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Original article

The effects of Tualang honey intake during prenatal stress on pain responses in the rat offsprings

Che Badariah Abd Aziz [a,*], Rozaziana Ahmad [a], Mahaneem Mohamed [a], Wan Nazirah Wan Yusof [b]

[a] *Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia Health Campus, Kubang Kerian, Kelantan, Malaysia*
[b] *Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia Health Campus, Kubang Kerian, Kelantan, Malaysia*

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Abstract

Introduction: The effects of honey administration during normal pregnancy and prenatal stress on pain behavior in the offspring are not known. Here we examined the effects of Tualang honey administration during pregnancy and prenatal stress on pain responses in male rat offspring.
Materials and methods: Pregnant dams were divided into four groups; control (C), honey (H), stress (S) and stress with honey (SH). Tualang honey (1.2 g/kg) or distilled water was administered orally throughout pregnancy. S and SH groups were subjected to restraining stress from day 11 of pregnancy until delivery. Intraplantar formalin injection and thermal stimulus were given to the male adult offspring (220–300 g). Behavior data due to formalin injection and thermal stimuli were analyzed using repeated measures analysis of variance (ANOVA) and one way ANOVA respectively and significance level was taken as less than 0.05.
Results: The study showed that the offspring from the H group had higher tail flick latency time compared to C and offspring from SH group had a significant reduction in the formalin test score in phase 1 and late phase 2 compared to S group.
Discussion: The nutrients in Tualang honey are beneficial to support neural development of the fetus and capable to modulate the offspring's nociceptive responses. The present study provides novel knowledge regarding the possible role of Tualang honey in fetal neural development which modulates pain responses in later life. Further studies are required to elucidate the mechanisms of action of honey on pain behavior in the rat offspring.

Keywords: Prenatal stress; Tualang honey; Formalin; Thermal stimulus

Introduction

During pregnancy, there is an increased requirement for vitamins and minerals which are crucial for the wellbeing of both mother and foetus alike [1]. Deficiency in these nutrients may lead to the abnormal development of foetal organs and complications, such as neuro-behavioural problems in the unborn child. Similarly, exposure to psychological stress during pregnancy has been shown to have adverse effects on the outcome of pregnancies [2] and could justifiably clarify the development of abnormal behaviour in later life regarding the offspring [3]. Studies have also revealed an association between prenatal stress and the alteration of the nociceptive responses in the offspring of rats [4–6] and an increase in the number of formalin-induced Fos-LI neurons in the lumbar dorsal horn [3].

Alteration of the nociceptive responses may be caused by various contributory factors. Hypercortisolemia which occurs during prenatal stress may cause permanent structural and functional changes during prenatal brain development and sexual differentiation predisposing to impaired reproductive systems and abnormal behaviour in both male and female offspring [7–9]. It is postulated that stress during early pregnancy impacts on offspring organ development while impending stress during late pregnancy impacts on neurobehavioral development [9]. The maternal stress leads to oxidative stress with consequent damage to the neurons, leading to neuronal loss in the brains of offspring during development [10].

Honey, is a renowned traditional remedy celebrated worldwide and has anti-oxidant properties which promote wound healing [11]. Studies have revealed that honey stimulates

* Corresponding author at: Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia. Tel.: +6 09 7676162; fax: +6 09 7653370.
E-mail address: badariah@kb.usm.my (C.B. Abd Aziz).

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antibody production during primary and secondary immune responses and also stimulates the proliferation of B and T lymphocytes in vitro. Other than that, honey stimulates the release of cytokines such as TNF-α, IL-1β and IL-6 which activates immune responses [12]. Human studies have shown that the oral administration of honey reduced plasma prostaglandin (thromboxane B_2, PGE_2 and PGF_{2a}), which is an illustrious mediator for both pain and inflammation [13,14].

To date, it is not known whether the consumption of honey plays a role of significance in foetal neural development which might modulate pain responses in the offspring of adults. In addition, there have been no recent investigations exemplifying whether or not there is a protective effect of honey against the prenatal stress-induced altered pain responses in the offspring of rats. Therefore, the aims of the present study were to elucidate the effects of the administration of Tualang honey during normal pregnancies and prenatal stress on pain behaviour and inflammatory responses following nociceptive stimulation in the offspring of male rats.

