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PERSICARIA MINOR (BIOKESUM®) AMELIORATES COGNITIVE DYSFUNCTION IN CHRONIC CEREBRAL HYPOPERFUSION-INDUCED RATS

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ABSTRACT

Vascular dementia (VaD) is the second most common cause of dementia, succeeding over Alzheimer's disease (AD) in the elderly, and is expected to double every 10-15 years. Chronic cerebral hypoperfusion (CCH) resulting from diverse cerebral vascular pathologies is a major cause of VaD. However, effective therapeutic approaches to cure VaD are still missing. The latest trends are seeing a shift in consumer preference to natural therapy for health and wellness with a high degree of convenience. *Persicaria minor*, commonly referred to as 'Kesum' in Malaysia is known for its potent antioxidant, anti-inflammation, anti-acetylcholinesterase, and cognitive-enhancing effects, which are beneficial in preventing aging and oxidative stress-related disease. However, the behavioral effect of *P. minor* in enhancing cognitive functions, especially following ischemic conditions, remains unknown. The present study evaluates the neuroprotective effects of a standardised aqueous extract of *P. minor* (Biokesum®) in CCH-induced rats. A permanent bilateral occlusion of common carotid artery (PBOCCA) surgery was performed to develop the CCH model in rats. The effects of *P. minor* (100, 200, and 300 mg/kg; orally) on motor and cognitive functions were evaluated using a series of behavioral tests. The behavioral assessment results have demonstrated that *P. minor* improves both short- and long-term recognition and spatial memories without affecting the motor functions of the PBOCCA rats. The present findings suggest the potential of *P. minor* in enhancing cognitive deterioration in CCH-induced rats.

Keywords: Vascular dementia, PBOCCA. Chronic cerebral hypoperfusion, cognition, *Persicaria minor*

INTRODUCTION

Vascular dementia (VaD), which is characterized by a progressive decline in cognition and behavior, is the second most common cause of dementia in the elderly, succeeding over Alzheimer's disease (AD) [1]. Chronic cerebral hypoperfusion (CCH) resulting from diverse cerebral vascular pathologies is a critical mechanism in developing vascular cognitive impairment and dementia. Advanced age and cardiovascular risk factors such as obesity, diabetes, hypercholesterolemia, arteriosclerosis, and smoking may affect the cerebral vascular system, resulting in moderate hypoperfusion and ischemic brain injuries, which lead to a progressive decline in cognitive and memory functions [2]. Although existing therapeutic agents such as memantine, galantamine, donepezil, and rivastigmine have achieved significant therapeutic advances in dementia, they are limited in the clinical application's safety and effectiveness. In searching for protective ways of CCH-induced cognitive dysfunction, much attention has been focused on the potential of natural antioxidants to attenuate neuronal injury, thus preventing vascular cognitive impairment and dementia.

Persicaria minor (*P. minor*) which is commonly known as 'kesum' in Malaysia, is an aromatic plant that belongs to the family Polygonaceae. It is extensively used as a spice and flavoring ingredient in culinary dishes or even consumed as a salad. *P. minor* has attracted particular attention as a nutraceutical because of its widespread health benefits. Traditionally, *P. minor* leaf decoctions are used to treat indigestion as a postnatal tonic and are applied to the scalp to treat dandruff [3]. Scientific reports have shown numerous biological activities of *P. minor*, including antioxidant, antimicrobial, anti-fungal, anti-inflammatory, anti-hyperlipidemia, antiproliferative, gastric cytoprotective activity, anti-hyperuricemic and neuroprotective effects, which were attributed to the presence of various phytoconstituents such as flavonoids, flavones, catechin, epicatechin gallate and terpenoids [4,5,6,7]. It

has also been reported to be rich in vitamins such as carotenes, retinol equivalents and vitamin C, α-tocopherol, and minerals such as calcium, phosphorus, iron, sodium, potassium, magnesium, copper and zinc, which are all major contributors to human nutrition [8]. Meanwhile, *P. minor* extract has shown superior antioxidant activity compared to other popular herbs such as '*pegaga*', '*ulam raja*', and curry leaves [3]. *P. minor* has also been reported to inhibit acetylcholinesterase, which is an enzyme that metabolizes acetylcholine, a neurotransmitter related to learning and memory in the brain [6]. The potent antioxidant and anti-acetylcholinesterase properties of P. minor may be attributed to its potential to prevent aging and other oxidative stress-related diseases [7]. To date, several studies have reported the ability of *P. Minor* extract to improve learning and memory in scopolamine-induced cognitive impairment in rodents [9]. However, these studies are insufficient to provide evidence-based use of the plant as a potential therapeutic agent for treating VaD.

