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Research Article

Pilot Study of the Efficacy and Safety of *Humulus lupulus* in the Treatment of Mild Anxiety

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Abstract

The ongoing coronavirus (COVID-19) pandemic is causing multiple psychiatric problems in several groups of the population, calling for the development of new approaches to manage these concomitant syndromes. After an in-depth review of the scientific literature of the European Medication Agency, it was observed that 14 plant species of the 408 (3%) documented by the Department of Environment and Territorial Policy of the Basque Government are approved for treatment of different pathologies at a European level. Among them, *Humulus lupulus* was selected for investigation as a medicinal plant with therapeutic application for anxiety. We plan to study the efficacy and safety of the plant by means of a randomized multicenter open clinical trial. Two treatment groups will be assessed in parallel: a group treated with the medicinal plant and another group receiving conventional treatment. The objective of the clinical trial is to describe the therapeutic effects of the medicinal plant *H. lupulus* administered in clinical practice in primary care, and to analyze its side effects and safety.

Keywords: *Humulus lupulus; Anxiety; Medicinal Plants; Hops; COVID-19*

Introduction

We are currently living through the COVID-19 pandemic, with information about this highly transmissible and often lethal disease constantly being updated and expanded [1].

COVID-19 is associated with multiple psychiatric problems in several groups of the population, including infected patients and the clinicians who care for them. In addition, the pandemic may adversely affect patients with pre-existing psychiatric disorders, who can have a poor understanding of the risk of infection and find it difficult to adhere to frequent handwashing and social distancing [1].

A bibliographic study [2] was carried out on the existing medicinal plant species of Urdaibai, a natural area of the Basque Country, in northern Spain. It concludes that it was observed that of the list of 408 vegetable species included in the Biodiversity Information System of the Department of the Environment, Territorial Planning, Agriculture and Fisheries, only 14 species (3%) have been included in the list of medicinal plants for human use registered and approved by the European Medicines Agency (EMA) up 2013. Furthermore, according to the results of this study, the use of Urdaibai medicinal plants coincide with the most common pathologies visited in primary care. For this reason, it was decided to focus the study in primary care. This study focuses on the treatment of one pathology of high prevalence in primary care: the moderate anxiety.

Humulus lupulus L. (Photograph 1) is a species of flowering plant in the plantain family, the Cannabaceae. A perennial herb, this climbing plant can reach 8 meters in height, and has palmate-lobed leaves of 3 to 5 serrated lobes. It produces characteristic pale green female flowers. In Urdaibai it is found growing alongside the *alisedas* (numerous streams that flow into the Oka River or directly to the Cantabrian Sea) [3].

Photograph 1: *Humulus lupulus.*

The part of the plant used as an herbal medicine are the dried, generally whole female inflorescences, known as hop strobile**.** The Committee on Herbal Medicinal Products of EMA concluded that, on the basis of their long-standing use, hop strobile preparations can be applied for the relief of mild symptoms of mental stress and to aid sleep [4]. The main constituents of hop strobile are [5]:

1. Bitter principles: Consisting mainly of prenylated phloroglucinol derivatives known as alpha-acids or humulones (2 - 12% of dried strobile), principally humulone (35 - 70%), and beta-acids or lupulones (1 - 10% of dried strobile), principally lupulone (30 - 55%).

2. Essential oil (0.5 - 1.5%): consisting mainly of myrcene (monoterpene) and beta-caryophyllene, humulene and farnesene (sesquiterpenes), which are found in freshly harvested hop strobile. The amount is higher in stored material, increasing to a maxi-

mum of about 0.15% of the dry weight (up to 20% of the volatile constituents) after 2 years due to degradation of humulones and lupulones.