Materials and methods

This was an experimental study conducted in the Physiology Department Laboratory, Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan. Experiments were performed between 09:00 and 16:30. This study consisted of two components i.e. behavioural assessment, and histopathology examination. Samples of tissue biopsy were stained using the haematoxylin and eosin staining. The research was conducted in accordance with the internationally accepted principles and animal usage had been approved by the USM Animal Ethical Committee (PPSG/07[A]/[2008,35]).

Animals

Forty adult female Sprague-Dawley rats, weighing between 200–250 g, which were obtained from Animal Research and Service Centre were maintained on a 12-h light: 12-h dark cycle (light phase 07:00–14:00) with standard laboratory food and water available ad libitum with an adaptation phase for 3–5 days.

Experimental groups

Forty female rats at proestrus were caged with a proven fertile male rat overnight at the laboratory. A vaginal smear was taken the next morning between 09:00 and 10:00 and if sperm was present, that day was considered as Day 0 of pregnancy [15]. The pregnant dams were kept in a standardized individual cage and were divided randomly into 4 groups: control (C), honey (H), stress (S) and stress treated with honey (SH).

Repeated restrain was used as a model of maternal stress [15] and the pregnant dams were restrained 3 times a day for 30 min at 08:00, 12:00 and 16:00 in a cylindrical restrainer measuring 23 cm (height) × 6 cm (diameter). Honey (1.2 g/kg body weight/day) was supplied by FAMA (Federal Agricultural Marketing Authority, Malaysia) and was given orally by giving it to the corresponding pregnant dams. The honey used in this study is a wild multi-flora honey which is known locally as Tualang honey. The rats in the control group were given distilled water. Following the delivery of the pups, male pups were kept until ten weeks old and their weight was between 220 g and 350 g before conducting the experiment.

Tail flick latency test

The tail flick test was conducted using the tail flick test device with an automated timer (IITC Life Science, USA). The rats were brought into the testing room and placed inside the Polyethylene tube at least 5 min before the determination of the tail flick latency test. Constant heat stimulation was applied to the dorsal aspect of the rat's tail [16] at three different stimulation areas marked at 40, 50 and 60 mm from the tip of the tail. The time was recorded at the moment the rat flicked or withdrew its tail from the heat source. The average latency time was taken for each animal. Stimulus cut off was set to 10 s to prevent possible tissue damage [17].

Formalin test

The formalin test was carried out in an observation chamber measuring 26 cm × 20 cm × 20 cm [18] with a mirror mounted at 45° below it, permitting an unhindered observation of the rat's paw. The rats were placed in the testing chamber for 15–30 min to allow for acclimatization before administering the formalin injection. The right hind paw was injected subcutaneously into the plantar surface with 50 μl of 1% formalin [19] using a 1 ml syringe with a 27-G needle [17]. The formalin dose chosen was based on the results from our previous laboratory work which showed that the injection of 1% formalin was sufficient enough to result in c-fos expression in the spinal cord [18]. The rat's behaviour was recorded immediately after the injection for 60 min using a video camera [20]. The tape was reviewed later and the pain score was determined at minute intervals and averaged at 5-minute intervals [18,21].

Sacrifice of animals and collection of samples

The rats were sacrificed four hours after the formalin injection with an overdose inhalation of diethyl ether. A section of the tissue including the skin was taken from the site of the formalin injection. The tissue was fixed in a 10% buffered formalin solution [22] and paraffin blocks were made. The sections were cut 4–5 μm thickness [23] from the paraffin blocks and stained with hematoxylin and eosin stains. Neutrophils were counted at different areas [23] surrounding the injection site using a light microscope at 400× magnifications and an image analyser (Olympus XC50, Japan). In this study, calculation of the neutrophil was implemented at 9 different areas surrounding the injection site and each area was estimated around 5740 μm^2 (Fig. 1). The total number of neutrophil for each rat was obtained and divided by nine to calculate the mean neutrophil number in each rat [23].