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The Effect of Honey bee Insect Body Extract on the Histological Structure and Sexual Ability in Male Rats

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Abstract
This study proved that honey bee worker insect body extract mixed with the food of male white rats by 10% led to an increase in the sexual ability and vitality of male white rats. The weight of the glands accessory to the male reproductive system increased from 0.09 mg to 0.11 mg for the seminal vesicle and from 0.10 mg to 0.18 mg for the prostate gland. There was no significant effect on the weight of the testicles and epididymis, but there was a narrowing in the diameter of the tail of the epididymis due to the increase in the number of animals. Sperm in the group treated with food mixed with honey bee body extract, where the increase in sperm count was, from 30.20 (X 106) to 37.20 (X 106), At the same time, this led to a reduction in congenital deformities in the tail and head of sperm from the normal state by 5%.

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Introduction

Insects have the potential to serve as healthy and sustainable alternatives due to their nutritional contents. Edible insects may have superior health benefits due to their high levels of vitamin B12, iron, zinc, fiber, essential amino acids, omega-3 and omega-6 fatty acids, and antioxidants. Adding edible insects such as locusts, crickets and bees to the human diet can provide a myriad of environmental and nutritional benefits, reduce agricultural use of land and water, improve the prevention and management of chronic diseases such as diabetes, cancer, and cardiovascular disease, and enhance immune function. Insects have the potential to be used as a meat substitute or food supplement (Abby *et al*. 2022).

Honey and honey bees are a rich source of nutrients. It contains sugars such as glucose and fructose, as well as minerals such as magnesium, potassium, calcium, sodium chloride, sulfur, iron, zinc, phosphate, and vitamins B1, B2, C, B6, B5, and B3 (Estevinho *et al*., 2008; Syazana, 2011) [8, 23].

Honey is the natural product of honey bees consisting of nectar collected from flowering plants. It is an alkaline food that contains components similar to those found in fruit, which become alkaline in the digestive system. Since ancient times, honey has been used as a natural sweetener and therapeutic agent for many ailments (Mahaneem *et al*., 2010) [15].

Male reproductive performance, especially sperm count, can be affected by environmental, genetic, behavioral and physiological factors. Honey is considered an aphrodisiac, increases sperm count, testosterone level, and sexual desire (Austin, 2011).

The US Food and Drug Administration (FDA) defines it as nectar and plant sugar secretions collected, transformed and stored in combs by honeybees. Honey contains water at a rate not exceeding 25%, sugar substances at a rate of approximately 70%, and the remainder consists of other substances such as proteins, vitamins, mineral salts, and other components (Al-Hasnawi, 2010) [2]. A study (Osaman, 2011) indicated that honey or honey bee derivatives play a major role in increasing fertility because it works to improve the quality and quantity of sperm present in the semen by improving the process of sperm formation as well as the hormones controlling it. Insects are an important source of bioactive compounds, such as bioactive peptides, chitin, etc., phenolic and fatty compounds, and amino acids, which provide health benefits (such as antioxidants, antihypertensives, anti-inflammatories, antimicrobials, immune modulation and increased fertility) when eaten.

Thus, edible insects are not only a good source of nutrients for human food, but can also be used as ingredients for nutraceuticals and functional foods (Jantzen 2020) [13].

The aim of the study: is to determine the extent of the effect of honey bee body extract on increasing sexual ability and vitality by increasing the fertility of male rats, in addition to knowing the histological effects on the structure of the testicle.

Materials and Methods of Work

Materials

1. Laboratory animals

Eight male white Sprague-Dawley rats were brought from the College of Veterinary Medicine at the University of Mosul. Their ages were between 10 and 12 weeks. They were raised under conditions of 12 hours of light and 12 hours of darkness in the animal house of the College of Veterinary Medicine at a temperature of 22±2 OC, good ventilation and humidity. Within the standard specifications, they were placed in plastic cages with a floor covered with sawdust and replaced twice a week. They were given food and water, and the food was fodder consisting of (yellow corn, wheat, barley, bran, protein, fiber, minerals, salts).

