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# Discussion of Melittin in Cardiovascular Treatments

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## ABSTRACT

In this research paper, we will go over details about melittin peptide, a cationic peptide that was derived from bee venom and is now being used in medicine to cure diseases. We will go over how it was derived, some of its uses in medicine, how it is helping in cardiology, and how it could affect the future of medicine. Recently, there has been a need for a more advanced level of treatment in cardiology, as it is one of the leading causes of death in the United States. That is where melittin is coming into play. Scientists are testing the molecule's efficacy of resolving issues in the heart by simulating those issues on mice. They then used melittin to see whether it would cure those complications, and they continually repeated those trials. Each time, they were able to see that melittin was able to remove those complications by using methods such as communicating with signaling pathways or even destroying certain molecules by itself.

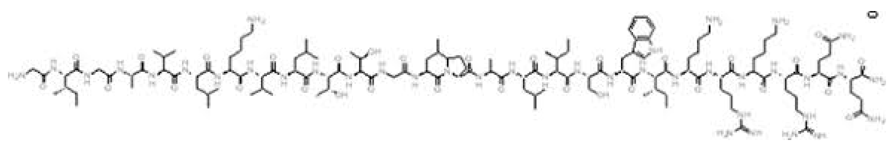

**Figure 1:** This is a model of a melittin molecule.

## Introduction

In today's world, there are more than 500 million people who are suffering from issues related to the heart. There have been many treatments in the world, but there has been an interesting development in a molecule that has been widely acknowledged, called melittin peptide. Melittin peptide is a cationic peptide that has 26 amino acids and is derived from honey bee venom. It has been used as a form of treatment for many complications, such as diabetes and viruses, but now, it is being used to help treat cardiovascular issues. This research is mainly intended to help spread more awareness of this type of molecule to be used in cardiology and it will also address other types of issues, including the vulnerability of the molecule being broken down due to temperature. In addition, the molecule in general can vary from different insects and as a result, it can affect some of the results. In this research, we are only going to study the most common type of melittin peptide, and that is the one derived from honey bee venom. This research will answer how melittin peptide can make treatments available to more people and how it can be used to advance treatments for people with issues relating to the heart. Research shows that some people have tried to test the effects of melittin peptide on the heart by simulating issues in mice's hearts. They injected CVB3 into their hearts to try to simulate viral myocarditis, and when they injected the peptide into the mice, they found that there were positive effects due to the molecule; what the peptide had done was initiate myocardial cell apoptosis, which is the death of cells responsible for myocarditis (*Melittin Ameliorates CVB3-induced Myocarditis via Activation of the HDAC2-mediated GSK-3β/Nrf2/ARE Signaling Pathway*, 2016). Throughout this research, more implications for this molecule will be explored and discussed.

