Scientific Research and Essays Vol. 6(26), pp. 5471-5474, 9 November, 2011
DOI: 10.5897/SRE10.1016
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2011 Academic Journals

Full Length Research Paper

Effects of nicotine and Gelam honey on testis parameters and sperm qualities of juvenile rats

Asiyah, H. A.[1], Syazana, N. S.[1], Hashida, N. H.[2]*, Durriyyah Sharifah, H. A.[3] and Kamaruddin, M. Y.[4]

[1]Applied Science with Islamic Studies, Academy of Islamic Studies, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Biology Division, Centre for Foundation Studies in Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
[3]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
[4]Department of Molecular Medicine, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

Accepted 18 October, 2011

The present study aimed to elucidate the effects of nicotine and Gelam honey on testis parameters and sperm qualities of rats. Sprague Dawley rats (4 to 5 weeks old) were divided into 4 groups with 7 rats for each group. Rats of the honey (H) and honey-control (HC) groups were force-fed daily with 1.0 ml/100 g body weight of Gelam honey and normal saline (0.9%), respectively. Rats in the nicotine (N) group were intraperitoneally (i.p.) injected with 5.0 mg/kg body weight of nicotine whilst the nicotine-control (NC) group received normal saline (0.9%) injection (i.p.) in similar doses as in the N group. After 60 days of treatments, the rats were sacrificed. Testicular parameters and sperm qualities were assessed for motility, vitality and morphology. There were no significant differences in weight, length and width gain of testis among the groups. The H group showed significantly higher sperm motility (18.85 ± 5.89 × 10^5/ml) and normal morphology of sperm (193.73 ± 1.03) than the HC group ($p \leq 0.05$). However, for the N group, lower sperm motility (17.80 ± 6.45 × 10^5/ml), lesser sperm with normal morphology (119.59 ± 5.70) and live sperm (156.80 ± 8.91) were observed as compared to the NC group ($p \leq 0.05$). This study suggested that i.p. injection of nicotine could adversely affect sperm qualities and Gelam honey was potentially useful in increasing the fertility of juvenile male rats by increasing sperm motility and number of morphologically normal sperm.

Key words: Sprague dawley rats, honey, nicotine, sperm quality.

INTRODUCTION

Biological and experimental data indicated that tobacco in cigarette smoking could lead to reproductive and infertility related problems in humans, especially for males. Tobacco smoking had been shown to reduce the male to female ratio of offspring born to smoking parents, even if only the father smokes (Fukuda et al., 2002). Specific adverse effects of cigarette smoking and passive smoking on sperm density, motility and morphology had been demonstrated (Stillman, 1989; Hull et al., 2000).

Unfavourable effects of cigarette smoking on fertility could possibly be due to the contents of cigarette smoke which includes nicotine, carbon monoxide and other recognized carcinogens and mutagens (Stillman et al., 1986). It had been reported that the reproductive capacity of nicotine injected rats was greatly reduced, and the effect was greater in males than in females (Riesenfeld and Olivia, 1988). Thus, there is an ongoing search for a protective substance from the many ailments of nicotine, including its adverse effects on reproductive health. One of the candidates is honey which contains sugars such as glucose and fructose; mineral such as potassium, calcium, iron, magnesium, sodium chloride, sulphur, and phosphates; as well as vitamins B1, B2, C, B6, B5 and B3 (Estevinho et al., 2008). Honey, one of the oldest remedies known for maintenance of health had also been proven to have antibacterial, antioxidant and wound healing properties (Aljady et al., 2000). Honey had been

*Corresponding author. E-mail: nhhpasum@um.edu.my. Tel: 603 - 7967 5981. Fax: 603 - 7957 6478.

Table 1. Testis parameters for nicotine and honey treated groups.

Treatments	Parameters	Testis		
		Weight (g) (mean±SEM)	Length (mm) (mean±SEM)	Width (mm) (mean±SEM)
Nicotine	Control (n = 7)	1.55±0.03[a]	11.98±0.06[a]	20.33±0.20[a]
	Treated (n = 7)	1.50±0.02[a]	11.89±0.15[a]	20.03±0.03[a]
Honey	Control (n = 7)	1.48±0.03[a]	20.14±0.29[a]	12.14±0.07[a]
	Treated (n = 7)	1.43±0.03[a]	20.08±0.48[a]	12.38±0.19[a]

[a]superscript in the column within the same treatment group shows no significant difference at P≤0.05.

reported to induce spermatogenesis in rats by increasing epididymal sperm count by 37% as well as the relative weight of the epididymis (Abdul-Ghani et al., 2008).