Therefore, the present study was designed to explore the neuroprotective effects of chronic 14-day administration of aqueous *P. minor* extract on improving cognitive deficits in rats induced by CCH. In this study, a widely accepted model of CCH induced by permanent bilateral occlusion of common carotid arteries (PBOCCA) in rats was used. PBOCCA is a classic and frequently used approach to imitate CCH in rats.

MATERIAL AND METHOD

Animals

Seven-week-old male Sprague Dawley (SD) rats (200–250 g) were obtained from the Laboratory Animal Facility and Management (LAFAM), Faculty of Pharmacy, UiTM Puncak Alam, Malaysia. They were housed in five rats per cage and kept in the animal transit room for a week for acclimatization before the animals were subjected to surgery. The animal room was maintained at a constant temperature (± 24 °C) with a 12-hour of light-dark cycle (light on at 7 am). Food and water were given ad libitum. All experimental procedures in this study have been reviewed and approved by the Animal Ethics Committee, Universiti Teknologi MARA (Ref. No.: UiTM CARE 3/2021/(340/2021).

Induction of CCH

CCH was induced in rats by permanent bilateral occlusion of the common carotid arteries (PBOCCA), as described previously [10]. Briefly, rats were anesthetised with an intraperitoneal (i.p) injection of ketamine hydrochloride (70 mg/kg) and xylazine hydrochloride (20 mg/kg). Fourty rats were randomly chosen for PBOCCA surgery. The common carotid arteries were exposed through a small ventral midline incision, isolated from the surrounding carotid sheath and the vagus nerves, and permanently doubly ligated with 6/0 surgical silk suture. The skin incision was closed, and the rats were kept in an air-conditioned room at 25 °C. The sham-operated rats (n = 8) were subjected to the same surgical procedure, except that the common carotid arteries were not occluded.

Plant materials

The standardized aqueous extract of *P. minor* (BioKesum®) leaves powder was obtained from Biotropics Malaysia Berhad (Batch No. KE200302). The extract contains bioactive compounds, which include quercetin-3-glucuronide (more than 0.45%), quercitrin (more than 0.15%), and total phenolic content (more than 100 mg GAE/gdE). A certificate of analysis was provided by Phytes Biotek Sdn. Bhd.

Treatment conditions

The rats were randomly divided into five treatment groups two weeks after surgery. Group 1: Sham rats received vehicle (distilled water) (n = 8), Group 2: PBOCCA rats received vehicle (n = 8), and Group 3, 4, 5 treated with *P. minor* extracts at doses 100, 200, or 300 mg/kg, respectively. The extracts and distilled water were administered to the rats via oral gavage.

Behavioral assessments

After 14 days of treatment, behavioral tests, including the open-field test, novel object recognition task, and Morris's water maze task were conducted to assess the animals' motor, learning, and memory functions. The motor function and exploratory behaviors of animals were measured using the open field test (OFT). The novel object recognition (NOR) test was conducted to assess short-term

and long-term recognition memory. The Morris water maze (MWM) task was used to evaluate the spatial learning and reference memory of the rats as previously described [10].

RESULTS AND DISCUSSION

P. minor does not affect locomotor activities and anxiety-like behavior in PBOCCA rats

The effects of *P. minor* on motor activity and exploratory behavior were measured using the open-field test. The treatments have no significant impact on the spontaneous locomotor and exploratory activities of the PBOCCA rats (Figure 1).

Expression of anxiety-related behavior in the open field test is related to the anxiety of open space and was indexed by the animal's reluctance to venture into the central zone. A greater time spent, as well as a higher number of entries into the central zone, indicate a decrease in state anxiety. However, there was no significant difference in the number of entries and time spent in the central zone among all groups (Figure 2).