2. Honey bee body extract

200 bees were collected from the Al-Tayseer apiary located in one of the suburbs of the city of Mosul in clean, well-sterilized plastic containers, then left to cool in the refrigerator for 24 hours until they die. Then we put the entire bodies of the dead bees in a ceramic bowl and mash and grind them while they are soft for an hour until we get on a paste made up of bee tissues, then we put the paste in a 500 ml glass beaker, then we put 100 ml of the organic solvent methanol and leave it on the Stirrer device for 24 hours with the glass beaker tightly closed to avoid the methanol volatilizing. Then we separate the extract resulting from dissolving the methanol using a funnel. The separation contains filter paper 1 using a vacuum device (Heakal and Marusich, (2020). Then, the methanol extract is placed in a sterile Petri dish and placed in the desiccator until the organic solvent dries completely. Then we weigh the remainder of the extract, which is the bee body extract. Just put it in the refrigerator for later use in making bee bread.

The method of work

1. Bee bread

Bee bread is made by adding 100 grams of dry bee body extract to the food that will be given to the rats. It is fodder (yellow corn, wheat, barley, bran, protein, fiber, minerals, salts) at a rate of 100 grams of bee body extract for every 900 grams. From fodder.

2- Feeding the rats: The rats are divided randomly into two groups A and B, four rats for each group, taking the average weight of the rats. Then group A is fed with regular feed as mentioned previously. Here it is considered the positive control group, while group B is fed with bee bread consisting of 90% feed and 10 % of bee body extract. The condition of the animals is monitored daily and the weights of the rats are recorded daily. The feeding period continues for 28 days for both groups in the same quantities and under the same conditions. After that, all the rats are killed by euthanasia. An autopsy is performed for all the rats and the testicles and reproductive organs are taken. The epididymis, seminal vesicle, and prostate gland are weighed and the results are recorded.

Sperm analysis and histological sections

For sperm count and morphological evaluations, the cauda epididymis was dissected with scissors in 2 ml of normal saline. Then, the mixture was filtered with an 80 mm nylon mesh and mixed with 2 drops of eosin Y. The mixture was left for 30 minutes. This mixture was used to determine sperm count and morphology. After 30 minutes, the epididymis content was diluted 20 times with normal saline using a ratio of 1:20. Then a drop of the solution was placed under the microscope at x 400 magnification. The sperm counting process began from 5 small squares selected out of 25 large squares. Sperm were counted by counting the sperm head per square. To obtain the original sperm sample concentration the dilution factor must be multiplied. Counting was repeated 5 times for each rat to reduce error (Haron *et al*., 2010) [12].

Sperm morphology is determined by observing an epididymal sperm slide under a light microscope. First, then drop one drop of the culprit epididymis mixture onto the slide, then leave the slide to dry for one day. The abnormal sperm morphology is then randomly counted among the 200 sperm observed from the slide and the results are recorded for each rat. (Haron *et al*., 2010) [12].

Then, tissue sections of the testicle, testicular wall, and epididymis are made and preserved in a 10% neutral buffered formalin solution. After that, a series of passes are made using ethyl alcohol, xylol, and paraffin wax, then casting into wax molds, cutting with a microtome, and staining the slides using the classic stain hematoxylin and eosin (H and E). (Luna, 1968) [14] Then it was examined with an optical microscope and photographed with a Japanese-made SONY digital camera.

Data analysis

Test experiments were conducted with three replicates for each treatment, and the standard deviation of the results represented by the diameter of each circle for each treatment was calculated. According to the program SAS 9 Version, SAS, JMP (2000). As for the differentiation between the equations at the probability level of .05, it was done with the Duncan test (Duncanes, 1955).

Results and Discussion:

Eating honey or its derivatives leads to an increase in the sexual ability of mammals in general. From Table (1) we note that there was no significant increase in the weight of the primary reproductive organs in the rats, group (A), compared to group (B), which is the testicle and epididymis; But the increase in weight and size was evident in the glands attached to the reproductive system, which are the seminal vesicle and the prostate gland. This means an increase in the secretion of semen and testosterone, and certainly an increase in the number of sperm. This study is consistent with the study (Fratellone *et al*., 2016) [11] in that honey bee derivatives increase of the vital capacity of mammals in general.

