The Effects of Honey Supplementation During Prenatal Stress on Reproductive System of Female Rat Offspring

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Abstract: Exposure to prenatal stress is associated with an impaired reproductive function in female rat offspring. Honey is traditionally used by the Malays for enhancement of fertility. The aim of this study was to determine the effect of honey on the reproductive system of female rat offspring exposed to prenatal restraint stress. Dams were divided into four groups (n = 10/group): control, honey, stress and honey + stress groups. Dams from honey and honey + stress groups received oral honey (1.2 g kg\(^{-1}\) body weight) daily from day 1 of pregnancy; meanwhile, dams from stress and honey + stress groups were subjected to restraint stress (three times per day) from day 11 of pregnancy until delivery. At ten weeks old, each female rat offspring was mated with a normal male. Female sexual behaviour and pregnancy outcomes were evaluated. Then, female rats were euthanised for assessment on reproductive parameters. Honey supplementation during prenatal restraint stress significantly increased oestradiol level and improved the number of lordosis and lordosis quotient in female rat offspring. In conclusion, this study might suggest that supplementation of honey during pregnancy seems to reduce the adverse effects of prenatal restraint stress on the level of oestradiol, the number of lordosis and lordosis quotient in female rat offspring.

Key words: Honey • Prenatal stress • Female reproductive system • Offspring • Pregnancy outcomes • Sexual behaviour

INTRODUCTION

Few studies have shown that prenatal stress has profound influences on the behaviour, endocrine and immune system, as well as on reproduction of offspring [1-4]. Mean body mass of pups from gestationally-stressed female rats is higher than control and most parturition events take place outside the core breeding period which is during light phase compared with control [3]. Females from stressed mothers also displayed irregular oestrus cycles compared with controls, which may suggest a delayed onset of puberty or a lengthier period to establish a regular cycle pattern [5]. It has also been demonstrated that elevating foetal glucocorticoid exposure, which occurs during gestational stress, inhibits placental glucocorticoid barrier and this can delay the time of vaginal opening, a marker of puberty onset in female rats [6].

Honey is a natural product of honey bees processed from nectar collected from various flowers. Honey contains water, monosaccharides, disaccharides, complex sugars and other substances, such as proteins, vitamins, enzymes, minerals and antioxidants [7, 8]. Flavonoids pinobanksin, pinocembrin, quercetin, chrysin, galangin, luteolin and kaempferol were reported to be present in honey [9]. However, the actual composition of these compounds varies widely according to the differences in floral types, climate and environmental conditions [10, 11, 12]. Honey also contains different quantities of macro mineral elements, such as potassium, calcium and...
sodium and trace minerals, such as iron, copper, zinc and manganese, with potassium being the most abundant element comprising approximately one-third of the total mineral content [13-15]. Potassium and sodium are the most abundant minerals in Malaysian honey [16]. Dark kinds of honey were found to have higher contents of trace elements than light honey [17]. Honey with higher antioxidant capacity was found darker [18-22]. Honey colour depends on the potential alkalinity and ash content, as well as on the antioxidant active pigments, such as carotenoids and flavonoids [18]. Honey is consumed as a nutrient and also with traditional belief to enhance fertility status. A study in ovarioctomised female rats suggests that honey may produce beneficial effects in female reproductive organs [23]. Administration of Tualang honey to ovarioctomised rats for two weeks significantly increases the weights of the uterus, increases the thickness of the vaginal epithelium, restores the morphology of the tibia bones and reduces the body weights compared with control rats without honey treatment [23]. It is suggested that high nutritional contents, mainly flavonoids may contribute to the beneficial effects of Tualang honey [24, 25].

**MATERIALS AND METHODS**

**Experimental Design:** Forty (40) ten-week-old, virgin female Sprague-Dawley rats with body weight 200-250 g, were procured from Animal Research and Service Centre, Universiti Sains Malaysia. Animals were housed individually in polypropylene solid-floor cages and maintained under standard laboratory conditions as well as had ad libitum access to animal chow and water.