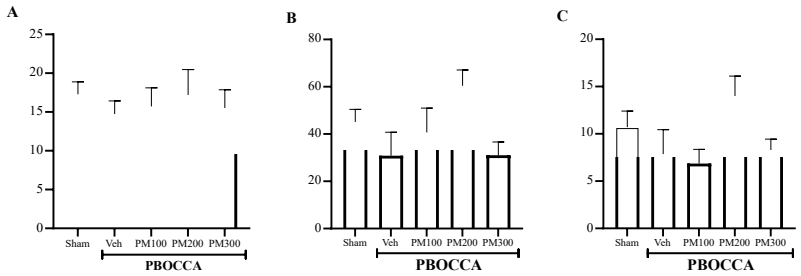

Figure 1: Effect of *P. minor* extract on (A) total distance traveled, (B) rearing activities, and (C) grooming activities of the PBOCCA rats in the open-field test. Statistical analysis using one-way ANOVA did not reveal significant group differences in the spontaneous locomotor and exploratory activity. Data are expressed as the mean±SEM (n=6-7).

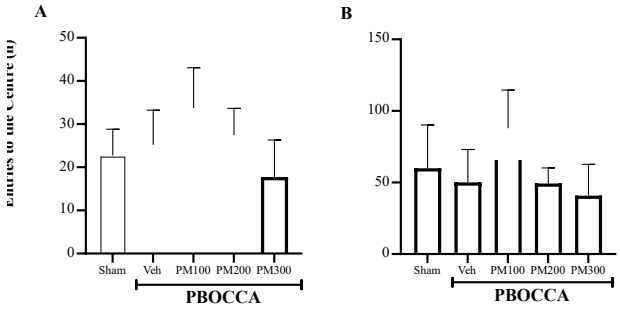

Figure 2: Effect of *P. minor* extract on anxiety-like behavior. (A) no. of entries to the center and (B) duration at the center. There is no significant difference among the groups analysed by one-way ANOVA. Data are expressed as the mean±SEM (n=6-7/group).

P. minor improves recognition memory deficits in PBOCCA rats

Figure 3 shows the effect of *P. minor* on short- and long-term recognition memories in the PBOCCA rats, presented by the recognition index (RI). PBOCCA rats exhibited a significantly lower RI value than sham-operated rats during both the short- ($p=0.0026$) and long-term ($p=0.0401$) test phases, respectively (Fig. 3A). Sham-operated rats with intact short- and long-term recognition memory spend more time exploring a novel object than a familiar object. PBOCCA rats did not recognise previously explored objects and spent almost equal time exploring familiar and novel objects during the short and long test phases. This indicates that CCH induces impairment of both short- and long-term recognition memory in rats. During the short-term phase, PBOCCA rats treated with *P. minor* (100, 200, and 300 mg/kg) exhibited significant ($p < 0.01$) higher RI values as compared to PBOCCA rats treated with a vehicle, which indicates the treatment with *P. minor* (100, 200, and 300 mg/kg) improve short-term recognition memory in PBOCCA rats. Meanwhile, during the long-term test phase, only the PBOCCA rats treated with 300 mg/kg of *P. minor* showed significant ($p < 0.01$) higher RI than PBOCCA rats treated with vehicle (Fig. 3B).

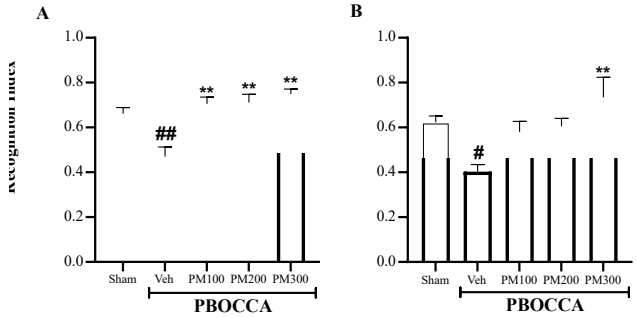

Figure 3: Effect of *P. minor* aqueous extract on (A) short-term and (B) long-term recognition memory in PBOCCA rats. Data are expressed as the mean±SEM (n = 6-7). The graph was statistically analysed by one-way ANOVA followed by Bonferroni post hoc test. # p < 0.05 vs. sham group; ## p < 0.01 vs. sham group; ** p < 0.01 vs. PBOCCA + vehicle group.

