ISSN: 2640-4133

Research Article

Advances in Bioengineering & Biomedical Science Research

Anti-Diabetic and Hemolytic Activity of the Antimicrobial Peptide Parapolybia-MP

Grace Vanathi M[1*] and R P Rajesh[2]

[1]*Centre for Nanomedical and Nanoscience, Sathyabama Institute Science and Technology, Chennai, Tamil Nadu*

[2]*Department of Biotechnology, University of Kentucky, Lexington KY, USA*

[*]Corresponding Author
Grace Vanathi M, Centre for Nanomedical and Nanoscience, Sathyabama Institute Science and Technology, Chennai, Tamil Nadu

Submitted: 2024 , Jan 08; **Accepted:** 2024, Jan 26; **Published:** 2024, Feb 12

Citation: Vanathi M, G., Rajesh, R. P. (2024). Anti-Diabetic and Hemolytic Activity of the Antimicrobial Peptide Parapolybia-MP. *Adv Bioeng Biomed Sci Res, 7*(1), 01-05.

Abstract

Parapolybia-MP is an antimicrobial peptide belonging to the family of Mastoparans. This peptide is composed of 14 amino acid residues, (INWKKMAATALKMI) and mostly resides in the venom of the Polistes wasp. It has a molecular weight of 1619.00KDa. Parapolybia-MP is a group of mast cell degranulating peptides possessing various activities such as anticancer, anti-inflammatory, and cytolytic properties. This peptide being a tetra deca-peptide is linear in configuration and, modifies itself into a cyclic one upon binding to the plasma membrane of the microbes. Diabetes foot ulcer is the leading cause of the lower leg amputation. Mainly 15% of patients with diabetes develop foot ulcers. Microbial species at the wound site tend to cause low wound healing capacity. To focus on this issue, a peptide with good anti-diabetic and hemolytic activity is required. Parapolybia-MP was found to have good anti-diabetic activity with an IC50 value of 70.31%. It also has negligible hemolytic activity as well.

Keywords: Parapolybia-MP, Cationic, Mastoparan, Mast cell, Cytolytic, Plasma Membrane.

1. Introduction

Antimicrobial peptides are a class of bioactive agents with multiple modes of action and appear as one of the most important drug candidates. Antimicrobial peptides were found to have broad-spectrum efficiency in destroying drug-resistant bacteria and bacterial biofilms. This activity is mainly due to their positive charge and hydrophobic residues that destroy the bacterial cell membrane. Mastoparans are amphiphilic α-helical peptides comprising about 14 amino acid residues. They possess several important biological characteristics such as antimicrobial properties, cytotoxic activity on tumor cells, increased mitochondrial permeability, and anticancer activity. Mastoparans are mainly found in the venom of the *Polistes* wasp. *Polistes major* is a neotropical wasp mostly found in the Caribbean region. Wasp venom constitutes various venom components composed of peptides, proteins, enzymes, and small molecules. Isolated chemical components from wasp venom have several biological activities, typically active against bacteria and fungi. Peptides isolated from Polistes major belonging to the Mastoparan family are called Parapolybia-MP or Mastoparan-MP [1]. It constitutes activities such as limited mast cell degranulation and has antimicrobial and hemolytic activity [2]. It initiates pore formation and modifies the intact cellular structure causing cytotoxicity and anti-proliferation. Parapolybia-MP binds to the 'ppGpp' an alarmone protein in bacteria. Stringent response signaling molecule "ppGpp" is an important signal in biofilm development. It is a secondary messenger, mainly activated during stress conditions. It regulates the expression of relevant genes associated with biofilm formation. These genes are associated with the formation of exopolysaccharides and adhesion molecules, leading to the synthesis of a biofilm matrix [3].

