Original Article

Impact of Rapid Eye Movement Sleep Deprivation on Pain Behaviour and Oxidative Stress in the Thalamus: Role of Tualang Honey Supplementation

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Abstract

Background: Insufficient sleep alters the body's physiological functions. This study investigated whether oxidative stress (OS) in the thalamus was correlated with the pain behaviour score in the rapid eye movement (REM) sleep-deprived rat model.

Methods: Four groups of Sprague-Dawley rats were included in the study (n = 6): i) control; ii) REM sleep-deprived rats for 72 h (REMsd); iii) REM sleep-deprived rats for 72 h pretreated with Tualang honey (REMsd-H) and iv) tank control (TC). Following the intervention, 1% formalin was injected on the right hind paw and pain behaviour was recorded for 1 h. OS markers of glutathione (GSH), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) in the thalamus were measured.

Results: REM sleep deprivation increased pain behaviour scores in phase II of the formalin test with decreased GSH, GR, SOD and CAT. The MDA level was significantly higher in the REMsd compared to REMsd-H. There was an inverse correlation between pain behaviour scores and GSH, GR and SOD levels. A positive correlation was found between the pain behaviour score and the MDA level.

Conclusion: OS levels were significantly correlated with the pain behaviour score in the REMsd rats. Tualang honey administration reduced pain behaviour score and OS in the thalamus.

Keywords: REM sleep deprivation, Tualang honey, pain behaviour score, oxidative stress biomarkers

Introduction

Sleep has a vital role in life, and it is involved in growth, neurotransmitter synthesis in the central nervous system, and modulation of immune and hormonal responses. Insufficient sleep may lead to difficulties in concentration, vision disturbances, slower reactions, poor memory (1) and may alter pain responses. The relationship between sleep disturbance and pain is bidirectional; pain can affect sleep quality and sleep disturbance can influence pain intensity. Human studies have shown that sleep deprivation is associated with increased pain perception (2–3). The severity of pain is affected by the degree of sleep disturbance in humans (4) and animals (5).

Potential mechanisms responsible for the alteration of pain responses following sleep disturbances might be related to the modulation of nociceptive pathways (e.g. opioid, monoaminergic and gamma amino butyric acid (GABA)ergic systems). Furthermore, hyper-reactivity of the hypothalamic-pituitary-adrenal axis to stressors (6) with upregulation of inflammatory mediators in various types of pain can be seen associated with sleep disturbances (7–8). Besides, sleep disturbance may cause an imbalance between the oxidant and antioxidant systems, resulting in oxidative stress (OS) (9). Although several studies demonstrated the association of OS with the degree of pain (10–11), its role in modulating pain responses in the central nervous system following sleep deprivation is not known.

Previous studies have shown that OS occurred in the hippocampus (12), hypothalamus, midbrain and hindbrain of rats (13) following rapid eye movement (REM) sleep deprivation. It is unknown whether OS occurs in pain modulating structures, such as the thalamus following REM sleep deprivation. If OS occurs in the thalamus, there is a correlation between the parameters and the pain behaviour score. The thalamus has been shown to modulate nociceptive responses in electrophysiological and imaging studies following thermal stimulation, inflammation, nerve injury and clinical pain (14–18).

An antioxidant can reduce OS by interrupting the propagation of free radicals or by inhibiting the formation of free radicals (19). Various types of antioxidants have been attributed to reducing OS such as vitamins C and E and glutathione (GSH) (20–21). In recent years, Tualang honey has been investigated for its potential therapeutic effects. Tualang honey is rich in phenolic acids and flavonoids. The phenolic components consist of gallic, syringic, benzoic, trans-cinnamic, p-coumaric and caffeic acids, whereas the flavonoid components consist of catechin, kaempferol, naringenin, luteolin and apigenin (22). Besides containing antioxidants, it has been reported to possess antimicrobial, anti-inflammatory and antitumor properties (23). Most recently, we have demonstrated that the administration of Tualang honey could reduce the pain behaviour score, improve the morphology of neurons, and reduce OS and N-methyl-D-aspartate (NMDA) levels in the thalamus in the offspring of prenatally stressed rats (24). Thus, it remains to be elucidated whether Tualang honey administration could provide a protective effect against the alteration of the thalamus due to REM sleep deprivation. The present study aimed to elucidate the impact of REM sleep deprivation on OS parameters and whether the parameters are correlated with the pain behaviour score in the formalin pain model. If the parameters are correlated, the administration of Tualang honey, as an antioxidant, is beneficial to reduce the impact of REM sleep deprivation.

Methods

Animal Study

Eight to ten weeks old Sprague-Dawley male rats (180 g–200 g) were bought from the Animal Research and Services Centre (ARASC), Universiti Sains Malaysia. The rats were divided into four groups (n = 6 per group): i) control; ii) REM sleep-deprived rats for 72 h (REMsd); iii) REM sleep-deprived rats for 72 h pretreated with Tualang honey (REMsd-H), and iv) tank control (TC) rats. Tualang honey (1.2 g/kg body weight) was the antioxidant used in the study and it was given by oral gavage for 4 weeks before adaptation. Rats were given standard laboratory food and water.

