Journal of Ethnopharmacology 133 (2011) 773–779

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Methanolic extract of *Hibiscus asper* leaves improves spatial memory deficits in the 6-hydroxydopamine-lesion rodent model of Parkinson's disease

Harquin Simplice Foyet[a], Lucian Hritcu[b,*], Alin Ciobica[b], Marius Stefan[b], Pierre Kamtchouing[c], Dumitru Cojocaru[b]

[a] Department of Agriculture, Cattle farming and Derived products, High Institute of the Sahel, University of Maroua, Cameroon
[b] Department of Biology, Alexandru Ioan Cuza University, Iasi, Romania
[c] Laboratory of Animal Physiology, University of Yaoundé I, Cameroon

ARTICLE INFO

Article history:
Received 15 September 2010
Received in revised form 27 October 2010
Accepted 2 November 2010
Available online 9 November 2010

Keywords:
Hibiscus asper extract
Antioxidant activity
6-Hydroxydopamine-lesion rat
Memory
Parkinson disease

ABSTRACT

Ethnopharmacological relevance: While the *Hibiscus asper* Hook.f. (Malvaceae) is a traditional herb largely used in tropical region of the Africa as vegetable, potent sedative, tonic and restorative, anti-inflammatory and antidepressive drug, there is very little scientific data concerning the efficacy of this.
Aim of the study: We investigated antioxidant activity and the effects of methanolic extract of *Hibiscus asper* leaves on neurological capacity of male Wistar rats subjected to unilateral 6-hydroxydopamine (6-OHDA)-lesion.
Materials and methods: Two model systems: 2,4-dinitrophenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and β-carotene bleaching inhibition assay were used to measure the antioxidant activities of the plan extract. We also investigated the neuroprotective effect of methanolic extract of *Hibiscus asper* leaves (50 and 100 mg/kg) in male Wistar rats subjected to unilateral 6-hydroxydopamine (6-OHDA)-lesion rat model.
Results: Methanolic extract of *Hibiscus asper* leaves showed potent antioxidant and free radical scavenging activity. Chronic administration of methanolic extract (50 and 100 mg/kg, i.p., daily, for 7 days) significantly reduce anxiety-like behavior and inhibit depression in elevated plus-maze and forced swimming tests, suggesting anxiolytic and antidepressant activity. Also, spatial memory performance in Y-maze and radial arm-maze tasks was improved, suggesting positive effects on memory formation.
Conclusions: Taken together, our results suggest that the methanolic extract of *Hibiscus asper* leaves have antioxidant effects and might provide an opportunity to management neurological abnormalities in Parkinson's disease conditions.

1. Introduction

Parkinson's disease (PD) is a common, slowly progressive, neurodegenerative disease resulting from the degeneration of neurons in the substantia nigra (SN), a region of the brain that controls movement. This degeneration results in a shortage of a neurotransmitter called dopamine (DA), therefore, causing impaired movement. Depression, memory and sleeping disturbance, speech impairments and dysphagia are the other symptoms of PD (Harish et al., 2010). PD is often complicated by a variety of cognitive symptoms that range from isolated memory and thinking problems to severe dementia. While the motor symptoms of PD are well-known (tremor, rigidity, slowness of movement, imbalance), the commonly seen deficits in memory, attention, problem-solving, and language are less understood. Studies have shown that over 50% of people with PD experience some form of cognitive impairment. About 20% have more substantial cognitive impairment. Memory problems in PD are typically milder than in Alzheimer's disease. In PD, the person may have difficulty concentrating, learning new information and recalling names (Hritcu et al., 2008a). Although the etiology of PD remains unknown, recent studies have suggested that also oxidative stress (OS) plays an important role in its pathogenesis (Valko et al., 2007; Hritcu et al., 2008a; Shim et al., 2009; Samoylenko et al., 2010). OS contributes to the damage to lipids, proteins, and DNA, and the cascade of events leads to the DA cell degeneration in PD. 6-Hydroxydopamine (6-OHDA) is a selective catecholaminergic neurotoxin that has

Abbreviations: DPPH, 2,4-dinitrophenyl-1-picryl hydrazyl; 6-OHDA, 6-hydroxydopamine; MAO-B, monoamine oxidase-B; DA, dopamine; RAM, radial arm-maze; PD, Parkinson's disease; SN, substantia nigra.

* Corresponding author at: Department of Biology, Alexandru Ioan Cuza University, B-dul Carol I, No. 20A, Iasi 700506, Romania. Tel.: +40 232201666; fax: +40 232201472.
E-mail address: hritcu@uaic.ro (L. Hritcu).

0378-8741/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2010.11.011

been widely used to produce PD models *in vitro* and *in vivo* because it induces apoptotic activity through OS (Hritcu et al., 2008b; Shim et al., 2009). There have been a number of indices of damage that have been studied in animals following unilateral destruction of dopaminergic motor tracts with toxins such as 6-OHDA (Hudzik et al., 2000; Yuan et al., 2007; Hritcu et al., 2008a).

