

THE SPERMICIDAL EFFECT OF THE HONEYBEE *APIS MELLIFERA* VENOM AND ITS MAJOR COMPONENT MELITTIN

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ABSTRACT

Several spermicide products are currently used containing detergent as the active ingredient. Those molecules showed a bad effect on normal vaginal epithelial cells and microflora. They also increase the possibility of cervical or vaginal infections or ulceration. In this study, the possible effects of crude bee venom (BV) of the honeybee (*Apis mellifera*) and its common bioactive peptides on human sperm motility were evaluated *in vitro*. Serially diluted BV and its bioactive peptides at noncytotoxic concentrations were tested for sperm immobilization by Sander-Cramer assay. The sperm viability test and the hypo-osmotic swelling were used to check the vitality and the integrity of the sperm membrane. Sperm morphological alterations were evaluated by a light microscope and scanning electron microscope. Results showed that BV at 3.0 µg/ml concentration was significantly immobilized and killed 100% (1.0 million) of the human sperm within 20 seconds. Similarly, the small peptide MLT showed immediate and complete sperm immobilization at 6.7 µg/ml. Moreover, apamin induced a moderate effect on sperm motility at 10.0 µg/ml, whereas mast cell degranulating peptide showed no effect on sperm motility up to 10.0 µg/ml. In conclusion, the current study indicates that BV and MLT have a spermicidal effect and exert their spermicidal activity through a direct lysis effect on the plasma membranes of sperm heads.

Keywords: Spermicides; Human sperm; Sperm motility; Bee venom; *Apis mellifera*; Melittin

1. Introduction

The world population is awaited to become more than 9.0 billion by 2050 [1]. However, more than 80% of the people will be in developing countries [2]. Thus, the current scenario of the world's population looks worrisome. Contraception combined with tough family planning programs is recommended as a way to confront this devastating problem. Therefore, there is an instant need for alternatives that are allowing women to avoid or delay their pregnancy [3]. Because of the unwanted side effects, and the failure rate or irreversibility resulting from the development several of contraceptive approaches, the acceptance of these methods was frequently restricted [4]. Currently, detergents are used as contraceptives. The main disadvantage of using surfactants is the effect of the detergent on the epithelial cells and normal vaginal microflora [5-9]. Accordingly, frequent use of

surfactants is correlated with vaginal and/or cervical ulceration [10]. This makes it susceptible to sexually transmitted diseases (STDs) [7]. Nowadays, researchers focused on natural products to find better alternatives with double characteristics effects for contraception and protection against STDs. In the past decade, natural products accounted for more than 25% of new drug models [11]. Animal venoms are highly rich natural sources of bioactive compounds that display comprehensive pharmacological activities [12-15]. They are rich in a variety of antimicrobial substances [16]. Hymenoptera venom therapy was performed a long time ago [17]. The BV is medically important due to the presence of numerous bioactive peptides such as, adolapin, apamin, mast cell degranulating peptide (MCD) & melittin (MLT), enzymes including phospholipase A2 (PLA2) [18, 19] and flavonoid components such as chrysin and pinocembrin [20, 21]. As a result, the BV showed

diverse biological activities as antiviral [22-24], antimicrobial [25, 26], and anti-inflammatory [19, 27-30]. The medical importance of BV is not just due to the property shown above, but also due to its allergenicity. Honeybee venom can induce allergenic properties ranging from local reactions to even death. To date and according to our knowledge, there is no information about the effects of BV on human sperm motility or viability. Therefore, the study has been designed to assess the possible action of several doses of the BV or its common purified peptides on the human sperm after *in vitro* phenotyping.

2. Materials and methods

2.1. Bee venom and peptides

Bee venom was purchased from the Faculty of Agriculture, El-Arish University, Egypt. Apamin, MCD, and MLT were purchased from Sigma (Sigma–Aldrich). BV and its peptides were dissolved in distilled water at a concentration of 1.0 mg/ml.

2.2. Reagents

The non-ionic detergent N-9 (IGEPAL CO-630) and pentoxifylline (PX) were purchased from Sigma (Sigma–Aldrich). FertiCult™ IVF, Sil-select stock, ALL Grad Wash, Vital Screen, Vitality stain, the eosin-nigrosin staining, and Spermac Stain were purchased from FertiPro (Beernem, Belgium). GM501 was purchased from SpermMobil (Gynemed, Lensahn – Germany). Ham's F-10 was purchased from cGMP-compliant (Grand Island, New York).

2.3. Human spermatozoa

Human semen samples were collected into a sterile cup for *in vitro* assays. Samples were obtained by masturbation from volunteers, and fertile donors, aged between 23 and 35 years at the centre Al Riyadh for Fertility and Reproductive Health Care, Giza, Egypt. The study protocol was accepted by the Research Committee Faculty of Science Al-Azhar University, Assiut, Egypt No: 5/2021. All the contributors were written informed consent to enrol in the study. Samples account above 100 million/ml, motility above 50% and viability above 60% with normal morphology were used for *in vitro* analysis. The samples were allowed to liquefy at 37°C for 20 min. The routine semen analyses of volume, pH, viscosity, and morphology were performed as per World Health Organization guidelines [31]. Briefly, human sperms were isolated by centrifugation by a discontinuous Sil-select gradient. Two millilitres of semen were carefully layered over a 45- 90% gradient of Sil-select and centrifuged at 1200 rpm for 20 min. Samples were resuspended and centrifuged again for

10 mins at 1200 rpm, and finally resuspended in (1.0 ml) ALLGrad Wash.

