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Dicentracin-Like from *Asian sea bass* Fish and Moronecidine-Like from *Hippocampus Comes*: Two Candidate Antimicrobial Peptides Against *Leishmanina major* Infection

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Abstract

Anti-Leishmanial drug therapy faces significant challenges related to cytotoxicity and drug resistance. Thus, new and efficient anti-Leishmanial drugs need to be identified. Due to their broad-spectrum antimicrobial and also immunomodulatory activities, antimicrobial peptides (AMPs) have attracted considerable attention. In this study, we comparatively assessed the anti-Leishmanial activities of two recently identified AMPs (dicentracin-like and moronecidine-like) and the well-known AMP piscidin from the hybrid striped bass. AMPs were first assessed against *Leishmania major* promastigotes using MTS. Subsequently, macrophages were infected with *L. major* and treated with AMPs to evaluate anti-amastigotes activity of AMPs, and non-infected macrophages were treated with AMPs to determine cytotoxicity against mammalian cells using MTS. The induction of factors limiting *L. major* growth (IL-12, TNF-α and reactive oxygen species (ROS)) by AMPs was measured by ELISA and dichlorofluorescin-diacetate (DCFH-DA) assay, respectively. Piscidin was more efficacious against *L. major* promastigotes as compared to dicentracine-like or moronocidin-like peptides, whereas, dicentracine-like and moronocidin-like peptide exhibited a higher activity against *L. major* amastigotes compared to piscidin. In turn, piscidin was most cytotoxic in non-infected macrophages compared to the other two AMPs. A direct association was observed between hydrophobicity of AMPs and their anti-promastigote and cytotoxic activities. Dicentracine-like or moronocidin-like peptides induced higher levels of IL-12, TNF-α and ROS in macrophages compared to piscidin. Collectively, our results suggest that dicentracine-like and moronocidin-like peptides represent potentially promising multi-functional therapeutic agents that might not only directly kill *L. major* but also induce anti-Leishmania factors that can limit *L. major* growth and intracellular survival.

Keywords *Leishmania* · Antimicrobial peptides · Anti-amastigote activity · Anti-promastigote activity · Cytotoxicity

Introduction

The genus *Leishmania* includes several species, which are the causative agents of either cutaneous, mucocutaneous or visceral leishmaniasis in mostly tropical countries (Ansari et al. 2016). More than 350 million people are at risk of infection, 2.4 million disability-adjusted life years (DALYs) are lost due to leishmaniasis, and 1-1.5 million cases of cutaneous and 500,000 cases of visceral leishmaniasis occur each year, with 20,000 to 40,000 deaths (Mitra and Mawson 2017; Gharavi et al. 2011a, b). Treatments to control leishmaniasis are mainly based on chemotherapy. Currently used drugs include pentavalent antimonial, amphotericin B, liposomal amphotericin B, miltefosine, paromomycin and pentamidine. However, adverse side effects such as nephrotoxicity and teratogenicity, as well as the prohibitive costs, long duration of treatment, and the potential for resistance formation, are important obstacles that impair treatment efficiency (Sundar and Chakravarty 2015; Ponte-Sucre et al. 2017).

Antimicrobial peptides (AMPs) are biologically active molecules of the innate immune system, particularly in invertebrates (Chiou et al. 2005; Sathyamoorthi et al. 2019;

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Shafiee et al. 2017; Zhang et al. 2009), that were first discovered in the 1980's to exhibit activity against invading pathogens such as bacteria, fungi and viruses (Chiou et al. 2002; Mohammad et al. 2015; Lei et al. 2019; Nayak et al. 2018). They are cationic and hydrophobic peptides, which can get incorporated into the negatively charged microbial membrane through electrostatic interactions, forming pores and leading to cell death(Chiou et al. 2002; Mohammad et al. 2015; Lei et al. 2019). It has been postulated that peptides kill pathogens by several mechanisms, including membrane destabilization, cytoplasmic leakage, collapse of metabolic pathways and also impairment of protein and nucleic acid synthesis (Guilhelmelli et al. 2013; Chifiriuc et al. 2014). Currently, several AMPs are being evaluated in preclinical and clinical trials to treat infectious diseases. Furthermore, two lipopeptides including polymyxin B and polymyxin E are currently used to treat topical infections and infection caused by multidrug-resistant gram-negative bacteria (Ngamprasertchai et al. 2018; Vaara 2019).