Currently, there is no established report on the mechanism behind the beneficial effects of honey on male reproductive system. Therefore, the present study was aimed to elucidate the detrimental effects of nicotine and the potential use of Malaysian Gelam honey on the testicular parameters and sperm qualities of rats.

MATERIALS AND METHODS

Twenty eight Sprague-Dawley male rats (4 to 5 weeks old) were randomly divided into 4 groups; nicotine (N), nicotine-control (NC), honey (H) and honey-control (HC) with 7 rats for each group. Rats of the H and HC groups were force-fed daily with 1.0 ml/100 g body weight of Gelam honey and normal saline (0.9%), respectively. Rats in the N group were intraperitoneally (i.p.) injected with 5.0 mg/kg body weight of nicotine whilst the NC group received normal saline (0.9%) injection (i.p.) in the same volume of nicotine that was given to the N group (Mahanem, 2006). The dose of nicotine and Gelam honey given were calculated according to animal's body weight on the week of the specified treatment. Anesthetized rats were sacrificed and their reproductive organs were removed after 60 days of treatment. General parameters of each testis measured were weight, length and width. Sperm cells from epididymis were then assessed for motility, vitality and morphology with five replicates for each rat. To study the morphology and vitality, the sperm cells were stained with eosin nigrosin staining method (NAFA and ESHRE-SIGA, Laboratory Manual, 2002) and observed under light microscope according to the World Health Organization (WHO) laboratory manual (WHO, 1999) which described the morphology of normal and abnormal sperm. The experiment was performed in accordance with the Guidelines for Animal Experiments of the Medical Centre Research Committee, University Malaya [PASUM/16/11/2010/NHH(R)].

Statistical analyses on the data obtained were performed on a microcomputer using the statistical package for social science (SPSS) program. Data were analyzed through one-way analysis of variance (ANOVA). Values with a confidence level of P≤0.05 were considered as significant.

RESULTS

There were no significant differences for weight, length and width of testis among the four groups studied (Table 1). However, sperm motility in the H group was significantly higher (18.85 ± 5.89 × 10^5/ml) than in the HC group (17.05 ± 5.27 × 10^5/ml). On the contrary, a significantly lower sperm motility of N group (17.80 ± 6.45 × 10^5/ml) was observed among rats of the N group as compared to the NC group (23.70 ± 4.87 × 10^5/ml) (Table 2). Observation on sperm vitality indicated no significant differences between the H and HC groups. However, the N group had more dead sperm (57.06 ± 8.83) than that observed in the NC group (11.18 ± 1.49) (Table 2). Based on WHO laboratory manual (1999) the sperm morphology was identified as being normal or having abnormal head and/or tail. Examples of morphologically abnormal sperm were headless sperm and/or sperm with crooked or bent tails. H group showed a significantly higher number of normal sperm (193.73 ± 1.03) than the HC group (190.06 ± 0.65). The N group showed significantly lower number of normal sperm (119.59 ± 5.70) as compared to the NC group (167.03 ± 4.84) (Table 2). However, there were no significant differences of sperm population with abnormal heads among the nicotine and honey treated groups.

In contrast, the N group showed a significantly higher number of abnormal sperm tail (94.74 ± 5.50) than that observed in the NC group (47.88 ± 3.97). The H group indicated a significantly lower abnormal sperm tail (5.43 ± 0.46) as compared to the HC group (7.09 ± 0.59) (Table 2).

DISCUSSION

The present results showed no significant difference for gross morphology parameters of the testis (weight, length and width) between control and Gelam honey groups; namely: the weight, length and width of the testis. This is in agreement with the previous preliminary studies (of similar dosage, treatment durations and age of rats) using another Malaysian honey (Tualang honey). It was reported that no significant effects of the Tualang honey were detected for the percentage of body weight gain, the absolute and relative weights of testis and male accessory organs (prostate gland, epididymis and seminal vesicles) (Mahaneem et al., 2006). However, the

Table 2. Sperm concentration, morphology and vitality of nicotine and honey treated rats.

