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Prevention of morphine dependence and tolerance by *Nepeta menthoides* was accompanied by attenuation of Nitric oxide overproduction in male mice

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ABSTRACT

Ethnopharmacological relevance: Repeated administration of morphine for chronic pain leads to dependence and tolerance that limits clinical usage. *Nepeta menthoides* is commonly known as Iranian Ustukhuddoos and are administered in traditional medicine for gastrodynia, bone pain, blood depurative and restlessness.
Aims of study: To investigate the effects of *Nepeta menthoides* on expression and acquisition of morphine dependence and tolerance in mice with regard to oxidative stress.
Materials and methods: Morphine dependence in mice was developed by administration of gradually increasing doses of morphine twice daily for 7 consecutive days. In experimental groups, administration of *Nepeta menthoides* (200 and 400 mg/kg), methadone and their combination were performed 60 min prior to each morphine injection (for acquisition) or the last injection of morphine on test day (for expression). Morphine tolerance was measured by the *tail-immersion test* before and after the administration of a single dose of morphine (100 mg/kg; i.p.) on the test day (8th day). Morphine dependence was also evaluated by counting the number of jumps after the injection of naloxone (5 mg/kg; i.p.).
Results: Nepeta menthoides, similar to methadone, significantly prevented the development (but not the expression) of morphine dependence, tolerance, and potentiated morphine antinociception and also reduced (23.23 ± 1.15) Nitric oxide (NO) overproduction (35.23 ± 3.36) (in compared with naloxone group (6.3 ± 0.52)). However, single and repeated application of the extract could not change high single-dose morphine analgesia.
Conclusion: It appears that *Nepeta menthoides* and methadone prevented morphine dependence and tolerance, partly through inhibition of the NO overproduction.

1. Introduction

Opioid drugs, such as morphine, clinically are the most powerful analgesic drugs for the treatment of acute and chronic pain (Ballantyne and Mao, 2003). However, development of morphine tolerance and dependence are two of the major problems associated with these drugs, limiting their effectiveness and usage (Tang et al., 2006; Haghparast et al., 2008; Hosseinzadeh et al., 2012). Withdrawal syndrome is typically observed following abrupt cut of morphine intake or precipitated by administration of a narcotic antagonist such as naloxone (Kest et al., 2001). Therefore, a search for alternatives or different analgesics, especially for the treatment of chronic pain, is the subject of intense investigation.

Despite extensive research efforts in the area of opioid tolerance and dependence, the exact mechanisms by which these phenomena occur, remain largely unknown. Some evidence suggests that nitric oxide (NO) is involved because NO synthase (NOS) inhibitors suppress the development of morphine tolerance and dependence (Babey et al., 1994; Abdel-Zaher et al., 2010). Furthermore, morphine was found to induce oxidative stress in the brain. Pretreatment with free radical scavengers attenuated the expression of morphine-induced withdrawal syndrome (Abdel-Zaher et al., 2010). Therefore, the efficacy of some

Abbreviations: NO, nitric oxide; NMDA, N- methyl-D-aspartate; NOS, NO synthase; WHO, world health organization; MDA, Malondialdehyde; i.p., intraperitoneal; GC/MS, Gas chromatography–mass spectrometry; MPE, maximal possible effect; TBA, thiobarbituric acid; S.E.M., standard error of mean; NS, normal saline; Nal, naloxone; Nep., Nepeta menthoides; MET, methadone; sGC, soluble guanylate cyclase; NR2A and NR2B, NMDA receptor subunit 2A and 2B; NR1, NMDA receptor subunit 1; LA, L-arginine; NOS1, neuronal NOS; L-NAME, N(G)-nitro-L-arginine methyl ester; MB, methylene blue; TLC, thin Layer Chromatogram; GAE, gallic acid equivalent; CE, catechin equivalent; HPLC, high performance liquid chromatography; RA, rosmarinic Acid; SAL B, salvianolic acid B; SAL A, salvianolic acid A; CA, caffeic acid

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substances with antioxidant properties in the therapy of morphine tolerance and dependence is expected. Thus, one of the main research trends in this area is the search for novel drugs with neuroprotective properties. The use of plants and plant extracts to treat diseases is a therapeutic modality. According to the world health organization (WHO), about three-quarters of the world population rely on traditional remedies (mainly herbs) for the health care of its people (Gilani and Rahman, 2005).

