., (2011) with some modifications, 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 minute. Results are 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. 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/minute $\pm$ standard deviation.

3. RESULTS AND DISCUSSION

3.1 Results.

3.1.1 Cytotoxicity and antiviral activity

Data recorded revealed that 100 % cellular viability was noticed on using *N. nigricollis*, *N. haje*, *E. carinatus* and *B. arietans* as 2 µg /0.1ml, 1 µg/0.1ml, 2 µg/0.1ml and 1 µg/0.1ml respectively (Figure 1).

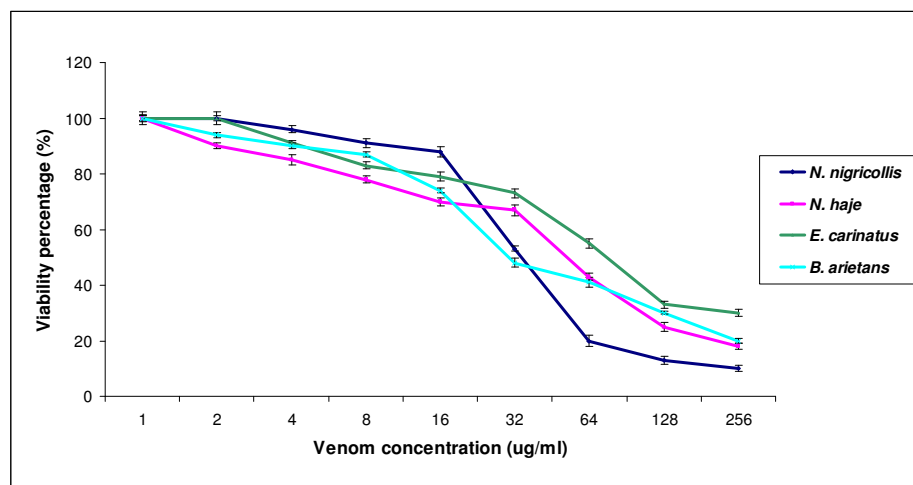


Fig. 1. Evaluation of cytotoxicity of test venoms to Vero cells using MTT assay. All the values are mean of three independent experiments, error bars show S.D.

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, *N. haje* venom didn't show significant viral depletion rate against test viruses at $P = .05$ (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 $\pm$ S.D			Mean depletion titer log (10)/ml	
		Untreated cells	Venom pretreated cells	1 h post viral infection	Venom pretreated cells	1 h post viral infection
<i>N. nigricollis</i>	HSV-1	5.3 $\pm$ 0.07	1.5 $\pm$ 0.15	3.2 $\pm$ 0.09	3.8*	2.1**
	RVFV	6.7 $\pm$ 0.05	3.5 $\pm$ 0.08	4.9 $\pm$ 0.14	3.2*	1.8**
	VSV	8.5 $\pm$ 0.1	6 $\pm$ 0.12	6.9 $\pm$ 0.16	2.5*	1.6**
<i>B. arietans</i>	HSV-1	5.1 $\pm$ 0.08	2.6 $\pm$ 0.06	3.6 $\pm$ 0.13	2.5*	1.5**
	RVFV	6.3 $\pm$ 0.11	4.5 $\pm$ 0.12	5.2 $\pm$ 0.08	1.8*	1.1**
	VSV	8.3 $\pm$ 0.13	7 $\pm$ 0.11	7.5 $\pm$ 0.12	1.3*	0.8**
<i>E. carinatus</i>	HSV-1	5.1 $\pm$ 0.14	3.2 $\pm$ 0.09	3.9 $\pm$ 0.08	1.9*	1.2**
	RVFV	6.5 $\pm$ 0.09	4.9 $\pm$ 0.1	5.7 $\pm$ 0.15	1.6*	0.8**
	VSV	8.4 $\pm$ 0.12	7.3 $\pm$ 0.07	7.7 $\pm$ 0.11	1.1*	0.7**
<i>N. haje</i>	HSV-1	5.2 $\pm$ 0.11	5.1 $\pm$ 0.03	5 $\pm$ 0.04	0.1	0.2
	RVFV	6.3 $\pm$ 0.1	6.1 $\pm$ 0.07	6.2 $\pm$ 0.02	0.2	0.1
	VSV	8.2 $\pm$ 0.13	8.1 $\pm$ 0.1	8 $\pm$ 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 value = .05

