

Effect of honey and intensity of swimming exercise on semen parameters of male albino Wistar rats

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ABSTRACT

Background: The impairment of male fertility has been linked to exercise in a volume-, intensity-, and modality-dependent manner. Infertility is a worldwide problem and male factor infertility is found to be increased. Chronic administration of honey results in elevating sorbitol dehydrogenase activity and decreases lactose dehydrogenase activity, which was found to be in abundance in spermatids and spermatozoa, and a decrease in this enzyme significantly affects the semen parameters and decreases ATP synthesis due to oxidative stress.

Objective: The study aimed to evaluate the effect of honey and intensity of swimming exercise on semen parameters of male albino Wistar rats.

Methodology: A randomized control trial study was adopted involving 50 sexually mature male Wistar rats (180 ± 20 g). The selected rats were divided into five groups of 10 rats each: group I served as normal control while group II was induced with honey only and served as study control. Groups III–V were study groups induced orally with 7.5 mL/kg of honey twice per week for 8 weeks and exposed to mild, moderate, and high-intensity swimming exercises 5 days/week for 8 weeks, respectively.

Results: Chronic oral administration of pure honey showed that motility, viability, sperm count, and semen morphology were significantly lower, and percentages of abnormal morphology were found to be significantly higher ($P < 0.05$) in group II compared with normal control rats (group I). There was a significant decrease in motility, viability, and morphology in group V when compared with groups I, III, and IV. However, they were significantly higher when compared with test control group. The decrease was found to be swimming exercise time-dependent.

Conclusion: Chronic consumption of pure honey has a deleterious effect on semen parameters, and mild, moderate, and intensity swimming exercises were found to have a positive effect of induced semen parameters of male albino Wistar rats.

Key words: Exercise; honey; intensity; semen; Wistar rats.

Introduction

Infertility is a worldwide problem that causes emotional and psychological distress in both men and women, and the absolute number of couples affected by infertility increased from 42.0 to 48.5 million.^[1] Male, female, and combined male and female factors play a role. Global data show that the percentage of infertility that is attributable to males ranged


between 20% and 70%.^[2] In developing countries, the situation is worse and has been reported that up to 30% of the couples are infertile.^[3,4] About 90% of cases of male infertility are

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due to low sperm count or poor semen quality.^[5] Exercise and physical activity promote overall health benefits that are hard to ignore. It is performed for various reasons, including increasing growth and development, preventing aging, strengthening the muscles, and cardiovascular system. It also boosts the immune system and helps prevent diseases of affluence such as cardiovascular diseases, type 2 diabetes, and obesity.^[6] It plays a role in regulating fertility health and augments an individual's sex appeal or body image, which has been found to be linked with higher levels of self-esteem.^[7,8]

It is, however, important to be aware of the impact of sports on the quality of semen.^[9] Some studies show that exercise may improve quality and quantity of sperm and also hormonal level in men who were previously sedentary.^[10] Despite the proven benefits of regular exercise, there is evidence that spermatogenesis may be hindered in physically active individuals.^[11] Some studies reported a decreased in seminal parameters and sex hormone levels in male athletes, and therefore, a possible impairment of male fertility has been linked to exercise in a volume-, intensity-, and modality-dependent manner.^[12] Therefore, knowing the right intensity of exercise to yield the beneficial effect is what matters. This is due to the fact that poor knowledge regarding the right exercise to be carried out can possibly lead to harmful effects on reproductive system and fertility.^[13]

Honey is a natural product of bees that is found to have several benefits in humans including antiseptic, antibacterial, wound healing, antifungal, anti-inflammatory, antioxidant, and also sex boosting properties.^[14,15] It also possesses an immune modulatory and also aphrodisiac properties because it has been reported to increase sperm count, testosterone, and libido level.^[16] Chronic consumption of honey has exhibited a significant reduction in semen parameters in rats with significant reduction in total count, percentage of motile spermatozoa, and also an increase in abnormal, dead, or none motile sperm cells. Also, none of the experimental animal was found to be fertile.^[17] Another study, however, showed an increase in sperm count, motility, and morphology.^[18] Most of these studies showed a significant reduction in semen parameters especially with chronic use of such agents. There are, however, no interventive measures taken to see whether the abnormalities can be reverted.

