Embryotoxicity and Teratogenicity of Honey Bee (*Apis mellifera*) Venom in Pregnant Female Mice during Organogenetic Period

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Abstract
Bee venom has a broad spectrum of therapeutic and cosmetic applications. However, the potential embryotoxicity and teratogenicity of bee venom exposure to women during pregnancy is unknown. Therefore, the aim of the present study was to investigate the effect of bee venom exposure on developing mice embryos during organogenetic period. Bee venom treatment was giving to pregnant female Swiss albino mice on three consecutive days: 12, 13, and 14 of pregnancy. The bee venom sting group was stung directly by one live honeybee; the bee venom injection group was injected subcutaneously with 200 µL of diluted bee venom; the normal control group was left without any treatment, and the vehicle control group was injected with 200 µL of physiological saline solution. All animals were dissected on the 18th day of pregnancy. The results of this study showed that bee venom exposure during pregnancy in mice led to a significant decrease in the number of fetuses, an increase of fetal atrophy, and appearance of congenital malformations. Bee venom has a harmful potential for embryotoxicity and teratogenicity and its use during pregnancy should be avoided.

Key words: Bee venom, pregnant mice, embryotoxcity, teratogenicity

Introduction
Bee venom of *Apis mellifera* is a bitter, slightly acidic, lucid, and odor less liquid produced by the worker honey bee [1, 2]. Bee venom is composed of complex mixture of peptides (melittin, apamin, adolapin, and mast-cell-degranulating peptide), enzymes (phospholipase A, hyaluronidase, phosphatases, and α-glucosidase), biogenic amines (histamine, dopamine, and norepinephrine), and other non-peptide components (carbohydrates, lipids, and free amino acids) [3-6]. Bee venom has been used by ancient civilizations of China, Egypt, and Greece [1, 4, 7, 8] as a therapeutic natural toxin to relieve pain and treat chronic musculoskeletal diseases, such as osteoarthritis, rheumatoid arthritis, and lumbar pain [3, 5, 6, 9, 10]. This practice has become known as Bee venom therapy (BVT) [3, 5, 6].

BVT is the application of live honey bee stings or purified bee venom injections to patients for therapeutic purposes. BVT is one of the most common therapeutic methods in complementary and alternative medicine. Recently, pharmacological studies have demonstrated that bee venom has a broad spectrum of therapeutic properties including analgesic [11], anti-inflammatory [12-14], anti-nociceptive pain medications[15-17], anti-apoptotic [18, 19], anti-fibrotic [20], anti-atherosclerotic [21], anti-cancer [22-25], anti-bacterial [26, 27], anti-fungal [28, 29], anti-viral [30], anti-mutagenic [31, 32], radioprotective [33], neuroprotective [34, 35], and in the treatment of many skin diseases [36, 37]. Moreover, BV has been added to many skin care cosmetic products as the main

topical cosmetic component for the treatment of acne [38, 39], alopecia [37, 40], melanoma [41], psoriasis [42], wound [43, 44], wrinkles [45], and vitiligo [46, 47]. There are several available topical cosmetics containing purified BV (e.g. BV serum and BV ointment) in the market.

However, despite the broad spectrum of therapeutic and cosmetic applications of BV the potential embryotoxicity and teratogenicity of bee venom exposure to women during pregnancy is unknown. Hence, studies on the effect of bee venom on fetal malformations are of great importance. Therefore, the aim of the present study was to investigate the effect of bee venom exposure on developing mice embryos during organogenetic period (i.e. at the stage of active organogenesis) to the possible appearance of congenital fetal malformations when pregnant female mice are directly stung by honeybees or injected by diluted bee venom.

Materials and Methods

Bee Venom

Worker honey bees of *Apis mellifera* were obtained from honey bee apiaries maintained at the Faculty of Agriculture, University of Tripoli, Libya. The collected bees were kept in aerated glass jars and were used on the same day to sting pregnant females with the natural bee venom.

Lypholized *Apis mellifera* Bee venom powder was obtained from Al-Harith Center for Alternative Medicine (Abu Salim / Tripoli) which was purchased from VACSERA, Egypt. Stock solution for bee venom injection was prepared by dissolving 1 mg of bee venom powder in 7 ml of physiological saline (NaCl 0.9%). Bee venom working solution was prepared by taken 1 ml of stock solution and diluted with 4 ml of physiological saline and kept in the refrigerator at 4°C until used. 200µL of working solution was injected subcutaneously to pregnant females, simulating the normal bee sting.