Studies are ongoing to find effective and novel anti-parasitic agents with low side effects in the host (Mor 2009; Torrent et al. 2012). The mechanisms of action of AMPs that lead to anti-Leishmania activities are being studied (Marr et al. 2012). The selectivity toxicity AMPs against *Leishmania* and the low host cell cytotoxicity renders them highly attractive as peptide-based drugs, either in combination with already approved therapeutics or as a monotherapy (Cobb and Denny 2010).

In our previous study, we characterized two novel antimicrobial peptides, called moronecidin-like (from *Asian sea bass* fish) and moronecidine-like (from *hippocampus comes)* peptides, which exhibited efficient antibacterial activity against both gram-positive and gram negative bacteria, and their antibacterial activities were somewhat dependent on electrostatic interactions with bacterial cell walls (Mohammadi et al. 2018; Taheri et al. 2018). In this study, we evaluated the anti-Leishmania properties of moronecidin-like and dicentracin-like AMPs against promastigotes and intracellular amastigotes of *L. major* cultured in macrophages, in comparison to the well-characterized AMP piscidin. The three AMPs exhibited considerable anti-Leishmanial activities, with piscidin being more efficacious against promastigotes, and dicentracine-like and moronecidin-like exhibiting higher and specific activities against the amastigote stage of *L. major* and low host cell toxicity. In macrophages, dicentracine-like and moronecidin-like induced the expression of IL-12, TNF-α and reactive oxygen species (ROS), which contributed to their anti-amastigote activities. This indicates that dicentracine-like and moronecidin-like AMPs should be further considered as potential multifunctional agents for the treatment of *Leishmania* infections.

Materials and Methods

Sequence Analysis of Peptides

To predict physicochemical features (Table 1) of AMPs, their amino acid sequence was submitted in the Prot Param (http://www.expasy.ch/tools/protparam.html). Furthermore, helical wheel projection (http://rzlab.ucr.edu/scripts/wheel/wheel.cgi).

was used to predict helical wheel and hydrophilic and hydrophobic interface on the secondary structure of the peptide.

Synthetic AMPs

The AMPs (dicentracine-like, moronecidine-like and piscidin) were synthesized by N-(9-fluorenyl) methoxycarbonyl (Fmoc) chemistry (pepmic, Suzhou, Co China). The peptides were C-terminally modified with amid group, and purified using RP-HPLC (SHIMADZU) employing an Inertsil ODS-SP (4.6 × 250 mm × 5 µm) column and elution with a 0–100% H2O/acetonitrile gradient containing 0.1% trifluoroacetic acid (TFA). The homogeneity of peptides was

Table 1 Physicochemical properties of AMPs peptides

Name	Dicentracin-like (P1)	Moronecidin-like (P2)	Piscidin (P3)
Sequence	FLRSLLRGAKAI-YRGARAGWRG	FFRNLWK-GAKAAFRA-GHAAWRA	FFHHIFRGIVH-VGKTIHRLVTG
Net charge(Ph7)/pI	6/12.18	5.1/12.30	3.9 /11.97
Theoretical/ Observed MW	2531.96/2531	2531.96/2531	2930.36/2930
Polar/non-polar residues (%)	50/50	40.91/59.09	54.55/45.45
Hydrophobic face residues	F G I L A A LWY L	F A A L G A F W F W	F L V I I I F V G F
Non-polar face hydrophobicity <H>	1.25	1.25	1.49
Hydrophobicity <H>/retention time	0.321 /20.518	0.375 /17.474	0.644 /21.588
Water solubility	Good solubility	Good solubility	Poor solubility
Hydrophobic moment <µH>	0.55	0.50	0.55

evaluated by HPLC, and the molecular masses were determined with mass spectrometry (MS) (Figs. S1, S2 and S3). The lyophilized peptides were suspended in peptide solution (0.01% acid acetic and 0.2% bovine serum albumin (BSA)), and stored at 20 °C as peptide stock (2 mg/ml).

Parasite Culture

All Culture Materials were Bought from Sigma Aldrich Co (UK)

Leishmania major promastigotes (MRHO/IR/75/ER) were cultivated at 26 °C in RPMI1640 Glutamax medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 0.25% hemin, 0.1% biotin, 10 mM adenine, 1% penicillin-streptomycin. For all experiments, stationary-phase *L. major* promastigotes was used because of high infectivity of *L. major* in this phase (Wozencraft and Blackwell 1987). For this purpose, *L. major* promastigotes has been cultured for 5 days, were harvested and washed in phosphate buffered saline (PBS) prior to use.

