PJN
ISSN 1680-5194
ansinet.com/pjn

PAKISTAN JOURNAL OF NUTRITION

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OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2023.88.94

Research Article
Histomorphology of Testes in Honey Treated Adult Male Albino Rats

[1]Anulika O. Onyemelukwe, [2]Rita I-O. Ogu, [1]Nkiruka C. Azubuike, [1]Cornelius O. Ogu, [1]Okechukwu S. Onwukwe, [1]Udoh P. Iniekong, [1]Basil C. Udeh, [1]Peter U. Achukwu and [1]Ikenna K. Uchendu

[1]Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, University of Nigeria, Enugu Campus, Enugu, Nigeria
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, University of Science and Technology, Enugu State, Nigeria

Abstract

Objective: Present study was conducted to examine the histomorphology of testes and serum testosterone levels of adult male albino rats fed with honey. **Materials and Methods:** The albino rats were divided into four groups of eight per cage. Controls received drinking water. Groups A, B and C were fed with 0.15, 0.3 and 0.6 mL kg^{-1} b.wt. of honey respectively twice a day, orally for four and eight weeks. Blood samples were obtained from 4 rats per group for testosterone estimation. Testes were excised for histomorphology. **Results:** After eight weeks, testes from control and honey-treated rats showed normal histoarchitecture, whereas four-week honey-treated rats showed decreased prominence of seminiferous tubules with prominent sertoli cells and reduction of leydig cells. After four weeks, honey-treated rats showed significant elevation in serum testosterone level ($p<0.05$), but after eight-week, no significant change ($p>0.05$) was observed, compared to controls. **Conclusion:** Honey consumption could have a short-term positive effect on testosterone secretion and may lead to hyperactive testicular cells.

Key words: Albino rats, honey, leydig cells, sertoli cells, testes, testicular tissues, testosterone

Citation: Onyemelukwe, A.O., R.I-O. Ogu, N.C. Azubuike, C.O. Ogu, O.S. Onwukwe, U.P. Iniekong, B.C. Udeh, P.U. Achukwu and I.K. Uchendu, 2023. Histomorphology of testes in honey treated adult male albino rats. Pak. J. Nutr., 22: 88-94.

Corresponding Author: Cornelius O, Ogu, Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, University of Nigeria, Enugu Campus, Nigeria Tel: +2348035387596

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Honey is a natural and biological product produced by bees from nectar obtained from flowering plants. It is among the most advantageous natural substance available to mankind for a long time[1]. Glucose and fructose are the two major sugars in honey along with enzymes, amino acids, proteins, flavonoids and phenolic acids[2].

Most herbal medicines use honey as a constituent since it is beneficial in repairing wounds and burns tissues, treating diabetic wounds and suppressing coughs[3]. There are many health benefits associated with honey, including hypoglycaemia, antioxidant, hepato-protective, reproductive, antihypertensive[4]. Honey is most useful as a topical agent for wounds and skin infections. In recent years, honey has become an important and active treatment agent. A number of its beneficial uses have been approved, including anti-inflammatory, anti-oxidant, antimicrobial as well as boosting immunity[5].

Globally, increased prevalence of male subfertility has become a major public health challenge[6]. Subfertility in males has been related to; environmental, genetic, nutritional, psychological, pharmacological and psychogenic factors[7]. Arab countries consider honey to improve men's capability. A previous study has reported that honey increased spermatogenesis in rats[8]. Procreation is the central theme of life.[9] There is a significant variation in the prevalence of infertility among men between countries due to differences in lifestyle[10]. In 2007, 72.4 million people were sub fertile and of these 40.5 million people visited hospital for fertility care.[6] Thirty percent of subfertility cases were caused by male factors[11]. Herbs and natural products have been used to curb subfertility over the years.

Testes are part of the male reproductive system or persons assigned male at birth (AMAB). Testes are also called male gonads and normally two in number each referred to as testis. Their major functions are to produce male sex hormones, mainly testosterone and spermatozoa. Testes are secured in sacs of skin called scrotum under the penile shaft. They are egg-shaped, 2-3 cm wide and 3-5 cm long in adult man[12]. Testicles enlarge during adulthood and then reduce in size later in life following natural fall in serum testosterone secretion. Temperature of the testes is about two degrees lower than that of the body and this temperature is suitable for spermatogenesis in the seminiferous tubules which takes about 72 days in man. In order to maintain a lower temperature, the testicles are suspended from the abdomen by the spermatic cord which is composed of nerves, blood vessels and tubes that enhance their function[12].