Ethical Approval

The experimental procedures in this study were performed according to the bioethical research guide established by the Libyan National Committee for Biosafety and Bioethics; which comply with the published guide “Principles of laboratory animal care” [48].

Animals

This study was conducted on female Swiss albino mice between the ages of 8-10 weeks and weighing between 20-25 gm. The mice were raised in a special room in the Zoology Department / Faculty of Science / University of Tripoli. They were placed in special plastic cages for raising laboratory animals, under adequate living conditions and access to standard rodent pellet chow diet and water *ad libitum*.

Mating

Mating was carried out by placing two adult virgin female mice with an untreated normal adult male in a cage overnight (to two extra nights). In the morning, the females were examined for the presence of vaginal sperm plug (i.e. a visible whitish mass in the vaginal

opening which indicate successful copulation and confirms pregnancy). Females with a vaginal sperm plug were considered to be in gestation day 0 (Gd 0) or Day 0 of pregnancy. After mating, the females were separated from males and divided randomly into four experimental groups with an approximately equal mean body weight. Each pregnant female was housed separately in individual plastic cages.

Experimental Groups

This research was conducted on 16 pregnant female mice, which were divided at random into four experimental groups (4mice/group): the normal control, the vehicle control, the bee venom sting, and the bee venom injection group.

Treatments

Bee venom treatment was giving to pregnant female mice on three consecutive days, from Gd 12, 13, and 14 of gestation period (i.e. pregnancy). The bee venom sting group was stung directly by one live honeybee on a shaved area of the thigh (Fig. 1A). The bee venom injection group was injected subcutaneously with 200 µL of diluted bee venom on a shaved area of the thigh (Fig. 1B). The normal control group was left without any treatment, and the vehicle control group was injected with 200 µL of physiological saline solution. During the experiment, no deaths or toxicity signs among pregnant females were observed but some redness of the skin was noticed at the stinging or injection area. All experimental groups were dissected on the 18th day of gestation period. The animals were anaesthetized using diethyl ether and killed by cervical dislocation. A surgical incision was performed along the abdominal midline to expose the maternal peritoneal cavity to evaluate fetal development. The uterine horns were examined for the number of gestational sacs and fetuses. Both uterine horns were excised, placed on a clean Petri dish, opened lengthwise to expose the gestational sacs, and the fetuses were removed out of the uteri, externally examined for any gross malformations and photographed.

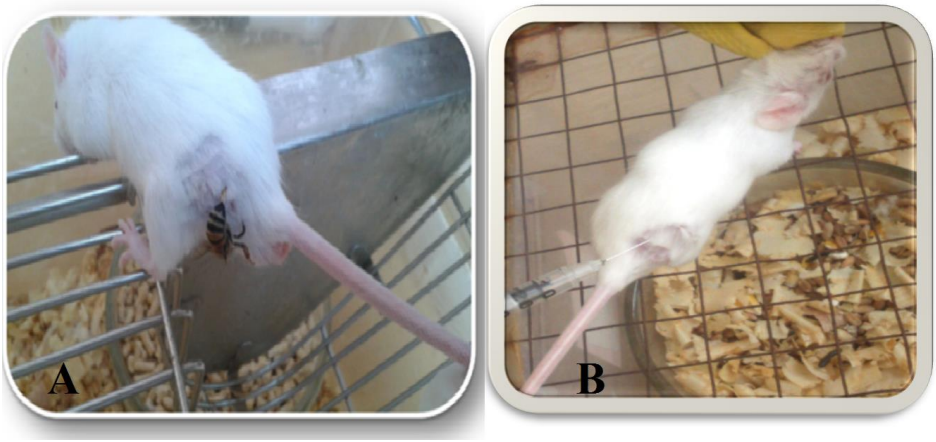

Figure 1. Bee venom treatment by a live bee sting (A) and by diluted bee venom injections (B).

Results

The results of this study showed that the bee venom of *Apis mellifera* had adverse toxic effect on embryonic development in mice. There was an apparent change on embryonic

developmental parameters such as number of live fetuses, number of atrophied fetuses and abortion in the bee venom treated groups compared to the control groups.

The normal and vehicle control groups showed normal pregnancy uteri (Fig. 2). Each uterine horn contained several gestational sacs (which appeared as a continuous chain of beads) with a live fetus in each sac. The fetus number per litter ranged from 9 to 11, the total number of fetuses in the normal control group was 45 and in the vehicle control group was 47, and no apparent fetal abnormalities were observed.