The macrophage cell line RAW264.7 was cultured in DMEM medium containing 10.00% FBS and penicillin-streptomycin at 37°C under 5% CO_2. Macrophages were collected at almost 80% confluence.

Evaluation of Anti-Leishmanial Activity of AMPs

L. major promastigotes were counted in a Neubauer hemocytometer and seeded into 96-wel microplates at 1×10^6 cells per well. After 24 h, parasites were treated with 15.5, 31 or 62 µM of peptides and incubated for 24 hours at 24 °C. The parasite viability was then determined using the MTS assay as described elsewhere (Lynn et al. 2011). The optical density was read at 570 nm and wells containing non-treated parasites were used as growth control. Each concentration was assessed in triplicate wells.

Microscopy

L. major promastigotes were exposed to 15.5, 31 or 62 µM of peptides in 96-wel microplates (1×10^6 cells per well) and incubated for 24 h at 24 C. The morphology of treated versus non-treated parasite was then assessed by light microscopy using an inverted microscope at 400 X magnification.

Cytotoxicity in RAW 264.7 Macrophage Cell Line

RAW 264.7 macrophages were seeded into 96-well microplates at 4×10^5 cells per well. Afterward, 15.5, 31 or 62 µM of peptides or peptide solution were added, and cells were cultured for 48 hours at 37 °C/5% CO_2. The viability of macrophages was assessed by MTS assay as previously described (Lynn et al. 2011). Each peptide concentration was assessed in duplicates. In this experiment, some wells without parasites were used as blank.

Measurements of Anti-amastigote Activity of AMPs

To assess anti-amastigote activity of AMPs, peritoneal macrophages were obtained from BALB/c mice. These animals were maintained under the animal license permit No. R.BPUMS.REC.1397.064 and experiments were approved by the ethics committee of Bushehr University of Medical Sciences. Macrophages isolated from Balb/c mice were placed into 8-well chamber slides (Thermo Scientific Nunc Lab-Tek, USA) (8×10^5 marcrophages/well) and were cultured in RPMI 1640 medium containing 10% FBS at 37°C/5% CO_2 for 5 h. Non-adherent cells were removed, and adherent macrophages were infected with stationary phase *L. major* promastigotes at a 1:5 ratio (macrophage/amastigote) and cultured for 5 h. Wells were washed with RPMI 1640 medium three times to remove non-phagocytosed parasites, and infected cells were treated with AMPs peptide (15.5 or 31 µM), ovalbumin-derived peptide (OVA peptide) as non-AMP peptide (15.5 µM) or peptide solution (as control infection) for 72 hr. Macrophages were then washed with PBS, fixed in methanol for 1 min and stained with 4', 6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, USA). The slides were analyzed with NucleoCounter® NC-200™ (Chemometec, Allerod, DK) and the number of intracellular parasites in 100 macrophages were counted and expressed relative to those within macrophages treated with peptide solution (control infection) multiply by100.

Measurement of Reactive Oxygen Species (ROS) Production in Macrophages in Response to AMP Treatment

RAW 264.7 macrophage cells (1×10^5 cells/ml) were cultured in 48-well microplate, treated with the AMP peptides (15.5 or 31 µM), OVA peptide as non-AMPs peptide (15.5 µM), the peptide solution as a negative control or LPS as positive control (100 ng/ml), and further cultured for 20 h. The production of ROS within macrophages was measured by 2′, 7′-dichlorofluorescin-diacetate (DCFH-DA) assay as described elsewhere (Fan et al. 2017). Briefly, the cells were treated with DCFH-DA, followed by incubation for 1 h and washed twice with PBS. Finally, intracellular fluorescence was quantified using the fluorescence micro plate reader (BioTek Synergy4, USA) at 485/530 nm.

Assessment of Cytokine Responses in Macrophages in Response to AMP Treatments

RAW264.7 cells were seeded at 2×10^4 cells/well in a 48-well plate overnight. Cells were exposed to AMPs (15.5 or 31 µM), ovalbumin peptide as non-AMP peptide (15.5 µM), peptide solution, or LPS as positive control (100 ng/ml), and then further cultured for 24 h at 37 C/5% CO2. Culture supernatants were collected and cytokine content (TNF-α, IL-12) was analyzed by indirect ELISA, according to the protocol provided by manufacture (Abcam, UK).

Statistical Analysis

The results and graphs are shown as mean the standard deviations (SD) as indicated. Differences between control and peptide-treated samples were analyzed with Prism GraphPad prism 0.8 software. A p-value of less than 0.05 was considered significant.