The study (Carpentier *et al*., 2004) [6] also confirms that increasing the sexual ability of humans, males or females, may depend on plant extracts originally, and this may not be completely consistent with this study on the one hand; But on the other hand, there may be plant compounds within the bee's body extract, such as pollen suspended in the bee's

body or nectar present in the intestines, and this is what was indicated by a study (Amanda *et al*., 2020), which confirmed the presence of approximately 0.3 grams of pollen. Attached to the feet of worker honeybees, adapted for this purpose, on each trip, is an amount of nectar from flowers. This proves that the bee's external body contains components of pollen. Pollen contains more than 250 biologically active substances, including proteins, carbohydrates, fats, fatty acids, and vitamins. And minerals, enzymes, and antioxidants. (Abu Al-Rab, 2021),

From Table (2) and Image (1), it is noted that there was a significant increase in the number of sperm or sperm in group (B) compared to group (A), which is the positive control group, as this study agrees with the study (Faryal *et al*., 2022), which confirmed that when mixing honey derivatives with rat food, this led to an increase in the number of sperm in males, an increase in the ovulation rate in females, and sexual desire in females as well. On the other hand, this study confirmed a decrease in the deformities occurring in the sperm, whether in the tail or head, and in general in shape. The morphology of the sperm in the group treated with bee bread or bee body extract (B) compared to the sperm in group (A), the positive control group, and this matches the study (Sofiane *et al*., 2018). We may also notice the number of sperm and the shape of the sperm in the picture (1) very clear.

This study confirmed that the bee body extract led to the stability of sperm in white male rats, and this is clearly observed in Figure (1). It is noticeable according to the figure that there was not a significant significant increase in the rate of sperm death or their stability, but this was observed in the deformities. The apparent difference in the shape of the head and the length of the tail in the sperm compared between groups (A) and (B) and this is in line with the study (Siti *et al*., 2021), which showed that adding 10% of honey and its derivatives to the milk's diet in general led to a clear increase in the condition of Sperm stability and fewer sperm deformities in terms of the shape of the tail and head.

Table 1: Average weights of the reproductive organs of male rats:

Weight of reproductive organs and appendages (g/100 g b.w)	The group B	The group A
Average testicle weight	1.02 ± 0.01	1.04 ± 0.02
Average weight of the epididymis	0.19 ± 0.02	0.20 ± 0.01
Average weight of seminal vesicle	0.11 ± 0.02	0.09 ± 0.02
Average weight of the prostate gland	0.18 ± 0.01	0.10 ± 0.02

Data are presented as mean ± SEM (n = 4 in each group). At a probability level of $p < 0.05$

Table 2: Average morphological condition and number of sperm in male rats:

	The group B	The group A
Morphological state *%*	1.02 ± 0.01	1.04 ± 0.02
Number of sperm (X 106(	37.20 ± 3.30	30.20 ± 3.20

Data are presented as mean ± SEM (n = 4 in each group). At a probability level of $p < 0.05$

