FACULTY OF MEDICINE - UNIVERSITY OF SÃO PAULO
UNIVERSITY OF SOUTHERN SANTA CATARINA
CRUZEIRO DO SUL UNIVERSITY
CITY OF SÃO PAULO UNIVERSITY

EFFICACY AND SAFETY OF AURICULAR ACUPUNCTURE IN DEPRESSION: A MULTICENTER RANDOMIZED CLINICAL TRIAL

Dr. Alexandre Faisal Cury (FM-USP)
Dr. Paulo Rossi Menezes (FM-USP)
Dr[d]. Daniel Maurício de Oliveira Rodrigues (FM-USP; UNISUL; UNICSUL; UNICID)
MSc[d]. Ana Elise Machado Ribeiro Silotto (FM-USP)
MSc[d]. Artur Heps (FM-USP)

São Paulo
2023

ABSTRACT

Depression, often under-diagnosed and under-treated, is the leading cause of disability worldwide and contributes significantly to the global burden of diseases. There is a growing demand for non-medicated forms of treatment for depression. In this sense, auricular acupuncture is considered a simple, low cost technique, well tolerated by patients. The Unified Health System (Sistema Único de Saúde –SUS) had this practice incorporated in 2006, but it lacks studies to verify its efficacy and safety. The main objective of this study is to evaluate the efficacy of auricular acupuncture to reduce depressive symptoms by comparing it to usual care and non-specific auricular acupuncture. It is a randomized clinical trial, blinded to the evaluator, participant and statistician. The study sample will be composed of 280 university students, divided equally into two groups: Experimental group - GE (auricular acupuncture) and Control group - GC (non-specific auricular acupuncture). The GE and GC participants will undergo 12 sessions of auricular acupuncture, 2 times a week. The primary outcome of the study will be the proportion of participants who present improvement of 50% or more in their symptoms, evaluated by PHQ-9, three months after inclusion. The secondary outcomes will be: improvement of 50% or more in their symptoms, after six months; quality of life (SF-36), sleep quality (PSQI), anxiety (STAI), change in the use of antidepressant medication, events and adverse effects, levels of Brain-derived neurotrophic factor (BDNF), Interleukin 1β, Interleukin-6 and TNF-α in blood plasma. The data will be analyzed aiming at treating according to the principles of *CONSORT*. A semi-structured interview will be applied to a sub-sample. These qualitative data will be analyzed using the Content Analysis method. The study will begin after approval by CEP-FMUSP.

INTRODUCTION

DEPRESSION AS A PUBLIC HEALTH PROBLEM

Depression is one of the most significant public health problems in the world. It is a severe mental disorder that has an episodic, recurrent, or persistent course over time and causes functional impairment in behavioral, social, family, educational, and occupational domains (HENDRICK et al., 1998). Several explanatory models have been developed to justify the syndrome, which can be psychogenic, organic, or both (BECK; ALFORD, 2011).

According to the World Health Organization (WHO), depression is the leading disabling factor worldwide. More than 300 million people (4.4% of the world's population) currently live with depression, an increase of over 18% between 2005 and 2015. Depression is more common among women (5.1%) than men (3.6%). The prevalence also varies according to age, income, WHO macroregions, countries, among other variables. Globally, depressive disorders are the leading cause of years lived with disability (7.8% of all years lost). In the worst-case scenario, depression can lead to suicide. Approximately 800,000 people die from this cause annually, and it is the second leading cause of death among adolescents and young adults (15 to 29 years). Despite high prevalence rates, less than 10% of those affected by the disease receive adequate treatment (WORLD HEALTH ORGANIZATION, 2017a, b).

In the United States of America, 8.1% of adults had depression between 2013 and 2016, almost twice as prevalent among women (10.4%) compared to men (5.5%). About 80% of adults reported difficulties with social activities due to depression symptoms (BRODY; PRATT; HUGHES, 2018). Another study with American women found a prevalence of 4.8% for major depressive disorder and 4.3% for mild depression, with only 32.4% of women with major depressive disorder and 20.0% with mild depression using antidepressants (GUO et al., 2018). In Brazil, the prevalence is one of the highest in the world: 5.8% of the Brazilian population suffers from depression, and 10.3% of the years of life lost by Brazilians are due to this disease (WORLD HEALTH ORGANIZATION, 2017a, b).

Depression is the most prevalent health disorder in the university environment: university students are at greater risk related to the pathology (NATIONAL

INSTITUTE OF MENTAL HEALTH, 2018). Regarding the prevalence of depression, a systematic review identified twenty-four articles on the prevalence of depression in university students. However, the results suggest that most articles have methodological flaws. The reported prevalence rates ranged from 10% to 85%, with a weighted average prevalence of 30.6%. The results suggest that university students have much higher rates of depression than those found in the general population (IBRAHIM et al., 2013).

According to the DSM-V (2014) (Diagnostic and Statistical Manual of Mental Disorders), there are several categories of depressive disorders, with the difference between them being the relationship between the frequency and severity of symptoms. Major Depressive Disorder (MDD), for example, is characterized by one or more depressive symptoms, a duration of two weeks with a depressed mood or loss of interest, and at least four additional symptoms of depression: significant weight loss or gain without dieting; insomnia or hypersomnia; psychomotor agitation or retardation; fatigue and loss of energy; feelings of worthlessness or excessive or inappropriate guilt; diminished ability to think or concentrate or indecisiveness; recurrent thoughts of death (not just fear of dying); recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide (AMERICAN PSYCHIATRIC ASSOCIATION, 2014).

There are various theories about the etiology of depression. One of the main neurobiological hypotheses states that the decrease in the expression of brain-derived neurotrophic factor (BDNF) contributes to depression. This hypothesis is supported by consistent findings of low levels of BDNF in the serum of depressed patients, compared to levels detected in non-depressed patients. Meta-analyses have confirmed abnormally low concentrations of BDNF in depressed patients and its normalization by antidepressant treatment. These findings appear to reflect peripheral manifestations, consistent with the neurotrophin hypothesis: depression is a consequence of altered BDNF expression in the brain (MOLENDIJK et al., 2014; BUS et al., 2015).

In the pathophysiology of depression, in addition to BDNF, it has increasingly been confirmed in recent years that the inflammatory immune system, especially the release of signaling molecules (cytokines), could influence many of the neurochemical changes induced by stress and contribute to the development of

depression, interacting with the neuroendocrine system and specific pathways related to mood (IRWIN; MILLER, 2007). According to recent studies, alterations in the levels of pro-inflammatory cytokines (Interleukin 1β, Interleukin-6, and TNF-α) are associated with an increased risk of depression, both in animal models and in patients with depression (RAISON; CAPURON; MILER, 2006; SIMON et al., 2008).

