Niger. J. Physiol. Sci. 30(2015) 011-016
www.njps.com.ng

Niger. J. Physiol. Sci.

Honey Attenuates the Detrimental Effects of Nicotine on Testicular Functions in Nicotine Treated Wistar Rats

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Summary: Effect of honey on reproductive functions of male rats exposed to nicotine was examined in this study. Thirty-two adult male wistar rats (n=8/Group) were grouped as Control (distilled water), Nicotine (1.0mg/kg bwt), Honey (100mg/kg bwt) and Nicotine with Honey. The animals were orally treated for 35 days consecutively. Epididymis sperm motility, viability, morphology and counts were estimated, serum Follicle Stimulating Hormone (FSH), Leutinizing Hormone (LH) and Testosterone were assayed using ELISA method and testicular histology were also assessed. Significant reduction in percentage sperm motility, viability, morphology and counts were observed in nicotine group *($p<0.05$)* compared to control. Serum FSH, LH and testosterone levels were significantly reduced in nicotine group($p<0.05$) when compared with the control. There was significant improvement in sperm motility, viability, morphology, counts, FSH, LH and Testosterone in group co-treated with nicotine and honey *($p<0.05$)* relative to nicotine group. Also, the degenerative seminiferous tubule architecture due to nicotine was improved by honey. In conclusion, honey may suppress nicotine toxic effect on reproductive functions in male Wistar rats.

Keywords*:* Nicotine, Honey, FSH, LH, Testosterone, Rats.

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Manuscript Accepted: December, 2015

INTRODUCTION

Nicotine is the principal alkaloid contained in tobacco which has been reported to have detrimental effects on male reproductive functions. Cigarette smoking and tobacco chewing are the major means which nicotine is consumed throughout the world (Abel, 1983). Nicotine is a highly toxic substance that can be absorbed quickly through the respiratory tract, oral mucosa and skin.

Cotinine is a nicotine metabolite which has a long half-life, both nicotine and cotinine adversely affected spermatogenesis, epididymal sperm count, motility, and the fertilizing potential of sperms (Aydos *et al.*, 2001). Oral administration of nicotine in male rats have been associated with testicular degeneration, disorganization of the cytoarchitecture and decreased serum testosterone levels, reduced sperm characteristics and fertility (Oyeyipo *et al.*, 2010; Oyeyipo *et* al.,2011).

Honey is a natural product of bees formed from nectar collected from flowering vegetation. It contains 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 (Gheldof *et al.*, 2002; Al- Waili, 2003; Yao *et al.*, 2004; Michalkiewicz *et al.*, 2008).

Its rich nutrients make it to boost and maintain health. It also possesses antibacterial, antioxidant and wound healing properties (Aljady *et al.*, 2000; Estevinho *et al.*, 2008). In Arab countries honey is considered to increase human male potency. Honey had been reported to increase sperm motility and spermatogenesis in rats and subnormal human (Abdul-Ghani *et al.*, 2008; Abdelhafiz and Muhamad, 2008).

Recently, Mahaneem *et al.*, (2011 and 2012) reported the protective effect of honey on toxic effects of cigarette smoke on testicular structure, antioxidant and spermatogenesis in male rats. Also, Noorhafiza *et al.*, (2013) showed that honey improved testicular structure and testosterone secretion in nicotine treated rats.

Since nicotine is one of the active compounds present in cigarette smoke, it is imperative to investigate the effect of honey on sperm motility,

viability counts, morphology and some reproductive hormones in adult male rats exposed to nicotine.

MATERIALS AND METHODS

Nicotine Preparation

Nicotine hydrogen tartrate was obtained from BDH Chemical Ltd Poole English. Nicotine stock solution was prepared at concentration of 1mg/ml and stored in foil-wrapped glass bottle 4 °C for no longer than ten days.

Honey

The honey used in this study was obtained from Department of Agriculture, University of Ilorin, Nigeria. It was concentrated (20% w/v water) by oven drying at 40 °C.100mg/kg of honey was administered to the rats. This dose was worked out relative to the local human consumption of honey which is 0.2 g/kg body weight daily. Honey at the dose of 1.0 g/kg body weight was freshly diluted with distilled water to prepare 0.5 mL of diluted honey for each rat. Then, 0.5 mL of the diluted honey was immediately administered to each rat by oral gavage.

