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LOCAL ANTINOCICEPTIVE EFFECT OF "SNOW MOUNTAIN GARLIC" IN AN EXPERIMENTAL FORMALIN MODEL

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Abstract

Medicinal plants are currently employed for curative purposes in developing and developed countries. One example is garlic (Snow mountain garlic, also called "Himalayan garlic"), used for its multiple therapeutic properties since ancient times. The local Himalayan population generally consumes it as a remedy for rheumatoid arthritis. This study aimed to evaluate the antinociceptive and anti-inflammatory effect of a "Snow mountain garlic" extract in a formalin test. Nociception was measured (number of paw flinches/shakes during one h for 5 min periods), under the administration of 50 µL with formalin 5%, saline solution 0.9%, Snow mountain essential oil 500 µg/kg, diclofenac 250 µg/kg, naproxen 400 µg/kg, and tramadol 500 µg/kg in independent groups. Snow mountain garlic extract has an antinociceptive effect on Phase 2 of the formalin test, which is associated with the inflammatory component of pain, and its behaviour is comparable to that of traditional NSAIDs used in the clinic.

Rezumat

Plantele medicinale sunt folosite în prezent în scopuri curative în multe țări. Un exemplu este usturoiul (usturoiul de munte, numit și „usturoiul de Himalaya"), folosit pentru multiplele sale proprietăți terapeutice încă din cele mai vechi timpuri. Populația locală din Himalaya îl consumă în general ca remediu pentru artrita reumatoidă. Acest studiu și-a propus să evalueze efectul antinociceptiv și antiinflamator al acestuia utilizând modelul experimental al formolului. S-a măsurat nocicepția (număr de tresăriri/agitări de labe pe parcursul unei ore, timp de 5 minute), după administrarea a 50 µL formol 5%, soluție salină 0,9%, ulei esențial *Snow Mountain* 500 µg/kg, diclofenac 250 µg/kg, naproxen 400 ug/kg și tramadol 500 ug/kg în grupuri independente. Extractul are un efect antinociceptiv în faza 2 a experimentului, care este asociat cu componenta inflamatorie a durerii, acest efect fiind comparabil cu cel al AINS clasice.

Keywords: antinociceptive effect, formalin test, garlic, medicinal plants, Snow mountain

Introduction

Today, medicinal plants have resumed for therapeutic purposes, and their extracts have been perfected [1]. The benefit of the active principles has led to the study of an increasing number of plant species reported as curative for treating numerous diseases [2]. The great vegetal diversity and the vast cultural wealth of Mexico have favoured the use of plants for medicinal purposes since pre-hispanic times [3]. Interest in research and commercialization of therapeutic flora increases due to rice revitalizing current consumption and the patented plant extracts. The validity of the use of medicinal plants in large sectors of the population of Mexico expresses the permanence of this cultural practice. It highlights the revaluation of traditional knowledge when solving health problems [4].

Garlic is known for its culinary applications and multiple therapeutic properties [5, 6]. It belongs to the genus *Allium,* which contains more than 300 species; among them is *Allium sativum,* the most studied species of the family *Liliaceae* and subfamily *Allioideae* [7]. Chemically, it contains different compounds to which various curative properties are attributed; between these compounds are sulphur compounds: alixin, garlicin, allicin, alliin, allyl methane thiosulphinate, diallyl disulfide, diallyl trisulphide, allyl methyl trisulphide, S-allyl mercaptocysteine, 2-vinyl-4H-1,2-dithiine and 5-allyl cysteine [8].

Garlic (*Allium sativum*) has a well-established reputation as a protective agent against cardiovascular disease, has antibacterial activity [9, 10], modifies immune response [11, 12], and inhibits the development of preneoplastic lesion [13]; among many other actions studied. In inflammatory bowel disease (IBD), garlic extract has an anti-inflammatory effect by IL-10 deregulation and reducing IL-12 production, preventing IL-12 from binding to its receptor on T and NK cells, causing inhibition of IFN-γ production [14]. While, about its cousin "Snow Mountain," also called "Kashmiri garlic" or "Himalayan garlic," nearly nothing is known. In ancient times, mountaineers used this garlic to raise their energy levels and detoxify their bodies in extreme cold weather conditions. Local people commonly consume it to remedy rheumatoid arthritis [15].

The study evaluated the antinociceptive and anti-inflammatory effect of a "Snow mountain garlic" (SM) extract in an experimental formalin model. The results compared to different commercial anti-inflammatory drugs.