Adaptation Period

For adaptation, each rat in REMsd, REMsd-H and TC groups was adapted individually in a dry tank with two inverted flowerpots (25–26), while each rat in the control group was placed individually in a standard dry cage for 72 h. The size of the tank used was 30 cm height, 30 cm length and 60 cm width. For REMsd and REMsd-H groups, two flowerpots (6.5 cm diameter) were placed in the dry tank. Meanwhile, a wider-diameter of flowerpot (13.5 cm diameter) was used for the TC group. To reduce stress due to social isolation, two rats underwent adaptation concurrently by placing two dry tanks side by side. The adaptation period was necessary so that the rats were adapted to the surroundings in the glass tank.

REM Sleep Deprivation Model

The glass tanks were filled with water up to 1 cm from the top of the platforms (6.5 cm diameter) in the glass tank. The water temperature was maintained at 30 ˚C. When the rats fell asleep, the muscles became atonic during the REM phase and they would fall into

the water. The water prevented the rats from sleeping and the rats would get back on the platform. The inverted flowerpot technique had been utilised by other studies to achieve REM sleep deprivation (25–28). Food and drinks were provided throughout the adaptation and experiment.

Control Groups

Each rat in the control group was placed in a cage with 22 cm in height, 56 cm in length and 38 cm in width. The cages were placed side by side to minimise social isolation stress. The glass tank for the TC group was also filled with water up to 1 cm from the top of the platform. The platforms used were larger in diameter (13.5 cm diameter) compared to REMsd and REMsd-H groups. The larger platforms were provided to ensure that this group of rats would be not REM sleep-deprived while examining the effect of exposure to the aquatic environment (25–26).

Formalin Test

The formalin test was chosen because it is considered a very powerful tool in pre-clinical research (29). Furthermore, this test allows the study of two different kinds of pain, i.e. acute peripheral pain mediated by the direct activation of nociceptors, and inflammatory and central nociceptive sensitisation (30).

Before formalin injection, each rat was acclimatised in a Perspex observation chamber (26 cm × 20 cm × 20 cm) for 15 min. One percent of formalin (50 µL) was given by subcutaneous injection on the right hind paw. The pain behaviour was recorded for 60 min and assessed later. Two hours following formalin injection, the rats were sacrificed using intraperitoneal injection of sodium pentobarbitone (31) followed by decapitation using a guillotine. The pain behaviour score was tabulated for every minute and the scores were averaged every 5 min. The pain behaviour score was given from 0 to 3 according to the scoring system as described below (31):

i) Score 0 (absence of pain): The injected hind paw was placed normally on the floor.

ii) Score 1 (mild pain): The hind paw rested lightly on the floor or the rat walked with a limping gait.

iii) Score 2 (moderate pain): The hind paw was raised without touching any surface.

iv) Score 3 (severe pain): The hind paw was licked, bitten and shaken.

In this study, the average score from minute 0 to 10 represented the pain behaviour score for phase 1. The average score from minute 15 to 35 represented the early phase 2, whereas the average score from minute 40 to 60 represented the late phase 2 of the formalin test (32).

Brain Removal and Harvesting

The brain samples were harvested and weighed using a digital analytical balance. The thalamus was identified using rat brain atlas (33) and fixed in 0.1 M phosphate-buffered saline (PBS, pH 7.4). Each brain was then homogenised in ice-cold PBS (10% w/v) and kept at −80 °C in individual tubes.

Quantification of Oxidative Stress Parameters

OS parameters in the thalamus were evaluated with the measurement of levels of GSH, glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) using ELISA kits from Bioassay (Bioassay System, Hayward, USA). Meanwhile, the levels of malondialdehyde (MDA) were measured using ELISA kits from Northwest (Northwest Life Science, Vancouver, Canada) ELISA kits.

Statistical Analysis

Normality test was performed to check the distribution of data before statistical analysis. Data entry and analysis were done using GraphPad Prism version 8.00. The pain behaviour score was analysed with repeated-measure analysis of variance (ANOVA) followed by post-hoc Bonferroni test. One-way ANOVA was used to compare the differences between OS parameters in the thalamus. The differences in the parameters between the groups were considered significant when $P < 0.05$. The data in this study are expressed as mean (standard deviation [SD]). Correlation analysis was conducted to determine the relationship between OS parameters and mean pain behaviour score. The strength of the correlation is indicated by the Pearson correlation coefficient (r), where $r < 0.25$ indicates a poor correlation, r between 0.26 and 0.50 indicates a fair correlation, r between 0.51 and 0.75 indicates a good correlation and r between 0.76 and 1.00 indicates an excellent correlation.

