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Harpagophytum procumbens extract potentiates morphine antinociception in neuropathic rats

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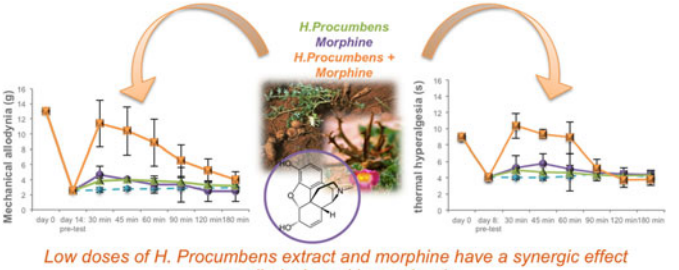

The association of opioids and non-steroidal anti-inflammatory drugs, to enhance pain relief and reduce the development of side effects, has been demonstrated. Given many reports concerning the antinociceptive and anti-inflammatory effects of *Harpagophytum procumbens* extracts, the aim of our study was to investigate the advantage of a co-administration of a subanalgesic dose of morphine preceded by a low dose of *H. procumbens* to verify this therapeutically useful association in a neuropathic pain model. Time course, registered with the association of the natural extract, at a dose that does not induce an antinociceptive effect, followed by a subanalgesic dose of morphine showed a well-defined antiallodynic and antihyperalgesic effect, suggesting a synergism as a result of the two-drug association. *H. procumbens* cooperates synergistically with morphine in resolving hyperalgesia and allodynia, two typical symptoms of neuropathic pain. The results support the strategy of using an adjuvant drug to improve opioid analgesic efficacy.

Keywords: hyperalgesia; allodynia; synergism; opioid; persistent pain; *Harpagophytum procumbens*

1. Introduction

Persistent pain, caused by peripheral nerve injury (neuropathic pain) is characterised by sustained hypersensitivity of neurons involved in pain perception and modulation at several levels along the pain-signalling pathway (Basbaum et al. 2009). Nociceptive and inflammatory

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mediators released from injured tissue can sensitise and excite nociceptors, resulting in a lowered firing threshold and ectopic discharge that lead to a sustained increase in the excitability (Ji & Strichartz 2004). As a result of these enhanced sensory processes, neuropathic pain is associated with hyperalgesia, which is an increased response to a stimulation that is normally painful, or allodynia, which results from a stimulus that does not normally provoke an algesic response (Sandkühler 2009). In the therapeutic approach to neuropathic pain, a major goal of the different drugs is to control pain but the mono-drug therapy is not always successful. The challenges produced by pain amplification processes may best be met by multitarget approaches (Argoff 2011; Parenti et al. 2013). In fact, while drugs directed at specific molecular targets are often found to be less effective at treating disease symptoms, the complexity of the molecular-biological pain mechanisms requires multitarget modulation. Most of clinically used opioid analgesics, such as morphine, elicit their effects through opioid receptor (Pasternak & Pan 2011) whose activation produces not only the analgesic effect but also a number of side effects (Benyamin et al. 2008). Hence the need to associate adjuvant pharmacological therapies, which consist in the association of opioid analgesics with a second non-opioid agent (Khan et al. 2011). This approach improves opioids-induced analgesia, enhancing pain relief and reducing the development of side effect (Khan et al. 2011; Orrù et al. 2014).

Non-steroidal anti-inflammatory drugs (NSAIDs) interfere positively on opioid analgesia for their anti-inflammatory and analgesic effect. One mechanism of action of NSAIDs involves the inhibition of the cyclooxygenases (COX) with the suppression of the synthesis of prostaglandins, which sensitise nerve endings at the site of injury (Déciga-Campos et al. 2003). Anti-inflammatory analgesic drug, ibuprofen, although is not amongst the first-line treatment for neuropathic pain, ameliorates mechanical hyperalgesia in rats by reducing central hyperexcitability (Zelcer et al. 2005; Redondo-Castro & Navarro 2014).