However, the bee venom sting group showed abnormal pregnancy uteri with few gestational sacs (Fig. 3A), or no gestational sacs just a swelling an indication of atrophied fetuses (Fig. 3B) or an empty uterus identified as abortion. The bee venom sting group exhibited a lower fetus number per litter compared with the untreated control groups which ranged from 0 to 3. The total number of fetuses was 10 and exhibited a large variation in their sizes (Fig. 4), as well as external fetal malformations such as an abnormal increase in the length of the tail, blood clotting under Skin, flattening of the skull, and limb defects (Fig. 4) especially forelimbs (Fig. 5A); two fetuses were totally deformed (Fig. 5B and 5C). Furthermore, in the bee venom injection group all females showed swollen uteri an indication of atrophied fetuses or abortion (Fig. 6).

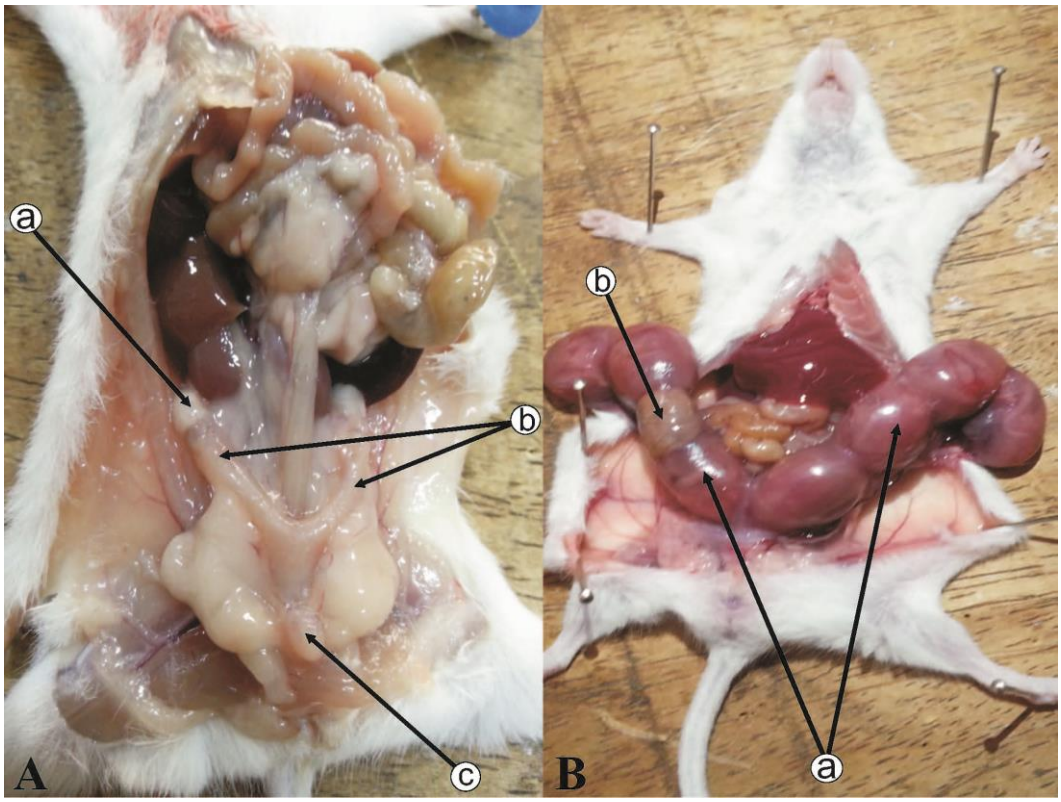

Figure 2. **(A)** Photograph of normal non-pregnant female reproductive organs showing ovary (a), uterus (left and right horns) (b), urinary bladder(c). **(B)** Normal control pregnant female uterus, showing a continuous chain of gestational sacs in the left and right uterine horns each sac contains a live fetus (a), dead fetus (b).