Alarmone executes action in a way similar to that of G-proteins receptors. G-protein receptors are present in all species and constitute Monomeric and Trimeric G-protein coupled receptors. G-proteins consists of αβδω subunits. It has a head, a transmembrane unit, and a tail. The transmembrane unit is a coiled structure and in-turn it is attached to the cell membrane. Alarmone and G-proteins executes action in a much similar way. Alarmone secretion is increased during bacterial starvation, and this in turn, if targeted, biofilm formation can be prevented. Parapolybia-MP mainly targets the alarmone, and this could effectively prevent biofilm formation [4]. Bacterial residence at the diabetic wound site is a common factor for preventing earlier re-epithelialization. To combat bacterial growth, biofilm formation must be prevented. And this in turn will promote earlier wound healing.

Diabetes is a chronic disorder. Diabetic foot ulcer occurs in 15% of patients with diabetes, and it is a leading cause of foot amputation. Diabetes associated with foot ulcers has various complications such as Neuropathy and Peripheral vascular occlusive disease. Nerve damage or Neuropathy causes null

sensations in diabetic patients and due to bacterial residence at the wound site healing doesn't occur [5]. Diabetic foot patients undergo leg amputation, due to healing inability at a much faster rate. This in turn is accompanied by opportunistic symptoms such as bacterial infections, necrosis, and loss of blood vessels. These will make the foot ulcer even more aggressive and finally lead to foot amputation. The global diabetic prevalence rate is estimated to reach up to 5.4/5 by 2025. This rate mainly corresponds to developing countries. To combat bacterial infections and prevent nerve damage, earlier angiogenesis is to be promoted for foot ulcer healing [6]. To heal diabetic wounds or ulcers, drugs with the potential to destroy microbes and their biofilm are essential. Drugs with good anti-diabetic activity are preferable. This mostly targets the amylase enzyme and reduces its activity in diabetes patients. Thereby, hyperglycemia could be effectively brought down and wound healing can be achieved.

Bacterial biofilm is one main challenge to conquer, it can be done by specifically targeting the biofilm signaling cascade protein. Alarmone or ppGpp is a biofilm signaling cascade molecule necessarily increased during the bacterial biofilm formation. If this protein alarmone is targeted, then bacterial biofilm formation at the wound site can be prevented, thus bringing up faster wound healing. Parapolybia-MP belongs to the family of antimicrobial peptide Mastoparans. Mastoparans collectively cause guanine nucleotide exchange by GTP binding regulatory proteins [7]. This is done in a way similar to that of G-protein coupled receptors. It enhances its action via trimeric G-protein reconstituting into lipid vesicles. Mastoparans are linear peptides, while binding to a phospholipid bilayer, it forms an α-helix lying parallel to the plane of the membrane with the hydrophobic layer facing towards the bilayer and the four positive charges facing outward. G-protein coupled receptors form clusters of positive charge displayed at the inner surface of the membrane. Mastoparans function as cellular probes and structural models of the G-protein activating domain [8]. Mastoparans facilitate nucleotide exchange by a mechanism similar to that of G-protein-coupled receptors. Regulation of G-proteins by mastoparans appears to be similar in many ways. Mastoparans found in the venom of the Polistes wasp, serves as a useful drug source for various pathologies such as cancer, autoimmunity, earlier wound healing, and immunocompromised disorders.

2. Materials and Methods

Parapolybia-MP sequence (INWKKMAATALKMI) was custom synthesized. 5mg of Parapolybia-MP peptide was weighed and dissolved in 1 ml of HPLC grade water, which is used as a stock solution having a concentration of 500µg/ml. From this stock solution, various concentrations 10, 20, 30, 40, 50, 60, 70, 60, 90, and 100µg/ml were prepared using HPLC-grade water.