Materials and Methods

Reagents

Sterile saline solution (SS) at 0.9% (NaCl) (Thermo Fisher Scientific), formaldehyde solution (FMN) (37 wt. % in H_2O) (Sigma-Aldrich), injectable diclofenac (DFC) solution (15 mg/3 mL) (Novartis Laboratories), naproxen (NPX) ≥ 98% (Sigma-Aldrich), and tramadol chlorhydrate (TML) ≥ 98% (Sigma-Aldrich), chloroform 99.97% (Chemical products of Monterrey).

Characterization by RAMAN spectroscopy

The specimen known as Snow mountain Garlic (*Allium sativum Kashmiri*) was characterized by Raman spectroscopy, using laser light with a wavelength of 785 nm (red light) at a power of 499 mW, travelling in optical fibre. This study used Ocean Optics equipped with a spectrometer with a CCD camera. The 499 mW laser-focused on the sample during the measurement and the Raman scattering spectra were obtained by Spectra suite software and further processed by Origin V8 software.

Each sample reading was collected in a simultaneous detection range of 2000 to 200 cm^{-1} in extended mode. The complete spectral measurement was performed with an integration time of 1s with approximately four spectral accumulations. Also, for the study, the whole specimen and essential oil of Snow mountain Garlic (*Allium sativum Kashmiri*) were used and measurements were at a temperature of 25°C [16, 17].

Snow mountain essential oil

A filter paper cartridge with 101.92 g of SM garlic was placed in a Soxhlet extractor to carry out the extraction in the boiling flask. 700 mL of chloroform were put together with boiling beads and heated at 60°C - 70°C. Water was circulated at 4°C by the refrigerant until obtaining a speed of 2 drops *per* second; the total extraction time was four hours.

When the time reached, it was allowed to cool and recover the essential oil by evaporating the solvent in a rot evaporator heated to 65°C.

Animals

The experimental models constantly were maintained under bioterium conditions. Male Wistar rats 6 to 8 weeks of age, with an approximate weight of 250 g, acclimatized at 25°C with regulated temperature, a 12 h/12 h light/dark cycle, and free access to food and water were used. Each experimental group constituted of n = 5. When the test concluded, sacrificed the animals according to the ethical guidelines for investigating pain in experimental animals of the International Association for the Study of Pain [18].

Measurement of nociceptive response

The animals were acclimatized for a 60-min period of three days and one hour before the experiment. Nociception was measured under formalin administration at 5% (50 µL), and the quantification of the number of paw flinches/shakes during one h, for 5-min periods, in two phases: the first, Phase 1 comprised 0 to 15 min, and the second, Phase 2, 15 to 60 min. The antinociceptive effect was evaluated by applying the treatments 20 min before the intraplantar administration of 5% formalin [19].

Experimental design

Animals were injected with a total solution of 50 µL *per* experiment, with the following compositions: formalin 5%, saline solution 0.9%, SM essential oil 500 µg/kg, DCF 250 µg/kg, NPX 400 µg/kg, and TML 500 µg/kg, in independent groups; additionally, a volume of 25 µL at the local level in the intra-plantar part of the hind leg of the rat.

Statistical analysis

Each group's average and standard error (SE) were calculated, with an n = 5 for the time courses. After this, applied the Shapiro-Wilks normality test, the one-way analysis of variance (ANOVA), followed by a post-hoc Tukey test ($p \leq 0.05$). It used Origin ver. 8.0 statistical software package to model the data.

Results and Discussion

This study evaluates the antinociceptive and anti-inflammatory effects of Snow Mountain Garlic (*Allium sativum Kashmiri)* using the formalin test. In that sense the extraction methods are favour of obtaining active principles from plants, providing compounds with a high therapeutic potential in the clinic. The yields are variable depending on the methodology used and the extraction cycles; thus, there are extraction methodologies based on steam, contact with organic solvents (Soxhlet), supercritical fluids, solid-liquid extraction, liquid-liquid by centrifugation, solid phase, or hydroalcoholic solutions that offer a range of extracted products with different effects and structures [20]. In this context, in 2005, Bekas *et al*. [21], reported an extract of 5 different types of garlic with the Soxhlet method using methanol at 60ºC, with yields from 14.36% to 10.49%. On the other hand, in 2017, El-Sayed *et al*. [22] obtained by hydrodistillation method yields of 0,8%, 0,4%, 0,6% and 0,2%. The oil yield obtained from SM garlic was 3.8% using the Soxhlet method and chloroform solvent, above the reported yields.

