



## EFFECT OF BEE VENOM ON HUMAN RED BLOOD CELL BREAKDOWN

Shahad Falah Abass <sup>a\*</sup>  
Mohammed Sabbar Hameed <sup>b\*</sup>  
Alaa Abduljabbar Abdulrazzaq <sup>c\*</sup>

<sup>a\*</sup>Department of Biology, College of Al-Rasheed, Baghdad, Iraq, E-mail : [Shahad.falah@alrasheedcol.edu.iq](mailto:Shahad.falah@alrasheedcol.edu.iq)

<sup>b\*</sup>Department of Biology, College of Al-Rasheed, Baghdad, Iraq, E-mail : [mohammed.sabbar@alrasheedcol.edu.iq](mailto:mohammed.sabbar@alrasheedcol.edu.iq)

<sup>c\*</sup>Department of Biology, College of Al-Rasheed, Baghdad, Iraq, E-mail : [alaa.abd@alrasheedcol.edu.iq](mailto:alaa.abd@alrasheedcol.edu.iq)

Article history:	Abstract:
<p><b>Received:</b> June 1<sup>st</sup> 2022 <b>Accepted:</b> July 1<sup>st</sup> 2022 <b>Published:</b> August 6<sup>th</sup> 2022</p>	<p>Red blood cells (erythrocytes) are the most abundant cells in human blood, which have been studied in numerous studies on a large scale within the framework of morphology, ultrastructure, biochemical and molecular functions. Therefore, erythrocytes are excellent cellular models in the study of bioactive compounds such as drugs and toxins on the structure and function of the cell membrane. The aim of this study is to know the effect of bee venom on the decomposition of red blood cells by increasing the concentration of the venom and thus increasing the active substances in the venom that cause this decomposition. And knowing the extent to which gender is affected by bee venom, as it was found that males are more affected by bee venom than females.</p>

**Keywords:** Bee venom, Erythrocytes, RBC, PLA2, MCD.

### INTRODUCTION

Red blood cells (RBCs), the most prevalent blood morphotic component, serve as crucial cell models for researching the effects of membrane-active substances <sup>(1)</sup>. in-depth and, in a number of works to date. Apitherapy has recently emerged as a promising field for the development of novel therapeutic <sup>(2)</sup>.

The active ingredient in honey bee venom (HBV, *Apis mellifera*) is a combination of proteins that both create localized inflammation and function as anticoagulants. HBV is a bitter, colorless liquid. It has been revealed that HBV contains the enzymes and is a complex mixture of low-molecular-weight polypeptides and enzymes phospholipase A2 (PLA2), hyaluronidase, phosphomonoesterase acid esterase,  $\alpha$ -D-glucosidase, lysophospholipase,  $\alpha$ -galactosidase,  $\alpha$ -acetylamino-deosiglucosidase and arylamidase. Bee venom (BV) compositions fluctuate between fresh and dried forms mostly in terms of their volatile components, although overall biological activity is comparable <sup>(3)</sup>.

Another peptide found in bee venom is called Apamin. Apamin is a member of the neurotoxic peptides, just like tertiapin. It has two disulfide links and 18 amino acids. Apamin primarily affects the brain and nerve system <sup>(4)</sup>. Smaller amounts of low-molecular weight substances that are found in nature, such as amino acids, catecholamines, carbohydrates, and minerals, are also present in BV. Although sugars have been found in

some BV preparations, if BV is gathered using a collector to exclude pollen and nectar contamination, it does not contain carbohydrates <sup>(5)</sup>.

Therefore, you should concentrate on identifying novel bioactivities of certain bee products, such as honey, pollen, propolis, royal jelly, and venom, which can be crucial in the development of biotechnology and pharmacology <sup>(6, 7)</sup>.

There are five different types of enzymes, which are proteins that catalyze certain reactions. Polypeptides are composed of two or more amino acids and have a lower molecular weight than enzymes. BV also contains a large number of polypeptides, the most important of which is melittin. This polypeptide has a wide range of advantageous biological effects and is largely nontoxic. Melittin has a molecular weight of 2.840 Daltons, but because it can also exist in tetrameric form, it can reach 12,500 Daltons <sup>(8, 9)</sup>.