The different types of depression are treated with specific medications, called psychopharmacological drugs. Their use is based on the already established alteration of neurochemical neurotransmitters. It is with such support that scholars explain the nature of the disease and the action of antidepressant medications (BARLOW; DURAND, 2008). Medications are the primary treatment for major depressive disorder, which explains why there is currently a wide variety of these products available. There is also another biological treatment that affects brain chemistry, known as electroconvulsive therapy (ECT) (AMERICAN PSYCHIATRIC ASSOCIATION, 2008).

Some healthcare professionals recommend psychotherapeutic interventions as a complementary treatment to psychopharmaceuticals, which may encompass different methods such as psychotherapy, psychodynamic therapy, interpersonal therapy, behavioral therapy, cognitive-behavioral therapy, group therapy, couples therapy, and family therapy, as well as interventions in lifestyle changes (SOUZA, 1999), creating new habits such as in nutrition, exercise practices, and therapeutic treatments from other eastern or western therapeutic systems, known as integrative and complementary practices in Brazil.

INTEGRATIVE AND COMPLEMENTARY PRACTICES AS A PUBLIC POLICY

Over the last three decades, significant discussions have been taking place in academic, political, and technical groups associated with the healthcare sector regarding the inclusion of other complex models of healthcare attention that are non-biomedical, referred to by the World Health Organization (WHO) as Traditional Medicine (TM) and Integrative and Complementary Medicine (ICM). These complex systems are widely used in both developed and developing countries, in both public and private settings. As such, the WHO established that member countries should implement therapeutic treatments composed of TM and ICM not belonging to the

biomedical model in healthcare systems (LUZ, 2005; WORLD HEALTH ORGANIZATION, 2013).

In this scenario, a significant event in Brazil was the creation, in 2006, of the National Policy on Integrative and Complementary Practices (PNPIC), to respond to the aspirations and demand for diverse forms of healthcare. The Integrative and Complementary Practices (ICP) regulated and implemented in the Brazilian Unified Health System (SUS) by this policy were Traditional Chinese Medicine/Acupuncture, Homeopathy, Medicinal Plants/Phytotherapy, and Social Thermalism/Crenotherapy. Later in 2006, Anthroposophic Medicine was also included as an ICP in the SUS through the approval and publication of Ministerial Ordinance No. 1600 (BRASIL, 2006).

In 2017, Ordinance 849 incorporated fourteen new practices in the PNPIC: art therapy, ayurveda, biodanza, circle dance, meditation, music therapy, naturopathy, osteopathy, chiropractic, reflexology, reiki, shantala, integrative community therapy, and yoga. Data from the National Registry of Health Establishments (CNES) demonstrate that 7,700 establishments offer some ICP, which is present in 1,708 Brazilian municipalities, covering all twenty-seven states and the Federal District, also present in all capitals. In the service network, they are mostly present in primary care (78%).

According to data from the 2nd cycle of the National Program for Improving Access and Quality of Primary Care (PMAQ-AB), 5,600 family health teams, out of the 30,000 evaluated, offer ICP services, and auricular acupuncture is offered in 908 teams, being the sixth most used ICP in the SUS. In 2016, according to e-SUS data, over two million ICP consultations were recorded in Basic Health Units (UBS), with 770,000 Traditional Chinese Medicine consultations, which includes various techniques, notably auricular acupuncture and acupuncture (BRASIL, 2017). In recent decades, there has been a significant increase in the use of Complementary and Integrative Practices (CIP) worldwide.

The growing demand for CIP has gained prominence in the health sector due to its differentiated approach, which seeks to understand the individual through a multidimensional, amplified, and singular perspective of the life-health-disease process, with the aim of improving the quality of life and well-being and promoting the

recovery and maintenance of the population's health (BRASIL, 2006; SABBAG et al., 2017; WORLD HEALTH ORGANIZATION, 2014).

Other factors that encourage the use of such practices include attentive listening, the establishment of a therapeutic bond, the individual's co-responsibility for their health, and the awakening of autonomy and empowerment (SABBAG et al., 2017).

In addition to these aspects, numerous studies demonstrate the effectiveness, safety, and mechanisms of action of CIP (WORLD HEALTH ORGANIZATION, 2013). The Cochrane, an independent, non-profit international organization that aims to gather the best evidence to support decision-making, has a reference center for CIP at the University of Maryland since 1996, producing the best evidence through systematic reviews and meta-analyses. Among the most commonly used and researched practices are those of Traditional Chinese Medicine (COCHRANE COMPLEMENTARY MEDICINE, 2018).

The history of Traditional Chinese Medicine (TCM) dates back 2,000 years. Various practices are used in TCM, in addition to acupuncture, a technique that is well-known in the West. Treatments include Chinese diet therapy and herbal therapy, Tai Chi Chuan, Lian Gong, cupping therapy, moxibustion, massage therapy, and auricular acupuncture (WORLD HEALTH ORGANIZATION, 2010).

The practice of auricular acupuncture, an ancient therapy, is according to Dal Mas (2004), the art of balancing the body through reflex points distributed in the auricular pavilion. Similar to reflexology, it is believed that the human body and all its organs and limbs are projected onto the ear, with each region corresponding to a specific point. Furthermore, it is believed that stimulation of specific points can produce systemic effects or effects on corresponding reflex regions.

EXPECTED RESULTS

The present study provides a better understanding of the efficacy and safety of auricular acupuncture in the treatment of depression, such as: efficacy in remission of depression symptoms in the short and medium term, safety related to adverse events and effects, changes in quality of life, sleep quality, and anxiety, and possible mechanisms of action related to biological markers.

In terms of benefits, the research procedures in the recruitment phase will identify patients with depression and possibly at risk of suicide - who, otherwise, would not have been identified - ensuring specialized care for severe depression or suicidal ideation. It is intended to prove the effects of a technique that has been used for millennia: if the technique's effects are positive, it could provide well-being to people suffering from depression and similar disorders.

This study will test a simple, low-cost, non-pharmacological, and easy-to-apply intervention for the treatment of depression. Therefore, it has the potential to generate great gains in public health, in terms of improving access to complementary treatments and reducing the treatment gap for depressive disorders. The knowledge gained may be crucial in influencing the agenda of collective and mental health policies in Brazil.

