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The Effect of Chronic Honey Intake on Sperm Parameters and Fertility Potential in Adult Male Wistar Rats

[1]W.N. Dare, [2]P.S. Igbigbi and [2]O.G. Avwioro

[1]Department of Human Anatomy, College of Health Sciences,
Niger Delta University, Wilberforce Island, Nigeria
[2]Department of Human Anatomy, College of Health Sciences,
Delta State University, Abraka. Nigeria

Abstract: This investigation was designed to determine the effects of administration of honey on sperm parameters and fertility potential in adult male Wistar rats. A total of forty five rats (thirty males and fifteen females) weighing between 225±5g were used for the study. The male rats were divided into 3 groups i.e., 2 experimental and 1 control groups of ten rats each, (n=10). The 15 female rats were used for fertility test. Experimental groups 1 and 2 male rats were administered with 5ml/kg body weight and 7.5ml/kg body weight of honey respectively through orogastric tube (gavage) twice a week for 10weeks. The above parameters were determined at the end of administration and after 10weeks of treatment rest, the results were compared with controls. Results revealed that chronic consumption of honey has exhibited significant reduction of sperm parameters. Total sperm count and percentage of motile spermatozoa were significantly depressed, but percentages of abnormal, dead/non-motile spermatozoa were increased significantly at ($P<0.05$). None of the experimental animals was fertile, while females mated with control males achieved pregnancy. Chronic consumption of honey appeared to have a deleterious effect on sperm parameters and fertility potential of male Wistar rats.

Key words: Excessive Honey · Sperm parameters · Fertility · Wister Rats

INTRODUCTION

Natural products such as honey and medicinal plants have been used in the treatment of diverse human diseases [1-3]. Since ancient times, honey has been used for its medicinal properties in many cultures. Currently, information on the use of honey for the treatment of many human diseases can be found in magazines, bee keeping journals [1] and natural products leaflets, suggesting a wide variety of unfounded properties. In contrast, medical reports supported by tests are few [4, 5]. Honey is also considered a part of traditional medicine [6]. It is effective in the healing of wounds, burns and the treatment of diabetic ulcers [7-10]

Honey is produced from many floral sources and its content and activity vary with its origin and processing technique. Histological studies of honey applied to wounds have been reported to be safe as it reduces inflammation in deep [11] and superficial [12] burns as well as in wounds [13]. At a concentration of 1%, it stimulates growth of monocytes in cell cultures to release cytokines, tumour necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, which activate the immune response to infection [14]. The proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture has also been stimulated by honey at concentrations as low as 0.1%; and phagocytes are activated by honey at concentrations as low as 0.1% [15]. Preliminary evidence showed that honey has an effect on reproduction [16]. Honey is said to have increased the sperm count in rats and monkeys and increased vaginal wall epithelium and muscle thickness, without showing any effect on circulating gonadotrophins or testosterone. Honey is used traditionally in post-menopausal women to treat vaginal atrophy and dryness. Honey was reported to enhance spermatogenesis in rats if given at the appropriate dose

Corresponding Author: W.N. Dare, Department of Human Anatomy, College of Health Sciences,
Niger Delta University, Wilberforce Island, Nigeria. E-mail: pastordarewn@yahoo.com.

[16] and to reduce the toxic effects of cigarette smoke on spermatogenesis [17]. Honey affects spermatogenesis by elevating sorbitol dehydrogenase (SDH) activity and by reducing lactate dehydrogenase (LDH) activity [6]. World Health Organization (WHO) sperm analysis [18] spelt out that concentration of $20x10^6$ sperms/ml or higher, percentage of normal sperm not lower than 30 and 50% of progressive motile sperm or more within 1hour of ejaculation are compatible with male fertility. The objective of the study was to determine the influence of bee honey on sperm parameters and fertility at the end of treatment and rest.

MATERIALS AND METHODS

Forty five adult Wistar rats were used for this study (thirty males and fifteen females). The rats were collected from the Animal House of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State Nigeria. They weighed between 225±5g and were acclimatised for two weeks, had access to water *ad libitum*. Feeds (growers) were obtained from Bendel Flour Mills Plc, Ewu Edo State and honey was purchased locally from M.C Super Market Ekpoma. The animals were caged separately for the purpose of identification.

Experimental Design

Male Rats: Twenty rats were used as experimental and ten as control. They were divided into three groups of two experimental and one control. The experimental rats were administered with two excessive varying doses of bee honey.

