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Original article

Gladiolus dalenii lyophilisate reverses scopolamine-induced amnesia and reduces oxidative stress in rat brain

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ABSTRACT

Learning and memory are the most important executive functions performed by the human brain, the loss of which is a prominent feature in dementia. *Gladiolus dalenii* is traditionally used to treat a number of illnesses such as epilepsy and schizophrenia in Cameroon. This study aims to investigate the anti-amnesia effect of *Gladiolus dalenii* in scopolamine-induced amnesia in rats and its possible antioxidant properties in this model. Morris water maze, novel object location and recognition tasks were used to assess spatial and working memory. Male rats were treated for 12 days with saline, *G. dalenii* or Tacrine. Experimental animals were co-treated with scopolamine once daily from day 9 to 12. Acetylcholinesterase activity was measured in the prefrontal cortex and hippocampus. Malondialdehyde and glutathione levels were measured in the hippocampus. *G. dalenii* reversed memory impairment induced by scopolamine in the Morris water maze, novel object location and recognition tasks. It decreased acetylcholinesterase activity in the hippocampus and prefrontal cortex. It also decreased the level of malondialdehyde and increased the level of glutathione in the hippocampus. The results of this study show that *G. dalenii* ameliorates the cognitive impairment induced by scopolamine, through inhibition of oxidative stress and enhancement of cholinergic neurotransmission. It can therefore be useful for treatment of conditions associated with memory dysfunction as seen in dementia.

1. Introduction

Dementia is a syndrome of progressive deterioration of cognitive abilities associated with psychiatric and behavioral disturbances and difficulties in carrying out daily functions [1]. Dementia affects almost 50 million people worldwide, manifesting as deterioration of cognitive functions, such as memory, thinking and behavior [2]. It can be caused by aging, physical and/or chemical injuries, or neurodegenerative diseases, which in most cases would affect the quality of learning and memory of the concerned individuals. Learning and memory are the most important executive functions performed by the human brain, the loss of which is a prominent feature in dementia [3]. One common feature seen in a number of dementia types, such as Lewy body dementia, Alzheimer's disease (AD) and dementias associated with Parkinson's disease (PDD) and cerebrovascular disease (CVD), are deficiencies in cholinergic neurotransmission in the brain [4]. Along with cholinergic neurotransmission, oxidative stress is another well-known causative factor in the pathogenesis of dementia among age-related neurodegenerative disorders [5]. In this regard, brain tissue in particular is more susceptible to the deleterious effects of reactive oxygen species (ROS). ROS then initiate lipid peroxidation which triggers neuronal degeneration especially the central cholinergic pathway, because it has a high rate of oxygen consumption, and reduced antioxidant defense systems [6,7].

Although conventional drugs used for most forms of dementia are effective in the early stages of the disease, long-term therapy has been associated with serious adverse effects [8,9]. Hence,

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because of the side effects of these medications, there is a need to search for alternative treatment. In this regard, over the last decade, in an attempt to discover new alternative therapies for the most common form of dementia, basic science has focused on the discovery of natural compounds as potential candidates that can protect neurons against various insults and exert beneficial effects on neuronal cells [9]. Currently, herbal medicine offers a kind of "built in" poly-pharmacology, because of the complexity of chemical content related to the diverse classes and multiple analogs within any class [10]. Therefore the plant's potential to yield new therapeutic agents has stimulated extensive research into identifying plant extracts with properties such as inhibition of acetylcholinesterase or with other mechanistic effects relevant to the treatment of dementia [11].

Gladiolus dalenii (GD) is traditionally used for the treatment of epilepsy and schizophrenia in Cameroon [12,13]. Previous studies have confirmed anticonvulsant properties of the aqueous extract of GD [12]. Antidepressant properties have been also observed in naïve mice and rats with temporal lobe epilepsy-associated depression [13,14].

This study aimed to investigate whether GD possesses anti-amnesia properties. We used scopolamine-induced amnesia in rats, a well-established animal model of memory dysfunction [15]. This experimental animal model of dementia has been extensively used in research to screen for drugs with potential therapeutic value in dementia [16]. Moreover, scopolamine-induced memory loss has been linked to increased oxidative stress in the whole brain, as well as specific structures associated with memory and learning [17].

Therefore in this study we investigated the anti-amnesia effect of GD in scopolamine-induced amnesic rats and its possible antioxidant properties in this model.

