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Original Article

Potential protective effect of honey against chronic cerebral hypoperfusion-induced neurodegeneration in rats

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ABSTRACT

Introduction: To investigate the neuroprotective potential of Malaysian Tualang honey in chronic cerebral hypoperfusion induced by permanent bilateral common carotid arteries ligation (2VO) in rats.

Methods: Rats were randomly divided into three groups ($n = 10$); sham control, honey-untreated 2VO group "2VO" and honey treated 2VO group "2VO + H". At 10th week of 2VO surgery, all the rat were sacrificed, brains were dissected out, the right hemisphere was processed for histological study, neuronal counts were performed on cresyl violet-stained sections, the number of viable neurons in CA-1 region of the hippocampus were analysed and counted.

Results: There were damaged, distorted, irregular cells with shrunken cytoplasm and dark pykonotic nuclei in "2VO" rats. Treatment of rats with honey restored the hippocampal cells to their normal structure and reduced loss of neurons in "2VO + H" rats as compared with "2VO" rats.

Discussion: This study shows that Malaysian Tualang honey might have therapeutic potential for the treatment of chronic cerebral hypoperfusion related neurodegenerative disorders.

1. Introduction

Neurodegenerative disorders are group of diseases affecting neurons of nervous system. It is characterized by loss of memory, progressive deterioration of intellectual and social functions, personality changes, inability for self-care and problems with communication and reasoning and irreversible loss of neurons from specific regions of the brain; the severity

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of cognitive deficits correlates best with synaptic loss and formation of neuro-fibrillary tangles,[1] as seen in Alzheimer's disease (AD) and particularly dementia which are associated with aging and reduced cerebral blood flow "CBF".[2] AD is the commonest neurodegenerative disorder of aging associated with destruction of hippocampal and cortical neurons.[3] There is a strong correlation between reduced CBF, cerebral micro-vascular pathology, cognitive impairment and neuronal degeneration in experimental animals.[2,4] Permanent bilateral occlusion of the common carotid arteries through two vessels occlusion (2VO) in rats results in a significant reduction of CBF in rats and cause severe histopathological damage in the CA1 region of the hippocampus and related behavioural deficits.[2]

Honey is a natural sweet substance made by bees; it has a long history in human consumption not only as a nutrient but also as an alternative medicine in various disorders.[5] The antioxidants that are naturally found in honey contribute to antioxidant capacity, anti-inflammatory, antibacterial, anti-viral, anti-ulcerous activities, anti-lipid and anti-cancer properties.[6] Consumption of antioxidant compounds can attenuate oxidative damage, improve cognitive performance in animals and slow the deterioration in memory and learning in old age people.[7]

Malaysian Tualang honey, mostly found in forests of Peninsular Malaysia, collected from the combs of Asian rock bees "*Apis dorsata*", the world's largest honey bees, which build their hives high up in the "Honey Bee Tree".[6] Tualang honey is used commonly as a medicinal product as well as food in Malaysia.[8] It has been used as therapy for low sperm count,[9] burn wounds,[8,10] hypoglycemic agent,[11] and treatment of hepatic damage in diabetes mellitus,[12] enhancing bone health,[13] and as anti-cancer.[14] Up to the authors' knowledge, there has been no published data concerning the effects of Tualang honey on the morphology of the CA1 area of hippocampus. The present study was conducted to investigate the effect of Tualang honey on chronic cerebral hypoperfusion induced neurodegeneration and to compare the histopathological changes in CA1 area of hippocampus rat brain between honey treated 2VO rats, 2VO rats and sham control rats.

2. Materials and methods

2.1. Animals

Thirty male Sprague–Dawley rats weighing 250A–300 gm were housed in cages (2 rats per cage) at temperature of 22 ± 1 °C and 12 h light/dark cycle. All animals were treated in accordance with the Guidelines for The Care and Use of Laboratory Animals of the National Institute of Health. The standard food pellets and tap water were allowed ad libitum.

2.2. Malaysian Tualang honey

Honey used in this study was kindly supplied by Federal Agricultural Marketing Authority (FAMA), Kedah, Malaysia. It has the following composition: total reducing sugar (64.3%) comprising of fructose (34.3%), glucose (26.2%), maltose (3.8%); fructose/glucose ratio (1.31) and water (22.0%).