Finally, this research will provide training for researchers and the publication of papers in scientific journals and international conferences, involving students with scientific initiation projects, undergraduate and postgraduate thesis, and dissertations. It will also contribute to the integration of teaching, research, and extension through the topics addressed in the project, which should be discussed in undergraduate and postgraduate courses and extension projects, as well as academic-institutional exchange between universities.

RESEARCH JUSTIFICATION

A search in the databases Pubmed, Medline, Lilacs, Ibecs, Cumed, Scielo, and Cochrane found 32 articles with the keywords in English, Spanish, and Portuguese: "ear acupuncture" or "acupuncture auricular" or "auriculotherapy" and "depression" or "brain-derived neurotrophic factor" or "Interleukin" or "IC-6" or "IC-1β" or "TNFα". Studies with animals, literature reviews, qualitative studies, case studies, or studies unrelated to the topic were excluded, leaving only three studies on depression. There is a predominance of scientific articles from China, with heterogeneity in data collection. The target audience of the studies were 90 women with breast cancer, 90 addicted men, and 24 obese women. Auricular acupuncture had significant results in depression scores in all three studies, but the studies had a low sample size and high risk of biases (XIAOAI; BEI; JING, 2015; SET; CAYIR; PIRIM, 2014; LIANG, et al., 2014).

Other studies have related the effects of auricular acupuncture in different situations and psychological outcomes: improvement in anxiety before dental treatment (KARTS et al., 2007); Generalized Anxiety Disorders, Decreased Anxiety in Pre-Hospital Transport (KOBER et al., 2003); improvement in sleep quality in individuals with post-traumatic stress disorder (HING, et al., 2015); improvement in stress in nurses (KUREBAYASHI; SILVA, 2014); improvement in stress, cortisol levels, and sleep quality in middle-aged women (CHA; PARK; SOK, 2017). However, no study has used auricular acupuncture to evaluate levels of BDNF, Interleukin 1β, Interleukin-6, and TNF-α in blood plasma with symptoms of depression.

Auricular acupuncture is one of the most widely disseminated complementary and alternative medicine practices today, as it is believed to yield rapid and effective results, with few reports of adverse reactions or contraindications. Research has shown that Australians and Americans report a preference for complementary and alternative medicine practices for depression (KESSLER et al., 2000; JORM et al., 1997; JORM, et al., 2000).

Another advantage of auricular acupuncture is its application in psychiatric patients. The patient does not need to undress and can remain seated, similar to psychotherapy. This allows the patient to maintain eye contact with the therapist

without being in an inferior and passive position, such as lying down without clothes (GEIB et al., 2015).

Despite reports of safety in practice, acupuncture and auricular acupuncture practices are not entirely free of adverse events. White (2001) reported an incidence of 684 adverse events per 10,000 consultations. The majority were minor events, such as bleeding, needle pain, or worsening of symptoms. In another prospective study, MacPherson et al. (2001) demonstrated that no serious adverse events were reported after 34,407 acupuncture treatments. This is important evidence for public health, as acupuncture practitioners perform approximately two million treatments per year in the UK. Comparing this rate of adverse events with the use of routinely prescribed medications in primary care suggests that acupuncture is a relatively safe form of treatment.

Several mechanisms may explain the therapeutic effects of acupuncture in depression. Strong evidence produced in the last thirty years has demonstrated that acupuncture acts on various neurotransmitters, predominantly the endogenous opioid mechanism, catecholamines, and serotonin, norepinephrine, and approximately 20 to 30 other neuropeptides (LEUNG et al., 2014). In addition, acupuncture has produced structural and functional changes demonstrated in magnetic resonance imaging and electroencephalography in areas of the anterior cingulate cortex, amygdala, hippocampus, hypothalamus, cerebellum, and other limbic structures (HUANG, et al., 2012; LIU et al., 2009). Dysfunctions in these brain areas have been linked to depressive disorders (HAMILTON et al., 2015).

Despite published studies on the mechanism of action of the technique, most studies on the efficacy and safety of different acupuncture modalities in depression have a high risk of bias. A systematic review by Cochrane on the subject (SMITH et al., 2018) concluded that most of the 64 studies had biases due to the low methodological quality of published clinical trials. Most of the articles included in this review used other acupuncture modalities (manual acupuncture, electroacupuncture, and laser acupuncture), or combined with auricular acupuncture: only one study using auricular acupuncture alone was analyzed in this review (XIAOAI; BEI; JING, 2015).

Therefore, based on the clinical experience of professionals in the field and the good acceptance of patients, auricular acupuncture has become a widely used

practice, also because it is economically accessible and easy to apply. However, there are few clinical studies with this technique, and most of these published studies have many biases, compromising the interpretation of the results. Thus, the purpose of this study is to evaluate the efficacy and safety of auricular acupuncture in depression.

OBJECTIVES

GENERAL OBJECTIVE

The primary objective is to estimate the effectiveness of auricular acupuncture in reducing depressive symptoms compared to usual care and non-specific auricular acupuncture, three months after participant inclusion.

SPECIFIC OBJECTIVES

a) To estimate the effectiveness of auricular acupuncture in reducing symptoms six months after participant inclusion;

b) To estimate the effectiveness of auricular acupuncture in quality of life, anxiety and insomnia symptoms, and antidepressant medication use before and after the intervention, three months after participant inclusion;

c) To evaluate occasional events and adverse effects of the use of auricular acupuncture technique in the treatment of depression;

d) To evaluate levels of BDNF, Interleukin 1β, Interleukin-6, and TNF-α in the blood plasma before and after the use of auricular acupuncture;

e) To analyze the perception of the influence of auricular acupuncture on depression, quality of life, anxiety, insomnia, and medication use.

PATIENTS AND METHODS

This research is classified as a quantitative study according to its nature, experimental according to the procedures, and explanatory according to the objectives (GIL, 2012). This study is characterized as a randomized, participant-blinded, assessor-blinded, and statistical analysis-blinded clinical trial. The Consolidated Standards of Reporting Trials (CONSORT) and its extension for acupuncture, Standards for Reporting Interventions in Clinical Trials Acupuncture (STRICTA), will be used for the implementation of this research. This clinical trial will be registered on the Clinicaltrials platform.