Results

Formalin Test

There was a significant difference between time and group interaction ($F(6, 70) = 4.762$; $P < 0.001$). The analysis on the pain behaviour score by one-way ANOVA demonstrated significant differences in early ($F(3, 36) = 11.87$; $P < 0.001$) and late phase 2 ($F(3, 36) = 23.06$; $P < 0.001$) when compared between all groups (Figure 1). The differences in phase 1 were insignificant. REM sleep deprivation was associated with increased pain behaviour score when compared to the control group in early and late phase 2; however, it was only significant in late phase 2 ($P < 0.001$). A significant difference was also seen when compared between REMsd and TC groups ($P < 0.05$). Oral administration of Tualang honey was associated with a reduction in the pain behaviour score in early and late phase 2 compared to the REMsd group ($P < 0.001$).
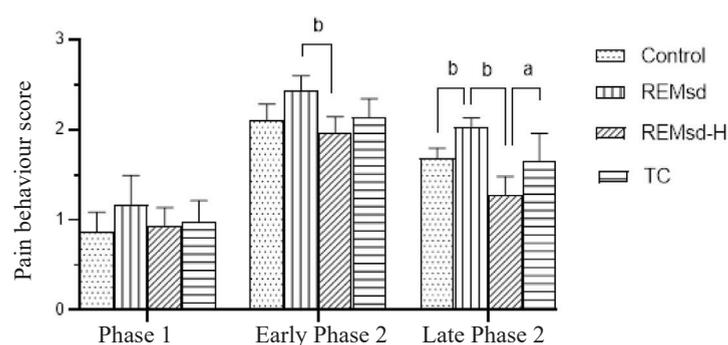

Figure 1. Effects of REM sleep deprivation and Tualang honey administration on pain behaviour score in each phase of formalin test ($n = 6$). REM sleep deprivation has significantly increased pain behaviour score in late phase 2 ([b]$P < 0.001$) compared to Control. There were significant differences when compared between REMsd-H and REMsd groups during early and late phase 2 ([b]$P < 0.001$). The difference was also significant when compared between REMsd-H and TC groups ([a]$P < 0.01$).

Level of Oxidative Stress Parameters

The differences in the level of GSH ($F(3, 20) = 24.76$), GR ($F(3, 20) = 16.62$), SOD ($F(3, 20) = 62.23$), CAT ($F(3, 20) = 23.13$) and MDA ($F(3, 20) = 67.19$) were found to be significant when compared between the groups ($P < 0.05$). The REMsd group showed a lower level of antioxidants (GSH, GR, SOD and CAT) and a higher level of MDA (Table 1) compared to other groups. Administration of Tualang honey was associated with a significantly higher level of GSH, GR, SOD and CAT, and a significantly lower level of MDA compared to the REMsd group ($P < 0.001$).

Table 1. Level of OS parameters in the experimental groups

OS parameters	Control mean (SD)	REMsd mean (SD)	REMsd-H mean (SD)	TC mean (SD)
GSH (μM)	54.16 (3.16)	36.02 (6.62)[a]	77.09 (4.41)[b]	53.85 (14.19)
GR (U/L)	31.78 (4.01)	18.24 (5.12)[a]	44.69 (9.73)[b]	31.76 (5.61)
SOD (U/mL)	2.47 (0.21)	1.27 (0.71)[a]	4.66 (0.42)[b]	2.40 (0.21)
CAT (U/L)	16.49 (4.07)	10.23 (1.98)[a]	27.67 (0.99)[b]	16.59 (5.75)
MDA (μM)	11.30 (2.54)	21.13 (0.59)[a]	8.13 (1.25)[b]	11.65 (1.71)

Notes: Data are presented as mean (SD), $n = 6$ rats in each group; [a]$P < 0.05$ when compared to REMsd-H; [b]$P < 0.05$ when compared to Control and TC groups

Correlation Study

Based on Pearson correlation (Figure 2), GSH, GR and SOD showed a good inverse correlation, whereas CAT showed a fair inverse correlation with the pain behaviour score. Meanwhile, MDA showed a good positive correlation with the pain behaviour score.
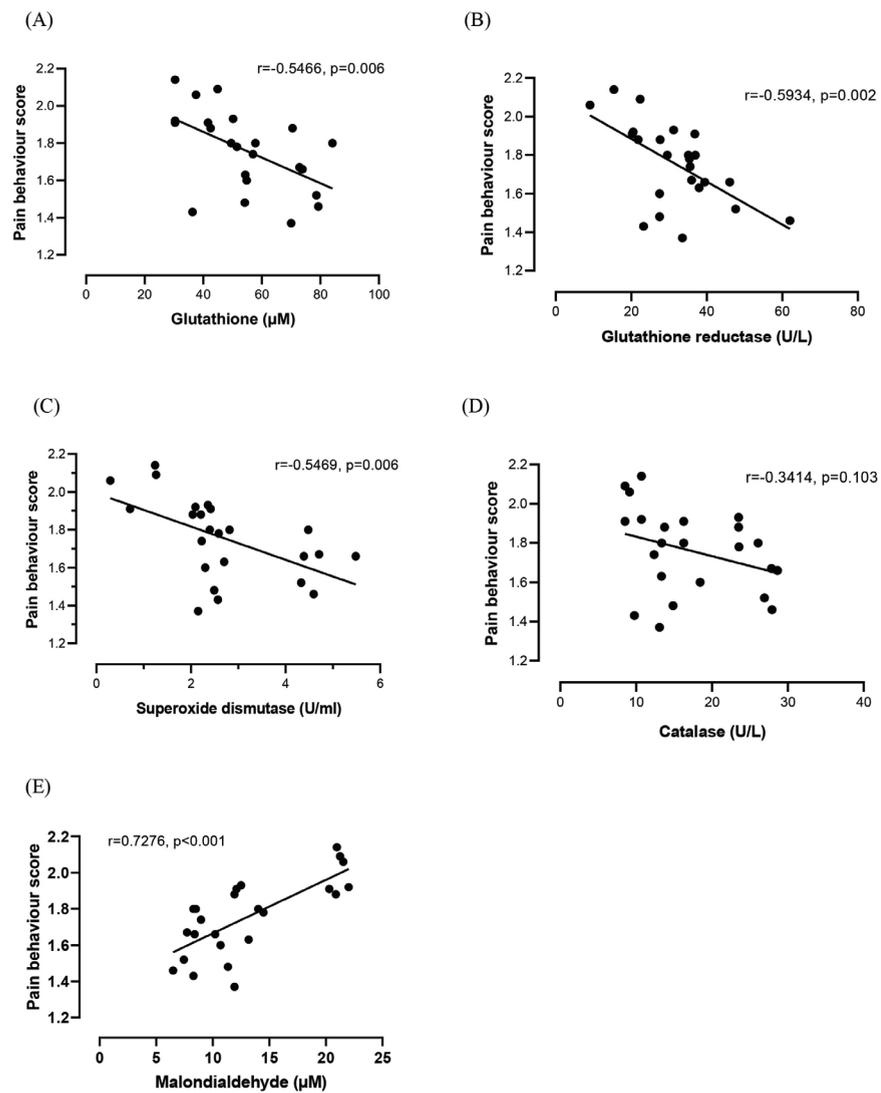
(A)