2.3. Experimental design

Animals were randomly divided into 3 groups ($n = 10$). Sham operated group; honey-untreated 2VO group "2VO" and honey treated 2VO group "2VO + H". Rats of honey treated 2VO group "2VO + H" received freshly prepared honey orally by gavage every morning (1.2 g/kg diluted with distilled water). The rats were anaesthetized intraperitoneally with ketamine 90 mg/kg and xylazine 20 mg/kg. The common carotid arteries were exposed via a ventral cervical incision, and separated from the carotid sheaths and vagal nerves, both arteries were doubly legated with silk sutures.

2.4. Removal of the specimen and tissue processing

Rats were sacrificed at 10th postoperative week; brains were quickly dissected out and transferred to an ice cold steel tray. The cerebellum was excised and the right and left cerebral hemispheres were separated. The fornix and corpus callosum of the right hemisphere were identified as the anatomical landmarks. The sample was dissected 3 mm posterior to the lower end of the fornix. The right hemisphere was fixed in 10% formal saline for 4 days, and processed for light microscopy. Sections of 5 μm thickness were stained by Cresyl Violet.

2.5. Neuronal counts

Neuronal counts were performed on Cresyl violet-stained sections; the number of viable neurons (cells of stratum pyramidal) in CA-1 region of the hippocampus was analysed and counted[15]; for standardization purpose, neurons were counted within 1 mm horizontal distance of CA1 hippocampus of all slides. Neurons with shrunken cytoplasm and dark pyknotic nuclei or naked nuclei (non-viable cells) were not counted.

2.6. Statistical analysis

Data were analysed using the Statistical Package for Social Science (SPSS) version 16.0 and are presented as mean ± S.E.M. One way ANOVA was used to assess the differences among groups. If the results were statistically significant, the differences between groups were assessed by post-hoc Tukey'test. A value of $p < 0.05$ was considered as statistically significant at 95% confidence interval.

3. Results

3.1. Morphological observation

The histopathological findings in the CA-1 region (Fig. 1A) of the hippocampus of untreated 2VO group demonstrated distinct morphological differences as compared with hippocampal sections of sham controls. In sham group, the viable neurons in CA1 region revealed the compact cellular structure of stratum pyramidal with well demarcated cellular membrane, clear cytoplasm and distinct nucleus (Fig. 1B). In contrast, most of CA1 stratum pyramidal in the untreated "2VO" group displayed the loose cellular arrangement with irregular, dark, shrunken cytoplasm with pyknotic nucleus (non-viable neurons). Only a

