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Original article

Antidepressant effects of standardized extract of *Centella asiatica* L in olfactory bulbectomy model

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ABSTRACT

Centella asiatica Linn leaves extract are reported to have promising antidepressant activity in acute animal models of depression. The aim of the present study is to evaluate the effects of standardized extract of *Centella asiatica* L leaves (INDCA) in rats using chronic stress medicated depression model in rats i.e. olfactory bulbectomy model. INDCA was extracted from *Centella asiatica* L. leaves and standardized to its bioactive constituent, asiaticoside, by HPLC. Effects of 14-day (subacute) oral pretreatment of INDCA (3, 10, or 30 mg/kg), imipramine (20 mg/kg), fluoxetine (30 mg/kg) and desipramine (15 mg/kg) were evaluated after behavioral depression induced by olfactory bulbectomy (OBX) in rats. Separate sham and vehicle control groups were also maintained. Food intake and body weight were measured 1 h prior to treatment (day 1, baseline), and on 7th day and 14th day of treatment. Behavioral and physiological parameters in open field and elevated plus maze were recorded on 14th day of treatment. Dose-dependent reversal of reduction of body weight, body temperature, and heart rate with the normalization of elevated food intake that was observed in OBX rats treated with INDCA. Hyperactivity shown by OBX rats in open field and elevated plus maze paradigm was significantly reversed by INDCA. In conclusion, INDCA exerts antidepressant effects in OBX model in rats.

1. Introduction

Depression is a common, debilitating, life-threatening illness with an increasing morbidity and mortality [1]. Depression is a heterogeneous illness that results from several neurotransmitters dysfunction on metabolic system [2]. Antidepressant drugs like tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase (MAO) inhibitors are available in clinic. However, the present antidepressants drugs have many disadvantages such as tardy onset of action, rather low response and many side effects. Therefore, the research and development of new type antidepressants is an urgent and unmet need [3,4].

Centella asiatica (Linn) Urban is a small herbaceous annual plant (family Mackinlayaceae). It is native to many Asian countries and used as a medicinal herb in Ayurvedic medicine, traditional African medicine, and traditional Chinese medicine. Botanical synonyms include *Hydrocotyle asiatica* L and *Trisanthus cochinchinensis* (Lour).

Asiaticoside (AS) is a major pentacyclic triterpenoid saponin of *Centella asiatica* L and shown to be promising antidepressant [5] and anxiolytic [6] agent in acute animal models. Recently, extract of *Centella asiatica* was reported as a promising anxiolytic agent in clinical study [7]. Total triterpenes of *Centella asiatica* L leaves showed promising antidepressant effects [8] in forced swim test (FST), an acute model of depression in laboratory animals.

However, management of clinical depression takes several days or weeks for evident therapeutic effect in patients [9–11]. Therefore, antidepressant agents need to be investigated in chronic model of depression with a good face validity with human depressive disorder. Olfactory bulbectomy (OBX) in rats is well-validated animal model and produces variety of physiological, endocrine, immune and behavioral changes in animals that resembles hyperemotional behavior in depressed patients in clinic [12]. Therefore, the present study is aimed at preparation of standardized *Centella asiatic*a extract (INDCA) and its evaluation in OBX induced depression in rats.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (250–270 g) were purchased from National Toxicology Centre (NTC), Pune. The animals were housed at a temperature of 25 ± 1 °C and relative humidity of 45–55%

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under 12:12 light:dark cycle. The animals had free access to feed pellets (Chakan Oil Mills Ltd., Sangli, Maharashtra, India) and tap water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune (Approval number - CPCSEA/49/10). All observations were recorded between 8:00 and 15:00 h and each animal was used only once. To avoid subjective bias, the observer was not aware about the given treatment (blind). Each experimental group consisted of six animals unless otherwise stated. Rats were transported from the animal house to the testing area in their housing cages and were allowed to adapt to the new environment before testing.

2.2. Drugs and chemicals

Imipramine hydrochloride (imipramine) and fluoxetine hydrochloride (fluoxetine) were obtained as gift samples from Torrent Pharmaceuticals Ltd and Cadila Pharmaceuticals, Gujarat, India respectively. Desipramine hydrochloride was purchased from Sigma-Aldrich, USA. Reference standard of asciaticoside was purchased from ChromaDex (Irvine, CA, USA, Purity 86.3%). The other chemicals were purchased from local vendors.