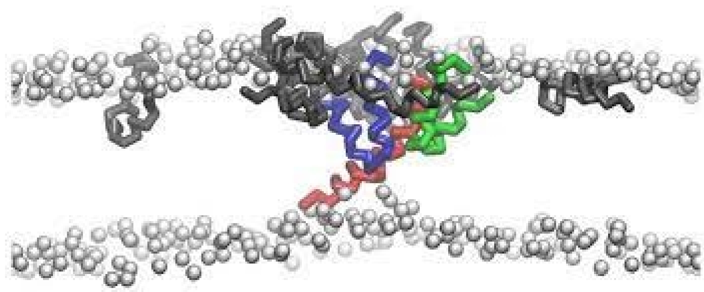

**Figure 2:** This is how melittin inserts itself into a cell membrane.

## Overview of Melittin Peptide

Melittin peptide is originally derived from bee venom and is primarily responsible for the activation of nociceptor cells, which is how pain is generated. This special type of venom is found and produced in the bee species *Apis mellifera L*. When those honey bees sting someone, they release melittin, which causes the red blood cells under your skin to undergo lysis. As a result, the skin also swells and it gives it a burning and itching sensation. It was originally discovered in 1970 when scientists were trying to study the composition of the venom of *Apis mellifera*. As stated before, this molecule is mainly constructed out of 26 amino acids, which include 6 positively charged residues of some amino acids. Since lysis results from when the peptide is introduced into an organism, it is also responsible for helping venom spread throughout the bloodstream of an organism. There are only two derivatives of this molecule because there are a lot of organisms that have melittin present in them. One of them is melittin-S, which is mainly found in African bees. The difference between that derivative and regular melittin is that one of the amino acids is replaced by the “Ser” amino acid; it still contains 26 amino acids and still has generally the same functions, but they will be amplified because of that new amino acid that replaced the old one. The second derivative of melittin peptide is called melittin-F. The reason why this derivative is different compared to melittin is that it only has 19 amino acids compared to the 26 in the regular melittin. However, that is basically the only significant difference between regular melittin and melittin-F. When looking at the chemical structure of melittin peptide, it is arranged in the pattern of amino acids that goes like this: Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln. The H and NH2 are used to signal the start and end of the amino acid chains.

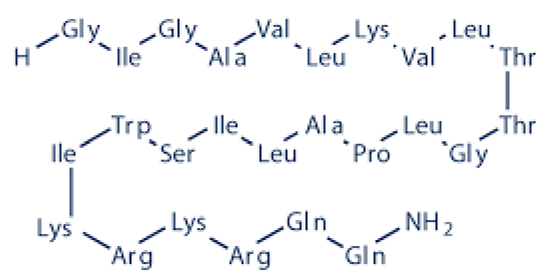


**Figure 3**: This is a picture of melittin’s chemical structure.

## Uses in Medicine

Recently, melittin has been acknowledged to be very helpful in therapeutic situations. Due to its quality of helping substances diffuse throughout the bloodstream, it could possibly help in diffusing substances to help cure any complication faster. There was an article published on PubMed that talked about a study that tried to prove this assumption.

The group of scientists tested this by injecting NADPH oxidase 4 into a group of mice's vascular smooth muscle cells (*NADPH Oxidase 4 Regulates Vascular Inflammation in Aging and Atherosclerosis*, 2016). NOX4 was primarily responsible for inhibiting vascular inflammation, atherosclerosis, and more. After they introduced NOX4 into the mice, they then injected a peptide that was obtained from melittin, and it resulted in a higher transfection rate and decreased cellular toxicity. For comparison, they used Lipofectamine, which was another type of transfection reagent, and they found that it had a lower efficiency rate than that of melittin peptide. Melittin peptide is used in many fields of medicine because many of the amino acids inside are important and can help actually attack those diseases and potentially cure them altogether. One important field of medicine that the peptide is being used in is oncology; it is useful in helping to cure cancers because those amino acids can target cancer cells and shut them down (Oršolić, 2012). They do that by punching holes in cell membranes so they lose their shape and all of their essentials, resulting in the death of those cells. Another field of medicine that melittin is popular is dermatology. There was a study that was conducted to try to find out just how effective melittin would be at trying to help cure acne (Pak, 2013). They collected a sample of skin cells with acne on it and they wanted to inject it with some purified bee venom, which is where the majority of melittin is found. The results of this study showed that there was a decrease in the number of acne on the skin cell sample which was placed in an agar. In addition, when using cosmetics with the purified bee venom, the amount of adenosine triphosphate also decreased in the cell by about 57.5%, which is a large difference compared to when cosmetics are used without any sort of melittin, which showed a 4.7% decrease in ATP. The reason why ATP is important here is that it helps in skin-moisturizing, but at the same time, it tries to do that by using natural oils that are produced in the skin. Due to that, if it lingers for too long before the person washes the oil off, it can then give way to generating acne. That is why it is important for the ATP to be kept at low amounts so the right amount of natural moisturization is still present. Another important field melittin is being used in is neurology. Going back to the issue of oxidative stress, it is still unclear as to how exactly melittin is actually helping neurological cells, but there has actually been a study to try to find out how effective melittin is at trying to cure Parkinson's disease, which is a neurological issue that is mainly caused because of a dopamine deficiency, among other symptoms. In the study, they wanted to see whether the cells that were responsible for the loss of those neurotransmitters would be stopped and removed by melittin, and the results confirmed that it was so. Bee venom has been known to be a very common substance where melittin is present, and as demonstrated by these studies, melittin has been proven to be an exceptionally effective molecule. However, there are still a few areas where melittin peptide is fallible.

