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# The Effects of Honey and Bee Bread on Bodyweight, Sperm Parameters and Reproductive Hormones in Male Mice

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## Abstract

Bee products (honey and bee bread) have been used for their nutritional and therapeutics properties since time immemorial. Honey and beebread contains numerous nutrients that have antioxidant properties that can be tested for improving male reproductive functions. The effect of Honey and beebread intake on male mice reproductive system has not been reported. Therefore the aim of this study was primarily designed to determine the effects of honey and beebread intake on bodyweight, reproductive hormones and spermatogenesis. Honey and beebread were harvested from bee hives and filtered to remove solid particles and concentrated (40% w/v water) by oven drying at 40ºC before use. A total of 30 male mice weighing between 12-30 grams were used for this study. The animals were randomly divided in a blinded fashion into 3 groups of 10 each, (Group A, B and C). Mice in group A served as control, which received oral administration of distilled water while mice in Group B and C received freshly prepared honey and beebread at dose rate of 1g/kg bodyweight orally respectively by gavage daily for 70 days according to Organization for Economic Co-operation and Development (OECD) guidelines. Parameters measured were bodyweights, serum hormonal levels (Follicle-stimulating hormone, Luteinizing hormone and Testosterone), sperm count, viability, motility and morphology. The result revealed that prolonged administration of honey and beebread found to be improved bodyweight and spermatogenesis in the treatment groups (B and C) as compared to the control (A), ($P<0.05$). Sperm concentration was found to be higher ($P<0.05$) in the honey treated group (18.47 ± 1.66) as compared to beebread treated group (14.42 ± 1.58) and control group (12.21 ± 0.97), while percentage motile sperm was also significantly ($P<0.05$) higher in the treatment groups (B: 78.41 ± 4.73) and (C: 75.06 ± 9.49) as compared to control group (A: 61.74 ± 4.78). The percentage of viable sperm was also significantly ($P<0.05$) higher in the treatment groups (B: 69.80 ± 1.48) and (C: 64.60 ± 2.51) as compared to control group (A: 59.20 ± 1.64) respectively. A significantly higher percentage of abnormal sperm was observed in the control group (A: 12.00 ± 1.58) as compared to treatment groups (B: 6.00 ± 0.71) and (C: 9.40 ± 0.55). The serum level of reproductive hormones (Follicle-stimulating hormone, Luteinizing hormone and Testosterone) were found to be non-significant ($P<0.05$) in all the groups. It was concluded that prolonged administration of honey and beebread in normal mice improves spermatogenesis but, the mechanism involved in the increase of spermatogenesis without altering the levels of reproductive hormones needs to be investigated further.

**Keywords:** Honey, Bee bread, Bodyweights, Follicle-stimulating hormone, Luteinizing hormone, Testosterone, Spermatozoa and spermatogenesis

## Introduction

Honey a sweet food made by bees using nectar from flowers has been proven to be of medicinal importance, It is a promising antitumor agent with pronounce anti metastatic and antiangiogenic effects. Antibacterial, anti-inflammatory, immune stimulant, antiulcer and wound burn healing effect Honey has been used by humans since ancient times, nearly 5500 years ago Most ancient population, including the Greeks, Chinese, Egyptians, Romans, Mayans, and Babylonians, consumed honey both for nutritional aims and for its medicinal properties [1].

Bee bread produced from bee pollen that acts as raw material reported that bees collected two principle of food which is nectar and pollen [2-5]. First, bees collect nectar to make an enzymatically activated food partially fermented food called honey; secondly they also collect pollen to make a lacto-fermented enzymatically activated food called bee bread. Honey serves as carbohydrate source for bee, and bee bread usually being their stable protein source [6-10]. Honey contains variety of biologically active compounds such as flavonoids, vitamins, antioxidants as well hydrogen peroxide [11-15].