Wistar rats have a long history in medical research: they were the first mammalian species specifically domesticated to be used in the laboratory. They have organs and body systems similar to humans and are also susceptible to some diseases that affect humans. Rats have been used to answer a wide range of basic science questions. Rats can swim and they enjoy water. They can swim up to half a mile on open

water.^[19] This study aimed to evaluate the effect of honey and intensity of swimming exercises on semen parameters of male albino Wistar rats with the hope that it will pave a way of conducting similar studies using different modalities of exercises in humans to see whether using simple life modification activity like exercise will improve abnormalities of semen in infertile men.

Methodology

Study design

A randomized controlled trial.

Animals

The experiment was performed in the Physiology Laboratory of Bayero University, Kano, after obtaining approval from the university ethical committee [Appendix]. A total of 50 sexually mature male Wistar strain albino rats, 2 months of age and weighing 180 ± 20 g,^[20,21] were bought from the Department of Biological Sciences, Bayero University, Kano. The honey was obtained from Dawakin Tofa Local Government, Kano State. The principles of laboratory animal care were followed throughout the experiment. The animals were provided with sufficient space and housed at room temperature, with a 12:12 h light–dark cycle, for 15 days prior to the experiment and thereafter throughout the experiment. Their cages were cleaned twice a week and were fed with natural food *ad libitum*^[22] transported from Kaduna state.

Exercise protocol

Fifty sexually mature male Wistar rats (180 ± 20 g) were randomly selected and divided into five groups of 10 rats each; group I served as normal control while group II was induced with honey only and served as study control. Groups III–V were study groups induced orally with 7.5 mL/kg of honey twice per week for 8 weeks and exposed to mild, moderate, and high-intensity swimming exercises 5 days/week for 8 weeks, respectively.

Group II served as the study control. This group was exposed to chronic administration of honey only, no exercise. They gave the semen parameters of male Wistar rats induced with honey.

Group III was exposed to chronic administration of honey and swimming exercise for 30 min/day, 5 days/week for 8 weeks.

Group IV was exposed to chronic administration of honey and swimming exercise for 1 h/day, 5 days/week for 8 weeks.

Group V was exposed to chronic administration of honey and swimming exercise for 1.30 h/day, 5 days/week for 8 weeks.

All the rats of the exercise group swam at the same time in separate water tanks with a calculated average of 300 cm² of water surface area for each rat and a depth of 60 cm at a water temperature of 35°C ± 1°C.^[23] An electric hair dryer was used to dry the body immediately upon removal from the water. The research assistants together with the researcher conducted the exercise protocol.

Euthanasia, collection of reproductive organ

At the completion of the exercise, a trained laboratory technician euthanized the rats. Animals of all groups were sacrificed by light ether anesthesia 24 h after the last day of exercise to avoid the acute effect of exercise. The epididymal sperm was collected by cutting epididymis into small pieces and flushing the sperm in 1 mL of normal saline. The prepared sperm sample was used to analyze the semen by the researcher under supervision of a microbiologist.

Analysis of semen parameters

Estimation of semen pH

The pH of the semen was measured using a specially treated calibrated paper blot that changes color according to the pH of the semen that it is exposed to.

Sperm count

Assessment of sperm count was carried out by diluting the semen (1 in 20) using sodium bicarbonate-formalin diluting fluid. The well-mixed diluted semen was then applied to an improved Neubauer ruled chamber and appropriately filled; it was then left for about 3 min for the spermatozoa to settle. The number of spermatozoa in an area of 2 mm² (i.e., 2 large squares) was counted. The number of spermatozoa in 1 ml of fluid was calculated by multiplying the number counted by 100,000.

Sperm viability

This was estimated using the improved one-step eosin staining technique. A fraction of each suspension of the sperm samples was mixed with equal volume of eosin stain and prepared on glass slides for each sample. After 2 min, the slides were examined under the microscope for percentage viability. Normal live sperm cells (viable) exuded the eosin, while dead sperm cells took up the stain. Percentage viability of the spermatozoa was counted using 40× objectives.