Medicinal plants could certainly represent potential agents in this therapeutic approach for the management of pain and, among them, one of the most studied is *Harpagophy procumbens* D.C. (Volk 1953; Mncwangi et al. 2012), a member of the Pedaliaceae family (commonly known as Devil's Claw). This medicinal, perennial, herbaceous plant originates from the Namibian steppes and Kalahari region of southern Africa (Mncwangi et al. 2012), whose drug, the water-storing secondary tuberous roots, has several potential therapeutic uses. The pharmacologically active components of the drug are considered to be the iridoid glycosides, including harpagoside, the major active constituent of the drug (Grant et al. 2007; Mncwangi et al. 2012) that, however, is not able to sufficiently explain the drug's effects (Grant et al. 2007). There have been many reports concerning the antinociceptive and anti-inflammatory effects of the plant extracts (Mahomed & Ojewole 2004; McGregor et al. 2005). Uchida et al. (2008) reported that *H. procumbens* reduced, in a dose-dependent manner, the times of licking/biting in both the first and second phases of formalin test in mice. Anti-inflammatory and analgesic effects of *H. procumben*s extract were also demonstrated in Freund's adjuvant-induced arthritis in rats (Andersen et al. 2004). Lim et al. (2014) showed an analgesic effect of *H. procumbens* extracts as measured by a reduction of ultrasonic distress vocalisations in plantar incision postoperative pain in rats and a decrease in allodynic response in animals with spared nerve injury.

The aim of this study was to determine whether the combined administration of *H. procumbens* extract with morphine could enhance the antinociceptive effect of the opioid in persistent pain. With this aim we used the chronic constriction injury (CCI) of rat left sciatic nerve, as a model of neuropathic pain, to investigate the efficacy of *H. procumbens* extract alone or with morphine, both at doses that are not capable to give an antinociceptive effect, towards two typical symptoms of persistent pain, thermal hyperalgesia and mechanical allodynia.

2. Results and discussion

2.1 *Mechanical allodynia*

The effects of *H. procumbens* were evaluated in rats that had undergone CCI of the left sciatic nerve. Following surgery, the animals developed progressive behavioural signs of mechanical sensitisation, quantised as a decrease in the paw withdrawal threshold (PWT) in response to stimulation with von Frey filaments. The allodynic threshold in CCI rats displayed a significant decrease 14 days after surgery (Figure 1(A)). *H. procumbens* extract was tested i.p. in ligated animals 14 days after surgery at 400, 600 and 800 mg/kg, resulting in a dose-dependent increase in the allodynic threshold values. In particular, while the dose of 400 mg/kg resulted in a slight attenuation of mechanical allodynia, doses of 600 and 800 mg/kg were significantly effective in determining a clear antiallodynic effect induced by CCI in the rat left hindpaw.