3. Flavonoids (0.5 - 1.5%): Including quercetin and kaempferol glycosides, and at least 22 prenylated or geranylated flavonoids, notably the chalcones xanthohumol (up to 1% of the dried strobile and 80 - 90% of total flavonoids), desmethylxanthohumol and dehydrocycloxanthohumol and the flavanones isoxanthohumol, 8-prenylnaringenin (25 - 60 mg/kg) and 6-prenylnaringenin (Rong., *et al*. 2000, Stevens., *et al*. 1997, 1999a, 1999b, Milligan., *et al*. 1999).

4. Other constituents include proanthocyanidins (2 - 4%), phenolic acids, proteins (15%), polysaccharides (40 - 50%) and minerals.

Hops are responsible for the bitter taste of beer and have been used for the preservation of beer for over 1000 years. Traditionally, it was observed that hop pickers easily became fatigued, apparently as a result of the accidental transfer of hop resin from their hands to their mouths [5].

Meissner and Häberlein (2006) in an *in vitro* experiment showed that the modulatory activity of xanthohumol (a constituent of *H. lupulus)* on the GABAA receptor was not mediated via an interaction with benzodiazepine receptors. The authors concluded that xanthohumol may play an important role in the sedative effects of hop preparations [6].

In a study on mice, various extracts of hops reduced locomotor activity and increased the sleep time induced by ketamine [7]. Franco., *et al*. (2012) concluded that 2 mg of hop extract, similar to the concentration in non-alcoholic beer, was more effective in reducing nocturnal activity than other doses, as well as in preserving the circadian activity/rest rhythm [8]. In another randomized, placebo-controlled, double-blind, cross-over study with young adults showing symptoms of depression, anxiety and stress, a significant reduction in the levels of all these symptoms was achieved by supplementation with hops, the results being significantly better compared to the placebo [9].

This project proposes to carry out a clinical trial on a native medicinal plant that will provide novel and useful results. A review of the EMA reference list demonstrates that there is no recent literature on the application of *H. lupulus* to treat anxiety disorders. An important aim of the study is to reduce the use of benzodiazepines, and therefore the risk of addiction to these drugs, by proposing a therapeutic alternative based on a medicinal plant. The results of the trial will increase knowledge of the medicinal properties of *H. lupulus*, thus favoring a suitable and responsible use of this plant. Additionally, the study will promote the exploitation of endemic resources, and potentially contribute to generating a more sustainable economic system.

Objectives of the Study

The principal objective of this project is to assess the efficacy and safety of the use of *H. lupulus* in the treatment of moderate anxiety in patients over 18 years of age who make primary care visits in Busturialdea (Bizkaia), Spain.

The secondary objectives are:

1. To determine the therapeutic effects of *H. lupulus* applying techniques commonly used in primary care.

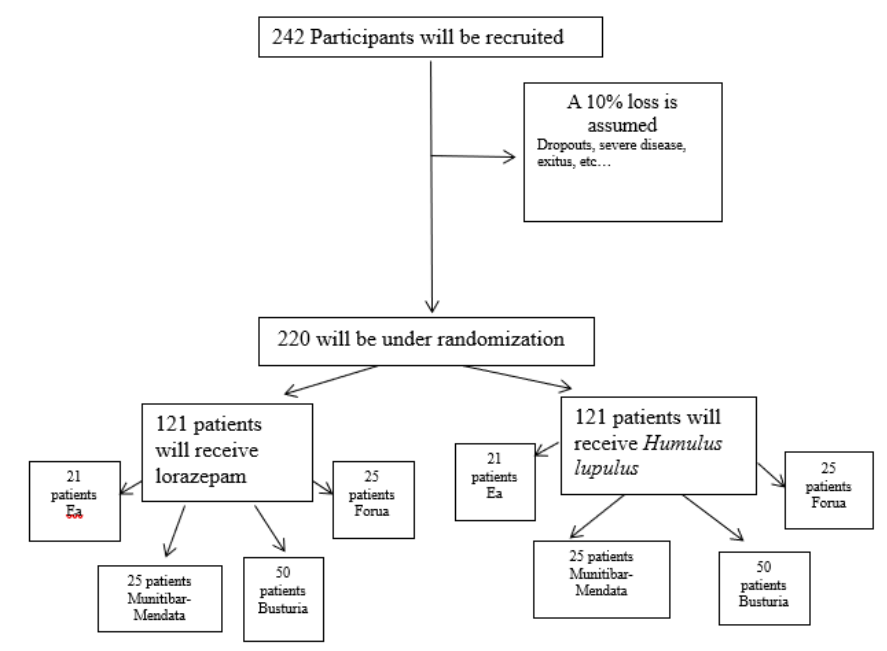
2. To evaluate the safety of *H. lupulus*.

