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# Evaluation of Analgesic Properties of *Piper Nigrum* Essential Oil: a Randomized, Double-blind, Placebo-controlled Study

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## ABSTRACT

**Objective:** Essential oils are complex mixtures of chemical compounds, extracted from a wide range of plants. The volatile fraction of essential oils is responsible for their characteristic aroma and presents diverse biological properties that have been studied over the years. In Traditional Chinese Medicine, *Piper nigrum* is considered to be pungent and hot. Although its chemical constituents and respective pharmacological properties have been described by several authors, the volatile fraction is still underestimated as a therapeutic agent. The aim of this study was to evaluate the analgesic properties of the volatile fraction of *Piper nigrum* essential oil, in patients presenting different types of pain.

**Methods:** Fifty-four patients presenting pain, were included in a randomized, double-blind, placebo-controlled study, over a 9-week period. The patients were randomly divided into two groups, and asked to inhale a vial containing *Piper nigrum* essential oil, or a vial containing a placebo (sesame oil), for 15 minutes. A numerical pain scale was applied before and after the inhalation.

**Results:** Results showed a statistically significant decrease in pain intensity in the patients that inhaled the black pepper essential oil, while the placebo group patients showed no significant change in pain intensity.

**Conclusion:** Although the results are preliminary due to the limited sample size and short inhalation time, the volatile fraction of the *Piper nigrum* essential showed promising results in reducing pain. In the Chinese medicine perspective, these results support the use of black pepper in different types of pain, since it warms the center and disperses cold.

**Key words:** *Piper nigrum*, essential oil, placebo, pain
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## INTRODUCTION

Essential oils are plant volatile compounds, which have been used throughout the world as therapeutic agents, in food flavoring and preservation, as insect repellents and in cosmetics. Essential oils are a complex mixture of low molecular weight compounds, from a variety of chemical classes, extracted from various parts of aromatic plants, such as stems, flowers, leaves and roots[1].

Jellinek, 1997, describes how constituents of the essential oil may influence behavior through the central nervous or endocrine system[2]. Small volatile molecules, such as terpenes, enter the blood stream through the nasal or lung mucosa. These compounds can also cross the blood-brain barrier and may act on the Central Nervous System, by binding into receptor sites or interacting with enzyme systems. Terpenes, such as linalool, have been found in the blood of rodents exposed to essential oils by inhalation[3].

Also Okuyama (1997) demonstrated the existence of the nose-brain route by spraying radioisotopes into the olfactory mucosa of anosmic patients and measuring the resulting cerebral radioactivity[4].

*Piper nigrum* is a member of the family *Piperaceae*[5], commonly grown in South India and Sri Lanka, and it can reach a height of 50–60 cm. Chemical analysis of *Piper* species have demonstrated that it contains diverse secondary metabolites, including lignans, neolignans, terpenes, chalcones, flavones, alkaloids, amides and propenyl phenols, corresponding to diverse biological activities such as antioxidant, immunomodulatory, anti-inflammatory, analgesic, antinociceptive, antipyretic, anti-coagulant, antifungal, anti-cancerogenous, gastroprotective, anxiolytic and anti-depressive[6–11].

β–caryophyllene is a sesquiterpene present in *Piper nigrum* essential oil, with anaesthetic activity[5]. It was recently identified as a natural agonist of the cannabinoid receptor 2 (CB2), which is peripherally expressed. Several studies show the CB2 involvement in the modulation of inflammatory and neuropathic pain responses[12–14].

Another study demonstrated the action of *Piper aleyreanum* essential oil as anti-inflammatory and gastric antiulcer in rodents. In this study, the chemical constituents caryophyllene oxide and β-pinene were among the major components of the essential oil and could be responsible for its activity[8].

Linalool is a monoterpene commonly found in aromatic plants and essential oils, including *Piper nigrum*[7]. Its traditional uses comprise analgesic and anti-inflamatory properties. Studies conducted with pain models in mice, demonstrated its action in anti-inflammatory pain, by activating the opioidergic and cholinergic systems[15–16].

Components such as β-pinene and linalool have an antidepressant activity and sedative effect, in rodents and humans[17].

Composition of black pepper essential oil comprises α-pinene, which is also present in essential oil extracted from the leaves of *Juniperus oxycedrus*. This chemical constituent presented anti-inflamatory effects in human chondrocytes, presenting potential anti-osteoarthritic activity. In this study, it was verified that α-pinene caused a potent inhibition of the IL-1β-induced inflammatory and catabolic pathways, namely NF-KB and JNK activation and the expression of the inflammatory (iNOS) and catabolic (MMP-1 and -13) genes[18].