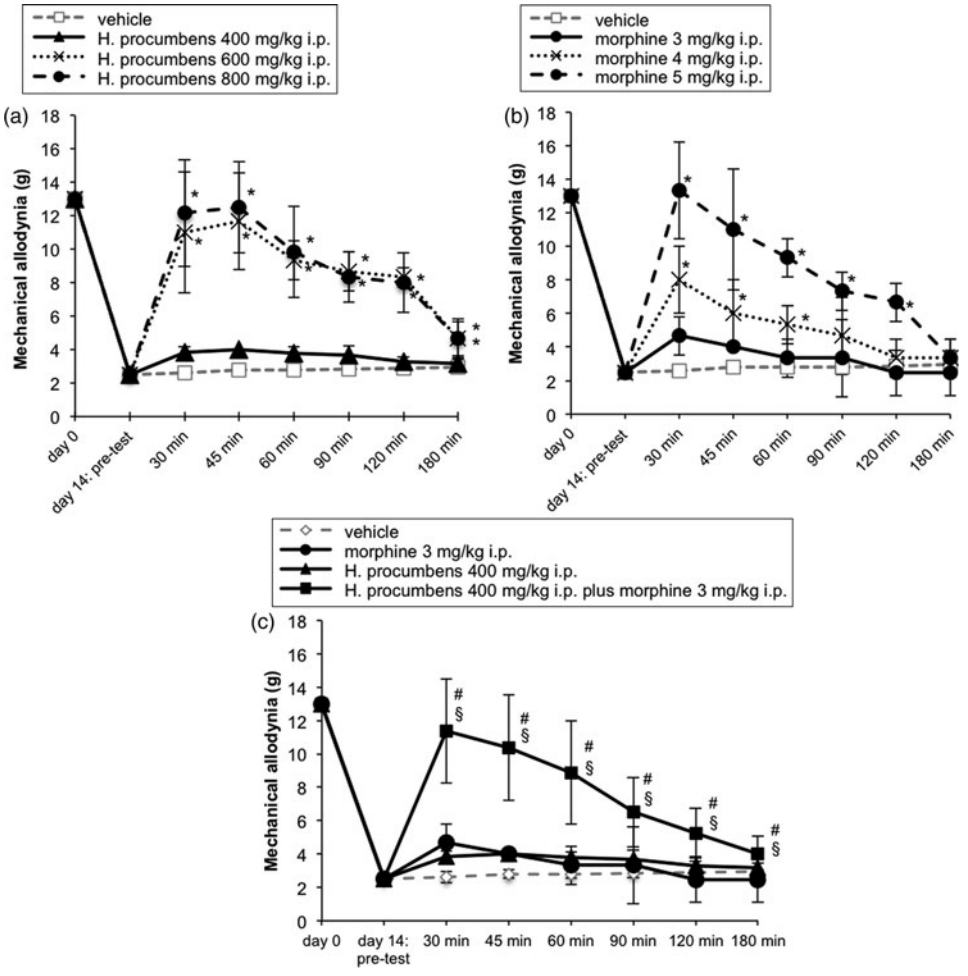

Figure 1. Effect of *H. procumbens* extracts and morphine association on CCI-induced mechanical allodynia. Time course of the effects of i.p. *H. procumbens* extracts (400, 600 and 800 mg/kg) (panel A), morphine (3,4 and 5 mg/kg) (panel B) and *H. procumbens* extract (400 mg/kg) plus morphine (3 mg/kg) (panel C), when tested in ligated animals 14 days after surgery, on mechanical allodynia measured with Von Frey's filaments. The results are expressed in grams (g). The data are mean ± SE from 8 to 10 rats. $^{*}p < 0.05$ vs. vehicle-treated rats; $\#p < 0.05$ vs. morphine (3 mg/kg)-treated rats; $\S p < 0.05$ vs. *H. procumbens* (400 mg/kg)-treated rats.

Values significantly different from the vehicle, from 30 until 120 min from *H. procumbens* administration, were observed, with the highest effect at 30 and 45 min (11.0 ± 3.6 g and 11.6 ± 2.8 g and 12.16 ± 3.1 g and 12.5 ± 2.8 g for 600 and 800 mg/kg, respectively). In the saline-treated group, the vehicle did not modify the basal mechanical thresholds.

Morphine (4 and 5 mg/kg i.p.) induced a strong attenuation of mechanical allodynia and the highest registered values at 30, 45 and 60 min were 8.0 ± 2.0 g, 6.0 ± 2.0 g, 5.33 ± 1.15 g and 13.33 ± 2.8 g, 11 ± 3.6 g, 9.33 ± 1.15 g, respectively, with 4 and 5 mg/kg i.p. (Figure 1(B)). The lower dose of 3 mg/kg caused only a slight increase in the allodynic threshold. After evaluating the *H. procumbens* extract or morphine individually, we chose the respective dose that did not result in a significant antiallodynic effect. So in Figure 1(C), besides these subanalgesic doses, we reported the time course of the association of 400 mg/kg i.p. of the natural extract followed by 3 mg/kg i.p. of the opioid. The registered values showed a well-defined antiallodynic profile (11.37 ± 3.11 g, 10.37 ± 3.15 g, 8.87 ± 3.09 g, 6.5 ± 2.07 g, 5.25 ± 1.48 g and 4.0 ± 1.06 g), suggesting a synergic effect as a result of the two combined drugs.

