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Research paper

Cell cycle arrest evidence, parasiticidal and bactericidal properties induced by L-amino acid oxidase from *Bothrops atrox* snake venom

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ABSTRACT

The present article describes an L-amino acid oxidase from *Bothrops atrox* snake venom as with anti-protozoal activities in *Trypanosoma cruzi* and in different species of *Leishmania* (*Leishmania braziliensis*, *Leishmania donovani* and *Leishmania major*). Leishmanicidal effects were inhibited by catalase, suggesting that they are mediated by H_2O_2 production. *Leishmania spp.* cause a spectrum of diseases, ranging from self-healing ulcers to disseminated and often fatal infections, depending on the species involved and the host's immune response. BatroxLAAO also displays bactericidal activity against both Gram-positive and Gram-negative bacteria. The apoptosis induced by BatroxLAAO on HL-60 cell lines and PBMC cells was determined by morphological cell evaluation using a mix of fluorescent dyes. As revealed by flow cytometry analysis, suppression of cell proliferation with BatroxLAAO was accompanied by the significant accumulation of cells in the G0/G1 phase boundary in HL-60 cells. BatroxLAAO at 25 μg/mL and 50 μg/mL blocked G0–G1 transition, resulting in G0/G1 phase cell cycle arrest, thereby delaying the progression of cells through S and G2/M phase in HL-60 cells. This was shown by an accentuated decrease in the proportion of cells in S phase, and the almost absence of G2/M phase cell population. BatroxLAAO is an interesting enzyme that provides a better understanding of the ophidian envenomation mechanism, and has biotechnological potential as a model for therapeutic agents.

1. Introduction

Snake venoms are complex mixtures of proteins such as phospholipases A_2, disintegrins, metalloproteases, serine proteases, L-amino acid oxidases, peptides, lipids, nucleotides and other substances [1,2]. L-amino acid oxidases (LAAOs) are widely distributed in the venomous snake families Viperidae, Crotalidae and Elapidae [3]. LAAOs have been isolated from sea animals, mucus of the giant snail *Achatina fulica* Férussac [4], ink of the sea hare *Aplysia californica* [5], bacteria, fungi and plants [6].

LAAOs are flavoenzymes catalyzing the stereospecific oxidative deamination of a wide range of L-amino acids, which generates the corresponding α-keto acids, H_2O_2 and ammonia [1,2,7]. LAAOs are amongst the most abundant proteins in ophidian, particularly in hemorrhagic venoms [6,8] and are capable of inducing the apoptosis of various cell types [9–12]. Recent studies showed that LAAOs are multifunctional enzymes exhibiting the induction or inhibition of platelet aggregation [13,14], stimulation of edema formation [15,16], hemorrhage [17,18], antibacterial, antiviral and leishmanicidal functions [19–22].

Apoptosis is a controlled and regulated form of cell death that plays an important role in the development and maintenance of higher organisms. It is defined by several morphological and biochemical hallmarks, like the exposure of phosphatidylserine to the outer leaflet of the plasma membrane, nuclear condensation, and chromatin cleavage into oligonucleosomal fragments [23].

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The influence of toxic agents can be studied in the analysis of the cell cycle. This refers to the growth and division of cells in a continuous and repetitive way. In the cell cycle, there are several points in order to check the genetic material at cell division, in which several proteins are present to repair DNA damage [24]. The DNA-repair regulation is usually dependent or determined by two different factors: the type of DNA lesion that must be repaired and the characteristics of the substrate associated with the cell cycle.

Apoptotic processes and cellular damage are mechanisms of action of some toxins, and several studies show potential applications of such substances as models for the development of chemotherapy and anticancer agents. This study will describe the toxic effect of BatroxLAAO in *Leishmania spp*., *Trypanosoma cruzi* and Gram-positive and Gram-negative bacteria. The cytotoxic activity of PBMC normal cells and HL-60 cancer cells was evaluated after treatment with BatroxLAAO. BatroxLAAO can provide important data for development of therapeutic strategies for more targeted action, such as chemotherapy and anticancer agent effectiveness.

2. Material and methods

2.1. Snake venom and reagents

The venom from *Bothrops atrox* was obtained from the Serpentarium SANMARU (São Paulo, Brazil) and Institute Butantã (São Paulo, Brazil). All chemicals used were of analytical grade.

2.2. Purification of L-amino acid oxidase from B. atrox snake venom

The purification of BatroxLAAO is in accordance to Alves et al. (2008).