Experimental Animals

Thirty-two male Wistar rats weighing between 180 to 200g were used. They were kept in the animal house of Madonna University, Elele campus, Nigeria under standard laboratory conditions with 12 hours light and 12 hours dark cycle. They were fed with standard laboratory chow and had access to water *ad libitum.* The animals were acclimatized for one week. The animal grouping is shown in table 1. The administration was through oral gavage for 35 days consecutively

Experimental Procedure

Blood samples were collected from the anesthetized animals through cardiac puncture, serum was obtained and used for FSH, LH and testosterone assays. Caudal epididymis was immediately dissected to obtain spermatozoa which were used for estimation of sperm motility, counts, viability and morphology. The testes were also dissected and fixed in 10% formalin for histology.

Sperm motility

As described previously by Kaur and Bansal (2004), the caudal epididymis was identified and its content squeezed into 1ml of normal saline at room temperature. One drop of the sperm suspension was charged into a Makler counting chamber and the number of motile and non-motile spermatocytes was counted in ten random fields. The number of motile spermatocytes was then expressed as a percentage of the total number of the counted spermatocytes (Mahaneem *et* al.,2011).

Table 1. Animal grouping

S/N	Groups (n =8)	Treatment
1	Control	Normal saline
2	Nicotine	1.0mg/kg of nicotine
3	Honey	100mg/kg of honey
4	Honey and Nicotine	1.0mg/kg of nicotine and 100mg/kg of honey

Sperm count

Sperm count was performed as reported earlier (Narayana *et al*, 2005) with minor modifications. Briefly, caudal epididymis was minced in 2 ml of normal saline to obtain sperm suspension, which was then stained with 2% eosin and sperm heads were counted using a Neubauer haemocytometer counting chamber.The sperm were counted and expressed as million per ml.

Sperm Viability

The caudal epididymis sperm was dropped on the slide and mixed with a drop of 0.5% eosin solution. After 2 minutes, the slide was examined under microscopy with 40X objective lens to count the percentage of viable (unstained) and non-viable sperm (stain red) (Cheesbrough, 2006).

Sperm morphology

This was determined by smearing a drop of the stained sperm suspension obtained during determination of sperm count on a glass slide; the smear was allowed to dry and subsequently examined under the light microscope at X400 magnification. For each sample, 200 spermatocytes were carefully observed and the percentage of total abnormalities of the spermatocyte head and tails were determined as described by Narayana et al (2005).

Reproductive hormones measurement

Testosterone, Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) in rat serum were measured by enzyme linked immunosorbent assay (ELISA) using commercially available kits form Endocrine Technologies, USA. Samples were run in the same assay to avoid inter-assay variations.

Testicular Histology

The testes of all the rats were fixed in 10% formalin, dehydrated stepwise in the graded ethanol, cleared in xylene and then embedded in paraffin wax. A section of 5pm thickness paraffin section was taken from the mid portion of each testicular tissue and stained with heamatoxylin and eosin, followed by examination under a light microscope with 200X magnification. Photomicrographs were taken as appropriate and analyzed by a pathologist with requisite experience. and their micrographs was taken.

Statistical Analysis

All statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the LSD post hoc tests for pair-wise comparisons were performed using SPSS 17.0 version. All data were expressed as Mean ± Standard Error of mean (SEM) and $p<0.05$ was considered significant.

RESULTS

Table 2 showed the effects of honey on sperm motility, counts, viability and morphology of rats exposed to nicotine. Results obtained indicate that administration of nicotine caused a significant diminution in percentage sperm motility, counts, viability and normal morphology with increased in abnormal heads and tails ($p<0.05$) as compared to control rats. Also, sperm counts and viability significantly reduced in group administered with honey compared to control group *(*$p<0.05$*)* while honey apparently, but insignificantly increased sperm motility and normal sperm morphology ($p<0.05$*).*

Co-administration of nicotine and honey caused a general and significant improvement in all the sperm parameters (sperm motility, counts, viability, and morphology) ($p<0.05$) when compared to nicotine group, but significant reduction *(*$p<0.05$*)* was observed in sperm motility, counts and viability when compared to control (Table 2).

Table 3 showed that FSH, LH and testosterone were significantly reduced in group treated with nicotine relative to the control group *(*$p<0.05$*)*, while the level of luteinizing hormone was significantly increased in honey group when compared with the control *(*$p<0.05$*)*. Furthermore, co-administration of nicotine and honey caused significant increase in the serum levels of FSH, LH and testosterone as compared to nicotine group ($p<0.05$), but significant reduction was observed when compared with control ($p<0.05$).

