Biomedicine & Pharmacotherapy 84 (2016) 387–394

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Neuroprotective effects of *Caralluma tuberculata* on ameliorating cognitive impairment in a D-galactose-induced mouse model

Muhammad zahid khan[a,b,*], Nagina Atlas[c], Waqas nawaz[d]

[a] Department of Pharmacology, China Pharmaceutical University, Nanjing 210009, China
[b] Department of Biotechnology, University of science and technology Bannu, K.P.K, Pakistan
[c] Department of Bioinformatics and Biotechnology, Faculty of basic and applied sciences. International Islamic university Islamabad, Pakistan
[d] School of basic medicine and clinical pharmacy, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 12 August 2016
Received in revised form 14 September 2016
Accepted 15 September 2016

Keywords:
Alzheimer's disease
Neuroprotection
Caralluma tuberculata
D-galactose
Oxidative stress

ABSTRACT

Cognitive deficiency and oxidative stress have been well documented in aging disorders including Alzheimer's disease. The aim of this study was to investigate the therapeutic efficacy of *Caralluma tuberculata* methanolic extract (CTME) on cognitive impairment in mice induced with D-galactose. In this study we assessed the therapeutic efficacy of CTME on cognitive impairment in mice induced with D-galactose by conduction of behavioral and cognitive performance tests. In order to explore the possible role of CTME against D-galactose-induced oxidative damages, various biochemical indicators were assessed. Chronic administration of D-galactose (150 mg/kg d, s.c.) for 7 weeks significantly impaired cognitive performance (in step-through passive, active avoidance test, Hole-Board test, Novel object recognition task and Morris water maze) and oxidative defense as compared to the control group. The results revealed that CTME treatment for two weeks (100, 200 and 300 mg/kg p.o) significantly ameliorated cognitive performance and oxidative defense. All groups of CTME enhanced the learning and memory ability in step-through passive, active avoidance test, Hole-Board test Novel object recognition task and Morris water maze. Furthermore, high and middle level of CTME (300 and 200 mg/kg p.o) significantly increased Total antioxidative capacity (T-AOC), Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) activity, neprilysin (NEP), and β-site AβPP cleaving enzyme 1 (BACE1) expression while Nitric Oxide (NO), Nitric Oxide Synthase (NOS) activity and Malondialdehyde (MDA) concentration, and the level of Aβ1-42 and presenilin 1 (PS1) were decreased. The present study showed that CTME have a significant relieving effect on learning, memory and spontaneous activities in D-galactose-induced mice model, and ameliorates cognitive impairment and biochemical dysfunction in mice.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia which is a serious loss of global cognitive ability [1–6]. In 2006, 0.40% of the global population (range 0.17–0.89%; absolute number 26.6 million, range 11.4–59.4 million) were affected by AD, and that the prevalence rate would triple and the absolute number would quadruple by the end of 2050 [7]. Currently there is no cure for AD, available treatments only offer small symptomatic relief and remain palliative. In Europe and the United States, Dementia, and particularly Alzheimer's disease are among the most expensive diseases for society [8,9]. The pathophysiology of AD is very complex, various deranged mechanisms such as Aβ production, mitochondrial dysfunction, hormonal imbalance, chronic oxidative stress, calcium mishandling, neurofibrillary tangles accumulation, inflammation, genetic components, may contribute in the disease process.

Caralluma belongs to the family Asclepiadaceae, its genus has about hundred species, dispersed in various countries including Spain, Saudi Arabia, Africa, Middle East, India, and Pakistan. *Caralluma tuberculata* plant is widely distributed throughout Pakistan, and traditionally used for the treatment of diabetic, rheumatism, leprosy, stomachic, carminative and central nervous system disorders [10]. Our previous studies demonstrated that *Caralluma tuberculata* methanolic extract CTME possess antioxidant properties [11,12]. D-galactose is a kind of reducing sugar,

* Corresponding author at: Department of Pharmacology, China Pharmaceutical University, No. 24 Tong Jia Xiang, Nanjing 210009, Jiangsu Province, China.
E-mail address: mzahidk786@hotmail.com (M.z. khan).

http://dx.doi.org/10.1016/j.biopha.2016.09.055
0753-3322/

which serves as a feasible brain aging model for animal. Injection of D-galactose can lead to a progressive deterioration in learning and memory ability. Nonetheless, the underlying mechanism of D-galactose-induced brain aging is remain unclear. Injection of D-galactose on long term basis in mice could lead to impairments in antioxidant capacity [13], mitochondrial function [14], calcium homeostasis [15], inflammatory response [16], neuronal apoptosis [17], and cholinergic degeneration [18] in the brain. It can lead to the formation of reactive oxygen species (ROS) by galactose oxidase at high levels. Oxidative damage and cellular injury are relevant to ROS generation in the neurodegenerative diseases [19]. Accumulating pieces of evidence show that D-galactose eventually damages learning and memory function and induces higher levels of Aβ in cortex and hippocampus [20]. The accumulation of Aβ in the brain is determined by the rate of production and degradation via several enzymes [21]. Aβ is generated by the cleavage of amyloid precursor protein (APP) through β- secretase (BACE1) and γ-secretase. Presenilin 1(PS1) is the catalytic component of the γ-secretase complex, and its mutations are associated with early-onset AD [22]. Neprilysin (NEP) is the most important Aβ peptide-degrading enzyme in the brain. Selective inhibition of NEP resulted in Aβ1-42 deposition in rat hippocampus [23].