## Shortcomings

One area where this molecule could be skeptical is its quality consistency. The reason why is that there are many different species of bees present in the world, and they have variations in their venom's chemical structures. Although *Apis mellifera L* is one of the only species in the world that has melittin present in adequate amounts, there could still be variations between different species. There was a study conducted by a group of scientists whose main goal was to try to find the differences in melittin content between four different species of honey bee, which are *A. dorsata, A. mellifera, A. florea,* and *A. cerana* (Pak, 2013). According to the data, the *A. dorsata* species had the highest levels of melittin peptide followed by *A. mellifera, A. florea,* and *A. cerana.* Looking at the actual numbers, they were measured as the percent of melittin content in their venoms, and they are shown in the graph below. There were slight discrepancies in the numbers, but they were minor as their average margin of error was around 2%.

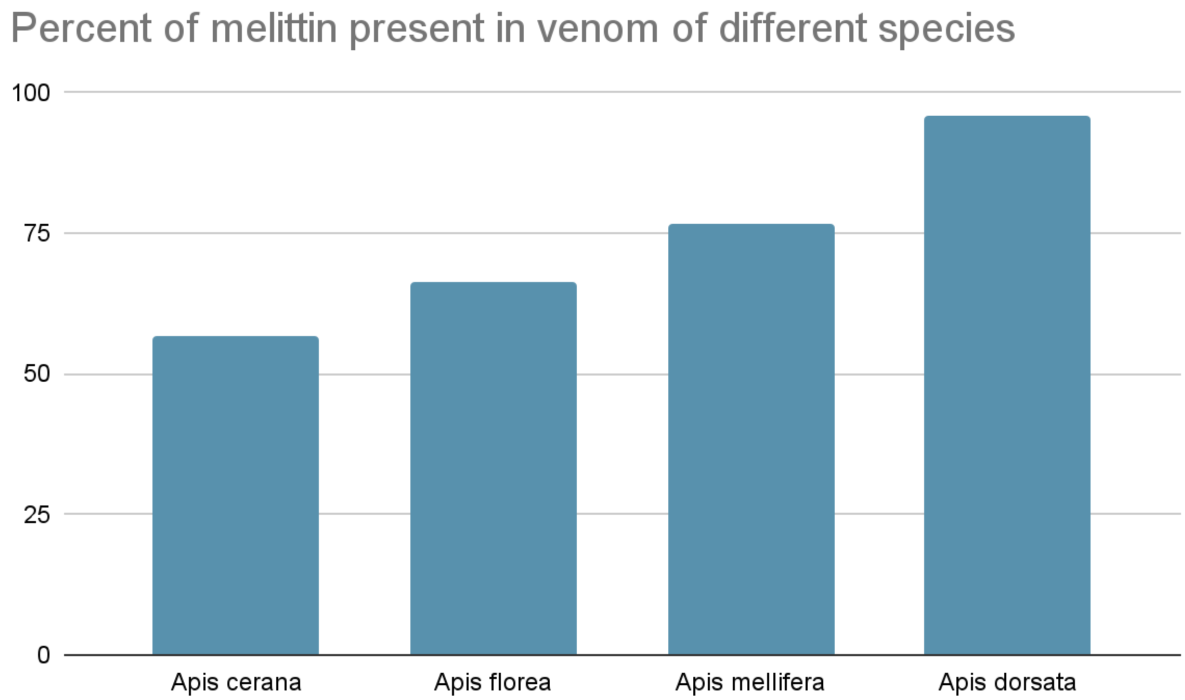


**Figure 4:** This graph highlights the comparison between the percent of melittin present in the four different species.

| Species | Percent melittin present in venom |
|---|---|
| Apis dorsata | 95.8 ± 3.2% |
| Apis mellifera | 76.5 ± 1.9% |
| Apis florea | 66.3 ± 8.6% |
| Apis cerana | 56.8 ± 1.8% |