Sperm motility

Semen samples from the different treatment groups were dropped on a glass slide and viewed under the microscope except the tail length which showed a significant increase. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 spermatozoa for each

rat. The count and percentages of sperm motility were taken.

Sperm morphology

A light microscope with the oil immersion objective was used to examine the stained cells and the percentage populations of normal, abnormal, dead, and none motile spermatozoa. The morphological abnormalities were divided into head and tail defects. Sperm abnormalities of the mid-piece were included as part of assessment of the sperm tail. The percentages of normal and abnormal shaped sperms were calculated.

Data collection and statistical analysis

Data were collected, cleaned, and coded and were entered into statistical software (SPSS version 23.0; SPSS, Chicago, IL, USA). The results were presented with the aid of tables and figures. The differences between the four groups were determined using one-way analysis of variance. The level of significance was set at 5% with a *P* value <0.05.

Results

The control group of male albino Wistar rats showed a pH of 7.21, sperm count of 53.40%, motility of 58.50%, a viability of 78.50%, and a normal morphology of 76.30%. However, the results obtained after 8 weeks twice daily of 7.5 mg/kg pure honey oral administration showed a pH of 7.20, sperm count of 42.00 × 10⁶/mL, motility of 18.50%, viability of 43.50%, and a normal morphology of 74.30% as shown in Table 1.

Following induction with honey and exposure to different intensities of exercises, the semen parameters of male albino Wistar rats that underwent low-intensity swimming exercise showed a pH of 7.18, sperm count of 47.00 × 10⁶/mL, a motility of 44.80%, and viability of 52.70 with a normal morphology of 64.50%. However, in the group that underwent moderate intensity exercise, the pH was found to be 6.96 with a sperm count of 48.10 × 10⁶/mL, a motility of 53.10%, viability of 56.00%, and normal sperm morphology of 67.80%. The results obtained following high-intensity swimming exercise showed a pH of 6.82 with a sperm count of 49.00 × 10⁶/mL, a motility of 38.40%, a viability of 37.40%, and normal morphology of 39.00% as shown in Table 1.

Discussion

The control group of male albino Wistar rats shows a pH of 7.2, count of 53.40%, motility of 58.50%, viability of 78.50%, and a normal morphology of 76.30%. The pH of the control group is similar to the World Health Organization (WHO) reference range PH, but other parameters of the control group are higher than the WHO 2010 reference values of 15 million

Table 1: Effect of honey and intensity of exercise in rats administered with 7.5 mg/kg of honey twice per week and daily swimming exercise for 8 weeks

Group/Doses	pH	Motility (%)	Viability (%)	Sperm count ($\times 10^6/ml$)	Semen morphology (%)
Group I (Normal Control)	7.21 \pm 0.018	58.50 \pm 3.16	78.50 \pm 1.67 ⁱ	53.40 \pm 0.81	76.30 \pm 3.35
Group II (TEST CONTROL) Administered with 7.5 mg/kg of honey for 8 weeks	7.20 \pm 0.039	18.30 \pm 1.29 [*]	43.50 \pm 3.33 [*]	42.00 \pm 1.60 [*]	74.30 \pm 3.73 [*]
Group III (Honey + Exercise) Administered with 7.5 mg/kg for 8 weeks twice per week and Swimming exercise for 30 min daily	7.18 \pm 0.04	44.80 \pm 5.29 ^a	52.70 \pm 1.98 ^k	47.00 \pm 0.69 ^p	64.50 \pm 2.47 ^c
Group IV (Honey + Exercise) Administered with 7.5 mg/kg for 8 weeks twice per week and Swimming exercise for 1 hour daily	6.96 \pm 0.04 ^t	53.10 \pm 2.47 ^b	56.00 \pm 2.49 ^m	48.10 \pm 0.69 ^p	67.80 \pm 1.51 ^d
Group V (Honey+Exercise) Administered with 7.5 mg/kg for 8 weeks twice per week and Swimming exercise for 1hour 30 min daily	6.82 \pm 0.03 ^r	38.40 \pm 2.06 ^s	37.40 \pm 2.53 ⁿ	49.00 \pm 1.30 ^p	39.00 \pm 1.59 ^e

