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Original article

Investigation of nepetolide as a novel lead compound: Antioxidant, antimicrobial, cytotoxic, anticancer, anti-inflammatory, analgesic activities and molecular docking evaluation

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ABSTRACT

In the present study, we describe various pharmacological effects and computational analysis of nepetolide, a tricyclic clerodane-type diterpene, isolated from *Nepeta suavis*. Nepetolide concentration-dependently (1.0–1000 μg/mL) exhibited 1,1-diphenyl,2-picrylhydrazyl free radical scavenging activity with maximum effect of 87.01 ± 1.85%, indicating its antioxidant potential, as shown by standard drug, ascorbic acid. It was moderately active against bacterial strain of *Staphylococcus aureus*. In brine shrimp's lethality model, nepetolide potently showed cytotoxic effect, with LC_{50} value of 8.7 μg/mL. When evaluated for antitumor activity in potato disc tumor assay, nepetolide exerted tumor inhibitory effect of 56.5 ± 1.5% at maximum tested concentration of 1000 μg/mL. Nepetolide at 20 mg/kg reduced carrageenan-induced inflammation ($P < .001$ vs. saline group) in rat paw. Nepetolide dose-dependently (100–500 mg/kg) decreased acetic acid evoked writhes, as exhibited by diclofenac sodium. *In-silico* investigation of nepetolide was carried out against cyclooxygenase-2, epidermal growth factor receptor and lipoxygenase-2 targets. Virtual screening through Patchdock online docking server identified primarily hydrophobic interactions between ligand nepetolide and receptors proteins. Enhanced hydrogen bonding was predicted with Autodock showing 6–8 hydrogen bonds per target. These results indicate that nepetolide exhibits antioxidant, antibacterial, cytotoxic, anticancer, anti-inflammatory and analgesic activities and should be considered as a lead compound for developing drugs for the remedy of oxidative stress-induced disorders, microbial infections, cancers, inflammations and pain.

1. Introduction

Nepeta genus belonging to the family Lamiaceae and is the largest genus contains approximately 250 species, found in Central and Southern Europe, the North Africa and Southern Asia. The plants of this genus are used in folk medicine as anti-septic, snake bite, scorpion bite, anti-asthmatic, anti-tussive, anti-spasmodic, diuretic and astringent agents (Newall et al., 1996). In Iranian medicine, they are used for treatment of various nervous, respiratory and gastrointestinal diseases (Amin, 1991). The genus *Nepeta* is named on the ancient Italian city of Nephi (Simonovic, 1959). About 67 species of the genus *Nepeta* are found in Iran and 58 in Pakistan. Phytochemical screening of plant reveals that *Nepeta* genus are rich fatty acids, flavones, flavone-glycosides, coumarins, steroids, iridoid-glycosides, monoterpenic lactones, eudesmane sesquiterpenoids, abietane diterpenoids, triterpenoids, and carbohydrates (Khan, 2010). Phytochemical investigation of *Nepeta Suavis* has led to the isolation of one new diterpene compound,

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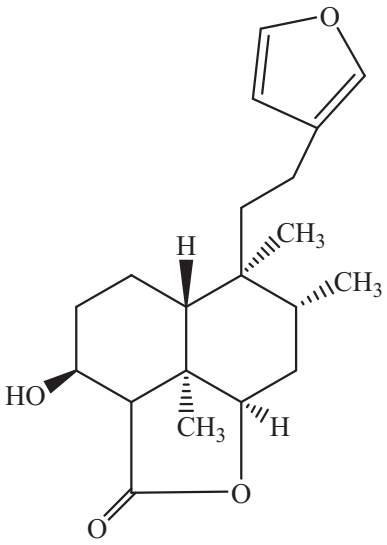

Fig. 1. Chemical structure of nepetolide: a diterpene compound isolated from *Nepeta Suavis.*

nepetolide (Fig. 1), as reported earlier (Hussain et al., 2008). We previously observed antispasmodic effect of nepetolide, mediated through Ca^{++} channel blocking mechanisms (Khan et al., 2016). The present research project is designed to investigate the nepetolide different pharmacological activities i.e. antioxidant, antibacterial, cytotoxic, antitumor, anti-inflammatory and analgesic as well as to evaluate it against cyclooxygenase-2 (Cox-2), epidermal growth factor receptor (EGFR) and 15-lipoxygenase-2 (Lox-2) targets, using molecular docking techniques.

2. Results

2.1. Effect on DPPH free radical-scavenging assay

The results of antioxidant activity of different concentrations of nepetolide are shown in Fig. 2. Nepetolide showed significant antioxidant activity when compared with the standard. Result are expressed as% DPPH free radical scavenging and represents the ability of nepetolide and standard to prevent free radical generation. Response of the test compound was in concentration dependent manner. Nepetolide at the test concentrations of 1, 3, 5, 10, 100, 300 and 1000 μg/mL showed 0.50 ± 1.76, 0.55 ± 2.87, 1.29 ± 3.56, 1.59 ± 2.47, 34.01 ± 2.71, 47.07 ± 2.35, 87.01 ± 1.85% free radical scavenging respectively. Ascorbic acid showed 3.27 ± 2.45, 16.65 ± 3.11, 21.36 ± 3.45, 40.95 ± 4.67, 44.48 ± 3.70, 51.76 ± 3.05 and 91.11 ± 1.65% free radical scavenging, at of 1, 3, 5, 10, 100, 300 and 1000 μg/mL respectively. Nepetolide showed maximum percentage scavenging of 87.01% ± 1.85% which is comparable to the free radical scavenging of ascorbic acid. EC_{50} value of ascorbic acid was 231.1 μg/mL while for nepetolide it was 330.0 μg/mL.

