THERAPEUTIC POTENTIAL OF MELITTIN PEPTIDE: MAJOR PAIN-PRODUCING SUBSTANCE OF EUROPEAN HONEYBEE (*APIS MELLIFERA*) VENOM AGAINST SEVERAL COMMUNICABLE AND NON-COMMUNICABLE DISEASES

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Abstract

Invertebrates have evolved to produce peptide compounds to ensure success in prey capture and defence against potential enemies. Some of these compounds have become highly potent therapeutic compounds used in medicine. Melittin, a peptide extracted from bee venom, has emerged as a promising therapeutic option that has the potential to obviate the disadvantages of existing therapeutics against several communicable and non-communicable diseases. This article reviews peer-reviewed journal articles to provide a conspectus of current research on the therapeutic uses of melittin. Melittin is predominantly an antimicrobial peptide and shows therapeutic potential against several non-communicable diseases, including cancers, inflammatory diseases and diabetes. Furthermore, it acts against a range of protozoan parasites that infect humans. The biological activities of melittin are mainly achieved via cytotoxicity and downregulation of certain metabolic pathways. The ability to conjugate with other compounds and nanoparticles to improve the effectiveness is an added advantage in melittin-based therapy. The antiparasitic properties and relatively shorter sequence enable the use of this molecule in biological control methods such as paratransgenesis. This is further facilitated by the ability to express in an inactive form to be activated later. Melittin is a peptide with a broad therapeutic potential. The supplementation of the existing knowledge with studies on effective and specific delivery mechanisms will enable the effective use of this peptide against many communicable and non-communicable diseases.

Keywords: Melittin, peptide, therapeutic

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Introduction

A plethora of animal-derived compounds is used for various purposes ranging from cosmetics and pharmaceuticals to candles, sticky resin, and polishes. Animals that use a cocktail of proteins to kill or paralyse their prey or a potential predator have been a major source of such compounds throughout history. Honeybees (*Apis* spp) are one such group that produce a range of compounds in their venom glands, among which melittin (MEL) stands out as one of the most widely studied insect-derived compounds.

Apitoxin (honeybee venom) has been used in venom therapy by Asians, Europeans and northern Africans in oriental medicinal applications since 1000-3000 BC (Zhang et al., 2018; Wehbe et al., 2019). Bee venom therapy has been improved since the 19th century with novel scientific (Carpena et al., 2020). However, precise knowledge of such venom's composition and mode of action was understood approximately 70 years ago (El-Aziz et al., 2019). Novel biochemical techniques such as high-performance liquid chromatography (HPLC), Gel permeation chromatography (GPC) and size-exclusion chromatography (SEC) were applied to identify the chemical nature of wasp venom and bee venom. Major components of honeybee venom are accurately and precisely characterised with Mass spectrometry (MS) and protein sequencing techniques, such as Edman degradation (Moreno & Giralt, 2015). Melittin is the main active pharmacological component of the venom of the European honeybee *Apis mellifera* (Memariani & Memariani, 2021), accounting for 40–50% of its total dry weight (Chen et al., 2016)—the first extraction of MEL from honeybee venom dates to 1952. Subsequently, a study showed that a penicillin-resistant isolate of *Staphylococcus aureus* was susceptible to bee venom and MEL fraction (Fennell et al., 1967). In 1989, Azambuja et al. demonstrated the cytotoxicity of MEL against trypanosomatids (Azambuja et al., 2005).

Bee venom therapy is used to manage patients with pain, skin diseases, arthritis, rheumatism and tumours (Hider, 1988). From previous studies, melittin is recognised as an antiparasitic, antibacterial, antifungal, anti-tumour and antiviral agent. It disrupts eukaryotic and prokaryotic cell membranes both chemically and physically (Rady et al., 2017). Studies elucidate the potential therapeutic uses of MEL. Melittin reduces cell viability time- and concentration-dependent by altering the cell membrane. This indicates the importance of MEL as an emerging, promising therapeutic candidate for anticancer therapy (Gajski & Garaj-Vrhovac, 2013). Treating with Honey Bee Venom (*Apis mellifera*) reduces blood glucose levels of Chinchilla rabbits by 14.9% -26.5% while High-Density Lipoproteins (HDL) levels increased by 2.5% - 26.25%, Low-Density Lipoproteins (LDL) levels lowered by 11.2%-14.2%, and blood cholesterol levels reduced by 12.5%- 19.1% after injecting 0.2-0.5 ml of *Apis mellifera* venom (Ts et al., 2015).

Although MEL is one of the most widely explored peptides, the development of clinical applications of MEL is still in the preclinical phase. Numerous studies explicate that MEL has impressive therapeutic properties. A collective understanding of all such studies would be useful in identifying research gaps and planning future studies. No attempts were made to summarise and review the outcomes of these studies to date. This comprehensive and concise review congregates the current knowledge of the medicinal uses of MEL with a special emphasis on antiparasitic uses.

Methodology

This is a narrative literature review on the therapeutic aspects of melittin, a compound found in European honeybee (*Apis mellifera*) venom. Literature searches with Google Scholar and PubMed using words [melittin] and [Antiviral] and [Antifungal] and [Cancer therapy] and [Anti-inflammatory] and [Anti-diabetic] and [Antiprotozoal activity] and [Protozoa] and [Leishmania] or [Drug

development] or [Biological control] or [Paratransgenesis] for papers published in English language until the end of 2021 were carried out by the authors. Collected data were correctly cited, and duplicates or articles that did not respond directly to the proposed objective were excluded. Based on the literature review, Biochemical properties, antimicrobial properties, antiviral properties, antifungal properties, anti-inflammatory properties, anti-diabetes activity, antiparasitic properties, and further studies (drug development and paratransgenic approach) of melittin are included in the review.

