Biomedicine & Aging Pathology 3 (2013) 75–81

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Original article

Protective effects of *Phoenix dactylifera* against oxidative stress and neuronal damage induced by global cerebral ischemia in rats

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ARTICLE INFO

Article history:
Received 22 March 2013
Accepted 24 April 2013

Keywords:
Phoenix dactylifera
Global cerebral ischemia
CA1 pyramidal neurons
Delayed neuronal death
Ischemia/reperfusion
Oxidative stress

ABSTRACT

Phoenix dactylifera (PD) has been claimed for its neuroprotective potential in the traditional system of medicine but has not yet been scientifically documented. Phytochemical reports of *Phoenix dactylifera* fruits have demonstrated the presence of polyphenols and flavonoids, which have already been documented to play major role in neuroprotection against various experimental models of cerebral ischemia/reperfusion. In the present study, we have investigated the neuroprotective as well as antioxidant properties of methanolic extract of *Phoenix dactylifera* fruits (MEPD) at 30, 100, 300 mg/kg p.o against global cerebral ischemia-induced oxidative stress and neuronal death. The global cerebral ischemia was induced by occlusion of bilateral common carotid arteries for 5 min followed by 24 h of reperfusion. Varied biochemical/enzymatic alterations, produced subsequent to the application bilateral common carotid artery occlusion (BCCAO) followed by reperfusion viz. increase in lipid peroxidation and decrease in glutathione, glutathione reductase, catalase, glutathione-*S*-transferase, glutathione peroxidase and superoxide dismutase, were markedly reversed and restored to near normal levels in the groups pretreated with 15 days. The pretreatment also reversed the histopathological changes induced by global cerebral ischemia in CA1 hippocampal region. The protective action, exhibited by MEPD against global cerebral induced brain injury, suggests its therapeutic potential in cerebrovascular diseases (CVD) including stroke. These findings are important because the present treatment strategies for CVD are far from adequate and PD with wide usage is known to be a safe natural product.

1. Introduction

Models of transient global cerebral ischemia have been extensively used in investigating the cellular mechanisms of ischemic cell death, as well as in studies on the pharmacology of cerebral ischemia [1]. Brief periods of global brain ischemia in rodents cause delayed cell death in hippocampal CA1 pyramidal neurons after reperfusion as among hippocampal neurons, CA1 region is most vulnerable to cerebral ischemia [2]. Death of CA1 hippocampal neurons following cerebral ischemia causes a variety of neurological dysfunction. Although the exact mechanism of neurological damage caused by cerebral ischemia has yet to be elucidated, recent works suggested the involvement of excitotoxicity, activation of voltage-gated calcium channels, inflammatory cytokines, and oxidative stress. Based on these potential neurotoxic mechanisms, various neuroprotective agents are being developed [3]. These include calcium antagonists, NMDA antagonists, glutamate release inhibitors, free radical scavengers, and leukocyte adhesion inhibitors. Considering the adverse effects of synthetic drugs, most of the world's population is looking for natural remedies, which are safe and effective. It is documented that 80% of the world's population has faith in traditional medicine, particularly plant drugs for their primary healthcare [4].

Phoenix dactylifera (PD), belonging to the family Arecaecea, is one of the widely used herbal medicines in most of the Arabian countries. Different parts of this plant are claimed to be used for the treatment of memory disturbances, fever, loss of consciousness and nervous disorders [5,6]. Several studies indicate that the consumption of fruits and vegetables is associated with the reduced risk of several chronic diseases.

The fruits of *P. dactylifera* have been documented to possess significant antioxidant potential. They also have been proved to prevent cancer, cardiovascular disorders and other degenerative processes involving oxidative stress [7]. Although *P. dactylifera* has been studied for its various pharmacological activities but it has not

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http://dx.doi.org/10.1016/j.biomag.2013.04.003

yet been studied for its neuroprotective potential against cerebral ischemia, insight of which the present investigation was carried out.

2. Materials and methods

2.1. Plant material

Fresh fruits of *P. dactylifera* were collected from local market and authenticated by Botanical survey of India (Voucher specimen number: BSI/WC/Tech/2009/674). The methanolic extract of dried fruit was prepared in the approved laboratories of Green Chem, Bangalore, India using the procedure mentioned below.

2.2. Preparation of extract

The *P. dactylifera* fruits were manually separated from the pits and dried at room temperature and ground into powder using a stainless-steel blender. This powder was then extracted with methanol–water (4:1, v/v), at room temperature (20 °C for 5 h using an orbital shaker). The extracts were then filtered and centrifuged at 4000 *g*, for 10 min and the supernatant was concentrated under reduced pressure at 40 °C for 3 h using a rotary evaporator to obtain the methanolic extract.