2.3. Bactericidal activity

The MIC values (the lowest concentration of the compound capable of inhibiting microorganism growth) of BatroxLAAO were determined in triplicate using the micro dilution broth method (NCCLS – 2001) [25] in 96-well microplates. Standard strains from the American Type Culture Collection of the following microorganisms were used: *Streptococcus mutans* (ATCC 25175), *Enterococcus faecalis* (ATCC 4082), *Sthaphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 14948). The samples were dissolved in saline (0.85%) to a concentration of 1 mg/mL, followed by dilution in tryptic soy broth to achieve concentrations ranging from 4 to 80 μg/mL. The final DMSO content was 4% (v/v), and this solution was used as negative control. The inoculum was adjusted for each organism to yield a cell concentration of 5×10^6 CFU/mL (colony forming units – CFU). One inoculated well was included as a control of the adequacy of the broth for organism growth. One non-inoculated well, free of antimicrobial agent, was also included to ensure medium sterility. Chlorhexidine dihydrochloride, streptomycin and penicillin were used as positive controls. The microplates (96 wells) were sealed with plastic film and incubated at 37 °C for 24 h. Next, an aqueous solution of resazurin (30 μL of 0.02%) was added to the micro plates to indicate microorganism viability. This procedure was based on the methodology described by Palomino et al. [26].

The special ability of LAAO to induce bactericidal activity against *E. coli* (ATCC 14948) and *S. aureus* (ATCC 29213) was also assayed by agar diffusion. The colonies were incubated with 24 and 48 μg of purified enzyme for 30 min and 60 min. The assay was carried out at the Laboratory of Pharmaceutical Biotechnology, Universidade Estadual Paulista (UNESP) according to the agar diffusion method described by Balows et al. [27], Soares et al. [28] and Stabeli et al. [15].

2.4. Trypanocidal activity

BatroxLAAO (0.5–32 μM) was tested *in vitro* against *T. cruzi* strain Y. Biological activity was evaluated by using the MTT colorimetric method [MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide – SIGMA] in a micro plate reader at 570 nm, as described by Muelas-Serrano, Nogal-Ruiz and Gómez-Barrio [29]. The negative control group containing solvent and medium, the negative control group containing medium, and groups containing the lignan compounds tested were all run in parallel. All the assays were performed in triplicate.

2.5. Cytotoxic effect of LAAO on Leishmania species viability

The direct cytotoxic effect of the BatroxLAAO on *Leishmania* species was measured. Briefly, four species of the *Leishmania* genus [1×10^6 promastigotes forms well^{-1} of *Leishmania braziliensis* (2904 P2), *Leishmania major* and *Leishmania donovani* (HU3 P6)] were incubated separately in M199 medium supplemented with 10% heat inactivated fetal calf serum (FCS) in the presence or absence of LAAO (0.5–32 μM) and catalase (0.5 mg/mL) for 24 h at 22 °C in a micro plate assay. Control groups without LAAO and with or without catalase were also tested. After the incubation time, 20 μL of the resazurin (SIGMA) solution (3 mM) was added and incubated for 5 h at 37 °C. The micro plates were read in a micro plate reader (Sunrise-TECAN) at 570 nm. All assays were performed in triplicate.

2.6. Cell culture

Human promyelocytic leukemia (HL-60) cells, obtained from American Type Culture Collection (ATCC), were grown in RPMI-1640, supplemented with 10% fetal calf serum and kept at 37 °C in a humidified atmosphere of 5% CO_2 in air. PBMC (peripheral blood mononuclear cell) cells were obtained from healthy individuals. Human blood care procedure, guidelines and experimental protocols were approved by Ethics Committee on Research (CEP) from University of São Paulo, USP, Brazil (Protocol number: 0024.0.212.000-08).

2.7. Apoptotic and necrotic analysis by epifluorescence microscopy

After the cells were exposed to BatroxLAAO, a morphological evaluation was performed in order to discriminate between the living cells and the necrotic or apoptotic ones. A solution with the mixture of the non-permeant dye propidium iodide (PI—5 μg/mL), fluorescein diacetate (FDA—15 μg/mL) and Hoechst 33342 (2 μg/mL) was prepared in phosphate buffered salt solution and then 40 μL of the fluorescent mix was added to the cells (2 mL final volume). After 5 min incubation at 37 °C in 5% CO_2 atmosphere, the cell suspension was washed and re-suspended in 1 mL of the medium [30]. Samples were dropped onto slides for observation using a fluorescent microscope (Olympus, Vanox AHBT3) with an ultraviolet light filter.