3. To elucidate the mechanism of action underlying its possible benefits.

Methods

Participants

Selected population: Patients over 18 years of age living in the geographical area of the Comarca de Busturialdea and Lea-Artibai (Bizkaia) in the north of Spain and registered as users in the automated files of the participating Health Centers (Ea Health Center, Health Center of Munitibar-Mendata, Forua Health Center, Busturia Health Center). As 110 patients are required for each treatment group, 220 in total, and assuming a loss of 10% (dropouts, etc.), 121 patients will be recruited per treatment, 242 in total, for this study.



Figure

Study population: The target population is defined by the following inclusion and exclusion criteria.

Inclusion criteria

Patients over 18 years of age who complete and sign the informed consent before randomization and agree to comply with all the procedures included in the protocol, and with *de novo* medical diagnosis of mild anxiety (score between 6 and 14) according to the Hamilton Test [8].

Exclusion criteria

- Pregnancy
- Autoimmune diseases or immunodeficiencies
- Panic disorder, aggression, depression, autolysis attempt or ideation
- COPD, asthma
- Heart disease
- Chronic renal failure

- Hepatic insufficiency
- Surgical interventions
- Medical or surgical emergencies
- Consumption of toxic substances
- Allergy to the plant
- Concomitant use of psychiatric medication
- Patients with a vital prognosis of less than one year.

Interventions

Patients will have a diagnosis of de novo anxiety clinically confirmed by the Hamilton Test. Two treatment groups of 125 patients each will be randomized.

Group I *Humulus lupulus*:

Oral dosage:

1. For a Hamilton score of 6 - 10: 1000 mg of flower crushed in 150 - 200 ml of water in an herbal infusion at night for 2 weeks.
2. For a Hamilton score of 10 - 14: 500 mg of flower crushed in 150 - 200 ml of water in an herbal infusion, 3 times a day for 2 weeks.

Group II Lorazepam 1 mg:

Oral dosage:

1. For a Hamilton score of 6 - 10: Lorazepam 1 mg 0-0-1 (Over 65a Lorazepam 1mg: 0-0-0.5).
2. For a Hamilton score of 10 - 14: Lorazepam 1 mg 1-0-1 (Over 65a, 0.5-0-0.5).

The drug will be prescribed by a doctor in compliance with the National System of Health of Spain.

Outcomes/Results

The study variables are:

- Main variables: Healing/Improvement (Yes/No), specifying the % of improvement.
- Secondary variables: Demographic data (age, gender, place of residence, etc.). Type of treatment (conventional/phytotherapy), Time to cure/improvement, Reason for consultation, Pathological history (HBP, obesity, diabetes, AMI, etc.), Signs and symptoms, Life habits (Toxic/alcohol consumption, diet, exercise, hobbies, etc.), Regular treatment and other unconventional treatments (Homeopathy, Osteopathy, Acupuncture, etc.).