Results

Biochemical properties of melittin polypeptide

Melittin is a basic 26 amino-acid polypeptide with a molecular weight of around 2846.4 g/mol and exists as a tetramer in aqueous salt solutions. Disulfide bridges are absent in the MEL structure. The stretch of charged molecules of MEL results in a hydrophobic N-terminal (residues 1–20), while the C-terminal (residues 21–26) is hydrophilic and chemically highly basic (Hossen et al., 2017). The distribution of charged amino acids and amphiphilicity makes MEL water-soluble and more reactive with biological membranes (Dempsey, 1990; Raghuraman & Chattopadhyay, 2007).

Arginine and Lysine amino acid residues affect the physiological pH of MEL (positive six). Similarly, the compound contains a significant amount of hydrophobic amino acids (Dempsey, 1990). The existence of detergent or lipid membranes MEL indicates an α-helical arrangement (Knöppel et al., 1979; Lauterwein et al., 1979; Brown et al., 1980). It converts to a coiled form in a low-concentration aqueous medium (Vogel, 1981; Vogel & Jähnig, 1986) and monomeric. It converts to a tetrameric structure under high ionic strength and higher pH (Hall et al., 2011). Melittin is capable of cell lysis by disrupting cell membranes via pore formation in natural and artificial lipid membranes (van den Bogaart et al. 2008).

Melittin is amalgamated in the secretory glands of the venom channels of both "worker" and "queen" castes of bees. The mRNA responsible for the synthesis of MEL has been isolated from queen bee venom glands. The mRNA produces 70 residual amino acids named pre-promelittin. This peptide sequence contains three main parts: (1) "Pre" sequence (21 amino acids), which leads to the promelittin translocation through membranes. This amino acid sequence consists of a hydrophobic N-terminal peptide sequence (2) "Pro" sequence (22 amino acids) (3) MEL sequence [26 amino acids] (Kreil et al., 1978; Hider, 1988) [Figure 1]. The precursor to product conversion is the presence of dipeptidyl-peptidase IV (DPP4) or Cluster of differentiation 26 (CD26). Also found is either Proline or Alanine in every succeeding region of "pro-segment" in the amino acid sequence of the precursor molecule.

The main biological functions of MEL are hemolytic activity (Habermann, 1972). Other than that, activation of phospholipase A_2, smooth muscle stimulation, blood coagulation reduction, central nervous system stimulation, retreating membrane surface tension and escalations of capillary permeability are other functionalities. Inhibition of H^+-K^+-ATPase, Na^+-K^+-ATPase transport pumps and Ca^{2+}-ATPase are the other extracellular activities (Cuppoletti et al., 1989; Cuppoletti & Abbott, 1990; Cuppoletti, 1990; Mahaney & Thomas, 1991; Voss et al., 1991; Mahaney et al., 1992). Moreover, MEL restrains gene expression related to apoptosis (Tipgomut et al., 2018), generates cytotoxicity in lymphocytes of human peripheral blood (Gajski & Garaj-Vrhovac, 2013), pledgees allergic reactions (Lee & Bae, 2016), and lyses erythrocytes (Tosteson et al., 1985; Alqarni et al., 2018). MEL/lipid and MEL/protein ratios indicate MEL–protein interactions affect the aggregation of membrane proteins, which subsequently result in the immobilisation of bacteriorhodopsin (Hu et al., 1985).
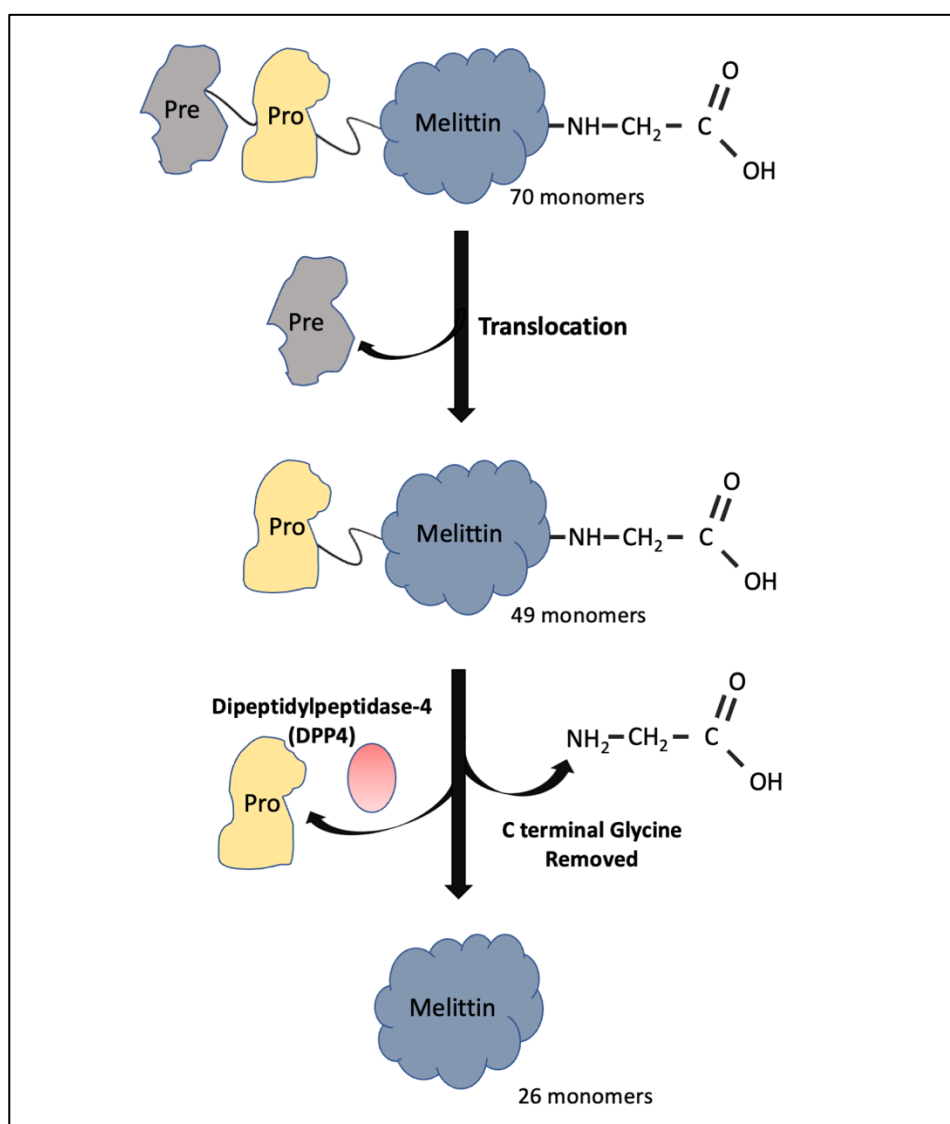
Figure 1: Conversion of pre-promelittin to melittin