2.8. Cell cycle analysis

Cells were treated with 5, 10, 25 and 50 μg/mL BatroxLAAO for 24 h. PBS buffer was used as negative control. Approximately 5×10^6 HL-60 cells were suspended for these experiments. Cells were treated with RNase-A (50 U/mL) and labeled with PI (20 μg/ml) for 3 h at room temperature. The percentage of cells in each

phase of the cell cycle was determined by flow cytometry in a FACSCanto (Becton Dickison, Franklin Lakes, NJ, USA) and analyzed using ModFit LT 2.0 1995-6 software (Verity Software House Inc.)

2.9. Statistical analysis

The statistical significance of the experimental results was determined by Student's *t*-test. For all analysis, $p < 0.05$ was accepted as a significant probability level.

3. Results and discussion

SV-LAAOs (LAAOs from snake venom) have been objects of great interest for pharmacological, molecular biology, and structural studies. BatroxLAAO is an acidic glycoprotein with a pI ~ 4.4 and Mr ~ 67,000 Da [12]. SV-LAAOs have been characterized showing distinct molecular mass, substrate preference, pro/anti-apoptosis, cytotoxicity, platelet aggregation, hemorrhage and edema as well as bactericidal, leishmanicidal and anti-HIV activities [6,11,13,19,22,31–35].

3.1. Bactericidal activity

In this work BatroxLAAO exhibited bactericidal activity against both Gram-positive and Gram-negative bacteria. BatroxLAAO (24 and 48 μg) displayed a bactericidal effect against *E. coli* and *S. aureus*. In both doses (24 and 48 μg) of BatroxLAAO, after 30 min of treatment, it did not present statistical difference ($p > 0.05$) in bactericidal activity against *E. coli* and *S. aureus* (Fig. 1). After 60 min of treatment of BatroxLAAO (24 and 48 μg), it demonstrated statistical difference ($p < 0.05$) compared to negative control *E. coli* and *S. aureus*, respectively (Fig. 1). Chlorhexidine dihydrochloride, streptomycin and penicillin were used as positive controls (Table 1). BatroxLAAO did not demonstrate efficient activity as bactericidal agent when compared to positives control.

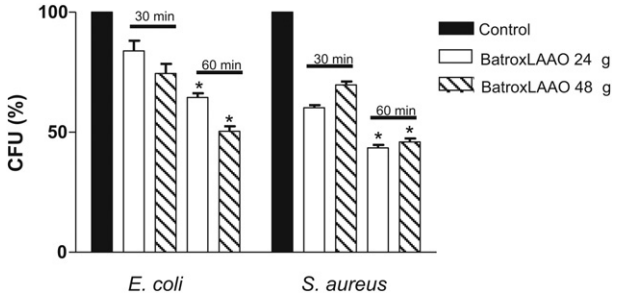

Fig. 1. This shows the bactericidal activity of the BatroxLAAO on the *E. coli* and *S. aureus*. Different concentration values (24 and 48 μg) of BatroxLAAO were incubated with 4×10^5 CFU for 30 min ($p > 0.05$ compared to non-treated group *E. coli* and *S. aureus*, respectively) and 60 min ($^*p < 0.05$ compared to a non-treated group of *E. coli* and *S. aureus*, respectively). Each bar represents the mean ± SD ($n = 3$).

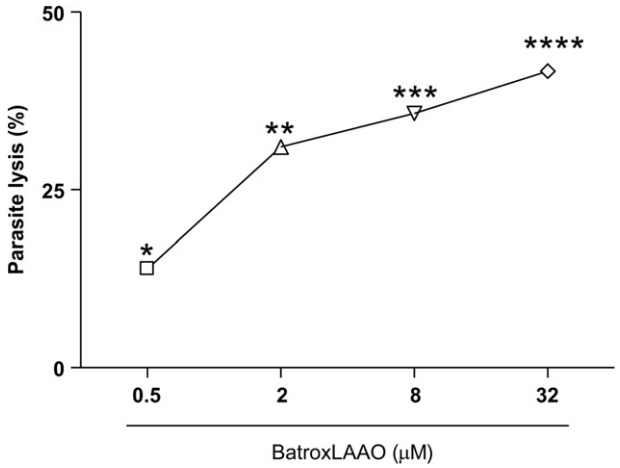

Fig. 2. *Bothrops atrox* LAAO parasiticidal effects. Trypanocidal dose-dependent effect induced by the BatroxLAAO enzyme on the *Trypanosoma cruzi* parasite. Statistical significance value means: *, **, ***($p < 0.05$) when comparing all treatments.