Results

Physicochemical Properties of Peptides

The piscidin peptide exhibited the lowest positive net charge and lower solubility relative to dicentracine-like or monorocidine-like AMPs (Table − 1). Furthermore piscidin had the most hydrophobic non-polar face as compared to dicentracine-like or monorocidine-like. The central residues of the nonpolar face of dicentracine-like and monorocidine-like (9 and 16 residues, respectively) are positioned by low hydrophobic residues including alanine and glycine, whereas the corresponding residues in piscidin are of higher hydrophobic residues (Isoleucine 9 and 16, respectively) (Fig. 1).

Evaluation of Anti-promastigote Activity

The ability of AMPs to kill promastigotes in the stationary-phase was evaluated to determine the direct cytotoxic activity against *L. major*. MTS assay and microscopic observation were used. MTS data showed (Fig. 2) that all peptides possess dose-dependent cytotoxic activity against *L. major* promastigotes. Dicentracine-like and piscidin exhibit greater activity against *L. major* as compared to morecidine-like with piscidin being the most efficacious of the three AMPs. Microscopy showed that piscidin, in comparison to the other AMPs, induced cellular deformation (rod-like shape) of promastigotes and inhibited cluster formation parasites, even at the lower peptide concentrations (15.5 or 31 µM). At the highest concentrations (62 µM), piscidin induced promstigote lysis (Figs. S4, S5, 6). Dicentracine-like exhibited a more pronounced ability to induce cellular deformation (rod-like shape) and inhibited cluster formation of promastigotes at 31 µM of peptide concentration.

Evaluation of cytotoxic activity of peptides on macrophage cell line

The cytotoxic activity of peptides against macrophages was determined using the MTS assay. As shown in Fig. 3, dicentracin-like and moronecidin-like did not exhibit cytotoxic activity against macrophages at 31 µM or lesser peptide concentration, but even stimulated macrophage proliferation at 31 µM peptide, whereas piscidin was cytotoxic against macrophages at this peptide concentration (31 µM).

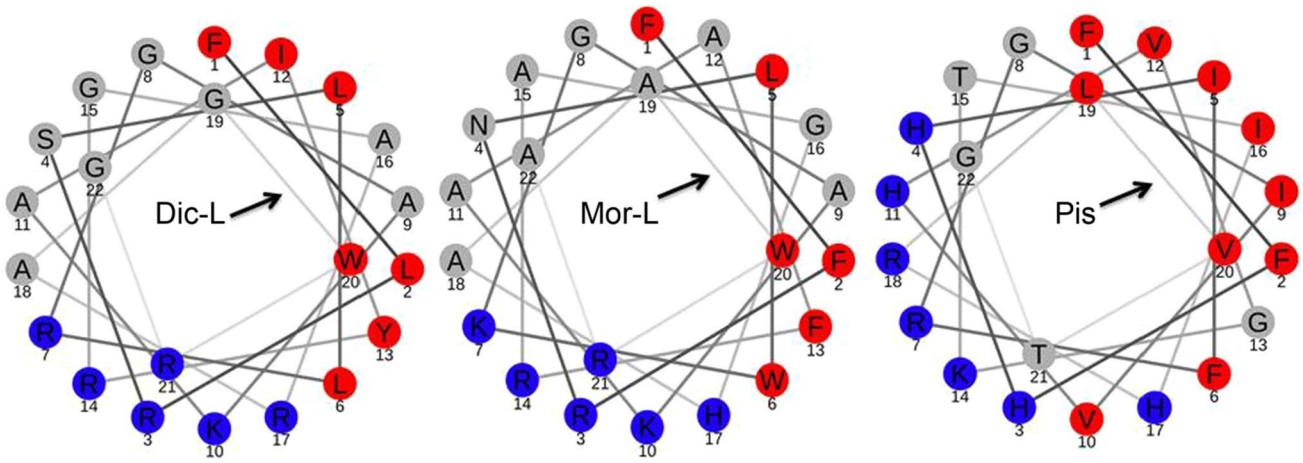

Fig. 1 Helical wheel projections of dicentracine-like (Dic-L) moronecidin-like (Mor-L) and Piscidin (Pis) peptides. Residues are numbered starting from the N terminus. Residues that are higlyh hydrophobic and positively charged are shown in red and blue, respectively. Arrows represents the central residues (A9, A16) of the non-polar face in the helical wheel model of AMPs