3.1.2 Antibacterial activity

It was found that *S. aureus* was the most susceptible strain to test venoms. *N. naja* and *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. On the other hand *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 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) ± S.D			
	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

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	–	–	–
<i>Pseudechis australis</i>	156	312.5	625	–
<i>Cerastes cerastes</i>	625	1250	1250	–
<i>Naja nigricollis</i>	312.5	625	–	1250
<i>Naja naja naja</i>	156	312.5	625	1250
<i>Vipera lebetina</i>	625	625	–	–
<i>Echis carinatus</i>	625	1250	–	–
Gentamicin (µg / ml)	0.5	0.25	0.25	0.5

3.1.3 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* and *P. australis* and *C. cerastes*. Moderate activity was recorded in *N. nigricollis* and *V. lebetina*. The highest PLA2 activity was identified in *B. arietans*, *N. naja*, *P. australis* and *N. nigricollis*. Moderate activity was noticed in *C. cerastes* and *V. lebetina*. On the other hand, *N. haje* showed the least LAAO and PLA2 activity (Figure. 2) and (Figure. 3).

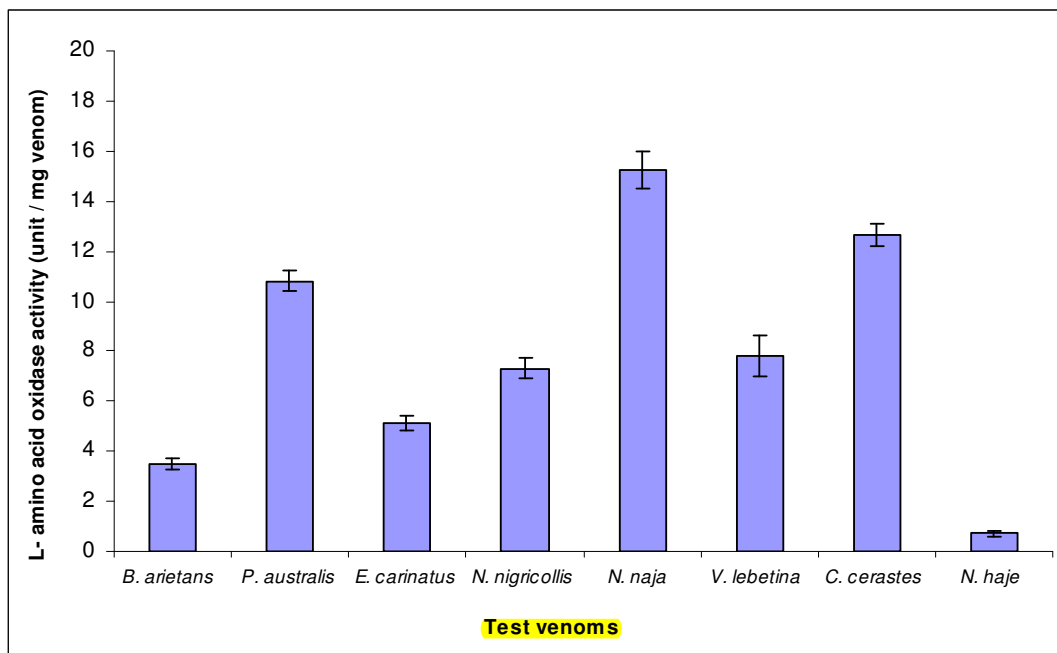


Fig. 2. Enzymatic activity of L-amino acid oxidase. All the values are mean of three independent experiments, error bars show S.D.

