Effect Of Nicotine And Goat Milk Co-Administration On Rat Testis And Sperm Parameters

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Abstract: The present study was conducted to observe the beneficial effect of goat milk on the sperm parameters of nicotine treated rats. Sprague Dawley juvenile rats (5 to 6 weeks old) were randomly divided into three groups. For nicotine (N) and goat milk (GM) groups, the rats were daily injected with 5.0mg/kg body weight of nicotine and force-fed with 20.0ml/kg body weight of goat milk, respectively. However, for nicotine with goat milk (N-GM) group, the rats were injected with 5.0mg/kg body weight of nicotine and force-fed with 20.0ml/kg body weight of goat milk. After 60 days of treatments, the rats were sacrificed and the reproductive organs were removed. Sperm were collected from epididymis and assessed for sperm count, viability and morphology. Goat milk (GM) group showed higher sperm count (40.30±13.39 x 10^5/ml), more live sperm (385.49±12.97) and more normal sperm (188.31±0.61) than N and N-GM groups (P<0.05). However, co-administration of nicotine with goat milk (N-GM) gave higher sperm parameters values as compared to that observe in N group (P<0.05). This study suggested that goat milk is potentially useful in increasing the fertility of nicotine treated male rats.

Key words: Goat milk; nicotine; sperm quality; Sprague Dawley rats

INTRODUCTION

Available data do not conclusively demonstrate that smoking reduces male fertility. However, with the increasing debates for the adverse effect of smoking on various semen parameters, smoking is regarded as a risk factor that causes infertility in males (Practice committee of American society for reproductive, medicine, smoking and infertility, 2008). Niu et al. (2010) reported that smoking more than 20 cigarettes daily or smoking greater than 10 years has a deleterious effect on semen volume, sperm motility and morphology in smokers. Tawadrous et al. (2011) reported that smoking increased sperm caspade-9 and DNA fragmentation in heavy smokers. Caspade activity in mature sperm would activate apoptotic machinery which lead to cell death (Paasch et al., 2004). The action of nicotine on the testis has been shown to reduce fertility in both experimental and clinical trials (Florek and Marszalek, 1999). There has been increasing concern over the decline of male reproductive health. It includes low sperm counts with concomitant decrease in semen volume and increases in male genital developmental abnormalities such as hypospadias and cryptorchidism (Andersen et al., 2000).

Sharpe and Skakkebaek (1993) reported that male fertility problem was also due to the changes in environment and lifestyle which include exposure to estrogens derived from dairy product. Milk is considered to be the major source of animal-derived estrogens in human diet (Hartmann et al., 1998). Several reports suggested that milk consumption is a risk factor for prostate cancer. Men with testicular cancer had consumed significantly more milk during adolescence than controls (Davies et al., 1996). The adverse effects of milk are rarely discussed in the literature and currently, there was no report on the effect of goat milk on the male reproductive performances. Goat milk is said to have more beneficial properties, which helps to prevent iron deficiency and subsequently, softening of the bones. Lower curd tension and different chemical and physical composition goat milk fat offers greater digestibility. Hence, the focus of this study was to show the potential effect of goat milk on the sperm parameters of nicotine treated rats.

Methods:

Sprague Dawley juvenile rats (5 to 6 weeks old) were randomly divided into three groups with nine rats for each group. The rats were housed under standard housing condition and fed with laboratory chow and tap water ad libitum. For nicotine (N) and goat milk (GM) groups, the rats were intraperitoneally (i.p) injected with 5.0mg/kg body weight of nicotine and force-fed with 20.0ml/kg body weight of goat milk, respectively for 60 consecutive days. However, for nicotine with goat milk (N-GM) group, the rats were injected (i.p) with 5.0mg/kg body weight of nicotine and force-fed with 20.0ml/kg body weight of goat milk. After 60 days of treatments, the rats were sacrificed and their reproductive organs were removed for analysis. General parameters
of each testis measured weight, length and width. Sperm from cauda epididymis were collected into Toyoda-Yokoyama-Hosi (TYH) medium and kept in CO₂ incubator at 37°C for 1 hour. Sperm cells were then assessed for motility using haemocytometer according to method suggested in NAFA and ESHRE-SIGA, Laboratory Manual (2002). Sperm morphology and viability were assessed by eosin nigrosin staining method (NAFA and ESHRE-SIGA, Laboratory Manual, 2002) under light microscope according to the WHO laboratory manual (WHO, 1999). The experiment was performed in accordance with the Guidelines for Animal Experiments of the Institutional Animal Care and Use Committee, University of Malaya [PASUM/20/09/2011/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 among the treated groups for body weight, testis weight, testis length and width, prostate gland and epididymis length of rat (P≥0.05) (Table 1). Significant differences with higher value were observed in GM and N-GM groups for sperm count (40.30±13.39 x 10⁵ and 33.41±8.06 x 10⁵, respectively) and live sperm (385.49±12.97 and 301.98±8.43, respectively) as compared to that observed in N group. Significantly different with higher normal sperm morphology was also observed for GM (188.31±0.61) and N-GM (183.12±0.63) than N (158.02±8.03) group (P<0.05) (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.