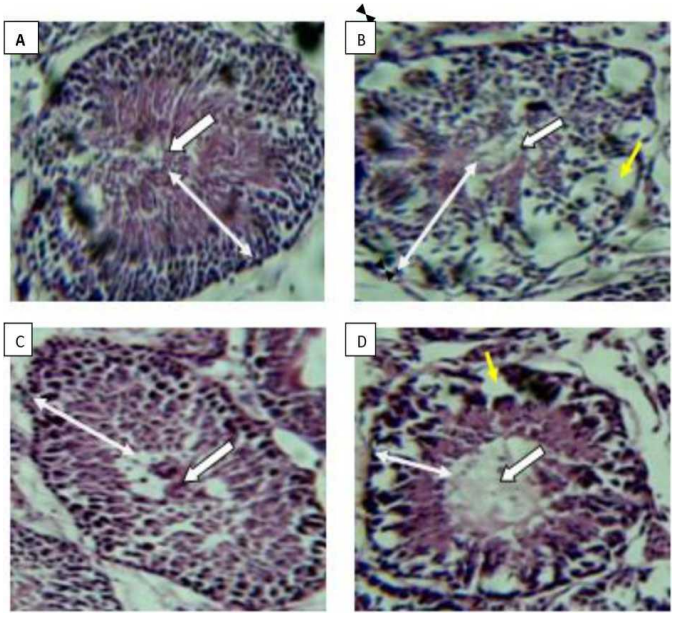

Figure 1: Testicular photomicrograph (H&E, 200X). Control and Honey groups (A &C) shows normal seminiferous tubule with normal germinal cell layer revealing spermatocytes muturation (spanned), the lumen (white arrow) shows spermatid and spermatozoa. Nicotine exposed group (B) showed one of numerous vacuolated (Yellow arrow) seminiferous tubules, the germinal cell layer is disorganized, no production of spermatids (spanned) the lumen (white arrow) is empty without spermatozoa. Nicotine with Honey treated group (D)shows re-organization of germinal cell layer that indicator repair of seminiferous tubule (spanned) with few vacuole (Yellow arrow).

DISCUSSION

This study was undertaken to investigate the effects of honey on reproductive functions in adult male rats exposed to nicotine. The present study showed that nicotine has negative effects on the sperm motility, sperm count, viability, morphology and increased the percentage of abnormal sperms. This finding is in support of the past studies that nicotine has ability to reduce reproductive capacity and has a mutagenic

Table 2: Effects of Honey on Sperm Motility, Counts, Viability and Normal Morphology of Rats Exposed to Nicotine

Groups	Sperm motility (%)	Sperm counts (million/ml)	Sperm viability (%)	Normal Sperm morphology (%)
Control	67.2 ± 1.59	39.0 ± 2.30	78.6 ± 0.93	79.0 ± 2.86
Nicotine	41.2 ± 1.07[a]	22.2 ± 1.16[a]	47.0 ± 0.95[a]	62.4 ± 1.86[a]
Honey	70.6 ± 1.98	33.4 ± 1.63[a]	66.8 ± 1.93[a]	79.4 ± 1.44
Nicotine + honey	55.4 ± 1.66[a,b]	25.4 ± 1.33[a,b]	58.2 ± 1.56[a,b]	73.4 ± 1.29[b]

Data are expressed in Mean ± SEM of 8 rats, [a, b] *Mean significant difference relative to control and nicotine respectively at* $p<0.05$

Table 3. Effects of honey on FSH, LH and Testosterone of Rats Exposed to Nicotine

Groups	FSH (mlU/ml)	LH (mIU/ml)	Testosterone (ng/ml)
Control	9.1 ± 1.24	9.4 ± 0.30	12.9 ± 0.09
Nicotine	0.7 ± 0.01[a]	2.6 ± 0.04[a]	11.9 ± 0.19[a]
Honey	7.6 ± 0.47	21.0 ± 0.24[a]	12.7 ± 0.01
Nicotine + honey	1.35 ± 0.16[a,b]	3.31 ± 0.07[a,b]	12.6 ± 0.04[a,b]

Data are expressed in Mean ± SEM of 8 rats, [a, b] *Mean significant difference relative to control and nicotine respectively at* $p<0.05$

consequences towards the germ cell production and maturation as well as the reproductive organ itself and accessory reproductive organs (Yamamoto *et al.,* 1998; Patil *et al.,* 1999; Oyeyipo *et al.,* 2011; Jana *et al.,* 2010, Seema *et al.,* 2007; Aruldhas *et al.,* 2005).