Group 1: Rats were administered with 5ml/kg body weight of bee honey through orogastric tube (gavage) once a day twice weekly for 10 weeks.

Group 2: Rats were administered with higher dose of 7.5ml/kg body weight, once a day twice a week for the same duration.

Group 3: Served as control with normal feed only.

Analysis of Sperm Parameters

Sperm Collection: At the duration of administration (10weeks), five from each group of both experimental and control animals were sacrificed by guillotine decapitation. Semen samples were collected from 10mm length (segment) of the distal epididymis, due to its role in storing mature sperm by careful exposure. Cut into several pieces, placed in specimen bottles containing 1ml of physiological saline and homogenised to release the spermatozoa. The specimen bottles and their contents were maintained at 37°C for 15minutes for appropriate quantity of sperm to diffuse into the saline. Semen was siphoned from the specimen bottles with a 1ml pipette and drops placed on cleaned slides and cover slips applied. The slides were placed under the light microscope and viewed for sperm parameters (count, motility and morphology) for both experimental and control animals and results compared.

Sperm Count: The new Improved Neubauer's Counting Chamber was used for sperm count. Drops of semen were placed on two edges of the chamber with cover slip applied focused with the light microscope and cells counted. The counting was repeated five times and average figure taken.

Sperm Motility: The motility of sperm cells was determined by random selection of some fields on the counting chamber and number of rapidly motile, sluggish and immotile/dead sperms were counted and percentages taken. The classification of the World Health Organization [18] was used in the assessment of sperm motility.

Sperm Morphology: Cells were stained and examined under the light microscope with the oil immersion objective and the percentage populations of normal, abnormal and dead/non-motile spermatozoa were determined and compared.

Fertility Potential Test: The fifteen female rats were prepared for this test.

Preparation: A daily lavage was done to determine the predominant epithelial cells present in the vaginal lavage, that is, 1ml of normal saline was introduced into the vagina with a pipette and vaginal swab was taken and drops were placed on clean slides and were viewed under the microscope. The predominant presence of uniformly large nucleated cells indicated pro-oestrus stage (the stage of the oestrus cycle which the female is receptive to the male). Cornified cells indicated oestrus (ovulation) and the predominant presence of leucocytes with or without epithelial cells signified dioestrus 1/dioestrus 2 stages. The female is not receptive to the male in the latter 3 stages.

Fertility Test: At the end of ten weeks treatment, the unsacrificed five rats from each group of the treated male experimental rats were mated with ten pro-oestrus females (five males to five females) over night. Five control males were also mated with five pro-oestrus females overnight. In the following morning, physiological saline was introduced into the vagina with a 1ml pipette and vaginal swabs were taken, drops were placed under the light microscope and viewed for the presence or absence of sperm. The presence of sperm in the vagina confirmed that mating had taken place and that day was taken as day zero of pregnancy. The results for the two experimental groups and control were compared.

Treatment Rest Study: After mating with the females, the unsacrificed male experimental rats, five from each group were maintained with normal feed for ten weeks, sacrificed and processed for sperm parameters.

Statistical Analysis: The parameters studied of both experimental and control groups were compared, using two-way analysis of variance (ANOVA) to test the observations,

RESULTS

Population (Count): There was gross significant depression on sperm count in the two experimental groups as compared with control. The depression was more pronounced in group 1 animals treated with 5ml/kg body weight than group 2 rats treated with 7.5ml/kg body weight as shown in Table 1.

Motility: Effect on sperm motility is shown in Table 1. There was a significant reduction in the percentage of motile spermatozoa in the experimental groups as compared with their respective control. However, minor difference was found between the two experimental groups. Both experimental and control had equal percentages of sluggishly motile spermatozoa.

Morphology: The percentages of normal and abnormal spermatozoa were the same in experimental group 1 and control. There was no significant ($P > 0.05$) difference between experimental group 1 and control but group 2 was significantly ($P < 0.05$) higher than group 1 and control. However, there was a significant difference in the percentage of dead/non-motile spermatozoa between the two experimental groups and control, higher in the experimental groups than control but found non-significant difference between the two experimental groups.

Total Sperm Count: Significant decrease in the experimental groups as compared with control.

Motility: Significantly lower in the experimental groups, however, minor difference was found between the two experimental groups.

Normal Sperm: The percentage of normal sperm population was higher in group 2 experimental than group 1 and control. No significant difference between group 1 and control.