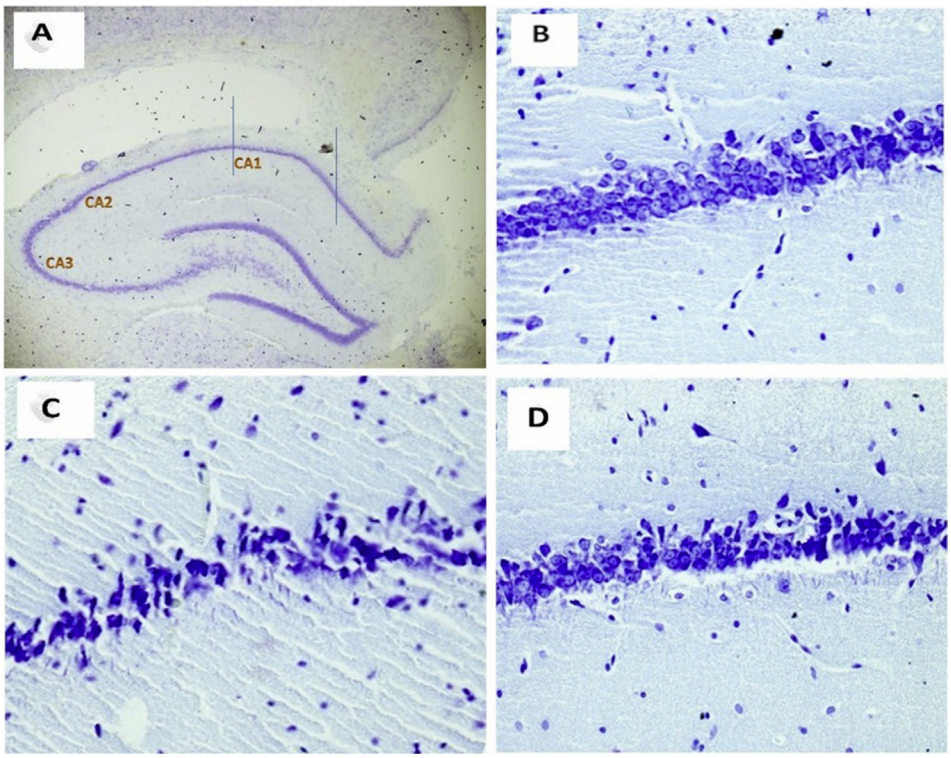

Fig. 1 – Photomicrographs of Cresyl violet-stained sections of the hippocampus. A; Coronal section of dorsal hippocampus shows the area of CA1, CA2 and CA (100×). B, C, D, (Sections of CA1 the hippocampal striatum pyramidal). B; Sham group showing the compact structure of normal pyramidal regular shaped cell with well-defined membranes and distinct nuclei (400×). C, 2VO group, representing the damaged, distorted, irregular cells with shrunken cytoplasm and dark pykonotic nuclei were seen as non-viable neurons (400×). D; 2VO + H group showing the preserved compact structure of CA1 striatum pyramidal and prevented pyramidal cell loss and no remarkable neuronal abnormalities were observed (400×).

few numbers of normal cells were with visible nucleus (Fig. 1C). Although, there was no much difference between the histological findings of 2VO + H groups and sham groups. The honey treated 2VO "2VO + H" animals preserved the compact structure of CA1 stratum pyramidal and prevented neuronal cell loss with less pyknotic nuclei which showed less neuronal cell damage than untreated 2VO group (Fig. 1D).

3.2. *Statistical study*

Statistical analysis demonstrated that the viable hippocampal CA-1 pyramidal cells were significantly reduced in the 2VO group (112.93 ± 11.09) as compared to the Sham group (235.25 ± 9.73); $p < 0.001$. The number of viable neuronal cells of the hippocampal CA-1 region of the "2VO + H" (216.5 ± 19.69) group was significantly higher than the untreated 2VO group (112.93 ± 11.09); $p < 0.001$. Meanwhile, there was no significant difference of viable neuronal cell between "2VO + H" group (216.5 ± 19.69) and Sham group (235.25 ± 9.73); $p > 0.05$ (Fig. 2).

4. Discussion

Honey possesses many beneficial properties such as neuroprotective, anti-oxidative, anti-inflammatory, antibacterial.[9,16] there has been very little research on the effect of honey on chronic cerebral hypoperfusion induced neurodegeneration in animal models; however honey bee venom has been shown to decreases the pathological changes in the spinal cord of female Lewis rat's experimental allergic encephalomyelitis.[17] Honey also attenuated lead-induced neurotoxicity and greatly improves AChE activity in male albino rats.[18] Until the present time, there is no effective treatment to stop or cure AD. However evidence-based research are conducted in therapeutic and preventive strategies that lead to small delays in Alzheimer's onset and its progression can significantly reduce the global burden of the disease.[3] There are several factors such as "mental and physical exercise, Omega-3 fatty acids and adherence to mediterranean diet" that may protect people from AD.[19] Currently, many herbal medicinal plants such as Coriander (*Coriandrum sativum* L.) have been used for prevention of neurodegeneration as they improve blood circulation to the head, impart mental concentration and memory capabilities,[20] the present study has demonstrated neuronal cell death in rats with Chronic cerebral hypoperfusion induced by permanent bilateral occlusion of the common carotid arteries (2VO) indicating that the hippocampus of brain is very sensitive to ischaemia. 2VO induced chronic cerebral hypoperfusion enhances the formation of Reactive Oxygen Species "ROS" in the brain.[21] It has been stated that oxidative stress has been linked to the neuronal cell death that is associated with neurodegenerative disorders like AD.[22]