The Snow Mountain Garlic contains a great variety of components, and the Raman spectroscopy offers a study through which we can know the functional groups. In our case, this technique shows displacements related to alin, allicin, and ajoene functional groups at 1777 cm^{-1} (C=O), 1623 cm^{-1} [C=C (diallyl-disulfide)], 1607 cm^{-1} (C=O and C=N), 1267 cm^{-1} [$(CH)_{-2}$], correlated to alin 869 cm^{-1} (CH); associated to adenosine at 1004 cm^{-1} (C-O) and 636 cm^{-1} (OH- and NH_2) shifts assigned to alin and adenosine; Figure 1, [16, 17, 23].

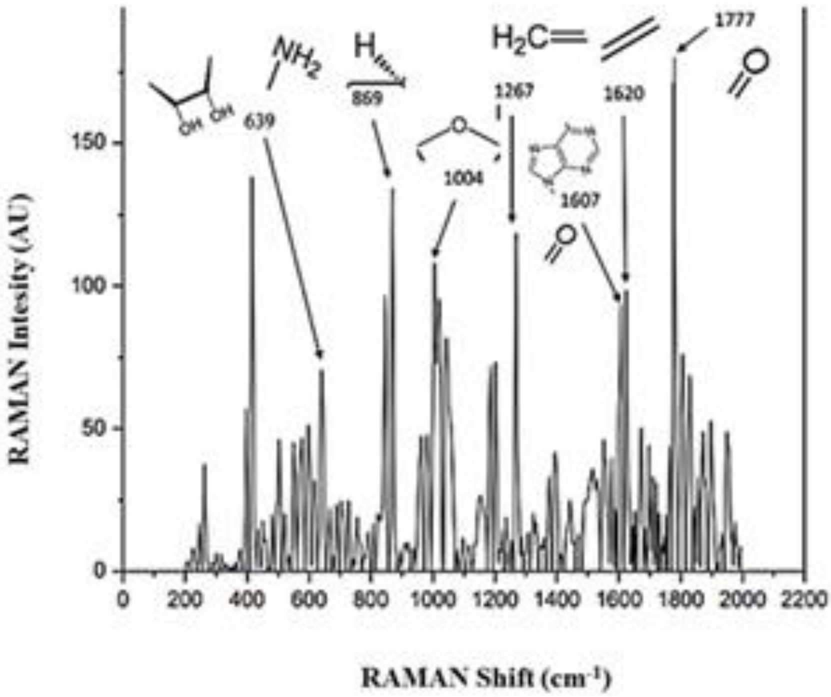

Figure 1.
Raman spectra of Snow Mountain Garlic (*Allium sativum* Kashmiri)

In particular, garlic is a popular medicine recognized for its results against pathological conditions. That effect is due to its composition, including allicin, ajoene and adenosine [24]. Allicin has shown analgesic and anti-inflammatory effects [25], as has ajoene [26] and adenosine [27].

To measure the antinociceptive potential of the Snow Mountain Garlic extract, we used the formalin test. This technique has been a widely used tool as a good model for measuring antinociception in rodents. From its first applications by Dubuisson in 1977 [28] and its multiple replications by other authors, it allowed observing the behavioural responses under continuous pain stimuli with 5% formalin at the intraplantar level (clinical pain). The phases represented in this test simulate neurogenic pain in Phase 1 (5 to 15 minutes) and 15 to 20 shaking appears; during the next 15 to 60 minutes (Phase 2), the number of shaking increases from 20 to 32 over the saline group (0.9%), (*$p \leq 0.05$ *vs*. saline group). Following the work done by Olimov *et al*. [29], and an investigation conducted in our laboratory by Lugo-Lugo *et al*. [30], this response is a product of the initial irritation known as inflammatory pain (Phase 1) and the secondary response to inflammation (Phase 2).

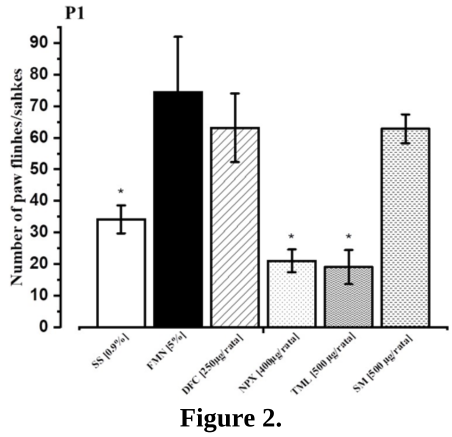

Figure 2.
Graphic of Phase 1 of the formalin test, the data representing $\dot{X} \pm$ SEM of an n = 5
*$p \leq 0.05$ *vs*. the 5% formalin group

When applying the SM garlic extract (500 μg/mL) in this work, was observed to produce a 20% decrease in the number of shaking during Phase 1 in addition for DFC (250 μg/mL) 19.5%, NPX (400 μg/mL)* 73.3% (Figure 2), TML (500 μg/mL)* 74.5% (*p ≤ 0.05 *vs*. FMN) (Figure 3), in Phase 2, 72% for DFC*, NPX* and SM garlic extract* and 81.8% for TML* (*p ≤ 0.05 *vs*. FMN) (Figure 3), the SM garlic extract has a more noticeable effect in this phase.