**Figure 5:** This graph gives more precise data given in the graph along with margins of error.

In addition, scientists have also found that the actual content in the bee venom to be different from those four species. They tried measuring four aspects of the bee venom of all of those different species, specifically the amount and composition of the lipids, proteins, carbohydrates, and alkali. They measured that the *Apis cerana* had the highest composition and amount of all four parts of the venom. *Apis mellifera* and *Apis florea* were the second and third highest, respectively, with *Apis dorsata* coming in last place having the least composition and amount of those four parts. All of these differences could mean that there could be some variation in the melittin structure, and as a result, it could result in inconsistencies in some of the beneficial qualities of the molecule. Another shortcoming of melittin peptide relates to the range of medical fields it is useful in. As it has already been explored, melittin is being used in most medical fields such as oncology and dermatology, but there are other fields where this molecule could play a very good role in helping to cure diseases. For example, there are many fields of medicine that can benefit from the rapid diffusive qualities that melittin has, but they have to be tested first in order to actually be effective. The reason why is because melittin's original purpose was to give a bee sting a more lethal effect by not only causing cells to lyse but also diffusing the venom to more places faster. If for some reason the melittin fails to attack harmful cells but instead causes other cells around it to lyse and destroy them, then a potential death could occur. There are very small details about melittin that cannot be neglected. Otherwise, the complications that were mentioned could potentially take place.

## Studies have confirmed that melittin peptide is helping cure complications within the heart.

Thankfully, there is one significant field of medicine where melittin could potentially change the medical field by introducing a more widespread form of treatment, and that is in cardiology. Cardiovascular issues are among the most common and most fatal ones because there are millions of people around the world suffering from those diseases, and even though there have been many treatments developed to try and ameliorate those issues, more mishaps have arisen. As a result, it has proven difficult for specialists to try to create more treatments for cardiovascular issues. With melittin, however, that will all change. There have been multiple studies that have talked about using melittin peptide to help cure issues related to the heart, and they have all ideally favored the hypotheses that were made. One particular study dealt with rats that were suffering from hypotensive hemorrhaging, a sequence of events that begin when blood reaches an extremely low level of pressure and cardiac output decreases as a result. According to an article published on PubMed, there were two scientists named Murat Yalcin and Vahide Savci whose main goals were to figure out the effects of melittin on those two conditions. They simulated those conditions in some mice by removing 2.2 mL of blood from them, resulting in the right conditions for hypotensive hemorrhaging (Savci, 2007). They even tried repeating some other experiments which will be explored in more detail later on to try to compare some of the results they would gather in this experiment. They tried using different amounts of melittin, with three different doses at levels of 1.5 μg, 3.0 μg, and 6.0 μg. After injecting melittin into the mice intracerebroventricularly, they found out that the largest dose of melittin fully reinstated the blood pressure the mice were at before the scientists wanted to simulate damaging heart conditions in the mice. In addition, because of the increase in blood pressure, melittin was also able to mitigate excessive hemorrhaging in the rats. The exact data regarding the efficacy of those different doses have not been properly disclosed in the article, however. After running those other experiments, they were able to confirm that melittin had positive effects on cardiovascular issues. However, the exact data is not clearly distinguished in the article the study was published in. There was another study published in an article on PubMed that talks about CVB3 inhibited viral myocarditis in rats (*Melittin Ameliorates CVB3-induced Myocarditis via Activation of the HDAC2-mediated GSK-3β/Nrf2/ARE Signaling Pathway*, 2016). Viral myocarditis is a disease in which the myocardium, the muscle of the heart, undergoes inflammation. This can cause a patient to suffer from arrhythmia and it can decrease total cardiac output. Scientists wanted to see whether melittin would be able to remove viral myocarditis, and they decided to test that by intraperitoneally injecting CVB3, which is an RNA molecule that is most commonly known for being a part of the inhibition of myocarditis. Once they injected CVB3, the mice's myocardia went under inflammation, and they used a dose of melittin and injected it into the affected rats. They found that melittin not only restored normal cardiac function, but also decreased the signatures of AST, CK, HBDH, and LDH, which are all molecules that are responsible for inhibiting viral myocarditis. It was able to do so by activating a special signaling pathway called the GSK-3ß/Nrf2/ARE signaling pathway, and it was mediated by HDAC2. This signaling pathway is well recognized in curing complications related to inflammation, oxidative stress, and protein stability. A third study was conducted that experimented with the effects of melittin on the cardiovascular system in general. They used normotensive conscious rats for this experiment (Erturk, 2006). They wanted to see what effect melittin really had on the cardiovascular system, and based on previous trends, they hypothesized that heart rate and arterial pressure would decrease and increase, respectively. When they conducted the experiment, they found that their hypothesis was correct and that melittin had decreased their heart rates and increased their arterial pressure. They tested this based on inhibiting TXA2 and its central receptors to see what would happen at an elevated heart rate.  Based on these studies, it can be established that melittin has a plethora of qualities that distinguish it from other molecules. It is able to cure basically any issue related to the heart and it can only get better from here. There are numerous studies that are still trying to test melittin on the heart, but based on the studies that have been analyzed here, it can reasonably be concluded that melittin is a good molecule to be used on the heart.

