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Original Article

Honey increases sperm count in male albino rats by enhancing testosterone production.

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ABSTRACT: We investigated the effects of different doses of honey (H) and testosterone (T) on sperm count and reproductive hormones in male albino rats. Thirty-five male albino rats were randomly divided in a blinded fashion into 7 groups of 5 rats each. Group 1 (control) was given 0.2 ml of distilled water. Groups 2, 3 and 4 were given 100 mg/kg, 200 mg/kg and 400 mg/kg of H orally respectively. Groups 5, 6 and 7 were given 2.5 mg/kg, 5 mg/kg and 7.5 mg/kg of T intraperitoneally respectively. All doses of H significantly (P<0.05) increased sperm count in rats, while all the doses of T significantly reduced sperm count in rats. Plasma T was increased and FSH was reduced (P<0.001) by all the three doses of H. On the contrary, LH was significantly reduced (P<0.05) by 100 mg/kg and 200 mg/kg of H but not by 400 mg/kg of H. All the three doses of T reduced the plasma T and LH in rats. Lastly, 5 mg/kg and 7.5 mg/kg but not 2.5 mg/kg of T reduced FSH in rats. The results suggest that honey enhanced sperm count in rat by increasing testosterone production.

KEYWORDS: Gonadotropins, Honey, Sperm count, Testosterone.

INTRODUCTION

Honey is a natural hive product with an extensive history of traditional human medicinal use in a large number of societies. It is widely available in most communities, and its therapeutic potential is grossly underutilized. The mechanism of action of several of its properties remain obscure, thus there is a need for further investigation (Zumla and Lulat, 1989).

Studies have shown that honey minimizes cellular injuries of the skin and post-radiotherapies mucosal trauma. Moreover, it possesses some biological properties such as antioxidant, antimicrobial, anti-inflammatory and immunomodulatory effects (Mohamed *et al.*, 2010; Estevinho *et al.*, 2008; Prakash *et al.*, 2008; Timm *et al.*, 2008; Perez *et al.*, 2006). The natural antioxidants, especially flavonoids, exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, anti-allergic, anti-thrombotic, and vasodilatory actions (Cook and Sammon, 1996). Other reported properties of honey include, anti-platelet and anti-nociceptive (Kamran *et al.*, 2006), prophylaxis against biofilm formation (Irish *et al.*, 2006), an aid or remedy to manage diversity of wound aetiologies (Gethin and Cowman, 2005) and dressing (Rahal *et al.*, 1984; Chirife *et al.*, 1983; Knutson *et al.*, 1981), dyspepsia and peptic ulcers (Al Somai *et al.*, 1994) and as a preservative for herbal medicines (Molan, 1998).

Honey is a natural product with very complex chemical composition. The composition of a particular honey sample greatly depends on the composition of nectar, where it originates. It contains more than 180 substances (White, 1979) including moisture; sugars such as glucose and fructose; enzymes such as catalase and glutathione reductase; trace essential elements such as iron, copper, zinc and calcium; vitamins such as vitamin A, C and E as well as some flavonoids and phenolic acids (Michalkiewicz *et al.*, 2008; Yao *et al.*, 2004; Al-Waili, 2003; Gheldof *et al.*, 2002). It is composed primarily of fructose and glucose but also contain 4-5% of fructooligosacharide which serves as prebiotic agent (Chow, 2002). Flavonoids of antibacterial activity including pinocembrin (Bogdanov, 1984; Villanueva *et*

al., 1970), kaempferol and quercetrin, as well as naringenin and pinocembrin were detected in sunflower honey (Sabatier *et al*., 1989). The presence of galangin and chrysin in several Swiss honeys has also been reported (Bogdanov, 1989). Thus, the composition of honeys varies with different floral sources as well as climatic and environmental conditions (Perez *et al*., 2007; Gheldof *et al*., 2002).

Traditionally, the local Malaysian and other population consume honey as a nutrient as well as for enhancement of fertility. Honey is farmed and used all over Nigeria. Initially local farmers harvested the honey from the wild but today apiculture is a growing industry in many parts of the country. Some studies on the healing effects and antimicrobial activity of honey collected from Nigeria on burns and wounds have been reported (Adesunkanmi and Oyelami, 1994). However, there is a paucity of data on the effects of honey collected from Nigeria on reproductive functions. The decline in male reproductive health and fertility in the last 30 years has been linked to environmental toxicants or xenobiotics (Sikka, 2008). This has led to increased interest to investigate the possible beneficial effect of honey in enhancing fertility in males.