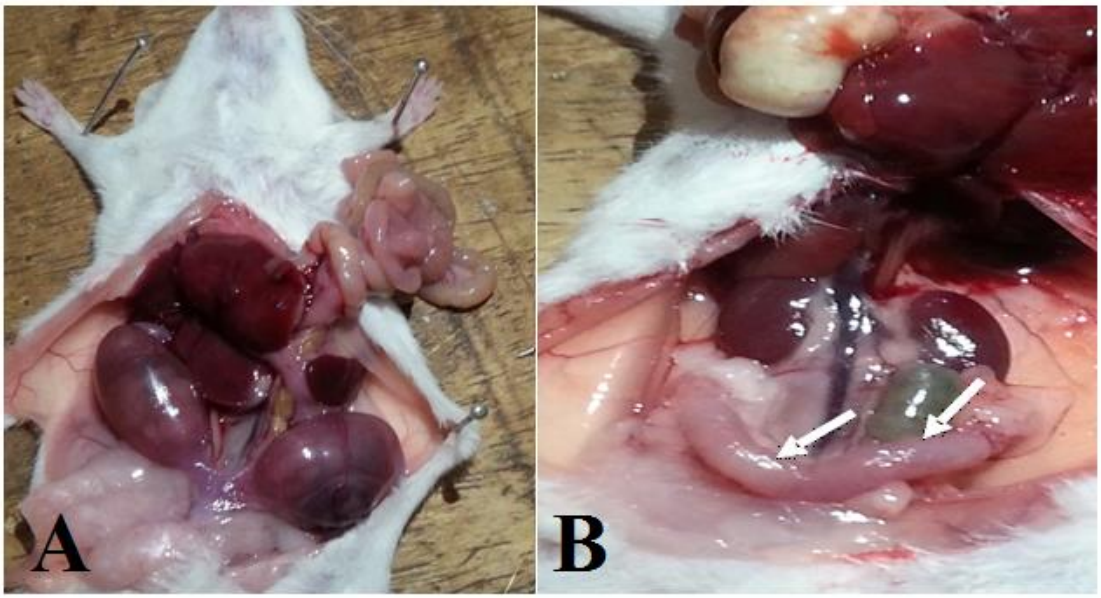

Figure 3. Photograph of bee venom sting pregnant female uteri. **(A)** Shows abnormal pregnancy where the left and right uterine horns each contain only one gestational sac. **(B)** Shows abnormal pregnancy and the left and right uterine horns are distended (white arrows) no gestational sacs (empty uterus) an indication of atrophied fetuses and abortion.

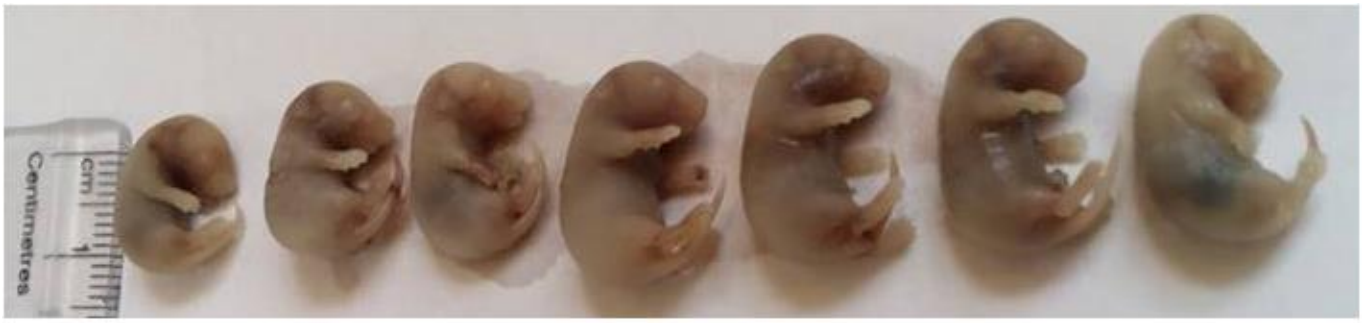

Figure 4. Photograph of fetuses of bee venom sting pregnant females showing large variations in their sizes and several external fetal malformations including abnormally long tail, flat skull, and deformation of limbs.

Figure 5. Photograph of some fetuses of bee venom sting pregnant females showing severe deformation of forelimb (A), severe body malformation (B), and total body malformation (C).