Each female with regular oestrous cycles was mated undisturbed overnight starting from the evening of prooestrus with a proven fertile male to induce pregnancy. In the following morning, the presence of sperm was checked by vaginal smear and a sperm-positive smear was considered as day zero (D0) of gestation. Then, pregnant rats (based on sperm positive smear) were randomised into control, honey, stress and honey plus stress (honey+stress) group (n=10 per group). Honey (1.2 g/kg body weight/day) was given by oral gavage to the corresponding pregnant rat from D0 of pregnancy until delivery. Honey was freshly prepared with distilled water to make 1 ml honey solution. This dose was calculated according to the local human consumption which is equivalent to one tablespoon or 0.2 g/kg body weight daily. Rats from control and stress groups were given 1.0 ml of distilled water daily. Rats in stress and honey+stress groups were restrained for 30 minutes, three times a day, starting from day 11 (D11) of pregnancy until delivery. This study was approved by Animal Ethics Committee, Universiti Sains Malaysia PPSG/07(A)/044/(2008) [35]. Following delivery, female pups were kept with their mother until weaning (postnatal Day 21). Then female pups were separated and their body weight was monitored weekly until the age of 10 weeks. Female offspring from each dam was randomly selected for the assessment of reproductive system.

Female offspring from each group were proceeded for the determination of oestrous cycle. The oestrous cycle regularity was determined by obtaining the vaginal smear daily for seven days. This was done by flushing the vagina with 0.9% normal saline using a blunt-ended eye dropper. Then, vaginal fluid was dropped on a ring glass slide and examined under the light microscope with the magnification of 100 and 400. Later, each female in oestrus phase was mated with proven fertile male with one to one (male: female) mating ratio. This was done by placing male first in a clear acrylic box (45 x 25 x 20 cm) and was allowed a 15 minutes adaptation period. Then, the female was introduced into the box and the sexual behaviour of the female was recorded for 30 minutes in a dark room using a video camcorder with a night shot. After the recording, the vaginal smear was performed to check for the presence of sperm which indicates that mating had taken place and considered as day zero (D0) of pregnancy. The sexual behaviour recording was evaluated [26].

On day 21 of pregnancy, females were anesthetised with ether and euthanised by cervical dislocation. Blood samples were collected from the inferior vena cava for hormonal assays. Gravid uterus and ovaries were removed and evaluated to assess pregnancy outcomes.

**Statistical Analysis:** Statistical analysis was done using IBM SPSS version 19. Numerical data with normal distribution and homogenous variance were analysed using one-way ANOVA followed by Tukey’s posthoc test and expressed as mean ± SEM. Meanwhile, statistical data with non-normal distribution and non-homogenous variance were analysed using Kruskal-Wallis test followed by Mann-Whitney U test and expressed as median (interquartile range). Statistical significance was accepted at p<0.05.
RESULTS

The results for reproductive hormone levels are presented in Table 1; meanwhile the results for oestrus cycle length, sexual behaviours and pregnancy outcomes are presented in Table 2.

Reproductive Hormones: The levels of serum FSH in stress and honey+stress groups were significantly lower than control and honey groups. Serum oestradiol levels in honey and stress groups were significantly lower than both control and honey groups. In honey+stress group, the level of serum estradiol was significantly higher than control and stress groups. Serum progesterone levels in honey, stress and honey+stress groups were significantly lower than control group. The level of progesterone in stress group was also significantly lower when compared with the honey group. However, there were no significant differences in serum LH levels between all groups.

Oestrus Cycle Length, Sexual Behaviour and Pregnancy Outcomes: There were no significant differences in the length of oestrus cycle among all groups. No significant differences were evident in both numbers of lordosis and lordosis quotient between control and honey groups. However, in stress group, both numbers of lordosis and lordosis quotient were significantly lower than control and honey groups. Numbers of lordosis and lordosis quotient in honey+stress group were not statistically different with other groups except with honey group whereby the numbers of lordosis and lordosis quotient were significantly lower in the honey+stress group compared with the honey group. No significant differences in litter size and percentage of resorption were observed between all groups.

DISCUSSIONS

The present findings of reduced FSH, oestradiol and progesterone levels in the stress group which might suggest that prenatal restraint stress can detrimentally affect the hypothalamic-pituitary-gonadal axis function of female rat offspring. Del Cerro et al. [27] similarly has reported that female offspring from mothers that were exposed to stress during the last week of gestation presented lower levels of progesterone and estradiol. Activation of HPA axis exerts an inhibitory effect on the female reproductive system. Previous study has shown that CRH inhibits GnRH secretion by the hypothalamus [28]. Animals exposed to prenatal stress typically have greater elevation of basal corticosterone [29, 30].

<table>
<thead>
<tr>
<th>Table 1: Reproductive hormone levels of female rat offspring in all experimental groups.</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>Follicle stimulating hormone (mIU/ml)</td>
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<td>Luteinising hormone (mIU/ml)</td>
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<td>Oestradiol (ng/ml)</td>
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<td>Progesterone (ng/ml)</td>
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Data are presented as mean ± SEM (n=10 per group). Significant differences determined by one-way ANOVA followed by Tukey’s posthoc test.