Treatment groups	Parameters	Motility (×10^5/ml) (mean±SEM)	Morphology of sperm			Vitality	
			Normal (mean±SEM)	Abnormality			
				Head (mean±SEM)	Tail (mean±SEM)	Live sperm (mean±SEM)	Dead sperm (mean±SEM)
Nicotine	Control (n = 7)	23.70±4.87[b]	167.03±4.84[b]	2.65±0.33[a]	47.88±3.97[a]	207.95±2.68[b]	11.18±1.49[a]
	Treated (n = 7)	17.80±6.45[a]	119.59±5.70[a]	3.71±0.51[a]	94.74±5.50[b]	156.80±8.91[a]	57.06±8.83[b]
Honey	Control (n = 7)	17.05±5.27[a]	190.06±0.65[a]	2.86±0.29[a]	7.09±0.59[b]	200±0.00[a]	0.00±0.00[a]
	Treated (n = 7)	18.85±5.89[b]	193.73±1.03[b]	2.17±0.33[a]	5.43±0.46[a]	200±0.00[a]	0.00±0.00[a]

[a,b]superscripts in the same column within the same treatment group shows significantly different at P≤0.05.

present results for the testis parameters between N and NC groups were in contrast to the report by Kasson and Hsueh (1985) which showed that nicotine decreased the size of testicles. A preliminary study had reported that rats treated with Malaysia Tualang honey had higher sperm and spermatid counts, lower percentage of abnormal sperm as well as slightly larger diameter of testicular seminiferous tubules and interstitial spaces (Mahaneem et al., 2006). In the current study, several sperm parameters were positively affected by the Gelam honey. Significantly, higher sperm motility and normal sperm were observed in the H group as compared to the HC group. Honey could possibly act as physiologic modulators of spermatogenic cells proliferation which influenced the spermatogenic cycle thus, increasing the sperm production. A possible mechanism would be an interaction with follicle stimulating hormone (FSH) and luteinizing hormone (LH), hormones which were shown to restore spermatogenesis of hypophysectomized rat (Garner and Hafez, 2000). Currently, there is little established reports concerning the use of honey in treating infertility of the human males. However, it had been reported that propolis (waxy resinous substance in bee hives) provided protection against infertility by improving sperm production, motility, sperm count and quality and increasing steroidogenesis process and, hence, testosterone production (Yousef and Salama, 2009). The present results showed that in addition to having negative effects on the sperm motility, nicotine also affected the vitality and morphology of normal sperm. The findings support past studies that nicotine, a component of cigarette smoke is a major toxic for reproductive health. Nicotine can reduce reproductive capacity and has a mutagenic consequences towards the germ cell production and maturation as well as the reproductive organ itself (Yamamoto et al., 1998) and accessory reproductive organs (Patil et al., 1999).

In the current study, sperm motility was significantly low, more dead sperm, lesser sperm with normal morphology and more sperm with abnormal tails were observed in the N than that of the NC group. Our results are similar to the findings reported by Kapawa et al. (2004) in which tobacco smoke reduced sperm concentration, sperm motility and fertilizing capacity in rats. However, according to Hung et al. (2009), semen quality and sperm function were not affected by environmental tobacco smoke (ETS) but sperm underwent metabolic changes with ETS exposure *in vivo*. The mechanism of negative effects of nicotine may involve reduced testosterone production. Inhibition of testosterone production by nicotine through its effects on acetylcholine receptors on cell membrane had been previously reported (Kasson and Hsueh, 1985). A drop in the testosterone level will lead to sterility of males since it plays a major role in spermatogenesis by being the main hormone for spermatogonia conversion and spermatids formation. Cotinine, the nicotine metabolite has effects on neurotransmitters released from the central nervous system. These in turn affect several enzymes including the ones that are involved in the synthesis of estrogen and testosterone (Benowitz, 1996). This study provided additional data on the adverse effects of nicotine on sperm quality. It is also providing evidence that a Malaysian honey (Gelam honey) is potentially useful in improving the fertility of juvenile male rats by increasing sperm concentration and number of normal sperm. However, further study is needed for a better understanding on the exact mechanism on the adverse effect of nicotine on spermatogenesis as well as the beneficial effects of honey on mammalian sperm.