Melittin may cause itchiness, localized response, and discomfort when administered to humans in high quantities, but when administered in moderate levels, it may have a beneficial, anti-inflammatory effect based on the inhibition of phospholipase A2 <sup>(10)</sup>.

It's interesting from a pharmacological and biotechnological perspective that significant antibacterial activities are caused by the interaction between melittin and cell membranes <sup>(11)</sup>.



The two most toxic components in BV are PLA2 and the mast cell degranulating (MCD) peptide. Due of the various effects BV has on the central and peripheral nervous systems (PNS), it can be utilized to treat patients with a variety of heart diseases. Individual BV components can be employed to accomplish specific biological effects. Multiple sclerosis (MS), Alzheimer's disease, and Parkinson's disease are just a few of the degenerative neurological disorders for which BV has been used in published reports <sup>(12-13)</sup>.

As compared to other human diseases, accidents, and other unusual cases, deaths from bee stings are rare, demonstrating that BV is exceedingly safe for the treatment of human disease. Most human deaths following one or a few bee stings are caused by allergic reactions, heart failure, or suffocation from swelling around the neck or the mouth. <sup>(14)</sup>.

Melittin has the potential to be exploited for a variety of applications, including antibacterial, cell-selective assault, and translocation of materials by altering the membrane permeability, because of its fascinating interaction with lipid membranes and its capacity to generate pores. <sup>(15-16)</sup>.

**METHODS**

**1 - Blood Samples**

Twenty venous blood samples from individuals of various ages and sexes were taken <sup>(17)</sup>.

**2 -Preparing the blood suspension**

The serum was isolated from 5 ml of blood by centrifuging it at 5000 revolutions per minute for 2 minutes before adding 2 ml of physiological salt solution and centrifuging it again at 5000 revolutions per minute for 2 minutes. Only red blood cells were obtained after three rounds of this procedure; 0.5 ml of this sample was then taken and the amount was increased to 50 ml with physiological salt solution <sup>(17)</sup>.

**3 - Preparation of antigen solution**

In physiological saline solution, half dilutions of crude bee venom ranged from 100 to 0.195. The produced blood suspension was then mixed with 1 ml of each dilution. As for the crude poison, the sample was taken without any dilution. A positive control factor was made by mixing 1 ml of the suspension with 1 ml of triton x 100, and a negative control factor was made by mixing 1 ml of physiological saline solution with 1 ml of blood. The samples were then incubated for 60 minutes at 37 °C before being centrifuged at 5000 rpm for 2 minutes to remove the unwanted material <sup>(17)</sup>.

4 - The results of the prepared samples were read using an optical absorber at a wavelength of 540 <sup>(17)</sup>.

**RESULTS**

In this study, a comparison was made of the effect of bee venom on the decomposition of red blood cells in humans for both sexes (males and females), to determine the extent of the effect of gender on the decomposition of red blood cells in bee venom. The results are shown in Table No. (1)

Table No. (1) Correlation between the concentration of bee venom and the decomposition of red blood cells in females

Conc. (%)	Mean ± Std.error	
	Female	
100	0.437	± 0.056
50	0.415	± 0.053
25	0.390	± 0.052
12.5	0.370	± 0.055
6.25	0.359	± 0.054
3.125	0.343	± 0.054
1.563	0.333	± 0.053
0.781	0.318	± 0.052
0.392	0.304	± 0.051
0.195	0.281	± 0.055
Control -	0.002	± 0.0001
Control +	0.916	± 0.03
LSD	0.1387	
<b>(P&lt;0.05) *</b>		

There is a correlation between the concentration of bee venom and the decomposition of red blood cells in females, as it was found that the concentration had a significant effect (0.1387) and this was demonstrated by increasing the degree of decomposition by raising the concentration, as it reached (0.437) at the concentration of 100 percent of bee venom.