Table 1: Showing ANOVA results of sperm parameters at the end of administration.

Sperm parameters	Group 1	Group 2	Group 3	F- ratio	Exact sig
Total count (10^6/ml)	69.8±3.49	106.4±16.32	221.8±20.91	131.9	0.00
Motility (%)	17.6*±2.88	23.1*±3.29	47.4±5.17	68.71	0.00
Normal sperm (%)	50.44*±3.14	72.1±3.05	53.22*±3.03	61.33	0.00
Abnormal sperm (%)	51.15±1.14	28.8±2.73	46.7±2.95	119.51	0.00
Dead/non-motile (%)	81.1*±3.44	75*±4.12	55.92±4.39	53.89	0.00

*The mean difference is not significant at 0.05 level, using Scheffe Post Hoc test.

Table 2: Showing ANOVA results of sperm parameters of post treatment (rest) for 10 weeks.

Sperm parameters	Group 1	Group 2	Group 3	F- ratio	Exact sig
Total count (10 /ml)	72.40±8.29	109.20±10.16	221.80±20.91	149.19	0.00
Motility (%)	0.00*±0.00	0.00*±0.00	47.4±5.98	313.79	0.00
Normal sperm (%)	70.40±8.88	85.84±4.26	53.22±3.03	37.63	0.00
Abnormal sperm (%)	29.60±8.88	14.40±4.56	46.7±2.95	36.17	0.00
Dead/non-motile (%)	100*±0.00	100*±0.00	55.92±4.39	505.16	0.00

* The mean difference is not significant at 0.05 level, using Scheffe Post Hoc test.

Abnormal Sperm: Significantly reduced in group 2 experimental rats as compared with control.

Dead/Non-Motile: The percentage was significantly higher in the experimental groups (1 and 2) but more in group 1, as compared with control.

Fertility Potential Test: It was observed that none of the female animals mated with the experimental males in both experimental groups (5ml/kg body weight and 7.5ml/kg body weight) archived pregnancy. All females mated with the control males became pregnant.

Post-treatment Effect (Withdrawal): The effect did not reverse after cessation of treatment for ten weeks. It rather grew worse (progressive) after 10 weeks of treatment rest as shown in Table 2.

Sperm Parameters: In general there was a significant difference in sperm count between the experimental groups and control was maintained after 10weeks of treatment rest. Significant difference was also observed in sperm motility between the two experimental groups and control; the experimental groups had one hundred percent (100%) non-motile spermatozoa. Normal sperm population was significantly higher in the experimental groups than control. There was also significant difference between the two experimental groups. Normal sperm population was higher in the experimental groups. Total sperm count significantly depressed in both experimental groups as compared with control. The effect was more in group 1 than group 2.

Motility: No motile sperm in both experimental groups (zero percentage motility)

Normal Sperm: Percentages of normal spermatozoa were significantly higher in the experimental groups as compared with control.

Abnormal Sperms: Percentages of abnormal spermatozoa were significantly less in the experimental groups as compared with control.

Dead Sperms/Non-Motile: All the sperms were dead in the two experimental groups.

DISCUSSION

Despite the many purported health benefits of honey for the treatment of bacterial infection cough suppression [19] diabetes [4]; not many works have been done on the effect of honey on sperm parameters and fertility in animals and man.

Therefore, present study focused on the effect of chronic intake of honey on spermatogenesis and fertility potential in male rats. The study showed that chronic intake of honey, an exogenous substance is armful to the reproductive system of male Wistar rats. Honey, when taken excessively, has negative influence on spermatogenesis, sperm motility and morphology. Previous investigators who established that honey enhances spermatogenesis did not consider its excessive intake on regular basis; the source of honey, duration of administration, dose and concentration which may affect expression on the body. Specifically, this study revealed that excessive ingestion of honey for a long period of time inhibits spermatogenesis, reduced the percentage of motile sperm and increased the percentages of dead and abnormal spermatozoa. The low sperm count affected the fertility potential of the experimental animals; none of the females mated with them archived pregnancy, but all the females mated with control males got pregnant. The infertility could be attributed to the reduced population of mature viable spermatozoa (oligospermia) and the depressed percentages of motile sperms.

CONCLUSIONS

This study has demonstrated that chronic and prolonged use of honey is harmful to the male reproductive system. It causes oligospermia, has spermicidal effect and consequent infertility in male rats. Males in their reproductive age who want to have children should refrain from prolonged ingestion of honey.

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