Melittin as an antimicrobial peptide (AMP)

Antimicrobial peptides (AMPs) have been widely explored as a treatment for drug-resistant infections, especially as an alternative to conventional antibiotics (Vila-Farres et al., 2015). First basic study on the antimicrobial properties of MEL against a penicillin-resistant isolate of *Staphylococcus aureus* (Fennell et al., 1967)**.** Melittin has shown strong antimicrobial activity in addition to the hemolytic activity and marked allergenic properties (Moreno & Giralt, 2015). Melittin interacts with lipid groups on the cell membrane. This interaction leads to antimicrobial activity and MEL's destruction of red blood cells (Blondelle & Houghten, 1991). Though the MEL is an effective AMP, its use is limited due to cytotoxicity, poor *in vivo* bioavailability caused by volatility, hydrophobicity, and the high cost of production (Soni et al., 2014). Modifying MEL by hybridising with cecropin A or shortening their amino acid sequence leads to low cytotoxicity and higher effectiveness than the unmodified amino acid state (Boman et al., 1989).

The antiviral capacity of the Melittin peptide

The antiviral activity of MEL is well demonstrated in several studies (Baghian & Kousoulas, 1993; Lee et al., 2012; Hood et al., 2013; Uddin et al., 2016). An amphipathic alpha-helical peptide containing 23-Amino Acid (HECATE) analogue to MEL has proven effective against herpes simplex virus-1 (HSV-1). Two mutations of HSV-1 (HFEM and MP) treated with MEL and HECATE completely inhibited the cell fusion and virus spread at 5 µg/ml concentration (IC_{90}). Viral infectivity was reduced by 2 to 28 folds at the multiplicity of infection (MOI) at 0.1 µg/ml without affecting the host cell (Baghian et al., 1997). In another study, two strains of Human Immunodeficiency Virus (HIV-1 NLHX and HIV-1 NLYU2) were exposed to MEL loaded Nanoparticles. It acted with an IC_{50} of 2.4 µg/ml (for HIV-1 NLHX) and 3.6 µg/ml (for HIV-1 NLYU2), and it did not affect the vaginal epithelial cells (Hood et al., 2013). Furthermore, *In-vitro* bioassays indicate that MEL can decrease the relative viral load of SARS-CoV-2 by half at 0.656 µg for MOI = 0.1 (EC_{50}) (Ghalib et al., 2021).

Antifungal mechanisms of melittin

Melittin acts as an antifungal against several fungal species including *Aspergillus fumigatus* (MIC 1.25 mg ∕ L) *Aspergillus parasiticus* (MIC 2.5 mg ∕ L) (Lee et al., 2012), *Fusarium graminearum* (MIC 8 mg ∕ L), *Fusarium moniliforme* (MIC 8 mg ∕ L), *Fusarium moniliforme* (MIC 8 mg ∕ L) and *Malassezia pachydermatis* (MIC more than 20 mg ∕ L) (Kim et al., 2001). In *Aspergillus fumigatus* and *Candida albicans,* melittin binds to the cytoplasmic membranes and alters the polarisation and permeability (Hwang et al., 2010). It leads to toroidal pore formation and induces K^+ leakages through the outer membrane (Choi & Lee, 2014; Hwang et al., 2010; Lee et al., 2002). In *Candida albicans,* melittin exerts antifungal activity by phosphatidylserine externalisation on the outer membrane (Chen et al., 2019; Conrad et al., 2018; Park & Lee, 2010), while in *Saccharomyces cerevisiae,* it alters the gene expression by DNA fragmentation in the nucleus. Those DNA fragments mostly control fungal membrane organisation, ribosome biogenesis, RNA processing and genetic repairing (López-García et al., 2002).

Melittin supports the increase of reactive oxygen species (ROS) in *A. flavus, A. fumigatus* and *C. albicans* (Conrad et al., 2018; J. et al., 2014; Moore et al., 2019) which leads to Calcium ion (Ca^{2+}) release from the endoplasmic reticulum (ER) and disruption of mitochondrial membrane potential. Both effects lead to mitochondrial Ca^{2+} overload, cytochrome c release, meta-caspase, and apoptosis (Lee & Lee, 2014). Further, MEL inhibits (1,3)-β-D-glucan synthase that is involved in the generation of beta-glucan in *A. fumigatus* (Lee, 1996), which is the main component of the fungi cell wall (Campbell et al., 1997). However, it has been reported that the fungicidal effect of MEL on different species, such as *Penicillium digitatum*, *S. cerevisiae*, *A. fumigatus* and *C. albicans*, is dependent on the tie of exposure and concentration (Muñoz et al., 2006; Lee et al., 2012b; Choi et al., 2013).

