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Phenylethanoid glycosides of *Pedicularis muscicola* Maxim ameliorate high altitude-induced memory impairment

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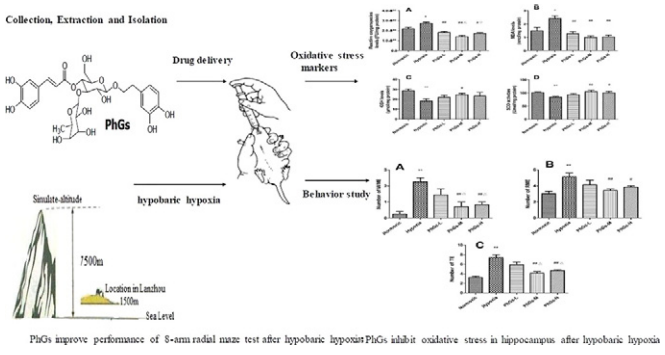
HIGHLIGHTS

- Hypobaric hypoxia results in hippocampal dependent memory impairment.
- PhGs supplement improves performance of 8-arm radial maze test and inhibits oxidative stress and neuronal degeneration and apoptosis in hippocampus after hypobaric hypoxia.

GRAPHICAL ABSTRACT



PhGs improve performance of 8-arm radial maze test after hypobaric hypoxia PhGs inhibit oxidative stress in hippocampus after hypobaric hypoxia

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ABSTRACT

Exposure to hypobaric hypoxia causes oxidative stress, neuronal degeneration and apoptosis that leads to memory impairment. Though oxidative stress contributes to neuronal degeneration and apoptosis in hypobaric hypoxia, the ability for phenylethanoid glycosides of *Pedicularis muscicola* Maxim (PhGs) to reverse high altitude memory impairment has not been studied. Rats were supplemented with PhGs orally for a week. After the fourth day of drug administration, rats were exposed to a 7500 m altitude simulation in a specially designed animal decompression chamber for 3 days. Spatial memory was assessed by the 8-arm radial maze test before and after exposure to hypobaric hypoxia. Histological assessment of neuronal degeneration was performed by hematoxylin-eosin (HE) staining. Changes in oxidative stress markers and changes in the expression of the apoptotic marker, caspase-3, were assessed in the hippocampus. Our results demonstrated that after exposure to hypobaric hypoxia, PhGs ameliorated high altitude memory impairment, as shown by the decreased values obtained for reference memory error (RME), working memory error (WME), and total error (TE). Meanwhile, administration of PhGs decreased hippocampal reactive oxygen species levels and consequent lipid peroxidation by elevating reduced glutathione levels and enhancing the free radical scavenging enzyme system. There was also a decrease in the number of pyknotic neurons and a reduction in caspase-3 expression in the hippocampus. These findings suggest that PhGs may be used therapeutically to ameliorate high altitude memory impairment.

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1. Introduction

Hypobaric hypoxia is a stressful paraphysiological condition that results in many adaptive physiological changes to promote survival [1]. However, excessive exposure to high altitudes results in Acute Mountain Sickness (AMS), High Altitude Pulmonary Edema (HAPE), High Altitude Cerebral Edema (HACE), and other neurophysiological abnormalities, such as sleep disorders, impacts on mood, and memory impairments [2]. The memory impairments induced by high altitude exposure are especially problematic because the ability to perform highly demanding mental functions is compromised. Previous studies have revealed that high altitude exposure results in decreased partial pressure of oxygen and increased formation of reactive oxygen and nitrogen species (RONS) at the hippocampal tissue level, which ultimately appears to decrease the activity and effectiveness of the antioxidant enzyme system [3]. The hippocampus is associated with learning and memory, and an insult to this brain region affects cognition. Recent studies have suggested that the oxidative, neurodegenerative, and dendritic plasticity effects of hypobaric hypoxia in hippocampus are associated with spatial memory impairments [4].

Pedicularis muscicola Maxim, belonging to the *Scrophulariaceae* plant of the genus *Pedicularis*, is one of the most popular traditional Chinese herbal medicines. Previous phytochemical studies on *Pedicularis* plants led to the discovery of alkaloids, flavonoids, and phenylpropanoid glycosides [5]. Among the identified components within *Pedicularis* plants, phenylethanoid glycosides were proven to exhibit distinct antioxidant, anti-apoptotic, anti-tumoral, anti-inflammatory, and neuroprotective activities [6, 7]. Acteoside (verbascoside), which is one of the main active phenylethanoid glycosides, has also been shown to have modest activities to directly scavenge reactive oxygen species and inhibit cholinesterases in vivo and vitro by inducing gene transcription of antioxidant enzymes [8, 9]. Recent studies reported that acteoside played a role in enhancing neuroprotective and memory enhancement effects in the senescent mouse model induced by a combination of D-gal and $AlCl_3$ [10]. Intragastrical administration of acteoside has also been shown to attenuate memory impairments and neurodegeneration in an animal model of Alzheimer's disease [11].