3.2. Trypanocidal and Leishmanicidal activities

The cellular viability of *T. cruzi* and *Leishmania* sp. was investigated after treatment with BatroxLAAO. The direct addition of BatroxLAAO to trypomastigotes forms of *T. cruzi* (Fig. 2), as well as to promastigotes of different *Leishmania* species resulted in a dose-dependent parasite killing (Fig. 3). BatroxLAAO presented low cytotoxicity effect in *T. cruzi*. BatroxLAAO (32 μM) showed 41.7 ± 2.4% of parasites death. All concentrations tested demonstrated significant statistical value of ($p < 0.05$). We investigated the cellular viability of *L. braziliensis*, *L. donovani* and *L. major* after incubation with BatroxLAAO. This protein induced a dose-dependent mortality of the promastigote forms. *L. major*, *L. donovani* were most susceptible to the toxic effect of BatroxLAAO. After treatment of BatroxLAAO (0.5, 2 and 8 μM), *L. braziliensis* demonstrated statistical significance ($p < 0.001$) when compared to *L. donovani* and *L. major*.

Briefly, the purified enzyme presented an EC_{50} of 23.34 μg/mL against *L. braziliensis*, 4.3 μg/mL against *L. donovani* and 4.5 μg/mL against *L. major*. *Leishmania spp*. had slightly different susceptibilities to LAAOs from snake venom, especially for BatroxLAAO, as observed from the EC_{50} of the purified enzyme. This could be explained by different enzymatic patterns in each strain or species [36]. *Leishmania* species were more sensitive to the action of this LAAO than *T. cruzi* (EC_{50} = 62.8 μg/mL). It is known that other ophidian LAAOs exhibit parasiticidal activities as a result of enzyme-catalyzed H_2O_2 production [19]. The toxic effect was almost completely abolished by the addition of catalase, suggesting that the release of H_2O_2 is directly involved with the parasiticidal effect of the enzyme. The addition of 0.5 mg/mL of catalase effectively abolished the effect of H_2O_2 produced by BatroxLAAO, resulting in a level of high viability of the parasites (Fig. 3).

Recently, it was demonstrated that the *Bothrops moojeni* LAAO caused a high mortality of promastigote forms of different species

Table 1
Sensitivity of gram-positive and gram-negative bacteria to positive controls.

	Escherichia coli 14948	*Enterococcus faecalis* 4082	*Staphylococcus aureus* 29213	*Pseudomonas aeruginosa* 27853	*Streptococcus mutans* 25175
Streptomycin[a]	5.9	NT	NT	>5.9	NT
Chlorhexidine[a]	NT	3.688	NT	NT	0.922
Penicillin[a]	NT	NT	0.3688	NT	NT

[a]Positive control; Controls concentrations: 0.0115–5.9 μg/mL; NT: non-tested.

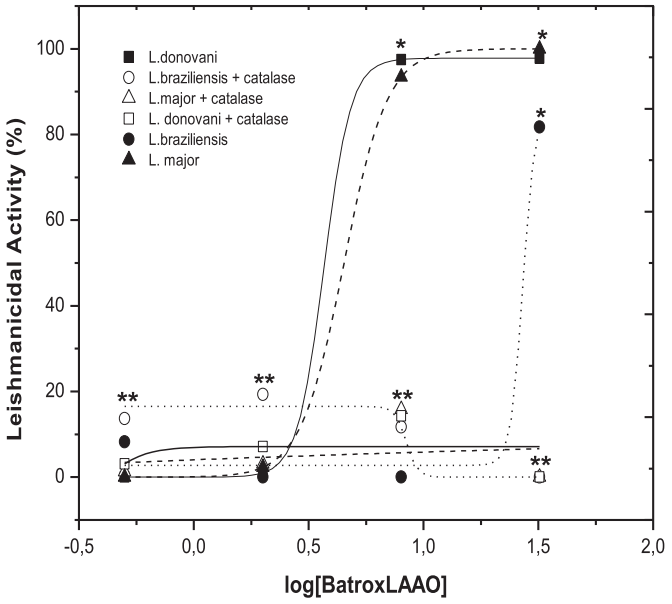

Fig. 3. *Bothrops atrox* LAAO parasiticidal effects. Leishmanicidal dose-dependent effect induced by BatroxLAAO enzymes on Leishmania spp. parasites. Statistical significance means: ($p > 0.05$). Leishmanicidal effect, after treatment with BatroxLAAO and catalase was (0.5 mg/mL). Statistical significance means *, ** ($p < 0.001$). Control wells were cultured in the presence of culture medium alone and with 1% DMSO. Data are expressed as the means ± SD ($n = 3$).

of *Leishmania* in vitro, suggesting a promising therapeutic strategy against leishmaniasis and other intracellular parasitic infections [37]. Ciscotto et al. [38] showed that crude venom from *Bothrops jararaca* and HTP1 (LAAO purified from *B. jararaca*) exhibited leishmanicidal activity against *L. amazonensis*. Upon incubation with *B. jararaca* whole venom, 69% viability was obtained, whereas 47.5% viability was observed after incubation with HTP1. In addition of catalase abolished the leishmanicidal activity of both crude venom and HTP1 [38].