2.2 *Thermal hyperalgesia*

The effect of *H. procumbens* was evaluated in rats that had undergone CCI of the left sciatic nerve. Following surgery, the animals developed progressive behavioural signs of thermal hyperalgesia, quantised as a decrease in PWT in response to a radiant heat stimulus with plantar test. Thermal PWTs decreased from 9.6 ± 1.3 s (baseline value) to 4.1 ± 0.9 s registered 8 days after surgery. *H. procumbens* was tested i.p. in ligated animals 8 days after surgery (Figure 2(A)) at 400, 600 and 800 mg/kg, and a dose-dependent increase in the registered thermal PWT was observed. In particular, the dose of 400 mg of the drug extract resulted in a slight increase in the hyperalgesic thresholds. With the doses of 600 and 800 mg/kg, the antihyperalgesic effect became significant for the entire period of observation, and the recorded values for the two doses were 7.7 ± 1.56 s, 7.9 ± 1.7 s, 6.93 ± 1.81 s, 6.8 ± 2.42 s, 4.0 ± 0.60 s and 3.93 ± 0.11 s; and 9.3 ± 2.43 s, 8.8 ± 1.05 s, 6.1 ± 3.07 s, 5.66 ± 0.57 s, 5.33 ± 0.98 s and 4.66 ± 0.57 s, respectively. In the control CCI rats, the vehicle (saline) did not modify the hyperalgesic thermal thresholds.

The administration of morphine to rats at doses of 3, 4 and 5 mg/kg i.p. caused a dose-dependent decrease in hyperalgesia induced by ligation of the sciatic nerve.

This decrease in hyperalgesia was significantly enhanced at 30, 45, 60, 90, 120 and 180 min (8.85 ± 1.48 s, 12.0 ± 0.8 s, 8.6 ± 2.54 s, 8.5 ± 2.4 s, 8.15 ± 1.2 s and 6.0 ± 1.2 s; Figure 2(B)) with the dose of 5 mg/kg; at 30 and 45 min (6.85 ± 0.3 s, 8.4 ± 2.26 s) with the dose of 4 mg/kg; and only slightly enhanced with the dose of 3 mg/kg with respect to vehicle-treated CCI rats (Figure 2(B)). After evaluating the *H. procumbens* extract or morphine indivually, we chose the respective dose that had a subanalgesic effect. Administration of the weak analgesic dose of the natural extract followed by the lower dose of the opioid resulted in a markedly antihyperalgesic effect that was significant 30 (10.33 ± 1.52 s), 45 (9.33 ± 0.57 s) and 60 min (8.9 ± 1.9 s) from the last administration, suggesting a possible synergistic effect between the two drugs.

These results indicate a synergistic antinociceptive effect between a low dose of *H. procumbens* extract and a subanalgesic dose of morphine. In fact, the association of *H. procumbens* and morphine induces a clear antiallodynic and antihyperalgesic effect in a CCI rat model of neuropathic pain. Damage to peripheral nerves can cause neuropathic pain, which involves mechanisms that are incompletely known (Ji & Strichartz 2004). Multiple inflammatory and nociceptive mediators, released from damaged tissues, increase the sensitivity of sensory neurons in the peripheral nervous system, producing ectopic discharge as well as

