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In-Silico Characterization of Apitoxin- Melittin

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Abstract
The Bee venom is used for treating a wide variety of conditions from acute tendonitis to chronic back pain to rheumatoid arthritis (RA) from ancient times. The use of honey bee products for medical purposes, this includes bee venom, raw honey, royal jelly, pollen, propolis, and beeswax, is called as apitherapy. Bee venom (apitoxin) is an active toxic substance, composed of only a few pharmacologically and enzymatically active components (18 active componenets), such as phospholipase A, hyaluronidase, histamines, melittin & apamin, a mast cell degranulating peptide (MCD). Melittin is one of the abundant active component of Bee venom (52% of bee venom) with biologically positive effects (wide antimicrobial, anti-inflammatory, hemolytic, cytolytic) having low toxicity which can be used to treat many diseases. The present investigation includes retrieval of amino acid information of melittin from major protein sequence database, sequence analysis of melittin was performed using Protoparam tool. The physiochemical parameters like amino acid propensity, molecular weight, isoelectric point, aliphatic index, hydropathicity were determined. The secondary structure of melittin was predicted using GOR method. The antigenecity region or peptide was predicted by using Kolaskar & Tongoankar method. Further *in silico* characterization of structural and functional elements of melittin can put insight into the new pharmacological applications of apitoxins emerging from oldest form of traditional medicines.

Keywords: *In silico*, Apitherepy, Apitoxin, Melittin.

1. Introduction

Use of honey and other bee products in human treatments traced back thousands of years and healing properties are included in many religious texts including the Veda, Bible and Quran [1]. Apitherapy is the use of honey bee products for medical purposes, this include bee venom, raw honey, royal jelly, pollen, propolis, and beeswax [1]. Honeybee (*Apis florea* L.) venom contains a complex mixture of therapeutic compounds, including antimicrobial peptides, allowing bees to defend their hives against predators and external threats. The chemical composition of these insect venoms is complex, encompassing, a mixture of many kinds of compounds, proteins, peptides, enzymes, and other smaller molecules. This mixture of biologically active substances can exert toxic effects, contributing to certain clinical signs and symptoms of envenomation. Human responses to stings include pain, small edema, redness, extensive local swelling, anaphylaxis, and systemic toxic reaction [1]. The amount of venom protein released in a sting varies between species, ranging between 50 and 140 micrograms for bees [2, 3]. It is composed of only a few pharmacologically and enzymatically active components, such as phospholipase A, hyaluronidase, histamine, melittin and apamin, a mast cell degranulating (MCD) peptide [4]. The anti-inflammatory properties of this venom, various forms of traditional bee venom therapy, including the administration of live stings, injection of venom, and venom acupuncture have been used to relieve pain and to treat chronic inflammatory diseases such as rheumatoid arthritis and multiple sclerosis [5, 6]. This traditional medicine also has been used for other diseases like cancer [5], skin conditions [7] and recently even for Parkinson's disease [8]. Bee venom therapy is the use of live bee stings (or injectable venom) to treat various diseases such as arthritis, rheumatoid arthritis, multiple sclerosis (MS), lupus, sciatica, low back pain, and tennis elbow etc. It refers to any use of venom to assist the body in healing itself.

Melittin is the principal toxic component in the venom of the honey bee and is a cationic, hemolytic peptide. It is a small linear peptide composed of 26 amino acid residues in which the amino-terminal region is predominantly hydrophobic whereas the carboxy-terminal region is hydrophilic due to the presence of a stretch of positively charged amino acids [8].The melittin shows the wide therapeutic effects shown in table 1 [9]. Although melittin is the most studied and known bee venom peptide, its development for clinical applications remains mainly in preclinical phases [10].

Table 1: Melittin & its therapeutic effects

Component & Total %	Effects
Melittin (Biologically active peptide) 50- 55%	**Main biologically active component** Membrane-active, diminishes surface tension of membranes Anti-inflammatory in very small doses; Stimulates smooth muscles; Increases capillary permeability increasing blood circulation and lowering the blood pressure, lowers blood coagulation, immunostimulatory and immunosuppressive, Radiation protective, influences the central nervous system.

2. Methods

i) Retrieval of protein sequence data of melittin

The melittin protein sequence (MELT) from *Apis florea* (Indian Honeybee) was retrieved from Uniprot database [11]. Uniprokb is public protein database which contains the amino

acid sequences of proteins. The sequence was retrieved & saved in FASTA file format with its Accession ID.

ii) **Analysis of Physico-Chemical composition**

Physicochemical properties of melittin was performed by using ProtParam analysis tool which on Expasy server. The ProtParam [12] computed parameters includes amino acid composition, molecular weight, theoretical pI, Instability index, Grand average of hydropathicity.

iii) **Prediction of Protein Secondary structure**

The secondary structure of melittin was predicted by GOR [13] secondary structure prediction method. The GOR method analyzes sequences to predict alpha helices, beeta sheets, turn or random coil secondary structure at each position based on 17 amino acid sequence windows.

iv) **Analysis of Functional Domains**

Domain is the most important factor governing the protein folding into the structure. The domain of the melittin protein was predicted from the Pfam [14] domain database which contains the information about protein families & domains.

v) **Prediction of Antigenicity**

The antigencity of melittin was performed by using Kolaskar & Tongaonkar [15] Antigenicity prediction method. This program predicts those segments from within a melittin protein from *A florea* sequence which are likely to be antigenic by eliciting an antibody response and the potent antigenic regions were highlighted with its residues.