According to Stux and Birch (2005), in the design of clinical trials in auricular acupuncture and acupuncture, several aspects should be considered: the most appropriate points for the situation, number of points, number and frequency of sessions, techniques and materials applied to each point, appropriate instruments, use of other interventions, experience and training of the professional, establishment of the control group and the sham auricular acupuncture, and the placebo effect. In addition to the difficulties of conducting a double-blind clinical trial, since complete blinding by the participant in PIC studies is challenging. Based on these considerations, the decision-making for the design will be based on preliminary studies and published guidelines.

RESEARCH SUBJECTS

The research subjects will be adults between 18 and 50 years old. The research will be advertised on university bulletin boards and invitation letters will be sent by email. The sample will be composed of individuals who reach the minimum scores and meet the inclusion and exclusion criteria of the study. Participants who agree to participate in the study will be required to sign an informed consent form (ICF). Afterward, a questionnaire with demographic, socioeconomic, health, and behavioral variables will be applied to determine if they are eligible to participate in the study based on the inclusion and exclusion criteria, and to identify individuals with depression symptoms using the Patient Health Questionnaire - PHQ-9. Participants who obtain moderate depression scores will be selected. The sample will be

composed of individuals who reach the minimum scores and meet the inclusion and exclusion criteria of the study. The ICF and all questionnaires will be administered through the REDCap platform to ensure the management of participant data according to the General Data Protection Law (GDPL).

RANDOMIZATION AND BLINDING

For the randomization process, a computer program will be used to perform the randomization in a 1:1 ratio for the two study groups: experimental group (auricular acupuncture - specific points for depression and usual care) and control group (auricular acupuncture - non-specific points and usual care). The allocation of participants will be concealed, meaning it will be done by a computer-generated random sequence, delivered in an opaque and sealed envelope, containing a letter informing the group to which the participant will be assigned.

Participants will be blinded to the group assignment of auricular acupuncture (specific and non-specific points for depression). They will be informed that there are two treatment protocols: an individualized auricular acupuncture based on Traditional Chinese Medicine diagnosis (experimental group) and a fixed point protocol for this study (control group - non-specific auricular points).

The evaluators of the study will be blinded to the groups. The auricular acupuncture applicators will not know the results of the participants' questionnaire scores. Finally, the statistician will also be blinded to the participants' origin when preparing the results reports.

SAMPLE SIZE

The sample size will be designed to detect a difference of 20% (experimental group 60% and control group 40%) in the recovery of depressive symptoms (PHQ-9 <10) between the arms. The minimum sample size is estimated to be 107 participants per group, considering the test as two-tailed, with 80% power and a significance level of 5%. An additional 30% will be added to account for possible dropouts, totaling 140 in each group, for a total of 280 participants. For secondary outcomes related to biological markers, a new randomization stage will be carried

out. This procedure aims to respect ethical principles related to non-maleficence, as well as the financial aspect of the proposal. The sample size for differences in means of biological markers was stipulated according to the standardized effect size magnitude indicated for comparisons between effect magnitudes of different variables. A standardized effect size magnitude of 0.7 will be used, considering α (bilateral) = 0.05 and β = 0.20. A minimum sample size of 34 participants per group is estimated (BROWNER; NEWMAN; HULLEY, 2008). For qualitative data, 20 participants will be randomly selected from each group.

ELIGIBILITY

The eligibility of the study will be assessed in a pre-screening process in the classrooms of the University. The inclusion and exclusion criteria of the study are as follows.

Inclusion Criteria

• Age between 18 and 50 years old;
• Obtaining minimum scores on the PHQ-9 for moderate depression;
• Availability of time for the sessions.

Exclusion Criteria

• Utilization of complementary therapies for at least 3 months;
• Risk of suicidal ideation assessed through the "Suicide-Risk Assessment Protocol" (S-RAP);
• Severe depression assessed through the PHQ-9 scores;
• Application of auricular acupuncture for depression previously;
• Pregnancy;
• Menopause;
• Allergy to metals and micropore;

• Lobes of the auricular pavilions inaccessible due to mutilation, cartilage deformation, perforation by wearing earrings or other artifacts that make it impossible to apply the auricular technique in the specific point.

STUDY SITE

The research will be conducted at the Clinical Schools of the University of Southern Santa Catarina (UNISUL), Cruzeiro do Sul University (UNICSUL), and Cidade de São Paulo University (UNICID).

PRIMARY OUTCOME:

The primary outcome will be the proportion of participants who show improvement of 50% or more in depressive symptoms, as evaluated by the PHQ-9, three months after inclusion. A follow-up score at 3 months demonstrating 50% improvement from baseline is considered successful or remission of symptoms.

SECONDARY OUTCOMES:

The study's secondary outcomes will be included to enrich the understanding of the primary outcome through a series of exploratory analyses, although the study was not designed to answer these scientific questions:

The proportion of participants who show improvement of 50% or more in depressive symptoms, in each treatment group, as evaluated by the PHQ-9, six months after inclusion;

Serum levels of BDNF, Interleukin 1β, Interleukin-6, and TNF-α, assessed at the beginning and end of auricular acupuncture applications (end of the sixth week of application);

Quality of life levels evaluated by the SF-36, sleep quality (PSQI), anxiety (IDATE) at the beginning, end of the sixth week of auricular acupuncture application, three and six months after inclusion;

Adverse events measured by the PHQ-9 and adverse effects;

Changes in medication (antidepressants, anxiolytics).

MATERIALS:

The materials used in the research will be: Patient Health Questionnaire - PHQ-9; Short Form Health Survey Questionnaire (SF 36); adverse effects questionnaire; Safety Monitoring Instrument S-RAP; Material for blood collection, disposable and semi-permanent auricular acupuncture needles, micropore, 70° alcohol, cotton, tweezers, electronic auricular point locator.

DATA COLLECTION INSTRUMENTS

Blood Collection

BDNF, Interleukin-1β, Interleukin-6, and TNF-α will be analyzed through blood plasma in the first and last session (twelfth), collected in the morning. The study participant will remain seated for 30 minutes before collection. Blood (4ml) will be collected in tubes with the anticoagulant EDTA, and after 15 minutes, the tube will be placed on ice and subsequently centrifuged for 20 minutes at a rotation of 3,000 rpm and a temperature of 8°C. The plasma will be separated, and 300 microliters will be pipetted into eppendorf and stored at a temperature of -80°C for later analysis of plasma levels. The markers will be evaluated by the ELISA method.

Evaluation of Depression Levels

The Patient Health Questionnaire - PHQ-9 (SANTOS et al., 2013) will be used to assess depression levels. It is a self-assessment instrument used worldwide. The PHQ-9 is a brief, validated instrument widely used in clinical research and sensitive to changes over time (LOWE et al., 2004; ELL et al., 2007).