2.1 Invitro Anti-Diabetic Activity

2.1.1 Alpha Amylase Activity

Alpha amylase enzyme is responsible for causing hyperglycemia, by inhibiting its activity. Diabetics people have low alpha-amylase activity for keeping their glucose level under control [9]. Alpha amylase inhibitors act by altering the digestive action of this enzyme as well as the proteinases. This in turn inhibits the normal digestive action in the gut of the insects [10]. Hence, alpha amylase inhibitors have a potential role in controlling the blood glucose level [11]. Alpha amylase activity of Parapolybia-MP with different concentrations was adapted by following the method of Sudha et al. (2011). 250µl of Porcine pancreatic α-amylase and 100µl of Parapolybia-MP at concentrations ranging from 15.6 to 250mg/L were added to a test tube. The mixture was pre-incubated at 37°C for 15min, before adding 250µl of 0.5% starch. This mixture was then vortexed and again incubated at 37°C for 15 minutes. This reaction was then terminated by adding 1 ml of dinitrosalicylic acid color reagent [13]. Test tubes were placed in a boiling water bath for 5min, then cooled to room temperature. 200µl of reaction mixtures were taken into the 96-well clear plate, and the absorbance was read at 540nm using the FLUOstar OPTIMA plate reader. Control sample containing α-amylase at 1U/ml without any incubator represented about 100% enzyme activity. Appropriate test extract controls containing the reaction mixture except enzymes were used for color interference. %Inhibition was calculated using the formula [14].

$$\% \text{ Inhibition} = [\text{ A control} - \text{A Test sample}/\text{ A control}] \times 100$$

2.1.2 In-Vitro Hemolytic Activity

Parapolybia-MP was evaluated for its hemolytic activity by carrying out the in-vitro hemolysis in a 96-well plate against the positive control TrixtonX-100 [15]. Chicken Whole blood was taken and used within 8hrs. Whole blood (4ml) was taken and mixed with equal volumes of histopaque (4ml) [16]. It was then centrifuged at 400rpm for 30 minutes. The supernatant containing the plasma was removed [17]. The pellet was washed twice using phosphate buffer saline. It was then centrifuged at 800rpm for 10mins, erythrocyte pellet was then suspended in PBS and used for the analysis [18]. From this, 2% RBC suspension was prepared by adding 1 drop of RBC and 49ml of PBS. A hemolytic assay was performed on a 96-well plate. Each well holds a final volume of 100µl of 2% erythrocyte. Parapolybia-MP at various concentrations of 10, 20, and 30µg/ml was added to the 2% RBC suspension and hemolysis was evaluated for every 10 minutes time interval. TrixtonX-100 was used as a positive control and 2%RBC suspension was used as a negative control. Lysis activity was measured for every 10 minutes interval and absorbance at 540nm was calculated using a Microplate reader.

3. Results and Discussions

In this present study, Parapolybia-MP was investigated for its anti-diabetic and hemolytic activity. Alpha amylase inhibitory activity was determined against the amylase enzyme. Parapolybia-MP had a dose-dependent inhibition of the alpha-amylase enzyme. Various concentrations such as 20, 40, 60, 80, and 100µg/ml of Parapolybia-MP were found to have good anti-diabetic activity compared to that of the control (amylase).