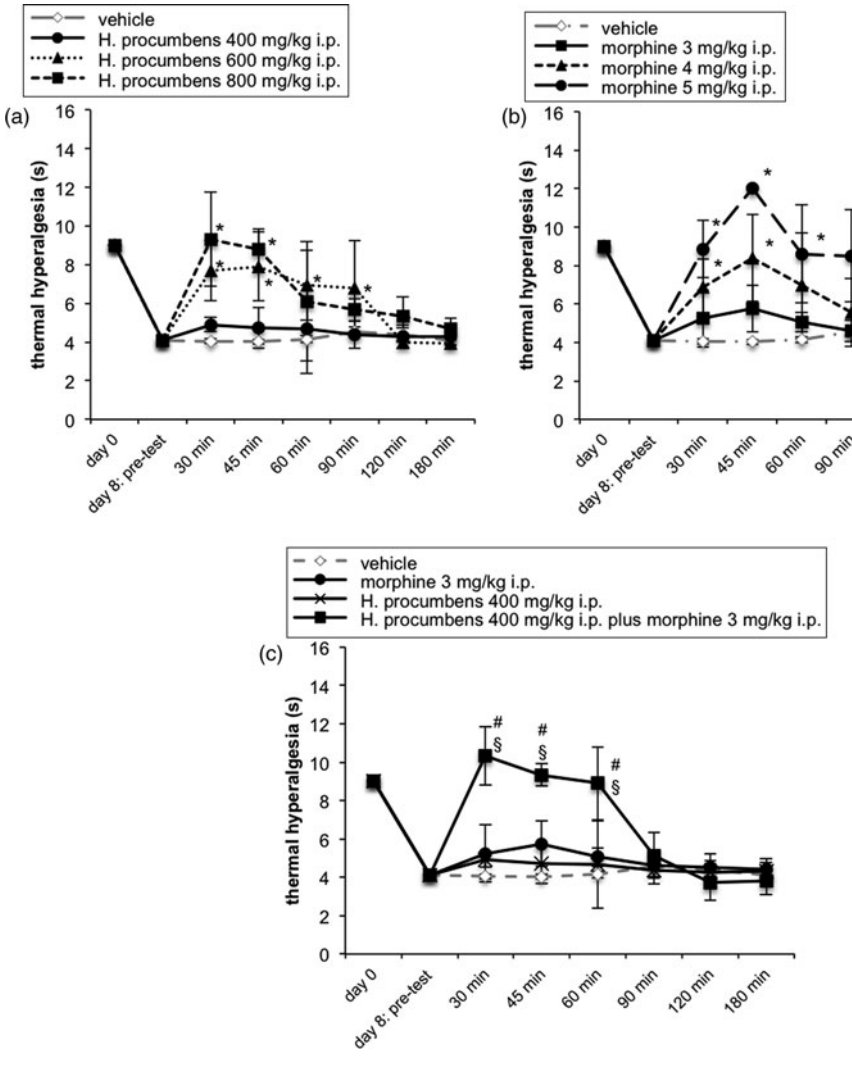

Figure 2. Effect of *H. procumbens* extracts and morphine association on CCI-induced thermal hyperalgesia. Time course of the effects of i.p. *H. procumbens* extracts (400, 600 and 800 mg/kg) (panel A), morphine (3, 4 and 5 mg/kg) (panel B) and *H. procumbens* extract (400 mg/kg) plus morphine (3 mg/kg) (panel C), when tested in ligated animals 8 days after surgery, on thermal hyperalgesia measured with the Plantar test. The results are expressed in seconds (s). The data are mean ± SE from 8 to 10 rats. $*p < 0.05$ vs. vehicle-treated rats; $\#p < 0.05$ vs. morphine (3 mg/kg)-treated rats; $\S p < 0.05$ vs. *H. procumbens* (400 mg/kg)-treated rats.