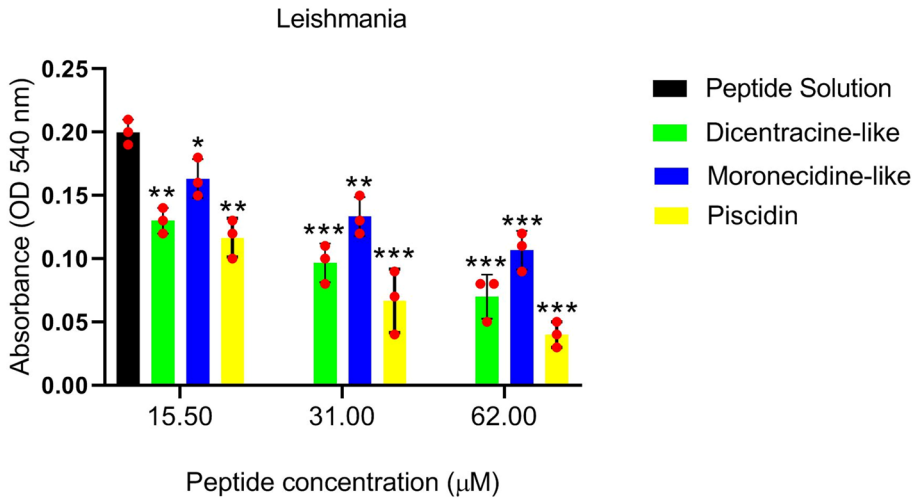

Fig. 2 Direct cytotoxic activity assessment of AMPs against *L. major* promastigotes. The promastigotes were exposed to 15.5, 31 or 62 μM or peptide solution. The parasite viability was determined using the MTS assay. Some well without parasites was used as blank, and some control wells with *Leishmania* and without peptide (peptide solution) are considered as 100% viable. Three independent experiments were performed, and the values are expressed as mean ± SD of those three independent experiments. Cytotoxic activity of AMP was compared to peptide solution using one-way analysis of variance (*ANOVA*). Differences at $p < 0.05$ were considered significant (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$)

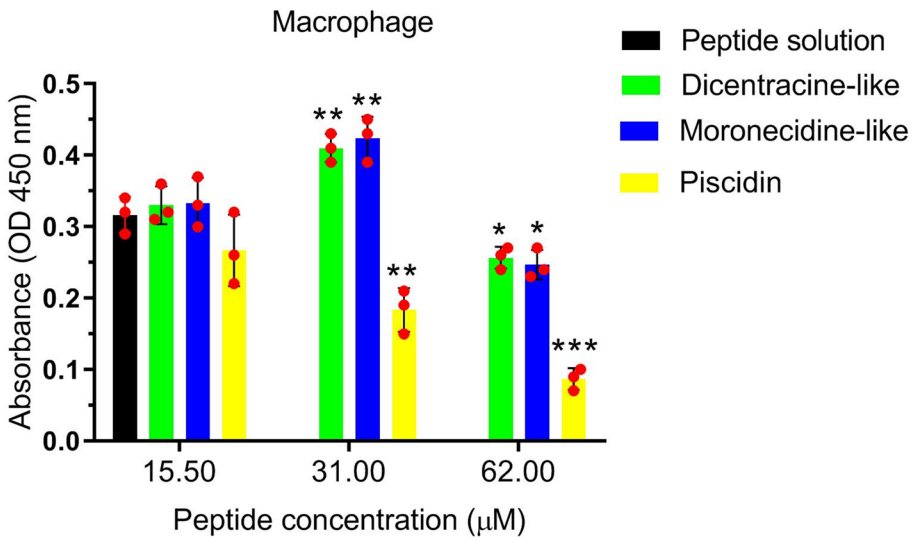

Fig. 3 Cytotoxic activity assessment of AMPs against the macrophage cell line RAW 264.7. RAW 264.7 macrophages were exposed to 15.5, 31 or 62 μM of AMPs or peptide solution. The viability of macrophages was determined by MTS assay. The values are expressed as mean ± SD for three independent experiments. Differences at $p < 0.05$ were considered significant (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$). Cytotoxic activity of each peptide was compared to peptide solution using *one-way* analysis of variance (*ANOVA*)

Evaluation of Anti-amastigote Activity of AMPs

Infected macrophages were treated with the AMPs, and counting of intracellular amastigotes revealed a decreased number of amastigotes in AMPs treated cells relative to those treated with non-AMP peptide. Interestingly moronocidin-like and dicentracin-like showed more pronounced, and virtually similar, anti-amastigote activity than piscidin, and the effect was dose-dependent (Fig. 4).