2.4. Assessment of spermicidal activity

The spermicidal test of the crude BV, MLT, apamin, and MCD were determined according to the modified Sander-Cramer method [32, 33]. In brief, tenfold serial dilution of BV and each peptide (protein equivalents) were prepared in physiological saline. Untreated sperm served as a control. A 10 µl sample of the sperm suspension (containing ~1 million sperm) was mixed with 90 µl of each dilution of BV or peptide in a microtube. Sperm motility was examined under a phase-contrast microscope Nikon Eclipse E200, Japanese (400x) after 20 s and 30 mins intervals for two hrs incubation after treatment and counted for motile spermatozoa. Commercial N-9 (100 µg/ml) was used as the positive control. The effective 100% inhibitory concentration (EC100) and the effective 50% inhibitory concentration (EC50) for immobilization of human sperm was calculated by SPSS software.

2.5. Hypo-osmotic swelling test

Control and BV-treated (3.0 µg/ml) human sperms were exposed to hypo-osmotic swelling (HOS) solution (1.351 g fructose, 0.735 g sodium citrate dehydrate combine in 100 ml of dH₂O) and incubated at 37 °C for at least 30 min. To assess changes in sperm membrane integrity a drop of the mixture was placed on a glass slide, stained, and covered with a coverslip [34]. The number of spermatozoa that displayed distinguishing tail curling was counted under a phase-contrast microscope Nikon Eclipse E200, Japanese (1000x). At least 200 spermatozoa were counted.

2.6. Sperm revival test

Spermatozoa were incubated with BV (3.0 µg/ml) for one hr at 37°C. To detect a reversal of sperm motility, the human sperms were washed twice in physiological saline and incubated once again in an ALL-Grad Wash® at 37°C for one hr. Immotile sperms were screened and the progressive motility to vibratory movement during or after incubation was considered revived.

2.7. Sperm viability test

The viability of sperms was evaluated by using the supravital staining (eosin-nigrosin) technique. Dead spermatozoa stained red or pink and live spermatozoa showed green in color. In brief, BV-treated (0.0003, 0.003, 0.03, 0.3, 3.0 and 30 µg/ml) and untreated human sperm samples were subjected to sperm viability evaluation under a phase-contrast microscope (400x). At least 200 spermatozoa were counted.

2.8. Pentoxifylline supplementation

Control and BV-treated (3.0 µg/ml) sperms were incubated in a CO₂ incubator (5%) at 37°C for two hrs. During incubation, 500 µg/ml of pentoxifylline was added to the medium at different time points. In another set of experiments, BV was premixed with pentoxifylline and then sperms were added to the mixture at different time points. The change in sperm motility was recorded after 20 s and then at 30 mins intervals for two hrs incubation.

2.9. BV inactivation treatment

2.9.1. Proteinase K digestion

To explore the nature of the responsible compound (protein or non-protein) in BV that can induce such spermicidal activity, BV (3.0 µg/ml) was incubated with proteinase K (1.0 mg/ml) (Sigma–Aldrich) and heated at 56°C for one hr. Proteinase - treated venom was then incubated with human sperms and motility was assessed as described above.

2.9.2. Heat treatment

Crude BV (3.0 µg/ml) was cooled at 4°C or heated at 95°C for 30 mins. Treated BV was mixed with sperms and subjected to mobility assay as described above.

2.10. Sperm morphological study

2.10.1. Light microscope

Treated human sperms with BV or MLT were subjected to morphological examination. The study of morphological human sperm was achieved using Spermac Stain (FertiPro NV Industriepark Noord 32, 8730 Beernem, Belgium). Stained sperms were examined under a phase-contrast microscope (1000 x) using oil immersion. At least 100 spermatozoa were counted.

2.10.2. Electron microscopic preparations

A scanning electron microscope (SEM) was performed for sperm morphology as previously described by [35]. In brief, BV or MLT-treated sperms were quickly fixed in 0.1 M phosphate-buffered fixative solution overnight at 4°C. Sperms were then washed in 0.1 M phosphate buffer, fixed for one hr in 0.1 M phosphate buffer containing 1% osmium tetroxide, and finally washed by the same buffer (3 times). Dehydrated samples in ascending series of ethyl alcohol were smeared, mounted on the coverslip, and dried for two hrs. The coverslip was coated with gold and examined by SEM (JSM-5600) at the electron microscope unit, Faculty of Agriculture, Mansoura University, Egypt.

2.11. MTT assay

The human vaginal epithelial cell line (ATCC PCS-480-010) was used for the evaluation of the cytotoxicity of BV, MLT, and N-9. Vaginal epithelial cells (cells density 1.2 – 1.8 × 10,000 cells/well) were seeded in triplicate of a 96-well plate and incubated for 24 hrs in a 5% CO₂ incubator and then treated with serial dilutions of BV, MLT, or N-9. MTT was added to the culture medium and incubated for five hrs. At the end of incubation, the supernatant was removed and DMSO was added. The absorbance was measured using a plate reader at a wavelength of 570 nm. The 50% cytotoxic concentration (CC50) was calculated by regression analysis.