Results are expressed as mean \pm SE, $n=10$. Values with superscript (*) are significantly different at $P<0.05$ when compared with the normal control group. Values in the same column with the same superscript are significantly similar at $P<0.05$ when compared with the test control

per mil sperm count or total count of 39 million in the entire sample, both progressive and none progressive motility of at least 32%, viability of 58%, and a normal morphology of at least 4%, and this may also explain the reason why rats have a high reproductive capacity [Table 1].

The results obtained after 8 weeks twice daily of 7.5 mg/kg pure honey chronic oral administration show no significant changes ($P > 0.05$) in pH, while motility, viability, sperm count, and semen morphology were found to be significantly lower, and percentages of abnormal morphology were found to be higher ($P < 0.05$) in test control rats (group II) when compared with the normal control rats (group I) [Table 1]. This result agrees with the findings of a study that was carried out in Delta state Nigeria, which revealed that chronic consumption of honey has resulted in significant reduction in sperm parameters with significant reduction in total sperm count and percentage of motile spermatozoa and significant increment in percentages of abnormal, dead/nonmotile spermatozoa ($P < 0.05$).^[19] It is also in keeping with the studies conducted in other places in Nigeria, Germany, Sri Lanka, and Saudi Arabia where *Anacardium occidentale*, Plantain fruit powder, Cimetidine, Iagactil, cadmium and diazinon, phenytoin sodium, and tamoxifen, 2,4-dichlorophenoxyacetic acid (2,4-D) were used to induce abnormal semen parameters in rats.^[21-24] A study that was carried out in humans showed that sperm motility, survival rate, sperm density, and normal sperm morphology rate of end-stage uremic patients were found to be significantly lower than those of the controls.^[25]

The semen pH at the dose of 7.5 mg/kg pure honey and 30 min swimming exercise (EG III) were lower ($P < 0.05$) when compared with test control rats, but statistically not significant ($P > 0.05$) when compared with the normal rats. However,

motility, viability, sperm count, and sperm morphology were found to be significantly higher ($P < 0.05$) when compared with test control but still lower when compared with the normal control [Table 1]. While at 7.5 mg/kg daily doses for 60 and 90 min swimming exercise, semen pH was found not to be significantly different ($P > 0.05$) between groups IV and V but significantly lower ($P < 0.05$) when compared with test control and normal groups. Similarly, motility, viability, and morphology were significantly different ($P < 0.05$) in group IV, while no significant change was observed in sperm count ($P > 0.05$) when compared with test control group (I). There was a significant decrease ($P < 0.05$) in motility, viability, and morphology in group V when compared with groups I, III, and IV. It was, however, significantly higher when compared with the test control group. The decrease was found to be exercise-time-dependent as it occurs with an increase in the time of swimming exercise by the rats. A study that was conducted in Spain links moderate physical activity in males with better hormone levels (follicle-stimulating hormone, luteinizing hormone, testosterone, cortisol, and T/C-testosterone cortisol ratio) and also better semen parameters compared with sedentary men.^[10] The same researcher also concluded that the semen parameters of elite sportsmen (triathletes and water polo players) are worse than in men who are just physically active.^[10] An alteration in sperm density, motility, and morphology was also reported in another study and therefore concluded that endurance training exercise is associated with subclinical modifications in semen characteristics. Similar findings were also reported in a study that was conducted among 286 subjects distributed to moderate and high- intensity exercises using treadmill running for 120 min, five sessions per week for 60 weeks. It shows a significant decrease in semen parameters in those in the high-intensity group compared with those exercising at moderate intensity.^[11] Multiple comparisons test after