(B)

(C)

(D)

(E)

Figure 2. Scatter plots of correlations between OS parameters: A) glutathione, B) glutathione reductase, C) superoxide dismutase, D) catalase and E) malondialdehyde and pain behaviour score

Discussion

The pain in phase 1 of the formalin test was contributed by formalin-induced inflammation in the periphery (34), whereas the pain in phase II was due to changes in the central nervous system as well as the persistence of peripheral-induced inflammation. A report has shown that prednisolone (glucocorticoid) significantly reduced the pain behaviour score in early phase 2 (32) and it was suggested that the inhibition was mediated by annexin 1, which reduced spinal prostaglandin E2 (35).

Sleep disturbance has been linked to increased pain responses in animal and human studies (2–5, 36). In this study, the group that

was given Tualang honey showed a reduction of pain behaviour score in phase 2, but the reduction was only significant in late phase 2. Kinin and other inflammatory mediators were shown to be released following formalin-induced inflammation in the periphery. The components of honey, including catechin and chlorogenic acid, were reported to inhibit kinin and other inflammatory mediators released peripherally (37–38), and this mechanism might contribute to the pain suppression in the Tualang honey group.

Another report has demonstrated that Tualang honey reduced inflammation in the central nervous system by decreasing the levels of TNF-α, IL-1, glial fibrillary acidic protein, allograft inflammatory factor 1 and COX-2 in the rats (39). A report has shown that the termination of pain behaviour at the later part of phase 2 was contributed by an endogenous inhibitory mechanism, which was not mediated by opioid receptors (40). There is a possibility that Tualang honey facilitates the inhibitory mechanism to reduce the pain behaviour score in late phase 2 of the formalin test. In this study, Tualang honey administration in the REMsd-H group was associated with decreased pain behaviour scores in phase 2 and not phase 1 of the formalin test. This suggests the central action of Tualang honey in modulating the pain responses, and in this study, we have chosen the thalamus as it is one of the structures in the pain pathway. Although increased pain behaviour in phase 2 may be contributed by modulation at the peripheral or central nervous system level, imaging studies have shown the altered activity in the thalamus following sleep deprivation (41–42).

In addition, a report has demonstrated that REM sleep deprivation increased the sensitivity to mechanical stimuli, with increased Fos protein in brain areas involved in nociceptive transmission (e.g., hypothalamus, periaqueductal gray and trigeminocervical complex (43). Several studies have demonstrated the presence of OS following sleep deprivation (9, 13). A few reports have shown the association of OS with the degree of pain (10, 11); however, its role in modulating pain is not much known. Furthermore, changes in OS parameters have not been reported in the thalamus following REM sleep deprivation. In this study, antioxidants GSH, GR, SOD and CAT were markedly reduced, while the MDA level was increased in the REMsd group.

Other studies have reported that OS contributes to the modulation of pain in various pain models [e.g. prenatal stress (44), cancer pain model (45), neuropathic (46) and arthritic pain (47)]. Accumulated reactive oxygen species (ROS) might contribute to increased neuronal excitability by reducing inhibitory transmission of GABAergic neurons in the spinal dorsal horn (48). However, ROS may reduce GABAergic transmission but did not decrease glycine transmission (48). With the persistence of peripheral inflammation, synaptic activity and neurotransmitter release (e.g. glutamate and glycine), increased. The binding of the neurotransmitters to NMDA receptors will modulate the nociceptive pathways leading to increased pain responses (49).

