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The auriculotherapy effect and safety on situational anxiety triggered by examinations.

Andreia Soares Vieira

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Instituto Ciências Biomédicas Abel Salazar

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The auriculotherapy effect and safety on situational anxiety triggered by examinations.

AUTHOR´S DECLARATION

Under paragraph 2a, Art. 31 of Decret-Law no. 65/2018, results from the publications listed below were used in this thesis. In compliance with the conditions above, the author declares she has participated in the study design, execution, and interpretation of the results. Moreover, the manuscripts formulated for this thesis are presented below, written under the name Vieira, A (Andreia Vieira).

"De acordo com o disposto no ponto nº 2, alínea a, do Art.º 31º do Decreto-Lei nº 65/2018, nesta tese foram utilizados resultados das publicações abaixo indicadas. No cumprimento do disposto no referido Decreto-Lei, o autor desta tese declara que interveio na conceção e na execução do trabalho experimental, na interpretação dos resultados e na redação dos manuscritos abaixo citados, sob o nome de Vieira, A (Andreia Vieira)."

Articles in international peer-reviewed journals:

- *Paper 1.* ***Vieira, A****.; Moreira, A. Machado, J. Outlining auriculotherapy in Anxiety as an Evidence-Based Medicine: A Brief Overview. Revista Internacional de Acupuntura, 202 .(review article accepted for publication - waiting publication).*
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"The good life is one inspired by love and guided by knowledge."

Bertrand Russell

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Abbreviations

AA: Auriculotherapy

AD: Anxiety disorders

AMSTAR: Assessment of Multiple Systematic Reviews

BNST: Bed nucleus of the stria terminalis

CI: Confidence intervals

CNS: Central nervous system

CONSORT: Consolidated standards of reporting trials

DSM-V: Diagnostic and statistical manual of mental disorders

EBMR: Evidence Based Medicine Reviews

fMRI: functional magnetic resonance imaging

GABA: Gamma-aminobutyric acid

GAD: Greater auricular nerve

GRADE: Grading of recommendations assessment development and evaluation

HPA: hypothalamic–pituitary–adrenal axis

I2: Heterogeneity

LC: Locus coeruleus

MD: Mean difference

NE: Norepinephrine

NRC: Nursing Reference Center

NTS: Nucleus tractus solitarius

PA: pharyngeal arches

PRISMA: Preferred reporting items for systematic reviews and meta-analyze

PsycINFO: Psychology and Behavioral Sciences Collection

RCT: Randomized controlled trials

RRs: Relative risks

SMD: Standardized mean difference

STAI: State-trait anxiety inventory

TCM: Traditional Chinese medicine

TN: Trigeminal nerve

VAS: Visual Analogic Scale for anxiety

VN: Vagus nerve

WHO: World Health Organization

WMD: Weighted mean differences

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Abstract

Background: Auriculotherapy is a therapeutic technique used for various conditions and could support standard treatment for anxiety disorders, but its effectiveness and safety remain undetermined. The aim of this thesis was: 1. Critically analyze published literature on auriculotherapy, 2. To provide an overview of this technique's effectiveness in managing health disorders; 3. Determine in which health conditions can auriculotherapy be used with efficacy; 4. To establish whether this technique is effective and safe for treating people with anxiety disorders. **Methods:** Firstly, through the search and analysis of pre-clinical and clinical research in PubMed/MEDLINE databases, related articles were used for narrative research. Secondly, the portfolio of systematic reviews based on meta-analysis with high methodological quality was gathered by evaluating well-known computerized databases. Later, randomized controlled trials showing auriculotherapy´ efficacy and safety in anxiety were also reviewed, and meta-analyses were conducted using the statistical software RevMan V5.4. Finally, a randomized, controlled pilot trial was conducted. The day before the exam, university students were randomly allocated to the auriculotherapy group or the waiting-list group. The outcomes were: i) the State-Trait-Anxiety Inventory; ii) quality of night sleep; iii) visual analogue scale for anxiety; iv) salivary cortisol, and v) adverse events. **Results:** The findings of our narrative review lend further support for auriculotherapy mechanisms of action to enhance mental health and serve as a robust resource for the general population. A total of 14 reviews focused on managing insomnia, smoking cessation, and pain was found. Besides that, nine of thirteen trials were included in the meta-analysis, and while auriculotherapy was effective in pre-operatory anxiety, there was a lack of evidence for students' anxiety. However, the auriculotherapy showed to reduce the outcomes compared with no treatment for students' anxiety and those students slept better. **Conclusions**: Auriculotherapy appeared safe and effective in reducing anxiety levels before university exams.

Keywords: auriculotherapy; anxiety; ear innervation parasympathetic nervous system;

brain modulation.

Resumo

Introdução: A auriculoterapia é uma técnica terapêutica utilizada para várias condições e pode apoiar o tratamento padrão na ansiedade, mas sua eficácia e segurança permanecem indeterminadas. O objetivo desta tese foi: 1. Analisar criticamente a literatura publicada sobre auriculoterapia; 2. Fornecer uma visão geral da eficácia desta técnica na saúde; 3. Determinar em quais condições de saúde a auriculoterapia pode ser utilizada com eficácia; 4. Descrever se esta técnica é eficaz e segura para o tratamento de pessoas com perturbações de ansiedade. **Métodos:** Primeiramente, realizou-se uma análise de pesquisas pré-clínicas e clínicas nas bases de dados PubMed/MEDLINE, para revisão bibliográfica. Em segundo lugar, realizamos um portfólio de revisões sistemáticas baseadas em meta-análises com alta qualidade metodológica utilizando bases de dados informatizadas de renome. Posteriormente, também foram verificados ensaios controlados e randomizados mostrando a eficácia e segurança da auriculoterapia na ansiedade. As metanálises foram realizadas usando o software estatístico RevMan V5.4. Finalmente, realizamos um estudo piloto randomizado e controlado. No dia anterior ao exame, os universitários foram alocados aleatoriamente no grupo de auriculoterapia ou no grupo da lista de espera. Procuramos o efeito nas seguintes escalas/medidas de avaliação: i) o Inventário Estado-Traço-Ansiedade; ii) qualidade do sono noturno; iii) escala visual analógica para ansiedade; iv) cortisol salivar, v) eventos adversos. **Resultados:** A revisão narrativa procura dar suporte adicional aos mecanismos de ação da auriculoterapia para melhorar a saúde mental e servem como um recurso robusto para a população em geral
Encontramos um total de 14 revisões focadas no tratamento da insônia, cessação do tabagismo e dor. Além disso, nove dos treze estudos incluídos na nossa parte qualitative foram incluídos na meta-análise e, embora a auriculoterapia tenha sido eficaz na ansiedade pré-operatória, houve falta de evidências para a ansiedade de estudantes. No entanto, com base no estudo piloto final, concluímos que a auriculoterapia mostrou-se eficaz em comparação com os alunos sem tratamento, e esses alunos dormiram melhor. **Conclusões:** A auriculoterapia é segura e eficaz na redução dos níveis de ansiedade antes dos exames universitários.

Palavras-chave: auriculoterapia; ansiedade; inervação da orelha; sistema nervoso parassimpático; modulação cerebral.

Overview

This thesis contemplates multiple study designs, searching for the best evidence-based practice for auriculotherapy in anxiety management. It comprises five chapters, each one begins with an introduction and finishing with a conclusion.

Chapter 1 (narrative review) provides an overview of the structure of the PhD thesis. Its relevance stands out in summarizing existing research and supporting future research using auriculotherapy for anxiety disorders.

Chapter 2 (overview of systematic reviews) explores the theoretical background and evidence published about auriculotherapy, which focuses mainly on managing insomnia, smoking cessation, and pain. Based on the overview performed, we did not find any systematic review in situational anxiety. Consequently, we designed a protocol for a systematic review to examine the effectiveness and safety of auriculotherapy for anxiety disorders. The protocol can be assessed in chapter 3.

As soon as the protocol was published, the team conducted a systematic review with metaanalysis (chapter 4) to determine whether auriculotherapy could be effective and safe for treating people with anxiety disorders. A moderate certainty of evidence and a positive effect was found in pre-operative anxiety. However, there was insufficient evidence of students' anxiety during the examinations despite two randomized controlled trials being included.

Chapter 5 delivers a pilot randomized controlled trial of auriculotherapy that appeared to be safe and effective in reducing anxiety levels before university exams.

CHAPTER 1

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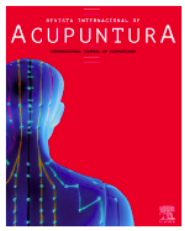

REVIEW

Outlining auriculotherapy in anxiety as an evidence-based medicine: A brief overview

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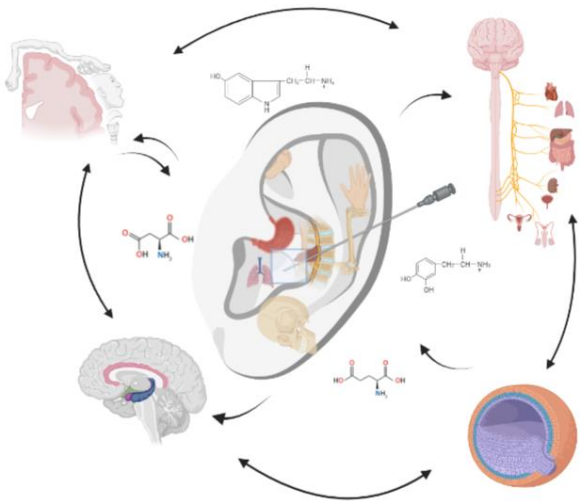
Outlining auriculotherapy in Anxiety as an Evidence-Based Medicine: A Brief Overview.

ABSTRACT

This review summaries the available research on nuclear magnetic resonance to show the Auriculotherapy (AA) effect in human brains. Through search and analysis of clinical research in the PubMed/MEDLINE database, available related articles up to January 2023 were used only in English. This overview is not comprehensive and is due to be predominantly informative. A total of 4 trials met our inclusion criteria and were included in the quality analysis. Electrical stimulations were more frequent (3 studies) than needles (1 study). The above research delivers insufficient evidence that the AA at different points led to other brain structures' modulation. Although, the brain structures activated by the AA seems to overlap territories belonging to the anxiety's neurobiology. The endeavour to link the AA mechanisms to the cranial nerves combined with an embryological approach is promising. Nevertheless, additional studies with imaging, different AA techniques, and auricular points are necessary to provide evidence for this effect on mental health.

Keywords: Brain modulation; Auriculotherapy; Ear innervation; Anxiety; Parasympathetic nervous

Graphic Abstract:



Abbreviations:

AA	**Auriculotherapy**
CAM	**Complementary and Alternative Medicine**
CNS	**Central nervous system**
DSM-5	**Diagnostic and Statistical Manual of Mental Disorders**
fMRI	**Functional magnetic resonance imaging**
GABA	**Gam-ma-aminobutyric acid**
GAN	**Greater auricular nerve**
HPA	**Hypothalamic-pituitary-adrenal**
LC	**Locus coeruleus**
NE	**Norepinephrine**
NST	**Nucleus tractus solitarius**
PA	**Pharyngeal arches**
PAG	**Periaqueductal gray**
RN	**Raphe nucleus**
SpV	**Spinal trigeminal nucleus**
TN	**Trigeminal nerve**
VN	**Vagus nerve**

1. Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), anxiety is the future anticipation of unbalanced, often associated with muscle tension and caution in preparation for future hazards ([APA], 2013). The excess may trigger severe psychopathological events disturbing an individual´s everyday life (Schneiderman, Ironson, & Siegel, 2005). Anxiety disorders are highly prevalent, with global estimations varying from 3.8 to 25% across countries, with incidence rates as high as 70% in people with chronic health conditions (Remes, Brayne, van der Linde, & Lafortune, 2016).

Succeeding the National Institute for Health and Care Excellence regarding pharmacological therapies for anxiety disorders, such as Benzodiazepines and antipsychotics, are associated with tolerance, dependence, and linked with several adverse effects. Therefore, they should not be used regularly to manage anxiety disorders [4]. Conventional medicine, such as psychotherapy and medication, has successfully treated

anxiety disorders; however, those patients with anxiety struggling to achieve complete remission of their condition feel the need to use Complementary and Alternative Medicine (CAM) [5].

The National Center for Complementary and Integrative Health classified CAM as respects: i) Nutritional (e.g., special diets, dietary supplements, herbs, probiotics, and microbial-based therapies), ii) Psychological (e.g., meditation, hypnosis, music therapies, relaxation therapies), iii) Physical (e.g., acupuncture, auriculotherapy, massage, spinal manipulation) and iv) combinations such as psychological and physical (e.g., yoga, tai chi, dance therapies, some forms of art therapy) or psychological and nutritional (e.g., mindful eating) [6].

Auriculotherapy (AA) is a therapy somewhat based on the ancient Chinese practice of body acupuncture, originating from a French physician's discoveries in the 1950s (Dr Paul Nogier and colleagues) demonstrating that specific areas of the external ear were associated with pathology in particular body parts (Nogier & Nogier, 1985; Terry Oleson, 2014a). Typically, AA involves the insertion of needles (Nogier & Nogier, 1985) (e.g., semipermanent or metal filiform needles and bloodletting) or pressure (Mehta, Dhapte, Kadam, & Dhapte, 2017) (e.g., using seeds, massage, or magnetic stones) or heating (e.g., moxibustion) (Jin et al., 2020) or by stimulation as laser therapy (Nogier & Nogier, 1985), and electrostimulation (Ulett, Han, & Han, 1998) in the auricular skin for therapeutic purposes. (Biaggioni & Kaufmann, 2014; Round, Litscher, & Bahr, 2013).

It is a technique known to diagnose and treat physical and psychosomatic dysfunctions by stimulating a specific point in the ear (Hou et al., 2015). In 2018, only fourteen systematic reviews with high methodological quality were published about AA, which focused on managing insomnia, smoking cessation, and pain (A. Vieira, Reis, Matos, Machado, & Moreira, 2018). Since then, exponential research has grown, describing it as a strategy capable of adjusting signal processing in the central nervous system (Romoli et al., 2014), and exploiting brain plasticity (Kaniusas et al., 2019), which is the perfect complementary technique to address psychological impairments. Curiously, AA evaluated with anxiety scales had a positive effect on pre-operative anxiety (Dellovo, Souza, de Oliveira, Amorim, & Groppo, 2019; Mafetoni, Rodrigues, Jacob, & Shimo, 2018; Michalek-Sauberer, Gusenleitner, Gleiss, Tepper, & Deusch, 2012; Usichenko et al., 2020; Valiee, Bassampour, Nasrabadi, Pouresmaeil, & Mehran, 2012; Wu, Liang, Zhu, Liu, & Miao, 2011; Wunsch et al., 2018). The salivary cortisol was reduced in students before an exam compared with the control group (Prado, Kurebayashi, & Silva, 2012; Usichenko et al., 2020; Andreia Vieira et al., 2022), and in post-caesarean woman (Kuo, Tsai, Chen, & Tzeng, 2016). Regarding generalized anxiety disorder, Rivadeneira et al. (2015) reported AA was more effective than conventional drug in decreasing the Self-Assessment Inventory and anxiety symptoms remission after four weeks, which could potentially reduce the use of

psychopharmacologic drugs (Díaz Rivadeneira, Díaz Cifuentes, González Hidalgo, Conteras Tejeda, & García Sánchez, 2015).

This narrative review aims to clarify the relationship between the possible auriculotherapy's neuromodulators' effects on anxiety management. As the current understanding of the mechanisms behind AA stands on the embryological hypothesis and the solid innervation of the ear, its relevance stands out in summarizing existing research and supporting future research using AA.

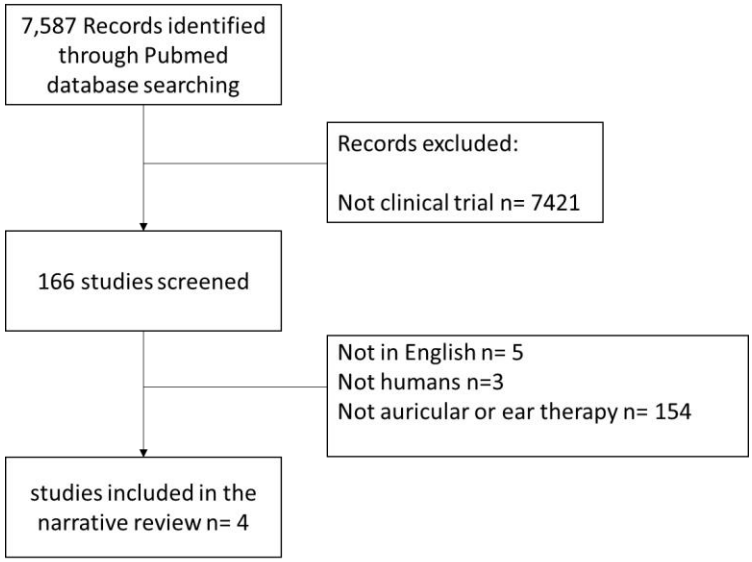
2. Methods

The authors first identified studies through keywords search "ear or auricular" and "fMRI" using Pubmed/Medline. Articles were collected from inception to January 2023, in English, and the outcome was the fMRI bold signal activation and signal reduction in humans. We excluded all guidelines for treatments, surveys, case series, case reports, interrupted time-series trials, qualitative trials, trials with missing or incomplete data, cohort studies, reviews, conference abstracts/posters, expert opinion, 'duplicate publications, newspaper articles, book reviews, 'mass media publications', health publications, general comments, or letters, due to their potential high risk of bias (Higgins et al., 2011). This overview is not comprehensive and is due to be predominantly informative.