*Piper nigrum* essential oil composition includes 1,8-cineol, which has been shown to possess pharmacological activities such as anti-bacterial and anti-inflammatory[19].

According to Chinese Medicine, *Piper nigrum* is pungent[5], and the nature of its temperature is hot, consequently its behavior is to warm up the body.

The objective of this work was to evaluate the analgesic properties of the volatile fraction of *Piper nigrum* (black pepper) essential oil, in patients presenting pain.

## METHODS

The study was conducted according to the principles of the Helsinki Declaration and approved by the ethics committee of the Hospital-School of the University Fernando Pessoa. All participants were volunteers and signed a written informed consent to participate in the study.

In this study, 54 patients presenting pain were recruited at the Physiotherapy Unit at the Hospital-School of the University Fernando Pessoa, between May 2015 and July 2015. A group of patients that inhaled essential oil and a placebo group were compared in a randomized, double-blind, placebo-controlled trial. The *Piper nigrum* essential oil and placebo (sesame oil) were placed in identical dark vials, marked with the code A and B, respectively. Both patient and investigator weren't aware of the code significance. The patients were allocated to the essential oil or placebo groups by drawing lots method.

The inclusion criteria were being over 18 years of age, presenting pain and the availability to participate in the study. The exclusion criteria were pregnant women or nursing mothers, pain medication in the last 8 hours, active pathologies of the superior respiratory tract, medication that could interact with the olfactory system (for example: quinolones).

After the patients have been randomly divided into two groups, they were informed of the objectives of the study and its possible risks, and asked to read and sign an informed consent. Afterwards, a basic questionnaire was applied in order to characterize the patient. For this study, a new questionnaire was designed, to facilitate the sample characterization and objectivity of evaluation. The first part of the questionnaire consisted of social-demographic characteristics (gender, date of birth), followed by questions related to the exclusion criteria (pain medication in the last 8 hours, active pathologies of the superior respiratory tract, medication that could interact with the olfactory system). Finally, the patient was asked to describe the location and intensity of the pain. For registering the level of pain, a numerical pain scale was used. This scale consists of a ruler divided into eleven equal parts, numbered successively from 0 to 10. The ruler can be presented to the patient horizontally or vertically. It was intended that the patient would make the equivalence between the intensity of their pain and numerical rating, with the rating 0 corresponding to "No pain", and 10 to classification "Maximum pain" (maximum intensity of imaginable pain)[20]. The numerical pain scale was applied before and after the inhalation. The numerical classification indicated by the patient was recorded on the questionnaire. It was also recorded the code of the vial attributed to each patient (A or B). Patients were asked to inhale the attributed vial for 15 minutes.

After data collection is necessary to interpret the information obtained to achieve the results and translate them in order to reach the study conclusions.

For this purpose, statistical analysis was performed with SPSS Statistics 22 (IBM Corp., Armonk, NY, USA). The hypothesis that the patients who inhaled *Piper nigrum* essential oil reported less pain than those who inhaled the placebo was evaluated using the non-parametric test Wilcoxon-Mann-Whitney. The Mann-Whitney test is a nonparametric test that compares two groups or treatments, without making the assumption that values are normally distributed. In this case, pain intensity was measured with a numerical pain scale, an ordinal scale of measurement. The null hypothesis stated that the pain intensity, before and after inhalation, in the essential oil group (A) was identical to the placebo group (B). The statistical analysis was performed with $\alpha = 0.05$.

## RESULTS

### Sample Characterization

The mean patient age was 39,9 years, with a standard deviation of ±15,3, the minimum was 18 and the maximum was 73 years. In group A (essential oil), the mean patient age was 43,0 with a standard deviation of ±18,5, the minimum was 21 years and the maximum was 73 years. In group B (placebo), the mean patient age was 37,2 years, with a standard deviation of ±11,6, the minimum age was 18 years and the maximum was 61 years (Table 1).

From the 54 patients, 35 (64,8%) were female and 19 (35,2%) were male. Group A was composed of 20 (80%) female patients and 5 (20%) male patients. Group B was composed of 15 (51,7%) female patients and 14 (48,3%) male patients (Table 1).