The activation of neuronal NMDA receptors has been shown to induce the release of superoxides-mediated OS in neighbouring neurons and astrocytes (50). The NMDA receptors are responsible for regulating the intracellular calcium level in the neurons. The mechanism for the increase in OS markers is believed to be due to calcium dysregulation as a result of NMDA receptor hyperactivity, which leads to neurotoxicity (51). In this study, Tualang honey administration was associated with a reduction in the pain behaviour score with higher antioxidant and lower MDA levels in the thalamus. Tualang honey is a natural product with a high concentration of phenolics and flavonoids that have important anti-inflammatory and antioxidant activities (32, 52–55). Sleep deprivation causes depletion of antioxidants and increases radical oxygen species that modified the membrane function and eventually damaged the cells in the brain, resulting in reduced neuronal number and abnormal histological features (55). The present study has also shown that the level of antioxidants in the thalamus is inversely correlated with the pain behaviour score, whereas the oxidant (MDA) is positively correlated with the score. These results suggest that OS in the thalamus contributed to the pain modulation following REM sleep deprivation.

Roles of signalling pathways in chemical-induced hyperalgesia have been reported. Extracellular signal-regulated kinase (ERK) is an important molecule in mitogen-activated protein kinase signalling pathways, which is known to play an essential role in the inflammatory pain. Furthermore, ERK can be phosphorylated (p-ERK) in the presence of noxious substances

that stimulate sensory neurons (56). Previous studies in mice have shown increased expressions of p-ERK in the ipsilateral dorsal horn of lumbar spinal cord in formalin-induced (57) and complete Freund's adjuvant-induced pain (58). Meanwhile, carrageenan-induced hyperalgesia in mice was demonstrated to involve nuclear factor kappa B (NF-κB) signalling pathway activation, which induces OS and cytokine production peripherally in the paw skin and centrally in the spinal cord (59). The carrageenan-induced animal pain model has been linked to defective cellular antioxidant systems, including SOD, CAT and glutathione peroxidase in the skin tissue oedema (60). This study demonstrated decreased antioxidants and increased MDA levels in the thalamus of REMsd rats. Thus, our next step is to evaluate the role of OS in the inflammatory response in the paw tissue induced by formalin injection.

Conclusion

In conclusion, REM sleep deprivation was associated with increased pain behaviour score and OS in the thalamus. The antioxidants in the thalamus showed a fair to good inverse correlation with the pain behaviour score, while MDA demonstrated a good correlation with the score. Tualang honey supplementation has reduced OS in the thalamus of REMsd rat models and thus modulated the pain behaviour in the formalin test.

Acknowledgements

Ethics of Study

This study was conducted after approval from the Universiti Sains Malaysia Institutional Animal Care and Use Committee [USM/IACUC/2019/(116)(964)].

Conflict of Interest

None.

Funds

Authors' Contributions

Conception and design: ASMS, CBAA
Analysis and interpretation of the data: ASMS, CBAA
Drafting of the article: ASMS, CBAA, LN
Critical revision of the article for important intellectual content: CBAA, IL, RS, LN
Final approval of the article: CBAA, IL, RS, LN
Provision of study materials or patients: ASMS, CBAA
Statistical expertise: CBAA, LN
Obtaining of funding: CBAA

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References

5. Silva R, Abilio V, Takatsu A, Kameda S, Grassl C, Chehin A, et al. Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. *Neuropharmacology*. 2004;**46(6)**:895–903. https://doi.org/10.1016/j.neuropharm.2003.11.032

6. Lacovides S, George K, Kamerman P, Baker FC. Sleep fragmentation hypersensitizes healthy young women to deep and superficial experimental pain. *J Pain*. 2017;**18**:844–854. https://doi.org/10.1016/j.jpain.2017.02.436

7. Schrimpf M, Liegl G, Boeckle M, Leitner A, Geisler P, Pieh C. The effect of sleep deprivation on pain perception in healthy subjects: a meta-analysis. *Sleep Med*. 2015;**16**:1313–1320. https://doi.org/10.1016/j.sleep.2015.07.022

8. Zelman DC, Brandenburg NA, Gore M. Sleep impairment in patients with painful diabetic peripheral neuropathy. *Clin J Pain.* 2006;**22**:681–685. https://doi.org/10.1097/01.ajp.0000210910.49923.09

9. Ajao F. Effects of rapid eye movement sleep deprivation on pain perception level in male Wistar rats. *Asian J Biomed Pharm.* 2011;**1(2)**:20–24.