The PHQ-9 consists of nine questions that evaluate the presence of each major depression symptom according to the DSM-IV. The frequency of each symptom in the last two weeks is evaluated on a Likert scale of 0 to 3 points, corresponding to the answers "not at all," "several days," "more than half the days," and "nearly every day," respectively. The questionnaire also includes a tenth

question that assesses the interference of these symptoms in the performance of daily activities, such as working and studying (SANTOS et al., 2013).

The distribution of scores ranges from 0 to 27 points, classified according to diagnostic levels of depression: absence of depression (0-4), mild symptoms (5-9), moderate symptoms (10-19), and severe symptoms (20-27) (LOWE et al., 2004; KROENKE; SPITZER; WILLIAMS, 2001). A PHQ-9 score of <10 is considered "recovered," since scores ≥ 10 are considered for a depression treatment plan.

Evaluation of Adverse Events and Effects

In this study, adverse events include: a) worsening of depressive symptoms, measured by the PHQ-9; b) appearance of suicidal ideation or self-injury, as evaluated by the S-RAP.

Interviewers may identify severely depressed or suicidal individuals during data collection, and specific actions to deal with these situations are included in the protocol. The PHQ-9 and S-RAP scores obtained will be analyzed by the research supervisor immediately after the evaluators collect the information. After identifying a participant at risk at any time during the study, a consultation with a psychiatrist researcher from the study will be scheduled for treatment of these symptoms, as well as an indication to seek treatment at the Psychology and Naturology School Clinic at the University.

Any worsening of depressive symptoms may not be related to the study itself. Considering that the proposed intervention is to reduce depressive symptoms, it is very unlikely to be associated with an increase in depression. According to a systematic review of clinical studies involving acupuncture and auricular acupuncture in depression, included studies confirm safety for depression treatment (SMITH et al., 2018). However, it is possible that depression may increase despite the intervention, and that is why routine evaluation of depressive symptoms and suicidal ideation throughout the intervention is necessary. This is related to the safety and monitoring plan to avoid inadvertent worsening of their conditions.

For the evaluation of adverse effects of auricular acupuncture, a questionnaire developed specifically for this study will be used in all sessions by the auricular acupuncture practitioners.

Quality of Life Assessment

The Short Form Health Survey Questionnaire (SF-36) is a generic measure of quality of life that is highly sensitive to individual improvement. It consists of 36 items, of which 35 are grouped into eight dimensions (physical functioning, pain, physical role functioning, emotional role functioning, social role functioning, mental health, vitality, and general health perceptions), while the last item evaluates changes in health over time in order to examine the participant's perception of their own health status. The higher the score, the better the individual's quality of life. Therefore, changes in quality of life can be assessed by a higher or lower score on the questionnaire resulting from certain treatments and assistance programs for groups of individuals with various health conditions. For each dimension, SF-36 items are coded, grouped, and transformed into a scale from zero (worst health state) to 100 (best health state). The questionnaire has been translated into Portuguese and adapted to the Brazilian culture (CICONELLI et al., 1997).

Sleep Quality Assessment

The Pittsburgh Sleep Quality Index (PSQI) questionnaire will be used to evaluate the levels of sleep disturbances. The Portuguese-translated version of the questionnaire, without changes from the original version, will be utilized and validated by Bertolazi et al. (2011). The questionnaire contains 19 questions about sleep quality and disturbances over the last month, assessing seven components of sleep: subjective quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleep medications, and daytime dysfunction. The score for each component ranges from 0 to 3, with a maximum score of 21 points (BERTOLAZI et al., 2011). The score ranges from 0 to 3, with 0 indicating "no difficulty" and 3 indicating "severe difficulty." The scores will be added to form a global value ranging from 0 to 21. Scores of 0-4 indicate good sleep quality, scores of 5-10 indicate poor quality, and scores above 10 indicate possible sleep disorders (LOURENÇO et al., 2012).

Anxiety Assessment

The IDATE questionnaire is an instrument for classifying individuals according to their level of anxiety. It has two stages, the State Anxiety and Trait Anxiety. Classification is based on the score, which ranges from 20 to 34 (low), 35 to 45 (moderate); 50 to 64 (high) and 65 to 80 (very high). The questionnaire was translate and validated for use in Brazil (BIAGGIO; NATALÍCIO; SPIELBERGER, 1977).

Participant Perception

Qualitative data will be collected through a semi-structured interview, which will be recorded and fully transcribed. For data analysis, the thematic content analysis technique systematized by Minayo (2014) will be used. For the qualitative data, 20 participants from each group will be randomly selected for analysis.

VARIABLES

There may be differences in the results among the participants of the study due to factors such as:

Independent variable

a) Experimental group (specific auricular acupuncture and usual care), control group (non-specific auricular acupuncture and usual care).

Dependent variables

a) Depression levels;
b) BDNF levels;
c) Interleukin-1β levels;
d) Interleukin-6 levels;
e) TNF-α levels;
f) Quality of life levels;
g) Sleep quality levels;
h) Anxiety levels;

i) Adverse effects;

j) Change in medication;

k) Participant perception.

Control variables

a) Medication;

b) Usual care.

PROCEDURES

The implementation of the present project will take place after approval by the Ethics and Research Committee of the School of Medicine at USP and related committees, subject to the availability of participants and researchers. Initially, potential participants will be instructed about the research objectives, as well as the respective ethical implications, through the Informed Consent Form. Then, a questionnaire will be administered to collect demographic, socioeconomic, health, and behavioral variables to determine eligibility according to inclusion and exclusion criteria. The PHQ-9 will be used to identify participants with symptoms of depression, and the SF-26 will be used to evaluate quality of life, sleep quality (PSQI), and anxiety levels (IDATE). All these instruments will be administered via tablets by researchers through the REDCap platform.

Study assessors will be trained to approach participants in the exact same manner to avoid recruitment biases. The study will have a protocol of questions to be used in all phases: pre-screening, screening, data collection, and protocol application. After randomization, blood collection will occur for all participants in the morning. Participants with severe depression (PHQ≥20) or suicidal ideation will not be included in the study and will be followed up by a psychiatrist researcher from the study and referred to the university's Psychology and Naturology clinic for treatment.