3. Results & Discussion

i) **Retrieval of protein sequence data of Melittin**

Melittin apitoxin protein [Uniprot ID: P01504] sequence from *Apis florae* (Indian Honeybee) was retrieved from Uniprotkb database, with its 26 amino acids and saved in FASTA format which shown as as below

>sp|P01504|MEL_APIFL Melittin OS=Apis florea GN=MELT PE=1 SV=1
GIGAILKVLATGLPTLISWIKNKRKQ

ii) **Analysis of physicochemical composition**

Physicochemical composition of melittin was analyzed by using ProtParam analysis tool which on Expasy server.The physiocohemical parameters were tabulated in table 2. As per table instability index is 35.59 classifies the melittin is stable, on the basis theoretical pI the melittin is basic in nature, as there are only presence of positively charged amino acids and the melittin is cationic in nature.

Table 2: Physicochemical parameters of Melittin

Parameter	Value
Number of amino acids	26
Molecular weight	2819.4
Theoretical pI	11.33
Instability index	35.59
Grand average of hydopathicity	0.308
No. of +vely charged amino acids	05
No. of - vely charged amino acids	00

iii) **Prediction of Protein Secondary structure**

The secondary structure of melittin was predicted by GOR Secondary Structure Prediction method. Secondary structural elements like alpha helix, Sheets, coils, etc were enlisted in table 3. The table shows the melittin has more number of coils as 50.00% followed by helices 26.92% & strands 23.08%

Table 3: Secondary structures of melittin

Secondary structure	No. of residues	Percentage
Alpha helices	7	26.92%
Extended strands	6	23.08%
Random coils	13	50.000%

iv) **Prediction of Domain**

The domain structure of melittin was predicted pfam domain database and the functional part was shown in Fig.1. The functional and conserved part belongs from melittin family has the haemolytic activity and it also inhibits well known transport pumps such as the Na^+-K^+-ATPase and the H^+-K^+-ATPase. It integrates into cell membranes and has multiple effects, probably, as a result of its interaction with negatively charged phospholipids.

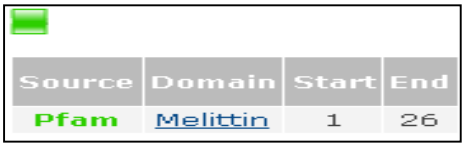

Fig 1: Domain of Melittin

v) **Prediction of Antigenicity peptides**

The antiegenic peptide of melittin was performed by using Kolaskar & Tongaonkar antigenicity prediction method. The potent antigenic region was highlighted with its residues is calculated in table 5 which shows the 16 residues peptide starts from position 4 to 19 is part of most potent antigenic. The antigenic plot is shown in Fig.2 where x-axis shows sequence number and y-axis shows average antigenic propensity. The average antigenic propensity of the melittin is 1.055.

Table 5: Antigenic region of melittin

No.	Start Position	End position	Peptide	Peptide length
1	4	19	AILKVLSTGLPALISW	16

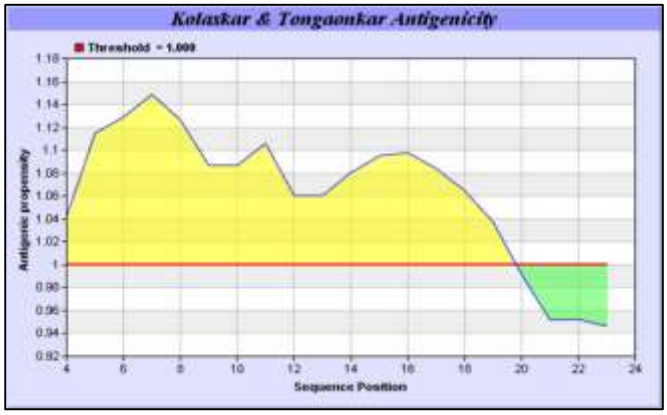

Fig 2: Antigenic plot of melittin

Summary and Conclusion

The present preliminary investigation mainly leads to understand the basic primary and secondary structure of melittin using various *in-silico* tools and techniques. The

primary structure illustrates that the melitin is a peptide with its 26 amino acids. The physicochemical properties depict that the melittin is alkaline, stable, amphiphilic & basic peptide in nature. The secondary structure reveals that melittin consist of a helix, a sheet and random coil structure within its short stretch of residues. Out of its 26 residues, 16 residues contribute for potent antigenic region of melittin, which confirms it as a potent bio-active peptide. This preliminary work and further detailed investigations on melittin regarding its structure and bio-active role, can prove it as a potent pharmacological & therapeutic agent and contribute to the new dimension of disease treatment strategies through immune therapy, vaccine designing & peptide drug designing using Bioinformatics.

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