BmarLAAO, an L-amino acid oxidase from *Bothrops marajoensis* caused parasitic death in a dose-dependent manner on promastigote forms of *L. amazonensis* and *L. chagasi*. The IC_{50} values were approximately of 1 μg/mL [22].

3.3. Cytotoxic activity

Venomous animals have evolved a vast array of toxins for prey capture and defense. Toxins as L-amino acid oxidases have been reported to show potent applications in pharmacology and cancer therapy [6]. As the balance between therapeutic potential and toxic side effects of a toxin is very important when evaluating its usefulness as a pharmacological drug, experiments were designed to investigate the *in vitro* cytotoxicity of BatroxLAAO against human cancer cells (HL-60).

Many LAAOs demonstrate apoptosis-inducing activity [12,19,23,39,40] and it is partially due to the generation of hydrogen peroxide. Hydrogen peroxide belongs to reactive oxygen species (ROS) and it is widely accepted that mitochondrial derangement has been associated with the increased production of ROS [41,42]. Many researchers have reported that L-amino acid oxidases have anti-proliferative effect on both leukemia and solid tumor [6,12,19,23].

In this work BatroxLAAO confirmed dose-dependent cytotoxicity in HL-60 by fluorescence microcopy as previously described by Alves et al. [12]. The morphological analysis with three different dyes (PI, DAF and Hoescht) in the HL-60 cell line is shown on Fig. 4. This assay enables to detect apoptotic, necrotic and also viable cells.

BatroxLAAO showed apoptotic and necrotic effects on HL-60 cells lines in a dose-dependent manner which was statistically significant compared to the negative control ($p < 0.05$) (Fig. 5A). At 50 μg/mL, BatroxLAAO was able to induce ~42.8% of total cytotoxicity (Fig. 5A). BatroxLAAO (50 μg/mL) presented apoptotic effect in 28.6 ± 0.4% of HL-60 cancer cells (Fig. 5B) and, at the same concentration, presented necrotic effect in 14.2 ± 0.5% of HL-60 cancer cells (Fig. 5C). The viable cells after being treated with BatroxLAAO (50 μg/mL) resulted in about 57.2 ± 0.75%. This result confirmed the cytotoxicity potential of this protein (Fig. 5D).

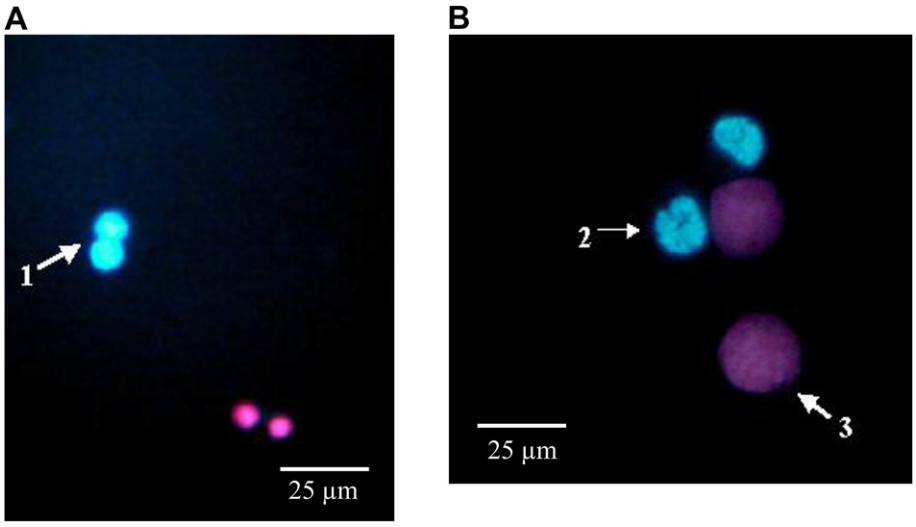

Fig. 4. The apoptotic and necrotic effects of the BatroxLAAO. The HL-60 cells were treated with BatroxLAAO at a concentration 50 μg/mL for 24 h, and collected for morphological assessment of cell death by fluorescence microscopy. (A) The arrow number 1 indicates normal living cells (Hoechst); (B) arrow number 2 indicates a dead cells population, either necrotic or apoptotic (PI); arrow number 3 indicates an apoptotic cell population with spotted nuclear bodies in blue (DAF). A representative figure from each group at 40× magnification is shown here. Scale bar equals 25 μM. Details of procedures are described in Materials and methods. Microphotographs were shown as representative results from three independent experiments.