Auricular acupuncture sessions will take place twice a week for 15 minutes in a private and confidential consultation room reserved for the research, subject to the availability of participants and researchers, for 6 weeks. The experimental group will consist of a protocol of points chosen according to the diagnosis of depression by

TCM. Six pre-established points will be used in all participants: Shen men, Sub-cortex, Heart, Lung, Liver, and Kidney (FOCKS, 2005; XIAOAI; BEI; JING, 2015; LIANG, et al., 2014).

International literature recognizes the difficulty in establishing protocols to be used as a control due to the high responsiveness and innervation of the auricular pavilion (PRADO, 2014). Therefore, the control group (auricular acupuncture - non-specific points) was chosen based on a previous protocol. The external ear and cheek/face area points (PRADO, 2014), and four non-specific points in the helix region (ZHONG, et al., 2016) will be used.

The first application will be performed on the right auricular pavilion of the participants, and subsequent sessions will alternate ears with each application. Participants will be instructed to keep the needles in and to stimulate the points manually for 30 seconds, three times a day (morning, afternoon, and night), every day. The research applicators will remove the needles applied in the previous session and apply them to the same points on the other auricular pavilion, ensuring constant stimulation of the free nerve endings. The research applicators will instruct the participants to manually stimulate each point for 30 seconds, three times a day (morning, afternoon, and night), every day.

All participants will respond to the PHQ-9, SF-36, and S-RAP questionnaires again in the fourth and sixth week of the study, after three and six months from the beginning of the study. Blood collections will take place at the beginning of the study and at the end of the application of the protocols so that analyses and comparisons of the participants' symptom evolution can be made.

In the final evaluation (6 months), each participant from the experimental (auricular acupuncture) and control group (non-specific auricular acupuncture) will be asked about which group they believed they were participating in, to evaluate the influence of this bias and whether the protocol applicator informed them of their group allocation. If it is found that the participant was not blinded or had access to other therapies, they will be classified as non-responders and excluded from the final analysis.

To ensure the blinding of evaluators, the protocol applicators will remove the needles from the participants' auricular pavilions prior to the data collection interviews. In addition to this procedure, study participants will be instructed not to

provide information about auricular acupuncture to the evaluators, thus avoiding unmasking the intervention. Evaluators will also be trained to avoid this bias.

The study protocol applicators will not be aware of the participants' levels of depression, quality of life, sleep quality, and anxiety. During the application, communication with participants will be limited to the necessary explanations about the study to avoid possible biases through suggestive observations. These researchers will be trained to avoid this type of interference. Professionals with specific training in the area and a minimum of three years of experience will be selected.

At the end of the research, control group participants will be invited to receive the same care protocol as the auricular acupuncture group if this protocol proves to be effective, without any cost to them, ensuring ethical principles of justice and equity.

This study will follow the criteria of good clinical practice according to the "The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use" of the International Conference on Harmonisation (ICH), including independent regular monitoring and the establishment of an independent data monitoring and safety committee. In addition to these guidelines, the ethical principles advocated in the Helsinki Declaration and National Health Council resolutions related to research ethics, especially CNS Resolution 466/2012, will be followed.

Human resources

The research will involve the participation of eight researchers: research supervisor (responsible for the training of evaluators and applicators and for monitoring events and adverse effects); research assistant (responsible for participant follow-up, scheduling, and follow-up); two evaluators (responsible for instrument application and blood collection); two applicators (responsible for protocol application); psychiatrist (responsible for the follow-up of participants at risk or with severe depression); statistician (responsible for randomization, allocation, and data analysis).

ADHERENCE AND FOLLOW-UP

Participants who do not return after the data collection instrument evaluations will be considered non-adherent to the study. Two non-consecutive absences during treatment sessions will be permitted, as the literature recommends weekly applications of auricular acupuncture (DAL MAS, 2004). Participants who do not return for evaluations will be considered lost to follow-up.

To ensure study retention, researchers will involve course coordinators and instructors to support the implementation of the study during class periods and the release of university students for data collection and auricular acupuncture protocols.

DATA ANALYSIS

The study data will be analyzed using Stata 14 software. The data analysis will be performed according to the CONSORT guidelines, primary analysis with intention-to-treat, emphasizing confidence intervals for between-group comparisons. Descriptive statistics will be used to determine any imbalances between the groups at baseline. A similar analysis, adjusting for baseline PHQ-9 scores and any observed imbalances between groups of possible predictor variables, will also be performed. Other secondary analyses will compare individuals according to the intervention received, accounting for any selection after random allocation. These analyses using mean comparisons will employ instrumental variable regression models. We will perform an exploratory subgroup analysis to investigate who benefits most from this intervention, but these analyses will be interpreted with caution because statistical power will be limited for interaction effects.

ETHICAL CONSIDERATIONS

For this research, the recommendations of Resolution n° 466/12 of the National Health Council (CNS) are adopted, as the study involves the participation of human subjects. Participants will be provided with one of two copies of the Informed Consent Form to ensure compliance with the ethical principles outlined in Resolution n° 466/12.

In addition, this research project ensures the confidentiality and anonymity of the participants, the return of research results to subjects and society, and access to research results by the public. The researchers declare no conflicts of interest related to the study's outcomes. The research project will be submitted to the Research Ethics Committee (CEP) of the University of Sao Paulo School of Medicine for evaluation. Once approved by the CEP, data collection will begin. Data will be collected only after approval and will be stored for five years before being discarded.

Participants with severe depression or suicidal ideation will not participate in the study due to the severity of their symptoms. These participants will be accompanied and medicated by a psychiatrist and researcher from the study according to their needs, in addition to being referred to the Psychology Clinic for psychological treatment and the Naturology Clinic at the University for complementary treatment.

If the treatment group's effects are clinically significant when compared to control groups I and II, the researchers will make the auricular acupuncture protocol available to interested participants.

On the risk-benefit balance:

Understanding that, according to Resolution CNS 466/12, all research with human beings involves risks of varying types and degrees, this research foresees minimal risks, but we will anticipate some circumstances in which they may appear.

During the recruitment phase, the screening process using the PHQ-9 questionnaire may be disturbing to some participants. The nature of the questions may induce certain emotional feelings, including sadness or crying. Others may worry about the PHQ-9 results, which suggest the presence of depressive symptoms or the need for other specialized care. We will train all research evaluators in ways to minimize these feelings and how to handle these situations.

From screening to any follow-up contacts, researchers may encounter severely depressed, suicidal, or otherwise severely mentally ill individuals. These participants will be excluded from the study analysis and referred to a psychiatrist who will take necessary measures according to each case.

Some participants may be concerned about the confidentiality of their data, particularly in relation to the possible exposure of opinions that could be embarrassing to peers. To minimize risks such as this, precautions will be taken to maintain confidentiality and anonymity.