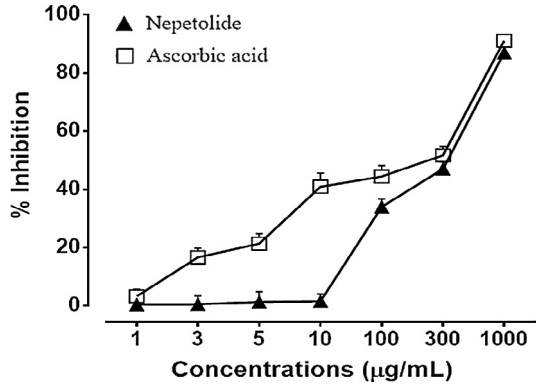

Fig. 2. Free radical scavenging activity of nepetolide and ascorbic acid measured as percent inhibition of 1,1-diphenyl-2-picryl hydrazyl radical. n = 3.

2.2. Bactericidal effect

The antibacterial potential of the nepetolide against four different bacterial strains are shown in Table 1. Nepetolide was only active against *Staphylococcus aureus* having the zone of inhibitions of 8.7 ± 0.5, 9.1 ± 1.7, 9.3 ± 1.4, 10.1 ± 2.1, 11.5 ± 2.5, 11.8 ± 0.9 and 12.2 ± 0.7 at 1, 3, 5, 10, 100, 300 and 1000 μg/mL. Nepetolide was ineffective against *Acinobacter*, *Escherichia coli* and Methicillin resistant *Staphylococcus aureus* at all the tested concentrations and hence no zone of inhibition was observed. The standard drugs ciprofloxacin showed activity against all four bacterial strains while negative control i.e. 5% DMSO showed no activity against any bacterial strain.

2.3. Fungicidal effect

Nepetolide at the test concentrations of 1, 3, 5, 10, 100, 300 and 1000 μg/mL did not show any inhibitory effect against *Candida albican* and *Aspergillus niger* (data not shown).

2.4. Effect on brine shrimp lethality

The results of cytotoxic activity at different concentrations of nepetolide are shown in Table 2. Mean percentage of larvae killed by nepetolide were 10 ± 5.77, 13.3 ± 8.82, 26.7 ± 6.67, 53.3 ± 13.33, 93.3 ± 6.67, 96.7 ± 3.33 and 100 ± 3.33% at of 1, 3, 5, 10, 100, 300 and 1000 μg/mL respectively. Etoposide killed 12 ± 4.5, 46.3 ± 3.5, 48.7 ± 3.5, 79.3 ± 2.7, 90.7 ± 5.5, 98.1 ± 0.1 and 100 ± 3.33% of mean larvae at 1, 3, 5, 10, 100, 300 and 1000 μg/mL respectively. The cytotoxic potential of nepetolide was found to be significant, having LC_{50} 8.70 μg/mL, which was comparable to that of standard drug etoposide having LC_{50} value of 5.64 μg/mL.

2.5. Effect on inhibition of tumor formation

Nepetolide was screened for their antitumor activity through 'potato disc tumor assay'. Table 3 represents the antitumor potential of the nepetolide. Antitumor activity of nepetolide was moderate. At different test doses of 1, 3, 5, 10, 100, 300 and 1000 μg/mL the percent tumor inhibition of nepetolide was 1.3 ± 1.5, 8.7 ± 1.5, 18.7 ± 1.5, 26.1 ± 1.5, 36.1 ± 1.5, 40.4 ± 1.5 and 56.5 ± 1.5% respectively, with LC_{50} value of 412.3 μg/mL. Standard drug vincristine at 250 μg/mL showed 91.3% tumor inhibition.

2.6. Effect on carrageenan-induced hind paw edema

Fig. 3 shows the response of carrageenan-induced paw edema model after the administration of test drug nepetolide. In case of control group, that was treated with 0.5 mL of normal saline, there was gradual increase in the paw thickness after the administration of carrageenan. The animals of saline group at 0, 1, 2, 3 and 4 h were having mean paw edema thickness of 4.22 ± 0.04, 5.58 ± 0.06, 5.77 ± 0.02, 5.74 ± 0.02 and 5.75 ± 0.02 mm respectively. In case of treated group there was inhibitory effect on the paw edema. Nepetolide at the dose of 20 mg/kg shows significant reduction ($P < .001$ vs. saline group) in edema after 2 h of carrageenan administration. Mean paw edema thickness in nepetolide tested group were 4.26 ± 0.02, 4.23 ± 0.01, 4.01 ± 0.02, 3.99 ± 0.03

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