The previous percentage means that SM garlic and the DFC (500 μg/kg and 250 μg/kg, respectively) decrease the number of flinches without reaching a difference concerning the group of FMN in Phase 1 (three to five flinches). However, at the indicated doses, NPX and TML treatments significantly decrease 55 flinches (*p ≤ 0.05 *vs*. FMN). Also, during Phase 2, DFC, NPX, TML and SM garlic extract substantially diminish the number of jolts from 72% to 75% (*p ≤ 0.05 *vs*. FMN), Figure 3.

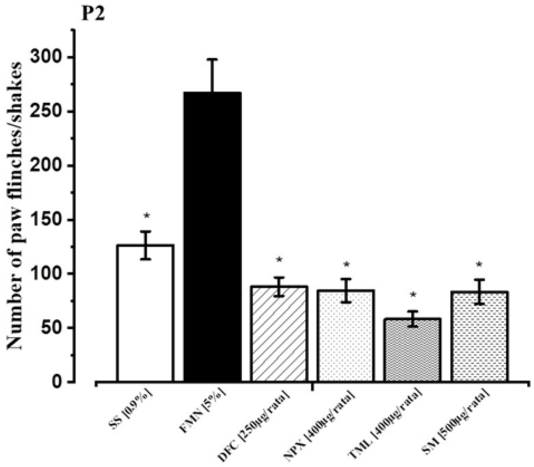

Figure 3.
Graphic of Phase 2 of the formalin test, the data representing Ẋ ± SEM of an n = 5
*p ≤ 0.05 *vs*. the 5% formalin group

Analysis of the effect of the treatments measured as % antinociception showed that NPX and TML treatments achieved a high percentage of antinociception during Phase 1 (71.81 ± 4.40% and 74.50 ± 6.62% respectively) and Phase 2 (68.31 ± 3.64% and 78.20 ± 2.39%; *p ≤ 0.05 *vs*. FMN); (Figure 3 and Figure 4). DFC showed 23.27 ± 8.71% antinociception during Phase 1 and 66.98 ± 3.22% during Phase 2, above the effect demonstrated by SM garlic extract in Phase 1 (15.66 ± 6.18%); however, SM during Phase 2 shows 66.73 ± 4.16% antinociception very similar to that produced by DFC, NPX and close to TML and consistently above the FMN group (5 %); *p ≤ 0.05; (Figure 4 and Figure 5).

The behaviour of SM garlic extract is more similar to the profile produced by DFC (250 μg/kg) compared to those of NPX (400 μg/kg) and TML (500 μg/kg) since the latter has a marked effect on both phases. In addition, at the experimental and clinical levels, NSAIDs are considered the best analgesics for acute, mild to moderate pain. DFC has been widely used to relieve pain in the first step of the WHO scale. However, the joint use of NSAIDs and low-dose opioids are recommended for moderate to severe pain.

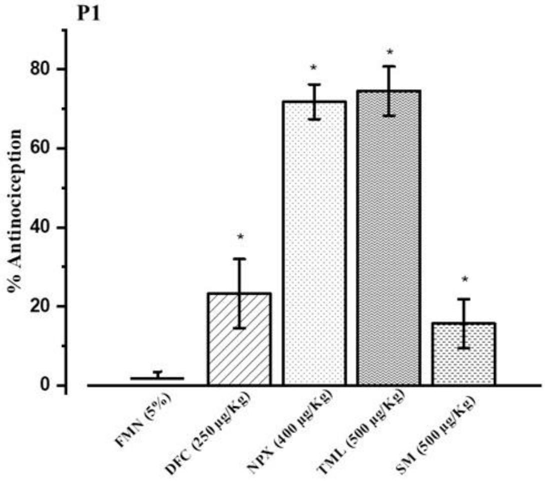

Figure 4.
Graphic of Phase 1 of the % antinociception, the data are representing Ẋ ± SEM of an n = 5
*p ≤ 0.05 *vs*. the 5% formalin group