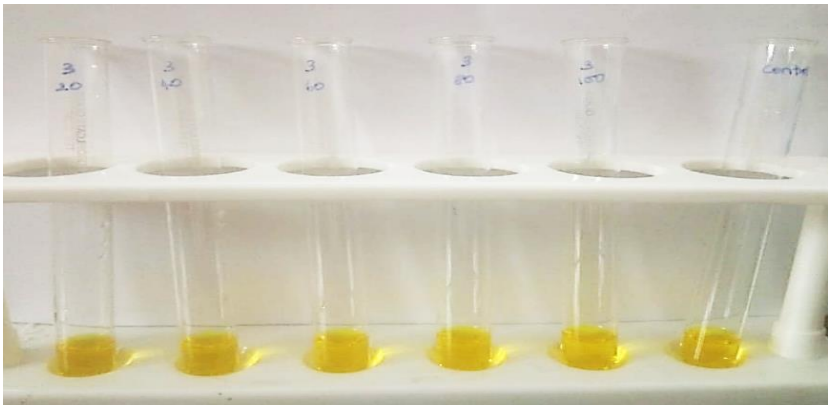


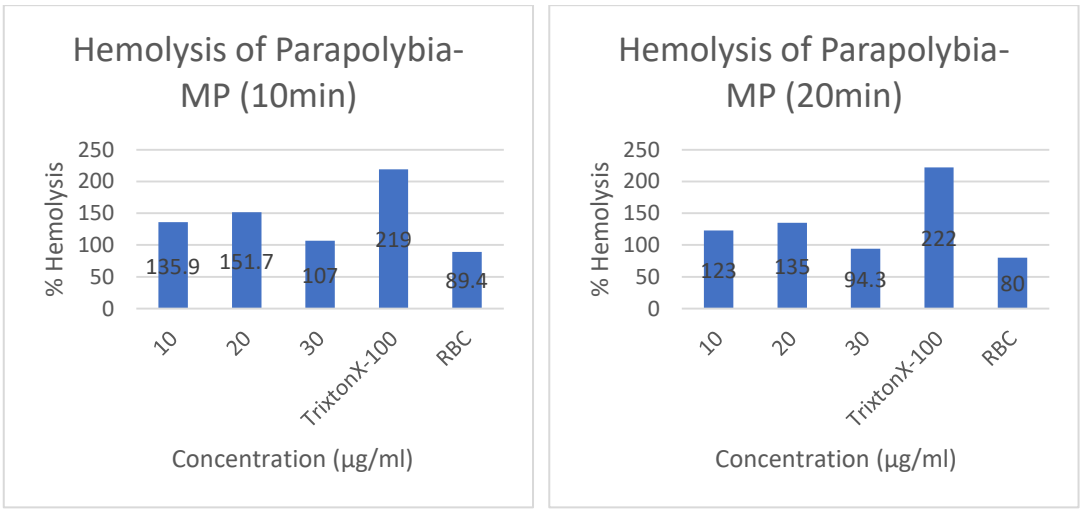
Figure 1: Invitro Antidiabetic activity (α-Amylase Inhibitory Action)

Conc. µg/mL	OD Value @540nm			Average	% of Inhibition
	I	II	III		
20	0.440	0.438	0.442	0.440	3.71
40	0.351	0.350	0.354	0.351	23.19
60	0.297	0.295	0.296	0.296	35.77
80	0.197	0.198	0.196	0.197	56.89
100	0.108	0.115	0.113	0.112	75.49
IC50					**70.31**

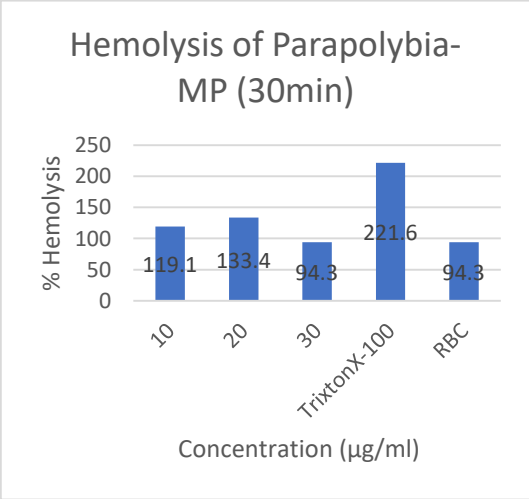
Table I: Measurement of Anti-diabetic activity at 20, 40, 60, 80, and 100µg/ml concentrations of Parapolybia-MP. Maximum alpha-amylase inhibition activity was observed at 100µg/ml

3.1 In-Vitro Hemolytic Activity

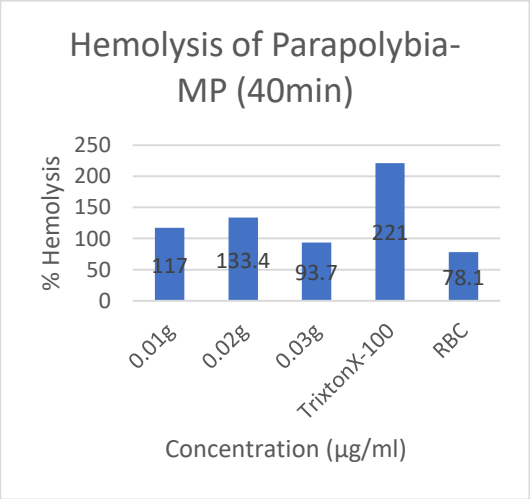
a) **b)**



c)



d)



e)