Table No. (2) Correlation between the concentration of bee venom and the decomposition of red blood cells in Males

Conc. (%)	Mean ± Std.error	
	Male	
100	0.765	± 0.040
50	0.740	± 0.039
25	0.724	± 0.041
12.5	0.707	± 0.041
6.25	0.689	± 0.043
3.125	0.666	± 0.043
1.563	0.651	± 0.043
0.781	0.579	± 0.047

<b>0.392</b>	<b>0.560</b>	<b>±</b>	<b>0.047</b>
<b>0.195</b>	<b>0.523</b>	<b>±</b>	<b>0.043</b>
<b>Control -</b>	<b>0.002</b>	<b>±</b>	<b>0.0001</b>
<b>Control +</b>	<b>0.916</b>	<b>±</b>	<b>0.03</b>
<b>LSD</b>	<b>0.1567</b>		
<b>(P&lt;0.05) *</b>			

The results here showed in Table (2) that bee venom has an effect on the decomposition of red blood cells in males, as the degree of decomposition increases with increasing concentration, reaching (0.765) at 100% concentration.

Table No. (3) comparison between males and females in the degree of lysis of red blood cells in bee venom

Con c. (%)	Me an ± Std.e rror		Me an ± Std.e rror		LSD
	Female		Male		
100	0.4 37	± 0.056	0.7 65	± 0.040	0.14 52
50	0.4 15	± 0.053	0.7 40	± 0.039	0.13 87
25	0.3 90	± 0.052	0.7 24	± 0.041	0.13 87
12.5	0.3 70	± 0.055	0.7 07	± 0.041	0.13 95
6.25	0.3 59	± 0.054	0.6 89	± 0.043	0.13 95
3.12	0.3 43	± 0.054	0.6 66	± 0.043	0.14 50
1.56	0.3 33	± 0.053	0.6 51	± 0.043	0.14 26
0.78	0.3 18	± 0.052	0.5 79	± 0.047	0.14 63
0.39	0.3 04	± 0.051	0.5 60	± 0.047	0.14 66
0.19	0.2 81	± 0.055	0.5 23	± 0.043	0.14 68
Cont rol -	0.0 02	0.000 ± 1	0.0 02	0.000 ± 1	
Cont rol +	0.9 16	± 0.03	0.9 16	± 0.03	
<b>(P&lt;0.05) *</b>					

Table (3) The table here shows a comparison between males and females in the degree of lysis of red blood cells in bee venom. The results showed that the degree of lysis of red blood cells in males was higher than in females at each of the concentrations used.

## DISCUSSION

There are three main bee venom compounds: melittin, tertiapin, and apamin. The focus on chemicals, proteins, and enzymes that destroy the cell wall is thought to be the cause of the rise in the decomposition of red blood cells. Melittin can operate as a natural surfactant by interacting with phospholipids, which can cause the lipid bilayer to be disrupted <sup>(18)</sup>.

Enzymes, proteins, peptides, and a variety of smaller molecules make up honey bees. Venoms from different stinging insects exhibit striking biochemical and pharmacological convergence. Most venoms can kill red blood cells and cause instant pain. They contain the enzymes phospholipases, hyaluronidase, and others. <sup>(19)</sup>. The main active ingredient of apitoxin (BV), melittin, is a potent PLA2 protein activator. Aldolase, metalloprotease, GTPase, and acyltransferase were the main enzymes implicated in these activities. RBC function can be severely disrupted by melittin <sup>(19)</sup>.

The superiority of males over females in the decomposition of red blood cells in bee venom is due to the higher proportion of hemoglobin in male blood, which reaches 13 to 17 g / ml, while females have 11 to 16 g / ml, and thus gives a greater amount of red blood cells for the decomposing enzymes found in the poison <sup>(20)</sup>.

## CONCLUSION

Bee venom is very effective in influencing red blood cells, and it caused the decomposition of these cells by the action of the active substances contained in the poison, which were previously mentioned. The aura in the poison

## REFERENCES

1. Bobrowska-Hägerstrand, M.; Wróbel, A.; Mrówczyńska, L.; Söderström, T.; Hägerstrand, H. Modulation of MRP1-like e<sub>ux</sub> activity in human erythrocytes caused by membrane perturbing agents. *Mol. Membr. Biol.* **2003**, *20*, 255–259.
2. Basa, B.; Wendimagegnehu, B.; Tilahun, A.; Teshale, A. Review on medicinal value of honeybee products: Apitherapy. *Adv. Biol. Res.* **2016**, *10*, 236–247.
3. Son DJ, Lee JW, Lee YH, Song HS, Lee CK, Hong JT. Therapeutic application of anti-arthritis, pain-releasing and anti-cancer effects of bee venom and its constituent compounds. *Pharmacol Ther.* 2007;*115*(2):246-70.
4. Moreno, M.; Giralt, E. Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: Melittin, apamin and mastoparan. *Toxins* **2015**, *7*, 1126–1150.