Finding from many studies also showed the ability of honey in controlling overweight and obesity when consumed orally, thus making it potential antiobesity agent [16-20]. Following their study of world population reported that 72.4 million people were infertile and of these 40.5 million people were seeking infertility medical care [21]. Over the years, curative measures have been applied to curb infertility; these measures include the use of herbs (Alfafa), vitamin supplements, drugs (Metformin, Spermomax, Manix) and medical procedures example *In Vitro* Fertilization and Embryo Transfer (IVF-ET) and Gamete Intrafallopian Transfer (GIFT). Honey is the natural product of bees (Honey bees) formed from the nectar collected from flowering vegetation addition of honey, as a supplement to the diet resulted in increased body weight in rats [22-29].

There are many factors contributing to male infertility including structural abnormality, hormonal imbalance, previous infection, environmental factor, immunological factor, genetic factor, systemic disease, erectile dysfunction, spermatogenic dysfunction, and idiopathic, renewed attention to alternative medicine and natural therapies has stimulated new wave of research interest into nutrition-based intervention since they are cheaper and have lesser side effects [30-39].

Previous researches have shown the effects of honey on body weights and spermatogenesis in rats and bonnet monkeys, but to date,

there is no research undertaken to determine the effects of honey and beebread on bodyweight and reproductive hormones in normal male mice reported. Therefore, this study was design to determine the effects of honey and beebread on reproductive hormones, spermatogenesis and bodyweight gain in male mice [40-49].

### Aims and objectives of the study

- To determine the effects of honey and bee bread on bodyweight in male mice.
- To determine the effects of honey and bee bread on reproductive hormones in malemice.
- To determine the effects of honey and bee bread on sperm parameters of mice.

## Materials and Methods

### Materials

Natural honey and bee bread were obtained from the Toungo Local Government Area, Adamawa State, Nigeria and are kept under sterile environment at room temperature. Both the honey and bee bread were. Measured 40 g poured on conical flask and distilled water was added make it to 100 mL, stirred, mixed well and filtered to remove the solid particles. The concentration of 40% w/v water was achieved by oven drying at 40°C before use [50-59].

**Experimental animals:** Thirty male mice weighing between 12-34 grams were used for the study. The mice are purchased at University of Jos. They were housed in standard laboratory cages at room temperature (25°C), humidity of 50%-64%, and maintained on a 12:12 hour light/dark cycle in the animal house. The animals were allowed to acclimatize to the environmental conditions and they were given pelletized feed for 2 weeks before the commencement of the experimental feeding procedures [60-69].

**Experimental design and feeding of the animals:** The animals were randomly assigned into 3 groups (A, B and C), consisting 10 each with a varied average weight per group at the onset of the experiment. The mice were handled according to International Guiding Principles for Biomedical Research (Council for International Organizations of Medical Sciences (ICOMS) and the International Council for Laboratory Animal Science (ICLAS, 2012). Commercial pellet food and water were given ad-libitun; Fresh diets for each group were giving every other day to avoid spoilage. The animals were allowed access to the diets and drinking water ad-libitum. The mice were aged 5 and 11 weeks at the time of commencement of feeding and at the time of sacrifice, respectively [70-79].

### Oral administration of honey and bee bread

In this study, mice were giving distilled water, honey and bee bread group with equal volume each A, B and C, respectively. Freshly diluted honey and beebread was administered to group B and C using distilled water (as a vehicle) to a 0.5 ml of solution with dose. 1 g/kg bodyweight and distilled water was administered to group A by orogastric tube (gavage) once daily for 70 days as described by Organization for Economic Co-operation and Development (OECD) guideline [80-89].

### Weighing of the experimental animals on weekly bases

All the mice were weighed before commencement of treatment and then weekly throughout the period of administration [90].

### Sperm head counts

The testes and epididymis from the humanely sacrificed mice were dissected out. The tunica albugenea was removed from the left testis before it is homogenized in 5 ml of normal saline. The head, body and tail of epididymis was separately homogenized in 2 ml of normal saline. The sperm head count per milliliter of the homogenate was done using a Haemocytometer [91]. The total sperm head count per homogenate was determined using the formula:

Sperm head count = volume of homogenate × count in 5 squares × 0.05 × 106 ml.

