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Protective effect of hesperetin in rat model of partial sciatic nerve ligation induced painful neuropathic pain: an evidence of anti-inflammatory and anti-oxidative activity

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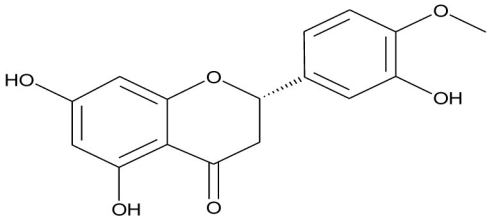
ABSTRACT

Behavioral, biochemical and gene expression changes were investigated in a rat model of partial sciatic nerve ligation (PSNL) after administration of hesperetin (20, 50 mg/kg; p.o.), pregabalin (10 mg/kg; p.o.) or vehicle (1 ml/kg, p.o.). Thirty-six animals were randomly divided into six groups. Left sciatic nerve was exposed and ligated, animals in the control and test groups were treated orally with respective drugs for fifteen days. Nociceptive threshold was assessed on 0 day and thereafter every three days. Three weeks later, sciatic nerve tissue homogenate was prepared and subjected for estimation of oxidative markers namely total protein, nitric oxide, lipid peroxidase, interleukins (IL-1β and IL-6) and TNF-α. Administration of hesperetin resulted in a dose dependent attenuation in PSNL-induced mechanical and thermal hyperalgesia, mechanical allodynia as well as down regulation of IL-1β, IL-6 and TNF-α, and biochemical markers. Consequently, it can be concluded that anti-hyperalgesic effect of hesperetin in rats after PSNL may be attributed to various oxidative markers as well as the pro-inflammatory mediators secreted at the injury site. Hesperetin appears to be a promising candidate for the development as a novel therapeutic for the patients suffering from the neuropathic pain.

1. Introduction

Isolated bioactive moieties from the class of flavonoids are considered as promising free radical scavengers, known to play key role in the amelioration of various diseases (Tapas et al., 2008). The hydrogen donating substituent attached to the aromatic ring structures of flavonoids allows the flavonoids to undergo a redox reaction enabling them to scavenge free radicals easily (Peng et al., 2003). Flavonoids are broadly distributed in higher plant such as citrus fruit, buckwheat, onions (Slimestad and Verheul, 2009) and reported for pharmacological properties. They serve as potential antioxidants (Nijveldt et al., 2001; Pietta, 2000; Ross and Kasum, 2002), anti inflammatory (Guardia et al., 2001; Kim et al., 2004), anti-diabetic (Vessal et al., 2003), immunomodulatory (Kuo et al., 2005; Lien et al., 2003), anticancer (Lopez-Lazaro, 2002; Ren et al., 2003), anti-nociceptive (Toker et al., 2004), and anti rheumatic (Chrubasik and Pollak, 2002) agents.

Hesperetin [(S)-2, 3-dihydro-5, 7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4one)] derived from the citrus fruit possess wide spectrum of activities including anti-inflammatory (Guardia et al., 2001; Yang et al., 2011, 2012), antioxidant (Choi, 2008; Leelavinothan and Kalist, 2011; Singh et al., 2004), and anti-rheumatic (Adams et al., 2009; Li et al., 2008, 2010). This agent is also neuroprotective in nature (Baluchnejadmojarad and Roghani, 2010; Choi and Ahn, 2008; Hwang and Yen, 2008). Hesperetin block the TNF-α induced activation of NFk-β and ERK (Kawaguchi et al., 2011; Yoshida et al., 2010).



Hesperetin IUPAC name: [(S)-2, 3-dihydro-5, 7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-benzopyran)]

Partial sciatic nerve ligation (PSNL) is a well established and globally accepted animal model for induction of neuropathic pain in laboratory animals. In PSNL, partial ligation is made around sciatic nerve for associated neuropathic symptoms such as pain-like behavior, allodynia, and hyperalgesia (Morani et al., 2012). In the light of reported antioxidant, anti-inflammatory, antinociceptive activities of hesperetin, the present study was designed to investigate the possible beneficial effect of hesperetin (PLH, 20 and 50 mg/kg, p.o.) in PSNL-induced neuropathic pain in rats by assessing behavioral, biochemical and gene expression parameters.

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2. Methods

2.1. Animals

Adult, Wistar rats of either sex, weighing 200–230 g were used in the present study. Animals were procured from the National Toxicological Centre, Pune, India. Animals were maintained at 24 ± 1 °C, with relative humidity of 45–55% and 12:12 h dark/light cycle and given free access to the food (Neutrivet Pvt. Ltd, Pune) and drinking water *ad libitum*. All experiments were carried out between 10:00 and 17.00 h. The experimental protocol was approved (Protocol approval no. SIOP/IAEC/2012/24) by the Institutional Animal Ethical Committee (IAEC) at the Sinhgad Institute of Pharmacy Narhe, Pune constituted as per the committee for purpose of supervision and control on the experimental animal [CPCSEA Reg. No 1139/a/07].

2.2. Drugs and chemicals

Hesperetin and bovine serum albumin were procured from Sigma Aldrich, St. Louis, USA. Pregabalin (Cipla, Patalganga), TNF-α, Interleukin-1β and Interleukin-6 (Quantikine, Elisa kit, U.S.A), Tris buffer, Sodium hydroxide, Folin phenol, Acetic acid, Copper sulfate, Phosphate buffer, Thiobarbituric acid, and Formalin (Research Lab, Mumbai), Sodium nitrite, Griesse reagent, Sodium dodecyl sulfate (Sri-chemicals, Mumbai), Sodium potassium tartarate (Poona chemical Lab, Pune), Saline solution (Ranbaxy), n-butyl alcohol (AA chemicals, Pune), Pyridine (Qualigens, Mumbai), Melanoldehyde (Acorus organics, Mumbai). All the reagents used in the present study were of analytical grade.