Use of melittin in cancer therapy

Cancer is one of the major non-communicable diseases affecting humans worldwide (Gajski & Garaj-Vrhovac, 2013). The available treatment approaches for cancer are surgery, radiation therapy, chemotherapy, gene therapy and/or hormonal therapy (Lai et al., 2012; Adhami et al., 2014). There is a growing demand for alternative therapeutics against cancer due to the side effects and unexpected outcomes of these mainstream methods. Melittin has been recognised as an alternative or complementary therapy for human cancers (Park & Lee, 2010; Park et al., 2014; Yang et al., 2014; Moreno & Giralt, 2015). Cytotoxicity on different cell types, such as effect on cell proliferation, cell cycle, growth inhibition and induction of apoptosis inhibition of angiogenesis (Gajski & Garaj-Vrhovac, 2013; Moreno & Giralt, 2015; Park & Lee, 2010; Shin et al., 2013; Yang et al., 2014). Renal cancers, lung cancers, liver cancers, prostate cancers, bladder cancers, breast cancers, and leukaemia can be

targeted by MEL (Moreno & Giralt, 2015). Melittin is known to activate caspases (Tu et al., 2008; Wang et al., 2009), death receptor-induced apoptotic cell death pathways (Jo et al., 2012), and induce apoptosis through downregulating signal pathways (Moon et al., 2008). Cell cycle arrest and angiogenesis are mainly achieved by the downregulation of metabolic pathways (Jeong et al., 2014; Shin et al., 2017)

In the later stages of the cancer progression, the tumour cells invade other tissues, further complicating the situation (Park et al., 2014). The evidence suggests that MEL affects the systemic migration of tumour cells by inhibiting metastasis mechanisms. A study on mice and in tumour cell cultures proposes that intravenous administration of MEL may inhibit metastases of mammary carcinoma cells to the lung (Orsolić et al., 2003). Melittin is also known to impact tumour invasion via the downregulation of the HIF-1α/VEGF, which is involved in tumour progression in human cervical carcinoma CaSki cells (Shin et al., 2013; Yang et al., 2014). This is achieved via the inhibition of extracellular signal-regulated protein kinases (ERK) and mTOR/p70S6K pathways (Shin et al., 2013) and by shortening the half-life (Shin et al., 2013).

As melittin has non-differentiated cytotoxicity, to achieve its therapeutic potential, a proper delivery mechanism such as MEL nanoparticles, which are capable of intravenously delivering a substantial quantity of MEL and targeting and executing specifically the cancer cells, is essential (Gajski & Garaj-Vrhovac, 2013).

Anti-inflammatory potential of the melittin peptide
Chronic inflammatory diseases
Several studies describe the anti-inflammatory mechanisms of MEL in different types of disease models (Lee & Bae, 2016). Though the MEL may cause itching, inflammation, and local pain in high doses, it produces anti-inflammatory effects in small doses (Raghuraman & Chattopadhyay, 2007). Furthermore, MEL suppresses signal pathways of nuclear factor kappa(κ)-B essential modulator (NEMO), Cluster of differentiation 14 (CD14), platelet-derived growth factor receptor beta(β) (PDGFRβ), toll-like receptors 2 (TLR2) and toll-like receptors 4 (TLR4). Inhibiting these pathways results in decreased inactivation of p38, extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), protein kinase B (PKB), phospholipase C gamma (γ) 1 (PLCG1), as well as translocation of nuclear factor kappa(κ)-light-chain-enhancer of activated B cells (NF-κB) into the nucleus. Finally, those inhibition pathways reduce inflammation in the skin, capillaries, joints, liver, and neuronal tissues (Lee & Bae, 2016).

Atherosclerosis
Atherosclerosis is a chronic inflammatory disorder of the arteries, one of the major causes of death in adults (Kobiyama & Ley, 2018). A plaque formation inside the artery is constructed of remnants of dead cells, immune cells, cholesterol, and triglycerides (Sloop et al., 1999). Helper T cells and macrophages are inflammatory immune cells recognised as the main components of producing inflammatory cytokines. Inflammatory cytokines are important in plaque growth (Sloop et al., 1999). The signalling of NF- κB is important in cell proliferation and apoptosis. Manipulation of the NF-κB signal leads to treatment for atherosclerosis as a potential drug target (Kucharczak et al., 2003; Pamukcu et al., 2011). Furthermore, tumour necrosis factor α (TNF-α) stimulates apoptosis and proliferation of vascular smooth muscle cells in the existence of MEL *in-vitro* (Cho et al. 2013: Son et al. 2007) and overturns atherosclerosis of mice *in-vivo* (Kim et al., 2011).

Skin inflammation

Acne vulgaris is caused by *Propionibacterium acnes*, a long-term skin disease of hair follicles in the face and upper trunk (Leyden, 2001). Antibiotics and other medicines have side effects in inflammation suppression of acne vulgaris (Aslam et al., 2015). It is important to overcome the side effects of anti-inflammatory drugs. Melittin-induced apoptosis and inflammation in human THP-1 monocytic cells and MEL treatment attenuated the increased phosphorylation of nuclear factor κb kinase (IKK), NF-κB, nuclear factor kappa B inhibitor (IκB), and p38 by heat-killed *P. acnes* in Cultured Human Keratinocyte (HaCaT) cells (Lee et al., 2014).