### Sperm motility

A drop of fresh semen was placed on a pre-warmed slide, covered with coverslip and examined under X 40, and subjective assessment was done by random selection of some fields on the counting chamber, and number of rapidly motile sperm was assessed (WHO, 2010) [92].

### Sperm morphology

A drop of the homogenate was placed on a slide and covered with slip, then a thin smear was made and air dried. The slide was stained with Eosin-Nigrosin stain and examined under oil immersion for morphology. At least 200 sperm were counted. The result was expressed in percentage [93].

### Sperm viability

One drop of fresh semen was added to one drop of Eosin-Nigrosin stain and allowed to stand for 30 seconds. Thereafter, a drop was placed on a slide and smear was made and air dried. The slide was examined under the oil immersion lens at x100; at least 200 sperm cells were counted. Sperm cells that pick up the stain were considered non-viable while the colorless cells were considered viable [94].

### Serum biochemical analyses

The mice were sacrificed at the end of procedure and whole blood were collected and submitted to university of Maiduguri Teaching Hospital for harvesting of serum and hormonal assay. Serum was carefully aspirated into sample tubes and stored frozen until used for biochemical analyses. Serum level of hormones was determined using mice assay kits of Testosterone (T), Estradiol (E), Progesterone (P), FSH, and LH. These were determined in serum by appropriate methods using commercial assay kits based on the principle of enzyme linked immunosorbent assay (Marburg, Germany). The assay procedures used were as contained in the kit manuals. Using appropriate wavelengths for each analyze, the optical density was taken and read with a micro titer plate reader, after which the corresponding concentration was calculated [95].

### Statistical analysis

All data were expressed as mean ± standard deviation of mean (± DM) to infer our finding to larger population. The SPSS software was used to analyse the data obtained in this study. The one-way Analysis

of Variance (ANOVA) was used followed by the least significant difference, Tukey HSD post-hoc comparison of means. Differences at $p \leq 0.05$ were considered statistically significant [96].

## Results

Result showed bodyweight of mice treated with distilled water (A), honey (B) and bee bread (C) comparing from day 0 to 70 Results on the effects of honey and beebread on the bodyweight showed that there was significant ($P<0.05$) difference in the weight gain observed in the treatment groups (B and C) compared to the control group, there was increased in weight numerically in the treatment groups, with highest weight gain recorded in the honey treated group, moderate weight gain in the beebread treated group and lowest weight gain in the control group was observed respectively [97] (Table 1).

| Days | Bodyweight in gram | | |
|---|---|---|---|
| | Control | Honey treated (g) | Beebread treated |
| 0 | 15.60 ± 2.51 | 27.60 ± 3.36 | 27.80 ± 3.42 |
| 7 | 19.40 ± 2.79 | 29.60 ± 3.71 | 26.60 ± 3.50 |
| 14 | 21.20 ± 3.03 | 31.40 ± 4.28 | 30.60 ± 3.50 |
| 21 | 24.00 ± 2.45 | 32.80 ± 4.09 | 30.80 ± 3.27 |
| 28 | 26.40 ± 2.07 | 34.40 ± 4.72 | 31.40 ± 4.34 |
| 35 | 27.20 ±1.92 | 34.80 ± 4.76 | 33.0 ± 3.87 |
| 42 | 28.00 ± 1.73 | 34.40 ± 4.50 | 33.40 ± 3.90 |
| 49 | 28.80 ±1.10 | 35.00 ± 4.18 | 34.60 ± 4.22 |
| 56 | 30.40 ± 1.67 | 35.60 ± 5.03 | 33.60 ± 3.44 |
| 63 | 30.60 ± 1.81 | 35.60 ± 5.03 | 34.40 ± 3.78 |
| 70 | 31.60 ± 1.14 | 35.60 ± 5.13 | 32.28 ± 4.20 |

**Table 1:** Result showed bodyweight of mice treated with distilled water (A), honey (B) and bee bread (C) comparing from day 0 to day 70 post-treatment.