Studies on the effect of honey on reproductive parameters are few and inconclusive. In a study using honey as vehicle for tamoxifen, a nonsteroidal antiestrogenic drug, honey was reported to increase sperm count without affecting other seminal parameters and reproductive hormones in male bonnet monkey (Gill-Sharma *et al*., 2003). A recent study showed that treatment with 5% solution of honey collected from Palestine for 20 days orally to adult male rats increased epididymal sperm count and testicular sorbitol dehydrogenase activity as well as reduced lactate dehydrogenase activity (Abdul-Ghani *et al*., 2008). Moreover, honey collected from Malaysia has recently been shown to increase sperm count without affecting other semen parameters and reproductive hormones in male rat (Mohamed *et al*., 2012). The mechanism underlying an increase in sperm count without any change in reproductive hormones is not clear and of interest to us. The observation of Mohamed *et al.* (2012) therefore raises a question on the possibility of an increase in sperm count without any change in reproductive hormones. Testosterone is needed for the growth and development of male reproductive organs (Mooradan *et al*., 1987) and in association with follicular stimulating hormone, it acts on the seminiferous tubules to initiate and maintain spermatogenesis (Christensen, 1975).

The study reported here was designed to evaluate the effects of different doses of honey on sperm count, testosterone and gonadotropins in male albino rats. To investigate whether the spermatogenic effect of honey is testosterone-related, we also studied the effect of different doses of testosterone on sperm count, testosterone level and gonadotropins in rats.

MATERIALS AND METHODS

Honey sample

The honey used in this study was obtained from the Federal College of Forestry, Ibadan. It was freshly diluted daily to the doses required using distilled water (as a vehicle).

Animals and treatment protocol

Thirty-five male albino rats (200-250 g) were used for the study. The animals were obtained from the animal house of the Department of Physiology, College of Health Sciences, University of Ilorin, Kwara State, Nigeria. They were housed at five per cage and provided with standard laboratory feed and water ad libitum. They were maintained in a well-ventilated room at 25 °C ± 2 °C on a 12-hour light/dark cycle. Study protocol and animal use were approved, prior to the beginning of the study, by our institutional research and ethical committee. All necessary protocols were followed to ensure the humane treatment of the animals.

The animals were randomly divided in a blinded fashion into 7 groups (5 rats per group). Group 1 (control) was given 0.2 ml of distilled water. Groups 2, 3 and 4 were given 100 mg/kg, 200 mg/kg and 400 mg/kg of honey respectively by oral gavage once daily for 4 weeks. Groups 5, 6 and 7 were given 2.5 mg/kg, 5 mg/kg and 7.5 mg/kg of Testosterone (T) (Laborate Pharmaceutical, India) intraperitoneally respectively once daily for 4 weeks. At the end of the study (24 h after the final treatment), laparatomy was performed under ether anaesthesia. Epididymis was removed and blood was collected into sample bottles and allowed to clot at room temperature and sera separated by centrifugation at 1000 rpm for 10 min and stored at -20 °C for hormone estimations.

Estimation of sperm count

The testes from each rat were carefully exposed and one of them was removed together with its epididymis. For each separated epididymis, the caudal part was removed and placed in a beaker containing 1 ml of normal saline solution. It was macerated with a pair of sharp scissors and left for few minutes to liberate the sperm cells into the normal saline. Semen drops were placed on a clean grease-free glass slide and two drops of warm 2.9% sodium citrate were added. The improved Neubauer counting chamber was charged with the semen solution and the number of sperm cells, appearing as black dots were counted in 25 small squares within the central counting area of the counting chamber as earlier described (Cheesebrough, 2000).

Estimation of reproductive hormones

Serum-free testosterone and gonadotrophins (luteinizing hormone and follicle-stimulating hormone) were measured by tube-based enzyme immunoassay (EIA) method (Raji *et al*., 2005) using commercial kits (IBL-Hamburg GmbH, Germany). The EIA is a standardized method used by WHO and part of its program for human reproduction research. The procedures for the assay as contained in the manufacturer's manual were strictly followed. The within assay variation was 8.1% and the sensitivity was 0.3 ng/ml. The optical density was read using a spectrophotometer (Jenway, 6300 spectrophotometer, UK) that was sensitive at wavelengths between 492 nm and 550 nm.