10. Devine JK, Bertisch SM, Yang H, Scott-Sutherland J, Wilkons A, Molina V, et al. Glucocorticoid and inflammatory reactivity to a repeated physiological stressor in insomnia disorder. *Neurobiol Sleep Circ Rhythms.* 2019;**6**:77–89. https://doi.org/10.1016/j.nbscr.2018.06.001

11. Besedovsky L, Lange T, Haack M. The sleep-immune crosstalk in health and disease. *Physiol Rev.* 2019;**99**:1325–1380. https://doi.org/10.1152/physrev.00010.2018

12. Haack M, Lee E, Cohen D, Mullington JM. Activation of the prostaglandin system in response to sleep loss in healthy humans: potential mediator of increased spontaneous pain. *Pain.* 2009;**145**:36–41. https://doi.org/10.1016/j.pain.2009.05.029

13. Pandey A, Kar SK. Rapid eye movement sleep deprivation of rat generates ROS in the hepatocytes and makes them more susceptible to oxidative stress. *Sleep Sci.* 2018;**11(4)**:245–253. https://doi.org/10.5935/1984-0063.20180039

14. Perrone S, Bellieni CV, Negro S, Longini M, Santacroce A, Tataranno ML, et al. Oxidative stress as a physiological pain response in full-term newborns. *Oxid Med Cell Longev.* 2017;**2017**:3759287. https://doi.org/10.1155/2017/3759287

15. Slater L, Asmerom Y, Boskovic DS, Bahjri K, Plank MS, Angeles KR, et al. *J Pain.* 2012;**13(6)**:590–597. https://doi.org/10.1016/j.jpain.2012.03.010

16. Ramanathan L, Gulyani S, Nienhuis R, Siegel JM. Sleep deprivationdecreases superoxide dismutase activity in rat hippocampus and brainstem. *Neuroreport.* 2002;**13**:1387–1390. https://doi.org/10.1097/00001756-200208070-00007

17. Mathangi DC, Shyamala R, Subhashini AS. Effect of REM sleep deprivation on the antioxidant status in the brain of Wistar rats. *Ann Neurosci.* 2012;**19**:161–164. https://doi.org/10.5214/ans.0972.7531.190405

18. Abd Aziz CB, Ahmad AH. The role of thalamus in modulating pain. *Malays J Med Sci.* 2006;**13(2)**:11–18.

19. Brooks JC, Nurmikko TJ, Bimson WE, Singh KD, Roberts N. fMRI of thermal pain: effects of stimuluslaterality and attention. *Neuroimage.* 2002;**15(2)**:293–301. https://doi.org/10.1006/nimg.2001.0974

20. Ladorola MJ, Max MB, Berman KF, Byas-Smith MG, Coghill RC, Gracely RH, et al. Unilateral decrease in thalamic activity observed with positron emission tomography in patients with chronic neuropathic pain. *Pain.* 1995;**63(10)**:55–64. https://doi.org/10.1016/0304-3959(95)00015-K

21. Kolhekar R, Murphy S, Gebhart GF. Thalamic NMDA receptors modulate inflammation-produced hyperalgesia in the rat. *Pain.* 1997;**71(1)**:31–40. https://doi.org/10.1016/s0304-3959(97)03334-4

22. Tu Y, Fu Z, Mao C, Falahpour M, Gollub RL, Park J, et al. Distinct thalamocortical network dynamics are associated with the pathophysiology of chronic low back pain. *Nat Commun.* 2020;**11(1)**:3948. https://doi.org/10.1038/s41467-020-17788-z

23. Tan BL, Norhaizan ME, Liew WP, Sulaiman Rahman H. Antioxidant and oxidative stress: a mutual interplay in age-related diseases. *Front Pharmacol.* 2018;**9**:1162. https://doi.org/10.3389/fphar.2018.01162.

24. Adwas AA, Elsayed A, Azab AE, Quwaydir FA. Oxidative stress and antioxidant mechanisms in human body. *J Appl Biotechnol Bioeng.* 2019;**6(1)**:43–47. https://doi.org/10.15406/jabb.2019.06.00173

25. Nawi A, Tengku Adnan TFA, Wan Ahmad WAN, Ab Aziz CB, Safuan S, Noordin L. Impact of REM sleep deprivation on endothelial function in rat model: role of vitamin C supplementation. *JSSM.* 2020;**15(8)**:22–33. https://doi.org/10.46754/jssm.2020.12.003

26. Mohamed ZBH, Alfarisi HAH. Tualang honey: composition, physiochemical properties and clinical importance. *Int Res J Pharm*. 2017;**8(9)**:1–5. https://doi.org/10.7897/2230-8407.089150

27. Firdaus A, Khalid, J, Yong YK. Malaysian Tualang honey and its potential anti-cancer properties: a review. *Sains Malays*. 2018;**47(11)**:2705–2711. https://doi.org/10.17576/jsm-2018-4711-14

28. Hasim H, Abd Aziz CB, Suhaimi SQA, Mohamed M, Long I, Zakaria R. Effects of Tualang honey on pain behaviour and oxidative stress in the thalamus of prenatally stressed rat offspring. *MJMHS*. 2020;**16(3)**:85–92.