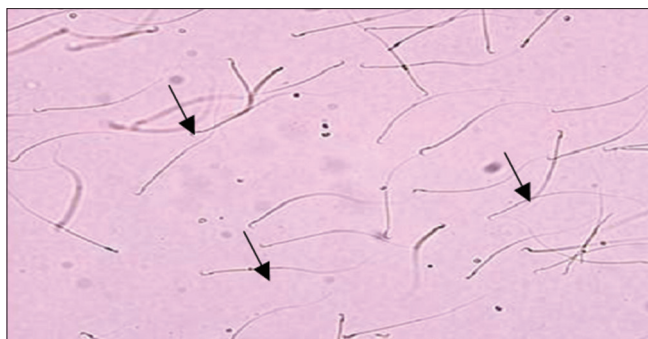


Figure 1: Photomicrographs of sperm obtained from dissected epididymis of rats in the normal Control, showing normal semen morphology with normal hook shape heads, and long tail

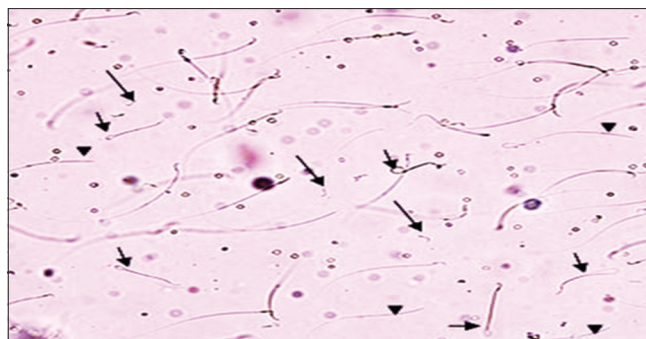


Figure 2: Photomicrographs of sperm obtained from dissected epididymis of rats induced with 7.5mg/kg pure honey for eight week (Group II) showing reduced head, greatly increase head size, reduced tail length ,bent tail, Abnormal shape and clump head

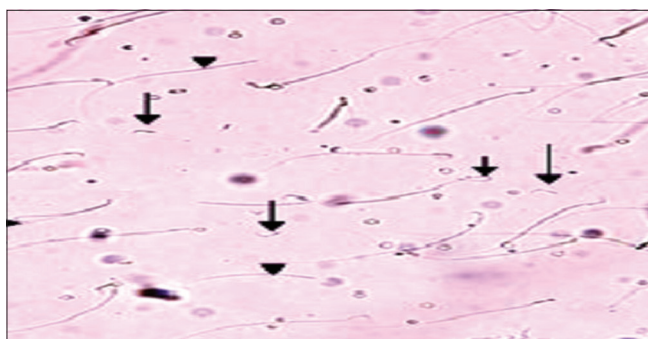


Figure 3: Photomicrographs of sperm obtained from dissected epididymis of rats Administered with 7.5mg/kg for eight weeks twice per week and Swimming exercise for 30min daily showing some reduction reduced head, greatly increase head size, reduced tail length ,bent tail, Abnormal shape and clump head

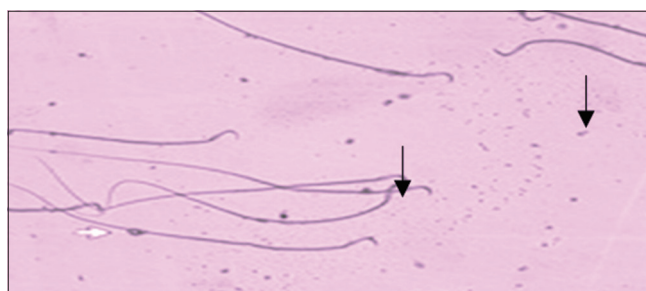


Figure 4: Photomicrographs of sperm obtained from dissected epididymis of rats Administered with 7.5mg/kg for eight weeks twice per week and Swimming exercise for 60min daily showing some significant reduction reduced head, greatly increased tail length, and tail bend, Abnormal shape and clump head

inducement with honey showed that the changes in motility, viability, sperm count, and morphology in group II were considered significant ($P < 0.05$) when compared with the normal group, while motility, viability, and sperm morphology of groups III–V were considered significantly lower ($P < 0.05$) while pH was found not to be significant when compared with the test control [Table 1]. Some studies, however, did not show any changes in semen parameters.^[25]