2.12. Statistical analysis

Data are presented as mean ± SEM taken from two independent experiments. To calculate the statistical significance, Student's *t*-test was used. *P* < 0.05 was considered significant.

3. Results

3.1. BV inhibits human sperm motility

The effect of BV on sperm motility was determined according to the modified Sander-Cramer method. BV diminished human sperm motility in a dose-dependent manner (Figs. 1a and 1b). The motility of the BV-treated spermatozoa was found to range from 0-80 % of control motility levels. All the BV concentrations tested (0.0003, 0.003, 0.03, 0.3 and 3.0 µg/ml protein equivalents) decreased motility compared to the control sample. The differences in the proportion of motile spermatozoa in all samples at different time points were found to be significant (*P* < 0.05) compared to control at 0.3 µg/ml. However, the differences were highly significant (*P* < 0.0001) at 3.0 µg/ml. The EC50 value or the amount of BV required to reduce the motility of spermatozoa by 50% in 20 s was calculated to be 1.7 ± 0.06 µg/ml (Fig. 1 b). Revival of motility was not noticed after subsequent two hrs incubation in Ham's F-10 medium at 37°C. Thus, these results indicated that BV possesses an immobilizing factor(s) that reduced motility.

3.2. BV affects sperm membrane integrity as evidenced by the HOS response test.

Under phase-contrast microscopy nontreated (control) human sperm were examined and showed swollen head and tail curling indicating a functionally intact membrane (Fig. 2 a, top). Upon exposure to BV (3.0 µg/ml), all sperms showed straight tails as a sign of non-HOS response (Fig. 2 a, middle). Approximately 94.5% of control human sperms were responsive to HOS, whereas all sperms treated with BV were completely non-HOS responsive (Fig. 2 b).

This suggests that after BV treatment, the overall loss of sperm-membrane physiology led to a weak response of the human sperm membranes to HOS responsiveness.

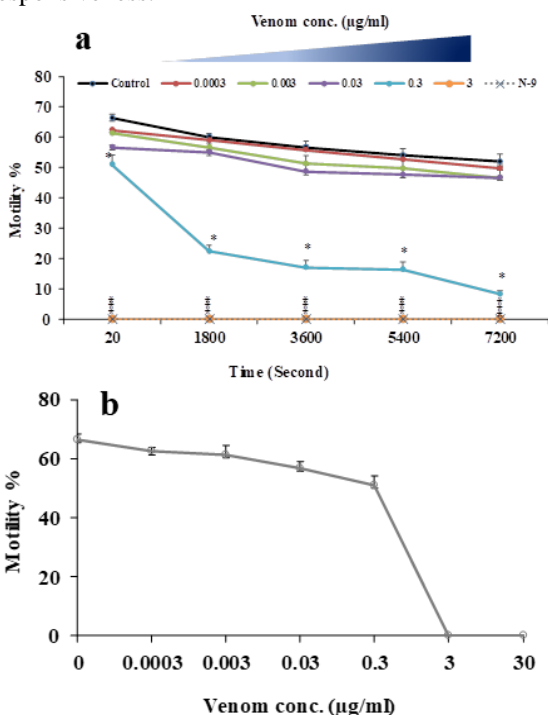


Fig. 1. Screening of sperm immobilizing concentration of the BV. (a) Human sperms were treated with ascending concentrations of the crude venom BV (0.0 - 3.0 µg/ml) at different time points (20 s up to two hrs). (b) Human sperms were treated with ascending concentrations of the crude venom BV (0.0003 - 3.0 µg/ml) for 20 s. N-9 (100 µg/ml) was used as the positive control. The data represents the mean \pm SEM of five independent experiments. *** $P < 0.001$, * $P < 0.05$

3.3. BV affects sperm viability

To check whether BV affects sperm viability, the sperms were mixed with BV (0.0003, 0.003, 0.03, 0.3, 3.0, and 30 µg/ml), examined after 20 s, and counted for vital spermatozoa. Untreated sperms were used as control. The viability of the sperms decreased immediately to zero after mixing with 3.0 or 30 µg/ml BV and remained the same over time (Figs. 3a and 3b). These results indicated that BV at the indicated concentrations could kill the sperm instantaneously.

3.4. Pentoxifylline could not enhance human sperm motility in the presence of BV.

Pentoxifylline is known to increase the motility of ejaculated spermatozoa *in vitro* from both normal and asthenozoospermic samples. To check whether pentoxifylline can enhance the motility of human

sperm in the presence of BV, pentoxifylline was added to the sperm/BV mixture at several time points (Fig. 4). Results indicated that pentoxifylline could not enhance the motility of human sperm in the presence of BV, confirming the spermicidal action of BV.