Statistical Analysis

Data were analysed using SPSS version 16.0 for windows. All values given were the mean ± S.E.M of the variables measured. Significance was assessed by the one-way analysis of variance (ANOVA), followed by a post-hoc Turkey multiple range test for multiple comparisons. P-Values of 0.05 or less were taken as statistically significant.

RESULTS

Effects of different doses of honey on sperm count in male albino rats

Effects of different doses of honey (H) on sperm count in male albino rats are shown in Figure 1. Oral administration of the three different doses of H significantly (P<0.05) increased sperm count in rats. The increases in sperm count were not dose-dependent as there was no significant change when the values of sperm count for the different doses were compared.

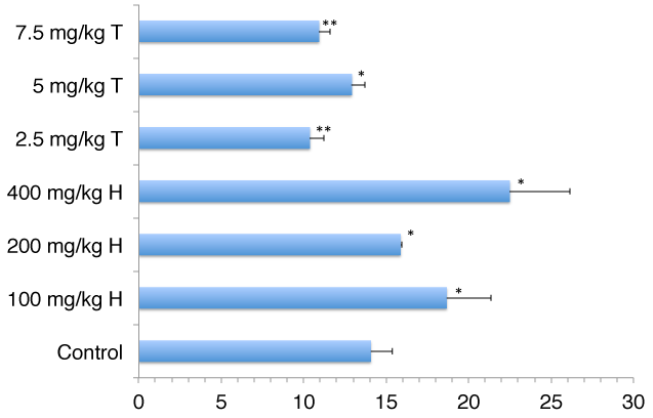

Figure 1: Effects of honey and testosterone administration on sperm count in male rats. H=Honey, T=Testosterne. Values are expressed as Mean ± S.E.M (n=5). $^{*}P < 0.05$, $^{**}P < 0.01$ vs control.

Table 1: Effects of honey (H) and testosterone (T) administration on plasma T, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in male rats. Values are expressed as Mean±S.E.M (n=5). *P<0.05, **P<0.01, ***P<0.001 vs control.

	T (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Control	3.76±0.05	2.75±0.16	8.26±0.52
100 mg/kg (H)	6.18±1.50***	2.57±0.05*	0.13±0.04***
200 mg/kg (H)	5.73±0.79***	2.27±0.07*	0.41±0.07***
400 mg/kg (H)	8.37±0.89***	2.78±1.38	0.46±0.13***
2.5 mg/kg (T)	2.88±0.1***	2.06±0.21*	6.84±0.66
5 mg/kg (T)	2.02±0.1***	1.58±0.2**	4.66±0.35***
7.5 mg/kg (T)	1.46±0.14***	1.14±0.13***	3.92±1.03**

Effects of different doses of testosterone on sperm count in male albino rats.

Effects of different doses of testosterone (T) on sperm count in male albino rats are shown in Figure 1. Oral administration of the three different doses of T caused significant reduction in sperm count in rats. The reduction was more significant (P<0.01) at 2.5 mg/kg and 7.5 mg/kg than 5 mg/kg (P<0.05).

Effects of honey on plasma testosterone and gonadotropins levels in male albino rats

The effects of different doses of H on T, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in male albino rats are shown in Table 1. Plasma T was significantly increased (P<0.001) by oral administration of all the three doses of H. On the contrary, plasma LH was significantly reduced (P<0.05) by oral administration of 100 mg/kg and 200 mg/kg of H but not by 400 mg/kg of H. Moreover, plasma FSH was significantly reduced (P<0.001) by all the three doses of H.

Effects of testosterone administration on plasma testosterone and gonadotropins levels in male albino rats

The effects of different doses of T on plasma T, FSH and LH in male albino rats are shown in Table 1. All the three doses of T significantly (P<0.001) reduced the plasma testosterone in rats. In addition, plasma LH was also significantly reduced by 2.5 mg/kg (P<0.05), 5 mg/kg (P<0.01) and 7.5 mg/kg (0.001) of T. Lastly, 5 mg/kg and 7.5 mg/kg but not 2.5mg/kg of T significantly reduced (P<0.001, P<0.01, P>0.05 respectively) the plasma FSH in rats.