3. Results

Our search identified 7,587 citations where only 166 titles were reviewed, as shown in the diagram flow (figure 1). A total of 4 trials met our inclusion criteria and were included in the quality analysis.

The 4 trials included two different type of AA interventions retrieved, where electrical stimulations were more frequent (Badran et al., 2018; Kraus et al., 2013; Zhang et al., 2019) than needles (Rong, Zhao, Wang, & Zhou, 2016).



Chapter 1. Figure 1 Study diagram flow

In two different sessions, the author´s Zhang et al. (2019) applied auricular electrical stimulation to twenty-six patients with migraines. The authors reported that the left cymba concha area produced significant fMRI signal reductions at the LC and improved signal in other brain areas (e.g., right temporoparietal junction, right para-hippocampus, left secondary somatosensory cortex, and left amygdala) compared to the left tail of the helix area (Zhang et al., 2019).

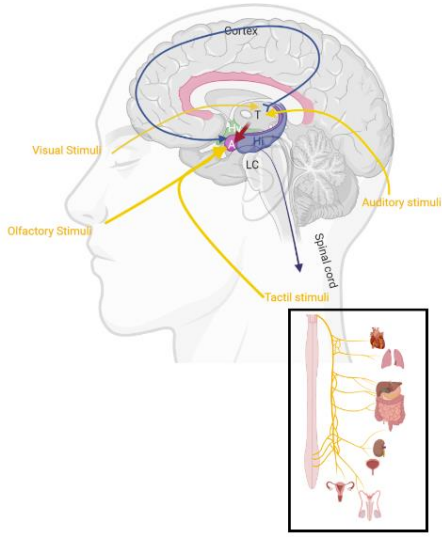
Badran et al. (2018) enrolled 17 healthy adults who received either left tragus or earlobe electric stimulation. They documented the tragus area activates the cerebral afferents of the vagal pathway (e.g., the Contralateral postcentral gyrus, bilateral insula, frontal cortex, right operculum, and left cerebellum), while the earlobe stimulation only produced activation in the contralateral postcentral gyrus (Badran et al., 2018).

Kraus et al. (2013) investigated fMRI effects in response to electrical stimulation of two zones in the left outer auditory canal (Kraus et al., 2013). Eight healthy subjects received electrical stimulation at the anterior wall area, and the other eight participants received it at the posterior side of their left external auditory canal, and both groups were later stimulated on their corresponding ear lobe (Kraus et al., 2013). The authors found the anterior part of the auricular stimulation decreased fMRI bold signal in the locus coeruleus and the solitary tract compared with the ear lobe region (Kraus et al., 2013). In contrast, the stimulation at the posterior wall leads to unspecific changes in the bold signal within the solitary tract (Kraus et al., 2013).

M Romoli et al. (2014) deliver preliminary evidence on the specificity of two auricular acupoints, the "Brain Stem Auricular Acupoint" and the "Thumb Auricular Acupoint" in 6 healthy volunteers who experienced two fMRI sessions (Romoli et al., 2014). These authors reported that the needle in the "Brain Stem Auricular Acupoint" of the left ear showed a pattern that largely overlapped regions belonging to the pain matrix (Romoli et al., 2014). At the same time, the "Thumb Auricular Acupoint" increased activation bilaterally in the parietal operculum, the territory of the secondary somatosensory area (Romoli et al., 2014).

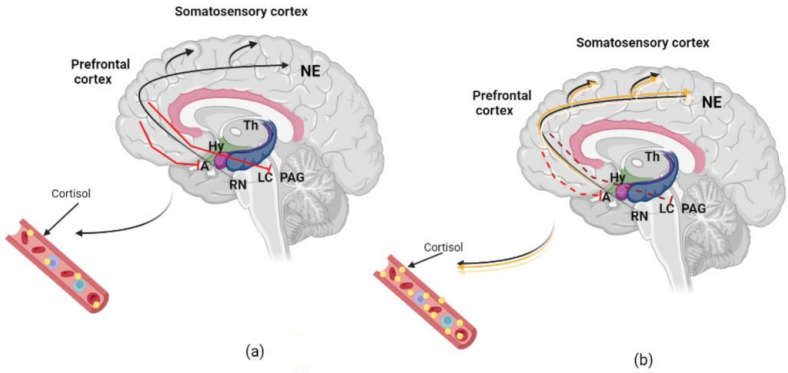
4. Discussion

Many neurotransmitters have been connected to anxiety´ neurobiology, including serotonin (Stefansdottir, Ivarsson, & Skarphedinsson, 2022), Gamma-aminobutyric acid (GABA) (Zacharopoulos, Sella, Cohen Kadosh, Emir, & Cohen Kadosh, 2022), Norepinephrine (NE) (Sun, Hunt, & Sah, 2015), and dopamine (DeGroot et al., 2020). Curiously, disbalances of any hormone may also affect the balance of activity in the brain's emotional centers in distinct ways (Karachaliou et al., 2021). However, cause and effect are rarely known, and it's often unattainable to distinguish between inadequate neurotransmitter balance (e.g because of life experiences) or underlying genetic predisposition (G. Liu et al., 2022; Martin, Ressler, Binder, & Nemeroff, 2009). Both can happen to anyone with anxiety; in some cases, a mix of both may be responsible for it. The limbic system processes sensory input from the external and internal environment (Figure 2) to determine, through memory and motivation, the emotional, autonomic, motor and cognitive responses essential for self-preservation and survival (McLachlan, 2009). The emotional circuit can be influenced by many chemical messengers implicated in anxiety psychopathology (such the co-release of neurotransmitters and neuropeptides) which are represented in limbic regions in the central nervous system (CNS) (Martin et al., 2009). One of those chemical messengers contain cholecystokinin, a peptide hormone found in the gastrointestinal system responsible for stimulating the digestion also responsible to inhibit the vagus nerve (VN) (Schatzberg & Nemeroff, 2009).



Chapter 1. Figure 2 Schematic illustration from the sagittal view of slice representing a normal limbic system midline connection after visual, olfactory, tactile, and auditory stimulus. Legend: LC: Locus coeruleus, A: Amygdala, Hy: Hypothalamus, T: Thalamus, Hi: Hippocampus. Schematic illustration created with BioRender.com.

By receiving projections directly from olfactory and tactile stimulus and indirectly (through thalamus or somatosensory cortex) by auditory and visual areas, the amygdala (Figure 3) mediates threats and other emotional responses including the regulation of the cortisol via hypothalamus (Davis, 1992). Potential threats identified by the sensory system (e.g., anxiety) appear to also trigger the locus coeruleus (LC) (Atzori et al., 2016), the main supplier of NE in the CNS (O'Donnell, Zeppenfeld, McConnell, Pena, & Nedergaard, 2012). The secretion of NE from the LC reaches the amygdala and the prefrontal cortex. This pathway affects HPA activation by producing cortisol, which elevates glucocorticoid distribution for a coordinated physiological reaction (Charmandari, Tsigos, & Chrousos, 2005) (Figure 3a). While the cortisol released from the adrenal cortex allows the body to persist on high alert (Lee, Kim, & Choi, 2015), the disproportionate concentration of these structures is associated with a prolonged anxious state (Ulrich-Lai & Herman, 2009). Combined with this amplified response, there can also be deficient HPA regulation, leading to an inability to control the physiological response (Figure 3b). Further functional magnetic resonance imaging (fMRI) will hopefully allow human LC assessment and a better understanding of its role in anxiety‘s neurobiology (Priovoulos et al., 2018).

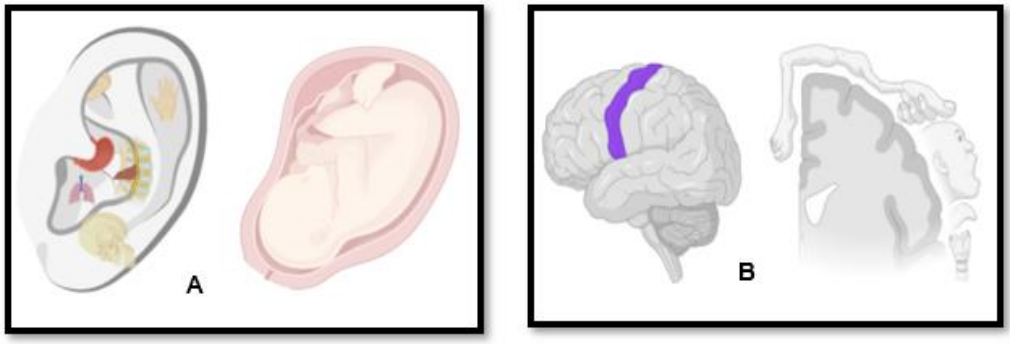


Chapter 1. Figure 3. a) Standard organism modulation response to acute physiological input. Legend: The red lines reveal the global and the Locus coeruleus (LC) regulation by the secretion of noradrenalin (NE) reaching the amygdala (Amy) and the Prefrontal cortex. This path affects Hypothalamic-pituitary-adrenal (HPA) activation by producing cortisol. (b) Abnormal organism reaction facing chronic physiologic input. Chronic anxiety can lead to less HPA regulation (dotted red lines). Therefore, increased NE in LC, amygdala, and hippocampus will deliver excessive cortisol production and contribute to reduced regulation of pathological anxiety. Figure created with BioRender.com based on Morris, McCall, Charney, & Murrough (2020) work (Morris, McCall, Charney, & Murrough, 2020).

AA has been applied more systematically in Europe since Doctor Nogier presented the inverted fetus map conception in 1957 (Nogier & Nogier, 1985). Nogier also found that the stimulation of the auricle leads to fluctuations in the radial artery (e.g., heart rate) known as "Vascular Autonomic Signal" (Gori & Firenzuoli, 2007; Round et al., 2013). Paul Nogier and later Frank Bahr explained that the ear has a so-called somatotopy, meaning that the whole person is represented on the ear (Gori & Firenzuoli, 2007; Round et al., 2013). Based on his AA experience, Paul Nogier began to take his patients' pulses while examining their ears. He discovered variations of the pulse when he touched different ear zones. This variation of pulse, or vascular autonomic signal, is a reaction from the CNS (sympathetic and parasympathetic) after auricular points stimulation (Wang, Yang, Zhao, Zhou, & Wirz-Ridolfi, 2016). The ear reflex map of a French physician, Paul Nogier, was introduced to China in 1958 by Dr. Ye Xiao-Lin (Rong et al., 2016). Since then, numerous reports arrived founding AA helpful for tooth extraction and sciatica management conducted by ear cauterization.

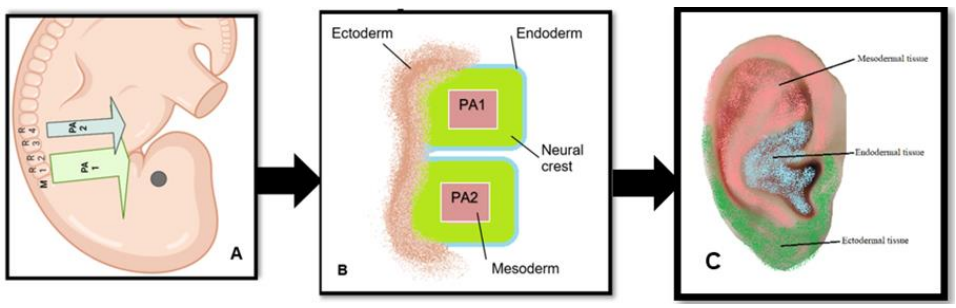
AA books sometimes refer to an embryological hypothesis which strives to explain how the ear's reflex map relates to the rest of the body (Alimi, Geissmann, & Gardeur, 2002; Hou et al., 2015; Beniamina Mercante et al., 2018; Terry Oleson, 2014b). On the one hand, the embryological view is based on the somatotopic organization of the human fetus theory (Nogier & Nogier, 1985) in the auricle (Figure 4A) and the adult ear's nerve network (Figure 4B). On the other hand, it uses a general anatomical understanding of how organs

and tissues grow from the fetus's three germinative layers.



Chapter 1. Figure 4: A. Schematic representation of a fetus in the intrauterine position projected on the pinna exemplifying Paul Nogier and Frank Bahr somatotopic theory, where the whole person (and its organs) is represented on the ear; B: Schematic representation of the regions of the human body reflected in the cerebral cortex - somatotopic map of the brain, simulating the Penfield homunculi theory. Combining both theories, when auriculotherapy is performed is believed the specific point chosen by the acupuncturist is correlated with the somatotopic cortex in the brain. Figure created with BioRender.com.

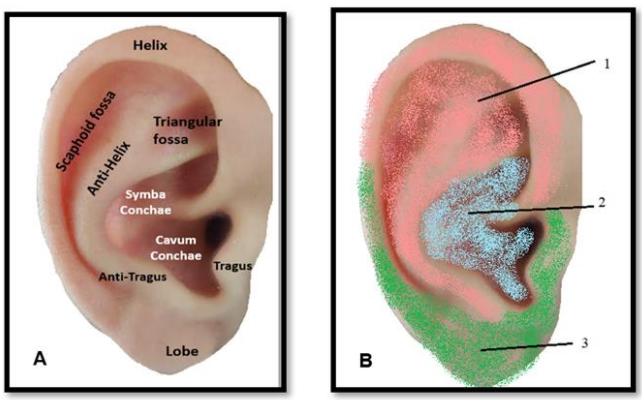
The ears are not an exemption; it originates from the proliferation of organized embryonic tissue of undifferentiated cells (Cheatham, 1993). In all vertebrates, the pharyngeal arches developed from the cephalic portion of the neural crest (e.g., strip of tissue that runs down the back of the origin and gives genesis to a large number of different organs) are responsible for producing the cartilage, bone, nerves, muscles, glands, and connective tissue of the face and neck (Anthwal & Thompson, 2016). The pharyngeal arches are banded externally by ectoderm and internally by endoderm and are filled with neural crest cells (Figure 5) surrounding a mesodermal core (Anthwal & Thompson, 2016; C. Liu, Jiang, Yu, & S, 2020; Terry Oleson, 2014b).



Chapter 1. Figure 5: Schematic illustration the early set-up of the ear. Legend: (A) The pharyngeal arches (PA) are filled by neural crest streams; the first PA1 is filled with neural crest cells from the midbrain (M), rhombomere (r1) and r2, while the second PA2 is filled with crest predominantly from r4. (B) The first and second PA are divided internally by the endodermal and externally by the ectodermal tissue. (C) division of the embryological cells in auricular area theory created based on Anthwal., N and Thompson.,H (2015); Liu C et al., (2020) and Terry Oleson (2014) works. Schematic illustration created with

BioRender.com.

These germinative layers become specialized into different types of organs. For example, the endoderm develops most internal organs: the stomach and intestinal system, lungs, tonsils, liver, pancreas, bladder, urinary system, thyroid, parathyroid and thymus represented in the concha (Figure 6A); innervated by the vagus nerve. The ectoderm creates the skin, the brain, the spinal cord, the subcortex, cortex and peripheral nerves, the pineal gland, the pituitary gland, kidney marrow, hair, nails, sweat glands, cornea, teeth, the mucous membrane of the nose, and the lenses of the eye, innervated by a branch of plexus cervicalis (Figure 6B), which has indirect connections with the cortex (C. Liu et al., 2020; Terry Oleson, 2014b). The mesoderm origins the skeletal muscles, smooth muscle, blood vessels, bone, cartilage, joints, connective tissue, endocrine glands, kidney cortex, heart muscle, urogenital organ, uterus, fallopian tube, testicles and blood cells from the spinal cord and lymphatic tissue, represented by the trigeminal nerve (TN) (Shafique & J, 2022). This reflects the scientifically validated organization and inverted orientation of the homunculus in the cerebral cortex as developed by Penfield (Penfield & Rasmussen, 1950). However, the body reflected in the brain does not illustrate the exact proportions of the actual body, such as Penfield's Homunculus. In 1937, Penfield and Boldrey described their work on the stimulation effects of the cerebral cortex in man. The homunculus can be considered as some form of "map" of human cortical representation, being more or less precisely in relation to actual brain areas identified at surgery (Schott, 1993). The same organization is thought to happen in the auricle, the acupuncture points corresponding to reflexes with body organs would be divided according to an organized anatomical arrangement. Thus, the head and hand occupy a larger area than they would if they were proportionate, while the thigh bone and arm reach a small space in the ear, precisely as in the somatotopic map of the brain. Consequently, the brain's somatotopic map is related to its functional importance rather than its actual physical size.



Chapter 1. Figure 6: Schematic illustration about the human auricle anatomy. Legend: (A) and auricular innervation (B), 1: pink area exemplifies the trigeminal, and auriculotemporal nerve

innervation; 2: includes the auricular branch of the vagus nerve (blue shading); 3: Green area is represented by the greater auricular nerve, desenvolved from Liu C et al., (2020) and Terry Oleson (2014) works.