Oxidative stress through generation of reactive oxygen species (ROS) had been proposed as one of the possible mechanisms of actions of detrimental effects of nicotine on male reproductive functions. It has been proven that nicotine increased the production of ROS by increase generations of testicular H_2O_2 and hydroxyl radicals in experimental rats (Bandopadhyay *et al.*,2008). Oxidative stress has been shown to lead to testicular damage following exposure to nicotine (Rajpurkar *et al.,* 2000).

In this study, it was observed that honey attenuated the detrimental effects of nicotine on the semen parameters. Honey has been reported to have some vitamins and antioxidants such as vitamins A, C and E (Al-Waili, 2003), flavonoids (Yao *et al.,* 2004) and phenols (Mohamed *et al.,* 2010). Therefore, it is plausible to suggest that the effect of honey in attenuating the nicotine induced impaired spermatozoa motility, viability, counts and morphology in this study could be partly mediated by its counteraction on oxidative stress within rat reproductive organs via its antioxidant properties.

The results of this study also showed the adverse effects of nicotine on testosterone, FSH and LH. Nicotine decreased the serum levels of testosterone, LH and FSH. The decreased in serum testosterone concentration observed in this study following nicotine administration may be attributed to disruption of testicular cytoarchitecture which may adversely affect leydig cells number and function, as well as reduction in LH concentration. Testosterone is secreted by the interstitial cells of Leydig cells in the testes but only when they are stimulated by LH from the anterior pituitary gland. Also, the decreased in serum testosterone in nicotine administered rats in this study may be attributed to the cholinergic agonist activity of nicotine which had been reported to inhibit testosterone secretion (Kasson and Hsueh, 1985). Testosterone plays a major role in spermatogenesis by being the main hormone for spermatogonia conversion and spermatids formation. Therefore, a drop in the testosterone level will lead to sterility in males. The decreased in the sperm count may be linked to decrease in testosterone level observed in this study.The findings in this study are in accordance with previous findings where it has been established that nicotine administration decreased serum testosterone and sperm counts in mature male Wistar rats (Yamamoto *et al.,* 1998; Oyeyipo *et al.,* 2010). Inhibition of FSH and LH by nicotine may be as a result of its negative effect on central nervous system that can inhibit the neural stimulus essential for the release of pituitary gonadotrophins (Reddy *et al.,* 1995), which lead to a lack of pituitary gonadotrophins essential for initiating and completing spermatogenesis and steroidogenesis in the testis (Aydos *et al.,* 2001).

There was an improvement in the levels of the reproductive hormones of the nicotine exposed rats when treated with honey. This shows that honey has a positive effect on the hormonal levels. The mechanism by which this is brought about may be due to its antioxidant properties. (Al-Waili, 2003; Gheldof *et al.,* 2002; Yao *et al.,* 2004). Recently, it was reported that honey contained antioxidant such as phenols and possess anti-radical and antioxidant properties (Mohamed *et al.,* 2010). Therefore, it is plausible to suggest that the effect of honey in attenuating the nicotine-induced impaired testicular functions in this study could be partly mediated by its counteraction on oxidative stress within rat reproductive organs via its antioxidant properties.

Mohamed *et al.* (2010) also observed that honey supplementation improved spermatogenesis in normal rats. Due to its antioxidant properties, it has been proved that honey is able to reduce testicular damage. This would suggest that honey has the potential healing properties against the toxic effects of nicotine.

In conclusion, administration of honey significantly attenuated the detrimental effect of nicotine on sperm counts, motility, viability, morphology, testosterone, FSH and LH levels in rats. This study indicates that honey may possess a protective effect against nicotine-induced impaired testicular functions in rats, but further research to elucidate its exact mechanism of action is essential.

Acknowledgements

The authors are grateful to Mr Ezeokafar Emmanuel, chief technologist of the Department of Physiology, Madonna University, Elele campus, Rivers State for his assistance in the sperm parameter analysis.

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