The aim of this study was to evaluate the effect of CTME on mice cognitive performance. A second aim was to investigate a possible mechanism for the improvement of cognition in CTME treated mice. The following tests (Morris water maze, Active-avoidance test, Passive-avoidance test, Novel object recognition test, and Hole-board test) of learning and memory were performed to measure cognitive functions known to be affected in AD followed by determination of couple of antioxidant indexes including Total antioxidative capability (T-AOC), Glutathione peroxidase (GSH-Px), Total superoxide dismutase (SOD), Nitric Oxide (NO), Nitric Oxide Synthase (NOS) levels and Malondialdehyde (MDA) concentration in mice brain. Furthermore, the activities of Aβ1-42, NEP, BACE1, and PS1 were measured by ELISA.

2. Materials and methods

2.1. Plant preparation and extraction

A fresh *Caralluma tuberculata* whole plant was purchased from local market district Bannu, Pakistan. The plant was identified by Prof. Abdur Rahman, Department of botany, post graduate college Bannu and submitted voucher specimen in the Herbarium of University of Science and Technology Bannu. Plant was chopped and kept under shadow for dryness. Then the plant was converted into fine powder with the help of mechanical grinder. 800 g powder was socked in 2 l of 80% methanol for seven days at room temperature and filtered. The filtrate was collected, and evaporated under reduced pressure in rotary evaporator. The crude extract was store at 4 °C for further analysis.

2.2. Experimental process and treatment

Male ICR mice (18–22 g) were kept at 25 °C on light/dark cycle. The mice were under free access to food and water. After 2 weeks of acclimatization, mice were randomly divided into six groups (each containing 12 mice): control group, model group, positive group, low dosage, middle dosage and high dosage of CTME groups. Except control group, mice were subcutaneously injected with 3% D-galactose at the dose of 150 mg/kg body weight once daily for 7 weeks while those of control group were treated with same volume normal saline. From the sixth week, To CTME groups, CTME was administrated intragastricaly with following three doses, 100, 200, 300 mg/kg body weight once daily for 2 weeks and to positive group Huperzine A was administrated intragastricaly with the dose of 0.3 mg/kg body weight once daily for 2 weeks. Same volume of normal saline was administrated to control group and model group. The treatment was given 30 min prior to daily injection of D-galactose at the sixth week.

2.3. Behavioral tests

The behavioral tests were performed in a silent, isolated room at the temperature of 22 ± 2 °C. The experimenter and the devices for data acquisition and analysis were located in an adjacent room. A video-camera viewing the experimental area was positioned on the vertical form at the center of arena and connected to a personal computer. Mice movements were tracked and analyzed with dedicative software (Any-maze™, Stoelting Co., Chicago, U.S.A.).

2.4. Step-through active avoidance test

The step-through active avoidance test was performed by using a two-compartment shuttle box (each 136 × 153 × 250 mm) adjacent to each other through an arch door (3 cm in diameter). The floor of the compartments was composed of 2 mm stainless-steel rods spaced 1 cm apart. In an acquisition trial, mice was initially placed in one compartment facing away from the door and allowed to acclimatize for 5 min. After the acclimatization, a buzzer was delivered in the compartments for 5 s. If the mice was still present in this compartment, then it was shocked by an electric foot current (10–20 mA, 10–15 Hz) through the grid floor in this compartment until they came into the other compartment. The average inter-trial interval was 60 s. Each test contained 20 buzzers and electric foot shocks. Each mouse was trained for 20 trials per day for 5 consecutive days. At the last day the final test results were recorded: (i) active avoidance response latency (AARL); (ii) escape response latency (ERL); (iii) active avoidance response times (AART) and (iv) escape response times (ERT).

2.5. Step-through passive avoidance test

The step-through passive avoidance test was performed by using a two-compartment shuttle box that consisted of an illuminated and a dark compartment (each 136 × 153 × 250 mm) adjacent to each other through an arch door (3 cm in diameter). The floor of the dark compartment was composed of 2 mm stainless-steel rods spaced 1 cm apart. In an acquisition trial, mice were initially placed in the illuminated compartment facing away from the dark compartment and allowed to acclimatize for 5 min. After the acclimatization, an electric foot shock (36 V) was delivered through the grid floor in the compartments. If the mice came into the dark compartment, they would be shocked and returned to the illuminated compartment immediately. Any mice that did not go to the dark compartment within 5 min suggested that they were not sensitive to light and were ruled out of further experiments. After the 5 min training, the electric shock was stopped. The mice were subject to the same conditions for the retention trial 24 h later, as soon as they went into the dark compartment and were shocked, the test was terminated. The time taken for a mouse to enter the dark compartment the first time was defined as the latency for the trials. The latency was recorded for up to 300 s.

2.6. Hole-board test

The hole-board test was conducted in a black chamber (25 cm × 25 cm × 25 cm). The floor of the base had 25 holes of 3 cm equidistant from each other. The mouse was placed on the hole-board and was allowed to explore freely for 5 min. The total number of head dips and crossings were recorded.