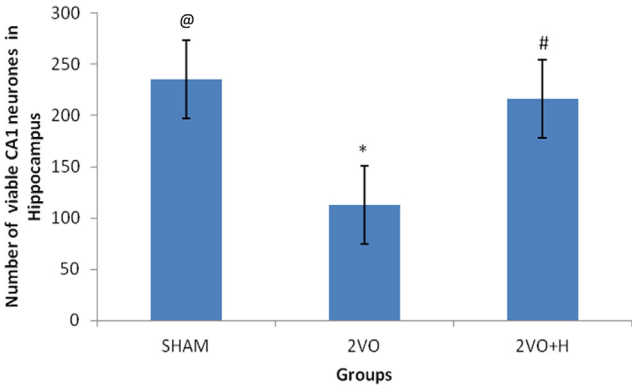

Fig. 2 – Number of hippocampal CA-1 neuronal cells in sham, "2VO" and "2VO + H" groups. Data represent the mean ± SEM [*$p < 0.001$ [sham vs 2VO]; #$p < 0.001$ [2VO vs 2VO + H]; @ $p > 0.05$ [Sham vs 2VO + H] pos hoc [Tukey].

The oxygen free radicals are found in brain regions affected by neurodegeneration, many studies have reported that free radicals are capable of mediating neuronal degeneration and death.[22] This mechanism may be involved in the pathogenesis of neuronal death in neurodegenerative disorders.[23] Impairment in memory and learning and deterioration in brain function during ageing has been related to the oxidative damage.[7] The present study demonstrated significant neuronal damage in the hippocampal CA1 region in 2VO rats as compared to sham controls. However, the daily supplementation of Malaysian Tualang honey to the 2VO rats for 10 weeks effectively improved the pathological changes in the hippocampus region and showed less neuronal cell loss i.e. significant increase in the viable neurons as compared with untreated 2VO rats. The current study, also demonstrated no morphological differences among honey-treated 2VO group "2VO + H" and sham control in CA-1 region of the hippocampus; this might be to potent antioxidant free radical scavenging and anti-inflammatory properties of honey, that play an important role in improving neuronal loss due to chronic cerebral hypoperfusion.

Consumption of antioxidants can attenuate oxidative damage, reduce anxiety and improve cognitive performance in animals.[7] In addition, honey can also enhance the body's antioxidants like blood vitamin "C" concentration by 47%, β-carotene by 3%, uric acid by 12%, and glutathione reductase by 7%.[16] The mechanism by which the constituents of honey attenuated neuronal cell death observed in the 2VO animal model needs further study. Nevertheless, antioxidant and anti-inflammatory properties of honey could be the underlying cause for the reduction in damaging effect of oxidative stress induced neurodegeneration and cognitive impairment.[10,16] To support this hypothesis; further studies are needed to investigate the mechanism of honey action on oxidative stress pathways, to determine the levels of oxidative stress markers in the rat brain; and also to explore the exact neuroprotective mechanisms or biologically active components of Malaysian Tualang honey.

Besides, the anti-inflammatory effects of honey, the effectiveness of honey as a therapeutic agent may be related to its controlling roll in the formation of free radicals released from the inflamed tissues.[24] Moreover, reduction of inflammation by honey could be due to its direct anti-inflammatory effect which has been supported in animal studies.[16] Mitochondrial failure is implicated as a crucial factor in the pathogenesis of mental disorders. Mitochondria are one of the enzymatic sources of reactive oxygen species (ROS) and could also be a major target for ROS-mediated damage; chronic increases in ROS production are associated with mitochondrial damage and dysfunction, which thus lead to a catastrophic cycle of mitochondrial functional decline, additional ROS generation, and cellular injury.[25] Mitochondrial abnormalities, particularly of cytochrome oxidase are found in the brain's cortical regions of subjects with senile dementia of the Alzheimer type.[26] Such abnormalities could be both the cause and result of oxidative stress and neurodegeneration. Electron microscopic investigation is in progress to study in detail the ultrastructural changes that occurred in the neurons of CA-1 region of the hippocampus induced by permanent bilateral occlusion of the common carotid arteries (2VO) in rats and the preventive role of honey administration.

5. Conclusions

The present study demonstrated that Malaysian Tualang honey attenuated the neuronal loss and significantly played a role in the inhibition of neuronal death in the hippocampus of rats induced by cerebral hypoperfusion. This study also shows that honey could be useful in prevention or disease progression in chronic cerebral hypoperfusion induced neurodegenerative disorders.

Conflicts of interest

All authors have none to declare.

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