



## Honey and Vitamin E Restore the Plasma Level of Gonadal Hormones and Improve the Fertilization Capacity in Noise-Stressed Rats

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### Abstract

**Objective:** Noise as a natural teratogenic factor affects the body systems including the reproductive organ to reduce the fertility rate and fetus health. Honey and vitamin E as natural antioxidants protects the sperm released from the reproductive system. This study was conducted to examine the efficacy of honey and vitamin E on fertilization capacity in noise-exposed rats by assessing plasma sexual hormones levels i.e., follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, altered in relation with noise stress.

**Materials and Methods:** This study was targeted the 24 male rats that randomly were divided into four equal groups including one control group (unexposed to noise stress) and three experimental groups pre-induced with noise stress for 50 days and then divided as: no treated, honey and vitamin E treated groups, respectively. Then, the blood samples of experimental and control groups were taken, and the serum level of the sexual hormones was analyzed. Finally, to investigate the fertility capacity of rats, the male rats of all groups were coupled with the female ones.

**Results:** Our results showed that FSH and LH level in noise stressed male rats raised, and the testosterone secretion decreased compared to the control group. Moreover, noise stress injury could reduce weight and the survival rate of the fetus. However, the honey and vitamin E improved the testosterone concentration, declined the plasma FSH and LH level in noise - exposed rats and enhanced the fertility rate.

**Conclusion:** These findings may also spell out a natural curative approach rather than pharmaceutical drugs to optimize of neuroendocrine gonadal axis and testicular integrity induced by pathogenesis stress, i.e., noise and enhance the male fertility capacity.

**Keywords:** Gonadal Hormones, Honey, Noise, Vitamin E

### Introduction

Stress is defined as a physiological response to the body's homeostasis disorders (1). Among environmental stresses, noise as a natural teratogenic agent is relatively more common and affects body systems including the reproductive organ to reduce the fertility rate and fetus health (2,3).

It has been shown that some stresses, e.g., immobilization, heat and water restrictions alter serum sex hormones levels, resulted to decrease in the fertilization rate and implantation (4,5). Furthermore, some researchers demonstrated that exposure to noise can change the stress hormones such as corticosteroid, norepinephrine, and adrenocorticosteroid and disrupts the testis integrity

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through decreasing the testosterone levels, led to reduce the number of healthy spermatozoa. Moreover, the long-term exposure to noise can highly increase the sperm sensitivity to oxidative stress and in turn affects the sperm capacity and acrosome reaction (6,7).

The testes normally have antioxidant factors, protecting their germ cells from the oxidative damage (8,9). Nevertheless, in the mature spermatozoa when the production of free radicals increases, the physiological system will not respond. Using of antioxidants in everyday life is very important, because many biological problems are caused by free radicals which induce oxidative damage to lipids and proteins in the cell membrane (10).

Natural antioxidants can prevent devastating changes in spermatozoa. Honey and vitamin E are principle antioxidants that are found in abundance in nature. Honey as an antimicrobial and anti-inflammatory food is known as a biological antioxidant and increase sperm number in rats without any affecting on the serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (10-12).

The antioxidant property of honey is due to its phenolic components (11) and others ingredients i.e., catalase, glucose and fructose, and vitamin C and B as well as minerals including magnesium, potassium and calcium (12-14). Vitamin E is another antioxidant that is found in cell membranes and protects the cell membranes against the hydrogen peroxides (15).  $\alpha$ -tocopherol is the natural form of vitamin E with high biological activity (16). Some reports indicate the key role of vitamin E in reducing if oxidative stress in the testis (17,18). Indeed, this vitamin has a protective effect on testicular function and increases male fertilization capacity (19).

A series of neurological, paracrine, and endocrine systems are involved in normal physiological functions of the reproductive system. However, less is known about the endocrinological response of the male reproductive organs to noise stress and also the role of the natural antioxidants in reducing stress and increasing the fertility outcome through modulation of sexual hormone level. Hence, this study aimed at evaluating honey and vitamin E effects on the sexual hormones levels and fertilization capacity in noise-exposed male rats. We found that both aforementioned components restore FSH, LH, and testosterone level to an optimum level led to improve fertilization capacity in noised-stressed rats.