Between the epidermis, dermis, and hypodermis are around 10.000 unique sensory receptors (including exteroceptors, interceptors and proprioceptors), at most 0.1 mm large (Moini, Avgeropoulos, & Samsam, 2021). These sensory receptors are mainly made of collagen fibers wrapped by a plasma membrane containing a negative electrical charge (low acid dissociation values of the lipid head group) (McLaughlin, 1989). Each auricular point includes a group of these receptors (Marzvanyan A & AF., 2022 Jan [Updated 2022 Aug 22]) served by the same nerve and blood vessel branch (majority from the external carotid artery) (Nguyen JD & H., 2022 Jan), which are activated by mechanical pressure (e.g., seeds, massage, needle), heat (e.g., moxibustion) and stimuli such as laser light and electrical stimulation(French & Torkkeli, 2009). According to Gate Control Theory by Melzack and Wall (Melzack & Wall, 1965), the receptors deliver the information to the brain based on the stimulation location, pressure-generation which triggers the small myelin nerves to conduct stimulations to spinal cord, midbrain, hypothalamus and pituitary axis where therefore, neuropeptides are released (Ezzo, Streitberger, & Schneider, 2006; Farivar, Malekshahabi, & Shiari, 2014).

The information provided by thermal, algic and proprioceptive stimuli is transmitted from the auricular pavilion by the fibers by a great deal of overlap between multiple nerves: i) auriculotemporal nerve; ii) auricular branch of the VN; iii) greater auricular nerve (GAN) and minor occipital nerve (sensitive branch of the cervical plexus)(Hou et al., 2015). The auriculotemporal nerve originates from the mandibular branch of the TN, which mainly supplies the antero-superior and antero-medial areas of the external ear. Auricular branch of the VN is the only peripheral branch of the vagus nerve, covering a considerable part of the auricular area and most of the region around the auditory canal. The minor and greater occipital nerve originates from the C-2 branch of the cervical plexus, being responsible for the sensitive innervation of the upper third of the auricle. Finally, the major auricular nerve originates from the C-2 to C-3 branches of the cervical plexus, being responsible for the innervation of the lower region of the auricle (B Mercante et al., 2015).

The VN is a mixed nerve composed of 20 % “efferent” fibers (sending signals from the brain to the body) and 80 % “afferent” (sensory) structures (carrying information from the body to the brain) (T. Oleson, 2018). The efferent cholinergic fibers are the main parasympathetic component of the autonomic nervous system, but a vital function of the VN is transmitting and mediating sensory information from throughout the body to the brain (Zagon, 2001). The VN is the most extensive cranial nerve from the brain stem to the abdomen (Hsieh, Chen, Wu, Yen, & Chai, 1998), with a somatic afferent distribution to the

external auricular area. The VN contains direct and indirect connections to the cortical-limbic-thalamic-corpus callosum neural circuit relevant to emotional and cognitive functions in psychological disorders (Howland, 2014; Ruffoli et al., 2011). The association between VN stimulation effects and AA was highlighted by procedures developed to electrically stimulate the VN through the AA points found in the auricle's concha region to treat depression (Conway et al., 2018; Sackeim, Dibué, Bunker, & Rush, 2020) and epilepsy (Aihua et al., 2014; Mandalaneni & Rayi, 2022). The VN stimulation seems to boost the NE release in structures responsible for regulating autonomic function and improve levels of neurotransmitters (e.g., GABA) in the brain and spinal cord while reducing glutamate levels (Marrosu et al., 2003).

On the other side, TN stimulation has also been suggested as an explanation via AA, providing anatomical support for physiologic results where the TN stimulation affects brainstem structures (B Mercante et al., 2015), and in turn, influence forebrain areas involved in the pathophysiology of specific CNS disorders.

TN is responsible for perceiving touch and pain between the anterior ear site to the CNS via the spinal trigeminal nucleus (SpV) (Shafique & J, 2022). Interestingly, unlike the VN, the TN does not contain autonomic fibers (Hsieh et al., 1998). However, the impulses from the Spv reach extra-trigeminal regions in the CNS, including the posterior parietal, auricular, occipital areas, and thalamus, essential for controlling many autonomic functions (Beniamina Mercante et al., 2018). Besides that, SpV transmits nociceptive and tactile stimulus to the thalamus reaching the somatosensory cortex. It also has projections for the nucleus tractus solitarius (NST), the LC and the Raphe nucleus (RN). In the brainstem, the vagus afferent fibers end in the NST which has fibers linking directly or indirectly different brain regions [83]. These regions include the dorsal raphe nuclei, locus coeruleus, amygdala, hypothalamus, thalamus, and orbitofrontal cortex. The NST, located in the dorsomedial medulla, is the first brain structure responsible for conducting visceral and taste afferents carried by the cranial nerves and has a critical role in the initiation and integration of a wide variety of reflexes controlling cardiovascular function, respiration, and gastrointestinal motility [84,85]. The RN which contain primarily serotonergic neurons, release serotonin, as well as synaptic connections with the limbic system [87]. Moreover, the RN receives catecholaminergic innervation from the periaqueductal gray (PAG) , amygdala, and hypothalamic nuclei, supporting those systems with mood regulation [88].

During the last years, some fMRI studies revealed different central nervous activations in distinct auricular areas accessed via electrical stimulation (Badran et al., 2018; Kraus et al., 2013; Zhang et al., 2019) and by titanium semi-permanent needles of the external ear.

Electrically, the stimulation on the inner side of the tragus showed by fMRI decreased activation in limbic brain areas (including the amygdala, hippocampus, and para-

hippocampal gyrus) and the middle/superior temporal gyrus but increased activation in the insula, precentral gyrus and the thalamus (Badran et al., 2018; Kraus et al., 2013; Ruffoli et al., 2011). Also, electrical stimulation at the cymba concha produced deactivations bilaterally in the hippocampus and hypothalamus (Zhang et al., 2019), and activation of the conventional vagal projections. While the stimulation led to unspecific activation patterns; precisely in the ventral region of the caudal medulla (Zhang et al., 2019), in the primary somatosensory cortex activation was consistent with the location of the face, and side/back of the head which goes in line with the Penfield homuncular map concept.

Interestingly, in M. Romoli and colleagues in 2014 study´s support the auricular points´ specificity and the Penfield homuncular map that the AA explanation relays. The needling of the auricular point found in the Scaphoid fossa, related in AA with the “Thumb” produced an increase in activation bilaterally in the parietal operculum, the region of the secondary somatosensory area. Furthermore, the needling of the auricular point in the antitragus area showed a pattern that essentially corresponds with regions fitting to the pain matrix, with local differences in the left amygdala, anterior cingulate cortex, and cerebellum. The above research provides preliminary evidence that the AA at different points led to other brain structures' activation or deactivation, which seems to overlap regions belonging to the anxiety's neurobiology (Breit, Kupferberg, Rogler, & Hasler, 2018; T. Oleson, 2018). Although most studies have shown several therapeutic effects induced by invasive VN and TN electrical stimulation, those can be reproduced without the electric current (Romoli et al., 2014). However, is still too early to make any recommendations for clinical practice and further randomized trials using fMRI to confirm the AA brain´s activation are needed.

5. Limitations and Future challenges

There are several limitations to this study. Firstly, our narrative review offers an overview of the possible mechanisms of AA in anxiety proved by fMRI; however, the used selection of publications is potentially biased since the present study is descriptive but not systematic. One of the primary biases of our work was not to limit the chosen keywords for anxiety. For example, we include an article from Zhang et al. (2019). This published paper reported the application of auricular electrical stimulation in 26 patients with migraines (Zhang et al., 2019) (not anxiety). We include this article due to the difficulty of finding papers demonstrating the nuclear magnetic resonance effect of AA in human brains.

Secondly, AA is commonly used in traditional Chinese medicine; the exclusion of Chinese databases and any unpublished trials may impact the results of this narrative review. Finally, only English language publications were retrieved, leaving out potential vital

data from other languages. Accordingly, we suggest further updated analysis in the future with the inclusion of Chinese databases and other languages to corroborate this work.

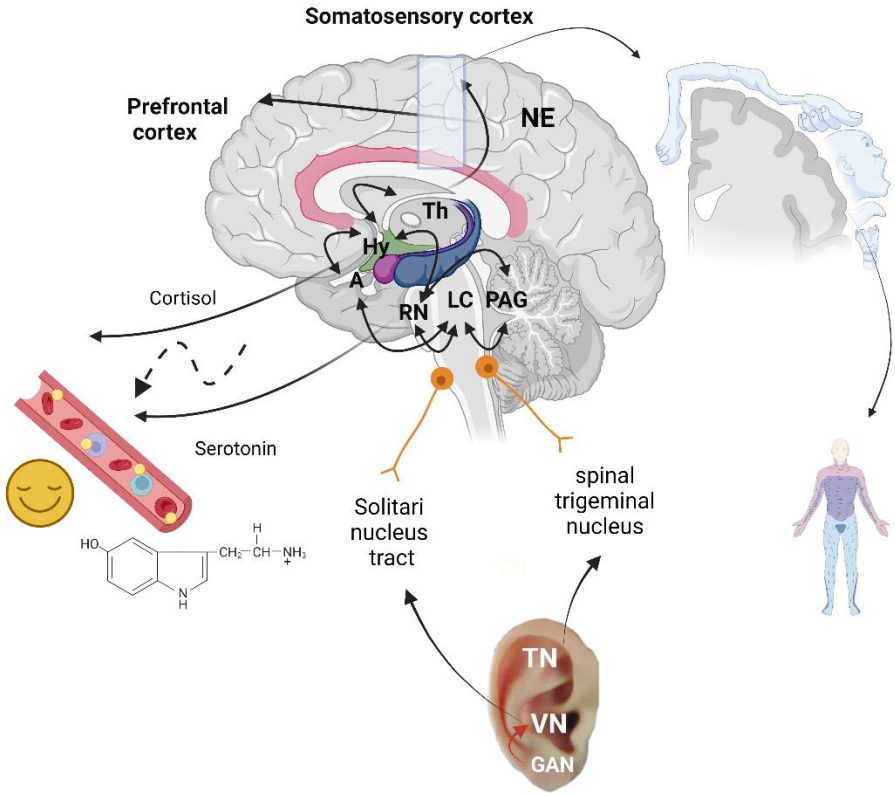
Tendentially, researchers are inclined to confirm the AA effects via the VN and TN stimulation. However, AA covers more areas, calling attention to the possible co-activation of the GAN when triggered through the auricular lobe area. The GAN communicates with several cranial nerves; for example, the anterior branch sends a tiny twig (or several small twigs) into the substance of the parotid gland, connects to the facial nerve (Ginsberg & Eicher, 2000), and the posterior branch communicates with the auricular branch of the VN (Berry, Bannister, & Stranding, 1995). So, it would make sense to study the AA effects under GAN stimulation in the future.

Generally, some articles refers to how AA evidence is limited (Chon et al., 2019; Stanton, 2018) and unclear (Jin et al., 2020); still, the diversity of AA modalities, such as invasive or noninvasive, may be associated with different treatment effects and physiological mechanisms, even if treating the same clinical condition. Also, the skin is composed of specific sensory receptors (e.g., exteroceptors, interceptors and proprioceptors), which could also be activated by different AA approaches showing supplemental pathways to reach the CNS. In this case, we raise the question if all noninvasive AA techniques performed with seeds, massage, moxibustion, laser light and electrical stimulation, could trigger exteroceptors and proprioceptors. At the same time, the invasive AA (with or without electrostimulation) could also reach interceptors and offer a prompter body response, regardless of noninvasive procedures could be a more practical way to use in young and elderly patients or those who are afraid of needles (Radmayr, Schlager, Studen, & Bartsch, 2001; Tan, Molassiotis, Wang, & Suen, 2014). Despite some adverse effects, such as transient dizziness, headaches, and fatigue, have been expressed with noninvasive AA (Quah-Smith, Williams, Lundeberg, Suo, & Sachdev, 2013), however, these noninvasive techniques also minimize the risk of adverse events, such as infection or bleeding complications (Chon et al., 2019; Correa et al., 2020; Klausenitz et al., 2016; Kurebayashi et al., 2017; Quah-Smith et al., 2013).

6. Conclusion

Invasive (needling) and non-invasive (electrical stimulation) AA at distinct auricular points led to the activation or deactivation of diverse brain structures. This effect appears to overlap regions belonging to the anxiety's neurobiology.

The attempt to link the AA mechanisms to the cranial nerves combined with an embryological approach is promising. Yet, more studies with imaging and different AA techniques and auricular points are necessary to provide evidence for this effect of AA. Meanwhile, we present a speculatory model in Figure 7.



Chapter 1. Figure 7: Schematic representation of auriculotherapy´s possible mechanisms of action. Legend: The trigeminal nerve (TN) sends input to the Central nervous system (CNS) and Thalamus (Th) via the spinal trigeminal nucleus; the vagus nerve (VN) via the solitaries' nucleus tract; and the greater auricular nerve (GAN) via facial and VN. Decreased activation in Locus coeruleus (LC) and in limbic brain areas, including the amygdala (A), hippocampus (Hy), and para-hippocampal gyrus leads to improvements in regulation of cortisol segregation. The increase activation in the parietal operculum and the secondary somatosensory area activation region aligns with the Penfield homuncular map concept. T. The catecholaminergic innervation from the PAG, amygdala, Raphe nucleus (RN) and hypothalamic nuclei towards the solitary nucleus tract supports the mood regulation and the release of serotonin. Schematic illustration created with BioRender.com.

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CHAPTER 2

Complementary Therapies in Clinical Practice
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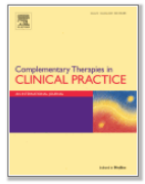

Does auriculotherapy have therapeutic effectiveness? An overview of systematic reviews

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ABSTRACT

Background and purpose: Auriculotherapy is a therapeutic technique used for a wide variety of conditions. Nevertheless, similarly to any health-related intervention, the clinical use of this therapy requires scientific evidence of effectiveness in order to support its rational use. The main goal of this article is to critically analyze published literature on auriculotherapy and to provide an overview of the effectiveness of this technique in the management of health disorders. **Methods:** The inventory of published reviews on this subject was carried out in November 2017, by assessing the following computerized databases: PubMed, MEDLINE, PsycINFO, EBMR, Cochrane Database of Systematic Reviews, CINAHL Plus NRC and Science Direct. Were only considered the systematic reviews based on meta-analysis with high methodological quality described according to AMSTAR (Assessment of Multiple Systematic Reviews). The eligible articles were systematically reviewed to find out in which health conditions auriculotherapy can be used with effectiveness. **Results:** A total of 14 reviews were eligible according to the inclusion and exclusion criterions. Those reviews were focused on the management of insomnia, smoking cessation, and pain, within the clinical scope of Neurology, Orthopedics and Rheumatology. **Conclusions:** Auriculotherapy has shown to have positive effects while associated to conventional treatments of insomnia, chronic and acute pain. Further well-designed studies are required to evaluate the effectiveness of this technique in the treatment of other health conditions.

Keywords: Auriculotherapy, Auricular Acupuncture, acupressure, insomnia, pain.

1. Introduction

Mammalian ear is a complex structure with origin in tissues of neural crest, mesoderm, endoderm and ectodermal. This anatomic structure includes ossicles, cartilage, muscles, nerves, blood vessels and epithelial membranes (Anthwal & Thompson, 2016). The current understanding of the mechanisms behind different reflex therapies, such as auriculotherapy, stands on the embryological hypothesis as well as on the strong innervation of the ear. In fact, the ear is one of few anatomic structures built up of tissue from each of the primary tissues found in an embryo. Therefore, this could be related to the representation of the human body in the ear reflexology charts (David Alimi, Geissmann, & Gardeur, 2002; Hou et al., 2015; Landgren, 2008; Mercante et al., 2018).

International standards and nomenclature were firstly developed in the nineties with the contribute of the World Health Organization (WHO). Most recently, Auricular Acupuncture Points (AAPs) were accepted as a biomathematical model of the brain's anatomical organization. This view come out while assessing the neurophysiological correlations between auricular zones and their brain correspondences (D. Alimi & Chelly, 2018).

The sensory innervation of vegetative nerve centers receives information from the internal organs by electrical impulses conducted through Alpha, Beta and Gamma fibers. In turn, these are disseminators of sensitive perceptions to touch, pressure, temperature and proprioception that reach the sensory nuclei of the cranial nerves and the posterior horn of the Spinal Cord (Bahr, 2010; Hou et al., 2015; Jenkins, 2017; Mercante et al., 2018; Rabischong & Terral, 2014; Strittmatter, 2011a). The information provided by thermal, algic and proprioceptive stimuli is transmitted from the auricular pavilion by the fibers of the following nerves: i) auriculotemporal nerve; ii) auricular branch of VN; iii) minor occipital nerve (sensitive branch of the cervical plexus) and iv) greater auricular nerve (Bahr, 2010; Hou et al., 2015; Jenkins, 2017; Mercante et al., 2018; Rabischong & Terral, 2014; Strittmatter, 2011a). The auriculotemporal nerve originates from the mandibular branch of the trigeminal nerve, which mainly supplies the antero-superior and antero-medial areas of the external ear. VN is the only peripheral branch of the vagus nerve, covering a considerable part of the auricular shell and most of the area around the auditory canal. The minor occipital nerve originates from the C-2 branch of the cervical plexus, being responsible for the sensitive innervation of the upper third of the auricle. Finally, the major auricular nerve originates from the C-2 to C-3 branches of the cervical plexus, being responsible for the innervation of the lower region of the auricle (Mercante et al., 2018). As well, the electrical

impulses are transmitted to other upper nervous systems structures such as the cranial nerve nuclei, the limbic system, the thalamus, the hypothalamus, the reticular formation, the cerebellum and the cerebral cortex. This signal processing and control is the base of the Sympathetic and Parasympathetic neurophysiological regulation (Strittmatter, 2011b).