There have been significant advances in PD therapy including surgical and pharmacological interventions. Although L-DOPA is considered as the best standard drug for treatment of PD, multiple complications such as motor fluctuations, hallucinations, and psychosis arise from long-term therapy (Simpkins and Jankovic, 2003). These allowed the usage of alternative molecules as dopamine receptor agonists, anti-cholinergic drugs, monoamine oxidase inhibitors, catechol-O-methyl transferase inhibitors, but also, the exploration of novel therapeutic molecules that prevent neurodegeneration. It has been clearly established that oxidative stress is among the major causative factors in the induction of many chronic and neurodegenerative diseases (Smith and Cass, 2007). Thus the use of scavenging peroxide molecules may be useful to attenuate oxidative neuronal damage associated with various neurodegenerative diseases (Baluchnejadmojarad et al., 2010). Previous studies suggested that many plant extracts have neuroprotective activity against 6-OHDA-induced toxicity through antioxidative and anti-apoptotic activities in PD models (RajaSankar et al., 2009; Shim et al., 2009).

Flavonoids are the largest group of polyphenols present in many plants, known to promote a number of physiological benefits, especially in cognitive function and memory impairment (Mandel et al., 2005) and also antioxidant activity.

Among plant species, *Hibiscus asper* Hook.f. (Malvaceae) is an important medicinal plant widely distributed throughout tropical Africa and in Madagascar. This species belongs to the genus *Hibiscus* represented by 250 species and characterizes by the presence of biological active compounds like flavonoids, phenolic acids, and polysaccharides (Vasudeva and Sharma, 2008). In the western region of the Africa, this plant is widely used by the traditional practitioners of the treatment of inflammation, anemia, jaundice, leucorrhoea, poison antidote, depression and dysmenorrhea (Schippers and Bosch, 2004). In the Western Region of Cameroon the leaves are used as a potent sedative, tonic and restorative. It is also use to treat male infertility and skin infection (Burkill, 1985). Furthermore, a preliminary phytochemical screening in our laboratory revealed the presence of polyphenols in the leaves extract of *Hibiscus asper*. Also, a recent study indicated that methanol extract of *Hibiscus rosa sinensis* had antioxidant effects, ameliorated anxiety and improved learning and memory processes in rats (Nade et al., 2010).

In the present study, we investigated antioxidant activity and behavioral recovery following chronic administration of methanolic extract of *Hibiscus asper* leaves using a unilateral 6-OHDA-lesion rat model of PD.

2. Materials and methods

2.1. Plant material and plant extract

Hibiscus asper leaves were collected in Fotouni (Western region, Cameroon) in May 2010 and identified by Dr. Focho Derreck, Department of Plant Biology (University of Dschang, Cameroon). Basically, the preparation of plant extract was the same as previously described (Foyet et al., 2008) but with modification. The leaves of *Hibiscus asper* were dried under shade and pulverized. 100 g of the pulverized leaves was macerated in 1 L of 90% methanol for 5 days at room temperature (25 °C). It was later filtered, and the solvents were separated from the residues by gravity filtration and then evaporated in vacuum. The yield of 12.5 g of crude organic extract was 12.5%.

2.2. Animals

30 Male Wistar rats weighing 230 ± 50 g at the start of the experiment were used. The animals were housed in a temperature and light-controlled room (22 °C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water *ad libitum*. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC). All behavioral evaluations were performed between 10:00 and 14:00 h.

2.3. Drugs

6-Hydroxydopamine (6-OHDA), ascorbic acid and sodium pentobarbital (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in an isotonic solution (0.9% NaCl). 2,4-Dinitrophenyl-1-picryl hydrazyl (DPPH), β-carotene, linoleic acid, chloroform, and Tween 20 were purchased from Prolabo (Paris, France).

2.4. In vitro experiment

Two model systems: 2,4-dinitrophenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and β-carotene bleaching inhibition assay were used to measure the antioxidant activities. In each assay, extracts were tested at the doses of 0.1, 1, 10 and 100 μg/mL. Ascorbic acid was used as standard antioxidant compound.

2.4.1. Antioxidant assay: DPPH assay method

The free radical scavenging activity of the methanolic extract of *Hibiscus asper* was evaluated as described by Mensor et al. (2001). Briefly, the test samples, prior dissolved in DMSO (Sigma), were mixed with a 0.3 mM 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) ethanol solution, to give final concentrations of 0.1, 1, 10 and 100 μg of extract per microliters of DPPH solution. After 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity. Ascorbic acid was used as a standard control. Each assay was repeated trice and the results, recorded as mean of the triplicated experiments.

2.4.2. β-Carotene bleaching inhibition assay

Antioxidant activity was determined using β-carotene bleaching test (Amin et al., 2004). Briefly, 1 mL of β-carotene solution (0.2 mg/mL in chloroform) was added to 0.02 mL of linoleic acid and 0.2 mL of 100% Tween 20. The mixture was then evaporated at 40 °C for 10 min by means of a rotary evaporator to remove chloroform and immediately diluted with 100 mL of distilled water. The water was added slowly to the mixture and agitated vigorously to form an emulsion. Five milliliters of the emulsion was transferred into different test tubes containing 0.2 mL of samples in 70% ethanol at different concentrations (0.1, 1, 10 and 100 μg/mL). 0.2 mL of 70% ethanol in 5 mL of the above emulsion was used as control. Standard (ascorbic acid) at the same concentration as samples was used for comparison. The tubes were then gently shaken and placed at 45 °C in a water bath for 60 min. The absorbance of the samples, standard and control was measured at 470 nm using a spectrophotometer against a blank, consisting of an emulsion without β-carotene. The measurement was carried out at initial time ($t = 0$) and after 120 min. All samples were assayed in triplicate and averaged. Antioxidant activity was calculated as percentage of inhi-