**Discussion:**

Nicotine plays an important inhibitory role on food intake and body weight in humans and animals (Guan et al., 2004). The present study showed that nicotine had no adverse effects on body weight, testis length and width, prostate gland and epididymis length of rat. Probably, the dosage of nicotine injected is not sufficient to cause metabolic changes which might reduce the food intake and increased in the amount of energy consumption as reported by Liu et al. (2003). Chronic nicotine used in mice provoke the reduction in testicular weight and atrophied male accessory sex glands, due to the androgenic depletion (Reddy et al., 1998).

Previously, it had been shown that nicotine was a major toxic for reproductive health and had toxic influences on sperm count and motility in adult ICR mouse (Kim et al., 2008). In the current study, N group indicated significantly lower sperm count, more dead sperm and lesser sperm with normal morphology than that of GM and N-GM groups. Our results were in agreement to the findings by Mahanem et al. (2006) in which the administration of nicotine reduced the epididymal sperm count, grade of motility and the percentage of normal sperm morphology. Kapawa et al. (2004) reported that tobacco smoke reduced sperm concentration, sperm motility, and fertilizing capacity in rat. However, semen quality and sperm function were not affected by environmental tobacco smoke (ETS) although sperm underwent metabolic changes with ETS exposure in vivo (Hung et al., 2009). Previous researcher claimed that nicotine involved in inhibiting testosterone production through its effects on acetylcholine receptors on cell membrane (Kasson and Hsueh, 1985). A drop in the testosterone level will lead to infertility of males due to its major role in spermatogenesis. Cotinine, the nicotine metabolite has effects on neurotransmitters released from the central nervous system. These in turn affect several enzymes, which involved in the synthesis of estrogen and testosterone (Benowitz, 1996). Our findings supported past studies that nicotine reduced reproductive capacity of male. It was reported that nicotine had 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).

The present study also recorded no adverse effects of goat milk on body weight, testis length and width, prostate gland and epididymis length of rat. Gammaa et al. (2004) reported no signs of toxicity on the organ and body weights of Wistar rats after treated with milk. Ma et al. (2009) also found no significant difference in the weight of testes and seminal vesicle in the control and cow milk treated mice. Goat milk (GM) group in the current study gave higher sperm concentration, live and normal sperm as compared to N and N-GM groups. Previous reports suggested that milk consumption was a risk factor for prostate cancer (La Vecchia et al., 1991). Davies et al. (1996) reported that men with testicular cancer had consumed significantly more milk during adolescence than control. According to Hartmann et al. (1998), the major source of animal-derived estrogens in human diet is milk and dairy products, accounting for 60% to 70% of animal derived estrogen. Administration of estrogen changed the migration of gonocytes towards the basal lamina, decreased cell proliferation and increased apoptosis which reduced spermatogonia and spermatocytes (Rosa Maria et al., 2006). Degeneration of gonocytes due to exposure to estrogen could be one of the causative factor to male infertility. A decrease in the number of gonocytes would decrease the number of spermatogonia, spermatid and finally sperm in the adult. However, current finding was contradicted to the result obtained by Ma et al. (2009) where, no significantly different was found in the mice sperm motility between control and cow milk treated groups.
Traditional, goat milk is believed as one of the best food medicines for rebuilding the brain and nervous system. It is one of the finest foods for regenerating the cells and healthy body. It is interesting to note that sperm concentration, live and normal sperm for N-GM group was significantly higher to that observed in N group. Probably, goat milk is useful in improving sperm parameters in nicotine treated rat. To the best of our knowledge, there is no established report concerning the use of goat milk to treat infertility problems in nicotine intake male. However, further study need to be established to support the present result. It is also important to know the protective mechanism of goat milk towards the toxic effect of nicotine on sperm parameters.

This study provides an additional data on the adverse effects of nicotine on sperm quality. It is also providing evidence that goat milk is potentially useful in improving the fertility of male rats by increasing the sperm count and number of normal sperm morphology. It is important in the future to study the mechanism on the adverse effect of nicotine on spermatogenesis as well as the beneficial effect of goat milk in improving male infertility.

**Conclusion:**

This study indicated that nicotine reduced sperm quality and goat milk had a protective effect against the toxic effect of nicotine. It can be suggested that goat milk can be used as supplement to improve fertility among male smokers due to the nicotine intake.

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REFERENCES


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