**Table 1.** Sample characterization.

| | Age | | | | Gender | |
|---|---|---|---|---|---|---|
| | Mean | SD | Min | Max | Female | Male |
| Total sample | 39,9 | 15,3 | 18 | 73 | 35 (64,8%) | 18 (35,2%) |
| Group A | 43,0 | 18,5 | 21 | 73 | 20 (80%) | 5 (20%) |
| Group B | 37,2 | 11,6 | 18 | 61 | 15 (51,7%) | 14 (48,3%) |

## Pain Characterization

In Chinese Medicine, the body is divided into 3 parts, the so-called tricaloric. In the Heidelberg Model, the tricaloric orb means that there are three sectors or regions of activity and functions, the upper, middle and lower caloric.

The upper caloric corresponds to everything that is in upper chest. The middle caloric corresponds to the area below the chest to the navel – the middle abdomen. The lower caloric goes from the navel to the feet – lower abdomen. The tricaloric function is to coordinate the energy flow equally in the different body sections[21].

As referred, in this study were included patients that presented pain, without selection of any type of pain. However, for analysis purposes, the diverse types of pain were grouped into the tricaloric classification (Table 2).

In this context, in the overall sample, 20 (37,0%) presented pain in the upper caloric, 29 (53,7%) patients presented pain in the lower caloric and 2 (3,7%) presented pain in the middle caloric. 3 (5,6%) patients presented pain in the upper and lower caloric. Group A integrated 10 (40%) patients with pain in the upper caloric, 10 (40%) with pain in the lower caloric, 2 (8%) with pain in the middle caloric and finally, 3 (12%) patients with pain in the upper and lower caloric simultaneously. Group B integrated 10 (34,5%) patients with pain in the upper caloric and 19 (65,5%) patients with pain in the lower caloric (Table 2).

Regarding the pain intensity before inhalation, taking into consideration all the patients, the median was 6,37, with a minimum value of 3 and a maximum value of 10. In group A, the median was 6, with a minimum value of 3 and a maximum value of 10. In group B, the median was 7, with a minimum value of 3 and a maximum value of 10 (Table 3).

Concerning the pain intensity after inhalation, taking into consideration all the patients, the median was 4, with a minimum value of 0 and a maximum value of 10. In group A, the median was 3, with a minimum value of 0 and a maximum value of 8. In group B, the median was 6, with a minimum value of 3 and a maximum value of 10 (Table 3).

**Table 2.** Classification of the pain according to the tricaloric system.

| | Tricaloric | | | |
|---|---|---|---|---|
| | Upper | Middle | Lower | Upper and lower |
| Total sample | 20 (37%) | 2 (3,7%) | 29 (53,7%) | 3 (5,6%) |
| Group A | 10 (40%) | 2 (8%) | 10 (40%) | 3 (12%) |
| Group B | 10 (34,5%) | 0 | 19 (65,5%) | 0 |

**Table 3.** Characterization of the pain intensity before and after inhalation.

| | Pain intensity before inhalation | | | Pain intensity after inhalation | | |
|---|---|---|---|---|---|---|
| | Median | Min | Max | Median | Min | Max |
| Total sample | 6,37 | 3 | 10 | 4 | 0 | 10 |
| Group A | 6 | 3 | 10 | 3 | 0 | 8 |
| Group B | 7 | 3 | 10 | 6 | 3 | 10 |

## Statistical analysis

The analysis of the differences between group A and B in terms of pain intensity was performed with the Mann-Whitney non-parametric test. This test is suitable for small samples with ordinal variables, and doesn't require a normal distribution of values[22]. The test was applied to the values of pain intensity before and after the inhalation.

The results presented in Table 4, demonstrate that, the null hypothesis should not be rejected (p>0,05), when comparing the pain intensity between the two groups (A and B), before the inhalation. This result expresses the similarity of the pain intensity between group A (essential oil) and group B (placebo), in the beginning of the study. This similarity between the two groups is also expressed by the Mean Rank values, which are 27,70 for group A and 27,33 for group B.

On the contrary, the results presented in Table 5 show that the null hypothesis should be rejected (p = 0.000), when using the Mann-Whitney test to compare the pain intensity between groups A and B, after the inhalation. Therefore, it may be concluded that the patients that inhaled *Piper nigrum* essential oil reported significant less pain than those that inhaled the placebo. This result is supported by the Mean Rank values, which is lower for group A (17,70) than group B (35,95).