leading to a sustained increase in the excitability of these sensory neurons. Hyperexcitability also develops in the CNS, for instance in dorsal horn neurons that in turn enhances the activation of spinal glia. The neuron–glia interaction involves positive feedback mechanisms and is likely to enhance and prolong pain even in the absence of ongoing peripheral external stimulation or injury (Ji & Strichartz 2004; Romero-Sandoval et al. 2008). Because pathologic mechanisms causing chronic neuropathic pain may be numerous and complex and are still not completely understood, a mono-therapy approach may not always achieve satisfactory pain relief. Despite the controversy on the opioids use in the therapy for persistent pain, there is a wide-ranging

agreement on the fact that opioids should be regarded not as a treatment modality by itself but as one part of multimodal pain management (Zelcer et al. 2005). So the strategy of using two or more agents at lower doses to achieve synergistic analgesic efficacy has been proposed (Khan et al. 2011; Galluzzi 2005).

In this study, we have proved that the administration of a subanalgesic dose of morphine preceded by a dose of *H. procumbens* (that at the utilised dose, in our model, does not induce an antinociceptive effect) is able to determine a clear antiallodynic and antihyperalgesic effect, in Von Frey and plantar tests.

Several hypotheses can be made to explain this ability of *H. procumbens* extract to positively modulate the antinociceptive effect of morphine in neuropathic rats. The major active constituents of *H. procumbens* are iridoid glycosides, harpagoside, harpagide, 8-coumaroylharpagide and verbascoside (Georgiev et al. 2013), which possess anti-inflammatory and antioxidant activities and that are believed to interact synergistically or antagonistically in modulating the enzymes responsible for inducing inflammation (Abdelouahab & Heard 2008). The validity of *H. procumbens* as an effective anti-inflammatory and analgesic (Baghdikian et al. 1997; Grant et al. 2007; Mncwangi et al. 2012; Hostanska et al. 2014) has been investigated in several animal studies from the earliest experimental investigations (Zorn 1958). Afterwards, it was reported that *H. procumbens* elicits a direct inhibitory effect on the COX-2 enzyme in mouse skin (Kundu et al. 2005) and in human breast epithelial cells (Na et al. 2004). Also of interest is the inhibitory effect of *H. procumbens* extract on the proinflammatory cytokine (TNF-α; Fiebich et al. 2001, 2012) and NF-kappa B activation (Huang et al. 2006).

Studies have shown evidence of free radical generation in neuropathic pain and have associated the reduction of hyperalgesia and allodynia with the administration of free radical scavengers (Kim et al. 2004). The significant anti-oxidant effect of *H. procumbens* was demonstrated in rat by Bhattacharya and Bhattacharya (1998), where they noted a dose-dependent increase in superoxide dismutase, catalase and glutathione peroxidase activities as well as a reduction in lipid peroxidation. Inhibition of lipid peroxidation induced by different pro-oxidants in a concentration-dependent manner was also demonstrated on brain homogenates and brain cortical slices (Schaffer et al. 2013). Of additional significance is the concentration-dependent suppression of nitrite release (80%) attributable to the transcriptional inhibition of iNOS expression (Kaszkin et al. 2004) and the reduction in the content of nitrites/nitrates (NO*x*) on the mouse spinal cord (Uchida et al. 2008) and the decrease of COX-1, COX-2 and NO production on whole blood (Anauate et al. 2010). The constituents responsible for the anti-oxidant activity in *H. procumbens* extracts may be flavonoids, known free radical scavengers (Dugas et al. 2000) and phenolics acting as hydrogen donors and/or oxygen radical scavengers (Sawa et al. 1999). Thus, the antioxidant ability of *H. procumbens* extract could explain its efficacy in our experiments.

3. Conclusions

In rats subjected to CCI, an experimental model of neuropathic pain, the combination of the natural well-known remedy *H. procumbens* and the classical analgesic drug morphine achieves a synergistic effect against allodynia and hyperalgesia evaluated at von Frey and plantar tests. These results support a multiple-therapeutic approach to improve the balance between analgesia and adverse effects in the treatment of neuropathic pain.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

The underlying research materials for this article can be accessed at http://dx.doi.org/10.1080/14786419.2015.1052069

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