In this scenario, auriculotherapy has been pointed out as a promissory method to treat conditions that go from substance abuse (Ahlberg, Skarberg, Brus, & Kjellin, 2016) to pain (Asher et al., 2010), obesity (Abdi et al., 2012), anxiety (Kurebayashi et al., 2017; Vieira, Hinzmann, Silva, Santos, & Machado, 2018; A Vieira et al., 2016), epilepsy (Kang, Shen, & Xia, 2013), and sleep disorders (Lan et al., 2015). Nonetheless, the effectiveness of this technique has only been tested in a relatively small number of evidence-based trials (Hou et al., 2015).

Given these arguments, the main goal of this work was the systematic study of the existing scientific evidence on the effectiveness of auriculotherapy as a therapeutic tool.

2. Methods

2.1. Inclusion and exclusion criteria

This systematic review followed the methodology guidelines detailed in the PRISMA checklist (Moher, Liberati, Tetzlaff, Altman, & Group, 2009). Our systematic review included: i) all systematic reviews with meta-analysis of high quality according to the criteria established by Assessment of Multiple Systematic Reviews (Shea et al., 2007); ii) analyzed variable: efficacy of auriculotherapy versus placebo, sham acupuncture or usual treatment, in any type of pathology; iii) any type of ear-acupuncture therapy or ear-acupressure therapy (such as needles inserted into ear acupoints, electric stimulation, seeds or magnetic pellets attached to ear acupoints, or prick blood-letting technique on ear acupoints).

Were excluded from this work all the narrative and systematic reviews of low methodological quality, according to the criteria established by Assessment of Multiple Systematic Reviews (AMSTAR) (Shea et al., 2007).

2.2. Search strategy

Two independent researchers aided by a health sciences librarian searched the electronic databases by stablishing key MESH terms, as well as free terms according to the PRESS standards (McGowan et al., 2016). The considered keywords are listed in the appendix file 1. The following electronic databases were assessed in November 2017: PubMed, MEDLINE,

PsycINFO (Psychology and Behavioral Sciences Collection), EBMR (Evidence-Based Medicine Reviews), the Cochrane Database of Systematic Reviews, CINAHL Plus, NRC (Nursing Reference Center) and Science Direct. The references listed in the output studies and reviews were also scanned for relevant literature. PubMed was firstly searched and the adopted strategy was adapted to the other databases. As well, PROSPERO was also searched for ongoing or recently completed systematic reviews.

2.3. Selection, data extraction and analysis

The titles and abstracts of the output articles were screened for eligibility. The full texts of the preliminarily selected articles were then reviewed to confirm that they met the inclusion criteria. Elected articles were further analyzed in order to extract the required information, which included first author name, year of publication and country, study characteristics such as sample size, adverse effects, methodological quality, participants' characteristics (target population, age and gender) and outcome measures. All doubts about the eligibility of certain studies were discussed between the authors of this study. The effectiveness criteria for auriculotherapy interventions was based on the statistical differences versus placebo, sham acupuncture or usual treatment (Tan, Suen, Wang, & Molassiotis, 2015; Zhang, Yang, Zhang, May, & Xue, 2014).

The AMSTAR checklist was used to assess the methodological quality of the included systematic reviews. The instrument is an 11-item questionnaire that asks reviewers to answer yes, no, can't answer or not applicable. All items scoring "yes" received one point, up to a maximum of eleven points for each review.

The criteria for AMSTAR includes 3 grades of evidence: i) score between 9-11 indicates that systematic review is of good quality; ii) score between 5-8 indicates that systematic review is of medium quality and iii) score between 0-4 indicates that systematic review is of low quality (Shea et al., 2007). See table 2-5 and the appendix file 2 for detailed information.

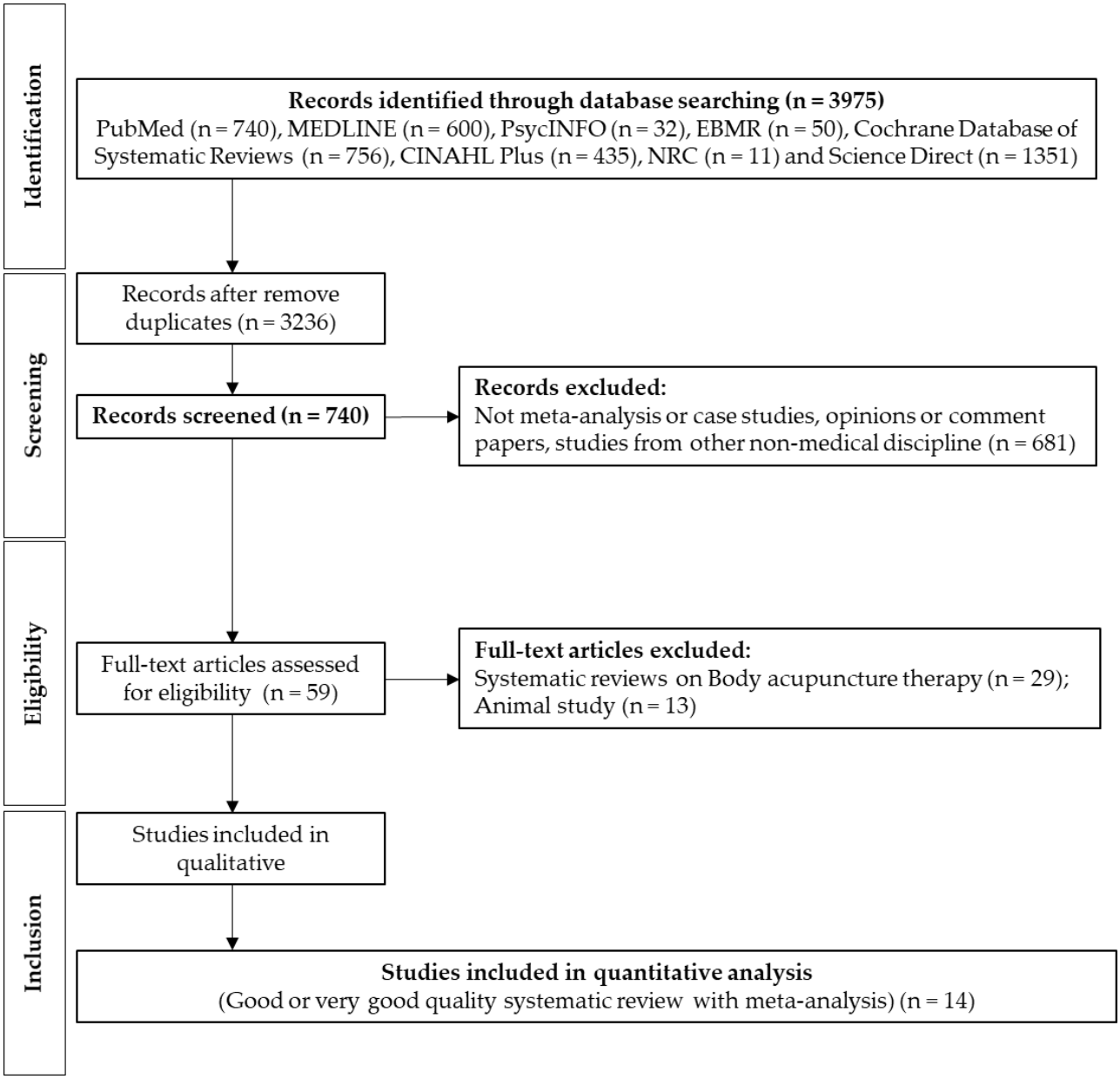
The statistical differences between the treatments and the sham or control groups were evaluated considering variables such as the standardized mean difference (SMD), the 95% confidence interval (CI), the mean difference (MD), the relative risk (RR) or the heterogeneity (I^2).

3. Results

3.1. Search output and selection

A total of 3975 articles were retrieved from the initial search. Among them, 59 studies were

considered after the preliminary screening evaluation. From these latter, 14 met the eligibility criteria and 45 were excluded (13 were in animals, 29 were on acupuncture and not exactly on auriculotherapy and 3 did not meet the quality requirements for inclusion). Figure 1 shows the diagram with the selection procedure, and Table 1 shows the selected high quality reviews grouped by medical specialty, as well as the level of effectiveness of auriculotherapy.



Chapter 2. Figure 1 PRISM diagram with the selection procedure (adapted from (Moher et al., 2009)).

Chapter 2. Table 0.1 High quality reviews on auriculotherapy grouped by medical specialty and level of effectiveness.

Medical Specialty and pathologies	Number of Reviews	Effectiveness	Lack of Effectiveness
Neurology: Insomnia.	4	Lan et al., 2015 (Lan et al., 2015); Yeung et al., 2012 (Yeung et al., 2012); Lee et al., 2008 (Lee, Shin, Suen, Park, & Ernst, 2008); Chen et al., 2007 (Chen et al., 2007).	-
Traumatology: perioperative pain; acute pain.	3	Murakami et al., 2017 (Murakami, Fox, & Dijkers, 2017); Yeh et al., 2014 (Yeh et al., 2014); Asher et al., 2010 (Asher et al., 2010).	-
Psychiatry: smoking cessation; cocaine dependence	3	Di et al., 2014 (Di et al., 2014); White et al., 2014 (White, Moody, & Campbell, 2007).	Gates et al., 2006 (Gates, Smith, & Foxcroft, 2006).
Rheumatology: Low back pain, chronic tension headache; chronic neck pain; Knee osteoarthritis; Rheumatoid arthritis; chronic non-specific spinal pain; posterior pelvic pain.	3	Asher et al., 2010 (Asher et al., 2010); Zhao et al., 2015 (Zhao, Tan, Wang, & Jin, 2015); Yeh et al., 2014 (Yeh et al., 2014).	-
Gastroenterology: constipation	1	Yang et al., 2014 (Yang et al., 2014)	-
Immunology: allergic rhinitis	1	Zhang et al., 2010 (Zhang et al., 2010).	-
Obstetrics: nausea and vomiting in early pregnancy	1	Matthews et al., 2014 (Matthews, Haas, O'Mathuna, Dowswell, & Doyle, 2014) Law et al., 2013 (Law & Li,	-

3.2. Effectiveness of auriculotherapy

Thirteen reviews presented in Table 1 point towards beneficial effects of auriculotherapy over the symptoms of the mentioned health conditions. One of the reviews concludes that auriculotherapy affects are not statistically different from placebo or from the usual treatment, and other one do not identify any effects in patients with cocaine dependence (Gates et al., 2006).

The studies corroborating the effectiveness of auriculotherapy over neurologic, rheumatic, traumatic injury, acute pain and even psychiatric disorders are shown in Tables 2-4. Data shown in Table 5 is concerned with several disorders, regarding which the reviews are based in a lower number of studies, reporting either the existence or the absence of effects.

The 4 systematic reviews mentioned in Table 2 show that auriculotherapy seems to be effective in the treatment of insomnia with rare adverse risks (e.g local pain). Considering the evaluation based on the quality of sleep parameters, one meta-analyze with 3 studies (n = 211) show that this technique is better as compared to conventional medication, as well as to the control group with 5 studies (n = 390), with sleep time increased to 6 hours (Chen et al., 2007). Other review with 2 studies (n = 122) suggests beneficial effects on sleep efficiency with magnetic pellets as compared to the sham group, as well as to the conventional medication group (Lee et al., 2008).

Regardless, Yeung et al., 2012 found that acupressure as monotherapy fared marginally better than sham control in one study (n=60) and that acupressure, reflexology, or auricular acupressure as monotherapy or combined with routine care was significantly more efficacious than routine care or no treatment in 1 study (n=10) (Yeung et al., 2012).

More recently, Lan et al., 2015 found that the participants who received auricular acupuncture were more likely to make an improvement in clinical effective rate, sleep duration, sleep efficiency, global score on PSQI, number of awakenings and sleep onset latency when compared to sham auricular acupuncture or placebo; while in auricular acupuncture VS medications comparison, a better effective rate, better sleep efficiency, lower PSQI score and less adverse effect can be seen also in auricular acupuncture group (Lan et al., 2015). In the included reviews, meta-analyses of included studies revealed a positive effect of auricular acupuncture for insomnia. However the poor methodological quality in all studies, mean that the evidence is not yet adequate to provide a strong support for the therapy in insomnia management.

Chapter 2 Table 0.2 Systematic reviews pointing to beneficial effects of auriculotherapy over Neurologic pathologies.

Author, year	Intervention/disorder	Results	Adverse effects	AMSTAR score
Lee et al., 2008 (Lee et al., 2008);	Semen vaccariae ear seeds and magnetic pellet for insomnia.	Auriculotherapy better than sham ($p < 0.05$): With MP, n = 122 (2 studies): MD = 7.5, 95% CI [2.2, 12.9] $I^2 = 0\%$ for sleep efficiency. Auriculotherapy better than conventional medication ($p < 0.05$): - 1 study reported significant beneficial effects of MP over sleep disorder as compared with diazepam, and 3 studies using SV presented significant improvements in nocturnal sleeping time. - 2 studies tested auriculotherapy with thumbtack-type needle *vs* no treatment and found beneficial effects on the sleep score and self-satisfaction scale, as compared with no treatment. **Methodological quality according to the adapted Jadad scale: Low quality.**	Adverse events such as local pain were mentioned in three studies.	10/11
Chen et al., 2007 (Chen et al., 2007)	Semen vaccariae ear seeds and magnetic pellet for insomnia.	Auriculotherapy better than control group ($p < 0.05$): RR= 1.93 (95% CI [1.40, 2.66]; n = 390 (5 studies). - Sleep time increased to 6 hours, RR= 2.64 (95% CI [1.22, 5.72] - SV were statistically favored as compared to controls RR = 1.80; 95% CI [1.42, 2.28]; n = 122 (2 studies). - Auriculotherapy better than diazepam, RR = 1.41 (95% CI [1.12, 1.77]; n = 211 (3 studies). **Methodological quality according to the Cochrane reviewers' handbook: Low**	No mentioned adverse effects.	9/11

		quality.		
Yeung et al., 2012 (Yeung et al., 2012)	Acupressure, reflexology, and auricular acupressure for insomnia.	Auriculotherapy better than sham ($p < 0.05$): MD = 52.7, 95% CI [16.9, 88.5], RR= 0.69, 95% CI [0.3, 1.1]; - Sleep efficiency: MD = 11.6%, 95% CI [5.5, 17.6], RR = 0.94, 95% CI [0.5, 1.4] n = 60 (1 study). Auriculotherapy better than no treatment ($p < 0.05$): MD = -2.5, 95% CI: [-4.2, -0.8], effect size: -0.82, 95% CI [-1.4, -0.3] n = 10 (1 study). **Methodological quality based on Jadad Score: Moderate quality.**	NR	10/11
Lan *et al*., 2015 (Lan et al., 2015)	Auricular acupuncture with seeds or pellets attachments for primary insomnia	Auriculotherapy better than sham ($p < 0.05$) n = 661 (5 studies): - Clinical effectiveness rate: RR = 1.40, 95% CI [1.07, 1.83]); - Sleep duration: MD = 56.46, 95% CI [45.61, 67.31]; - Sleep efficiency: MD = 12.86, 95% CI [9.67, 16.06]; - Global score on PSQI: MD = -3.41, 95% CI [-3.93, -2.89]; - Number of awakenings: MD = -3.27, 95% CI [-6.30, -0.25]); - Sleep onset latency: MD = -10.35, 95% CI [-14.37, -6.33]. Auriculotherapy better than convencional medications ($p < 0.05$) n = 575 (8 studies): - RR = 1.24, 95% CI [1.15 to 1.34]; - Sleep efficiency MD = 21.44, 95% CI [16.30, 26.58]; - Lower PSQI score: MD = -3.62, 95% CI [-4.59, -2.65]. **Methodological quality based on grade system: Low quality.**	Less adverse effect:, RR = 0.11, 95% CI [0.04, 0.26]	11/11

Abbreviations: 95% CI - 95% Confidence Interval; MD - Mean Difference; RR - Relative Risk; I2 -

Heterogeneity; SV - Semen Vaccariae; MP - Magnetic Pearls; PSQI - Pittsburgh Sleep Quality Index; NR - Not Related. AMSTAR (Assessment of Multiple Systematic Reviews) grades of evidence: High (9-11); Medium (5-8); Low (0-4).