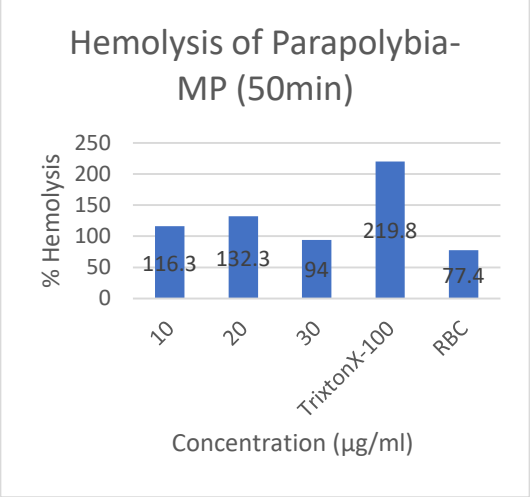


Figure 2: Shows the hemolysis graph of Parapolybia-MP at various time intervals of 10,20,30,40 and 50 minutes. Parapolybia-MP at concentrations ranging from 10µg/ml, 20µg/ml, and 30µg/ml were evaluated.

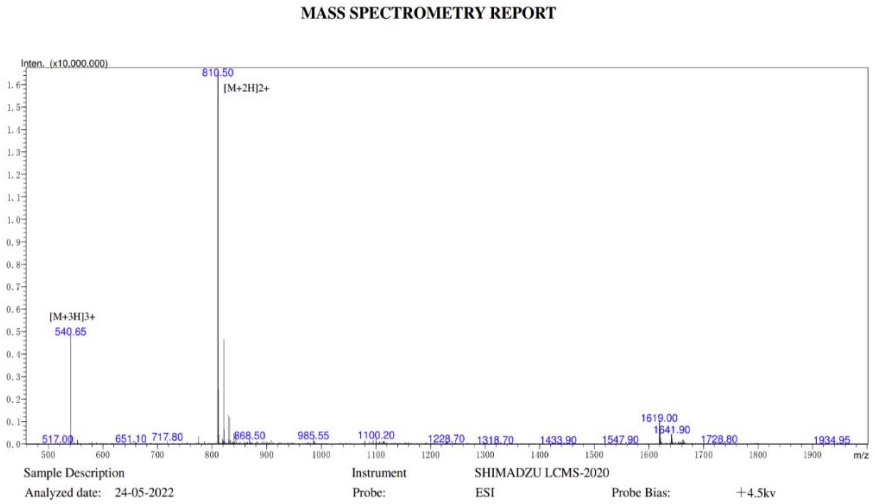


Figure 3: Mass Spectrometry report of antimicrobial peptide Parapolybia-MP

4. Conclusion

Parapolybia-MP (INWKKMAATALKMI), a 14 amino acid peptide significantly showed good anti-diabetic activity with an IC50 value of 70.31%. This being significantly higher compared to that of the control (alpha amylase). In-vitro hemolytic activity of this peptide was evaluated for every 10-minute interval and was found to have lesser RBC denaturation capacity compared to that of the positive control TrixtonX-100. The outcome of this research confirms that the antimicrobial peptide Parapolybia-MP from Polistes wasp showed good anti-diabetic activity and significant hemolytic activity.