2.3. Chemicals and drugs

Thiobarbituric acid and trichloroacetic acid were purchased from SD fine chemicals, Mumbai, India, nicotinamide adenine dineuleotide phosphate (NADPH), oxidised glutathione and reduced glutathione were purchased from Vijay chemicals, Pune, India, bovine serum albumin was purchased from Spacelab, Nashik India, phenazine methosulphate, nitroblue tetrazolium, 1-chloro 2,4 dinitrobenzoic acid were purchased from Anand agencies, Pune, India, NADH, Folin–Ciocalteau reagent were purchased from Bansal sales, Pune, India. The other chemicals and solvents used were of analytical grade purchased from commercial suppliers.

2.4. Animals

Swiss Albino mice weighing 25–30 g were used. They were caged in a room under standard laboratory conditions (temperature 23 ± 10 °C, relative humidity 55 ± 5% and lighting 08:00–20:00).The rats were fed on a pelleted diet (Amrut feed, Pune, India) and water ad libitum. The rats were transferred to the laboratory at least 1 h before the start of the experiment. The experiments were performed during day (08:00–16:00).

2.5. Ethical clearance

All the studies were carried out in accordance with the guidelines given by the Indian Council for Medical Research and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India) and the Institutional Animal Ethical Committee approved the study (Approval No.: CPCSEA/IAEC/PC-04/07-2K8).

2.6. Preliminary phytochemical analysis

The extract was subjected to preliminary screening, for various active phytochemical constituents, such as alkaloids, carbohydrates, steroids, protein, tannins, phenols, flavonoids, gum and mucilage, glycosides, saponins and terpenes by standard procedures [8].

2.7. Preliminary acute toxicity test

Healthy adult male Albino mice (18–22 g) were subjected to acute toxicity studies as per the guidelines (AOT 425) suggested by the organization for economic co-operation and development (OECD-2000). The mice were observed continuously for 2 h for behavioural and autonomic profiles and for any sign of toxicity or mortality up to a period of 7 days [9].

2.8. Experimental protocols

The animals were divided into five groups of six each. First group served as sham-operated control group and received vehicle i.e. distilled water. Second group also received distilled water and served as global cerebral ischemia control group. Third, fourth and fifth groups served as test groups i.e. drug-treated global cerebral ischemia groups and received MEPD at doses of 30, 100 and 300 mg/kg, respectively. Animals were treated for 15 days. On the last day 60 min after the last dose, all the rats except sham-operated underwent 5 min of global cerebral ischemia followed by 4 days reperfusion. Then 3 h after ischemia reperfusion, rats were assessed for neurological function deficits according the neurological score of Mcgraw (1997) in the following manner:

- 0 = no deficits (no symptoms)
- 1 = hunched posture or hair roughed up
- 2 = ptosis
- 3 = circling behaviour
- 4 = splayed-out hind limb
- 5 = seizures.

On the fourth day all animals were sacrificed and their brains were removed and subjected to histopathological evaluation and biochemical analysis [10].

2.9. Surgical procedure for global cerebral ischemia

Surgical procedures were performed between 8:00 and 13:00. All the surgical equipments and surgical pad were disinfected with 70% ethanol before the surgery to avoid any kind of infection and sepsis. Overnight-fasted animals were anesthetised with an intraperitoneal injection of 100 mg/kg ketamine and supplemented as needed. A median incision was performed in the skin of the ventral part of the neck and the subcutaneous adipose tissue was dissected avoiding the thyroid. The omohyoid muscle was cut through a median incision and a dissection was made between the sternocleidomastoid and the sternohyoid muscles parallel to the trachea. Each carotid artery was freed from its adventitial sheath and vagus nerve, which was carefully separated and maintained. The induction of global cerebral ischemia was performed by occluding bilateral common carotid arteries with microaneurysmal clips (Bulldog Clamp, 25 mm straight) for 5 min. At the end of the ischemic period, the clamps were removed, blood flow was restored and the patency of carotid arteries was checked by direct visualization. The skin was then sutured with 3-0 silk suture. Sham control animals received the same surgical procedures, except bilateral common carotid arteries were not occluded. Core temperature (rectal) was maintained at 37 ± 0.5 °C throughout the surgical process using a heating lamp. After the surgery, the antiseptic Povidone iodine was applied to the neck incision area of all the animals to avoid infection and they were returned to their home cages with free access to feed and water [10].