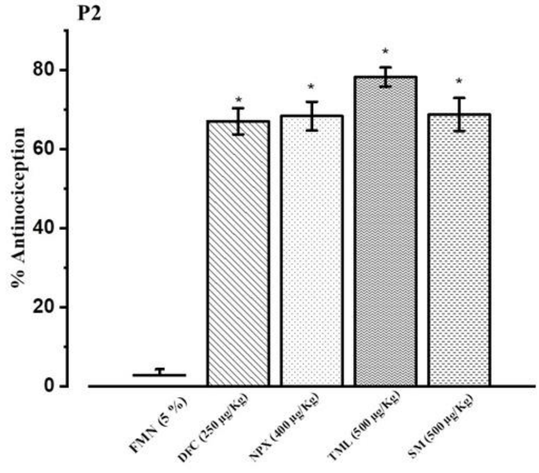

Figure 5.
Graphic of Phase 2 of the % antinociception, the data are representing Ẋ ± SEM of an n = 5
*p ≤ 0.05 *vs*. the 5% formalin group

It is fundamental to mention that using opioids for moderate to severe pain has a good effect, such as tramadol. For severe pain, interventionist techniques are considered [31, 32]. On the other hand, Picazo, *et al*. [33] demonstrated that DFC is good analgesia in the formalin model, decreasing nociceptive behaviour and favouring synergistic behaviour in combination with gabapentin. Also, Hasani *et al*. [34] showed that the intraperitoneal administration of diclofenac (10 mg/kg) favours decreasing nociceptive behaviour and preventive analgesia in the thermal model of inflammation-induced, after minute 10th of DFC administration. However, natural compounds have also shown a crucial antinociceptive effect. For example, eugenol (clove oil) has been studied with a good margin of success as shown by the works of Kurian *et al*., [35]; Apparecido *et al*. [2]; Dal Bó *et al*., [37]; Lugo-Lugo *et al*. [30] and González-Lugo *et al*., [36]. Other examples are the aguacate bone, alacrancillo grass, arnica, canastilla, cedar bark, the plant of the chilagogue, colcolmeca, cornizuelo, crucetillo, cuajilote, epazote, garlic (*Allium sativum*), etc. They are effective for inflammation, muscle and visceral pain [38].

Olimov *et al*. [29] reported that garlic could be a good anti-inflammatory. Also, Owoyele *et al*. [39], when studying the aqueous extract of *Allium ascalonicum* at a concentration of 200 mg/kg, observed that there is a decrease in Phase 1 and Phase 2 of the formalin test; Jayanthi and Jyoti [40] showed that *Allium sativum* powder completely abolished Phase 1 and Phase 2 of the formalin test at a concentration of 150 mg/kg and 300 mg/kg respectively.

The data obtained in this study are consistent with the findings of Olimov *et al*. [29] since the garlic variety SM garlic extract in Phase of the formalin test, nevertheless, 4.5 times below the effect achieved by NPX and TML and 1.5 times below the effect achieved by DFC; Figure 4. In the Phase 2 SM garlic extract (500 µg/kg) achieves the same effect as DFC (250 µg/kg), NPX (400 µg/kg) and TML (µg/kg,) around 70% antinociception on average for the groups (*$p \leq 0.05$ *vs*. FMN) (Figure 5).

Suresh *et al*. [41] described that garlic sprout extract at 250 mg/kg and 500 mg/kg reduced NaCl-induced writhing episodes (4%). The best inhibitory effect was at the 500 mg/kg dose in hot plate and tail-flick; this dose is close to that used in our laboratory for SM extract and with the component in Phase 1 and Phase 2 similar to DFC. Moreover, Jayanthi and Jyoti [40] discussed that *Allium sativum* (variety powder) had analgesic activity at the central level due to opioid receptors; a measure of this is the effect produced by the thermal component of the hot plate at the central level. At the peripheral level, the effect is due to phytocomposites that exert NSAID-like actions on cyclooxygenases and prostaglandins. In our laboratory, we use formalin as a substance that promotes nociception. The behaviour in the rodent implies an inflammation phase where afferent fibres are affected due to the irritation of the C fibres, and the late phase is the product of an inflammatory reaction [42]. SM garlic extract (500 µg/kg) does not significantly alter Phase 1 but does alter Phase 2, suggesting an effect at the inflammatory level compared to that of DFC (250 µg/kg), NPX (400 µg/kg), and TML (500 µg/kg).

Conclusions

Snow Mountain garlic has an antinociceptive effect in Phase 2 of the formalin test, which is associated with the inflammatory component of pain. Its behaviour is comparable to that of traditional NSAIDs used in the clinic as the DFC and NPX, which mediates its effects mainly by inhibiting cyclooxygenases. However, it is necessary to carry out more studies about this garlic variety's activity and effects that clarify all its antinociceptive potential.

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest.

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