29. Nawi A, Eu KL, Faris ANA, Wan Ahmad WAN, Noordin, L. Lipid peroxidation in the descending thoracic aorta of rats deprived of REM sleep using the inverted flowerpot technique. *Exp Physiol*. 2020;**105(8)**:1223–1231. https://doi.org/10.1113/EP088667

30. Siran R, Ahmad AH, Abd Aziz CB, Ismail Z. REM sleep deprivation induces changes of down regulatory antagonist modulator (DREAM) expression in the ventrobasal thalamic nuclei of Sprague–Dawley rats. *J Physiol Biochem*. 2014;**70**:877–889. https://doi.org/10.1007/s13105-014-0356-x

31. Mendelson W, Guthrie RD, Guynn R, Harris RL, Wyatt RJ. Rapid eye movement (REM) sleep deprivation, stress and intermediary metabolism. *J Neurochem*. 1974;**22**:1157–1159. https://doi.org/10.1111/j.1471-4159.1974.tb04353.x

32. Maloney KJ, Mainville L, Jones BE. Differential c-fos expression in cholinergic, monoaminergic, and gabaergic cell groups of the pontomesencephalic tegmentum after paradoxical sleep deprivation and recovery. *J Neurosci*. 1999;**19**:3057–3072. https://doi.org/10.1523/JNEUROSCI.19-08-03057.1999

33. Mogil JS. Animal models of pain: progress and challenges. *Nat Rev Neurosci*. 2009;**10(4)**:283–294. https://doi.org/10.1038/nrn2606

34. Lopez-Cano M, Fernandez-Duenas V, Llebaria A, Ciruela, F. Formalin murine model of pain. *Bio-protocol*. 2017;**7(23)**:e2628. https://doi.org/10.21769/BioProtoc.2628

35. Hayati AA, Zalina I, Myo T, Badariah AA, Azhar A, Idris L. Modulation of formalin-induced fos-like immunoreactivity in the spinal cord by swim stress induced analgesia, morphine and ketamine. *Ger Med Sci*. 2006;**6**.

36. Ismail CAN, Che Hussin CM, Mohamed M, Abd Aziz CB. Preemptive effects of administration of Tualang honey on inflammatory responses in adult male rats. *J Pharm*. 2017;7:6–12. https://doi.org/10.6000/1927-5951.2017.07.01.2

37. Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. 5th ed. Amsterdam: Elsevier Academic Press; 2014.

38. Mullington JM, Hinze-Selch D, Pollmächer T. Mediators of inflammation and their interaction with sleep: relevance for chronic fatigue syndrome and related conditions. *Ann N Y Acad Sci*. 2011;**933**:201–210. https://doi.org/10.1111/j.1749-6632.2001.tb05825.x

39. Ayoub SS, Yazid S, Flower RJ. Increased susceptibility of annexin-A1 null mice to nociceptive pain is indicative of a spinal antinociceptive action of annexin-A1. *Br J Pharmacol*. 2008;**154**:1135–1142. https://doi.org/10.1038/bjp.2008.166

40. Pan F, Tian J, Cicuttini F, Jones G. Sleep disturbance and its association with pain severity and multisite pain: a prospective 10.7-year study. *Pain Ther*. 2020;**9**:751–763. https://doi.org/10.1007/s40122-020-00208-x

41. Noguchi Y, Fukuda K, Matsushima A, Haishi D, Hiroto M, Kodera Y, et al. Inhibition of Df-protease associated with allergic diseases by polyphenol. *J Agric Food Chem*. 1999;**47**:2969–2972. https://doi.org/10.1021/jf9812073

42. Dos Santos MD, Almeida MC, Lopes NP, De Souza GE. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biol Pharm Bull*. 2006;**29(11)**:2236–2240. https://doi.org/10.1248/bpb.29.2236

43. Sairazi NSM, Sirajudeen KNS, Mustapha M, Mummedy S, Asari MA, Sulaiman SA. Tualang honey reduced neuroinflammation and caspase-3 activity in rat brain after kainic acid-induced status epilepticus. *Evid. Based Complement Altern Med*. 2018:1–16. https://doi.org/10.1155/2018/7287820

44. Azhdari-Zarmehri H, Mohammad-Zadeh M, Feridoni M, Nazeri M. Termination of nociceptive bahaviour at the end of phase 2 of formalin test is attributable to endogenous inhibitory mechanisms, but not by opioid receptors activation. *Basic Clin Neurosci.* 2014;**5(1)**:48–54.

45. Chee MW, Tan JC, Zheng H, Parimal S, Weissman DH, Zagorodnov V et al. Lapsing during sleep deprivation is associated with distributed changes in brain activation. *J Neurosci.* 2008;**28(21)**:5519–5528. https://doi.org/10.1523/JNEUROSCI.0733-08.2008

46. Ma N, Dinges DF, Basner M, Rao H. How acute total sleep loss affects the attending brain: a meta-analysis of neuroimaging studies. *Sleep.* 2015;**38(2)**:233–240. https://doi.org/10.5665/sleep.4404

47. Kim SH, Park JY, Shin HE, Baek Lee S, Ryu DW, Kim TW, et al. The influence of rapid eye movement sleep deprivation on nociceptive transmission and the duration of facial allodynia in rats: a behavioral and Fos immunohistochemical study. *J Headache Pain.* 2019;**20(1)**:1–9. https://doi.org/10.1186/s10194-019-0977-0

48. Abd Aziz CB, Ahmad Suhaimi SQ, Hasim H, Ahmad AH, Long I, Zakaria R. Effects of Tualang honey in modulating nociceptive responses at the spinal cord in offspring of prenatally stressed rats. *J Integr Med.* 2019;**17(1)**:66–70. https://doi.org/10.1016/j.joim.2018.12.002