ROS Production in Macrophages After Exposure to AMPs

The ability of the peptide to induce ROS production in macrophages was evaluated by DCFH-DA assay. Macrophages treated with moronocidin-like and dicentracin-like produced significantly high amounts of ROS as compared to those treated with piscidin, non-AMP peptide or peptide solution(Fig. 5). An increase in the concentration of

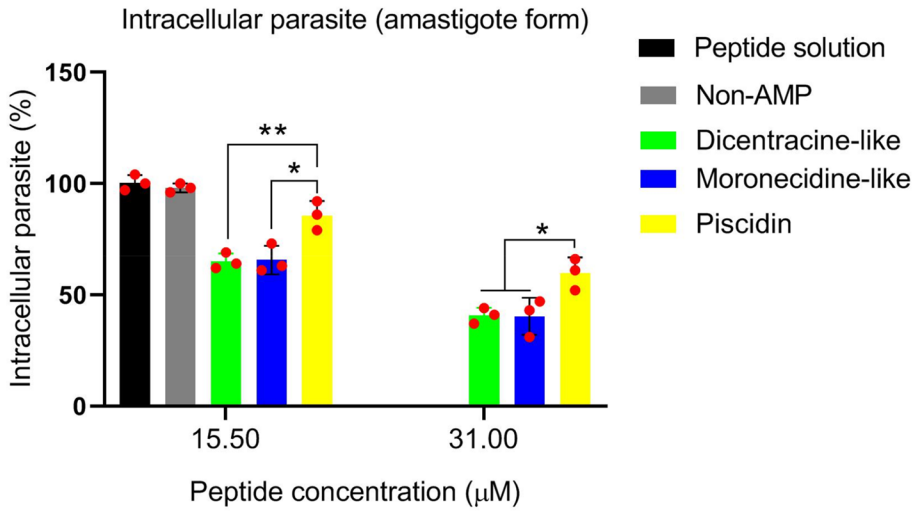

Fig. 4 Cytotoxic activity assessment of the AMPs against intracellular *Leishmania* amastigotes. Adherent peritoneal macrophages were infected with *L. major* promastigotes and cultured for 5 h, followed by treatment with AMPs (15.5, 31 μM) or peptide solution. Macrophages were fixed and stained with DAPI, and intracellular amastigotes were quantified. The values are expressed as mean ± SD for three independent experiments. Differences at $p < 0.05$ were considered significant (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$). The anti-amastigote activity of each peptide was compared to piscidin peptide using one-way analysis of variance (ANOVA).

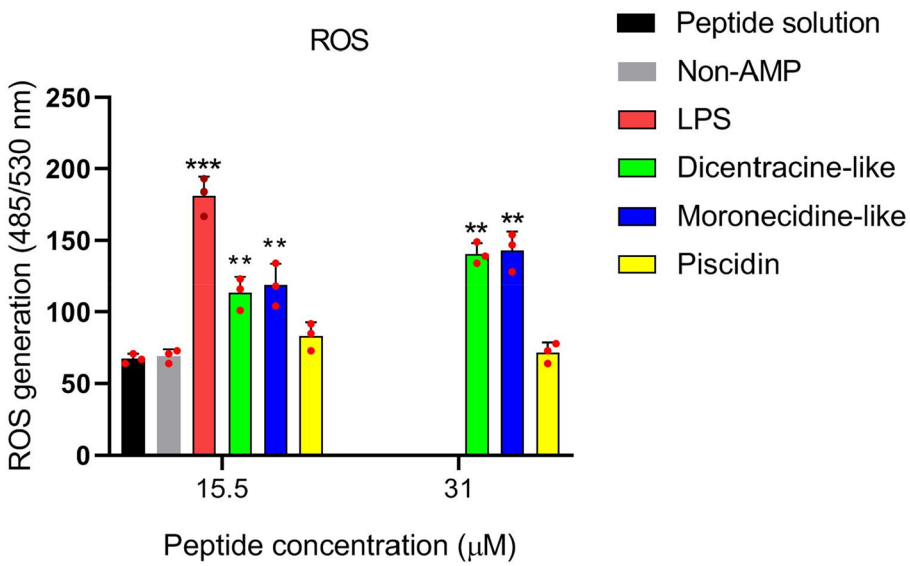

Fig. 5 Measuring ROS generation in macrophages. RAW 264.7 macrophages were treated with AMPs (15.5 or 31 μM), non-AMP peptide, peptide solution or LPS, and then intracellular ROS were measured using the fluorescence-based DCFH-DA assay at 485/530 nm. The values are expressed as mean ± SD of three independent experiments. Differences at $p < 0.05$ were considered significant (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$. The levels of ROS generation of each peptide were compared to peptide solution using *one-way* analysis of variance (*ANOVA*)

moronocidin-like and dicentracin-like peptides up to 31 μM induced the increased ROS production, while no statistically significant change was observed in the production of piscidin-induced ROS.