Each testis consists of multiple compartments connected by narrow seminiferous tubules. Sperm cells are produced in the seminiferous tubules through a process of spermatogenesis. As sperm cells develop and mature, they move through seminiferous tubules to rete tests, then to the epididymis, where they are stored and mature completely.

A hormone called testosterone is responsible for secondary men features such as; facial and body hairs and muscular enlargement. Testosterone also promote sex urge (libido), sexual activities and men fertility[12]. Leydig cells secrete testosterone and are located between the seminiferous tubules in the testicles. Absence of testosterone results in infertility because testosterone stimulates the maturation of sperm cells. Men have the ability to produce millions and billions of sperm cells daily. However, complete maturation of sperm cells to active cell takes several months. Maturation of sperm cells commence in the testicles but completion occurs in the epididymis where they are stored.

Congenital testicular disorders

Cryptorchism: In Cryptorchism testicles fail to descend after few months of birth and this condition is corrected by surgery known as orchiopexy. Orchiopexy help to prevent the testes from prolonged exposure to higher abdominal temperature. The higher abdominal temperature is a risk factor for infertility or testicular cancer[13]. Cryptorchism is a common congenital disorder of male babies[14].

Retractile testes: Retractile testes are a condition where the testes move between the scrotum and abdomen. This doesn't require treatment if the testes are in the scrotum.

Polyorchidism: Polyorchidism is a congenital condition where a baby boy has more than two testes at birth[15].

Transverse testicular ectopia: It occurs when both testes descend into one scrotum. This normally occurs with another defect such as hypospadias (when the urethral opening is not at the tip of penis[16]. A congenital disorder of the testicles is not orchitis, varicocele, testicular torsion and cancer of the testes[17-19.]

Histological functions of the testes: The reproductive system of male consists of the testicles, genital cord, accessory glands and penis. The testes are comprised of the fibrous tunica albuginea, testicular lobes, seminiferous tubules, septae, rete testis, epididymis, ductus deferens.

Sperm cells are produced in the testicular, seminiferous tubules. Capsule gives rise to the septa which divides the testicles into around 250 partial lobules. In the posterior border, the septa meet along a thickening called the mediastinum testis of the tunica albuginea. Each lobule comprises of one to four seminiferous tubules enclosed in a connective tissue stroma. The seminiferous tubules have a length of 30-70 cm.

Spermatozoa production is enhanced by leydig and sertoli cells. Gametogenesis (Spermatogenesis) occurs in male at puberty age. It occurs in the seminiferous tubules and controlled by testosterone secreted by the leydig cells. The process of Gametogenesis (Spermatogenesis) involves two stages of meiotic division from one diploid cell to four haploid cells.

The germinal or seminiferous epithelium of the seminiferous tubules contains spermatogenic and sertoli cells. The spermatogenic cells (spermatogonium) multiply mitotically to form 2 sparmatogonia. First and second meiotic divisions will form gametes (primary and secondary spermatocytes, respectively) from the spermatogonia formed during mitosis. The secondary spermatocytes will then undergo spermiogenesis to form the spermatids and spermatozoa. Until spermatozoa are formed, developing cells are attached to the cytoplasmic bridge[20].

Sertoli cells: Seminiferous tubules are covered by two different groups of cells, spermatogenic cells and sertoli cells. Spermatogenic cells develop and mature into spermatozoa and sertoli cells function as supportive and nutrient supplying cells. Sertoli cells are simple columnar cells, which extend from the basement membrane to the lumen of the cell. Germ cells multiplying and differentiating are surrounded by Sertoli cells, which form pouches around them. Sertoli cells supply nutrients and phagocytose excess cytoplasmic substances from the spermatids (immature spermatozoa or immature sperm cells). These phagocytosed cytoplasmic substances are not necessary in maturation of the sperm cells. Sertoli cells are connected by continuous tight junctions, forming blood-testis barrier that does not allow big molecules to move between the adluminal and basal compartments. To prevent antibody formation against sperm cells, blood-testis barriers prevent maturing spermatogonia, spermatocytes and spermatids and mature sperm cells from entering bloodstream. As epithelial cells, sertoli cells absorb nutrients from nearby capillaries to nourish their germ cells. Sertoli cells also produce testicular fluids which contain proteins that bind and concentrate testosterone making it available for the spermatozoa development. As germ cells develop, sertoli cells move towards the lumen to engulf degenerating germ cells and excess cytoplasm.

