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Effect of *Salvia officinalis* Hydroalcoholic Extract on Vincristine-induced Neuropathy in Mice

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[ABSTRACT] AIM: Vincristine is one of the most commonly used chemotherapeutic drugs to treat a variety of malignant diseases, including leukemia and lymphoma. Studies have shown that vincristine cause painful effects, whereas *Salvia officinalis* (SO) showed analgesic and anti-inflammatory effects. The aim of this study is to investigate the effects of the SO hydro-alcoholic extract on vincristine-induced peripheral neuropathy in mice in comparison with morphine. **METHODS:** Experiments were performed on 60 NMRI male mice weighing 25–30 g divided into six groups. The individual groups received normal saline, SO hydro-alcoholic extract, vincristine, SO hydro-alcoholic extract and vincristine (12 days before formalin test), morphine, and vincristine and morphine, respectively. The injected hind paw biting and licking was measured in a 5-minute interval for one hour. **RESULTS:** The results showed that formalin induce significant ($P < 0.05$) pain responses (the first phase: 0-5 min and the second phase: 15-40 min after injection). Administration of SO extract before formalin test showed significant ($P < 0.05$) decrease of pain response in the second phase. Administration of vincristine caused significant ($P < 0.05$) increase in the second phase of pain response. Injections of SO extract and vincristine showed that SO significantly ($P < 0.05$) decrease the second phase of vincristine-induced pain. Morphine decreased vincristine-induced pain in the first and second phase of formalin test significantly ($P < 0.05$). In comparison, morphine showed analgesic effects in the first phase and SO extract showed significant ($P < 0.05$) anti-inflammatory effects in the second phase of formalin test. **CONCLUSION:** Both SO and vincristine showed analgesic and painful neuropathic effects, suggesting that SO extract could be useful in the treatment of vincristine-induced peripheral neuropathic pain.

[KEY WORDS] *Salvia officinalis*; Vincristine; Pain; Morphine; Mice

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1 Introduction

Painful peripheral neuropathy is one of the main side effects induced by diverse classes of chemotherapeutic agents including vincristine [1-2]. Vincristine is one of the most commonly used chemotherapeutic drugs to treat a variety of malignant diseases including leukemia and lymphoma, and to prevent tumor cell replication through the alteration of cytoskeletal structure and disorientation of microtubules [3-4]. However, vincristine may also induce painful peripheral neuropathy. The chief clinical manifestation of vincristine-induced peripheral neuropathy is to disturb both sensory and motor functions [5-6]. Sensory disturbances range from mild tingling to spontaneous painful burning paresthesia and hypersensitivity to painful stimuli [7]. Vincristine-induced painful peripheral neuropathy is the major dose-limiting side-effect and requires discontinuation of treatment, with great affecting the survival of cancer patients [8]. Moreover, the resulting symptoms, which frequently include moderate to severe pain, often caused disability and significant loss of functional abilities, and decreased the quality of life [9].

Unfortunately, neither prophylactic strategies nor symptomatic treatments of this chemotherapy-induced peripheral neuropathy (CIPN) have proven useful yet. Aspirin, ibuprofen and celebrex are commonly prescribed to patients to treat CIPN yet with limited efficacy [10]. Furthermore, gabapentin, lamotrigine, nortriptyline and amitriptyline were disappoint-

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ing in treating CIPN [11], and there have been no trials of opioids in patients with CIPN.

Data concerning the effectiveness of opioids on neuropathic pain were controversial [12]. However, animal models [13] and controlled patient trials [14] suggest that μ-opioid receptor agonists are effective in attenuating neuropathic pain, but with some side-effects.

Nowadays herbal treatment including supplement usage and total extract is common around the world. An increasing number of patients are using medicinal herbs or seeking the advice of their physicians regarding their uses. More than one third of Americans use herbs for health purposes, yet patients (and physicians) often lack accurate information about the safety and efficacy of herbal remedies [15]. Previous studies have shown that herbal medication could be useful in chemotherapy-induced painful neuropathy [16].