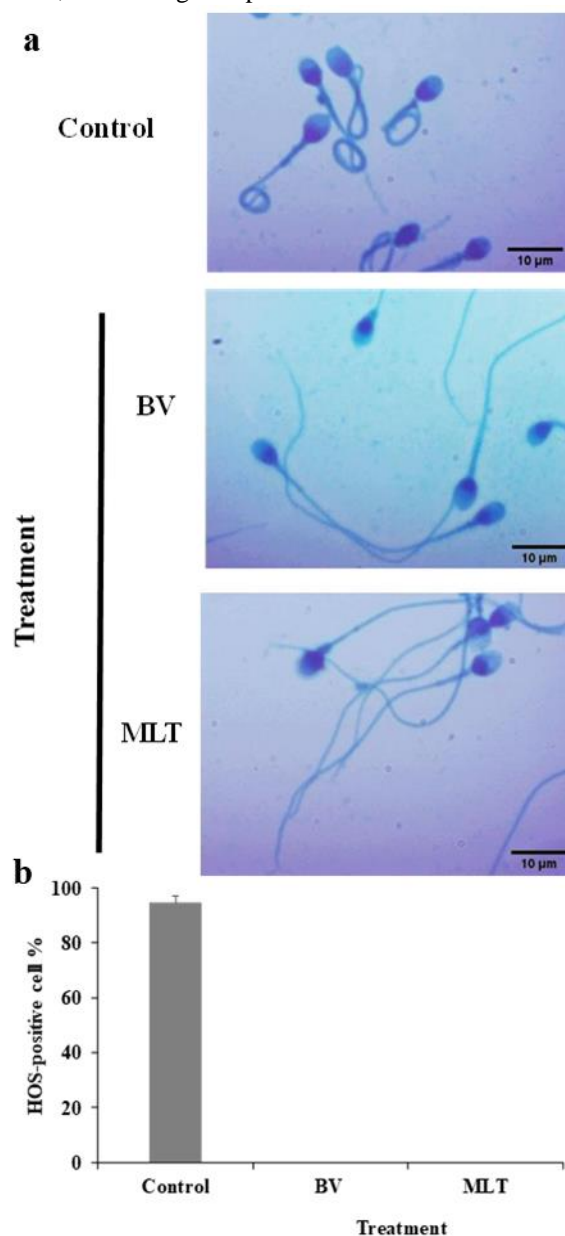


Fig. 2. Effect of BV and MLT on the HOS responsiveness of the human sperm. Control and BV (3.0 µg/ml) or MLT (7.0 µg/ml) treated human sperm were exposed to HOS solution for at least 30 mins at 37°C. The number of sperms displays distinguishing tail curling was counted. Control sperms display distinguishing tail curling however BV and MLT-treated sperms were non-HOS responsive (a). Over 90% of control sperms were responsive to HOS, whereas all sperms treated with BV or MLT were non-

HOS responsive (b). The data represents the mean \pm SEM of three independent experiments. §: below the detection limit.

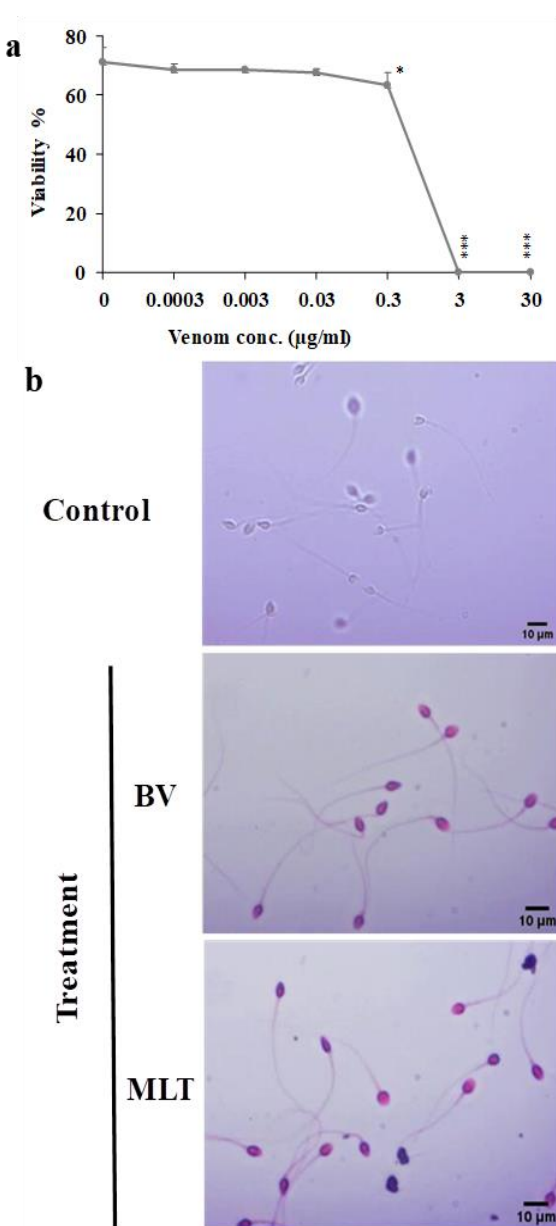


Fig. 3. Effect of BV and MLT on sperm viability. The sperm viability was assessed by the eosin–nigrosin staining technique. Sperms were treated with tenfold ascending concentrations of the crude venom BV (0.0–30.0 µg/ml) for 20 s or left untreated as a control (a). The data represents the mean \pm SEM of five independent experiments BV-treated (3.0 µg/ml) or MLT-treated (7.0 µg/ml) spermatozoa showed red or pink color indicating death and control showed live spermatozoa green in color (b). *** $P < 0.001$, * $P < 0.05$