Leydig cells: Leydig cells are found between the tubules as interstitial cells and the cytoplasm is pale due to high cholesterol-lipid droplets used for testosterone synthesis. In response to a hormone produced by pituitary gland called luteinizing hormone (LH) the leydig cells synthesize and secrete testosterone. After the onset of LH secretion, testosterone enhances the production of spermatozoa, secretions from accessory sex glands and male secondary character development. Sertoli cells are stimulated by follicular stimulating hormone (FSH) to secrete androgen-binding protein (ABP) into the lumen. The ABP bind testosterone and make it locally available for spermatogonia. Sertoli cells possess active FSH receptors, whereas leydig cells possess LH receptors[20].

The production and secretion of men sex hormones are controlled by negative feedback mechanisms at different stages. Hypothalamus is the first point where testosterone is produced and therefore, spermatozoa are produced. Hypothalamus is part of the brain which composes neuroendocrine cells which can secrete a substance called Gonadotropin-releasing hormone (GnRH). GnRH influences the basophilic cells of the adenohypophysis, through the "portal system" to secrete two mediating hormones within the men sex hormone cycle.

Inhibin: Inhibin is secreted by sertoli cells when there are fewer nutrients to feed developing spermatozoa. Inhibin acts in a negative feedback style, goes to brain to reduce the secretion of Follicular Stimulating Hormone and Gonadotropin-Releasing Hormone. Inhibin is released by sertoli cells in the testicles which are responsible for monitoring the development and health of spermatozoa. The sertoli cells of the testes releases inhibin when spermatozoa levels are high, causing nutrients for their development to be scarce. The inhibin moves through the bloodstream to the brain, where it halts the production of GnRH. In the absence of GnRH, Follicular Stimulating Hormone and Luteinizing Hormone levels fall and sperm production slows down. If the spermatozoa count falls to 20 million mL^{-1}, the sertoli cells stops the secretion of inhibin and the spermatozoa count rises. This is one of the main ways through which men sex hormones are maintained at relatively constant levels[21].

An investigation into the histomorphological effects of honey on the testicles is still being carried out to build up adequate knowledge about reproductive fertility. Although,

previous studies have reported a wide variety of the therapeutic and nutritional values of honey[5,22] but to date, there is paucity of information concerning the medicinal effects of honey on reproductive performance with respect to testicular morphology. Hence, the present study was carried out to evaluate the histomorphology of the testes and its relationship with serum testosterone level in honey-fed adult male albino rats.

MATERIALS AND METHODS

Procurement of honey: A fresh, original bottle of honey was obtained from a town of South-East Nigeria and authenticated at Plant Sciences Department of a higher institution.

Preparation of the Honey: Honey was freshly diluted every day with double dilution, 10 mL of the honey was mixed with 10 mL of clean drinking water in a sterile universal container and was swirled for homogeneous mixture to make a 1:2 dilution for easy pipetting.

Animal housing: A total of 32 adult male albino rats, weighing approximately 200 g were obtained from an animal house in the Department of Anatomy, College of Medicine of a higher institution. The rats were kept in clean gauzed cages in groups according to approximate body weight. They were fed on standard feed pellets (Guinea feed® Nigeria Plc). Feed and clean water were provided *ad libitum*. The rats were acclimatized for two weeks and handled in accordance with approved protocols by Institutional guidelines on Animal Care and use Committee and conform to guidelines set by National Institutes of Health on experiments involving the use of animals. Research approval and identification number (FVM-UNN-IACUC-2019-0249) were obtained from Veterinary Medicine Faculty of a higher institution.

Experimental design: A total of 32 adult male albino rats were divided into four: Group-A, group-B, group-C and group-D of eight rats per group and cage according to approximate body weight before commencing treatment. Group D served as control and received only feed and water, while groups A, B and C received 0.15, 0.3 and 0.6 mL kg^{-1} b. wt., of honey respectively twice a day for four weeks and eight weeks. After the first four weeks, four rats were selected at random per cage and weighed. Blood samples were obtained via retro-orbital puncture from the four rats for testosterone estimation. Their testes were excised under chloroform anesthesia, weighed and preserved in 10% formal saline for further histomorphology tissue processing. After the second four weeks (eight weeks), the remaining four rats per cage were weighed. Blood samples were obtained via retro-orbital puncture from the remaining four rats per group for testosterone estimation. Their testes were excised under chloroform anesthesia, weighed and preserved in 10% formal saline for further histomorphology tissue processing. The testicles were professionally handled following standard protocol for tissue preservation and processing in Histopathology laboratory. The tissues were stained with Hematoxylin and Eosin staining technique[23]. Microscopy and photomicrography were done by Histo-Scientist and Pathologist.

Testosterone assay: The testosterone levels in the serum were estimated using the standard Accu Bind® ELISA Microwells with Testosterone code system product code: 3725-300, by Monobind Inc. Lake Forest, CA 92630, USA. It applied the principle of Competitive Enzyme immunoassay.