Cytokine Responses in Macrophages After Exposure to AMPs

Cytokine response of macrophages was measured after exposure of cells to AMPs or LPS. High levels of TNF-α and IL-12 were measured in the culture supernatants of macrophages treated with moronocidin-like and dicentracin-like peptides, as compared to those treated with piscidin, non-AMP peptide or peptide solution (Fig. 6). Furthermore no statistically significant difference was observed in the level of piscidin-induced TNF-α and IL-12 as compared to those induced with non-AMP peptide or peptide solution. An increase in the concentration of moronocidin-like and dicentracin-like peptides up to 31 μM similarly induced the increased levels of TNF-α and IL-12, while no statistically significant change was observed in the levels of TNF-α and IL-12 produced by piscidin.

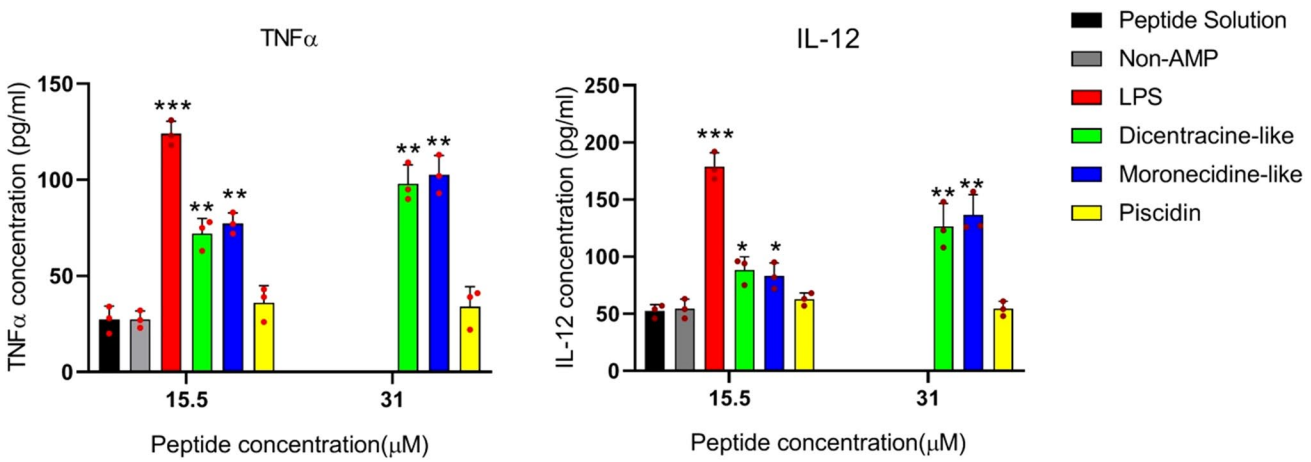

Fig. 6 Secretion of tumor necrosis factor (TNF alpha) and IL-12 by RAW 264.7 macrophages in response to peptides. RAW 264.7 macrophages were treated with AMPs, non-AMP peptide, LPS or peptide solution, and then the presence of TNF-α or IL-12 in culture supernatants was quantified by ELISA. The values are expressed as mean ± SD for three independent experiments. Differences at $p < 0.05$ were considered significant (***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$). Cytokine concentrations of medium supernatants from AMP treated macrophages were compared to peptide solution using *one-way* analysis of variance (*ANOVA*)

Discussion

Cutaneous leishmaniasis, caused by *L. major* and *L. tropica*, is a main health problem in various provinces of Iran (Gharavi et al. 2011a, b). There is no approved vaccine for prophylaxis of leishmaniasis in the endemic regions, and treatment is suboptimal, causing side effects. Consequently, there is an obvious and urgent need for developing affordable, safe and efficacious drugs against leishmaniasis (Fuertes et al. 2008).