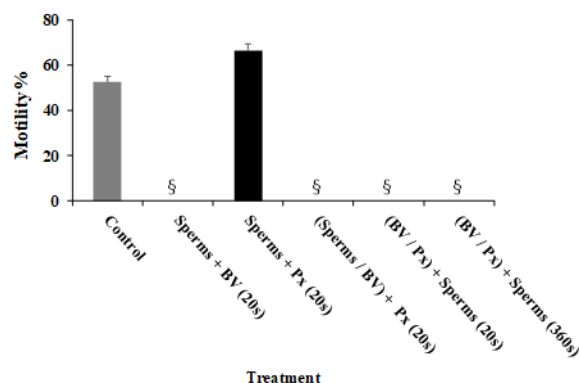


Fig. 4. Effect of PX addition on the spermicidal action of BV. The ability of PX to improve the motility of human sperm in the existence of BV (3.0 µg/ml) was checked. PX was added to the sperm/BV mixture or mixed with BV at the indicated time points. In all experiments, PX could not enhance the motility of human sperm in the presence of BV, confirming the spermicidal action of BV. Data represents mean \pm SEM. of the data obtained from two independent experiments. §: below the detection limit.

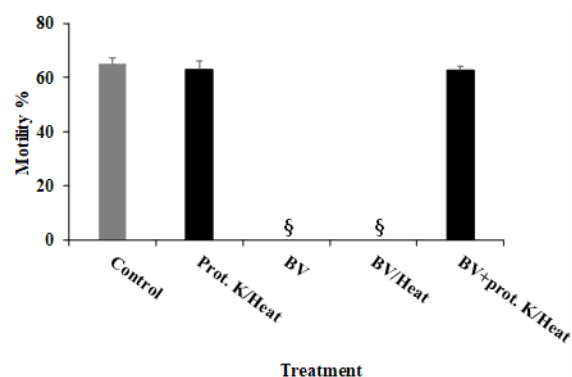


Fig. 5. Effects of protein digestion on the BV spermicidal action. BV (3.0 µg/ml) was treated with proteinase K. Obviously, treatment of BV with proteinase K prevents its activity against sperm motility. Additionally, sperms were either incubated with pre-heated proteinase K or heated at 56°C for one hr. Heated proteinase K or heat by itself did not affect sperm motility. Data represents mean \pm SEM of the data obtained from two independent experiments. §: below the detection limit.

3.5. Protein digestion abolishes BV spermicidal activity

Bee venom contains a mixture of constituents such as non-peptide, peptides, components, and enzymes [36]. To figure out the nature of the compound (s) (i.e. proteins or non-protein) that are responsible for the spermicidal activity, BV (3.0

$\mu\text{g/ml}$) was incubated with proteinase K (1.0 mg/ml) at 56°C for one hr. The proteinase K-treated BV was mixed with human sperm for one hr at 37°C and the motility of human sperm was assessed. Results indicated that proteinase K, heated at 56°C did not affect sperm motility by itself. Moreover, human sperms showed normal motility as same as untreated sperms (control) after incubation for one hr with proteinase K pre-treated BV (Fig. 5). These results indicated that protein elimination abrogated the BV spermicidal activity.

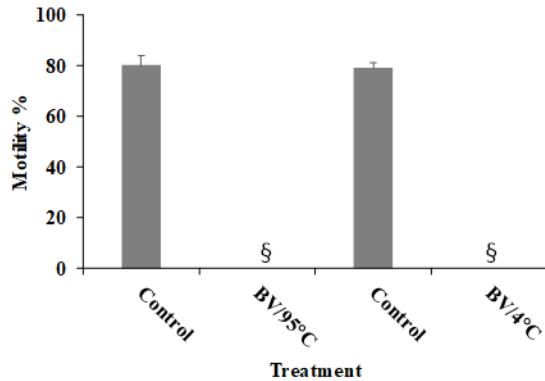


Fig. 6. Effects of different temperatures on the spermicidal action of BV. $3.0 \mu\text{g/ml}$ of BV heated at 95°C for 30 mins or cooling at 4°C for 30 mins. Treated BV was incubated with sperms and motility was checked within 1 min. Pre-heated or pre-cooled BV showed spermicidal activity. Data were obtained from two independent experiments and represented as mean \pm SEM. §: below the detection limit.

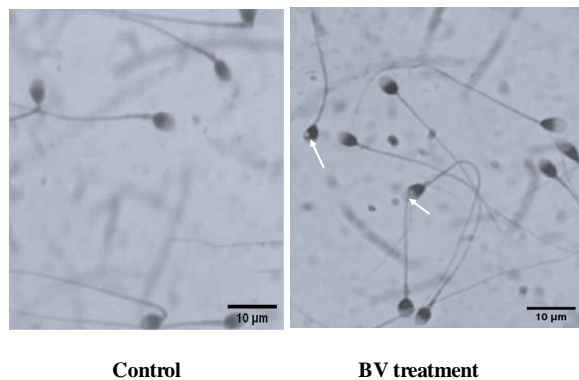


Fig. 7. Effect of BV treatment on sperm morphology by light microscope. Sperms were treated with $3.0 \mu\text{g/ml}$ of BV for 20 s - 60 min, untreated sperms served as control. When examined by light microscope and staging method, untreated control group sperms showed normal morphology with oval heads and intact plasma membranes. In contrast, in the BV-treated group, some gaps (vacuoles) were observed -indicated by white arrows- in the head of $\sim 40\%$ of sperms.