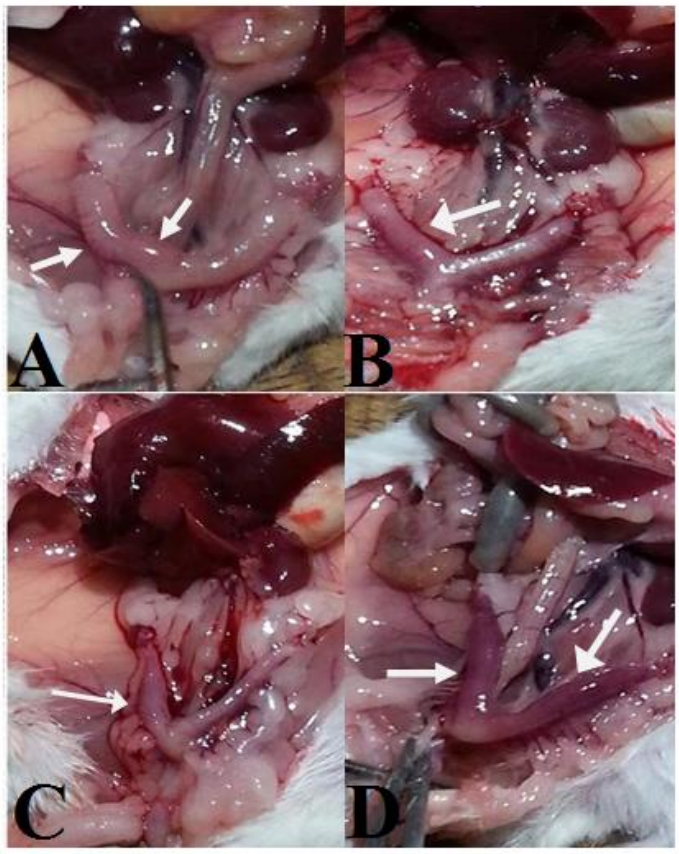

Figure 6. Photograph of bee venom injection pregnant females showing abnormal pregnancy uteri with no gestational sacs and presence of swelling (white arrows) an indication of fetal atrophy or empty horn uteri an indication of abortion.

Discussion

Studies on using bee venom for therapeutic and cosmetic purposes are very numerous. However, studies on the effect of bee venom on embryo development are very scarce; only one published study had investigated bee venom potential toxic effects on embryo development [49] the article is in Bulgarian with an English abstract. Therefore, this study investigated the potential risk of embryotoxicity and teratogenicity associated with the administration of bee venom to pregnant female mice. Bee venom was administered to pregnant female mice via bee sting or subcutaneous injections to determine the effect of bee venom on fetal development. The pregnant female mice were sacrificed on day 18 of pregnancy and the uterus and fetal development were examined.

The results of this study showed that treatment of pregnant female mice with bee venom during the period of organogenesis on days 12, 13, and 14 of pregnancy leads to fetal atrophy and the appearance of external abnormalities including abnormally long tail, flat skull, blood clotting under skin, and deformation in the limbs. These results provide evidence for bee venom embryotoxicity and teratogenicity. These results are in agreement

with the results published by [49] who previously reported similar embryotoxic effects of bee venom in rats. They found injecting pregnant rats with whole bee venom and its low and high weight molecular fractions daily between the 6th and 14th day of gestation resulted in autolysis (atrophy) of embryo and dead rats in the uterus of the treated animals; however, there were no teratogenic alterations. In addition, the low weight molecular fraction of the bee venom showed more manifestations of embryotoxicity.

Furthermore, bee venom potential toxic effects on embryo development correspond to the effect of other animal venoms such as scorpions and snakes. In a study conducted by Ismail et al. [50] on the effect of injecting scorpion *A. amoreuxi* venom into female rats on days 9, 10, and 11 of pregnancy resulted in a flattening of the skull and the absence of the first cervical vertebra. When females were injected with this venom on pregnancy days 7 to 14, there was total atrophy of all the fetuses. In another study by Spadacci-morena et al. [51] who injected snake *bothrops jararaca* venom into mice on day 8 of pregnancy resulted in increased appearance of atrophied embryos. Hmed et al. [52] injected rats with scorpion *Buthus occitanus* venom on day 7 to 13 of pregnancy, resulted in the emergence of embryos with organs of small sizes such as eyes, brain, kidney, spleen, tails, and the appearance of blood clots under the skin.

Moreover, the results of this study showed that the lypholized *Apis mellifera* bee venom powder diluted injections had a stronger effect than bee stinging venom, as all the subjects injected with the diluted powder solution led to total atrophy of the embryos; perhaps this is due to a difference in the ratio of the components of the bee venom powder compared to the natural stinging venom. Young and Roh [53] studied the analysis of components of natural bee venom and clinically used bee venom powder in different concentrations using High performance liquid chromatography (HPLC). They showed a difference in the components of natural bee venom (Bees) and venom powder (apitoxin). The components of bee venom have changed depending on the method of collection and concentration. Therefore, it is necessary to test the composition of the venom for its safe and effective use.

Conclusion

The results of this study indicate that bee venom exposure during pregnancy in mice led to a significant decrease in the number of fetuses, appearance of congenital malformations, and a higher incidence of fetal atrophy (autolysis). Therefore, bee venom could be considered embryotoxic because it induced fetal atrophy and decrease the number of fetuses and teratogenic because it induced malformations in mice fetuses. Bee venom has a harmful potential for embryotoxicity and teratogenicity and its use during pregnancy should be prohibited.

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Conflict of interest

The authors declare that there is no conflict of interest.

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