<table>
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<th>Table 2: Oestrus cycle length, sexual behaviour and pregnancy outcomes of female rat offspring in control, honey, stress and honey+stress groups.</th>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>Oestrus cycle length (day)</td>
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<tr>
<td>Number of lordosis</td>
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<tr>
<td>Lordosis quotient (%)</td>
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<tr>
<td>litter size</td>
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<td>Foetal weight (g)</td>
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<td>Resorption (%)</td>
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<td>Gross congenital abnormalities (%)</td>
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Data are presented as median (Interquartile range) (n=10 per group). Significant differences determined by Kruskal-Wallis test followed by Mann-Whitney U test.

*Data are presented as mean ± SEM (n=10 per group). No significant differences (p>0.05, one way ANOVA) were observed among all the groups.

p<0.05 compared with control group, *p<0.05 compared with honey group.

@ p<0.05 compared with stress group.
In addition, corticosterone suppresses the female gonadal axis function at the hypothalamic, pituitary and uterine level [31, 32]. Hence, it is suggested that reduced FSH, oestradiol and progesterone levels in stress group of female rat offspring might be due to the activation of HPA axis. Interestingly, no significant differences were observed in LH level among all groups. The reason for this lack of expected response is unclear. Clearly, more studies will be needed to examine this observation. With the supplementation of honey, the oestradiol level was improved in honey+stress group. This might suggest that honey has a protective effect on oestradiol level in female rat offspring exposed to prenatal restraint stress.

In the present study, prenatal restraint stress did not have any significant effect on oestrus cycle of adult female rat offspring. The normal oestrus cycle is an ovulatory cycle which can be four to five days in length [33]. Baker et al. [5] reported that 25% of female rats from stressed mother had experienced a delayed onset of puberty or a lengthier period to establish a regular oestrus cycle pattern, but the percentage of rats with normal cycle pattern in stress group was increased on subsequent observation weeks at or above the level of control group. The lack of statistically significant effect in the present study might be due to the duration and intensity of stress used in this study. It is possible that longer duration and higher intensity of stress might have produced more significant effects on oestrus cycle of female rat offspring.

However, in the sexual behaviour assessment, the number of lordosis and lordosis quotient (LQ) were significantly lower in stress group compared with control and honey groups. Lordosis behaviour is a sexually receptive posture made by female rats in response to mounting by a male. LQ has been the most commonly assessed parameter of female sexual behaviour to measure female receptivity for successful mating to occur. Lordosis reflex requires oestradiol; meanwhile, progesterone enhances the probability that the reflex will occur [34]. Ovariectomised Fischer female rats show high levels of lordosis responding when primed with oestradiol benzoate and progesterone [35]. In the present study, low levels of oestradiol and progesterone were also observed in stress group. Therefore, it is plausible to suggest that the reduction in the number of lordosis and LQ in stress group could be due to the presence of low oestradiol and progesterone levels. With the supplementation of honey, the number of lordosis and LQ in honey+stress group were significantly higher than stress group. Enhanced lordosis has been reported in rats by daily injection of oestradiol [36-39]. In the present study, the level of oestradiol was increased in honey+stress group. Therefore, this could explain why the number of lordosis and LQ were higher in the honey+stress group.

Pregnancy outcomes were assessed in this study by measuring the litter size, foetal weight, percentage of resorption and gross congenital abnormalities. All parameters showed no significant changes between all groups. Similarly, Gotz et al. [3] has demonstrated that female rats from mother exposed to social stressor during pregnancy showed no alteration in the number of pups compared with controls. Although the levels of FSH, oestradiol and progesterone, as well as the number of lordosis and LQ in stress group, were significantly lower, these changes did not provide evidence of any significant alterations in pregnancy outcomes of female rat offspring. Once again the reason for this lack of expected response is unclear, nevertheless, seems to suggest that further studies are needed to examine the effect of prenatal restraint stress on pregnancy outcomes in female offspring.

In conclusion, prenatal restraint stress did not affect the oestrus cycle and pregnancy outcomes, but it significantly lower the percentage of LQ in stress group compared with control group. Prenatal restraint stress also significantly decreased serum FSH, oestradiol and progesterone levels compared with control group. Honey significantly improved the oestradiol, number of lordosis and lordosis quotient. This study demonstrated that supplementation of Tualang honey during pregnancy seems to have a protective effect against the adverse effects of prenatal restraint stress on the reproductive system of female rat offspring. This effect is probably due to antioxidant property of Tualang honey which may work by reducing the increase of corticosterone level and oxidative stress that lead to adverse effects on the reproductive system.

REFERENCES