Liver inflammation

Melittin prevents thioacetamide (TAA)-induced liver fibrosis by inhibiting liver inflammation and fibrosis, the mechanism of which is the interruption of the NF-κB signalling pathway (Park et al., 2011). In a subsequent study, results indicate MEL-protected galactosamine (GalN)/lipopolysaccharide (LPS)-induced acute hepatic failure through the inhibition of inflammatory cytokines and apoptosis (Park et al., 2012). Furthermore, MEL possesses a potent suppressive effect on apoptotic responses in TNF-α/Actinomycin-D (Act D)-treated hepatocytes via the NF-κB pathway (Park et al., 2014)

Neurodegenerative diseases

In-vitro assays have shown the capability of MEL in the prevention of neurodegenerative diseases (Lee & Bae, 2016). Moon *et al.* suggested that MEL may potentially treat neurodegenerative diseases accompanied by microglial activation by its potent oppressive effect on proinflammatory reactions of BV2 microglia cells (Moon et al., 2007).

Progressive neurodegenerative disorder, Amyotrophic lateral sclerosis (ALS), is a disease that affects motoneuron in the central nervous system (CNS), resulting in weakness and progressive degeneration (shrinkage or nerve tissue) of muscles (Zarei et al., 2015). A previous study has shown that MEL treatment improves the anti-neuroinflammatory ability of proteasomes in the CNS of ALS model mice (Yang et al., 2011). Another study has explored the pharmacological effects of MEL in mice, specifically on dopaminergic-related behaviours (Dantas et al., 2014). Melittin shows antipsychotic properties with fewer side effects than neuroleptic medicines. It also has the potential to be used in psychotic disorder treatments (Lee et al., 2016).

Arthritis therapy

Any disorder that causes swelling or inflammation of joints of the human body is defined as Arthritis (CDC, 2019). Transcriptional factor p50 (NF-kB1) is important in immune response, cell proliferation and immune response (Yu et al., 2009). Melittin and bee venom directly bind to the p50 subunit and inactivate NF-κB. This suggests a possible antiarthritic mechanism. The transcription of the inflammatory gene is downregulated by inhibiting LPS-induced p50 translocation into the cell nucleus (Hye et al., 2004).

Potential use of melittin peptide as an anti-diabetic agent

Melittin acts on several metabolic pathways and significantly lowers blood glucose and lipid levels, as demonstrated by animal models (Hossen et al., 2017). It increases the insulin excretion from the β-cells (of the pancreas) and enables glucose uptake to reduce blood glucose levels (Morgan & Montague, 1984). Furthermore, it alleviates complications of diabetic Mellitus by ameliorating lipid profiles. Melittin moderates blood glucose levels by various mechanisms involving depolarisation of β-cell membranes, accumulating the extracellular calcium concentration with stimulating calcium channels (Simonsson et al., 2000; Ts et al., 2015), activating cytosolic phospholipase A2 (Heisler, 1989),

increasing glucose transporter lipid uptake into adipose tissues (Khulan et al., 2016), and suppression of β-cell inflammation (Park et al., 2008)

Antiparasitic properties and potential applications of the melittin peptide

Insect-borne diseases are responsible for severely affecting human life around the world, causing substantial morbidity and mortality (Coutinho-Abreu et al., 2010). According to the World Health Organization, vector-borne diseases caused by viruses, bacteria, and parasites cause more than 700,000 deaths and over 1 billion cases, which is 17% of all infectious diseases (WHO, 2020). Protozoan infections endanger the lives of almost one-third of the world's population (Memariani & Memariani, 2021). Malaria, visceral leishmaniasis (kala-azar), Chagas disease (American trypanosomiasis), and sleeping sickness (African trypanosomiasis) are still debilitating to human life, especially in tropical and sub-tropical regions (Laura Sbaraglini et al., 2016; Norman et al., 2020). According to the available literature, the interest in exploring the biological effects and modes of action of MEL against different protozoan parasites has received more attention.

The first evidence of anti-protozoan activity of the MEL was documented in a study carried out in the late 1980s in which the MEL was shown to be cytotoxic to trypanosomatid protozoan parasites (Azambuja et al., 2005). However, the Antiparasitic activity of MEL has been proven to kill different parasites such as *Leishmania*, *Plasmodium*, *Toxoplasma*, and *Trypanosoma in-vitro* (Memariani & Memariani, 2021). The primary mechanism of action of MEL identified as an anti-protozoan agent is the direct membrane-disruptive activity (Memariani & Memariani, 2021). Furthermore, the ability to destabilise calcium homeostasis reduces mitochondrial membrane potential, disorganises the kinetoplast DNA, instigates apoptotic cell death, and induces autophagy in protozoan pathogens (Memariani & Memariani, 2021). Here, we have summarised the potential applications of the MEL peptide as an antiprotozoal agent against different groups of parasites.

Leishmania **parasites.**

Leishmaniasis is one of the main vector-borne diseases caused by unicellular parasites of the genus *Leishmania,* which is transmitted by sand flies (WHO, 2019). *Leishmania* parasites have a dimorphic life cycle between an invertebrate vector and a mammalian host. The pathogen exists as extracellular flagellated promastigotes within the digestive tract of the sand fly, and intracellular nonmotile amastigotes live and proliferate inside the host's phagocytes (Steverding, 2017).