Although the effects of hypobaric hypoxia on memory function at different altitudes and durations are well documented [12], to date, there are no effective medications to ameliorate hypobaric hypoxia induced memory impairment. A recent report by Baitharu et al. [13, 14] demonstrated that inhibition of corticosterone synthesis and/or signaling had positive effects on memory impairment during hypobaric hypoxia exposure. However, side effects similar to those of systemic glucocorticoids may occur after a period of administration. Phenylethanoid glycosides act as antioxidants and also enhance memory, but it is unknown whether PhGs can prevent hypobaric hypoxia-induced memory impairment. The present study aims to explore the effects of PhGs on hypobaric hypoxia induced memory impairment. The study further attempts to unfold the molecular mechanisms underlying the neuroprotective effects of PhGs.

2. Materials and methods

2.1. Collection, extraction and isolation

Pedicularis muscicola Maxim was collected in July of 2014 in the Tianzhu Tibetan autonomous counties, Wuwei, Gansu Province (East longitude 103°47′, latitude 37°23′, altitude 3240 m), China. The species was authenticated by Dr. Zhigang Ma, School of Pharmacy, Lanzhou University. The air-dried leaves and twigs of *Pedicularis muscicola* Maxim (2.0 kg) were extracted three times with distilled water (1:8) using a water decoction method. The mix of filtrates was subjected to column chromatography with a macroporous resin (50–60 mesh, 500 g) using 30% methanol as the eluent and using a UV detector. Fractions (absorbance ≥ 0.2) were concentrated by the rotavapor under reduced pressure and at a controlled temperature (50–60 °C). The solvent of the concentrated solution was removed using vacuum freeze-drying equipment and yielded 40.58 g (2.02% by dry weight). The purity of PhGs was checked by high-performance liquid chromatography (HPLC). More detailed experimental procedures were described in our previous work [15]. When acteoside (Fig. 1) was used as the reference substance, the sample was found to have 45.82% purity by HPLC analysis (Fig. 2).

2.2. Animals

Male Wistar rats (180–230 g, clean class, certificate No. 62000800000023, Experimental Animal Center, Lanzhou University) were maintained on a 12 h light/dark cycle at a room temperature of 25 ± 2 °C. The amount of food was adjusted daily so that body weight was maintained at 80–85% of the free feeding level throughout the experiment. Water was given ad libitum. All experiments were conducted according to the guidelines of the Committee on the Care and Use of Laboratory Animals of Lanzhou University.

2.3. Experimental design

Rats (n = 60) were trained in the 8-arm radial maze test. Among them, 50 rats were successfully trained and therefore were included in the study. After training, the rats were divided randomly into five groups by the method of random number table: normoxic group (n = 10), hypoxia group (n = 10), low dose group (PhGs-L, n = 10), middle dose group (PhGs-M, n = 10), and high dose group (PhGs-H, n = 10). Low, middle, and high dose group rats were supplemented with 50 mg/kg, 200 mg/kg, and 400 mg/kg of PhGs orally for a week, respectively, and normoxic and hypoxia group rats were administered distilled water. The experimenter(s) who performed the behavioral tests and tissue analysis was/were blinded for the treatment.

2.4. Exposure to simulated hypobaric hypoxia

On the fourth day of drug delivery, hypoxia, low, middle, and high dose group rats were exposed for three days to a simulated altitude of 7500 m in a specially designed animal decompression chamber that reduced the barometric pressure (oxygen partial pressure 8.1–8.0 kPa and temperature 25 °C). The normoxic rat group was kept at a normal atmospheric pressure with controlled temperature and humidity that were similar to the conditions in the hypoxic chamber to control for the effects of enclosed housing on memory functions. The beginning rate of ascent to the desired altitude was 10 m/s over a period of 30 min. To avoid secondary oxygen-enriched injury in rats, the chamber was brought down to the altitude of 4000 m at 9:00 AM for 1 h every day to replenish food, water, and drug treatment. Cognitive assessments and specimen collections were also conducted at the altitude of 4000 m.

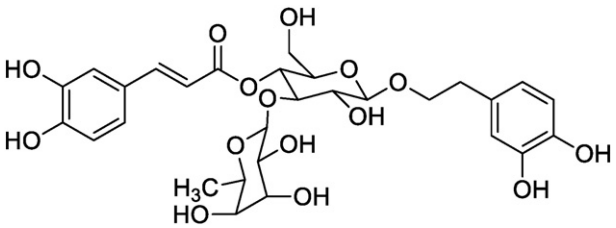

Fig. 1. The chemical structure of acteoside.