P. minor attenuates spatial reference and learning memory deficits in PBOCCA rats

The ability of *P. minor* to attenuate spatial learning and reference memory deficit in PBOCCA rats was assessed via the MWM task. During acquisition, a two-way repeated measures ANOVA analysis revealed significant treatment effect ($F_{4,26} = 11.82$, $p < 0.0001$), test day effect ($F_{4,104} = 77.92$, $p < 0.0001$), as well as significant treatment x test day effect ($F_{16,104} = 0.81$, $p = 0.6688$) (Fig. 4A). The escape latencies during five days of training were significantly increased in the PBOCCA rats received vehicle (PBOCCA + vehicle) in comparison with the sham group. P. minor extract (200 and 300 mg/kg) treatment for 14 days significantly improved the spatial learning impairment in PBOCCA rats. This was evident by the significant reduction ($p < 0.05$) in the escape latencies starting from day 1 to day 5 (200 mg/kg) and from day 2 to day 5 (300 mg/kg) as compared to sham. However, treatment with a lower dose of *P. minor* (100 mg/kg) did not show an apparent difference ($p > 0.05$) as compared with that of the sham group across five days of training.

Retrieval of spatial memory was explicitly tested with the probe trial performed 24 h after the last day of the training trial. As shown in Fig. 4B, CCH rats induced by PBOCCA spent less time in the target quadrant as compared to the sham group ($p < 0.05$). PBOCCA rats treated with 300 mg/kg of *P. minor* extract spent significantly ($p < 0.05$) more time in the target quadrant compared to the PBOCCA + vehicle group. In addition, the visible platform trials in the water maze task did not reveal any significant difference ($p > 0.05$) in escape latency among the groups (Fig. 4C). This implies that the group differences during the acquisition and probe trial were not due to visual deficits.

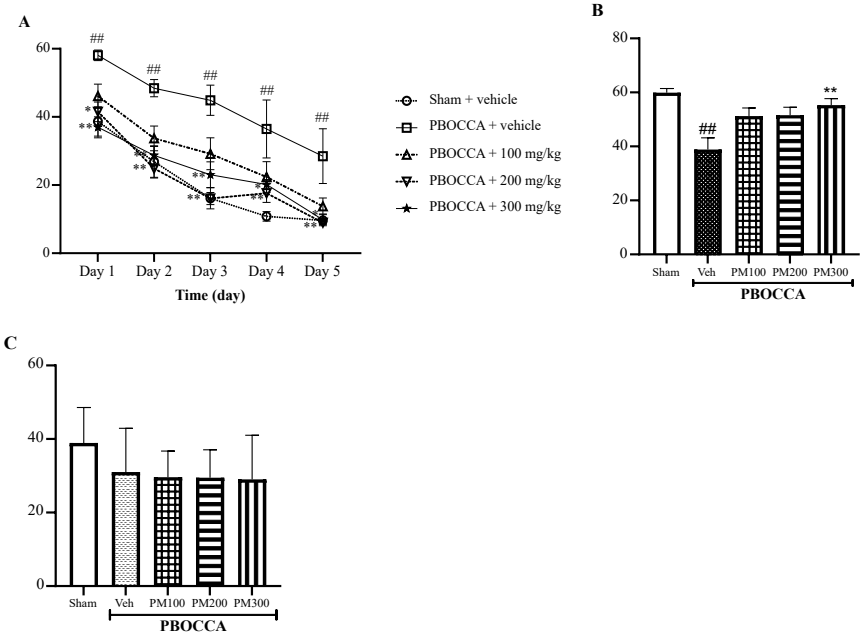

Figure 4: Effect of *P. minor* on spatial reference learning deficits in PBOCCA rats. (A) Escape latency time during acquisition (Day 1-5). Data are expressed as the mean±SEM (n=6-7), analysed by two-way ANOVA followed by the Bonferroni post hoc test. (B) Percentage time spent in the target quadrant during the probe trial. (C) Escape latency time with a visible platform. Data are expressed as the mean±SEM (n=6-8), analysed by one-way ANOVA followed by Bonferroni post hoc test. ## p < 0.01 vs. sham group; * p< 0.05 vs. PBOCCA + vehicle group; ** p < 0.01 vs. PBOCCA group.

CONCLUSION

The findings demonstrated an improving effect of *P. minor* extract on memory in the CCH rat model, suggesting that *P. minor* extract could be a potential treatment for vascular dementia and Alzheimer's disease patients as well as a preventive healthcare measure in preventing the rapid decline of cognitive function associated with aging.

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