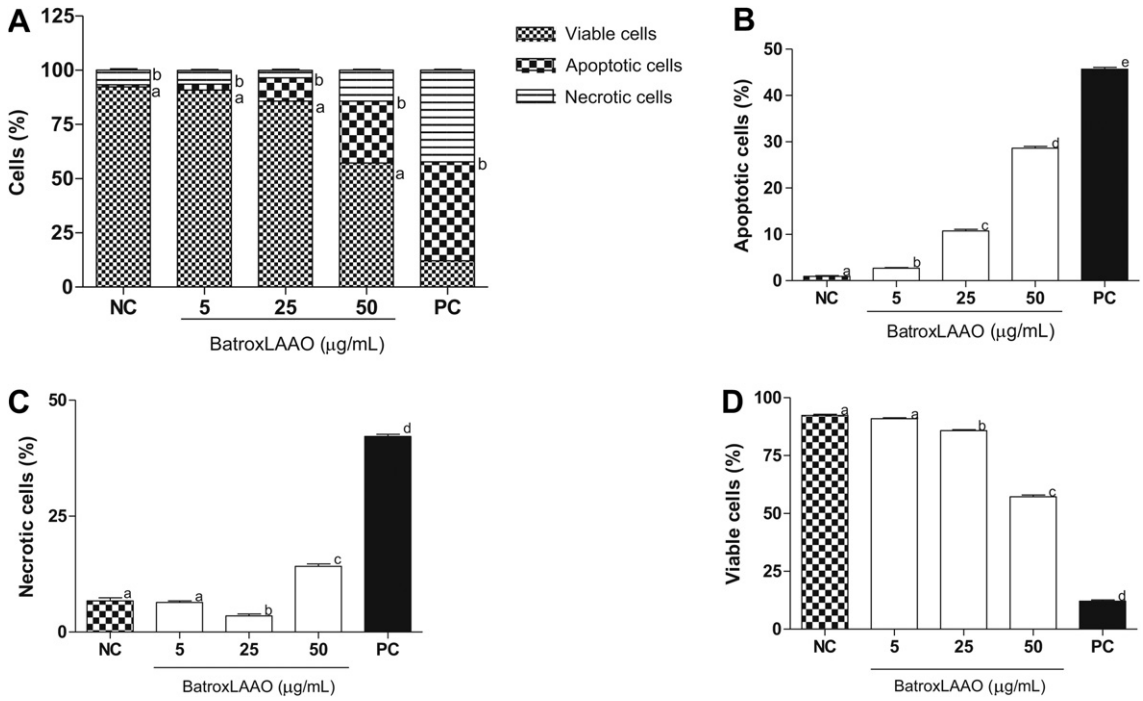

Fig. 5. Cytotoxicity effects in HL-60 cells lines. A) Apoptotic, necrotic and viable cells after exposure to BatroxLAAO (5, 25, 50 μg/mL); (for different letters $p < 0.001$, compared to negative control; for the same letters $p > 0.05$, compared to NC); B) Apoptotic cells (for different letters $p < 0.05$, compared to NC); C) necrotic cells (for different letters $p < 0.001$, compared to NC) and D) viable cells after treatment with BatroxLAAO (for different letters $p < 0.001$, compared to NC). NC = negative control. PC = positive control (camptothecin – 10 μM/mL). Values are expressed as ± S.D. for three independent experiments, in which each measurement was performed in triplicate.