In the current study, we compared the anti-*L. major* activities of the three AMPs dicentracine-like, moronecidin-like, and piscidin (Mohammadi et al. 2018; Taheri et al. 2018). The three AMPs exhibit dose dependent activity against *L. major* promastigotes, with piscidin being the most efficacious one as compared to the other two peptides, and induced promastigote lysis at a high peptide concentration. The most likely explanation for the high cytotoxicity of piscidin against promastigotes may be owing to its high hydrophobic nonpolar face relative to the other two peptides. Prior studies demonstrated that peptides with highly hydrophobic nonpolar face possess more propensities to the cell membrane, which in turn leads to increased cytotoxicity (Irazazabal et al. 2016; Taheri et al. 2019). In addition, piscidin also exhibited the highest cytotoxic activity against the macrophage cell line. This is consistent with earlier studies that had demonstrated the high cytotoxicity activity of piscidin against mammalian cells such as Hela cell and human red blood cells. (Kim et al. 2010; Kumar et al. 2016). In a previous study, we demonstrated that a reduction of hydrophobicity at the nonpolar face of piscidin through amino acid substitution resulted in decreased cytotoxicity (Taheri et al. 2019).

In contrast to what was seen with promastigotes, moronecidine- like and dicentracine-like were more potent than piscidin against *L. major* amastigotes. This is consistent with the observation that moronecidine- like and dicentracine-like peptides promoted the proliferation of macrophage cells. T helper1(Th1)-associated cytokines (especially interferon-γ, TNF-α and IL-12) are known as the key factors in the induction of protective immunity against L. major infection (Dayakar et al. 2019), this cytokines are up-regulated upon signaling of NF-κB and MAPK pathways (Wang et al. 2019).

Given that AMPs enhance macrophage function through TLR4-induced NF-κB and MAPK signaling (Wang et al. 2019), we studied the influence of AMPs on the expression and induction of limiting factors on *L. major* growth (IL-12, TNF-α and ROS) in macrophages.

Dicentracine-like and moronecidin-like peptides were capable to induce IL-12, TNF-α and ROS, while non-AMP peptides did not. This result was consistent with previous study that demonstrated immunomodulatory activities of AMPs through the production of type 1 T helper (TH1) -polarizing cytokines (IL-12 and TNF-α) (Agier et al. 2015). Furthermore, Zheng et al. (Zheng et al. 2007) demonstrated that Cathelicidin LL-37 (a human AMPs), induced the generation of ROS in macrophages. AMPs-induced IL-12 and TNF-α cytokines could activate NK cells to produce interferon (IFN)-γ, a key cytokine for polarization of T cells towards a Th1 immune response, and also activate macrophages cells to produce ROS and nitric oxide (NO), thereby protects the host cells through killing of intracellular amastigotes(Dayakar et al. 2019; Roma et al. 2016).

In this study, dicentracine-like and moronecidin-like treatements induced higher levels IL-12, TNF-α and ROS

compared to piscidin, and this might be due to the high cytotoxic activity of piscidin against macrophage, leading to the reduction of piscidin-induced productions(IL-12, TNF-α and ROS). Considering that IL-12, TNF-α and ROS act as key factors for intracellular *L. major* amastigote growth inhibition (Roma et al. 2016), the greater anti-amastigote activity of dicentracine-like and moronecidin-like peptides than piscidin peptide is mainly linked to their ability to induce the production of IL-12, TNF-α and ROS (indirect anti-amastigote activity). This function corresponds to the well-known role of AMPs in the innate immune system (Diamond et al. 2009). Furthermore, considering that piscidin peptide was disable to induce IL-12, TNF-α and ROS production, the greater anti-amastigote activity of piscidin peptide compared to non-AMP peptides could be due to its direct cytotoxic activity against intracellular *L. major* amastigote (direct anti-amastigote activity), following the internalization of piscidin into macrophage cells. This result was consistent with the great cytotoxic activity of piscidn peptide against promastigote form. In agreement with this hypothesis, previous studies demonstrated intracellular antiviral activity of AMPs, via the internalization into infected cell and interruption of viral replication(Chessa et al. 2020).

Taken together, these AMPs could act as multifunctional agent against *L. major* not only through direct killing of *L. major*, but also modulation of proinflamatory cytokines and the activation of macrophage that might synergistically control leishmania infection. However, further experiments will be required to verify the anti-Leishmania activity of theses AMPs in the mouse model.

Conclusions

In the current study, we characterized two peptides with negligible toxicity (dicentracine-like and moronecidine-like), that not only exhibited direct cytotoxicity against *L. major* but also induced limiting *L. major* growth (proinflamatory cytokines and ROS) in macrophage cells. These two peptides suggested as attractive and efficient multi-functional therapeutic agent again leishmaniasis.

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Availability of Data and Material The authors confirm that the data supporting the findings of this study are available within the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest regarding the publication of this article.

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