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Study of the effect of bee venom (HBV, Apis mellifera) on human plasma proteins and blood clotting rate

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Abstract:
The present study was conducted at the National Center for Hematology Research and treatment in (2023) and it demonstrates the effect of bee venom (HBV, Apis mellifera) on human plasma proteins and blood coagulation rate. The effect of bee venom on blood clotting in humans was compared for both sexes (males and females) to determine the extent of the effect of gender on blood clotting time. The results indicated a correlation between the concentration of bee venom and the duration of blood clotting in females, where the highest significant effect was recorded for the concentration of bee venom at 100% at the 0.05 level, for a time period of 25 minutes.
Keywords: Honeybee, venom, clotting time, blood coagulation, anti-coagulants

Introduction:
Bee venom, especially BV, is a complex mixture of several proteins, including phospholipase and melittin, which influence the process of blood coagulation and blood clot formation. (Adewole et al., 2013)
As mentioned by (Sun et al., 2007), bee venom is a clear liquid, and its active component consists of a group of proteins that cause local inflammation and act as anticoagulants. While (Bogdanoy et al., 2015) indicated that it is a complex mixture of enzymes and peptides with small molecular weights. The substance includes phospholipase PLA2 and hyaluronidase. Bee venom has been used in ancient Chinese and Korean medicine, as well as by the Egyptians and Greeks to treat various diseases.BV bee venom, sometimes referred to as Api-toxin, is extensively utilized in the management of diverse inflammatory conditions such as rheumatoid arthritis or multiple sclerosis.

Furthermore, it is widely recognized that BV can enhance the wound-healing process. The substance possesses capabilities that combat inflammation, oxidation, fungal infections, viral infections, and microbial growth (Badr et al., 2016). Honeybee venom, also known as apitoxin, is produced in the venom gland located in the abdominal region of honeybees. It is used by adult bees as a primary means of protecting the colony. The composition of this substance includes a variety of biologically active compounds such as peptides, enzymes, amines, amino acids, phospholipids, minerals, and carbohydrates (Al-Shaeli et al., 2022). In addition to certain volatile constituents. Melittin and phospholipase A2 are crucial constituents of BV, possessing significant therapeutic potential such as anticancer, antibacterial, anti-inflammatory, anti-arthritic, anti-nociceptive, and other properties. Thus, in the field of medicine, BV has been employed for millennia to combat a range of ailments including arthritis, rheumatism, back pain, and numerous inflammatory illnesses (Akhtari et al., 2024). Currently, bacterial vaginosis virus or its constituents are being utilized individually in many nations as a natural remedy for treating diverse ailments, with few adverse effects. The study was conducted by Choi K et al. in 2014...

Consequently, scientists and several pharmaceutical businesses are endeavoring to acquire a fresh comprehension of bacterial vaginosis, including its constituents and its behavior, to utilize this natural therapy more efficiently in contemporary medicine (El-Seedi, et al., 2020).

Honeybee venom is currently employed in the treatment of various ailments in both humans and animals, including but not limited to alterations in the neurological system, arthritis, circulatory disorders, tumors, skin conditions, and certain immune-related abnormalities (Ullah et al., 2023). The current research on the safety of honeybee venom is inconclusive and conflicting. Honeybee venom can occasionally lead to allergic and anaphylactic reactions, which vary depending on the individual's immune system and the dosage delivered (Frangieh J. al., 2019).

Administration of bee venom at a concentration of 100 µg/ml to sensitized individuals can lead to severe consequences including limb paralysis, discomfort, difficulty breathing, nausea, loss of consciousness, and loss of lymphocytes due to phospholipase A2 (PLA2), melittin, and hyaluronidase, the main allergenic components present in bee venom (Pucca et al., 2019). Bee venom can have a local or systemic effect on the host body. Local reactions manifest themselves in the form of redness, inflammation, and fluid

accumulation. Systemic reactions are determined by the effect of the allergens present in the venom. These allergens can lead to various symptoms in sensitized individuals, such as angioedema, urticaria, vomiting, itching, and diarrhea (Fitzgerald and Flood, 2006). Hence, clinical practitioners should obtain updated information on the safety of honeybee venom to prevent any significant side effects and negative consequences associated with this vital substance for bees (Ullah et al., 2023).

Aim of the Study

The aim of this study was to investigate the mechanism by which honeybee venom affects human plasma proteins and its effect on blood coagulation.

Materials and working procedures

Honeybee colonies were obtained from private apiaries in Baghdad. Bee venom was collected from 500 worker bees, which were captured upon entering the colony and rendered immobile by rapid freezing at -°20C.

The individuals were dissected, their stingers removed and placed in a tube with 2.5 ml of dH2O. The tube was then centrifuged at 12,000 × g for 5 min at °4C, resulting in the collection of the upper juice as raw venom. (Ali et al. 2012).

The steps of the work included:

1- Creating the toxic solution: By preparing the bee venom solution by dissolving 1 mg of bee venom in 1 ml of normal saline and mixing them for 1 min. It was then stored in the refrigerator at °4C until use.

2-formulating toxicological preparations: Physiological saline solution was used to generate decimal dilutions of bee venom. Subsequently, 0.5 ml of each dilution was combined with 0.5 ml of straight venous blood without an anticoagulant. The resulting mixture was then compared to the control factor, which represented the blood without any additions.

Statistical analysis:

The statistical analysis was conducted using the software package SPSS IBM Corp., Released 2021. IBM SPSS Statistics for Windows, Version 28.0. (IBM Corp., Armonk, NY). Demographic data were characterized using descriptive statistics T test and person correlation (r). Estimated p-values <0.05 were considered significant.