The other minimal risks relate to minor discomforts associated with auricular acupuncture, such as painful sensations at the auricular point and the possible emergence of some other physical or emotional reaction resulting from the technique, or some physical discomfort with blood collection. Although researchers may not be able to identify other risks, if they are identifiable, they will be controlled.

In terms of benefits, the research procedures during the recruitment phase will identify patients with depression and possibly at risk for suicide who would not have been identified otherwise, ensuring specialized care for severe depression or suicidal ideation or a millenary treatment for participants in the experimental group. It is intended to prove the effects of a technique that has been used for millennia, and if the effects are positive, this technique could provide well-being to people suffering from depression and similar disorders.

This study will test a simple, inexpensive, non-pharmaceutical, and easy-to-apply intervention for depression. Therefore, it has the potential to generate significant gains in public health in terms of improving access to complementary treatments and reducing the treatment gap for depressive disorders. The knowledge acquired may be essential in influencing the agenda of collective and mental health policies in Brazil.

Furthermore, the study results will be made available to participants by email or in the manner the participant chooses, after completion of the study.

Thus, this research is justified in its risk-benefit balance, as the potential benefits outweigh the possible risks. Since auricular acupuncture is widely used worldwide, considered a technique that is easy to apply, low-cost, and with minimal side effects.

On the process of free and informed consent:

In order to ensure proper respect and human dignity, this study provides for a process of free and informed consent. Therefore, the participants will be contacted by

the researchers, either in person or by telephone, to invite them to participate in the research. The best time, condition, and location suitable for the participant's privacy, as determined by the participant, will be identified. Sufficient time will be provided for reading, understanding, and reflection. The informed consent form will be drafted in a clear and accessible language for the participants, which must be read and understood before granting their free and informed consent. Only after signing the informed consent form will the questionnaires be provided. The completed questionnaires will be placed in a paper envelope, and only the researchers of this study will have access to the data.

On the Informed Consent Form:

All recommendations of Resolution CNS 466/12 will be followed in drafting this form. It will be prepared in duplicate, with both copies signed by the researchers and participants, and the participant will retain the 2nd copy with the respective protocol number. The informed consent form will also include contact information for the researchers, CEP-USP, and participating centers.

REFERENCES

AMERICAN PSYCHIATRIC ASSOCIATION. **Diretrizes para o tratamento de transtornos psiquiátricos**: compêndio 2006. Porto Alegre: Artmed, 2008.

_____. **Manual diagnóstico e estatístico de transtornos mentais**: DSM-5. Porto Alegre: Artmed, 2014.

BARLOW, D.H; DURAND, V.M. **Psicopatologia**: uma abordagem integrada. São Paulo: Cengage Learning, 2008.

BECK, A.T.; ALFORD, B.A. **Depressão causas e tratamento**. 2. ed. Porto Alegre: Artmed, 2011.

BRASIL. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. **Política Nacional de Práticas Integrativas e Complementares no SUS - PNPIC.** Brasília: Ministério da Saúde, 2006.

_____. Departamento de Atenção Básica. **Portaria amplia oferta de PICS**. 2017.

BRODY, D.J.; PRATT, L.A.; HUGHES, J.P. Prevalence of depression among adults aged 20 and over: United States, 2013-2016. **NCHS Data Brief**, n. 303, p. 1-8, Feb. 2018.

BROWNER, W.S.; NEWMAN, T.B.; HULLEY, S.G. Estimando o tamanho da amostra e o poder estatístico: aplicações e exemplos. In: HULLER, S.G et al. **Delineando a pesquisa clínica**: uma abordagem epidemiológica. 3. ed. Porto Alegre: Artmed, 2008.

BUS, B. A. et al. Chronic depression is associated with a pronounced decrease in serum brain-derived neurotrophic factor over time. **Mol Psychiatry**. v. 20, n. 5, p. 602-8. May. 2015.

CHA, N. H.; PARK, Y.K. SOK, S.R. Effects of auricular acupressure therapy on stress and sleep disturbance of middle-aged women in South Korea. **Holist Nurs Pract.** v. 31, n. 2, p. 102-109. Mar/Apr. 2017.

CICONELLI, R.M. et al. Tradução para a língua portuguesa e validação do questionário genérico de avaliação de qualidade de vida SF-36 (Brasil SF-36). **Rev Bras Reumatol**., v. 29, n. 3, p. 154-50. 1997.

COCHRANE COMPLEMENTARY MEDICINE. **About us.** 2018.

DAL MAS, Walter Douglas. **Acupuntura auricular na doutrina brasileira**. São Paulo: Roca, 2004.

ELL, K. et al. Managing depression in home health care: a randomized clinical trial. **Home health care services quarterly**, v. 26, n. 3, p. 81-104. 2007.

FOCKS, C. **Atlas de acupuntura**: com sequência de fotos e ilustrações, textos didáticos e indicações clínicas. Barueri: Manole, 2005.

FU, W.B et al. Depressive neurosis treated by acupuncture for regulating the liver-a report of 176 cases. **J Tradit Chin Med.,** v. 29, n. 2, p. 83-6, Jun. 2009.

GEIB, J et al. (2015) Introduction of auricular acupuncture in elderly patients suffering form major depression: protocol of a mixed methods feasibility study. **Biomed Res Int.,** p. 1-6, May. 2015.

GUO, N. et al. Prevalence of depression among women of reproductive age in the United States. **Obstet Gynecol**., v. 131, n. 4, p. 671-679, Apr. 2018.

HAMILTON, J.P. et al. Depressive rumination, the default-mode network, and the dark matter of clinical neuroscience. **Biological Psychiatry**, v. 78, n. 4, p. 224-30. 2015.

HUANG, W. et al. Characterizing acupuncture stimuli using brain imaging with FMRI – a systematic review and meta-analysis of the literature. **PLoS One**, v. 7, n. 4. 2012.

HENDRICK, V. et al. Evaluation of mental health and depression during pregnancy. **Psychopharmacol Bull,** v. 34, p. 297-9, 1998.

HING, H.C. et al. Auricular acupuncture for sleep disturbance in veterans with post-traumatic stress disorder: a feasibility study. **Mil. Med**., v.180, n. 5, p. 582-90, 2015.

IBRAHIM, A.K. et al. A systematic review of studies of depression prevalence in university students. **J Psychiatr Res**., v.47, n. 3, p. 391-400, Mar. 2013.

