Journal of Ethnopharmacology 122 (2009) 561–566

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Proapoptotic and prepulse inhibition (PPI) disrupting effects of *Hypericum perforatum* in rats

Mariane G. Tadros[a,*], Mohamed R. Mohamed[b], Amal M. Youssef[c], Gilane M. Sabry[b], Nagwa A. Sabry[d], Amani E. Khalifa[a]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt
[b] Department of Biochemistry, Faculty of Science, Ain Shams University, Cairo, Egypt
[c] Department of Physiology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
[d] Department of Clinical Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 30 April 2008
Received in revised form 1 December 2008
Accepted 2 January 2009
Available online 9 January 2009

Keywords:
St. John's wort
Prepulse inhibition (PPI)
Antiamnestic
Hyperforin
Apoptosis

ABSTRACT

Ethnopharmacological relevance: St. John's wort extract is commonly used as a wound healing, anti-inflammatory, anxiolytic, diuretic, antibiotic, antiviral and cancer chemoprotective agent. It also has nootropic and/or antiamnestic effects.
Aim of the study: Prepulse inhibition (PPI) of startle response is a valuable paradigm for sensorimotor gating processes. A previous study indicated that single administration of St. John's wort extract (500 mg/kg) caused PPI disruption in rats. The effect of antiamnestic doses of the extract on PPI has not been investigated despite the coexistence of impaired memory and PPI deficit in some neurological disorders.
Materials and methods: The effects of acute (500 mg/kg) and chronic (200 mg/kg for 3 days) administration of St. John's wort extract were investigated for its antiamnestic activity. The effects of administration of the antiamnestic dose of the extract and hyperforin, its main active component, were tested on PPI of an acoustic startle response in rats. This study also investigated the proapoptotic effect of hyperforin in animals, demonstrating PPI deficit, by electrophoresis of DNA isolated from selected brain areas.
Results: Disruption of PPI resulted after treatment of rats with an antiamnestic dose of the extract (200 mg/kg for 3 days) and with hyperforin. Gel electrophoresis showed DNA fragmentation of the cortices of hyperforin-treated animals exhibiting PPI deficit.
Conclusions: The exacerbating effect of St. John's wort extract on PPI deficit may provide a limitation for using the extract to manage cognitive disturbance in psychotic and Huntington's disease patients manifesting PPI deficit.

1. Introduction

Hypericum perforatum Linn, commonly named St. John's wort, is a herbaceous perennial plant of Hypericeae family. The extract of this plant is used as a wound healing and an anti-inflammatory agent (Di Carlo et al., 2001) and as anxiolytic, diuretic, antibiotic, antiviral (Miller, 1998) and cancer chemoprotective agent (Schwarz et al., 2003). It is also used for the treatment of mild to moderately severe depressions (Whiskey et al., 2001). Recently, St. John's wort extract demonstrated an inhibitory effect on locomotor hyperactivity, stereotyped behaviors, tremors and audiogenic seizures during ethanol withdrawal in ethanol-dependent rats (Coskun et al., 2006). Recent reports imply that St. John's wort may be effective in the treatment of tobacco and alcohol abuse (Uzbay, 2008). Another study reported an inhibitory effect of St. John's wort on caffeine-induced locomotor hyperactivity in mice (Uzbay et al., 2007).

Previous work in our laboratory demonstrated that acute administration of St. John's wort extract (4, 8, 12 and 25 mg/kg, p.o.) to mice enhanced retrieval memory of one-trial passive avoidance conditioning at dose levels equivalent to the ones used clinically for depressed patients (Khalifa, 2001). Such properties of the extract were supported by Klusa et al. (2001) who showed that administration of the German extract to rats (50 mg/kg day^{-1}), p.o.) for 2 days had cognitive enhancing properties. In addition, Indian *Hypericum perforatum* extract administered at a dose of 100 and 200 mg/kg once daily for 3 consecutive days demonstrated nootropic effects in a number of paradigms testing memory function and antagonized scopolamine-induced deficit of passive avoidance retention (Kumar et al., 2000). Therefore, St. John's wort extract is

* Corresponding author. Tel.: +20 10 6038263.
E-mail address: mirogeogo@yahoo.com (M.G. Tadros).

0378-8741/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2009.01.009

potentially beneficial in neurological disorders associated with impaired memory.

Prepulse inhibition (PPI) is the suppression of the startle reflex when a startling stimulus is preceeded by a weak stimulus at lead times of 20–500 ms (Kodsi and Swerdlow, 1995). In some neurological disorders, sensorimotor gating deficits, manifested as disruption of PPI of acoustic startle reflex, co-exist with impaired memory. Examples of such neurological disorders are Huntington's disease and psychosis. Previous work in our laboratory showed that significant disruption of PPI of acoustic startle reflex resulted after treatment of rats with 500 mg/kg of St. John's wort extract (Khalifa, 2005). Given the reported antiamnestic properties of the extract, its potential use to ameliorate cognitive deficit in neurological disorders of sensorimotor gating associated with impaired memory may be risked by the exacerbating effect of the extract on PPI deficit. Nevertheless, such proposition has not been studied yet. Therefore, the aim of this study was to investigate the effect of an antiamnestic dose of the extract on scopolamine-induced PPI deficit.