2. Methods

2.1. Plant collection, identification and extract preparation

The corms of *G. dalenii* used in this study were harvested during the dry season in Babadjou (West Cameroon). Botanical identification was performed at the National Herbarium of Yaoundé. A voucher specimen was deposited at the Yaoundé Herbarium (reference number 25742/SRF/Cam). The macerate was prepared according to the method used by traditional healers who administer it orally to patients. The corms were selected and crushed at room temperature. A total of 1 kg of the Paste (crushed corms) was macerated in 10 L of distilled water for 5 h. The supernatant (macerate) was then collected, filtered through Whatman No. 1 filter paper, and frozen. The frozen extract was lyophilized at 0 °C. The dry residue obtained from the lyophilization was 153.88 g. The yield of the extraction was 15.39%. The following doses were used: 15 and 150 mg/kg based on previous studies of the anticonvulsant and antidepressant properties of the extract [12–14].

2.2. Experimental animals

Male Wistar albino rats ($n = 35$), weighing 150–200 g at the beginning of the experiment, were obtained from the Laboratory of Animal Physiology and Phytopharmacology (LAPHYPHA) of the University of Dschang, Cameroon. The animals were housed in polyacrylic cages (7 animals/cage) under a 12-h light/dark cycle. Food and water was available ad libitum. Prior to the treatment, the animal were fasted for 12 h to avoid any interaction with food when treated [18]. However, all animals were allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of the Cameroonian bioethics committee (reg N°. FWA-IRB00001954) and in accordance with NIH-Care and Use of Laboratory Animals manual. Efforts were also made to minimize animal suffering and to reduce the number of animal used in the experiment.

2.3. Drugs and treatments

The following drugs were used in the study: Scopolamine (1 mg/kg, Sigma–Aldrich, St. Louis, USA) and Tacrine (10 mg/kg, Sigma–Aldrich, St. Louis, USA) [19] were given by intraperitoneal injection (i.p.) while saline and *G. dalenii* were administered by oral gavage. A total of 35 animals were used in the experiments.

Group I received saline (0.9% NaCl solution) and served as the control. Group II received saline and served as the dementia model group. Group III received Tacrine (10 mg/kg, i.p.). Group IV and V received *G. dalenii* at doses of 15 and 150 mg/kg, respectively, by oral gavage. Group II, III, IV and V were co-treated with scopolamine (1 mg/kg) from day 9 to 12, 30 min before the behavioral test. From day 9 to 11, regarding the novel object recognition and object location tasks, animals were put in the open field for familiarization before any scopolamine administration used during MWM training session. Scopolamine was administered 30 min prior to the training session in the MWM. The same set of animals was subjected to NORT and MWM. MWM was performed 1 h after the novel object recognition and object location tasks. All the groups received the different treatments for 12 days. Behavioral testing was done from day 9 until day 12 (Fig. 1).

2.4. Morris water maze

The Morris water maze was used to evaluate spatial learning and memory in experimental rodents. It is a circular tank (diameter 185 cm and height 60 cm), which was filled with water and maintained at 25 °C. The tank was divided into four equal quadrants. A platform (10 cm^2) centered in one of the four quadrants of the pool was submerged approximately 1 cm below the surface of water. The position of the platform and visual cues (4) fixed in 4 sides of the surrounding walls dividing the pool into equal quadrants were kept constant throughout the training sessions. Each animal was subjected to four consecutive acquisition training sessions on each day with a session lasting 60 s and an interval of 5 min between sessions. During each training session, the rats were allowed to locate the hidden platform and to remain there for 20 s. If the animal was unable to locate the hidden platform within 60 s, it was gently guided by hand to the platform and allowed to remain there for 20 s. During each trial, the latencies of rats to locate the hidden platform were recorded and the latency was considered to be an index of acquisition and learning. On the 4th day, the platform was removed and each rat was allowed to explore the pool for 60 s. The latency to enter the target quadrant and the total time spent in the target quadrant were noted as indices of retrieval. Rats received *G. dalenii* for 12 days. All the animals were treated with scopolamine (1 mg/kg) 30 min before tested.

2.5. Novel object recognition task

The *novel object recognition task* (NORT) was conducted to assess the rat's ability to recognize a novel object, determined by their exploratory behavior. Familiarization of the animals with the experimental procedures occurred on the first 3 days, where the animal was placed in an empty open field, and they were tested on the 4th day.