The genus *Salvia*, is one of the most important genus of Lamiaceae family and is widely used in flavoring and folk medicines all around the world [17]. Fifty-eight species of this genus are documented in the Flora of Iran; 17 of which are endemic [18]. The plants of the genus *Salvia*, which consist of about 900 species [19], are generally known for their multiple pharmacological effects, such as analgesic [20], hepatoprotective [21], hypoglycemic [22], and antiischemia activities [23]. Many *Salvia* species and their isolated constituents possess significant antioxidant activity in enzyme-dependent and enzyme-independent systems [24-27]. A number of these have exhibited effects relevant to potential treatment of CNS-related disorders [28]. The flavonoid apigenin for example has been shown to protect neurons against Aß-induced toxicity [29]. In addition to antioxidant activity, many *Salvia* species and their isolated constituents showed anti-inflammatory properties [28, 30]. Therefore, finding new analgesic agents, especially herbal drugs, is popular because of side-effects of synthetic drugs [31-33].

The aim of this study is to investigate the effects of SO hydro-alcoholic extract on vincristine-induced peripheral neuropathy in mice in comparison with morphine.

2 Methods and Materials

2.1 *Animals*

Experiments were performed on 25−30 g adult NMRI male mice of 8−9 weeks, purchased from Razi Institute. Animals were housed six per cage in a temperature and humidity-controlled environment under a 12-h light/dark cycle (lights on at 7 AM). Food and water were available ad libitum. The National Institutes of Health guidelines for care and use of animals were followed, and the protocol was approved by the Committee on Animal Research of Tehran University and International Guiding Principles for Biomedical Research Involving Animals (1985). All efforts were made to minimize the number of animals used and their suffering degree.

The vincristine-induced peripheral neuropathy model induced by intravenous (IV) injection was used in this experiment. Animals subsequently received daily IV injections of vincristine sulfate (100 μg/kg/day), saline (0.1 ml/kg/day) and SO hydroalcoholic extract (100 mg/kg) intraperitoneally, immediately following behavioral testing. The treatment paradigm consisted of five daily injections, followed by a 2-day interval where no injections were administered, followed by five subsequent daily injections, as described previously [34].

Animals were randomly divided into six groups, the first group received normal saline (saline group), the second group received SO hydroalcoholic extract (100 mg/kg/IP) (SO group) [35], the third group received vincristine (100 μg/kg/IV/day) (Vin group) [36], the fourth group received SO hydroalcoholic extract and vincristine (SO & Vin group), the fifth group received morphine (10 mg/kg/IP 30 minutes before formalin test) (Morphine group) and the sixth group received vincristine and morphine (Morphine & Vin group).

2.2 *Formalin test*

Behavioral experiments were conducted in a quiet, temperature-controlled (20−22 °C) room between 10 AM and 4 PM. Formalin test was preformed based on Dubission & Dennis method. After weighing, the animals were placed in the observation container for 15 min to get used to the new environment, then 20 μL of attenuated formalin (5%) was injected in hind paw skin with insulin syringe in restrainer. After injection, the animals were returned to observation container immediately and were observed, the time of injected hind paw biting and licking were measured in a 5 min interval for 1 h [16].

2.3 *Chemicals*

Formalin was purchased from Merck Company. Vincristine was purchased from Tocris Cookson Ltd., Bristol, Avon, UK.

2.4 *Administration of test agent*

Vincristine (100 $\mu g \cdot kg^{-1}$) was administered intravenously via tail vein. Formalin used in the study was administered into the hind paw via the intradermal route. Agents used in the study were dissolved in normal saline, and the volume was adjusted to 1 $mL \cdot kg^{-1}$ for intravenous and 5 mL/paw for intradermal administration, respectively. Dose selection of each agent was based on the results of previous studies [37-40].

2.5 *Extracting method*

SO leaves (*Salvia officinalis*) were purchased from Esfahan (Iran) Pharmaceutical Company, and identified by Dr Ebrahm Mirshkari, an assistant professor of agriculture and specialist in herbal plants in Islamic Azad University of Tabriz, and drench method was used for extraction. For this purpose, the leaves were mildly powdered, and 20 g of SO leaves powder was mixed with 200 mL of 80% ethanol for 48 h (container were shaked for 5 min with 12 h withdrawal time). The mixture was leached and ethanol was extracted by rotary adjusted in 70 °C. The caliginous fluid was spread on a plain sheet in 50 °C oven and after drying the powder was gathered and used in this experiment [30].

2.6 *Data Analysis*