2.3. Preparation of INDCA

The extraction and standardization of test compound, INDCA, was performed in the facilities of Indus Biotech Private Limited, Pune as per following procedure. The plant, *Centella asiatica* L, was collected freshly in month of May 2009 from Cochin region in India. It was authenticated at Dr A.S. Upadhye, Scientist, Agharkar Research Institute, Pune and voucher specimen was deposited at that Institute (Voucher specimen number is WP-80). Clean and dry leaves of the *Centella asiatica* were pulverized to a size and passed through 20-mesh size. One kg of dried material was extracted with 5 L of isopropyl alcohol in a fixed bed counter current extractor repeatedly for 8 h at 30 °C. The extract was filtered to clear all suspended matters. The filtrate was concentrated to a semisolid mass in a rotary evaporator under vacuum. The concentrated mass, was further diluted with 3 L of deionised water to obtain a homogenous liquid. The liquid was washed twice with 2 L of hexane. The bottom aqueous layer was separated and extracted twice with 1 L of methylisobutylketone. Then, aqueous layer was passed through an adsorbent resin Amberlite XAD 180 (400 ml) bed maintaining a flow rate of 25 ml per minute and the outflow was monitored for the absence of terpenoid glycosides. The column was washed with 5 L of demineralised water until the washings were colourless. The adsorbent column was eluted with ethyl alcohol until the thin layer chromatography (TLC) test showed absence of terpenoid glycosides. The resultant elute was passed through a column comprising of 100 g of activated charcoal and 250 g of silica gel (60 mesh). The resultant elute was collected, the column washed thoroughly with ethyl alcohol, and all the washings combined with elute and, concentrated by vacuum distillation to obtain powder. This powder was dissolved in 300 ml demineralised water to prepare clear solution. The solid content was spray-dried in a co-current indirect hot air (inlet temperature: 140 °C; outlet temperature: 80 °C) spray-dryer. The obtained dried product was labeled as INDCA (Yield: 30 g; pale yellow, water-soluble powder).

2.4. Standardization of INDCA by HPLC

The INDCA was standardized for the content of marker compound, asciaticoside by HPLC. Sample solution of INDCA and reference standard of asciaticoside was prepared by dissolved 10 mg of INDCA powder in 100 ml demineralized water. Separate chromatograms were obtained using following HPLC conditions: Column: Chromadex Reverse Phase, C-18 (250 × 4.6 mm), Particle size: 5 μ., detector: UV, wavelength: 20 nm, Flowrate: 1.4 ml/min, Mobile phase: Acetonitrile: water (Initial 75:25, 30 min: 45:55, and 40 min: 75:25).

2.5. Preparation of drug solutions

The test drug (INDCA) and standard drugs (imipramine, desipramine and fluoxetine) were dissolved in distilled water immediately before use and administered once daily orally 1 h before the experiments.

2.6. Bilateral olfactory bulbectomy surgery

The procedure reported by days Redmond et al. [13] was followed. The male rat was anaesthetized with ketamine (80 mg/kg i.p.). The animal was placed in streotaxic frame (Inco, India). Head was shaven and midline scalp sagittal incision (1 cm) was made and bilateral burr holes (2 mm diameter) were drilled 8 mm anterior from bregma and 2 mm lateral from midline. Both main and accessory olfactory bulbs were aspirated through both burr holes using a blunt hypodermic needle attached to water pump without damaging frontal cortex. The burr holes were then plugged with a haemostatic sponge to control bleeding. Povidone iodine solution was applied to the wounds and the rat was allowed to recover for 14 days.

2.7. Treatment schedule in olfactory bulbectomized (OBX) rats

Group I rats were treated as sham control (had surgery but no OBX) and were administered saline. After recovery period of 14 days, OBX rats were divided into following groups of six rats each and received oral drug treatments as follows. Group II was OBX control and received distilled water. The OBX rats in Group III, IV, V were were administered with imipramine (20 mg/kg), fluoxetine (30 mg/kg), and desipramine (15 mg/kg) respectively. Group VI, VII and VIII OBX rats were treated with INDCA (3, 10, 30 mg/kg). All the treatments were administrated orally once daily for 14 days.

2.8. Effect on body weight and food intake

The rats in all the groups were weighed and were placed individually in polypropylene cages. During the study period, the rat's body weight, and food intake were measured 1 h before drugs administration, on day 1 (baseline), 7 and 14 after OBX as per reported procedure [14].

2.9. Effects on behavioural and physiological parameters during open field activity

The animals were placed in the centre of open field apparatus. The animals were observed for the period of 3 min. Number of ambulation, rearing and grooming during this 3 min observation period was counted [15]. The body temperature (rectal) was measured immediately after five-minute intervals of the open field activity with the help of telethermometer (Electrolab, India) and The heart rate was measured at 30 min after open field activity using Powerlab® data acquisition system (ADInstruments, Australia).

2.10. Effect on behavioural parameters in elevated plus maze

The rats which have undergone olfactory bulbectomy were tested in the elevated plus maze apparatus for anxiety measurement [16]. Thirty-minute after administration of respective treatment, each rat was placed individually in the centre of maze,