Relative organ weight: After four and eight weeks of honey administration, the testicles of the rats were harvested under chloroform anesthesia and cleaned. Gross observation was carried out on the testes for the presence of lesions or any signs of abnormality. The testes of each rat were weighed for determination of the Relative Organ Weight (ROW) using the following equation:

$$\text{Relative organ weight (testis) (\%)} = \frac{\text{Weight of testis (g)}}{\text{Weight of rat (g)}} \times 100$$

The statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 20.0. Data were reported as Mean±Standard Error of Mean (SEM). One-way Analysis of Variance (ANOVA) and Post Hoc Tukey HSD (Honestly Significant Difference) test was used for comparisons among means. Differences of $p \leq 0.05$ were considered statistically significant.

RESULTS

Effects of honey on the relative organ weight (ROW) of testicles of adult male wistar rats: Table 1 shows the relative organ weight (ROW) of the testicles of rats. After four weeks, the mean relative organ weight of the testes of honey-treated rats were significantly higher in group A ($p = 0.006$), group B ($p = 0.043$) and group C ($p = 0.046$) compared to the control group. After eight weeks, there was no significant increase in

Table 1: Relative testicular weights (%)

Groups	Mean±SEM (%) after 4 weeks	p-values	Mean±SEM (%) after 8 weeks	p-value
Control	0.673±0.169		1.103±0.045	
Group A	1.623±0.087	0.006**	1.187±0.068	0.213
Group B	1.263±0.154	0.043*	1.063±0.083	0.366
Group C	1.147±0.078	0.046 *	0.983±0.045	0.089

p<0.05: *Significant, p<0.01: **Highly significant and p<0.001: ***Very highly significant

Table 2: Serum testosterone level (ng mL^{-1})

Groups	Mean±SEM (ng mL^{-1}) after 4 weeks	p-values	Mean±SEM (ng mL^{-1}) after 8 weeks	p-value
Control	0.23±0.02		4.00±1.09	
Group A	0.97±0.87	0.20	2.60±1.84	0.24
Group B	2.43±0.83	0.04*	5.27±0.16	0.15
Group C	2.90±0.53	0.01**	4.97±0.35	0.21

p<0.05: *Significant and p<0.01: **Highly significant

the mean relative organ weights of the testes of honey-treated rats, in group A (p = 0.213) and there was no significant decrease in relative testicular weight in group B (p = 0.366) and C (0.089) compared to the control group.

Effects of honey on the serum testosterone level of adult male wistar rats: In comparison to the control group, honey-treated rats in groups B (p = 0.04) and C (p = 0.001) showed a significant increase in testosterone levels after four weeks, however, no significant difference was observed between group A (p = 0.2) and control group, after four weeks of treatment. However, after eight-weeks of treatment, there was no significant difference in serum testosterone level between group A and control, whereas compared to the control group, neither group B (p = 0.15) nor group C (p = 0.21) showed a significant increase (Table 2).

Effects of honey on the histomorphological of the testicular tissues of adult male wistar rats: According to Fig. 1, testicular tissues from the control group showed normal testicular histoarchitecture or microanatomical structure whereas, Fig. 2 showed that honey treated rats showed significantly reduced spermatogenesis, a decrease in number of germ cells with prominent sertoli cells and a reduction in leydig cells compared with control group, after four weeks. After eight weeks, honey treated rats showed no obvious differences in testicular histoarchitecture compared to the control group (Fig. 3).

DISCUSSIONS

In honey treated rats, relative organ weight of the testes increased significantly after four weeks, possibly due to increased testosterone levels after four weeks in honey treated rats. This result contradicts with a previous study which showed no significant difference in the relative organ weight

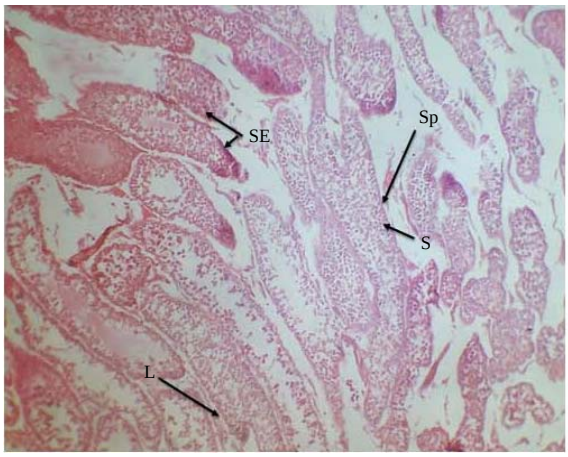

Fig. 1: A photomicrograph of testis from control group shows normal histoarchitecture, H and E staining; x400
SE: Sertoli cells, L: Lerdig cells, SP: Spermatogonia, S: Spermatocyte