REFERENCES

Abdul-Ghani AS, Dabdoub N, Muhammad R, Abdul-Ghani R, Qazzaz M (2008). Effects of Palestinian honey on spermatogenesis in rats. J. Med. Food, 11(4): 799-802.

Aljady AM, Kamaruddin MY, Jamal AM, Mohd Yassim MY (2000). Biochemical study on the efficacy of Malaysian honey in infected wounds: An animal model. Med. J. Islamic Acad. Sci., 13(3): 125-132.

Benowitz NL (1996). Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol. Rev., 18: 188-204.

Estevinho L, Pereira A, Moreira L, Dias L, Pereira E (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chem Toxicol., 46: 3774-3779.

Fukuda M, Fukuda K, Shimizu T, Andersen CY, Byskov AG (2002). Parental peri-conceptional smoking and male: female ratio of newborn infants. Lancet., 359: 1407-1408.

Garner DL, Hafez ESE (2000). Spermatozoa and seminal plasma. In Hafez, B. & Hafez, E.S.E (ed). Reproduction in Farm Animals. 7th Edition. Lippincott Williams and Wilkins, New York, pp. 365-375.

Hull MG, North K, Taylor H, Farrow A, Ford WC (2000). Delayed conception and active and passive smoking: The Avon Longitudinal Study of Pregnancy and childhood study team. Fertil Steril., 74: 725-33.

Hung PH, Froenicke L, Lin CY, Lyons L, Miller MG, Pinkerton KE, VandeVoort CA (2009). Effects of environmental tobacco smoke in vivo on rhesus monkey semen quality, sperm function and sperm metabolism. Reprod. Toxicol., 27: 140-148.

Kasson BG, Hsueh AJW (1985). Nicotinic cholinergic agonists inhibit androgen biosynthesis by cultured rat testicular cells. Endocrinology, 117: 1874-1880.

Kapawa A, Giannakis D, Tsoukanelis K, Kanakas N, Baltogiannis D, Agapitos E (2004). Effects of paternal cigarette smoking on testicular function, sperm fertilizing capacity, embryonic development, and blastocyst capacity for implantation in rat. Andrologia, 36: 57-68.

Nordic Association for Andrology (NAFA) and European Society of Human Reproduction and Embryology (ESHRE) - Special Interest Group on Andrology (SIGA) (2002). Manual on basic semen analysis.

Mahaneem M, Siti Amrah S, Yatiban MK, Hasnan J (2006). Effect of 'Tualang Honey' on Spermatogenesis in Rats. *Proceedings of 1st International conference on the medicinal uses of honey (from hive to therapy).* Kota Bharu Kelantan, Malaysia.

Mahanem MN, Nor Asmaniza AB, Phang HT, Muhammad HR (2006). Effects of nicotine and co-administration of nicotine and vitamin E on testis and sperm quality of adult rats. Malays. Appl. Biol. 35(2): 47-52.

Patil S, Patil S, Bhaktaraj B, Patil SB (1999). Effect of graded doses of nicotine on ovarian and uterine activities in albino rats. Indian J. Exp. Biol., 37: 184-186.

Riesenfeld A, Olivia H (1988). Effects of nicotine on the fertility, cytology, and life span of male rats. Acta Anat., 131(2): 171-176.

Stillman RJ (1989). Seminar in reproductive endocrinology: smoking and reproductive health. New York Thieme Medical Publishers.

Stillman RJ, Rosenberg MJ, Sachs BP (1986). Smoking and reproduction. Fertil Steril., 46: 545-566.

World Health Organization (1999). WHO Laboratory Manual for Examination of human Semen and Semen-Cervical Mucus Interaction. 4th Edition. The Press Syndicate of the University of Cambridge. Cambridge, UK.

Yamamoto Y, Isoyama E, Sofikitis N, Miyagawa I (1998). Effects of smoking on testicular function and fertilizing potential in rats. Urol Res., 26: 45-48.

Yousef MI, Salama AF (2009). Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem. Toxicol., 47: 1168-1175.