49. Calixto-Campos C, Corrêa MP, Carvalho TT, Zarpelon AC, Hohmann MS, Rossaneis AC, et al. Quercetin reduces Ehrlich tumor-induced cancer pain in mice. *Anal Cell Pathol.* 2015;**2015**:1–18. https://doi.org/10.1155/2015/285708

50. Rostami Z, Ghasemi S, Farzadmanesh H, Safari M, Ghanbari A. Sex difference in trigeminal neuropathic pain response to exercise: role of oxidative stress. *Pain Res Manag.* 2020:2020:1–9. https://doi.org/10.1155/2020/3939757

51. Kumar VL, Roy S. Calotropis procera latex extract affords protection against inflammation and oxidative stress in Freund's complete adjuvant-induced monoarthritis in rats. *Mediators Inflamm.* 2007;**2007**:47523. https://doi.org/10.1155/2007/47523

52. Yowtak J, Lee KY, Kim HY, Wang J, Kim HK, Chung K, et al. Reactive oxygen species contribute to neuropathic pain by reducing spinal GABA release. *Pain* 2011;**152(4)**: 844–852. https://doi.org/10.1016/j.pain.2010.12.034

53. Gao Y, Zhou S, Pan Y, Gu L, He Y, Sun J. Wnt3a inhibitor attenuates remifentanil-induced hyperalgesia via downregulating spinal NMDA receptor in rats. *J Pain Res.* 2020;**13**:1049–1058. https://doi.org/10.2147/JPR.S250663

54. Reyes RC, Brennan AM, Shen Y, Baldwin Y, Swanson RA. Activation of neuronal nmda receptors induces superoxide-mediated oxidative stress in neighboring neurons and astrocytes. *J Neurosci.* 2012;**32(37)**:12973–12978. https://doi.org/10.1523/JNEUROSCI.1597-12.2012

55. Brittain MK, Brustovetsky T, Brittain JM, Khanna R, Cummins TR, Brustovetsky N. Ifenprodil, a NR2B-selective antagonist of NMDA receptor, inhibits reverse $Na^+/^{Ca2+}$ exchanger in neurons. *Neuropharmacology.* 2012;**63(6)**:974–982. https://doi.org/10.1016/j.neuropharm.2012.07.012

56. Kishore RK, Halim AS, Syazana MN, Sirajudeen K. Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. *Nutr Res.* 2011;**31**:322–325. https://doi.org/10.1016/j.nutres.2011.03.001

57. Khalil MI, Alam N, Moniruzzaman M, Sulaiman SA, Gan SH. Phenolic acid composition and antioxidant properties of Malaysian honeys. *J Food Sci.* 2011;**76**:921–928. https://doi.org/10.1111/j.1750-3841.2011.02282.x

58. Bashkaran K, Zunaina E, Bakiah S, Sulaiman SA, Sirajudeen K, Naik V. Anti-inflammatory and antioxidant effects of Tualang honey in alkali injury on the eyes of rabbits: experimental animal study. *BMC Complement Altern Med.* 2011;**11**:90. https://doi.org/10.1186/1472-6882-11-90

59. Zhang L, Guo HL, Zhang HQ, Xu TQ, He B, Wang ZH, et al. Melatonin prevents sleep deprivation-associated anxiety-like behavior in rats: role of oxidative stress and balance between GABAergic and glutamatergic transmission. *Am J Transl Res.* 2017;**9(5)**:2231–2242.

60. Zhang L, Yin JB, Hu W, Zhao WJ, Fan QR, Qiu ZC, et al. Analgesic effects of duloxetine on formalin-induced hyperalgesia and its underlying mechanisms in the CeA. *Front Pharmacol*. 2018;**9**:317. https://doi.org/10.3389/fphar.2018.00317

61. Tabata-Imai A, Inoue R, Mori H. Increased sensitivity to inflammatory pain induced by subcutaneous formalin injection in serine racemase knock-out mice. *PLoS ONE*. 2014;**9(8)**:e105282. https://doi.org/10.1371/journal.pone.0105282

62. Fang JQ, Fang JF, Liang Y, Du JY. Electroacupuncture mediates extracellular signal-regulated kinase 1/2 pathways in the spinal cord of rats with inflammatory pain. *BMC Complement Altern Med*. 2014;**14(1)**:1–12. https://doi.org/10.1186/1472-6882-14-285

63. Ruiz-Miyazawa KW, Zarpelon AC, Pinho-Ribeiro FA, Pavao-de-Souza GF, Casagrande R, Verri Jr WA. Vinpocetine reduces carrageenan-induced inflammatory hyperalgesia in mice by inhibiting oxidative stress, cytokine production and NF-κB activation in the paw and spinal cord. *PLoS ONE*. 2015;**10(3)**:e0118942. https://doi.org/10.1371/journal.pone.0118942

64. Ben Khedir S, Mzid M, Bardaa S, Moalla D, Sahnoun Z, Rebai T. In vivo evaluation of the anti-inflammatory effect of Pistacia lentiscus fruit oil and its effects on oxidative stress. *ECAM*. 2016;**2016**:1–12. https://doi.org/10.1155/2016/6108203