Differently, BatroxLAAO showed low cytotoxicity toward PBMC cells (Fig. 6A). At 50 μg/mL, BatroxLAAO caused a cytotoxicity of ~35.32% (Fig. 6A). At 50 μg/mL of BatroxLAAO the apoptotic cells were 18.16 ± 0.44%, which was statistically significant compared to the negative control ($p < 0.05$) (Fig. 6B). BatroxLAAO (50 μg/mL) presented necrotic effect in 17.16 ± 0.72% of PBMC cells (Fig. 6C). The viable cells, after treatment with BatroxLAAO (50 μg/mL) made about 64.66 ± 0.92% PBMC (Fig. 6D). This protein (5 and 25 μg/mL)

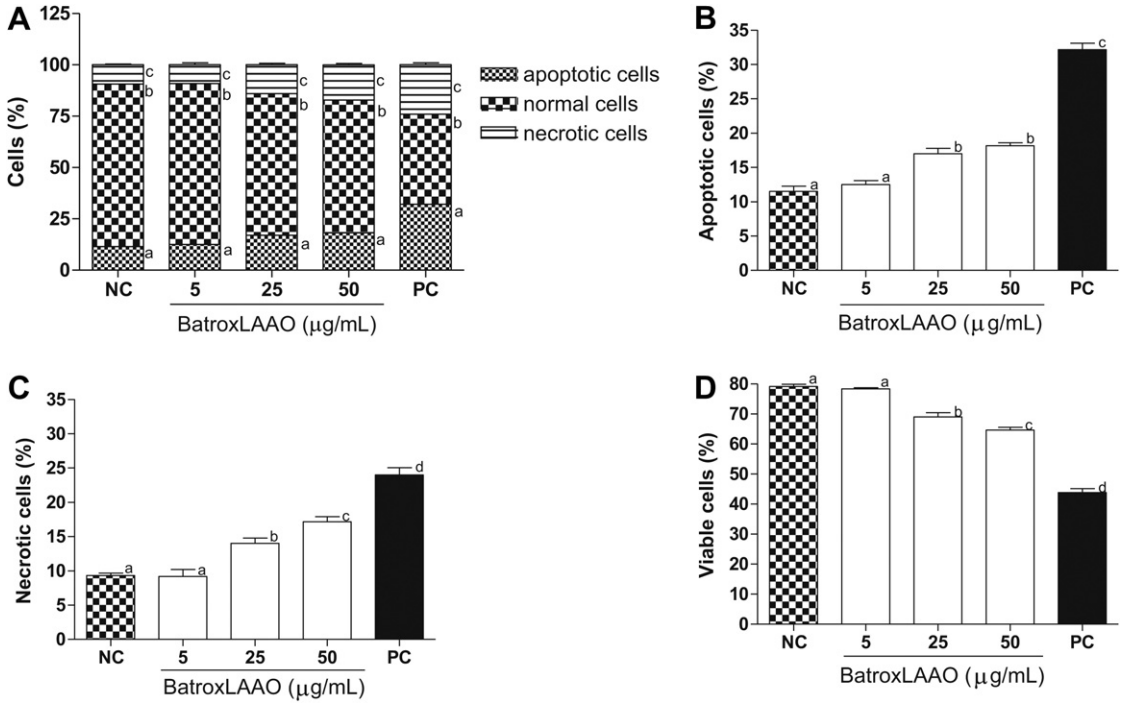

Fig. 6. Cytotoxicity effects in PBMC cells. A) Apoptotic and necrotic effects in PBMC cells after exposure to BatroxLAAO (5, 25, 50 μg/mL); (for different letters $p < 0.001$, compared to negative control; for the same letters $p > 0.05$, compared to NC); B) Apoptotic cells (for different letters $p < 0.001$, compared to NC), C) necrotic cells (for different letters $p < 0.001$, compared to NC) and D) viable cells after treatment of BatroxLAAO (for different letters $p < 0.001$, compared to NC). NC = negative control; PC = positive control (camptothecin – 10 μM/mL). Values are expressed as ± S.D. for three independent experiments, in which each measurement was performed in triplicate.

presented apoptotic effect in 12.5 ± 0.57% and 17.00 ± 0.76 of PBMC cells which was not statistically significant compared to the negative control ($p > 0.05$) (Fig. 6B). Comparing to HL-60 cytotoxicity BatroxLAAO presented low cytotoxicity in PBMC cells.

LAAO from *Bungarus pirajai* induced cytotoxic effects for S180 tumor cells, human breast (SKBR-3) cells, acute T cell leukemia (Jurkat) cancer cells, and Erlich ascitic tumor (EAT) cells. No significant cell death was observed for macrophages [16]. ACTX-6, an L-amino acid oxidase from *A. acutus* snake venom induced A549 cell death *in vitro* [39]. L-amino acid oxidase from *Bungarus fasciatus* snake venom exhibited a cytotoxic effect on A549 cells and caused up to 41.2% apoptosis of A549 cells following 12 h incubation period [43]. LAAO from *Vipera berus berus* induced apoptosis in K562 cells after 24 h of incubation [44].