The *Nepeta* genus (Lamiaceae) known as "Poone-sa" in the Persian language, comprises about 400 species, most of which grow wild in Central and Southern Europe, the North Africa and Central and Southern Asia (Miceli et al., 2005). Iran is one of the centers of origin of this genus with 75 species and approximately 53% endemics (Jamzad et al., 2003). A lot of species of this genus (such as *Nepeta bracteata* Benth, *Nepeta cataria* L, *Nepeta racemosa* Lam, *Nepeta micranta* Bunge, *Nepeta ispahnica* Boiss, *Nepeta crispa* Wild, *Nepeta binaloudensis* Jamzad, *Nepeta pungens* (Bunge) Benth, *Nepeta pogonosperma* Jamzad & Assadi, *Nepeta menthoides* Boiss. & Buhse, *Nepeta glumerulosa* Lam) are used in folk medicine for nervous, respiratory and gastrointestinal diseases. They are administered in asthma, pulmonary infections, sinusitis, bronchitis, tuberculosis, pertussis, dyspnea, common cold, headache, migraine, bone pain, rheumatism, anxiety, obsession, colic, flatulence and gastrodynia (Zargari, 1995; Amin, 1992; Hooper and Field, 1937; Amini, 1997; Nadjafi et al., 2009; Miceli et al., 2005; Amiri and Joharchi, 2013; Naghibi et al., 2005). In regard to antiseptic and astringent properties, some species are used as a topical remedy in children cutaneous eruptions, snakes and scorpion bites. Also, they are utilized as anti-tussive, antispasmodic, febrifuge, diuretic, nerves tonic, cardiac tonic, blood depurative and expectorant (Miceli et al., 2005; Zargari, 1995; Amin, 1992; Amini, 1997; Amiri and Joharchi, 2013). Some of the species are administered in high blood pressure and goiter (Amini, 1997).

There is documented evidence for anti-inflammatory (Miceli et al., 2005; Hussain et al., 2012; Ali et al., 2012a, 2012b), anti-nociceptive (Hussain et al., 2012; Ali et al., 2012a, 2012b; Taviano et al., 2007), anti-bacterial, fungicidal, and antiviral (Sonboli et al., 2009; Saxena and Mathela, 1996; Bourrel et al., 1993; Sattar et al., 1995; Cigremis et al., 2010) effects of several different *Nepeta* species. Moreover, there are some reports about sedative, anti-convulsant (CNS depressant) (Galati et al., 2004; Bhat et al., 2012; Taviano et al., 2007), and antioxidant (Cigremis et al., 2010; Salehi et al., 2012; Gkinis et al., 2010) properties of several *Nepeta* species.

Nepeta menthoides Boiss. & Buhse or *Nepeta menthoides* is one of the endemic species of Lamiaceae in Iran which is distributed in the several areas of the country (Rechinger, 1963-, 2010; Jamzad, 1991; Jamzad, 2013; Amin, 1992; Naghibi et al., 2005; Joharchi and Amiri, 2012). Azerbaijan, Sabalan Mount located in Ardabil province, and the areas around Marand are recognized as some of the distributional areas for Iranica flora. *Nepeta menthoides* is also known as "Yarpuz" in Ardabil (Rechinger, 1963-, 2010). It is a gramineous, perennial, rising and sturdy plant, which normally reaches 15–40 cm height with violet flowers (Amin, 1992; Zargari, 1995). *Nepeta menthoides* is commonly known as "Ustukhuddoos" in Iranian folk medicine (Amin, 1992; Zargari, 1995; Naghibi et al., 2005; Joharchi1 and Amiri, 2012; Kahkeshani et al., 2014). Ustukhuddoos has been prescribed for a number of nervous disorders such as epilepsy and melancholy, chronic pain and restlessness (Avicenna, 1988). *Nepeta menthoides* are administered traditionally in gastrodynia, high blood pressure, bone pain, rheumatism, blood depurative, nervous disorders such as anxiety and depression (Amin, 1992; Amini, 1997).