Melittin has been explored for its anti-leishmanial properties in several studies using several *Leishmania* species. The very first study found in 1998, for the R9 strain of *L. donovani,* was performed using an MTT assay to analyse the viability of promastigote cells and found a high killing activity of MEL against promastigote (LD$_{50}$:0.3µm) (Díaz-Achirica et al., 1998). In another study, promastigotes of *L. donovani* S-2strain were tested by evaluating Calcium influx by fluorescence measurements. They found a dose-dependent induction of Calcium influx across the plasma membrane and inhibition of MEL-induced calcium influx by 3-(4-octadecyl)-benzoylacrylic acid (Catisti et al., 2000). It was reported that MEL could modulate Th1 and Th2 immune responses in Swiss Cluster of Differentiation 1 (CD1) mice upon *Leishmania* infection. MEL-adsorbed attenuated *Leishmania* loping anti-leishmanial vaccine (Memariani & Memariani, 2021). Furthermore, the direct inhibition of both amastigotes and promastigotes and indirect inhibition of intracellular amastigotes of *Leishmania infantum* by immunomodulatory effects on macrophages by MEL were also evidenced (Pereira et al., 2016). The observation that MEL improves the immune effect against *Leishmania* spp. is also consistent with other studies.

Pérez-Cordero et al. performed a study for promastigotes of both *L. major* and *L. panamensis* to assess the Cell viability against MEL using microplate Alamar blue assay. He describes the key findings of the study as induction of death in 50% of *Leishmania significant* promastigotes at 74.01 ± 1.27 μg/mL and the ineffectiveness of MEL in killing 50% of *L. panamensis* promastigotes at > 100 μg/mL (Pérez-Cordero et al., 2011).

Anti-malaria activity by melittin.

Plasmodium spp. are the causative agents of Malaria, which is a hazardous mosquito-borne disease that inflicts a massive burden in many tropical countries (Talapko et al., 2019). During the past 20 years, 1.5 billion malaria cases and 7.6 million deaths related to Malaria have been reported around the globe. Furthermore, 229 million new cases have been reported in 2019, and among them 490, 000 succumbed to death (WHO, 2021). *Plasmodium falciparum, P. malariae, P. ovale, and P. vivax* species have been considered parasites of humans causing Malaria (Memariani & Memariani, 2021).

Plasmodium berghei is a frequently used species for human malaria studies (Goodman et al., 2013). A study was carried out by Carter et al. for *the Plasmodium berghei* ANKA strain. It analysed the effects of MEL on ookinetes (*in vitro*) and sporogony stages (*Anopheles stephensi*) of the parasite, where they found a complete obliteration of ookinetes after 30 min, a significant reduction in both infection prevalence and infection intensity compared to those in control mosquitoes (Carter et al., 2013). In the same study, an analysis of the effects of MEL on the sporogony stages of *Anopheles gambia*e was done using Gametocytes of *P. falciparum* NF54 strain and found significant decrements in both infection prevalence and infection intensity compared to those in control mosquitoes (Carter et al., 2013).

Another study in which again the Gametocytes of *P. falciparum* NF54 strain were used to analyse the effect of MEL and multi-MEL arrays on sporogony stages of *Anopheles coluzzii* revealed that there is a significant reduction of infection intensity in mosquitoes fed on cultured *P. falciparum* spiked with MEL (native or modified peptide) compared to those in control mosquitoes (Habtewold et al., 2019). In this context, there is a promising place for transgenic mosquitoes that express MEL to control Malaria.

Toxoplasma spp

Toxoplasma gondii, the causative agent of toxoplasmosis, is an obligate intracellular protozoan parasite (Portes et al., 2020). Felids (mammals in the cat family) are known to be the only definitive hosts and intermediate hosts, including birds, mammals and, possibly, fish. Human *T. gondii* seroprevalence is high and is associated with hazardous health outcomes among the Inuit population in Canada (Francia et al., 2016; Reiling & Dixon, 2019). One-third of the world's population who are infected with *T. gondii* remain asymptomatic, whereas immunodeficient individuals manifest severe disease, which may result in chronic complications such as blindness or neurological abnormalities (Flegr et al., 2014). Although toxoplasmosis is self-limiting, severe or persistent disease is treated with a combination of drugs such as pyrimethamine and sulfadiazine, which inhibits parasite folate metabolism (Rajapakse et al., 2013). Though there is a lack of effectiveness of these medications may be due to the long-term treatment and risk of relapsing (Alday & Doggett, 2017), alternative therapies have yet to be explored.

One study revealed that MEL induces cytosolic β-galactosidase release and cell lysis of extracellular tachyzoites of lacZ transgenic strain of *Toxoplasma gondii* (RHβ strain) (Seeber, 2000) This finding suggested that MEL directly eradicates extracellular *T. gondii* tachyzoites through disruption of plasma membrane integrity. However, further research is required to assess the effects of MEL to develop as a better therapeutic option.

Trypanosoma spp.

Trypanosoma brucei is the causative agent for Human African trypanosomiasis (HAT) or sleeping sickness, which is a life-threatening insect-borne disease found in rural parts of sub-Saharan Africa, where it is transmitted by the bite of Tsetse fly (Bukachi et al., 2018). Two subspecies of *T. brucei* are responsible for the disease in humans, namely *T. brucei gambiense*, which gives rise to slow-onset chronic illness in western and central Africa, whereas *T. brucei rhodesiense* is responsible for the more acute form of HAT in southern and eastern Africa (Malvy & Chappuis, 2011). Only four medications are currently registered for treating early- and late-stage HAT: Pentamidine, Suramin, Melarsoprol, and Eflornithine. Nifurtimox is another medication used in combination with Eflornithine for the second stage of HAT due to *T. brucei gambiense* (Malvy & Chappuis, 2011).