DISCUSSION

Previous studies have reported that honey collected from Palestine and Malaysia increases sperm count in rat and monkey (Mohamed *et al*., 2012; Abdul-Ghani *et al*., 2008; Gill-Sharma *et al*., 2003). The observed increase in sperm count following administration of honey in the present study is consistent with these previous reports. However, critical observation of the data of Mohamed *et al.* (2012) may suggest a contradictory finding as they reported that 200mg/kg of honey obtained from Malaysia did not significantly increase the sperm count in rat, whereas it did in our own study with honey sourced from Nigeria.

Previous studies using honey that originated from Malaysia or Palestine also reported no significant effects of honey on reproductive hormones including testosterone (T), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in rat (Mohamed et al., 2012) and monkey (Gill-Sharma *et al*., 2003). The present study however showed that honey from Nigeria increased T but decreased LH and FSH in rat. The content and composition of honeys vary with different floral sources as well as climatic and environmental conditions (Perez *et al*., 2007; Gheldof *et al*., 2002). The variations between the present study and those previous studies may be a result of the difference in the source, and of climatic and environmental conditions of the honey used for the studies. In addition, we used wistar (albino) rats in this study; Mohamed *et al.* (2012) used Sprague-Dawley rats while Gill-Sharma *et al.* (2003) used bonnet monkey. We speculate that species difference could be another cause of the discrepancy in the findings.

The significant effect of honey on the reproductive hormones in this study is of great interest. 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 sorbital dehydrogenase activity and reduces lactate dehydrogenase activity (Abdul-

Ghani *et al*., 2008). However, we were able to establish another fact 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. Expectedly, the increased plasma testosterone caused reduction in plasma LH and FSH through the previously well-established negative feedback mechanism (Kellis and Vickery, 1984; Prasad *et al*., 1996; Meeuwen *et al*., 2007; Koehler *et al*., 2009).

To support our hypothesis that the sperm count boosting potential of honey is testosterone dependent, we studied the effect of exogenous administration of different doses of testosterone. The observed reduction in sperm count, testosterone and gonadotropins following exogenous administration of testosterone in this study is comparable with previous reports (Awoniyi *et al*., 1989; 1992; Airkin *et al*., 1989; Sun *et al*., 1989; Sharpe *et al*., 1988; Robaire *et al*., 1979). Moreover, testosterone withdrawal has also been shown to cause spermatogenic cell degeneration (Kerr *et al*., 1993). Though we did not measure the testicular testosterone and gonadotropins levels, we speculate that reduction in testicular testosterone level following its exogenous administration could have led to reduction in spermatogenesis, and subsequently low sperm count.

A study on the chemical and physical characterization of honey sourced from different sources within Nigeria showed that it is quite rich in minerals such as K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br and Rb, as well as carboxylic acids, aldehydes, alkynes, nitrites, alkenes and ethers (Adebiyi *et al*., 2004). Recently, Nurul Syazana *et al.* (2013) and Odeh *et al.* (2007) respectively identified 35 and 30 volatile compounds in honey collected from Malaysia and Pakistan. These compounds include acids, aldehydes, alcohol, ketones, terpenes, hydrocarbons, furans, etc. However, the specific component(s) of the honey used in this study that exerted effects different from those sampled from Malaysia or Palestine (Gill-Sharma *et al*., 2003; Abdul-Ghani *et al*., 2008; Mohamed *et al*., 2012) is a subject of interest and further investigation. Better knowledge of this will shed more light into the specific testosterone-dependent mechanism by which honey increases sperm count.

However, it is noteworthy that chrysin (5,7-dihydroxyflavone), a natural flavonoid has been reported to be present at high levels in honey, propolis and many plant extracts (Wang and Morris, 2007). The study of Ciftci *et al.* (2012) clearly showed that treatment with chrysin significantly increased serum testosterone levels in rats. Similarly, Jana *et al.* (2008) found that testosterone production was dramatically enhanced in primary cultures utilising Leydig cells isolated from mouse testis when the cells were treated with chrysin. Other investigators have also drawn attention that aromatase inhibition by chrysin could block the conversion of androgens into oestrogens with a consequent increase in testosterone (Jeong, 1999; Le Bail, 1998; Kellis and Vickery, 1984). However, whether or not chrysin or any other agent (s) capable of boosting testosterone level is present in the honey samples used in this study is not known. Further phytochemical analysis of honey to reveal the active ingredient (s) that is/are capable of boosting testosterone will be worthwhile.

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