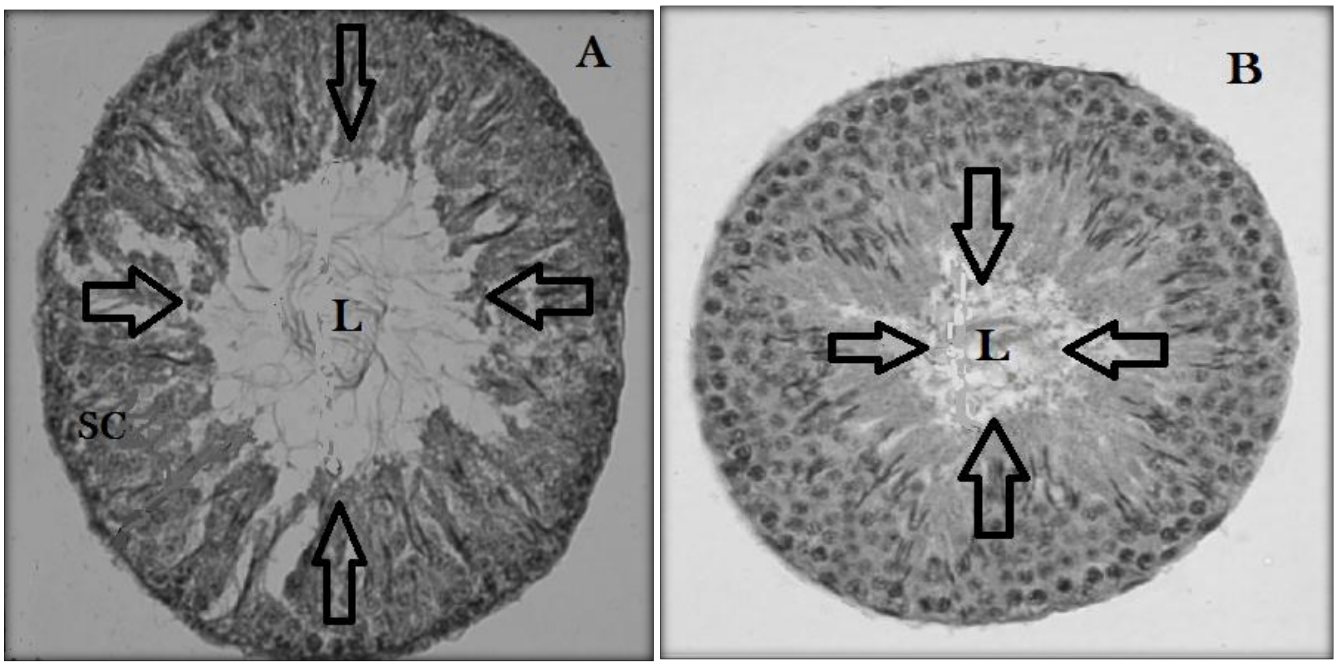

Fig 1: A section of one of the epididymal ducts (A), the control group (B), the group treated with bee body extract food (L), the internal area of the epididymal duct.

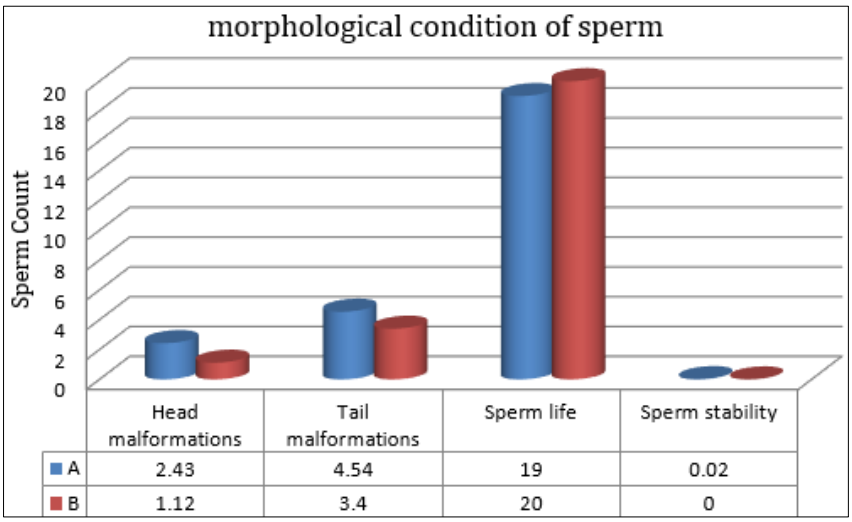

Fig 2: Morphological status of sperm, as (A) the control group and (B) the group treated with bee body extract.

Conclusions

1. Honey bee body extract mixed with the food of white rats led to an increase in the secretion of semen and the secretion of the accessory glands in the reproductive system.
2. A significant increase in the number of sperm in rats treated with bee body extract mixed with food.
3. A significant decrease in congenital abnormalities in the shape of sperm in rats fed on bee body extract.

Recommendations

1. Conducting other extensive studies on bee body extract specifically.
2. Trying to use bee body extract on humans and finding out the extent of its ability to increase human sexual ability.
3. Using bee body extract to increase the sexual ability of laboratory and non-laboratory animals, as it is economically feasible.

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