According some reviews, auriculotherapy shows positive effects over pain in all included studies with few adverse effects, such as local pain, tiredness, bleeding, headache, intraoperative bradycardia, nausea and dizziness. Newly, Murakami et al., 2017 found In 6 studies (n = 159) the effect of auriculotherapy was superior as compared to sham treatment and conventional analgesic medication up to 48 hours after surgery keep up with strong methodological quality of studies include (Murakami et al., 2017). Yeh et al., 2014 represent the efficacy of auricular therapy for pain relief, with moderate- to high-strength evidence in 13 studies (n = 806) and found efficient in both acute and chronic pain as compared to the control group and sham (Yeh et al., 2014).

The effectiveness over acute pain (2 studies, n = 111) as well as over chronic pain (5 studies, n = 261), is reflected by the reduction of pain intensity in relation to the sham group, as well as in the reduction of conventional analgesics in perioperative pain (5 studies, n = 412) follow poor (41%) to good (35%) strength evidence. (Asher et al., 2010).

Various types of chronic pain can benefit from this therapy (4 studies, n = 206), especially chronic low back pain (4 studies, n = 193) and headaches (2 studies, n = 150) caused by muscle tension. When compared to control and sham groups, the intervention groups show differences in pain relief within 0 to < 3 weeks (7 studies, n = 363), 3 to < 6 weeks (6 studies, n = 327), > 6 weeks (4 studies, n = 181), and higher follow-up periods (5 studies, n = 293), but with methodological flaws identified in the analyzed trials (Zhao et al., 2015).

Chapter 2. Table 0.3. Systematic reviews pointing to beneficial effects of auriculotherapy over orthopaedic and rheumatic pathologies.

Author, year	Intervention/disorder	Results	Adverse effects	AMSTAR score
Asher et al., 2010 (Asher et al., 2010)	Auricular needles, electrical stimulation of either indwelling auricular needles or transcutaneously without needles, laser stimulation and acupressure for pain management	Auriculotherapy better than control group ($p < 0.05$) n = 466 (8 studies): - Pain intensity SMD = 1.56; (95% CI): 0.85, 2.26]. - For perioperative pain, auriculotherapy reduced analgesic use: SMD = 0.54; 95% CI [0.30, 0.77]; n = 412 (5 studies). - Acute pain, MD = 1.35; 95% CI [0.08, 2.64] n = 111	Ear pain (n = 16) and tiredness (n = 16); local minor bleeding (n = 2), dizziness and nausea (n = 1), and headache (n = 1).	10/11

		(2 studies); - Chronic pain, MD = 1.84; 95% CI [0.60, 3.07] n = 261 (5 studies); Removal of poor quality studies did not alter the conclusions. **Methodological quality based on the criteria of the United States Preventive Services Task Force and the National Health Service Centre for Reviews and Dissemination (United Kingdom criteria): 35% were good, 24% were fair, and 41% were of poor quality.**		
Yeh et al., 2014 (Yeh et al., 2014)	Auricular acupuncture, auricular electroacupuncture and auricular acupressure for pain management	Auriculotherapy better than sham ($p < 0.05$): - SMD = 1.59; 95% CI [-2.36, -0.82]); - Pain relief: MD = -1.81, 95% CI [-2.92, -0.70], p = 0.001) n = 806 (13 studies). Auricular eletro-stimulation for pain reduction: SMD = -0.39; 95% CI [-1.05, 0.26] n = 494 (7 studies); - Acupressure for pain relief: MD = -1.85; 95% CI [-3.35, -0.35] n = 275 (4 studies). **Methodological quality of include evidence based on Methodological Quality: good quality.**	NR	10/11
Zhao et al., 2015 (Zhao et al., 2015)	Ear plaster with vaccaria seeds, electroacupuncture and auricular bloodletting for chronic pain	Auriculotherapy better than sham ($p < 0.05$): - Chronic pain: (4 studies, n = 206); MD = -3.76; 95% CI [-4.97, -2.54] in pain relief; Chronic low back pain (4 studies, n = 193); MD = -1.70; 95% CI [-2.83, -0.56]; Chronic tension headaches (2 studies, n = 150); MD = -0.63; 95% CI [-0.95 to -0.30]; Acupressure (3 studies, n = 180); MD =-0.75; 95% CI [-1.26, -0.25]; Auricular electro-stimulation (4 studies, n = 131); MD = -3.29, 95% CI [-5.87, -0.72]; Chronic neck pain showed no statistically significant change between groups (2 studies, n = 83); MD = -3.03; 95% CI [-9.61 to 3.56]. Acupressure plus bloodletting therapy also reported significant positive effects of the auriculotherapy intervention for pain relief. However, sub-group analysis showed no difference between groups in chronic pain management. Duration of pain relief: - Short-term (0 to < 3 weeks: 7 studies, n = 363); MD = -2.15; 95% CI [-3.29, -1.01]; - Mid-term (3 to < 6 weeks: 6 studies, n = 327); MD = -2.05; 95% CI [-3.20, -0.89];		10/11

		- Long-term (> 6 weeks; 4 studies, n = 181); MD = -2.47; 95% CI [-4.72, -0.22]. Follow-up periods: - One month: 5 studies, n = 293; MD = -2.46; 95% CI [-4.32, -0.60]; - 3 months: 3 studies, n = 160; MD = -0.97; 95% CI [-2.81, 0.87]. **Methodological quality based on Cochrane Handbook for Systematic Review for Intervention: Low quality**		
Murakami et al., 2017 (Murakami et al., 2017)	Ear acupuncture for immediate pain relief	Auriculotherapy better than sham group ($p < 0.05$): - Ear acupuncture was superior in pain intensity (3 studies, n = 204); MD = -0.96; 95% CI = [-1.82, -0.11], but the MD was small. - Analgesic requirements, ear acupuncture was superior (6 studies, n = 159); MD = -1.08; 95% CI = [-1.78, -0.38]. **Methodological quality based using the Physiotherapy Evidence Database (PEDro) Scoring System: good or excellent**	Local pain, bleeding, headache, intraoperative bradycardia, nausea and dizziness in 6 studies.	9/11

Abbreviations: 95% CI - 95% confidence interval; SMD - standardized mean differences; MD - mean difference. AMSTAR (Assessment of Multiple Systematic Reviews) grades of evidence: High (9-11); Medium (5-8); Low (0-4).

Regarding the framework of psychological conditions, two reviews show positive effects concerning smoking cessation. One of them shows some adverse effects such as sore ears, tenderness, sensation around residual needles, bruising, facial swelling, headache, dizziness, nausea, giddiness, vomiting, euphoria, decreased migraine and insomnia. This Review concluded that, at end of the treatment, auriculotherapy was superior to control group with cessation rates of 22.7% and 12.6%, respectively in 10 studies (n=1386). In a three months follow-up study the cessation rates were 15.8% (intervention) vs. 10.1% (control), with an improvement of 12.5% confirmed by biochemical methods. After 6 months the cessation rates were still considerable different with values of 12.4% (intervention) vs. 6.1% (control) (Di et al., 2014). The other Review shows with moderate-strength evidence that, in three studies (n = 325), true acupressure was more effective in short-term cessation than sham acupressure (White et al., 2007). Biochemical verification of tobacco cessation (analysis of urine, blood and saliva samples) has been used to validate self-report smoking status (Noonan, Jiang, & Duffy, 2013). Subjects responses regarding their recent tobacco use are mainly affected by recall bias

resulting in inaccurate reporting (Jain et al., 2015). Therefore, biochemical validation is recommended in smoking cessation studies (Cha, Ganz, Cohn, Ehlke, & Graham, 2017).

Chapter 2. Table 0.4. Systematic reviews pointing to beneficial effects of auriculotherapy over physical, emotional, and psychological pathologies.

Author, year	Intervention/disorder	Results	Adverse effects	AMSTAR score
Di et al., 2014 (Di et al., 2014)	Ear-acupuncture, ear-acupressure and auriculotherapy for cigarette smoking cessation	Auriculotherapy better than control group ($p < 0.05$): - Cessation rates: were 22.7% (intervention) *vs.* 12.6% (control); RR = 1.77; 95% CI [1.39 to 2.25], I^2 = 53% n = 1386 (10 studies); - 3 month follow-up (RR = 1.54; 95% CI [1.14, 2.08], I^2 = 11%) and the cessation rates were 15.8% (intervention) *vs*. 10.1% (control), with 12.5% improvement confirmed by biochemical methods - 6 month follow-up (RR = 2.01, 95% CI [1.23, 3.28], I^2 = 0%) with cessation rates of 12.4% (intervention) *vs.* 6.1% (control), RD 6.3%; without biochemical confirmation. **Methodological quality based on Cochrane Risk of Bias: Not described in article**	Sore ears, tenderness, sensation around residual needles, bruising, facial swelling, headache, dizziness, nausea, giddiness, vomiting, euphoria, decreased migraine, insomnia.	9/11
White et al., 2014 (White et al., 2007)	Acupressure for smoking cessation	Auriculotherapy better than sham group ($p < 0.05$): RR = 1.69, 95% CI [1.32, 2.16], n = 1155 (14 studies); - Subgroup analysis showed an effect for continuous acupressure (7 studies, n = 496) RR = 2.73; 95% CI [1.78, 4.18]; - Acupressure in correct points was more effective for short-term cessation than placebo group (3 studies) n = 325; RR = 2.54; 95% CI [1.27, 5.08] **Methodological quality based on GRADE Working Group grades of evidence: Moderate quality.**	NR	11/11

Abbreviations: 95% CI - 95% confidence interval; SMD - standardized mean differences; MD - mean

difference. AMSTAR (Assessment of Multiple Systematic Reviews) grades of evidence: High (9-11); Medium (5-8); Low (0-4).

Reviews focused in other pathologies included a lower number of studies reporting either the existence or the absence of effects. Only one Review show 1 of 37 patients and 2 of 60 patients had slight localized redness and damage in the skin and 7 of 39 patients had mild, tolerable, and short-term itchiness of the ear points. In the field of Ophthalmology, one review included the results of one study (n = 33) on the treatment of glaucoma. In this study, after four weeks, the differences in intraocular pressure presented by the acupressure group was significantly lower than the sham group. Nevertheless, these differences were not statistically different at any other follow-up time, including the longest one, at eight weeks (Law & Li, 2013).

In the Immunology field, specifically in the treatment of allergic rhinitis, 2 studies (n = 508) showed that auriculotherapy has a significantly higher short-term effect (4 weeks) when compared to Chinese herbal medicine, and significantly better long-term effect (6 months) than anti-histamine medication in 1 study (n=150) but not than body acupuncture or anti-histamine (cetirizine) in a 4 weeks (Zhang et al., 2010). Regarding the gastroenterology field, one review with 15 studies (n = 1324) points towards improvements in constipation, however no effects over other symptoms associated with this disorder (e.g., abdominal distension) (Yang et al., 2014). The effects of acupuncture over nausea and vomiting in early pregnancy is referred in 41 reviews (n = 5449) (Matthews, Haas, O'Mathuna, & Dowswell, 2015). Nonetheless, just one study on auriculotherapy shows differences between the intervention and sham groups. Those differences were noticed on day 6 (3 days after the beginning of the treatment), however no differences were found in the number of used anti-emetic drugs. This reviews showed some evidence of auriculotherapy but the true benefit is yet to be determined due to poor quality of the included studies as well lack of clinical trials.

Chapter 2. Table 0.5 Systematic reviews pointing to beneficial effects of auriculotherapy over in Gastroenterology, Immunology, Ophthalmology and Gynaecology pathologies.

Author, year	Intervention/disorder	Results	Adverse effects	AMSTAR score
Yang et al., 2014 (Yang et al., 2014)	Semen vaccariae or magnetic pellets for constipation in Adults.	Auriculotherapy better than control group ($p < 0.05$): RR = 2.06; 95% CI [1.52, 2.79] with total effective rate: RR = 1.28; 95% CI [1.13,	1 of 37 patients and 2 of 60 patients had slight localized redness and	9/11

		1.44]; n = 1324 (15 studies). - For other symptoms associated with constipation, such as abdominal distension, the results of the meta-analyses showed no statistical significance. **Methodological quality based on the Cochrane risk of bias tool: 2 studies were of high quality, 15 were of moderate quality and 1 study was of low quality.**	damage in the skin, 7 of 39 patients had mild, tolerable, and short-term itchiness of the ear points.	
Law et al., 2013 (Law & Li, 2013)	Auricular acupressure for glaucoma	Auriculotherapy better than sham group ($p < 0.05$): - Intraocular pressure after four weeks -3.70; 95% CI [-7.11, -0.29] for the right eye; -4.90; 95% CI [-8.08, -1.72] for the left eye, but was not statistically different at any other follow-up time, including the longest one at eight weeks, n = 33 (1 study). **Methodological quality based on: 1 study - Low risk for random sequence generation, low risk for allocation concealment; low risk for blinding of participants and personnel; high risk for detection bias and low risk for attrition bias; unclear risk for reporting bias, and low risk for other bias.**	NR	10/11
Zhang et al., 2010 (Zhang et al., 2010)	Ear-acupressure for allergic rhinitis	Auriculotherapy better than fitotherapy group ($p < 0.05$): n = 108 (1 study) RR = 1.32; 95% CI [1.09, 1.59] and n = 400 (1 study) RR = 2.48; 95% CI [1.95, 3.15]; but not than body acupuncture: n = 66 (1 study) RR = 0.98; 95% CI [0.89, 1.08] or anti-histamine (cetirizine): n = 150 (1 study) RR= 0.96; 95% CI [0.88, 1.04] in a short term (4 weeks). However, it has a significantly better long-term (6 months) effect than anti-histamine medication: n = 150 (1 study) RR= 3.02; 95% CI [1.54, 5.93]. - Auriculotherapy combined with body		9/11

		acupuncture had effects that were superior to those from body acupuncture alone: n = 80 (1 study) RR= 1.22; 95% CI [1.04, 1.43]. Auriculotherapy had better effects than body acupuncture treatment for subjects with Lung and Spleen Qi deficiency syndromes. However, in subjects with phlegm-heat and blood stasis the effects were worst when compared to body acupuncture: RR= 0.66; 95% CI [0.44, 0.98]. When subjects of the subgroups are combined, the two treatments showed similar clinical outcomes: RR= 1.01; 95% CI [0.79, 1.28] n = 66 (1 study) **Methodological quality based on Jadad Scale: low quality.**	
Matthews et al., 2014 (Matthews et al., 2014)	Auriculotherapy for nausea and vomiting in early pregnancy	Auriculotherapy better than sham group ($p < 0.05$): - Nausea/vomiting score combined Rhodes Index score on day 6 (3 days after the beginning of the treatment): 1 study, n = 91; MD = -3.60; 95% CI [-6.62, -0.58] for overall effect: 2.34, but no differences in number of used anti-emetic drugs on day 6. **Methodological quality based on GRADE approach: 1 study - Low risk for random sequence generation, unclear risk for allocation concealment, high risk for blinding of participants and personnel, low risk for detection bias and low risk for attrition bias, low risk for reporting bias and unclear risk for other bias.**	11/11

Abbreviations: 95% CI - 95% Confidence Interval; MD - Mean Difference; RR - Relative Risk; I2 - Heterogeneity; SV - Semen Vaccariae; MP - Magnetic Pearls; PSQI - Pittsburgh Sleep Quality Index; NR - Not Related. AMSTAR (Assessment of Multiple Systematic Reviews) grades of evidence: High (9-11); Medium (5-8); Low (0-4).

4. Discussion

The current understanding of the physiological mechanisms beyond auriculotherapy treatments is still in progress (Hou et al., 2015; Rabischong & Terral, 2014). Still, a significant amount of work has been performed to assess auriculotherapy efficiency in the management of several health conditions.

The present work aimed to establish the current state of the scientific evidence on the therapeutic use of auriculotherapy and, therefore, to promote the development of hypothesis to explain the physiologic mechanisms of action.

Currently, more than 200 acupoints have been identified in the ear. Those are in the basis of the ear diagnosis, which by means of inspection, palpation, and electrical skin conductance of relevant areas on the ear, is believed to allow the identification of disorders elsewhere in the body. So, based on this idea, when a disease or disorder is present, some related auricular acupoints show a higher or lower resistance as compared to nearby reference auricular points (Colbert A.P., 2011). Our results point towards positive effects of auriculotherapy as a therapeutic tool, particularly for pain relief. Nevertheless, more research is needed to corroborate our findings, as well as to encourage standardization, systematization and follow-up studies.

According to Juan Muñoz-Ortego (2016), systemic acupuncture is often used in pain management, such as in the treatment of tension headaches and migraines, osteoarthritis, low back pain, ankle sprains as well as in sore throat (Munoz-Ortego, Solans-Domenech, & Carrion, 2016). Based on the reviews included in our study, auriculotherapy is shown to be efficient in the treatment of insomnia.

Although some reviews report therapeutic indications of acupuncture in the area of neurology, more studies with cross-methodologies involving systemic acupuncture and auriculotherapy are needed to make clear its usefulness (Munoz-Ortego et al., 2016).