Specifically, the variables will be:

a) Sociodemographic. 1.1. Age (date of birth); 1.2. Gender (male/female); 1.3. Civil status; 1.4. Education level; (Primary Education, Secondary Education, Bachiller, Formation Professional, University); 1.5. Place of residence (population/district).

b) Clinics: 2.1. Baseline situation: Hamilton Anxiety Score, defined according to the Hamilton Anxiety Scale; 2.2. Diagnosis of Anxiety (Golberg Scale score); 2.3. The 2nd visit (score): the clinical situation is assessed at 7 - 10 days according to the Hamilton Anxiety Scale; 2.4. The 3rd visit: Healing/Improvement (Yes/No), defined according to the Hamilton Anxiety Scale at 30 days; 2.5 Type of treatment (*H. lupulus*/conventional); 2.6. Pathological background (HBP, obesity, diabetes, etc.); 2.7. Habits of life (Toxic/alcohol consumption, diet, exercise, hobbies, etc.); 2.8. Current treatments; 2.9. Other non-conventional treatments (Homeopathy, Osteopathy, Acupuncture, Psychotherapy, etc.). 2.10. Time to cure. 2.11. Side effects.

Sample size

There are two treatment groups (lorazepam/*H. lupulus*) and the main variable is the healing/improvement on the 3rd visit (Yes/No), defined according to a scale of evaluation of signs and specific physical examination designed for the present study. A loss of 10% is assumed, as well as a statistical power of 80% and a level of significance of 0.05. If it is assumed that 80% of cures are in the control group and there is an increase of 13% in the cure/improvement between the treatment group (*H. lupulus*) and the control treatment group (lorazepam), the study requires each group of treatment to have 110 patients, 220 in total; taking into account a 10% loss (dropouts, etc.), 121 patients will be required per treatment, 242 in total.

Randomization

A randomized list of both treatments (conventional/herbal medicine) has been prepared by the research department, maintaining a proper allocation concealment, which will be delivered to the nurses station. In the list, each patient is indicated by a number and a treatment. Once the doctor has made the diagnosis and filled out the data collection sheet, nursing will deliver a treatment according to the list, without the knowledge of the corresponding doctor.

Masking

At the time of the evaluation, the data collection sheet will contain the number of each patient from the randomization list without the treatment received. In this way, the doctor will blindly evaluate the patients.

Statistical methods

A database will be built to store all the information collected in the study. This information will be anonymized and controlled, following the current legislation.

To calculate sample size, the proportions comparison procedure of the power prop.test () function of the R package See 3.4.2 has been used. See Power Calculations for Two-Sample Test for Proportions, Peter Dalgaard, work by Claus Ekstrøm, https://stat.ethz.ch/R-manual/Rdevel/library/stats/html/power.prop.test.html.

Before the statistical analysis, the quality control of the information will be checked, comparing it with the clinical information of the patient. Also, a Statistical Analysis Plan will be established, detailing the statistical methods, tables and results of the final results report, as well as the suitability of analyzing different subgroups or subpopulations following the indications of the study protocol.

Descriptive analyses of all the variables will be carried out according to their nature.

For comparison between treatment groups, a statistical analysis will be performed by means of a suitable test to compare the Hamilton Scale and drowsiness between the baseline visit and each subsequent visit. Thus, if the variables are measured on an ordinal scale (0, 1, 2, 3, 4, etc.), the Mantel-Haenszel chi-square test will be used for comparison, and if it is done on a binary scale (presence/absence), the chi-square test will be used. When the frequencies observed are less than 5%, other alternative tests should be applied besides the chi-square test, such as Fisher's exact test.

All statistical analyses will be performed with the SPSS package for Windows (version 21) and with R package v.3.1.

Discussion and Conclusion

Infection with SARS Cov-2 may cause psychiatric symptoms, including anxiety, depression, insomnia, and post-traumatic stress disorders [1]. In the acute phase of the illness, patients frequently show a neuropsychiatric syndrome. A study [1] on 144 patients with COVID-19 found that anxiety occurred in 35 percent and depressive symptoms in 28 percent. Another study conducted on 4,872 patients in China warns of the risks for children of over-information about the coronavirus through the internet and social networks, which can produce symptoms of anxiety and depression [10].