The results obtained from the morphological assessment of sperm such as reduced head, greatly increased head size, reduced tail length, bent tail, abnormal shape, and clump head indicated that the percentage of greatly increased head size was significantly higher in group II when compared with control nonhoney-induced group I, and reduced head, reduced tail length, bent tail, abnormal shape, and clump head were not seen in group I [Figure 1]. However, in group II, a majority of abnormalities seen were reduced head, greatly increased head size, reduced tail length, bent tail, abnormal shape, and clump head [Figure 2]. However, rats in groups III–IV administered with 7.5 mg/kg and swimming exercise for 30 and 60 min daily, respectively, showed significant reduction ($P < 0.05$) in all the abnormalities as the time

of exercise increases. Except the tail length which showed a significant increase [Figures 3-5]. Other studies where high-fat diet, phenytoin sodium, and 2,4-D were used as the inducing agents also reported increment in abnormal sperm morphology. However, in studies where cadmium and diazinon and also seeds of *Garcinia kola* were used as the inducing agents, the percentage of sperms with abnormal morphology was found to be lower.^[19] The use of 10% honey as a cryopreservative agent was, however, found to be associated with increment in normal morphology of sperms cells.^[26] Cimetidine and ranitidine were, however, found to have no effect on sperm morphology.^[26]

Conclusion

Male factor infertility is found to be on the increase especially in developing countries and nothing much is done to address the problem. In addition, chronic consumption of honey has a deleterious effect on semen parameters, and honey is one of the agents of abuse especially in northern part of Nigeria. Mild and moderate intensity swimming exercises were found to have a positive effect on induced semen parameters of male albino Wistar rats as seen in this study. There is the need to educate the public about the harmful effect of the chronic use

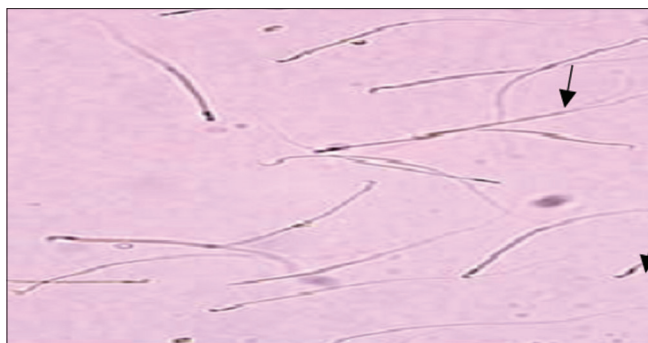


Figure 5: Photomicrographs of sperm obtained from dissected epididymis of rats Administered with 7.5mg/kg for eight weeks twice per week and Swimming exercise for 90mins daily showing some significant reduction in reduced head, greatly increase head size, reduced tail length ,bent tail, Abnormal shape and clump head

of honey, educate the public on the right intensity of exercise to yield a beneficial effect, and also conduct a research on the effects of exercise on semen parameters of male partners with abnormal semen parameters to see whether exercise, a simple life modification activity, will play a role in improving abnormal semen parameters in infertile men.

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Nil.

Conflicts of interest


There are no conflicts of interest.

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Appendix


BAYERO UNIVERSITY, KANO
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16th August, 2017

Dr. Hauwa Musa Abdullahi,
Department of Obstetrics and Gynaecology,
College of Health Sciences,
Bayero University,
Kano.

Dear Ma,

RESEARCH ETHICS APPROVAL

Sequel to your application dated 20th June, 2017, seeking for approval to conduct research. **THE EFFECT OF HONEY ON INTENSITY OF SWIMMING EXERCISE AND SEMEN PARAMETERS OF MALE ALBINO RATS.**


The College Research Ethics Committee (CHS-REC) has reviewed your proposal and made observations ,which you adequately addressed.

In view of the above ethics approval is hereby granted to conduct the research.

You are required to submit a report on the progress of the study and its completion to the CHS-REC.

Accept the assurance of my highest regard.

Best wishes.


Ummi M. Gwadabe
FOR: Chairman

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