Results and discussion

The effect of bee venom on blood clotting in humans was compared for both sexes (males and females) to determine the extent of the effect of gender

on blood clotting time. The results of Table (1) indicated a correlation between the concentration of bee venom and the duration of blood clotting in females, where the highest significant effect was recorded for a concentration of 100% venom at the 0.05 level, and for a time period of 25 minutes.

There is a correlation between the concentration of bee venom and the duration of blood fortification in females. The concentration of venom has a statistically significant impact (p-value of 0.05), as evidenced by the prolonged cultural suppression time (25 minutes) observed at 100% concentration of cardamom venom

Table 1: The impact of bee venom on the level of blood coagulation in females

Concentration %	Clotting time (CT) sec Mean ± SD		P – value
	Female N=10	Control N=10	
100	122.4±25	10±2.0	0.001**
50	109.3±21.8	10 ±2.0	0.001**
25	98.2±13.9	10±2.0	0.001**
12.5	87.3±25.5	10 ±2.0	0.001**
6.25	75.6±20.5	10±2.0	0.001**
3.12	65.2±18.6	10 ±2.0	0.001**
***0.05 significant differences**			

The results of the study in Table (2) show that bee venom has a significant effect on blood clotting time in men, where with an increase in the concentration of bee venom and an increase in the exposure period of 19 minutes at a concentration of 100%, the highest significant effect of bee venom was achieved.

The data presented in Table (2) demonstrates that bee venom has a significant impact on the duration of blood clotting in men. As the concentration of bee venom increases, the clotting time progressively increases, reaching a duration of 19 minutes at 100% concentration.

Table (2): The effect of bee venom on the degree of blood clotting in male

Concentration %	Clotting time (CT) sec Mean ± SD		P – value
	Male N=10	Control N=10	
100	113.7±19.0	9.5±1.77	0.001**
50	103.9±17.2	9.5±1.77	0.001**
25	90.8±18.1	9.5±1.77	0.001**
12.5	73.7±19.0	9.5±1.77	0.001**
6.25	65.1±19.2	9.5±1.77	0.001**
3.12	84.8±13.2	9.5±1.77	0.001**
***0.05 significant differences**			

The data presented in Table (1&2), The increase in blood clotting time with increasing dose concentration is attributed to the high concentration of anticoagulant proteins found in bee venom. These proteins possess potent anticoagulant activity, significantly prolonging the time it takes for a clot to form. They achieve this by affecting the clotting factor responsible for prothrombin formation and influencing various other clotting factors. The coagulation cascade is a topic discussed in the study conducted by Isidorov et al. in 2023.

Table 3 presents a comparative analysis of bee venom clotting time between males and females. The results indicated that there was no significant difference at the 0.05 level in clotting time. Perhaps in some cases females may show longer clotting times than males.

The duration of bleeding time is influenced by multiple factors, including platelet and endothelial cell functions, as well as the specific coagulation techniques used. Deficiency or absence of clotting factors increases clotting time (CT). The presence of estrogen in females increases clotting time, which subsequently reduces platelet activity. The higher CT in females compared to males is attributed to higher estrogen levels in females. Estrogen prolongs CT scan time and reduces the amount of fibrinogen in plasma. On the other hand, BT is lower in males due to increased platelet activity and aggregation. A comparative analysis of behavioral therapy (BT) and cognitive therapy (CT) on gender differences (Adhana et al., 2018).

Table (3): Comparison between males and females in blood clotting time with bee venom

Concentration %	Clotting time (CT) sec Mean ± SD		P – value
	Male N=10	Female N=10	
100	113.7±19.0	122.4±25	0.393
50	103.9±17.2	109.3±21.8	0.547
25	90.8±18.1	98.2±13.9	0.446
12.5	73.7±19.0	87.3±25.5	0.195
6.25	65.1±19.2	75.6±20.5	0.253
3.12	84.8±13.2	65.2±18.6	0.036
***0.05 significant differences**			

shows the direct relationship between the concentration of the poison and the clotting time (4) Table

Correlations			
		CT* (sec)	Concentration (%)
CT (sec)	Pearson Correlation	1	+0.710**
	Sig. (2-tailed)		0.0001
	N	140	140
Concentration (%)	Pearson Correlation	0.710**	1
	Sig. (2-tailed)	0.0001	
	N	140	140
**** Correlation is significant at the 0.01 level.** **CT: clotting Time**			

The table (4) demonstrates a positive link between the concentration of the poison and the time it takes for blood to clot. More precisely, as the level of toxicity rises, the duration required for blood clotting also increases. his is because the higher the concentration of the poison, the greater the number of anticoagulant proteins, such as phospholipase and melittin, which affect platelet functions and hinder the work of clotting factors, thus increasing blood clotting time.

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دراسة تأثير سم النحل (HBV, Apis mellifera) على بروتينات البلازما البشرية ومعدل تخثر الدم

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المستخلص:

أجريت الدراسة في المركز الوطني لبحوث وعلاج امراض الدم في (2023) حيث اظهرت تأثير سم النحل (HBV, Apis mellifera) على بروتينات البلازما البشرية ومعدل تخثر الدم، وتمت مقارنة تأثير سم النحل على تخثر الدم لدى البشر لكلا الجنسين (ذكور وإناث) لتحديد مدى تأثير الجنس على زمن تخثر الدم، وأشارت النتائج إلى وجود ارتباط بين تركيز سم النحل ومدة تخثر الدم لدى الإناث، حيث تم تسجيل أعلى تأثير معنوي لتركيز سم النحل بنسبة 100% عند مستوى 0.05، لفترة زمنية مقدارها 25 دقيقة.

الكلمات المفتاحية:نحل العسل، سم، زمن التخثر، تخثر الدم، مضادات التخثر،
ملاحظة: هل البحث مستل من رسالة ماجستير او اطروحة دكتوراه؟ نعم: **كلا :** ✔