Several studies have shown convincing evidence that MEL rises Calcium ion concentration ($[Ca^{2+}]$) in *Trypanosoma brucei brucei* (Ruben et al., 1996; Xiong et al., 1997; Ridgley et al., 1999; Catisti et al., 2000). Initial experiment on melittin-induced Ca^{2+} influx across the parasite plasma membrane (Ruben et al., 1996). According to the results, MEL had no impact on intracellular $[Ca^{2+}]$ in a buffered salt solution comprising 3 mM ethylene glycol-bis (β-aminoethyl ether) - N, N, N′, N′-tetraacetic acid (EGTA; for chelating the extracellular Ca^{2+}). However, the peptide caused an elevation of intracellular $[Ca^{2+}]$ in the same solution containing 2 mM Ca^{2+}. This observation is supported by another study using Mn^{2+} quench experiments on *T. brucei. brucei* cells loaded with calcium-sensitive dye Fura-2 (Catisti et al., 2000).

It has been identified that most of the Ca^{2+} that entered the cell across the plasma membrane or was liberated from the acidocalcisome transiently accumulated into mitochondria during the signalling process induced by MEL. In response to MEL, the acidocalcisome is thought to maintain Ca^{2+} homeostasis (Xiong et al., 1997). Furthermore, exposure of *T. b. brucei* procyclic trypomastigotes to MEL in a Ca^{2+}-free medium led to an appreciable increase in intracellular $[Ca^{2+}]$ (Catisti et al., 2000).

Trypanosoma cruzi is the causative agent of Chagas disease or American trypanosomiasis, which was previously considered confined to Latin America and more recently became a neglected worldwide disease with a high morbimortality rate and associated social impacts. The primary route of transmission of the disease is via contamination of the bite site or intact mucous membranes by infected Triatomine bug faeces and, less commonly, via blood transfusions, organ transplantation, and transplacental transmission can occur. Congestive heart failure, oesophagal dilatation, and enlargement of the colon are serious complications associated with the chronic disease (Bern, 2015). Though both available medications exhibit significant adverse effects and reduced effectiveness in adults, Benznidazole and nifurtimox are the only medications with proven efficacy against Chagas disease.

Several studies with evidence state the antiparasitic effects of MEL on *T. cruzi* (Memariani & Memariani, 2021). The Ca^{2+} influx by fluorescence measurements and found inhibition of MEL-induced Ca^{2+} influx by OBAA (Catisti et al., 2000). Another study was performed using Trypomastigotes of *T.cruzi* macro phagotrophic Tehuantepec strain by light, fluorescence, and electron microscopies, evaluation of trypanocide activity, and measurement of β-galactosidase release (before and after parasitic infection). Though there was inhibition of the parasite motility, disruption of the plasma membrane and reduction of the parasite infectivity, a reduction in the growth of intracellular parasite was not found. Investigation on the determination of lethal concentration, evaluation of T. *cruzi* killing by dual peptide treatment, and recovery of AMP-treated cells after transfer to non-AMP containing media came up with the findings of high killing activity against *Trypanosoma cruzi*, synergistic and additive antiparasitic effects of MEL in combination with certain AMPs and the inability

of the parasite to recover after treatment with 10 μM of MEL. Another two subsequent studies were done by Adade et al. using the CL Brener clone of *T. cruzi* to evaluate the parasite viability (Adade et al., 2013; Adade et al., 2012). Mwllitin induced a dose-dependent decrease in the number of T. *cruzi* cells, increased permeabilisation of the protozoan cell membrane (High percentages of PI-labeled epimastigotes and trypomastigotes) and inducted autophagy (epimastigotes) and apoptosis (trypomastigotes) (Adade et al., 2012).

The subsequent study found MEL has effects on induction of growth inhibition or killing of developmental forms of the parasite (< 2.5 μg/mL), induction of structural changes (plasma membrane blebbing, mitochondrial swelling, and nuclear alterations), induction of alterations in mitochondrial membrane potential ($\Delta\Psi_m$), disorganisation of the kinetoplast DNA filaments, induction of alterations in flagellar structure, permeabilisation of the cell membrane and induction of apoptosis and autophagy (Adade et al., 2013). According to these study findings, MEL, either alone or combined with other effector molecules, would seem to be a promising nominee for use in novel techniques to control Chagas disease transmission.

Discussion

Melittin, the principal component of European honeybee venom, has garnered significant attention in recent years due to its diverse applications in various fields, particularly in antiparasitic, antimicrobial, and therapeutic approaches. This peptide, known for its potent cytolytic properties, has demonstrated promising potential in developing novel strategies to combat parasites and infectious microorganisms.

One of the notable applications of melittin lies in its antiparasitic properties. Research have shown that melittin exhibits significant activity against various parasites, including protozoa and helminths. Its ability to disrupt the integrity of the parasitic cell membrane makes it a promising candidate for developing antiparasitic drugs. Biological control approaches against parasitic diseases have also been shown to be more effective, including vector transgenesis and paratransgenesis, which are novel strategies that have been explored toward reducing vectorial capacity and eliminating pathogen transmission (Coutinho-Abreu et al., 2010). In paratransgenesis, genetically modified insect symbionts are used to express antimicrobial molecules within the vector that are harmful to the pathogens they transmit (Ogaugwu & Durvasula, 2017).

In the paratransgenic approach, several antimicrobial peptides (AMPs) have been explored as effector molecules (Mylonakis et al., 2016; Giovati et al., 2018). The major challenge of such antimicrobial compounds is their efficacy against parasites and their detrimental effects on the symbiotic bacterium. However, MEL has obtained the attention of researchers as an effector molecule to use as an antiparasitic agent due to the proven efficacy of MEL in killing different protozoan parasites such as *Leishmania*, *Plasmodium*, *Toxoplasma*, and *Trypanosoma in-vitro* (Memariani & Memariani, 2021; Wijerathna et al., 2020). Furthermore, the ability to secrete this compound in an inactive form and activate it later makes this a good solution for having detrimental effects on the symbiotic bacterium. The bacterium will produce the inactive version that maintains a buildup without affecting themselves. Once a blood meal with parasites enters the insect gut, the compound will become active against parasites.