2.3. Induction of peripheral neuropathic pain

The rats were anesthetized with thiopental sodium (35 mg/kg, i.p.) and half of the left sciatic nerve was ligated at the upper thigh level using an 8-0 nylon suture. Sham surgery was done by exposing the sciatic nerve without ligation. Behavioral parameters were conducted every 3 days till three weeks (Morani et al., 2012).

2.4. Experimental protocol

Animals were divided into following six groups (n = 6).

Group-I (Normal): No treatment/ligation.
Group-II (Sham): Vehicle (1 ml/kg, p.o.) for 15 days. Left sciatic nerve was exposed without ligation.
Group-III (Control): Surgical exposure and ligation of sciatic nerve.
Group-IV (PREG): Pregabalin 10 mg/kg, p.o.
Group V (PLH 1): Hesperetin 20 mg/kg, p.o.
Group VI (PLH 2): Hesperetin 50 mg/kg p.o.

All drugs were freshly prepared before administration. Test and Standard drugs were administered for 15 days after ligation. Nociceptive thresholds were assessed on days 0, 1, 4, 7, 10, 13, 16, 19 and 22. After three weeks, the rats were sacrificed by cervical dislocation, sciatic nerve was immediately isolated, and the tissue homogenate was prepared in 0.1 M Tris–HCl buffer (pH 7.4) for biochemical estimation. For gene expression studies sciatic nerve was removed and stored in liquid nitrogen.

2.5. Measurement of behavioral parameters

2.5.1. Radiant heat hyperalgesia test

Thermal hyperalgesia threshold was assessed with a plantar test apparatus (Ugo Basile Biological Instruments, Italy) as described previously (Hargreaves et al., 1988) with slight modifications. Briefly, each rat was placed on the glass platform, under an inverted clear acrylic box (18 × 8 × 8 cm) open at bottom. After habituation to the test apparatus, a 50 W radiant heat stimulus projected through an oval shaped aperture (5x10 mm) on to heel of the left hind paw. A photocell attached light beam turned off when the paw was moved with a maximum cut off time of 15 s. The latency of paw withdrawal was recorded.

2.5.2. Cold allodynia test

Cold allodynia test was performed as per the method described by Naik et al. (2006). In this method, the left hind paw of the rat was gently submerged in ice cold water (4 ± 1 °C) in a beaker. The paw withdrawal latency was observed with a maximum cutoff time of 20 s.

2.5.3. Static mechanical hyperalgesia test (Randall Selitto)

Mechanical (static) nociceptive threshold, an index of mechanical hyperalgesia, was assessed by pressure stimulation method as described by Randall and Selitto (1957). The nociceptive flexion reflex, expressed in grams, measured by applying increasing pressure to the left hind paw of the rat was quantified using the Randall Selitto paw pressure device (UGO Basile, SRL Biological Research Apparatus, Italy). Withdrawal of left paw or vocalization response was used to assess the nociceptive threshold. The cutoff pressure of 450 g was maintained.

2.5.4. Mechano-tactile allodynia test (Von-Frey hair) (VFH)

Mechano-tactile allodynia (non-noxious mechanical stimuli) was assessed by previously described method of Chaplan et al. (1994). Briefly, animals were placed on wire mesh covered by an inverted transparent plastic box (18 × 8 × 8 cm) open at bottom so that calibrated VFH monofilament (IITC, Woodland Hills, USA) of 18 g could be applied to plantar skin of the left hind paw. The brisk withdraw of the paw was considered as a positive response.

2.5.5. Tactile mechanical hyperalgesic test (pinprick)

Mechanical (tactile) hyperalgesia was assessed by the pinprick test as described by Ze et al. (1990). The plantar surface of the injured left hind paw was touched with the point of the bent 18 gauge needle (at 90° angle) at an intensity sufficient to produce a reflex withdrawal response in normal, non-operated animals, but at an intensity which was insufficient to penetrate the pin in the skin. The duration of the paw withdrawal was recorded in seconds with a maximum cutoff time of 20 s.

2.5.6. Motor co-ordination test (Rota-rod)

Motor co-ordination (grip muscle strength) was evaluated by a rota-rod device (Techno, Lucknow, India) as described by Jones and Roberts (1968). Briefly, rats were placed individually for one minute on the rotating rod (25 RPM). Fall off time from the rotating rod during one minute period was recorded.

2.5.7. Spontaneous locomotor (exploratory) test

Photoactometer test was employed to assess the effect of drug treatment on spontaneous motor (exploratory) activity by using actophotometer (INCO, India). Each animal was observed for a period of 5 min in a square closed field area (30 × 30 × 30 cm) equipped with 6 photocells in the outer wall. Interruptions of photocell beam (locomotor/exploratory action) were recorded by digital counter (Bushnell, 1988).

2.5.8. Motor nerve conduction velocity test (MNCV)

The animals were anesthetized using thiopental sodium (35 mg/kg, i.p.) for electrophysiological recording. The dorsal side of rat paw was shaved and cleaned using a moist cotton plug. MNCV was recorded on the last day of study (22nd day) by stimulating the sciatic and tibial nerves at sciatic and tibial notch respectively by 200 μs square pulse delivered through a pair of monopolar needle electrodes (1.0–1.5 mA, 2.0 mV/D) using a stimulator. Responses were recorded from the

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