In the USA, a study [11] with 62,354 patients without prior diagnosis of psychiatric disorders suffering from COVID-19 observed a higher incidence of a first psychiatric diagnosis in the following 14 to 90 days compared with six other health events (HR 2.1, 95% CI 1.8 - 2.5 vs influenza; 1.7, 1.5 - 1.9 vs other respiratory tract infections; 1.6, 1.4 - 1.9 vs skin infection; 1.6, 1.3 - 1.9 vs cholelithiasis; 2.2, 1.9 - 2.6 vs urolithiasis and 2.1, 1.9 - 2.5 vs fracture of a large bone; all $p < 0.0001$). The hazard ratios were greatest for anxiety disorders, insomnia, and dementia. Also, individuals in quarantine during the COVID-19 pandemic may develop a wide range of psychiatric symptoms [1], including anger, anxiety, boredom, confusion, fear, depression, emotional exhaustion, frustration, irritability and stress.

Health care workers are also prone to suffering psychiatric symptoms. A meta-analysis of 25 studies [1] concluded that clinically significant psychological stress was more likely to occur in health care workers exposed to the virus than in controls (odds ratio 1.7, 95% CI 1.5 - 2.0). The frequency of mental health problems in health professionals in the emergency caused by the SARS epidemic was particularly high and with a tendency to be long term: anxiety symptoms (45%), followed by depression (38%), acute stress (31%), professional burnout syndrome (29%) and post-traumatic stress (19%) [10].

COVID-19 may also produce symptoms or residual pathologies that can last for weeks or months after the initial recovery, which has come to be called long-COVID or long-haul COVID. A meta-analysis [9] study investigating the long-term effects of COVID-19 estimated that 13% of patients suffer from anxiety. Thus, the management of patients with COVID-19 should be tackled by a multidisciplinary approach, including neuropsychiatric support, with professional and/or pharmacological therapy [12].

In general, anxiety is one of the most common pathologies among patients in primary care and is frequently treated with benzodiazepines. They are the most habitually prescribed medicines by psychiatric physicians (Moore and Mattison, 2017), with more than 1 in 20 people in the U.S. filling a prescription each year (Bachhuber., *et al*. 2016). They are also the third most consumed illicit drug among adults and adolescents in the U.S. ([CBHSQ], 2018b; Johnston., *et al*. 2018) [7].

The numerous side effects of benzodiazepines are well-established in the scientific community, and there is a growing public health problem associated with their intake. In particular, benzodiazepine-related overdose deaths in the U.S. increased by more than 400% from 1996 to 2013 (Bachhuber., *et al*. 2016) and emergency department visits related to benzodiazepine ingestion rose by more than 300% from 2004 to 2011 (Jones and Aninch, 2015). These trends are concurrent with the rising rates of benzodiazepine prescription,

which grew by 67% from the mid-1990s to 2013, a period when the quantity prescribed increased by more than three-fold (Bachhuber., *et al*. 2016) [7]. In the urgent search for an alternative therapy, *H. lupulus* is a promising candidate.

Traditional medicine has long used *H. lupulus* for its sedative effects, but its therapeutic value needs to be established by scientific evidence, hence the design of this clinical trial.

The main limitation of the proposed study is the impossibility of implementing a double-blind clinical trial, as we will be working with a *H. lupus* infusion. The difficulty of finding a placebo with the same galenic presentation means there is a risk that the patient will be influenced in their perception of symptoms and thus bias the efficacy of both the conventional and herbal-based treatments. This is particularly relevant in a pathology such as anxiety, where symptoms predominate over signs of the disorder, and it is not possible to evaluate its evolution with objective tests.

Therefore, it is suggested that in future studies, tablets of *H. lupulus* extracts are administered, as they can be compared with placebo tablets, eliminating observational bias and resulting in more statistically significant data.

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