3.6. BV spermicidal responsible factor is not heat sensitive.

It is known that enzyme activities are temperature-dependent. We decided to check whether the temperature has any effect on BV spermicidal activity. BV ($3.0 \mu\text{g/ml}$) was treated with (i) heating at 95°C for 30 mins, and (ii) cooling at 4°C for 30 mins. BV pre-heated at 95°C or pre-cooled at 4°C for 30 mins was mixed with human sperms and incubated for 30-60 mins. No motile sperms were detected during incubation either with pre-heated or pre-cooled BV (Figs. 6 a and 6b). This indicates that the activity of a bioactive compound that affects human sperm motility in BV is not heat-sensitive.

3.7. BV induces defects in human sperm morphology

To check the effect of BV on human sperm morphology. Human sperms were incubated with $3.0 \mu\text{g/ml}$ of BV for 20 s - 60 mins. When examined by light microscope and staining method, no morphological changes were found in the tail or midpiece but some gaps (vacuoles) in the head of 43% of sperms at 20 s exposure (Fig. 7 right). However, when examined with SEM, most sperm heads showed an irregularity, lysis, and partial removal of the plasma membrane's external surface. After a longer incubation time of one hr, vacuoles, broken plasma membranes, and damaged acrosomal caps were observed ranging from holes and vacuoles to complete fragmentation in most sperms (Fig. 8).

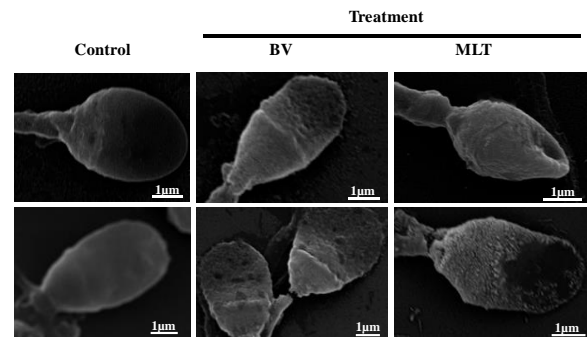


Fig. 8. Effect of BV and MLT treatment on sperm morphology by SEM. Sperms were treated as indicated above. When examined by SEM, untreated spermatozoa showed intact heads and tail regions. On the contrary, BV ($3.0 \mu\text{g/ml}$) and MLT ($7.0 \mu\text{g/ml}$) treated groups showed irregular folding, lysis, and partial distraction of the plasma membrane in the head region.

3.8. Spermicidal activity of BV is mediated by MLT

Following, we tested the action of commonly active peptides in BV, such as apamin, MCD, and

MLT. Of each peptide, 10.0 $\mu\text{g/ml}$ was added individually to the washed sperms and incubated at 37°C for one hr, and mixtures were checked for sperm motility. Results showed that MLT can inhibit sperm motility. Apamin has a mild effect (reduce motility) on sperm motility. Whereas MCD does not affect sperm motility (Fig. 9 a & Table 1). Tenfold serial dilutions of MLT were prepared and incubated with washed sperms. A substantial dose-dependent manner of spermicidal activity of MLT was noticed (Fig. 9 b) with EC_{50} being $3.3 \pm 0.12 \mu\text{g/ml}$. Accordingly, human sperms treated with MLT (7.0 $\mu\text{g/ml}$) were non-HOS responsive (Fig. 2). In addition, the SEM of MLT-treated sperms showed similar defects as that observed in BV-treated sperms. When incubation extended to one hr, approximately 90% of sperms' midpiece region were separated from the sperm head, and fragmented plasma membrane with an injured acrosomal cap occurred in most sperms as well (Fig. 8).