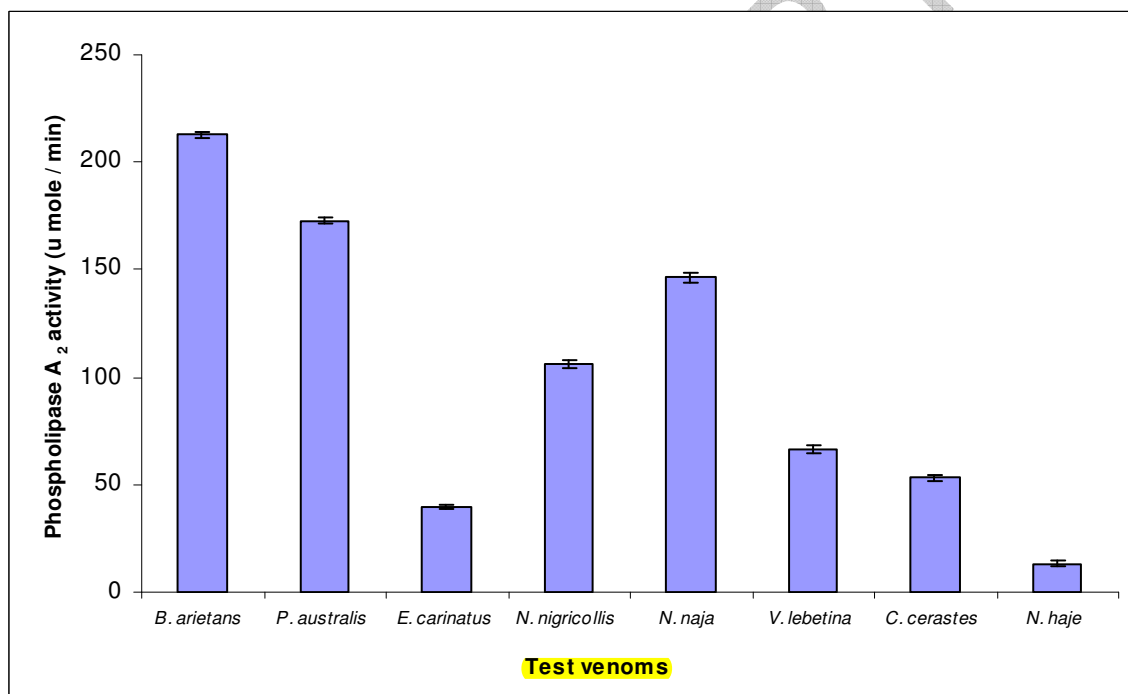


Fig. 3. Enzymatic activity of phospholipase A₂. All the values are mean of three independent experiments, error bars show S.D.

3.2 Discussion

In order to evaluate the non toxic concentrations of the tested venoms, their cytotoxicity to Vero cells was first assessed. The non toxic concentrations of *N. nigricollis*, *N. haje*, *E. carinatus* and *B. arietans* were examined against virus 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 cells. These results were 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 cellular 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 hours 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). 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* showed the highest susceptibility to the test venoms, followed by *E. coli* then *P. aeruginosa*. On the other hand, *S. typhimurium* exhibited the least susceptibility to test venoms. These results were in agreement with San et al., (2010) who reported that Gram negative bacteria were more resistant to tested crude venoms. In addition, resistance against Gram negative bacteria could possibly be due to outer membrane charged lipopolysaccharides of Gram negative bacteria. These 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. 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.

In an attempt to investigate the bioactive components of the snake venoms, L- amino oxidase and phospholipase A2 activities of crude venoms were assessed. Enzymatic assay of LAAO and PLA2 in test venoms revealed variable contents. Test venoms with the highest enzymatic activities, also exhibited peak antibacterial activity. Moreover, *N. haje* venom that showed minor enzymatic activities compared to other snake 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). 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. We gave a glimpse on a part of the enzymatic components of these crude venom. Further studies are in progress to purify the active antimicrobial components and to examine their activities over a wide range of microbial models. It is also recommended to sequence these antimicrobial proteins, use them as a prototype in drug design to present a new strategy for antimicrobial therapy depending on snake venoms.

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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