Despite numerous studies about general Ustukhuddoos (Lavender), there is little research about *Nepeta menthoides*. Nonetheless, there are reports about the positive effects of *Nepeta menthoides* on depression and memory (Firoozabadi et al., 2015; Ahmadian-Attar et al., 2014; Sarahroodi et al., 2012). Also, some studies suggest anti larvicidal and antibacterial effects of *Nepeta menthoides* (Mahnaz et al., 2012; Sonboli et al., 2009). Anti-nociceptive and anti-inflammatory effects of *Nepeta menthoides* has been attributed to its Nepetalactone and 1, 8-cineole components (Asadi Balsin Sharif Abadi et al., 2013). Chemical analysis of the essential oil obtained from the aerial parts of *Nepeta menthoides* by our laboratory (unpublished), shows that its main ingredients are,1, 8-cineole (50%), alpha -terpineole (7.9%), alpha -linalool (4,72%), beta-pinene (4.66%), alpha-Pinene (2.10%). From structure-activity relationships of the pairs morphine+cineole and naloxone+beta-pinene, it was shown that similarities exist in the stereochemistry and in the respective atomic charges of these molecules (Liapi et al., 2007). Some studies have shown that α-Terpineol and linalool reduce nociceptive behavior from one side (Quintans-Júnior et al., 2011; Sakurada et al., 2011), and attenuate morphine-induced physical dependence and tolerance from another side (Parvardeh et al., 2016; Hosseinzadeh et al., 2012). Therefore, the present study was designed for the first time to investigate the effects of *Nepeta menthoides* extract on acquisition and expression of morphine tolerance and dependence and to compare with methadone and the combination of *Nepeta* and methadone. Also, in regard to some evidence about antioxidant effects from one side (Cigremis et al., 2010) and pro-oxidant activity of some species or parts of the *Nepeta* plant on the other side (Gkinis et al., 2010), in this study, the effects of *Nepeta menthoides* extract on brain Malondialdehyde (MDA) and NO were also investigated in morphine dependence and tolerance.

Methadone is a mu-opioid receptor agonist best known for its utility as a treatment for opioid addiction. In addition, methadone is a potent analgesic and is often used for the treatment of chronic malignant pain, most often in the context of opioid rotation (He et al., 2009).

2. Materials and methods

2.1. Animals

Two hundred and sixty adult male albino NMRI mice weighing 25–30 g (Razi Institute, Karaj) were used in our experiments. Mice were housed in groups of 5 mice / home cage at constant temperature (20–22 °C) and humidity (30–40%). They were housed under a 12-h light/dark cycle with lights on at 6 a.m., and with standard pellet food and tap water available ad libitum. Animals were allowed to habituate in the colony room for 1 week before the experimental manipulations were undertaken. All protocols of the study were approved by Institutional Animal Ethics Committee of Shahed University, Iran (approval No.: P/A/22/88), which followed the NIH guidelines for care and use of animals.

2.1.1. Animals classification

240 male mice were randomly divided into 4 equal groups based on acute or chronic morphine and pretreatment administrations. Chronic morphine treated groups: (1) with acute pretreatments, (2) with chronic pretreatments. Acute morphine treated groups: (3) with acute pretreatments, (4) with chronic pretreatments. Also, each group was divided into 6 subgroups (10 mice per subgroup) based on the type of pretreatment. Our pretreatments include: normal saline (positive control), different doses of *Nepeta menthoides* (200 and 400 mg/kg) (Rahmati et al., 2013; Sarahroodi et al., 2012), methadone (5 mg/kg) (Hołuj et al., 2013) and co-administration of methadone (5 mg/kg) and *Nepeta* (200 and 400 mg/kg). Two other subgroups are naloxone and intact groups. It is worth to cite the work of (Asadi Balsin Sharif Abadi et al., 2013) in which nontoxic effect of hydroalcoholic extract of *Nepeta menthoides* has been shown.

The naloxone group only received naloxone, while the intact group received a single dose of morphine 2 h before the injection of naloxone on the test day. Notice that, in our experiments, the intact group is intact only prior to the test day. Also, since the administration of a high single dose of morphine (100 mg/kg) on the test day in the intact and single-dose groups may lead to single dose morphine-induced depen-