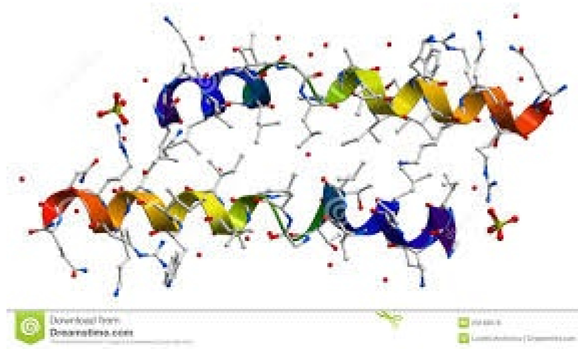

**Figure 6:** Here is a more colorful representation of the molecule.

## Future Discussion

While it is important for people to know about how melittin has been proven to be effective in curing heart-related issues, it is also important for them to know about how the molecule could evolve in the future to support more fields of medicine and eventually become a more feasible option for treatment. As has been discussed, one of the shortcomings of melittin was that it wasn't fully available to all medical fields. However, things could change in the years to come thanks to advances in technology and testing methods. Scientists will be able to try to test melittin on more medical complications to try to expand the range of medical fields it is effective in. For example, there are scientists that have already tested the effectiveness of bee venom on breast cancer cells, and they found that within an hour, all of the cancer cells were completely destroyed. In fact, other types of cancer cells can be destroyed using melittin, including but not limited to prostate, liver, and lung. However, that is only the beginning. In addition, the possibility of creating new treatments with melittin has sparked interest in the medical field. Bee venom is the most common substance where melittin is present, so theoretically, scientists could create a new type of medicine where they will be able to combine the effects of honey and honeybee venom into one single liquid or pill that a person could take. All of this will cost some money to create, however, and there is no data to go in-depth into how scientists can take more cost-saving measures to ensure that the masses can get the proper treatment and that production can run smoothly. Implementing this molecule into medicines or pills will be a momentous achievement because there are so many implications for this molecule in cardiology and it has proven to be a very effective molecule in most fields, and it is no different here. Doctors can use bee venom since they are very high in melittin concentration, but scientists could theoretically create a drug with just the melittin contents and as a result, it would translate to a raw melittin treatment. However, only time can tell where all of this is going to go, but medical professionals and scientists are already taking the step towards using that molecule.

## Conclusion

In conclusion, melittin is a molecule that has proven to be very helpful in a lot of medical fields, especially in cardiology. Its diverse chain of amino acids contributes to the number of functions it has and its efficacy in relieving medical complications. They are able to communicate with multiple molecules around them and are able to perform many actions that previously required individual molecules. Melittin is also commonly found in bee venom, and it was originally meant to act as a trigger for nociceptor cells, which is why a person's skin lyses and gets hot and red when a bee stings someone. Many more studies will be conducted to test and verify the effects of melittin on more cardiovascular issues, but there is already a wide range of complications this molecule can be used in. Cardiology is one of the most vital fields of medicine because heart issues are very common among some people; in fact, one person dies every 36 seconds due to cardiovascular disease in the United States, which makes it one of the leading causes of death. With melittin, however, those numbers could decline sharply. However, there are still many requirements that must be fulfilled in order for those numbers to decline, such as mass-producing a drug with the molecule present. The reason why is because while people are able to find bee venom in large quantities, with the caution of bees of becoming endangered species, they are not trying to use much of the bee venom. Instead, there are multiple sources that are selling melittin in varying quantities, though they can be quite expensive. For example, a 1 mg sample of melittin goes on sale for about $71, which is a large premium for that sample size. However, with the everlasting advancements of technology, that price could also decrease, leading to more melittin treatments being manufactured. There is a wide range of possibilities for this molecule, as it has the potential of becoming one of the most effective treatments for cardiovascular issues, but only time can tell what can be done with the molecule.