## Materials and Methods

A total of 24 adult male Sprague-Dawley rats weighing  $200 \pm 20$  g were acclimated to  $22 \pm 1^\circ\text{C}$  and maintained under conditions of 12-h periods of the light and dark with free access to tap water and commercial rat food. All procedures were approved by international guidelines and by the Institute Research Ethics and Animal Care and Use committee of Tabriz University of

Medical Sciences. Every effort was made to minimize the number of animals used and their suffering. The rats randomly divided into four groups (six rats per group) and classified as follows:

Control group that was not exposed to noise and experimental groups 1, 2, and 3 consisting no treated, honey and vitamin E treated groups, respectively. All experimental groups were exposed to 90-130 dB of noise with a frequency of 300-350 Hz daily from 7 pm to 7 am for 50 days. Then, the male rats were transported to a room with dimensions of  $3 \text{ m} \times 4 \text{ m} \times 3 \text{ m}$  lagged by wood and acoustic segments (anti-loud voice). In this room, the set of white noise was prepared at 19 O'clock with frequency of 300-350 Hz and intensity of 90-120 dB by the speaker (20) and the timer had been set so that after 1 h of operation, the noise was turned off for a few minutes (from 15 to 60 min) and then re-operated. This situation was led to prevent animal's compatibility. Automatically, intensity and frequency of noise were changed in the district of minimum and maximum every 2-3 min by the noise producing device, aided in non-compatibility condition again (21). For evaluating the amount and intensity of noise, the noise level meter was used (22).

Group 2 and 3 noise-exposed rats received honey (0.2 ml of 5 % honey dissolved in water) and vitamin E (75 mg/kg/day dissolved in corn oil) by gavage with a custom-made instrument containing a tube with an approximate diameter 2 mm, once a day at 18:00 h for 50 days without delaying upon noise pollution.

After 50 days, the blood sample obtained from each rat's tail and serum FSH, LH, and testosterone level were measured using enzyme-linked immunosorbent assay (ELISA) technique (CUSABIO kit, Japan).

Both in experimental and control groups, each male rat was coupled with two females for 1 week. Then, vaginal plug-positive females were considered as being pregnant on day 0. Finally, prior to delivery, the pregnant female rats were killed with an anesthetic drug and then, weight and number of living, dead, and absorbed fetuses were calculated.

## Statistical analysis

All experiments were replicated at least three independent times and data were presented as a mean  $\pm$  SD and analyzed by one-way ANOVA followed by Tukey's *post-hoc* multiple group comparison test. A difference between groups was considered as statistically significant if  $P < 0.050$ .

## Results

### Sexual hormone level

To investigate whether noise pollution changes sexual hormones levels, male rats were induced by noise for 50 days and our results showed that serum FSH and LH levels demonstrated to be  $8.06 \pm 0.9$  and  $7.46 \pm 0.8$  mIU/ml which was significantly different from control group calculated  $2.69 \pm 0.9$  and  $2.35 \pm 0.3$  mIU/ml, respectively ( $P < 0.001$ ) (Figure 1A and B). Moreover, measurement of testosterone

level in the control group was shown to be  $8.61 \pm 0.33$  ng/ml but noise stress could significantly decrease it to  $3.25 \pm 0.28$  ng/ml.

Administration of vitamin E and honey diminished gonadotropin hormones levels to  $2.97 \pm 0.6$  and  $4.33 \pm 0.4$  mIU/ml (for FSH) and  $3.39 \pm 0.36$  and  $4.6 \pm 0.35$  mIU/ml (for LH), respectively compared to noise-stress group ( $P < 0.001$ ) (Figure 1A and B). contrasting to serum gonadotropin hormones, the plasma testosterone secretion was significantly raised to  $7.56 \pm 0.55$  and  $5.4 \pm 0.75$  ng/ml in vitamin E and honey administered rats, respectively, compared to noise-stressed rats ( $P < 0.001$ ) (Figure 1C).