All values were expressed as mean ± standard deviation (S.D) values with different superscript within the column abc are significantly different at $P<0.05$

### Analysis of sperm parameters

**Sperm count:** The epididymal sperm count was found to be significantly higher ($p<0.05$) in the honey treated group compared to the control and beebread treated groups. The honey treated group had the highest sperm count; the beebread treated group has moderate sperm count while the control group had lowest sperm count (Table 2). Testicular sperm count were also significantly higher ($p<0.05$) in the honey treated group as compared to the control and beebread treated groups. The honey treated had highest sperm count, the beebread treated group has moderate sperm count while the control group had lowest sperm count.

**Sperm motility:** The percentage of motile spermatozoa was significantly ($p<0.05$) higher in the treated groups (B and C) as compared to the control. The highest percentage of motile sperm was recorded in the honey treated group, moderate percentage was recorded for the beebread treated group and the lowest percentage was recorded in the control group.

**Abnormal sperm percentage:** The percentage of abnormal spermatozoa was significantly ($p<0.05$) higher in control group as compared to all treated groups, moderate percentage was recorded in the beebread treated group and lowest percentage of abnormal sperm was recorded in the honey treated group.

**Viable sperm count:** The percentage of viable spermatozoa was significantly ($p<0.05$) higher in the treatment groups as compared to the control. The highest percentage of viable sperm was recorded in the honey treated group while the lowest percentage was recorded in the control group [98].

| Sperm parameters | | | | | |
|---|---|---|---|---|---|
| Groups | Epididymis count × 106 /ml | Testicular sperm count × 106 /ml | Motility % | Abnormal % | Viability % |
| Control (A) | 12.21 ± 0.97b | 10.58 ± 0.29 | 61.74 ± 4.78 | 12.00 ± 1.58 | 59.20 ± 1.64 |
| Honey (B) | 18.47 ± 1.66 | 14.96 ± 0.35 | 78.41 ± 4.73 | 6.00 ± 0.71 | 69.80 ± 1.48 |
| Beebread (C) | 14.42 ± 1.58 | 11.42 ± 0.08 | 75.06 ± 9.49 | 9.40 ± 0.55 | 64.60 ± 2.51 |

**Table 2:** Results Effects of honey and beebread on sperm counts, sperm motility, sperm morphology and sperm viability in the control and experimental groups at the end of administration.

Means in the same Colum with different superscripts differ significantly ($p<0.05$) The Results Effects of honey and beebread on male mice reproductive Hormones (Testosterone, Follicle stimulating Hormone and Luteinizing hormone). Results Effects of honey and beebread on male mice reproductive Hormones (Testosterone, Follicle stimulating Hormone and Luteinizing hormone) study revealed that there was no significant ($P<0.05$) difference on the of reproductive hormones (Testosterone, FSH and LH) in all the groups [99] (Table 3).

| Parameters | Various treatments groups | | |
|---|---|---|---|
| | Control group | Horney Treated group | Beebread Treated group |
| Testosterone (ng/mL) | 0.86 ± 0.79 | 0.72 ± 0.71 | 1.36 ± 1.35 |
| FSH (miU/mL) | 0.16 ± 0.09 | 0.22 ± 0.21 | 0.52 ± 0.77 |
| LH (miU/mL) | 4.06 ± 1.44 | 4.52 ± 1.77 | 3.56 ± 1.14 |

**Table 3:** Results of male mice reproductive Hormones (Testosterone, Follicle stimulating Hormone and Luteinizing hormone) comparing control and treatment group.