**Table 4.** Mann-Whitney test results for comparison of the pain intensity in the essential oil group (A) with the placebo group (B), before inhalation.

| | Ranks | | | |
|---|---|---|---|---|
| | Group | N | Mean rank | Sum of ranks |
| Pain intensity before inhalation | A | 25 | 27,70 | 692,50 |
| | B | 29 | 27,33 | 792,50 |
| | Total | 54 | | |

| Test Statistics[a] | |
|---|---|
| | Pain intensity before inhalation |
| Mann-Whitney U | 357,500 |
| Wilcoxon W | 792,500 |
| Z | –0,088 |
| Asymp. Sig. (2-tailed) | 0,930 |

[a]Grouping Variable: Group

**Table 5.** Mann-Whitney test results for comparison of the pain intensity in the essential oil group (A) with the placebo group (B), after inhalation.

| | Ranks | | | |
|---|---|---|---|---|
| | Group | N | Mean rank | Sum of ranks |
| Pain intensity after inhalation | A | 25 | 17,70 | 442,50 |
| | B | 29 | 35,95 | 1042,50 |
| | Total | 54 | | |

| Test Statistics[a] | |
|---|---|
| | Pain intensity after inhalation |
| Mann-Whitney U | 117,500 |
| Wilcoxon W | 442,500 |
| Z | –4,295 |
| Asymp. Sig. (2-tailed) | 0,000 |

[a]Grouping Variable: Group

## DISCUSSION

Pain is essential for human survival and wellbeing. Individuals who don't experience pain cannot engage in protective behavior in order to guarantee their survival[23]. Pain is a multidimensional experience, in which sensory mechanisms and emotions play a fundamental role[24].

As previously discussed, essential oils present a wide variety of biological activities, including anti-inflammatory and analgesic activities.

*Piper nigrum* is one of the most popular spices in the world, and its essential oil has been used in traditional medicine systems for centuries. The presence of active volatile compounds such as cariophyllene, 1,8-cineol, sabinene, linalool, among others, appoint this oil as an important ally of modern medicine.

In this study, a group of patients that inhaled *Piper nigrum* essential oil and a placebo group were compared in a randomized, double-blind, placebo-controlled trial, at the Hospital-School of the Fernando Pessoa University, Portugal.

The sample was constituted mainly by women (64,8%), and patients ranged from 18 to 73 years of age (Table 1).

Patients presented diverse types of pain, which was mainly located in the upper and lower caloric parts of the body (Table 2). The pain intensity before inhalation varied extremely in the diverse patients, with the mean intensity of 6,37, a minimum value of 3 and a maximum value of 10 (Table 3).

From statistical analysis, it was demonstrated that patients that inhaled *Piper nigrum* essential oil presented superior decrease in the intensity of pain than the placebo group (Table 4 and 5).

This result is in consonance with the analgesic properties of *Piper nigrum* essential oil, since it may interact with diverse pain receptors in the human body, such as opioid and cannabinoid receptors.

Pain receptors are distributed in the surface of the skin and in some tissues such as internal periosteum, arterial walls and joint surfaces. In other deep tissue, innervation is scarce, but an extensive injury can also cause there a continuous, chronic pain[25]. There are diverse types of receptors involved in inflammatory pain. For this study, the emphasis was made on the peripheral opioid and cannabinoid receptors, due to their relation with essential oils analgesia.

Opioid receptors are present in peripheral tissues. Opioid analgesia is mainly due to the activation of opioid receptors on peripheral sensory neurons[26]. Studies have demonstrated the analgesic potential of linalool, a chemical constituent of *Piper nigrum* essential oil, and its relation with opioid analgesia[16, 27].

Cannabinoid receptors are another promise in inhibiting peripheral sensitization. Activation of one or the two types of cannabinoid receptors, CB1 and CB2, produces an antinociceptive response. The first is expressed on central and peripheral neurons, while the latter may act on the inhibition of immune cells. This type of receptors may act synergetically with the opioid receptors[28], potentiating the final effect.

β-caryophyllene, a chemical constituent of *Piper nigrum* essential oil, has proven to be an agonist of cannabinoid receptor 2 (CB2) by Klauke and colleagues in 2014[14].

It is important to refer that, due to time constrains, this study was performed with a small sample size and a short inhalation time, limitations that should be adjusted in further studies. However, the results were very promising and opened new perspectives regarding the therapeutical use of essential oils.

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