The reviews included in our study relating auriculotherapy to mental health show that smoking cessation can be benefited by this technique. Moreover, systemic acupuncture is only reported with success in the management of depression and schizophrenia (Munoz-Ortego et al., 2016). However, according to Gates (2006) auriculotherapy do not has positive effects in the management of cocaine dependence. The author highlights the low methodological quality of two studies involving 1,433 participants, suggesting further randomized trials to overcome those limitations.

With regard to other pathologies, such glaucoma, constipation, allergic rhinitis or nausea and vomiting in early pregnancy, it is difficult to draw reliable deductions from available data to

support the use of auriculotherapy. We need understand the ethical considerations, RCTs comparing auriculotherapy alone with standards treatments or placebo are unlikely to be justified in countries where the standard of care has already been established. subsequently, most patients currently cared for by medical specialties do not use nontraditional therapy, so, clinical practice decisions will have to be based on physician judgments and patient preferences, given this lack of data in the literature (Law & Li, 2013).

We are aware of the limitations of our review, which is inevitably subjected and integrates the limitations of the included studies. Nevertheless, according to the criteria established by AMSTAR, the included 14 systematic reviews were classified as high quality reviews. Those found that some studies had several methodological limitations and flaws, such as the heterogeneity, loss of information on important outcomes, inappropriate subgroup analyses, conflict with new experimental data, and duplication of publication (Gopalakrishnan & Ganeshkumar, 2013).

The selected reviews in our study excluded some clinical trials with a small sample size, lack of randomization, low heterogeneity, poor literature review and laboratory experiments on animals. Although this strategy excluded several studies with positive results it, has the advantage of just include high-quality articles.

The diversity of auriculotherapy modalities such as auricular acupuncture/acupressure, auricular electro-acupuncture, as well as the use of semi-permanent needles may be associated with different treatment effects, even if treating the same clinical condition. This represents a challenge in research, who requiring further systematization. Actually, in the mentioned studies, each approach used a specific cartography, which may lead to subjectivity in the selection of points for a given indication. As well, placebos should be indistinguishable from the true interventions, and most importantly, should be inert, which means that they should only stimulate non-specific physiological and psychological effects (Dincer & Linde, 2003; Tan et al., 2015; Zhang et al., 2014). The selection of sham ear points should always consider the dermatomes interaction as we can see in our previous study (Vieira et al., 2018; A. Vieira et al., 2016). Moreover, with regards to the technique safety the mild, short-term adverse effects reported in our study only affected a very small proportion of the patients, and no serious adverse events associated with Auriculotherapy occurred in the analyzed studies. Subsequently, auriculotherapy is one of the most popular complementary therapies, and it can be viewed as an easily administered and safe intervention, also supported by other systematic reviews (Zhao et al., 2015).

Following this critical sense, researchers should follow the Consolidated Standards of Reporting Trials (CONSORT) statement (Moher, Schulz, & Altman, 2005), and the Revised Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) guidelines (MacPherson et

al., 2010).

5. Conclusions

This systematic review shows that auriculotherapy has enough scientific evidence to be indicated in the treatment of chronic pain, mainly headaches, migraines, back pain, neck pain and osteoarthritis. More research is needed in order to find out if this technique can be used as an effective therapeutic tool in the management of other heath conditions.

Our findings suggest that auriculotherapy may be used as an adjunctive therapy in pain management, as well as in the treatment of insomnia and in smoking cessation interventions, reducing the use of conventional medications, minimizing the potential adverse effects, and reducing the required time to achieve positive results.

Conflict of interest: None

Authorship: All authors made significant contributions to the study design, acquisition of data, drafting of the article, and final approval of the article.

Competing interests: None.

Financial support: None.

Supplementary Material

Additional File 1: Search strategies

Identifying and removing duplicate records from systematic review searches.

Additional File 2: AMSTAR

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Additional File 1 - Search strategies

Identifying and removing duplicate records from systematic review searches

Pubmed Search their inception to 8 November, 2017

Auriculotherapy [Subject Heading]

“ear acupuncture”/ OR “auricular acupuncture”/

“auricular acupressure”/ OR “ear acupressure”/

1 OR 2 OR 3

"Meta-analysis"/ OR "Evidence synthesis"/ OR "Systematic review"/

4 AND 5

Limit 6 to Humans.

	Search Details	Search Results
1	"auriculotherapy"[MeSH Terms] OR "auriculotherapy"[All Fields]	448
2	"ear acupuncture"[All Fields] OR "auricular acupuncture"[All Fields]	594
3	"auricular acupressure"[All Fields] OR "ear acupressure"[All Fields]	98
4	("auriculotherapy"[MeSH Terms] OR "auriculotherapy"[All Fields]) OR ("ear acupuncture"[All Fields] OR "auricular acupuncture"[All Fields]) OR ("auricular acupressure"[All Fields] OR "ear acupressure"[All Fields])	740
5	"Meta-analysis"[All Fields] OR "Evidence synthesis"[All Fields] OR "Systematic review"[All Fields]	203024
6	(("auriculotherapy"[MeSH Terms] OR "auriculotherapy"[All Fields]) OR ("ear acupuncture"[All Fields] OR "auricular acupuncture"[All Fields]) OR ("auricular acupressure"[All Fields] OR "ear acupressure"[All Fields])) AND ("Meta-analysis"[All Fields] OR "Evidence synthesis"[All Fields] OR "Systematic review"[All Fields])	52
7	(("auriculotherapy"[MeSH Terms] OR "auriculotherapy"[All Fields]) OR ("ear acupuncture"[All Fields] OR "auricular acupuncture"[All Fields]) OR ("auricular acupressure"[All Fields] OR "ear acupressure"[All Fields])) AND ("Meta-analysis"[All Fields] OR "Evidence synthesis"[All	39

Additional File 2 – AMSTAR

1. Was an “a priori” design provided?

The research question and inclusion criteria should be established before the review.

- □ Yes
- □ No
- □ Can’t answer
- □ Not applicable

2. Was there duplicate study selection and data extraction?

There should be at least two independent data extractors and a consensus procedure for disagreements.

- □ Yes
- □ No
- □ Can’t answer
- □ Not applicable

3. Was a comprehensive literature search performed?

At least two electronic sources should be searched. The report must include years and databases used (e.g. Central, EMBASE, and MEDLINE). Key words and/or MESH terms must be stated and where feasible the search strategy should be provided. All searches should be supplemented by consulting current contents, reviews, textbooks, specialized registers, or experts in the particular field of study, and by reviewing the references in the studies found.

- □ Yes
- □ No
- □ Can’t answer
- □ Not applicable

4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?

The authors should state that they searched for reports regardless of their publication type. The authors should state whether or not they excluded any reports (from the systematic review), based on their publication status, language etc.

- □ Yes
- □ No
- □ Can’t answer
- □ Not applicable

5. Was a list of studies (included and excluded) provided?

A list of included and excluded studies should be provided.

□ Yes
□ No
□ Can't answer
□ Not applicable

6. Were the characteristics of the included studies provided?

Data from the original studies should be provided in an aggregated form such as a table. Data should include the sample characterization (including e.g. age, race, sex, relevant socioeconomic data, disease status, duration, severity, or other diseases), interventions and outcomes.

□ Yes
□ No
□ Can't answer
□ Not applicable

7. Was the scientific quality of the included studies assessed and documented?

"A priori" methods of assessment should be provided (e.g., choice to include only randomized, double-blind, placebo controlled studies, or allocation concealment as inclusion criteria); for other types of studies alternative items will be relevant.

□ Yes
□ No
□ Can't answer
□ Not applicable

8. Was the scientific quality of the included studies used appropriately in formulating conclusions?

The methodological accuracy and scientific quality should be reflected in the analysis and conclusions of the review, and explicitly stated in the formulating of recommendations.

□ Yes
□ No
□ Can't answer
□ Not applicable

9. Were the methods used to combine the findings of studies appropriate?

For the pooled results, a test should be done to ensure the studies were combinable, to assess their homogeneity (i.e. Chi-squared test for homogeneity, I^2). If heterogeneity exists a random effects model should be used and/or the clinical appropriateness of combining should be taken into consideration (i.e. is it sensible to combine?).

□ Yes
□ No
□ Can't answer
□ Not applicable

10. Was the likelihood of publication bias assessed?

An assessment of publication bias should include a combination of graphical aids (e.g., funnel plot, other available tests) and/or statistical tests (e.g., Egger regression test).

□ Yes
□ No
□ Can't answer
□ Not applicable

11. Was the conflict of interest stated? Potential sources of support should be clearly acknowledged in both the systematic review and the included studies.	□ Yes □ No □ Can't answer □ Not applicable

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CHAPTER 3

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Protocol

Is auriculotherapy effective and safe for the treatment of anxiety disorders? – Protocol for a systematic review

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Is auriculotherapy effective and safe for the treatment of anxiety disorders? – Protocol for a systematic review

Abstract

Introduction: The side effects associated with standard anxiety medications can limit their use. Therefore, a non-pharmacological approach such as auriculotherapy could play an important role in anxiety management. The main aim of this protocol for a systematic review will be to examine the effectiveness and safety of auriculotherapy for anxiety disorders (AD).

Methods: The authors will conduct searches on the following databases: Cochrane Central Register of Controlled Trials, MEDLINE Ovid, Stephens Company, PubMed, Latin American and Caribbean Health Sciences Literature Database, British Library's table of contents, World Health Organization, Clinical Trials. We will conduct a systematic review for randomized controlled trials (RCTs) evaluating the effectiveness and safety of auriculotherapy compared with placebo, waitlist treatment, routine care, or alternative treatment. To assess the evidence's quality, we plan to use the Grading of Recommendations Assessment, Development and Evaluation tool while, for risk assessments, we will use the statistical software RevMan V 5.4. Data collection and analysis will be performed by two reviewers independently.

Discussion: If effective and safe, then auriculotherapy may be an appropriate alternative to pharmacological treatment. Registration number: PROSPERO ID: CRD42021254503.

Keywords: Anxiety disorder, Auriculotherapy; Auricular Acupuncture, acupressure, brain stimulation. Systematic review

1. Introduction

Anxiety is one of the most natural body responses, resulting in a sign of fundamental alert in humans to respond to a given threat (Notebaert et al., 2020; World_Health_Organization, 2017). However, individual differences in physical and emotional health impairment can lead to hormonal changes, and anxiety can become pathological (Dean, 2016). These hormonal changes can affect the individual's immune system, resulting in various conditions such as cancer, chronic musculoskeletal pain, respiratory diseases, cardiovascular and infections (Kolesnikov et al., 2006; Moons & Shields, 2015). Anxiety Disorder (AD) is pathological when anxiety's intensity, duration, or frequency interferes with a person's functioning. (American_Psychiatric_Association, 2014). Following the Diagnostic and Statistical Manual of Mental Disorders (DSM-V, 2014), there are several types of anxiety disorders such as Separation AD, Social phobia, selective mutism, Specific phobia, Panic Disorder, Agoraphobia, Generalized AD, AD Induced by Substance/Medicine, AD due to other Medical Condition, and Other Specified or non-specified AD (American_Psychiatric_Association, 2014).

AD can affect around 3.6% (264 million people) of the global population (World_Health_Organization, 2017). Potential healthcare costs from AD include reduced productivity, absenteeism from work, increased drug prescriptions, hospitalization, emergency care and suicide,(Francois, Despiegel, Maman, Saragoussi, & Auquier, 2010; World_Health_Organization, 2017). Conventional medicine often relies on treatments for AD, such as benzodiazepines(Lader & Kyriacou, 2016), antidepressants (Popovic, Vieta, Fornaro, & Perugi, 2015), barbiturates(Invernizzi & Vitali, 1969) and antihistamines (Charlton, 2005). However, the risk of side effects and resistance to pharmacological treatment affects approximately one in three patients with AD (Durham, Higgins, Chambers, Swan, & Dow, 2012). Despite that, some studies show positive findings using auriculotherapy in AD treatment, but there is insufficient research evidence to draw solid conclusions. (Amorim et al., 2018; Correa et al., 2020; Pilkington, Kirkwood, Rampes, Cummings, & Richardson, 2007; A. Tan et al., 2020).

Auriculotherapy is a technique derived from traditional acupuncture developed into a distinct treatment system of its own (Asher et al., 2010). Auriculotherapy is the introduction of needles (auricular acupuncture) (Vieira, Hinzmann, Silva, Santos, & Machado, 2018) with or without percutaneous electrical nerve stimulations (Fallgatter et al., 2003) (electroacupuncture) and auricular bloodletting (Ni et al., 2018), or pressure with Vaccaria seeds (acupressure) (Asher et al., 2010) or laser-auriculo-stimulation (Suen, Yeh, & Yeung, 2016) into the human auricular

area.´

The induction of auricular areas endorses responses from the reticular formation and through the sympathetic and parasympathetic nervous systems. (Mohrsen) Such information coming through the thermal, Algic and proprioceptive stimuli are transmitted from the auricular pavilion by the fibres of the trigeminal nerve, auricular magnum, and minor occipital (sensitive branch of the cervical plexus), and the vagus nerve. ("Correction: Cervical plexus and greater occipital nerve blocks: controversies and technique update,"). The vagus nerve is responsible for the parasympathetic innervation of the lungs, heart, stomach, and small intestine, as well as the pharynx and larynx muscles, and it also sends information to essential brain regions in the regulation of anxiety (locus coeruleus, orbitofrontal cortex, hippocampus, and the amygdala). In turn, the trigeminal nerve mainly controls the mastication muscles and facial sensitivity. Finally, the cervical plexus innervates the neck, diaphragm, and thorax ("Drugs to Treat Anxiety Disorders and Obsessive Compulsive Disorder (OCD)," 2022). Recordings of vagus somatosensory evoked potentials from the scalp(Fallgatter et al., 2003) revealed the feasibility of auriculotherapy as an effective therapeutic strategy for managing several clinical disorders, including pain (Vieira, Reis, Matos, Machado, & Moreira, 2018), epilepsy (Wu, Wang, Zhang, Yao, & Zhang, 2020), depression (Li et al., 2019), migraine (Straube, Ellrich, Eren, Blum, & Ruscheweyh, 2015), substance dependence (Gates, Smith, & Foxcroft, 2006; Lui, Li, Xia, & Terplan, 2009) and tinnitus (Kim et al., 2012). Regarding anxiety, there is some limited evidence in favour of auricular acupuncture for perioperative anxiety (Dietzel et al., 2020; Pilkington et al., 2007), for AD disorders and major depressive disorders (de Lorent, Agorastos, Yassouridis, Kellner, & Muhtz, 2016), for situational anxiety primary school examinations (Usichenko et al., 2020; Vieira, Hinzmann, et al., 2018; Vieira et al., 2016), and the reduction of state anxiety before dental treatments (Michalek-Sauberer, Gusenleitner, Gleiss, Tepper, & Deusch, 2012). Although, there are no systematic reviews to prove auriculotherapy effectiveness in AD treatment.

Concerning the adverse events related to auriculotherapy, Correa et al., 2020 reported that auriculotherapy on stress, anxiety and depression in adults and older adults could cause headaches and bleeding at the needle application site (Klausenitz et al., 2016) and local pain (Kurebayashi et al., 2017). Nevertheless, the author´s (Correa et al., 2020) had used the Jadad scale to determine the studies' quality. In our review, we aimed to perform the assessment using the risk of bias tool. Furthermore, in 2014, Tan Jing et., al; reported that the most frequently reported adverse events in auriculotherapy was local skin irritation, discomfort, mild tenderness or pain, and dizziness for acupressure. The same authors found that minor infections could happen in auricular bloodletting therapy (J. Y. Tan, Molassiotis, Wang, & Suen, 2014).

The most recently published review of auriculotherapy interventions for anxiety was a historical and narrative overview in which the authors included a range of study designs. The authors had

mixed a variety of psychological disorders (e.g., stress, anxiety, and depression), making it harder to conduct a risk of bias assessment or a meta-analysis (Correa et al., 2020). Consequently, we expect to bring an up to date on the possible adverse events caused by auriculotherapy in anxiety management.

The question which guided our study is: Is auriculotherapy effective and safe for the reduction of levels of anxiety in children's, adult, and older adult patients compared to placebo, waitlist/no treatment, standard or routine care, or alternative treatment? In this way, the main goals of this study have been formulated to:

- Assess the effectiveness of auriculotherapy on remission of AD.
- Calculate the effectiveness of auriculotherapy in anxiety symptoms evaluated through psychometrically robust measures of anxiety symptoms.
- Evaluate the effectiveness of auriculotherapy in the reduction of cortisol in saliva samples.
- Describe changes in symptoms according to TCM (Traditional Chinese Medicine) diagnosis or general physical examination (e.g., temperature, heart rate, blood pressure or respiratory rate).
- Determine the comparative efficacy of auriculotherapy alone or plus usual care with other forms of auriculotherapy (e.g., auricular acupuncture versus acupressure).
- Assess the safety of auriculotherapy.
- Describe the frequent points used in auriculotherapy for anxiety trials.

2. Methods

This work is a systematic review of the literature based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyze (PRISMA) recommendations. The PRISMA (Moher, Liberati, Tetzlaff, Altman, & Group, 2009) checklist is available in supplementary file, and the protocol is at PROSPERO with registration number/ ID: CRD42021254503.