The biological activity of St. John's wort extract was first attributed to the naphthodianthrones hypericin, pseudohypericin, protohypericin and protopseudohypericin (Meruelo et al., 1988). Recent studies revealed that the phloroglucinol hyperforin and its derivative adhyperforin, also contribute to the biological activity of St. John's wort extract. Hyperforin has already been reported to be the active constituent responsible for the antidepressant activity of the extract by inhibition of the reuptake of serotonin, noradrenaline and dopamine (Medina et al., 2006). Therefore, hyperforin may be responsible for the disruptive effect of the antiamnestic dose of St. John's wort extract on PPI, knowing that elevated monoamines levels have a role in PPI disruption (Alsene et al., 2006). Hence, another objective for this study was to investigate the effect of hyperforin on PPI of acoustic startle reflex.

Hyperforin has been shown, in a number of studies, to induce apoptosis of tumor cells (Schempp et al., 2002; Hostanska et al., 2003; Dona et al., 2004). In diseases, such as Huntington's disease, that manifest disrupted PPI response, apoptosis also occurs and has been shown to contribute as a main mechanism in the pathophysiology of the disease (Norenberg and Rao, 2007). Therefore, this study investigated whether the dose of hyperforin causing PPI deficit has proapoptotic properties. This was determined by assessing DNA fragmentation of selected brain areas in animals treated with PPI deficit-inducing dose of hyperforin.

2. Materials and methods

2.1. Animals

In this experimental study, male albino Wistar rats (200–250 g) and male Swiss albino mice (20–30 g) were used. They were kept in a temperature of 25 °C with alternating 12 h light and dark cycles and allowed free access to food and water. On the day of the experiment, animals were brought to the experimental room and allowed to habituate to the environmental conditions for approximately 60 min before the beginning of the experiment. Handling and experimentation were conducted in accordance with the international ethical guidelines concerning the care and use of laboratory animals and the experimental protocol was approved by Ain Shams University Faculty of Pharmacy Review Committee for the use of Animal Subjects.

2.2. Drugs

Chinese *Hypericum perforatum* was grown in Hebei province and was harvested in August. The aboveground parts (leaves, flowers, and stem) were dried before extraction with 80% ethanol (v/v). The herb-to-extract ratio is 12:1 for a 100% native extract. The dried extract was obtained from China National Corporation of Traditional & Herbal Medicine, Beijing, China. The extract solutions were prepared according to Good Manufacturing Practice rules and the quality and identity of the constituents were checked by thin-layer chromatography. The naphthodianthrone content of the herb is reported to be 0.1–0.15% (w/w) [0.3% hypericin and 0.7% pseudohypericin]. The hyperforin content is 3% (w/w), whereas the flavonoid content is more than 20% (w/w).

Hyperforin (250 μg/ml in methanol) and scopolamine were purchased from Fluka, Chemie Gmbh, Germany. The rest of the chemicals and reagents used were of the highest commercial grade available.

Hypericum perforatum extract was prepared by dissolving with 0.3 ml dimethyl-sulfoxide (DMSO) and completed to the final volume with saline (NaCl 0.9%) to give DMSO concentration of 3% (v/v) and was administered orally in a volume of 10 ml/kg. Scopolamine was dissolved in saline and subcutaneously injected in a volume of 2 ml/kg. Control animals received respective solvent injections, and they were run concurrently with drug-treated groups.

2.3. Passive avoidance

2.3.1. Apparatus

A step-through passive avoidance apparatus was used (Ugo Basile, Italy). It consisted of a Plexiglas box divided into two compartments. One compartment is white and illuminated by a light fixture, featuring a 24-V, 10-W bulb, fastened to the compartment lid. The second compartment is dark and made of black Perspex panels. The two compartments are separated by an automatically operated sliding door. The apparatus included a steel-rod grid floor, which consisted of 40 parallel bars (0.3 cm in diameter, set 1.2 cm apart). The bars of the dark compartment floor are wired to a constant current high-precision eight-pole scrambling circuit located in the controller.

2.3.2. Experimental procedures

Training session: Each rat was trained by gently placing it in the light compartment and when the animal stepped through the dark compartment putting all its paws on the grid floor, the door automatically closed and electric shock (1.5 mA) was delivered for 3 s (Babar et al., 1994; Kozlovskaya et al., 2001). The animal was then returned to its home cage.

Test session: Twenty-four hours after training, each rat was introduced to the light compartment and the latency to step-through to the dark compartment was recorded as a passive avoidance behavior indicating memory level. Electrical shock was not delivered during this test session. An upper cutoff time of 300 s was set and all tests were run between 10:00 and 15:00 h (El-Sherbiny et al., 2003).

A group of rats ($n = 8$) received St. John's wort extract (500 mg/kg, p.o.). One hour after extract administration and 30 min before training sessions, rats were subcutaneously injected with scopolamine (7 mg/kg). Twenty-four hours after training, animals were tested for task acquisition. Two more groups ($n = 8$) served as amnestic and normal controls receiving scopolamine or saline with respective solvents, and they were run concurrently with drug-treated groups.

If animals did not acquire the task, another dose of St. John's wort extract was tested, instead, where the test group of rats ($n = 8$) received 200 mg/kg of the extract orally for 3 days. One hour after the last dose and 30 min before training sessions, rats were subcutaneously injected with scopolamine (7 mg/kg). Twenty-four hours after training, animals were tested for task acquisition. Two more groups ($n = 8$) served as amnestic and normal controls receiving scopolamine or saline with respective solvents, and they were run concurrently with drug-treated groups.