3.4. Cell cycle arrest

Emerging evidence has demonstrated that the anticancer activity of certain chemotherapeutic agents is involved in interruption of the cell cycle, which is one of the preferred means of managing cancer. In order to decipher the suppressive mechanisms of BatroxLAAO on HL-60 cell lines, we monitored the changes in cell cycle distribution by cytometry flow. Treatment with BatroxLAAO resulted in a dose-dependent increase in the distribution of cells in G0/G1 phase; with a decrease in S phase (Fig. 7). BatroxLAAO (5 and 10 μg/mL) showed 52.5% and 52.71% of cells in G0/G1 phase, 42.78% and 44.56% in S phase, 4.72% and 2.71% of cells in G2/M phase, respectively. BatroxLAAO (25 μg/mL) promoted 58.49% of cells in G0/G1, 34.91% in S phase and 6.56% in G2/M phase. BatroxLAAO (50 μg/mL) presented 68.12% of cells in G0/G1, confirming the arrest in this phase of cell cycle. The value of cells in G0/G1 after treatment with BatroxLAAO (50 μg/mL) presents a statistically significant difference in value ($p < 0.05$) when compared with cells in S and G2/M phases. The G1/S checkpoint prevents initiation of DNA replication in cells that have damaged DNA. Cell cycle progression is driven by phosphorylation events mediated by cyclin/cdk complexes [45]. These cyclin/cdk complexes are the main targets of the effectors of the G1/S checkpoint [45,46].

Studies by Zhang et al. [39] demonstrated that ACTX-6, an L-amino acid oxidase from *A. acutus* induced markedly increased accumulation of sub-G1 phase [39]. Bengalin, a protein from an Indian black scorpion which is responsible for anti-proliferative and apoptogenic activities against human leukemic cells denominated U937 (histiocytic lymphoma) as well as the K562 (chronic myelogenous leukemia). Inhibition of U937 and K562 cell proliferation occurred by apoptosis as evidenced from cell cycle arrest at sub-G1 phase [47]. Unlike the cell cycle arrest in G1, other compounds present apoptotic potential evidenced by arrest in G2. A basic polypeptide from *Naja naja atra* venom (CTX-III) exerts its specific anti-proliferative effects of solid tumor cells HepG2 via S phase cell cycle arrest [48]. Ochratoxin A (OTA), a mycotoxin commonly found in several food commodities worldwide could induce GES-1 cells arrested in the G (2)/M phase [48].

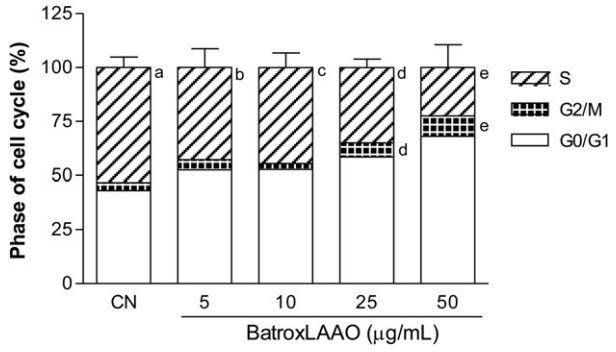

Fig. 7. Inhibition of cell cycle progress in HL-60 cells by treatment with BatroxLAAO for 24 h. Cell cycle arrest in G0/G1 after treatment with BatroxLAAO (25 and 50 μg/mL). Control: G0/G1 43.08%, S 53.3%, G2/M 3.61%. 5 μg/mL BatroxLAAO: G0/G1 52.5%, S 42.78%, G2/M 4.72%. 10 μg/mL BatroxLAAO: G0/G1 52.71%, S 44.56%, G2/M 2.71%. 25 μg/mL BatroxLAAO: G0/G1 58.49%, S 34.91%, G2/M 6.56%. 50 μg/mL BatroxLAAO: G0/G1 68.12%, S 22.45%, G2/M 9.42%. Cells were fixed with ethanol and stained with PI, and then cell cycle distribution was analyzed by flow cytometry. ^{d,e} $p < 0.05$ compared to cells in G0/G1 after treatment with BatroxLAAO 25 μg/mL and 50 μg/mL, respectively.

4. Conclusion

The present study will influence the future anticancer drug development from snake venom and its mechanism of action. L-amino acid oxidases could effectively trigger cell apoptosis *in vitro*. Further studies will be necessary to be carried out to discover the structure and the sites of activity of BatroxLAAO.

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