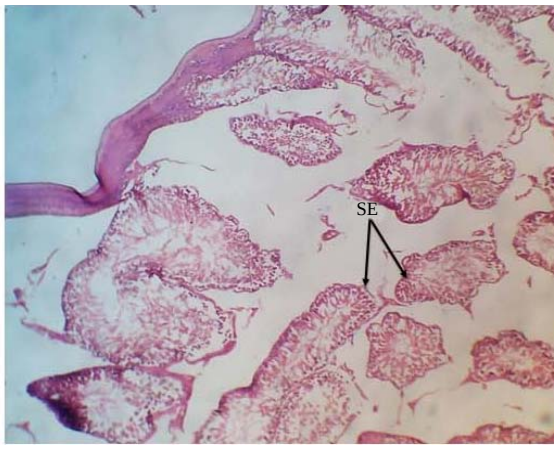

Fig. 2: Group C- (4 Weeks) decreased germ cell population, prominent Sertoli (SE) cells. The leydig cells are markedly reduced and there is decreased tubular density H and E staining: x400

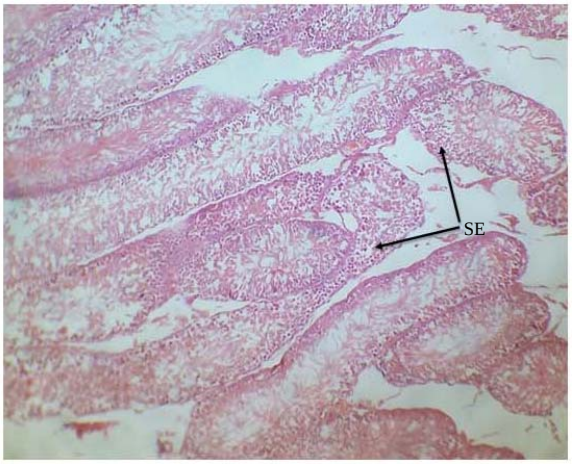

Fig. 3: Group C (8 weeks) sertoli cells (SE) present. No obvious difference in the testis histoarchitecture compared to the control

of the testes of honey-treated rats when compared to controls, though their honey was Obudu natural honey and on long term of twenty-nine weeks[24] but correspond with the result of the present study after eight weeks of honey administration which revealed no significant increase and decrease in the relative testicular weight of wistar rats.

A significant increase in testosterone level was observed in honey treated rats who received 0.3 mL kg^{-1} b. wt. and 0.6 mg kg^{-1} b. wt. honey after four weeks, but there were no statistically significant changes after eight weeks. There is a correlation between this raised level and the relative testicular weight and histoarchitecture of the testis. Previous studies also reported increase in serum testosterone levels of honey-treated rats[25-27]. Testosterone is a steroid hormone that is produced in the leydig cells within the testes. It is the male sex hormone required for spermatogenesis. Spermatogenesis occurs as a result of the stimulation by testosterone[28].

In the present study, the histomorphology of the testes of honey-treated rats after four weeks showed decreased spermatogenesis, prominent sertoli cells and reduced leydig cells. There is a possibility that honey stimulated leydig cells to secrete more testosterone which through a negative feedback mechanism, reduced leydig cell secretion and spermatogenesis by interacting with luteinizing hormone. The result of this research is in accordance with a previous study conducted by Tesarik *et al.*[28], who reported reduced serum level of Luteinizing Hormone and Follicular Stimulating Hormone of honey-treated male wistar rats. However, a comparison between honey treated rats and control rats after eight weeks showed no obvious difference in testes histoarchitecture, hence long term oral intake of honey had no effect on testosterone secretion and testicular histoarchitecture.

Regular use of honey can be counter-productive or non-productive as shown in Table 2 and Fig. 1 since histomorphology of the testes after four weeks showed a significant increase in ROW compared to control unlike after eight weeks of treatment. Similarly, a significant increase in serum testosterone was seen after four weeks compared to the control group unlike after eight weeks of treatment as reported in previous study[24]. There were histomorphological changes in the cellular components and densities in the testicular tissues after four weeks of honey treatment but not so after eight weeks of honey treatment.

CONCLUSION

Honey consumption could exert short term positive effect on testosterone secretion and can lead to hyperactivity of testicular cells. Moderate consumption of honey may be recommended for male reproductive fertility. However, aside from the numerous benefits of honey, it also has positive reproductive effects on the male reproductive system.

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