### Male's fertilization outcome

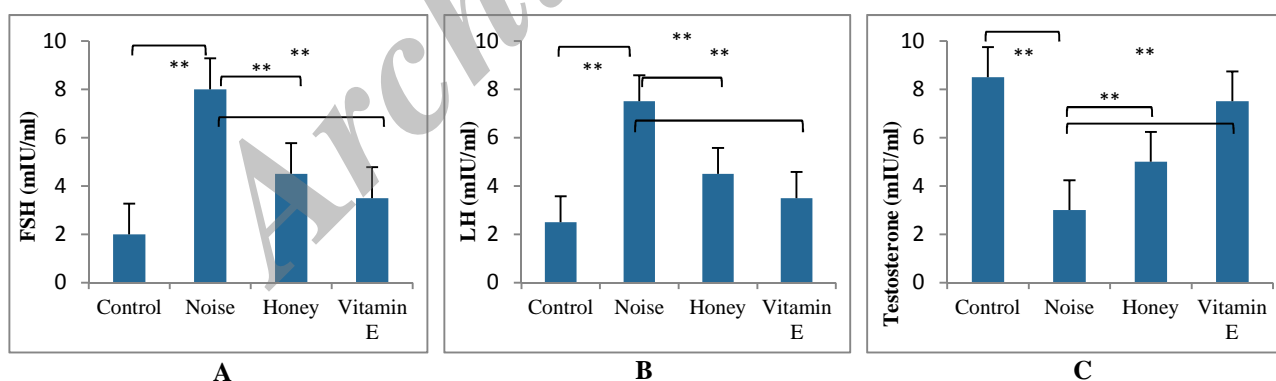
As shown in table 1, noise-exposed male rats found to generate six living and one dead fetuses weighing  $3.35 \pm 0.33$  g upon mating with female rats compared to control group that delivered nine living fetuses weighing  $4.51 \pm 0.48$  g. Whether honey and vitamin E can improve fertility rate in male noised stressed rats, our results showed that these antioxidants could restore fertilization potential to male rats to produce 11 and 9 living fetuses weighing  $5.29 \pm 0.88$  g and  $5.29 \pm 0.31$  g, respectively (Table 1).

### Discussion

Normally, FSH and LH secreted from the hypophysis gland control the primary function of the testis through adjusting the spermatogenesis and testosterone production by Leydig's cells. However, some stress factors i.e., noise pollution and forced swimming stress may influence on this balance and sperm parameters (2,23-25).

In present study, we showed that the noise stress changed physiological secretion of sexual hormone in rat plasma and honey and vitamin E restored their levels to an optimum rate affected on animal fertilization potential. In accordance with the current result, Swami et al. (2) also found that the serum FSH and LH levels raised multi-folds in the male rats exposed to noise stress (100 dB) that in turn is more resulting in decreased the plasma testosterone level. In addition, the intermittent scrotal hyperthermia stress was resulted in plasma FSH and LH raising and serum testosterone decreasing (22). Therefore, it concepts that noise pollution may disrupt the steroid hormones concentration and the neuroendocrine gonadal axis resulting in cellular damage of testicular tissue caused to fall sharply secretion of testosterone serum. Furthermore, it seems that every factor that can trigger reduce or even inhibit gonadotropin and sex hormones secretion, normally can lead to induction of deleterious in germinative and somatic cells of testes parenchyma (26). Indeed, reduction in the plasma testosterone level may be back to disorganization of Leydig's cells, as stated in the previous study (12).

Some of these troubles could probably be diminished using the natural curative agents, protecting reproductive system within the stress period. The current study also pointed out that honey and vitamin E improved the serum testosterone concentration while declined the plasma FSH and LH secretion in noise-exposed rats. Some studies also confirmed this finding so that Abdul-Ghani et al. reported that honey augments the spermatogenesis process without any disturbance in steroids hormones secretion, i.e., gonadotropins and estradiol (10).



