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Original article

Tualang honey ameliorates restraint stress-induced impaired pregnancy outcomes in rats

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Abstract

Introduction: Exposure to stress during pregnancy has been shown to have adverse effects on pregnancy outcomes such as low birth weight, spontaneous abortion and premature delivery. Honey has been shown to improve testicular function in rats exposed to cigarette smoke. The aim of this study was to determine the effects of honey on corticosterone level, adrenal gland histomorphometry and pregnancy outcomes in pregnant rats subjected to restraint stress.

Materials and methods: Rat dams were divided into four groups ($n = 10$/group), i.e. control, honey, stress and honey plus stress (honey + stress) groups. Rats from honey and honey + stress groups received Tualang honey (1.2 g/kg body weight/day) orally by gavage from Day 0 of pregnancy until delivery. Rats from stress and honey + stress groups were subjected to stress by repeated restraining (three times/day) from Day 11 of pregnancy until delivery. Following delivery, pregnancy outcomes were assessed and dams were euthanized at postnatal Day 21 for assessments on serum corticosterone level, adrenal gland histology and rate of resorption.

Results: Rats from stress group had significantly higher corticosterone level, zona fasciculata thickness and duration of pregnancy as well as decreased litter size when compared with control and honey groups. These parameters were significantly improved in rats receiving honey (honey + stress group).

Conclusion: Supplementation of honey has a protective effect against increased corticosterone level and zona fasciculata thickness as well as impaired pregnancy outcomes in rats subjected to restraint stress.

Keywords: Maternal stress; Tualang honey; Pregnancy outcomes; Corticosterone; Restrain stress; Adrenal histology

Introduction

Exposure to stress during pregnancy has been shown to have adverse effects on pregnancy outcome such as low birth weight, spontaneous abortion and premature delivery in human as well as in animal experimental studies [1]. The exact mechanism of maternal stress leading to disturbances in pregnancy outcome is not yet clearly understood. However it is postulated that during chronic stress, there is a prolonged activation of maternal hypothalamic–pituitary–adrenal (HPA) cortex and sympathetic-adrenal medulla axes which leads to high blood levels of cortisol and catecholamine, which in turn might result in reduced birth weight, spontaneous abortion and premature delivery. In animal studies, maternal stress has been shown to affect pregnancy length, reduce offspring body mass at birth, and alter the offspring's sex ratio [2–5].

Honey is a natural product generated from nectar and contains moisture, sugars such as glucose and fructose, enzymes such as catalase and glutathione reductase, and essential elements such as iron, copper, zinc and calcium. It also contains

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vitamins such as vitamins A, C and E, as well as flavonoids and phenolic acids [6–9]. Studies have demonstrated some beneficial effects of honey such as gastrointestinal [10,11] and reproductive protections [12], as well as hypoglycaemic [13], antihypertensive [7], antibacterial [14], antifungal [15], anti-inflammatory [16] and antioxidant [12,17] effects. Honey has been used as a traditional nutrient by local Malaysian population to enhance fertility and vitality. A recent study has shown that consumption of Malaysian honey namely Tualang honey at 1.2 g/kg body weight/day for 28 days resulted in a higher sperm count in rats [18]. Administration of honey is also able to attenuate the toxic effects of cigarette smoke on spermatogenesis and testosterone level in rats [12]. It is postulated that the effect of honey in attenuating impaired testicular function induced by cigarette smoke is mediated via its antioxidant properties [12].

However, whether supplementation of honey may also have a role to prevent or reduce the adverse effects of maternal stress on pregnancy outcomes has yet been reported. Therefore, the aim of this study was to determine the effects of honey on corticosterone level, adrenal gland histomorphometry and pregnancy outcomes in rats subjected to restraint stress.

Materials and methods

Experimental design

Forty ten-week old virgin female Sprague Dawley rats with body weight between 190 and 210 g were procured from Animal Research and Service Centre, Health Campus, Universiti Sains Malaysia. Animals were housed individually in polypropylene solid-floor cage and maintained on a 12-h light/dark cycle at 20–24 °C. They also had ad libitum access to animal chow and water. This study protocol was approved by the Animal Ethics Committee, Universiti Sains Malaysia [PPSG/07(A)/044/(2008)(35)]. The oestrous cycle regularity of the virgin rats was determined by obtaining the vaginal smear daily for ten days. This was done by flushing the vagina with 0.9% normal saline using blunt-ended dropper. Then, vaginal fluid was dropped on a ring glass slide and examined under a light microscope with the magnification of 10 and 40. Females with regular oestrous cycles were mated with proven fertile male to induce pregnancy. The rats were left to mate undisturbed overnight starting from the evening of proestrus. Two to one (female:male) mating ratio was used in this study. The following morning, vaginal smear from each female was checked for the presence of sperm and a sperm-positive smear was considered as Day 0 of pregnancy.

Then pregnant rats were divided by a simple randomisation into control, honey, stress only and honey plus stress (honey + stress) groups ($n = 10$/group). Rats in honey and honey + stress groups were given Tualang honey orally by gavage at a dose of 1.2 g/kg body weight daily from Day 0 of pregnancy until delivery. This dose of honey was calculated according to the local human consumption which is equal to one table spoon or 0.2 g/kg body weight daily. Rats from control and stress groups were given 1.0 ml of distilled water daily. Repeated restraint was used as a model of maternal stress. Rats in stress and honey + stress groups were restrained for 30 min, three times a day starting from Day 11 of pregnancy until delivery [19]. Maternal body weight was monitored weekly. Following delivery, the pregnancy outcomes were assessed and alive pups were kept with their mother until weaning (postnatal Day 21). At postnatal Day 21, each dam was euthanized and blood was taken to collect the serum for corticosterone level measurement. Adrenal glands were also carefully dissected for assessment of absolute and relative weights (in comparison to body weight) as well as for histomorphometric analysis.

Assay of corticosterone level

Serum corticosterone was measured in duplicate using enzyme-linked immunosorbent assay kit from IBL International GmbH, Germany. The inter-assay and intra-assay coefficient of variations were 4.08% and 5.54%, respectively.

Histological assessment of adrenal gland

Adrenal glands were immersed-fixed in 10% formalin. After 24 h of immersion, each adrenal gland was sliced and placed inside a histology cassette, dehydrated and embedded in paraffin. Solidified paraffin-embedded tissues were cut into 5 μm thick sections on a microtome (Thermo Electron Corporation, USA) for microscopic examination. The sections were then stained with haematoxylin and eosin. The slides were examined for the presence of lipofuscin pigment and any morphological cell differences between control, honey, stress and honey plus stress groups. Thickness of zona glomerulosa, zona fasciculata, zona reticularis and medulla of adrenal gland was measured using an image analyser (Olympus Corporation, Japan) at 40× magnification. The thickness was taken at two different regions and expressed as mean of the two measurements.

Assessment on pregnancy outcomes

The parameters being assessed for the pregnancy outcomes were gestation period, litter size, birth weight, gross congenital abnormality and 21-day survival index of the pup as well as percentage of resorption. The 21-day survival index was calculated based on the percentage of live born pups that survived until 21 days. The percentage of resorption was calculated based on the percentage of difference between number of implantation site and litter size.

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 post hoc test and expressed as mean ± SEM. Meanwhile, numerical 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 $p < 0.05$.