2.1 Search strategy

Searches will run on the following databases using relevant keywords, subject headings (controlled vocabularies), and search syntax, appropriate to each resource from inception until the 30th of June 2021: Cochrane Central Register of Controlled Trials

(CENTRAL); PubMed; MEDLINE Ovid; Elton B. Stephens Company (EBSCO); Latin American and Caribbean Health Sciences Literature Database (LILACS); British Library's table of contents (ZETOC); We also intend to search the international trial registries (including US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (clinicaltrials.gov) and the World Health Organization International Clinical Trials Registry Platform (apps.who.int/trialsearch/)) to identify additional ongoing and unpublished studies.

Full details of the search strategy, including Mesh terms, can be found as a supplementary file.

The study selection is planned to be independently performed by two reviewers (AV and AM) using the inclusion/exclusion criteria given, followed by discussion and consensus within their search team (JM, NR and XH). The first stage of selection is through the titles and abstracts, the second stage is the full text of the papers. Moreover, the authors will only compare the same type of anxiety in participants (e.g., the same kind of AD with AD or contrast the same situational anxiety) as described in the eligibility criteria.

2.2 Eligibility criteria

Included studies

The population will consist of all participants with AD following DSM-V diagnosis and situational anxiety: perioperative anxiety, anxiety before school examinations, anxiety post-abortion, postpartum-specific anxiety/ breastfeeding, or anxiety before dental treatment.

Regarding the intervention, we will include studies that had used auriculotherapy to treat AD. Precisely, we will include auricular acupuncture, auricular electroacupuncture, auricular acupressure, auricular moxibustion, auricular laser therapy, or auricular bloodletting therapy. We aim to compare auriculotherapy with placebo/ waiting list control or with anxiety usual care (e.g., cognitive-behavioural therapies, music therapy, hypnosis, relaxation techniques).

Excluded studies

The authors plan to exclude all RTC that, according to the diagnostic criteria of the

Diagnostic Statistical Manual DSM V, the Study participants were not assessed for AD diagnosis or those who did not report the situational anxiety as stated in our included standards. Also, we will exclude guidelines for treatment, reports of possible adverse events, surveys, case reports/series, qualitative studies, conference abstracts/posters, cohort studies, reviews, case reports, experimental studies, expert experience, study's data missing/ incomplete, duplicate publications, newspaper articles, book reviews, 'mass media publication' health publications, general comments, or letters, Quasi-RCTs, crossover trials, controlled before and after studies, interrupted time-series studies, and non-experimental studies due to their potential high risk of bias. We intend to exclude RTC´s dated before January 2011 following Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines (Moher et al., 2012).

The primary outcomes are:

Remission/proportion of participants with the absence of all diagnoses for AD post-treatment, made by reliable and valid structured interviews as defined by DSM-V.

Reduction in anxiety symptoms post-treatment: measured using psychometrically robust measures of anxiety symptoms that yield symptom scores on continuous scales (Julian, 2011), such as:

State-Trait Anxiety Inventory (STAI) (Marques, 2009; Spielberger, 1970).

Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983).

Anxiety Visual Analogue Scale numerical rating scale (0-10/100) (VAS) (Facco et al., 2011).

Beck Anxiety Inventory (Beck, Epstein, Brown, & Steer, 1988; Marques, 2009).

Hamilton Rating Scale for Anxiety (Hamilton, 1959; Marques, 2009)).

The secondary outcomes are:

Reduction in Cortisol in Saliva Samples (Kirschbaum & Hellhammer, 1994).

Changes in symptoms according to TCM diagnosis or general physical examination (e.g., temperature, heart rate, blood pressure or respiratory rate).

Adverse events (AEs) reported by the number and type of reported adverse events

during the trial, from randomization to post-treatment assessment. In this study, an adverse event is “any untoward and unintended responses to the trial intervention, at any dose administered, including all AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to the trial intervention” (Coomarasamy A, 2016).

This systematic review will include randomized controlled trials (RCTs). The study will consist of English, French, Spanish, German, Portuguese, and Italian trials.

2.3 Data extraction and management

Two review authors (AV, AM) will independently screen the titles and abstracts of all studies identified. The trials are going to be coded as "retrieve" (eligible or potentially eligible/unclear) or "do not retrieve". We plan to retrieve the full‐text study, and the same review authors expect independently screen the full texts to identify studies for inclusion and identify and record reasons for excluding the ineligible studies. If required, the authors will resolve any disagreements by consulting a third review author (JM, NR, XH). The same authors (AV and AM) intend to independently extract the study characteristics and outcome data from the included studies as follows:

Methods: study design, total duration of the study, details of any 'run‐in' period, withdrawals, and study date).

Participants: number, mean age, the severity of the condition, diagnostic criteria, comorbidities, inclusion criteria, and exclusion criteria.

Setting: number of study centres and location, study setting.

Interventions: type of intervention, how long the intervention was for, comparison, concomitant medications, excluded medications, delivery format, therapist contact time, who delivers intervention and description of qualification/years of experience.

Outcomes: primary and secondary outcomes specified and collected, and time points reported.

After data collection and agreement on the data extracted, we will enter it into Review Manager 5.4 (Collaboration, 2020).

2.4 Risk of bias assessment

The risk of bias for each study will be assessed independently by two review authors (AV and AM). We will use the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins JPT, 2020). We intend to discuss any disagreements involving another review author (JM). The same authors plan to perform the risk of bias assessments according to the following domains:

- Random sequence generation: Each included study needs to report the method used to generate the allocation sequence.
- Allocation concealment: Each included study will need to describe the method used to conceal allocation to interventions before the assignment.
- Blinding:
    - Blinding of participants and personnel: Each included study will need to report the methods used to blind study participants and personnel.
    - Blinding of outcome assessment: Each included study will need to report the methods used to blind outcome assessors from knowing which intervention the participant received.
- Incomplete outcome data.
- Selective outcome reporting.
- Other bias, including therapy integrity: Checking for bias due to problems not covered by (1) to (5) above.
    - We expect to judge each potential source of bias as low, high, or unclear risk of bias and provide a supporting quotation from the study to justify our judgment in the “Risk of bias” table.

2.5 Measures of treatment effect

Data from the assessment administered immediately after treatment (or the assessment closest to the end of therapy) will be used to assess post‐treatment outcomes. We intend to collect dichotomous data (remission of primary anxiety diagnosis) and continuous data (anxiety scales, cortisol analyses, and general physical examination) using standardized measures to assess

post-treatment outcomes. These measures are expected to evaluate the maintenance of treatment effects at a series of follow‐up time points (≤ 6 months post‐treatment, > 6 months post‐treatment but ≤ 12 months post‐treatment, and > 12 months post‐treatment). Studies reported follow‐up data at multiple time points within one category (e.g. one‐month and three‐month follow‐up), and we plan to use data from the longer follow‐up period.

Dichotomous data

Odds Ratios (ORs) and 95% confidence intervals (CIs) based on the random‐effects model, with pooling of data via the inverse variance method of weighting, are expected to be employed and the estimate of significance set at P<0.05. The authors will calculate the number needed to treat an additional beneficial outcome (NNTB) with 95% CIs, along with a summary statistic of all those responding to treatment reported as a percentage of the total number of participants for each comparison.

Continuous data

The continuous data will be analyzed as mean difference (MD) or standardized mean difference (SMD) and data presented as a scale with a consistent direction of effect.

Skewed data is meant to be narratively described and reported as medians and interquartile ranges and analyses continuous data based on the random‐effects model, with pooling of data via the inverse variance method of weighting. We will use the SMD to pool continuous data measured in different ways across studies but conceptually the same (i.e. measuring anxiety or cortisol or global functioning). We plan to present the endpoint data where both endpoint and change data will be available for the same outcome. We estimate and have set the significance at P < 0.05.

Dealing with missing data

We intend to contact all Investigators or study sponsors to verify key study characteristics and obtain missing numerical outcome data where possible. We plan to document the correspondence with study authors and which study authors respond to our queries. The authors intend to include studies that have not provided statistical data for risk of bias, but we won't accept them for meta-analyses.

Missing participants

If data allows, we plan to conduct an intention‐to‐treat (ITT) analysis. When analyzing dichotomous data, we plan to assume that all non‐completers in the auriculotherapy group are treatment failures, and non‐completers in the control group are treatment successes.

For dichotomous outcomes, we also will undertake completer analysis, using only data from participants who completed post‐treatment assessments.

Assessment of heterogeneity

We intend to assess clinical heterogeneity by comparing differences in the distribution of essential participant factors between studies (e.g., age, gender, specific diagnosis, duration and severity of the disorder, associated comorbidities). The authors expect to assess methodological heterogeneity by comparing trial factors (randomization, concealment, blinding of outcome assessment, losses to follow‐up). We will use the Chi^2 test and the I^2 statistic to assess heterogeneity and set the significance at $P < 0.1$. The Cochrane Handbook for Systematic Reviews of Interventions recommends using a range for the I^2 statistic and a guide to interpretation. If we found either moderate heterogeneity (I^2 around 30% to 60%) or substantial heterogeneity (I^2 in the range of 60% to 90%), we plan to perform subgroup and sensitivity analyses where possible.

Data synthesis

We intend to conduct meta‐analyses where this is meaningful, that is, if the treatments, participants, and the underlying clinical question are similar enough for pooling to make sense.

We will carry out separate analyses to identify whether auriculotherapy was more effective post‐treatment than waiting list/no treatment; treatment, as usual, alternative therapies or placebo; and whether auriculotherapy in combination with standard care is more effective than usual care alone or sham group.

Sensitivity analysis

The authors predict carrying out sensitivity analyses to test the robustness of decisions made in the review process. We aim to provide sensitivity analyses with evidence of significant heterogeneity (we plan to inspect forest plots and examine each study in turn to determine the source of any significant heterogeneity). For the trials with either high RoB2 in selection bias or allocation concealment, we will run a sensitivity analysis and investigate the source of heterogeneity.

The critical need to improve access to treatment for AD means that it is imperative to explore the efficacy of approaches that may help maximize treatment efficiency. As outlined above, there are also unanswered questions concerning the benefits of auriculotherapy. Therefore, we expect to explore the efficacy of different types of auriculotherapy delivery and briefer and shorter interventions. We also would like to examine treatment effects amongst participants with AD using subgroup analyses. Specifically, where it is possible and meaningful to do so, we will undertake subgroup analyses to examine differences between:

Types of auriculotherapy delivery (e.g., auriculotherapy with needles, laser, electrotherapy, or seeds).

Auriculotherapy interventions with a varying number of sessions (1 session, $\geqslant$ 5 sessions and, $\geqslant$ 10 sessions).

Studies specifying different Healthcare practitioners (e.g., licensed acupuncturists, physicians, physiotherapists, nurses, or allied health professionals) with those studies without description about how performed the auriculotherapy treatment.

To examine differences between age groups by evaluating the differences between studies where all participants were age 17 or younger ($\leqslant$ 18 years) with all participants were age 18 or older ($\geqslant$ 18 years).

Assessment of publication biases

The authors anticipate investigating publication bias for a minimum of 10 studies included using

funnel plots. If we find any asymmetry, we intend to perform a statistical investigation using Egger's test.

Tables and figures

Data will be transferred and collected into Review Manager 5.4 (Collaboration, 2020) and presented graphically, and the area to the left of the line of no effect indicates a favorable outcome for auriculotherapy. The authors expect to deliver either the included studies characteristics, and the excluded studies list in a table. We intend to summarize the risk of bias and the PRISMA chart in figures type.

We plan to create a summary of the findings table for primary and secondary outcomes. We expect to use the five Grading of Recommendations Assessment, Development and Evaluation (GRADE) considerations (study limitations, consistency of effect, imprecision, indirectness, and publication bias) to assess the quality of evidence. We will use the methods and recommendations described in the Cochrane Handbook for Systematic Reviews of Interventions, employing GRADEpro GDT software (GRADEpro GDT: GRADEpro Guideline Development Tool [Software]. McMaster University). Where appropriate, the author plans to justify all decisions to downgrade or upgrade the quality of studies using footnotes and comments to aid the reader's understanding of the review.

3. Discussion

Anxiety disorders are common, perhaps disabling, conditions that mostly begin during childhood, adolescence, and early adulthood (Craske & Stein, 2016; World_Health_Organization, 2017). Psychological treatments, particularly cognitive behavioural therapy (Tolin, 2010), and pharmacological treatments (particularly selective serotonin-reuptake inhibitors and serotonin-noradrenaline-reuptake inhibitors), are effective (Thibaut, 2017). However, cognitive behavioural therapy is considered too time-consuming (Dietzel et al., 2020), and the risk of side effects and resistance to pharmacological treatment is high (Durham et al., 2012). For the above reason, we need more research to develop personalized treatments (Craske & Stein, 2016; Thibaut, 2017).

A recent protocol for a systematic review (Dietzel et.al, 2020) suggests that auriculotherapy might become an effective, safe, and easy-to-perform treatment for preoperative anxiety but that there

were insufficient systematic evaluations of Auriculotherapy in AD to fill this gap. Moreover, Corrêa et al., 2019 had identified evidence in the scientific literature about the effects of auriculotherapy for treating stress, anxiety and depression in adults and older adults and had found a positive impact of available evidence. However, the above author did not perform meta-analyses, and there was no information about which type of anxiety Auriculotherapy has effectiveness for treatment and safety.

Subsequently, we aim to perform a new review, analyzing the RCTs based on Auriculotherapy for AD treatment. The results of this review will offer the source for which type of anxiety auriculotherapy has effects and is safe, as might help to develop appropriate methodological protocols for future RCT´s in this field.

4. Limitations

This systematic review may be affected by excluding Chinese databases where auriculotherapy is widely used in traditional medicine(Dietzel et al., 2020). However, we aim to perform a follow up systematic review in the future and include Chinese databases to corroborate expectable results.

Authors' contributions

All authors contributed to this study.

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There is no financial or other support or sponsors.

Declaration of competing Interests

Nicola Robinson is Editor in Chief of this journal, and Xiaoyang Hu sits on the journal's editorial board. There are no other conflicts of interest to declare.

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Data availability

The authors supply the manuscript search strategy.

Search strategies

Cochrane Central Register of Controlled Trials

ID	Search Hits
#1	MeSH descriptor: [Anxiety Disorders] explode all trees
#2	MeSH descriptor: (Bas-Hoogendam et al.) explode all trees
#3	MeSH descriptor: [Agoraphobia] explode all trees
#4	MeSH descriptor: [Panic Disorder] explode all trees
#5	MeSH descriptor: [Anxiety, Separation] explode all trees
#6	MeSH descriptor: [Phobic Disorders] explode all trees
#7	MeSH descriptor: [Phobia, Social] explode all trees
#8	MeSH descriptor: [Patient Health Questionnaire] explode all trees
#9	anxiety
#10	(#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9)
#11	MeSH descriptor: [Auriculotherapy] explode all trees
#12	MeSH descriptor: [Acupuncture, Ear] explode all trees
#13	(#11 or #12)
#14	(#10 and #13)

MEDLINE Ovid

Search detail:

1	anxiety {Including Limited Related Terms
2	ANXIETY DISORDERS {Including Limited Related Terms}

3 AGORAPHOBIA or ANXIETY, SEPARATION or PANIC DISORDER {Including Limited Related Terms}

4 generalized anxiety {Including Limited Related Terms}

5 GAD or separation anxiety {Including Limited Related Terms}

6 1 or 2 or 3 or4 or 5 {Including Limited Related Terms}

7 Auriculotherapy {Including Limited Related Terms}

8 Ear Acupuncture {Including Limited Related Terms}

9 7 or 8

10 1 and 9

11 2 and 9

12 10 or 11

PubMed

Article type: Clinical Study, Clinical Trial, Randomized Controlled Trial

Search detail:

(((anxiety[MeSH Terms]) OR (anxiety[Title/Abstract]) or anxiety or ansiedade or Anxious[Title/Abstract] OR "Panic Disorder"[Title/Abstract] OR "Social Phobia"[Title/Abstract]) and (Auriculotherapy [MeSH] Auriculotherapy [Title/Abstract] or auriculoterapia or Auriculo acupuncture [Title/Abstract] or acupressure [Title/Abstract] or ear therapy [Title/Abstract] or ear acupuncture [Title/Abstract])))

Latin American and Caribbean Health Sciences Literature Database)

Databases selected: LILACS; BDENF – Nursing; CUMED; MOSAICO - Integrative health; Coleciona SUS; PIE

Search detail:

(("auriculotherapy" OR "auriculoterapia" OR "acupuntura auricular" OR "auricular acupuncture" OR "ear acupuncture" OR "acupuntura orelha" OR "acupressure") AND ("anxiety" OR "ansiedade" OR "anxious" OR "ansioso" OR "Anxiety Disorders" OR "agoraphobia" OR

"agorafobia" OR "Phobia Social" OR "Fobia Social" OR "Panic Disorder" OR "Pánico" OR "Anxiety, separation" OR "ansiedade separação" OR "Phobic disorders")) AND (db:("LILACS" OR "BDENF" OR "CUMED" OR "MTYCI" OR "colecionaSUS" OR "PIE")) AND (year_cluster:[2011 TO 2021])

British Library's table of contents (ZETOC)

Records for: any: auricular acupuncture and anxiety

Elton B. Stephens Company (EBSCO) – Search Databases:

MLA International Bibliography with Full Text, MLA Directory of Periodicals, Academic Search Ultimate, Business Source Ultimate, EconLit with Full Text, Communication Abstracts, Criminal Justice Abstracts, eBook EngineeringCore (EBSCOhost), eBook Collection (EBSCOhost), Applied Science & Technology Index (H.W. Wilson), MathSciNet via EBSCOhost, Education Source, ERIC, Fonte Acadêmica, Humanities Abstracts (H.W. Wilson), Historical Abstracts, APA PsycArticles, APA PsycBooks, APA PsycInfo, Regional Business News, Psychology and Behavioral Sciences Collection, Sociology Source Ultimate, Library & Information Science Source, GreenFILE, Teacher Reference Center, The Serials Directory

Search detail:

S16 S14 AND S15

S15adverse effects

S14 (S1 OR S2 OR S3) AND (S12 AND S13)

S13 S1 OR S2 OR S3

S12 S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11

S11 anxiety

S10 anxiety separation

S9 agoraphobia

S8 anxiety disorders

S7 generalized anxiety disorder or gad

S6 social anxiety disorder

S5 posttraumatic stress disorder

S4 panic disorder

S3 acupuncture, ear

S2 auriculoterapia

S1 auriculotherapy

Organization International Clinical Trials Registry Platform (ICTRP) and ClinicalTrials.gov search strategy:

Terms and Synonyms Searched:

Auricular Acupuncture for Anxiety

Auricular Acupuncture

ear acupuncture

acupuncture auricular

Anxiety

anxious

Angst

Anxiousness

Acupuncture

Auricular

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Systematic review

Is auriculotherapy effective and safe for the treatment of anxiety disorders? – A systematic review and meta-analysis

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Is auriculotherapy effective and safe for the treatment of anxiety disorders? – A systematic review and meta-analysis

Abstract

Introduction: Auriculotherapy (AA) could support standard treatment for anxiety disorders (AD), but its effectiveness and safety remain undetermined. The aim of this systematic review was to determine whether AA was effective and safe for treating people with AD.