References

1. Abd El-Wahed, A., Yosri, N., Sakr, H. H., Du, M., Algethami, A. F., Zhao, C., ... & El-Seedi, H. R. (2021). Wasp venom biochemical components and their potential in biological applications and nanotechnological interventions. *Toxins, 13*(3), 206.
2. Moore, A. J., Beazley, W. D., Bibby, M. C., & Devine, D. A. (1996). Antimicrobial activity of cecropins. *Journal of Antimicrobial Chemotherapy, 37*(6), 1077-1089.
3. Higashijima, T., Burnier, J., & Ross, E. M. (1990). Regulation of Gi and Go by mastoparan, related amphiphilic peptides, and hydrophobic amines. Mechanism and structural determinants of activity. *Journal of Biological Chemistry, 265*(24), 14176-14186.
4. Jiale, Z., Jian, J., Xinyi, T., Haoji, X., Xueqin, H., & Xiao, W. (2021). Design of a novel antimicrobial peptide 1018M targeted ppGpp to inhibit MRSA biofilm formation. *AMB Express, 11*(1), 1-14.
5. de Santana, C. J. C., Pires Júnior, O. R., Fontes, W., Palma, M. S., & Castro, M. S. (2022). Mastoparans: A Group of Multifunctional α-Helical Peptides With Promising Therapeutic Properties. *Frontiers in Molecular Biosciences, 9*, 824989.
6. Choi, M. B., & Lee, Y. H. (2020). The structure and antimicrobial potential of wasp and hornet (Vespidae) mastoparans: A review. *Entomological Research, 50*(7), 369-376.
7. ČeŘovský, V., Pohl, J., Yang, Z., Alam, N., & Attygalle, A. B. (2007). Identification of three novel peptides isolated from the venom of the neotropical social wasp Polistes major major. Journal of Peptide Science: *An Official Publication of the European Peptide Society, 13*(7), 445-450.
8. Raheem, N., & Straus, S. K. (2019). Mechanisms of action for antimicrobial peptides with antibacterial and antibiofilm functions. *Frontiers in microbiology, 10*, 2866.
9. Zinjarde, S. S., Bhargava, S. Y., & Kumar, A. R. (2011). Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC complementary and alternative medicine, 11*(1), 1-10.
10. Sekhon-Loodu, S., & Rupasinghe, H. V. (2019). Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants. *Frontiers in nutrition, 6*, 53.
11. Chaudhari, M. G., Joshi, B. B., & Mistry, K. N. (2013). In vitro anti-diabetic and anti-inflammatory activity of stem bark of Bauhinia purpurea. *Bulletin of Pharmaceutical and Medical Sciences (BOPAMS), 1*(2), 139-150.
12. Govindappa, M., Hemashekhar, B., Arthikala, M. K., Rai, V. R., & Ramachandra, Y. L. (2018). Characterization, antibacterial, antioxidant, antidiabetic, anti-inflammatory and antityrosinase activity of green synthesized silver nanoparticles using Calophyllum tomentosum leaves extract. *Results in Physics, 9,* 400-408.
13. Adisakwattana, S., Sompong, W., Meeprom, A., Ngamukote, S., & Yibchok-Anun, S. (2012). Cinnamic acid and its derivatives inhibit fructose-mediated protein glycation. *International journal of molecular sciences, 13*(2), 1778-1789.
14. Shivanna, R., Parizadeh, H., & Garampalli, R. H. (2015). Screening of lichen extracts for in vitro antidiabetic activity using alpha amylase inhibitory assay. *Int J Biol Pharm Res, 6*(5), 364-367.
15. Holtz, B. S., Toll, M. D., Adam, D., Burkholder, M. D., Susan, W., Carlin, B. A., ... & Tina, B. (2009). Establishing the Purity of Mononuclear Cell Preparations Using Morphology and Flow Cytometry.
16. Malagoli, D. (2007). A full-length protocol to test hemolytic activity of palytoxin on human erythrocytes. *Invertebrate Survival Journal, 4*(2), 92-94.
17. Evans, B. C., Nelson, C. E., Shann, S. Y., Beavers, K. R., Kim, A. J., Li, H., ... & Duvall, C. L. (2013). Ex vivo red blood cell hemolysis assay for the evaluation of pH-responsive endosomolytic agents for cytosolic delivery of biomacromolecular drugs. JoVE *(Journal of Visualized Experiments), (73),* e50166.
18. Greco, I., Molchanova, N., Holmedal, E., Jenssen, H., Hummel, B. D., Watts, J. L., ... & Svenson, J. (2020). Correlation between hemolytic activity, cytotoxicity and systemic in vivo toxicity of synthetic antimicrobial peptides. *Scientific reports, 10*(1), 13206.