IRWIN, M. R.; MILLER, A.H. Depressive disorders and immunity: 20 years of progress and discovery. **Brain Behav Immun.**, v. 32, n. 4, p. 374-83, May. 2007.

JORM, A.F. et al. Mental health literacy: a survey of the public's ability to recognise mental disorders and their belief about the effectiveness of treatment. **Medical Journal of Australia***, n.* 166, p. 182-6. 1997.

JORM, A.F. et al. Public beliefs about the helpfulness of interventions for depression: effects on actions taken when experiencing anxiety and depression symptoms. **Australia and New Zealand Journal of Psychiatry,** *v.34, p.* 619-26. 2000.

KARST, M. et al. Auricular acupuncture for dental anxiety: a randomized controlled trial. **Anesth Analg**. v. 104, n. 2, p. 295-300. 2007.

KESSLER, R.C.; SOUKUP, J.; DAVIS, R.B. The use of complementary and alternative therapies to treat anxiety and depression in the United States. **American Journal of Psychiatry,** n. 158, p. 289-94, 2000.

KOBER, A. et al. Auricular acupressure as a treatment for anxiety in prehospital transport settings. **Am Soc Anesthesiol.,** v. 98, p.1328-32. 2003.

KROENKE, K., SPITZER, R.L.; WILLIAMS, J.B. The PHQ-9: validity of a brief depression severity measure. **J Gen Intern Med**. v.16, n. 9, p.606-13. 2001.

KUREBAYASHI, L.F.S.; SILVA, M.J.P. Eficácia da acupuntura auricular chinesa para o estresse em equipe de enfermagem: ensaio clínico randomizado. **Rev. Latino-Am. Enfermagem**, v. 22, n. 3, p. 371-378, Jun. 2014.

LEUNG, M.C. et al. Mechanisms underlying the effect of acupuncture on cognitive improvement: a systematic review of animal studies. **Journal of Neuroimmune Pharmacology**, v. 9, n. 4, p. 492-507. 2014.

LIANG,Y. et al. Comparative study on effects between electroacupuncture and auricular acupuncture for methamphetamine withdrawal syndrome. **Zhongguo Zhen Jiu**, v. 34, n. 3, p. 219-24, Mar. 2014.

LIU, P. et al. Combining spatial and temporal information to explore function-guide action of acupuncture using fMRI. **Journal of Magnetic Resonance Imaging,** v. 30, p. 41-6. 2009.

LÖWE, B. et al. Monitoring depression treatment outcomes with the patient health questionnaire-9. **Medical care**, v. 42, n.12, p.1194-201. 2004.

LUZ, M.T. Cultura Contemporânea e Medicinas Alternativas: Novos paradigmas em saúde no fim do século XX. **Physis**: Revista Saúde Coletiva. Rio de Janeiro, 15(Suplemento):145-176, 2005.

MACPHERSON, H. et al. The York acupuncture safety study: prospective survey of 34,000 treatments by traditional acupuncturists. **BMJ**, v.323, p.486-7, 2001.

MOLENDIJK, M.L. et al. Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). **Mol Psychiatry,** v. 19, n. 7, p.791-800, Jul. 2014.

NATIONAL INSTITUTE OF MENTAL HEALTH. Depression and College Students. 2018. https://www.nimh.nih.gov/health/publications/depression-and-college-students/index.shtml

PRADO, J.M. **Aplicação da acupuntura auricular verdadeira e sham no tratamento do estresse em enfermeiros**. 2014. 98 p. Dissertação (Mestrado em Enfermagem na Saúde do adulto), Universidade de São Paulo, São Paulo, 2014.

RAISON, C. L.; CAPURON, L.; MILLER, A.H. Cytokines sing the blues: inflammation and the pathogenesis of depression. **Trends in Immunology**, v. 27, n. 1, p. 24-31, 2006.

SABBAG, S.H.F. et al. A. Origem e desenvolvimento da Naturologia no Brasil: aspectos conceituais, históricos e políticos. **Cad. Naturol. Terap. Complem**, v. 6, n. 11, p. 59-68, Set. /Abr., 2017.

SANTOS, I. S. et al. Sensibilidade e especificidade do Patient Health Questionnaire-9 (PHQ-9) entre adultos da população geral. **Cad. Saúde Pública**, Rio de Janeiro, v. 29, n. 8, p. 1533-1543, Aug. 2013.

SET, T. CAYIR, Y. PIRIM, A.B. Effects of ear acupuncture therapy for obesity on the depression of obese women. **Acupunct Med.,** v. 32, n. 5, p. 427-9, Oct. 2014.

SIMON, N. M., et al. A detailed examination of cytokine abnormalities in Major Depressive Disorder. **European Neuropsychopharmacology,** v.18 , n. 3, p. 230-233, 2008.

SMITH, C.A. et al. Acupuncture for depression. **Cochrane Database of Systematic Reviews,** n. 3, 2018.

SOUZA, F.G.M. Tratamento da depressão. **Rev. Bras. Psiquiatr**, v. 21, n.1, Maio.1999.

STUX, G. BIRCH, S. Padrões de tratamento por acupuntura propostos para estudos clínicos. In: STUX, G. BERMAN, B; POMERANZ, B. **Basics of acupuncture**. Berlin: Springer, 2005.

WHITE, A. et al. Survey of adverse events following acupuncture (SAFA): a prospective study of 32, 000 consultations. **Acupuncture in Medicine**, v.19, p. 84-92, 2001.

WORLD HEALTH ORGANIZATION. **Benchmarks for training in traditional /complementary and alternative medicine**: benchmarks for training in traditional Chinese medicine. 2010. Genebra: Who: 2010.

_____. **WHO Traditional Medicine Strategy 2014-2023**. Genebra: WHO. 2013.

_____. **Atlas de Medicina Complementares e Tradicional**. 2014. Genebra: OMS.

_____. **Depression and Other Common Mental Disorders:** Global Health Estimates. Genebra: OMS, 2017a.

_____. Mental Health. **Depression:** let's talk. 2017b.

XIAOAI, L.V.; BEI, W.A.N.G; JING, Y.E. Clinical observation of depression after breast cancer operation treated with auricular point sticking therapy. **Zhongguo Zhen Jiu**, v. 35, n. 5, p. 447-50, May. 2015.

ZHONG, L.L.D. et al. The combination effects of body acupuncture and auricular acupressure compared to sham acupuncture for body weight control: study protocol for a randomized controlled trial. **Trials,** n. 17, n. 346. 2016.