In addition to its antiparasitic potential, melittin has demonstrated remarkable antimicrobial activity. Its membrane-disrupting properties make it effective against a broad spectrum of bacteria, including both Gram-positive and Gram-negative strains. Melittin's ability to selectively target bacterial membranes

while sparing host cells makes it an attractive option for combating drug-resistant bacterial infections. The antimicrobial activity of melittin has been explored in various contexts, ranging from wound healing to the development of alternative antimicrobial agents.

Furthermore, melittin shows promise in therapeutic applications beyond its antiparasitic and antimicrobial properties. Research suggests that melittin may have anti-inflammatory and anticancer effects. Its ability to modulate immune responses and induce apoptosis in cancer cells opens avenues for developing therapeutic interventions against inflammatory diseases and certain types of cancer. However, further research is needed to fully understand the mechanisms and optimise melittin's therapeutic potential.

Drug development has several steps, starting from basic scientific discovery followed by preclinical and clinical studies (Ator et al., 2006; Mohs & Greig, 2017; Gooneratne, 2019). Furthermore, regulatory reviews are also required once the drug is approved to be used. Melittin has considerable potential as a drug against a multitude of diseases. However, most studies are still at the laboratory level and have yet to proceed beyond *in vitro* experimentation. Thus, it needs to proceed through several steps before being available as a drug. Future studies should be focused on *in vivo* studies and preclinical and clinical level assessment of this compound against the diseases mentioned herein.

Another major caveat for the use of MEL as a drug against non-communicable diseases is the difficulty in transporting the specific target site with a high level of specificity (Son et al., 2007). For instance, the use of MEL in a diabetic is majorly hindered by the challenges in subcutaneous drug delivery (Hossen et al., 2017; Shimpi et al., 2016). The same applies to cancer therapy, as the precautions for non-specific cytotoxicity must be avoided through specific drug delivery (Mosmann, 1983). Therefore, it is paramount to focus on developing a secure drug delivery mechanism without any adverse effect on non-target tissues such as skin or healthy cells.

Forming biofilms on the inner and outer surfaces of surgical equipment, glass surfaces, contact lenses, and other surfaces that may cause health concerns is one of the major challenges faced by medical practitioners and manufacturers of such equipment. Melittin stands out as a reliable and effective antimicrobial peptide to be used against biofilm formation due to its higher activity. The possibility exists for the use of this peptide as a coating in the manufacture of contact lenses (Hui, 2017; Musgrave & Fang, 2019). This could be further extended to be used for medical equipment as well.

Silico experimentations have emerged as one of the most effective techniques for understanding the functional potential of molecules. Furthermore, numerous algorithms and machine learning methods in modelling molecular-level processes have shown significant progress in the last few years. The functional potential of MEL could be further investigated using such advanced methods more cost-effectively.

Cytotoxicity, genotoxicity, allergic reactions, and size of the peptide are major disadvantages of MEL, as described below. Computational chemistry, polymer science, and nanotechnology help find new drug delivery techniques to a specific location in humans to overcome major disadvantages. Perfluorocarbons (PFC) demonstrate promising results as a drug releaser in cancer therapy. The size of PFC and MEL conjugation (100-200 nm) makes feasible drug delivery in anticancer treatments (Lee et al., 2011; Matsunaga et al., 2012). Correspondingly, combining MEL with enzymes makes it possible to construct even smaller (12 nm), less hemolytic activity and less cytotoxic conjugate of MEL (Matsunaga et al., 2012; Luo et al., 2013; Jallouk et al., 2015). Most importantly, MEL transporting

polymer should not affect other organs (such as the liver and kidney) in the human body. Further studies on MEL-delivering polymers should focus on the nephrotoxicity and hepatotoxicity of the encapsulate. Furthermore, the integration of MEL with nanoparticles is likely to show better results in most of the aspects in which MEL is used, especially in antimycotic medication (Adade et al., 2012; de Cesare et al., 2020) and in its use as a fungicidal spray in agriculture (Keymanesh et al., 2009; Badosa et al., 2009). However, the available knowledge is purely based on *in vitro* studies. Therefore, further studies are required to confirm these effects.

While the applications of melittin are promising, challenges such as potential toxicity and the need for targeted delivery systems must be addressed for successful clinical translation. Nevertheless, the multifaceted properties of melittin position it as a versatile candidate for developing innovative solutions in parasitology, microbiology, and therapeutics. In conclusion, melittin, the major pain-producing component of European honeybee venom, transcends its initial role in envenomation by offering diverse applications in antiparasitic, antimicrobial, and therapeutic contexts. Exploring melittin's unique properties holds the promise of addressing critical challenges in medicine, ranging from parasitic infections to antibiotic-resistant microbes and even cancer. As research in this field advances, the transformative potential of melittin may pave the way for innovative and effective approaches to combat various diseases and improve human and animal health.

Conclusion

The current work reviews a wide range of therapeutic and disease-control applications of MEL. Opportunities exist for further development with more studies, especially about antipathogenic potential. The ability to be secreted in an inactivated form makes it an ideal candidate for paratransgenesis-based disease control. Furthermore, studies beyond *in-vitro* studies are required before the therapeutic application of MEL against other non-communicable diseases. With appropriate studies to fill the existing knowledge gaps, MEL could be utilised in many areas with promising results.

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