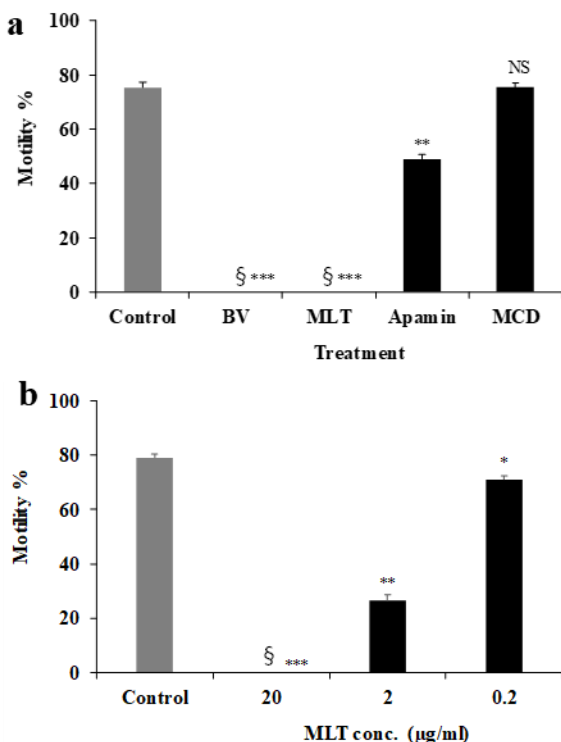


Fig. 9. Effect of apamin, MCD, and MLT peptides on sperm motility. A Fixed concentration (10 $\mu\text{g/ml}$) of each peptide; apamin, MCD, MLT, or BV (3.0 $\mu\text{g/ml}$) was mixed with human sperms for 20 s and then the sperm motility was checked. MLT inhibited sperm motility, apamin moderately reduced sperm motility while MCD did not affect sperm motility (a). Tenfold dilutions of MLT (20, 2, and 0.2 $\mu\text{g/ml}$) were prepared and mixed with human sperms for 20 s, and then the sperm motility was checked. A dose-dependent

manner of spermicidal activity of MLT was noticed (b). Data represents mean \pm SEM of data obtained from two independent experiments. §: below the detection limit. *** $P < 0.001$, * $P < 0.01$, $P < 0.05$, $NS > 0.05$

Table 1. Spermicidal potential, cytotoxicity and selectivity index of BV, MLT, apamin, MCD and N-9.

Treatment	EC_{100} ($\mu\text{g/mL}$)	EC_{50} ($\mu\text{g/mL}$)	CC_{50} ($\mu\text{g/ml}$)	SI	Safety versus N-9
BV	3	1.7	74.29	43.7	20.8
MLT	6.7	3.3	21.6	6.54	3.11
Apamin	>5.0	>5.0	NA	NA	NA
MCD	>5.0	>5.0	NA	NA	NA
N-9	486.82	78.34	163.7	2.1	1

EC_{50} ; 50% effective concentration.
 CC_{50} ; 50% cytotoxic concentration.
 SI; Selectivity index ($\text{CC}_{50}/\text{EC}_{50}$).

3.9. BV and MLT showed no detectable cytotoxicity to the human vaginal cell line (PCS-480-010) at EC_{100} concentration.

The human vaginal epithelial cell line (ATCC PCS-480-010) was treated with serial dilutions of BV, MLT, or N-9 to evaluate their cytotoxic effects using an MTT assay. CC_{50} was calculated for the three tested compounds. Results revealed that CC_{50} of BV, MLT, and N-9 against cells was 74.29 ± 4.04 , 21.6 ± 3.09 , and $163.7 \pm 8.86 \mu\text{g/ml}$ respectively. SI was also calculated and indicated that BV and MLT have higher SI compared to N-9 (Table 1).

4. Discussion

Previous reports showed that BV had shown useful anti-cancer activity against prostate and ovarian cancer, as well as anti-viral potential against HIV and HCV [37-41]. In the present study, we investigated the extent to which BV could affect fresh sperm functional parameters, i.e., motility and vitality. Our data showed that incubation of human spermatozoa with crude BV decreased sperm motility and increased dead spermatozoa with increasing venom concentration and time of incubation. These changes in sperm motility and viability were accompanied by severe deformation of spermatozoan plasma membranes. Interestingly, BV at a noncytotoxic concentration caused markedly immobilization of all human sperm motility immediately (20 s). Most probably BV can induce human sperm death due to distraction from the integrity of the plasma membrane which is a natural and indispensable matter for effective sperm movement during transport and fertilization. The plasma membrane is a highly important structure for the physiology of sperm. It is mainly responsible for

the fusion with the outer acrosomal membrane and controlling calcium influx during the sperm cell and the oocyte binding [42]. An intact plasma membrane acts as a barrier between the extracellular and intracellular medium and preserves cellular osmotic balance. Therefore, membrane injuries would lead to cell death [42]. The plasma membrane also plays a critical role during acrosome reaction by making a continuous membrane structure during fertilization [43]. The notion of the sperm membrane functional integrity was lost upon exposure to BV is supported by the observation of the HOS test, the supravital staining technique (eosin-nigrosine), and SEM. The latter showed irregular plasma membranes, lysis, and partial removal of the plasma membranes external surface of most sperm heads. All these observations support that BV can disrupt sperm membrane integrity leading to loss of sperm motility and death. Several animal venoms were tested against human sperms to find new compounds that can regulate sperm physiology including sperm motility. In this regard, dermaseptins (DSs) were isolated as an antimicrobial peptide from the skin of a South American frog *Phyllomedusa sauvagei* [44]. Snake venoms of *Oxyuranus scutellatus* and *Daboia russellii* were found to be highly potent inhibitors of sperm motility [45]. This is most probably due to snake venoms being rich in neurotoxins and phospholipase enzymes. The effects are mainly induced by high K^+ in the venom suggests that direct stopping of K^+ currents alters the properties of the plasma membrane, leading to the entry of Ca^{++} [46]. The main common components of BV are PLA2, MLT, adolapin, and apamin [18, 19, 28]. Therefore, we assessed the spermicidal activity of apamin, MCD, and MLT. An MLT showed an almost similar effect as crude BV and seems to be the responsible factor for the BV spermicidal activity. Previous studies proposed that MLT affected the lipid organization of the viral envelope during interaction with the phospholipid bilayer [47, 48]. Furthermore, MLT may produce pores in the cell membranes through which the contents could diffuse [49] or creates ion-permeable channels in the pathway of the voltage-gated 'pore' [50]. MLT hinder transport pumps, such as $(H^+ + K^+)$ ATPase [51] and $(Na^+ + K^+)$ ATPase [52] and accelerate the permeability of cell membranes to ions. It induces aggregation and inhibition of membrane proteins [53, 54], and also MLT stimulates the activity of phospholipase A2 [55]. Plasma membrane lipid composition is directly associated with sperm motility [56]. Fluidity of the sperm membrane which is crucial for sperm motility providing is maintained mainly by cholesterol and polyunsaturated fatty acids [57, 58]. Additionally, human sperm motility positively correlates to the presence of docosahexaenoic fatty acid in the sperm membrane [59]. Accordingly,