**Figure 1.** Sexual hormones levels in different groups; Compared to control group: (A) follicle-stimulating hormone (B) luteinizing hormone levels were increased in noise-exposed rats but honey and vitamin E decreased these gonadotropin hormones levels, (C) compared to control, noise declined significantly testosterone level which was increased again in honey and vitamin E consumed rats

\*\* Versus noise-stressed rats,  $P < 0.001$

**Table 1.** Number of living and dead fetuses and their weights in different groups

Groups	Total fetus	Number of living fetus (%)	Number of dead fetus (%)	Fetus weight (g) (mean $\pm$ SD)
Control	9	9 (100)	-	$4.51 \pm 0.48$
Noise	7	6 (85)	1 (15)	$3.35 \pm 0.33$
Honey	11	11 (100)	-	$5.29 \pm 0.88$
Vitamin E	10	9 (90)	1 (10)	$5.29 \pm 0.31$

SD: Standard deviation

Mahanem et al. declared that if a suitable amount of honey was given to the male rats, the spermatogenic cells lineage and also rates of sperms were increased (27). Naseem et al. reported that vitamin E enhanced the sperm parameters (28). Indeed, with notice to above-mentioned studies, the only thing that can be concluded from this finding is that vitamin E and also with more efficiently honey, due to rich of enzymatic and non-enzymatic antioxidants, done this efficacy, by neutralizing of the injuries patterns of testes integrity (may due to the destructive effects of noise stress) or by regulating of anti-apoptotic patterns. Therefore, in general, each factor that affects the function of interstitial space cells (Leydig and myoid cells) and as well as epithelium germinal cells (sertoli and germ cells), it consequently may lead to suffer a harmful effect on the neuroendocrine gonadal axis and in turn on the spermatogenic cell lineage.

With considering above information, it can suppose that honey and vitamin E by impression on both neuroendocrine gonadal axis and testicular cells may be created condition that the deleterious process (i.e., apoptosis or necrosis) modify or even decrease in the cells. The second assumption is honey and vitamin E; due to enhance steroid hormones levels to sertoli cells, can have a positive effect on nutrition of germ cells. This study also suggests that honey and vitamin E can be useful for keeping the cells in aliveness which suffer from deleterious factors such as noise pollution. The data of this study reinforced the previous studies (29,30). However, Asiyah et al. reported that honey has no more positive influence on the sex hormones concentration in men (31). Therefore, further studies need to elucidate whether honey and vitamin E have a direct effect on sexual hormones production or decreased level of FSH and LH may be as a compensatory mechanism to elevate testosterone level.

In the present study, to show the fertility rate of the noise-exposed rats treated with honey and vitamin E, the male rats were coupled with females. Surprisingly, it was observed that the continuing effects of noise stress injury could reduce the weight of the fetus and the number of the living fetus and survival rate of the fetus. Previously, it has been shown that honey and vitamin E facilitate the recovery of spermatogenesis process by increasing the motility and production of spermatozoa in the epididymis that finally resulted in enhanced fertilization rate in rat (12,18). It was also confirmed that the level of sorbitol dehydrogenase, as a key enzyme in the spermatogenesis, increased following to the honey consumption (10). Honey has also a protective effect on sertoli cells and nourishes them to enhance mature spermatozoa count and affect overall sperm quality. Mahanem et al. indicated that in male rats exposed to cigarette smoking, the serum LH, FSH and testosterone levels changed to optimum levels upon receiving honey (27). In another study, Kimmel et al. (32) observed that in the pregnant rats exposed

to 100 dB noise the number of living fetus and survival rate of fetus were decreased because of increased fetal resorption rate. It seems that the noise pollution has harmful effects on the fertility of male. Also these findings may spell out a natural curative approach rather than pharmaceutical synthetic drugs to optimize of both neuroendocrine gonadal axis and testicular integrity induced by pathogenesis stress, i.e., noise and as well as enhance fertility rate in men.

### Conclusion

According to these studies, the noise have a negative impact on male hormones and natural path of sperm development, leading to infertility, and honey and vitamin E as antioxidants reduce the negative effects of noise pollution and enhance the number of living fetuses.

### Ethical issues

Ethical of this research work was approved by Ardabil University of Medical Sciences, Iran.

### Conflict of interests

We declare that we have no conflict of interests.

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