5. Shkenderov S, Ivanov T. Pcelni produkti, the bee products (in Bulgarian). Bulgaria: Zemizdat; 1983. 1-238 p.
6. Woźniak, M.; Mrówczyńska, L.; Waśkiewicz, A.; Rogoziński, T.; Ratajczak, I. The role of seasonality on the chemical composition, antioxidant activity and cytotoxicity of Polish propolis in human erythrocytes. *Brazilian J. Pharmacogn.* **2019**, 29, 301–308.
7. Woźniak, M.; Mrówczyńska, L.; Waśkiewicz, A.; Rogoziński, T.; Ratajczak, I. Phenolic profile and antioxidant activity of propolis extracts from Poland. *Nat. Prod. Commun.* **2019**, 14, 1–7.
8. Habermann E, Jentsch J. Über die struktur des toxischen bienengiftpeptids melittin und deren beziehung zur pharmakologischen wirkung. *Naunyn Schmiedebergs Arch Pharmacol.* 1966;253(1):40-1.
9. Habermann E, Zeuner G. Comparative studies of native and synthetic melittins. *Naunyn Schmiedebergs Arch Pharmacol.* 1971;270(1):1-9.
10. Pratt, J. P. *et al.* Melittin-induced membrane permeability: a nonosmotic mechanism of cell death. *In Vitro Cell. Dev. Biol. Anim.* **41**, 349–55 (2005).
11. Takahashi, T.; Nomura, F.; Yokoyama, Y.; Tanaka-Takiguchi, Y.; Homma, M.; Takiguchi, K. Multiple membrane interactions and versatile vesicle deformations elicited by melittin. *Toxins* **2013**, 5, 637–664.
12. Kim JI, Yang EJ, Lee MS, Kim YS, Huh Y, Cho IH, *et al.* Bee venom reduces neuroinflammation in the MPTP-induced model of Parkinson's disease. *Int J Neurosci.* 2011;121(4):209-17.
13. Lee SM, Yang EJ, Choi SM, Kim SH, Baek MG, Jiang JH. Effects of bee venom on glutamate-induced toxicity in neuronal and glial cells. *Evid Based Complement Alternat Med.* 2012;2012:ID368196.
14. Abdu Al-Samie MA. Studies on bee venom and its medical uses. *IJOART.* 2012;1(2):1-15.
15. Boman, H. G., Wade, D., Boman, I. A., Wöhlin, B. & Merrifield, R. B. Antibacterial and antimalarial properties of peptides that are cecropin-melittin hybrids. *FEBS Lett.* **259**, 103–106 (1989)
16. Soman, N. R. *et al.* Molecularly targeted nanocarriers deliver the cytolytic peptide melittin specifically to tumor cells in mice, reducing tumor growth. *J. Clin. Invest.* **119**, 2830–42 (2009).
17. Mortari, M. R.; Cunha, A. O. S.; de Oliveira, L.; Gelfuso, E. A.; Vieira, E. B. And dos Santos, W. F. (2005). Comparative toxic effects of the venoms from three wasp species of the genus *polybia* (Hymenoptera Vespidae). *Journal of Biological Sciences.* 5(4): 449-454.
18. Kallarackal, A.J.; Simard, J.M.; Bailey, A.M. The effect of apamin, a small conductance calcium activated
19. potassium (SK) channel blocker, on a mouse model of neurofibromatosis 1. *Behav. Brain Res.* 2013, 237, 71–75.
20. Gajski, G.; Garaj-Vrhovac, V. Melittin: A lytic peptide with anticancer properties. *Environ. Toxicol. Pharmacol.* 2013, 36, 697–705
21. Bryk, A.H.; Wiśniewski, J.R. Quantitative analysis of human red blood cell proteome. *J. Proteome Res.* 2017, 16, 2752–2761