asthenozoospermic sperm cells in men were found to contain low concentrations of such fatty acid [56]. BV and MLT cytotoxic effects on human vaginal epithelial cell line *in vitro* culture were assessed. In this connection, EC100 of both BV and MLT showed potent and immediate sperm immobilizing properties without affecting the viability of the human vaginal epithelial cell line for up to 24 hrs. It is worth mentioning that the cytotoxicity effects of BV and MLT on the human vaginal epithelial cell line was significantly lower than that of N-9. Interestingly, the selectivity index of crude bee venom is much higher than that of melittin. In addition, the EC_{50} is twice as low. This suggests that either there is a different spermicidal agent in bee venom of greater potency and selectivity, or there is some synergistic effect.

Conclusion

Throughout this study, we demonstrated that BV could quickly and irreversibly immobilize sperms at low and noncytotoxic concentrations. The spermicidal action seems to be by disrupting the plasma membrane integrity. The main player, which is engaged in such a spermicidal effect is MLT.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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تأثير سم نحل العسل "أبيس ميليفرا" ومكونه الرئيسي ميليتين في قتل الحيوانات المنوية

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الملخص:

الغرض الرئيسي من هذه الدراسة هو إيجاد مركبات قادرة على تثبيط حركة الحيوانات المنوية بشكل فعال , في هذه الدراسة قمنا بدراسة تأثير السم الخام لنحل العسل و بعض من مكوناته الرئيسية مثل الميليتين, الأبامين والام سي دي على حركة الحيوانات المنوية للإنسان. تم جمع عينات السائل المنوي من الذكور المتطوعين الذين ليس لديهم ايه مشاكل تتعلق بالخصوبه في كوب معقم وتراوحت أعمارهم بين 23 و 35 سنة بمركز الرياض للخصوبة وعقم الإنجاب والرعاية الصحية. (الجيزة , القاهرة .مصر). تم أخذ عينات السائل المنوي عن طريق الاستمناء بعد 3-5 أيام من الامتناع عن الجماع. وفقاً لمنظمة الصحة العالمية لتحليل السائل المنوي تم تحديد اختبار الحيوانات المنوية المعالجة بالسم أو المركبات المعزولة وذلك بطرية سانر كرايمر . تم فحص حركة الحيوانات المنوية بعد إضافة سم النحل الخام بتركيزات متصاعدة على مدار فترات زمنية 20 ثانية و 30 دقيقة حتى 120 دقيقة أثناء فترة حضانة الخليط عند درجة حرارة 37 درجة مئوية وتم رصد وعد الحيوانات المنوية المتحركة والغير قادرة على الحركة. أظهرت النتائج قدرة سم النحل عند تركيز 3 ميكروجرام/ مللى على قتل جميع الحيوانات المنوية أثناء الفحص فى أقل فترة زمنية و هى 20 فانية. تم استخدام اختبار حيوية الحيوانات المنوية والانتفاخات للتحقق من حيوية وسلامة غشاء الحيوانات المنوية. كما تم تقييم التغيرات المورفولوجية للحيوانات المنوية بواسطة المجهر الضوئي والمجهر الإلكتروني الماسح والتي أظهرت قدرت سم النحل على تدمير الغشاء البلازى لرءوس الحيوانات المنوية. وللكشف عن اى هوية المركب الفعال فى سم النحل تم اجراء سلسلة من التجارب التى اشارت الى وجود مركب بيبتيدي غالباً هى المسئول عن تلك التأثيرات ومن ثم فقد اظهر البيبتيد الصغير الميليتين تثبيطاً فورياً وكاملاً للحيوانات المنوية عند 6.7 ميكروجرام / مل و تسبب الأبامين في تأثير معتدل على حركة الحيوانات المنوية عند 10.0 ميكروجرام / مل ، بينما لم يظهر البيبتيد ام سي دي أي تأثير على حركة الحيوانات المنوية حتى 10.0 ميكروجرام / مل. في الختام، تشير الدراسة الحالية إلى أن سم النحل والمركب النشط الرئيسي المكون له -الميليتين- لهما تأثير قاتل للحيوانات المنوية ويمارسان نشاطهما في قتل الحيوانات المنوية من خلال القدرة على تحليل مباشر لأغشية البلازما فى رؤوس الحيوانات المنوية.