## Discussion

This study focused on the effect of prolonged administration of honey and beebread on the bodyweight, male reproductive hormones and spermatogenesis in mice. The study showed that prolonged administration of honey and beebread had no significant ($P<0.05$) effect on the plasma level of testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in all the groups which was in consonant with Previous studies using honey that originated from Malaysia that no significant effects of honey on reproductive hormones including Testosterone (T), Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) in rat and Bonnet monkey, who reported significantly increased sperm count in the experimental groups without altering the levels of testosterone and gonadotropins as compared to control monkeys supplemented with 2 ml of honey for a period of 30 days [100].

However, excessive and prolonged use of honey inhibits testosterone production in male Wistar rats but promoted the production of follicle stimulating hormone, luteinizing hormone, progesterone and prolactin reported that honey from Nigeria increased Testosterone but decreased LH and FSH in rat which was inconsistent with our findings despite the fact that honey and beebread used in this study is also from Nigeria. The content and composition of honeys varies with different floral sources, climatic and environmental conditions as well as methods of preparation. The variations between the present study and those of previous studies may be as a result of the differences in the source, and or climatic and environmental conditions of the honey used for the studies used Wistar (albino) rats, used Sprague-Dawley rats while used Bonnet monkey, Another suggestion for the discrepancy in the findings of this study could be attributed to the specie used for the study. reported that excessive consumption of honey appears to have a deleterious effect on serum levels of testosterone but enhances the production of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and prostate-specific antigen. Chronic consumption of natural honey and sweetened foods has been linked to alteration in plasma levels of many hormones.

In the present study the sperm count was considerably increased in the treatment groups (B and C) compared to the control group (A). The observed increase in sperm count following administration of honey and beebread in the present study is consistent with the findings of. It has been suggested that honey from Palestine enhances epididymal sperm count by possibly affecting the key enzymes in spermatogenesis and sperm maturation such as increases sorbitol dehydrogenase activity and decrease lactate dehydrogenase activity. However, reported that honey increases sperm count by increasing the testicular production of testosterone, which was evident from the elevated plasma testosterone following treatment with all doses of honey. However, in the present study the level of reproductive hormones was not significantly different among the groups, a finding that was contrary to what was reported by reported that 200 mg/kg of honey obtained from Malaysia did not significantly increase the sperm count in rats, whereas in the present study, with honey sourced from Nigeria significantly increase sperm count in mice.

Previous work by reported that honey administered at a dose of 1 ml per 100 g of body weight also caused increases in sperm count, sperm motility and sperm morphology in the experimental rats. Previous works done by on the effect of honey on sperm quality in experimental rats which was all concurred with the results of this study in showing that honey improves the semen quality in experimental mice.

In this study, it was found that the administration of honey and beebread at 1 g/kg body weight showed there was significant ($P<0.05$) difference in the weight gain observed in the treatment groups (B and C) compared to the control group, there was increased in weight numerically in the experimental groups, with highest weight gain recorded in the honey treated group, moderate weight gain in the beebread treated group and lowest weight gain in the control group was observed respectively. This study was corroborated with previous findings reported that addition of honey as a supplement to the diet resulted in increased bodyweight; honey also enhanced weight gain in fructose-fed rats reported that the administration of honey at 1 ml/100 g of body weight increases the mean body weight of rats and also reported an increase in mean body weight following administration of 1 ml/100 mg bodyweight in rats which could be attributed to the presence carbohydrate and simple sugars in honey.

However these observations was not agreed with the findings reported by Wistar rats fed high fat diet treated with honey had non-significant greater body weight than chow fed rats for a period of 6 week reported that honey administration to healthy rats resulted in lower body weight and weight gain which was not corroborated with the present findings [101].

## Conclusion

This study showed that prolonged administration of honey and bee bread at dose of 1 g/kg resulted in improved bodyweight and spermatogenesis in the treatment groups (B and C) compared to control group (A) ($P<0.05$). But the serum hormonal levels of testosterone, follicle stimulating hormone and luteinizing hormones was not significant by different among groups ($P<0.05$).

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