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## References

A, M., & C, W. (1980). *The effects of honeybee (Apis mellifera L.) venom and two of its constituents, melittin and phospholipase A2, on the cardiovascular system of the rat*. PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/7210027/

*Applications and evolution of melittin, the quintessential membrane active peptide*. (2021, November). PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/34543656/

*Astragalus polysaccharide from Astragalus Melittin ameliorates inflammation via suppressing the activation of TLR-4/NF-κB p65 signal pathway and protects mice from CVB3-induced virus myocarditis*. (2019, April 1). PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/30586589/

Bitar, M. S. (2012, July 1). *The GSK-3β/Fyn/Nrf2 pathway in fibroblasts and wounds of type 2 diabetes*. NCBI. Retrieved January 9, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3609099/

Comparative Study of Antimicrobial Properties of Bee Venom Extracts and Melittins of Honey Bees. (2021, December 8). *antibiotics*, 14.

Conformations and Dynamic Transitions of a Melittin Derivative That Forms Macromolecule-Sized Pores in Lipid Bilayers. (2018, June 22). *Langmuir*, 7. https://pubs.acs.org/doi/abs/10.1021/acs.langmuir.8b00804

Erturk, M. (2006, June). *The role of the central thromboxane A2 in cardiovascular effects of a phospholipase A2 activator melittin administrated intracerebroventricularly in normotensive conscious rats*. PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/16524625/

*Figure 2*. (n.d.). Retrieved January 18, 2022, from https://www.genscript.com/peptide/RP10290-Melittin.html

*Heart Disease Facts | cdc.gov*. (2021, September 27). CDC. Retrieved January 9, 2022, from https://www.cdc.gov/heartdisease/facts.htm

Hong, J., Lu, X., Deng, Z., Xiao, S., Yuan, B., Yang, K., & Dosio, F. (2019, May 7). *How Melittin Inserts into Cell Membrane: Conformational Changes, Inter-Peptide Cooperation, and Disturbance on the*

*Membrane*. NCBI. Retrieved January 9, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6539814/

Hong, T. (2007, August). *Melittin, a major bioactive component of bee venom toxin, inhibits PDGF receptor beta-tyrosine phosphorylation and downstream intracellular signal transduction in rat aortic vascular smooth muscle cells*. PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/17654254/

*How Melittin Inserts into Cell Membrane: Conformational Changes, Inter-Peptide Cooperation, and Disturbance on the Membrane*. (n.d.). MDPI. Retrieved January 16, 2022, from https://www.mdpi.com/1420-3049/24/9/1775/htm

*Melittin = 85 HPLC 20449-79-0*. (n.d.). Sigma-Aldrich. Retrieved January 9, 2022, from https://www.sigmaaldrich.com/US/en/product/sigma/m2272

*Melittin ameliorates CVB3-induced myocarditis via activation of the HDAC2-mediated GSK-3β/Nrf2/ARE signaling pathway*. (2016, November 4). PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/27693786/

*Melittin has an inhibitory effect on TNF-α-induced migration of human aortic smooth muscle cells by blocking the MMP-9 expression*. (2012, November). PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/22926441/

*NADPH oxidase 4 regulates vascular inflammation in aging and atherosclerosis*. (2016, December 14). PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/27986445/

Oršolić, N. (2012, January). *Bee venom in cancer therapy*. PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/22109081/

Pak, C. (2013, September). *Effects of cosmetics containing purified honeybee (Apis mellifera L.) venom on acne vulgaris*. PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/24063779/

Savci, V. (2007, December). *Cardiovascular effects of centrally injected melittin in hemorrhaged hypotensive rats: the investigation of peripheral mechanisms*. PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/17897713/

Savci, V. (2009, December). *Cardiovascular effect of peripheral injected melittin in normotensive conscious rats: Mediation of the central cholinergic system*. PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/19910175/

Seymour, L. (2001, December 14). *Melittin enables efficient vesicular escape and enhanced nuclear access of nonviral gene delivery vectors*. PubMed. Retrieved January 9, 2022, from https://pubmed.ncbi.nlm.nih.gov/11600500/