Methods: Searches were conducted on eight databases for randomized controlled trials (RCT) evaluating the effectiveness and safety of AA compared with placebo, waiting list treatment, routine care, or alternative treatment. Searches were run from inception until the 30th of June 2021. Methodological quality of included studies was assessed using the Cochrane risk of bias assessment tool and quality of evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation tool. Meta-analyses were conducted using statistical software RevMan V5.4. The protocol was published and registered PROSPERO ID: CRD42021254503.

Results: Thirteen trials met the inclusion criteria for quality and of these nine were included in the meta-analysis. AA (n=386) reduced anxiety levels compared with placebo (n=382) standardized mean difference (SMD): -0.44 95% of Confidence Intervals (CI), 9 studies, for AA compared with a waiting list (n=360), 8 studies SMD (-0.55; 95% CI [-0.70, -0.41]). Certainty was graded as moderate and with unlikely publication bias. There was moderate certainty of evidence for an AA (n=130) intervention for pre-operatory anxiety levels when compared with placebo (n=129) SMD -1.40 95% CI [-2.54, -0.26], 3 studies and when compared with a waiting list group (n=98) Mean difference (MD) -5.02 95% CI [-8.15, -1.90], 2 studies. Few studies reported adverse events and other important secondary outcomes such as salivary cortisol and vital signs.

Conclusion: AA may be effective as a complementary treatment for situational anxiety. There is still an evidence gap regarding its safety and efficacy. The type and frequency of AA used for anxiety treatment requires further exploration.

Keywords

Anxiety Disorders; Auriculotherapy; Auricular acupuncture; Acupressure; Brain stimulation; Systematic review

1. Introduction

Anxiety is one of the most natural body reactions in response to a given threat (Notebaert et al., 2020; World_Health_Organization, 2017). However, individual differences in physical and emotional health impairment can lead to hormonal changes, and anxiety can become pathological (Dean, 2016). Although effective treatment approaches (e.g., pharmacotherapy, psychotherapy, and their combination) for anxiety are available, epidemiological studies have revealed that its prevalence has been increasing over time, particularly during the coronavirus pandemic(Santabarbara et al., 2021). In light of this, novel preventive and treatment strategies need to be explored (Wong et al., 2022). Some trials have shown encouraging findings using acupuncture for AD treatment, but there is insufficient research evidence to reach solid conclusions. (Amorim et al., 2018; Correa et al., 2020; Pilkington, Kirkwood, Rampes, Cummings, & Richardson, 2007; Tan et al., 2020).

AA is a technique derived from traditional acupuncture that developed into a distinct treatment system (Asher et al., 2010). The stimulation of auricular areas appears to be associated with the reticular formation through the sympathetic and parasympathetic nervous systems (Mangold & J, 2022; Yates, Bolton, & Macefield, 2014). Such information coming through the thermal, algic and proprioceptive stimuli are transmitted from the auricular pavilion by the trigeminal nerve fibres, auricular magnum, and minor occipital (sensitive branch of the cervical plexus). The vagus nerve (Rabischong & Terral, 2014) is responsible for the parasympathetic innervation of the lungs, heart, stomach, and small intestine, as well as the pharynx and larynx muscles, and it also sends information to essential brain regions in the regulation of anxiety (locus coeruleus, orbitofrontal cortex, hippocampus, and the amygdala) (Kenny & Bordoni, 2022). The trigeminal nerve mainly controls the muscles involved in mastication and facial sensitivity (Sanders, 2010). Finally, the cervical plexus innervates the neck, diaphragm, and thorax, where its rootlets diverge from the spinal accessory nerve after its exit from the jugular foramen and subsequently the course through the vagus fibres (Jobe., F., Martinez., & Weller., 2021). Recordings of vagus somatosensory have been shown to evoke potentials in the scalp (Fallgatter et al., 2003) and have revealed the feasibility of AA as an effective therapeutic strategy for managing several clinical disorders, including pain (Asher et al., 2010; Sant'Anna, Sant'Anna, Chao, & Sant'Anna, 2021), epilepsy (K. Wu, Wang, Zhang, Yao, & Zhang, 2020), depression (X. J. Li et al., 2019), migraine (Straube, Ellrich, Eren, Blum, & Ruscheweyh, 2015), and substance dependence (Gates, Smith, & Foxcroft, 2006; Lui, Li, Xia, & Terplan, 2009) and tinnitus (Kim et al., 2012).

Regarding anxiety, as per the protocol previously published, there is some limited evidence in favour of auricular acupuncture for perioperative anxiety (Dietzel et al., 2020; Pilkington et al., 2007), for AD disorders and major depressive disorders (de Lorent, Agorastos, Yassouridis, Kellner, & Muhtz, 2016), for situational anxiety primary school examinations (Usichenko et al., 2020; A. Vieira, Hinzmann, Silva, Santos, & Machado, 2018), and the reduction of state anxiety before dental treatments (Michalek-Sauberer, Gusenleitner, Gleiss, Tepper, & Deusch, 2012). Although, there have been no systematic reviews to demonstrate AA´s effectiveness for AD treatment (Andreia Vieira, Moreira, Machado, Robinson, & Hu, 2022)

Regarding the adverse events associated with AA, Correa et al., 2020 stated that AA used for the treatment of stress, anxiety and depression in adults and older adults could cause, headaches and bleeding at the needle application site (Klausenitz et al., 2016) and local pain (Kurebayashi et al., 2017). However, the authors (Correa et al., 2020) used the Jadad scale to determine the trials' quality, our systematic review aimed to appraise included studies using Cochrane's risk of bias tool and update the possible adverse events caused by AA in anxiety management.

Therefore, the primary aims were to:

I) Assess the effectiveness of AA on remission of AD.

II) Calculate the effectiveness of AA in anxiety symptoms evaluated through psychometrically robust and validated measures for anxiety symptoms.

Our secondary aims were to:

III) Evaluate the effectiveness of AA in reducing cortisol in saliva samples.

IV) Describe changes in symptoms according to TCM (Traditional Chinese Medicine) diagnosis or general physical examination (e.g., temperature, heart rate, blood pressure or respiratory rate).

V) Determine the comparative efficacy of AA alone or plus usual care with other forms of AA (e.g., auricular acupuncture versus acupressure).

VI) Assess the safety of AA.

VII) Describe frequent points used in AA for anxiety trials.

2. Methods

This is a systematic review of the literature based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyze (PRISMA) recommendations (Moher, Liberati, Tetzlaff, Altman, & Group, 2009). The PRISMA checklist is available in our protocol previously published (Andreia Vieira et al., 2022) registered on PROSPERO, registration number/ ID: CRD42021254503.

Search strategy

Searches were run from inception until the 30th of June 2021 on the following databases: Cochrane Central Register of Controlled Trials; PubMed; MEDLINE Ovid; Elton B. Stephens Company; Latin American and Caribbean Health Sciences Literature Database; British Library's table of contents; Scopus, and ScienceDirect. We also searched the international trial registries (including US National Institutes of Health Ongoing Trials Register – ClinicalTrials.gov (clinicaltrials.gov) and the World Health Organization International Clinical Trials Registry Platform (apps.who.int/trialsearch/)) to identify additional ongoing and unpublished trials. The trial selection was independently performed by two reviewers (AV and AM) using the inclusion/exclusion criteria given below, followed by discussion and consensus with a third author (JM). The first stage of selection was conducted by identifying potentially relevant papers through the titles and abstracts and at the second stage the full text of the papers was appraised.

Full details of the search strategy, including Mesh terms, can be found in our protocol´ supplementary file (Andreia Vieira et al., 2022).

Eligibility criteria

Included trials

The population consisted of all participants with AD following the Diagnostic and Statistical Manual of Mental Disorders, (DSM-V) diagnosis and situational anxiety: perioperative anxiety, anxiety before school examinations, anxiety post-abortion, postpartum-specific anxiety/

breastfeeding, or anxiety before dental treatment.

Regarding the intervention, we included trials that had used AA to treat AD as auricular acupuncture, auricular electroacupuncture, auricular acupressure, auricular moxibustion, auricular laser therapy, or auricular bloodletting therapy. AA was compared with waiting list control or with anxiety usual care (e.g., cognitive-behavioural therapies, music therapy, hypnosis, relaxation techniques). The primary outcomes were:

i) Remission/proportion of participants with the absence of all diagnoses for AD post-treatment, made by reliable and valid structured interviews as defined by DSM-V.

ii) Reduction in anxiety symptoms post-treatment: measured using psychometrically robust measures of anxiety symptoms that yielded symptom scores on continuous scales (Julian, 2011), such as:

   a. State-Trait Anxiety Inventory (STAI) (Marques, 2009; Spielberger, 1970).
   b. Anxiety and Depression Scale (Zigmond & Snaith, 1983).
   c. Anxiety Visual Analogue Scale numerical rating scale (0-10/100) (VAS) (Facco et al., 2011).
   d. Beck Anxiety Inventory (Beck, Epstein, Brown, & Steer, 1988; Marques, 2009).
   e. Hamilton Rating Scale for Anxiety (Hamilton, 1959; Marques, 2009)).

The secondary outcomes were:

i) Reduction in cortisol in saliva samples (Kirschbaum & Hellhammer, 1994).

ii) Changes in symptoms according to TCM diagnosis or general physical examination (e.g., temperature, heart rate, blood pressure or respiratory rate).

iii) Adverse events (AEs) reported by the number and type of reported adverse events during the trial, from randomization to post-treatment assessment. In this trial, an adverse event was “any untoward and unintended responses to the trial intervention, any dose administered, including all AEs judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to the trial intervention” (Coomarasamy A, 2016).

This systematic review only included RCTs in English, French, Spanish, German, Portuguese, or Italian.

Excluded trials

The authors excluded all RCTs that, according to the diagnostic criteria of the Diagnostic Statistical Manual DSM-V; trial participants who were not assessed for AD diagnosis or those who did not report the situational anxiety as stated in our included standard criteria. Also, excluded were; guidelines for treatments, surveys, case series, case reports, Quasi-RCTs, crossover trials, interrupted time-series trials, experimental and non-experimental trials, qualitative trials, trials with missing or incomplete data, cohort studies, reviews, conference abstracts/posters, expert opinion, 'duplicate publications, newspaper articles, book reviews, 'mass media publications', health publications, general comments, or letters, due to their potential high risk of bias. RCTs dated prior to January 2011 following the Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines (Moher et al., 2012).

Data extraction and management

Two review authors (AV, AM) independently screened the titles and abstracts of all identified trials. The trials were coded as either "retrieve" (eligible or potentially eligible) or "do not retrieve". The trial characteristics and outcome data were extracted as follows:

- Methods: trial design, total duration of the trial, details of any 'run-in' period, withdrawals, and trial date).
- Participants: number, mean age, the severity of the condition, diagnostic criteria, comorbidities, inclusion criteria, and exclusion criteria.
- Setting: number of trial centres and location, trial setting.
- Interventions: type of intervention, length of the intervention, comparison group, excluded medications, delivery format, therapist contact time, person delivering the intervention and description of their qualification/years of experience.
- Outcomes: primary and secondary outcomes.

Risk of bias assessment

The risk of bias for each trial was assessed independently by two review authors (AV and AM), employing the criteria summarized in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins JPT, 2020). Any discrepancies were discussed with another review author (JM). The same authors had judged each potential source of bias as low, high, or unclear risk of bias and had provided a supporting quotation from the trial to justify their judgment in the “Risk of bias” table. The risk of bias assessments were performed according to domains available in our protocol previously published (Andreia Vieira et al., 2022)

Measures of treatment effect

Data from the assessment administered immediately after treatment (or the assessment closest to the end of therapy) was used to assess post-treatment outcomes. Dichotomous data (remission of primary anxiety diagnosis) and continuous data (anxiety scales, cortisol analyses, and general physical examination) were collected using standardized measures to assess post-treatment outcomes.

Effects of interventions:

RevMan 5.4.1, the standard software provided by the Cochrane Collaboration, was employed to analyze the results of the RCTs. We performed a meta-analysis when the patients, interventions, controls, and outcomes were similar, and the corresponding data were sufficiently homogeneous. Continuous outcomes were expressed as weighted mean differences (WMDs) and dichotomous data as relative risks (RRs) with 95% confidence intervals (Cis). If there was significant heterogeneity, we explored the possible reasons by conducting a sensitivity analysis.

Heterogeneity was identified across the trials using both Chi-squared tests as well as I2.

To minimize bias in our findings and recommendations, we graded and assessed the available evidence using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Profiler (pro), with four levels of evidence: high, moderate, low, and very low (GRADEpro GDT: GRADEpro Guideline Development Tool [Software]. McMaster University).

Dichotomous data

Odds Ratios (Ods) and 95% CI based on the random-effects model, with pooling of data via the inverse variance method of weighting, were employed and the estimate of significance was set at P<0.05. The authors calculated the number needed to treat an additional beneficial outcome with 95% CI, along with a summary statistic of all those responding to treatment reported as a percentage of the total number of participants for each comparison.

Continuous data

The continuous data was analyzed as MD or SMD and data was presented as a scale with a consistent direction of effect.

Skewed data was narratively described and reported as medians and interquartile ranges and analyses continuous data based on the random-effects model, with pooling of data via the inverse variance method of weighting. We use the SMD to pool continuous data measured in different ways across trials but conceptually the same (i.e. measuring anxiety or cortisol). We presented the endpoint data available for the same outcome with significance at $P < 0.05$.

Dealing with missing data

All investigators or trial sponsors were contacted via email to verify key trial characteristics and obtain missing numerical outcome data. All included trials that had not provided statistical data were suitable for estimating risk of bias, but not for meta-analyses.

Missing participants

The authors assumed all participants non-completers in the AA group were treatment failures, and non-completers in the control group were treatment successes. For dichotomous outcomes, we undertook completed analysis, using only data from participants who completed post-treatment assessments.

Publication bias assessment and sensitivity analysis

The authors conducted Publication bias by inspecting funnel plots and performing Egger's regression test (Egger, Davey Smith, Schneider, & Minder, 1997) in the presence of more than 5 studies as recommended (Higgins JPT, 2020). The funnel plot and the Egger´s test was

performed in the comparison of AA compared to placebo and waiting list controls.

The sensitivity analyses were also performed by examining each study to determine the source of any substantial heterogeneity (Higgins JPT, 2020). To determine the existence of heterogeneity, we used the Chi^2 test and the I^2 statistic with a significance of $p < 0.1$. The Cochrane Handbook for Systematic Reviews of Interventions recommends sensitivity analyses if either moderate heterogeneity (I^2 around 30% to 60%) or substantial heterogeneity (I^2 in the range of 60% to 90%)(Higgins JPT, 2020) is found. Specifically, where it was meaningful to do so, we undertook subgroup analyses to investigate differences between:

i) Types of AA (e.g., auriculotherapy with needles, laser, electrotherapy, or seeds).

ii) AA interventions with a different number of sessions (1 session, ≥ 5 sessions and≥ 10 sessions).

iii) Studies specifying different healthcare practitioners (e.g., licensed acupuncturists, physicians, physiotherapists, nurses, or allied health professionals) with those studies without a description of who performed the auriculotherapy treatment.

Data synthesis

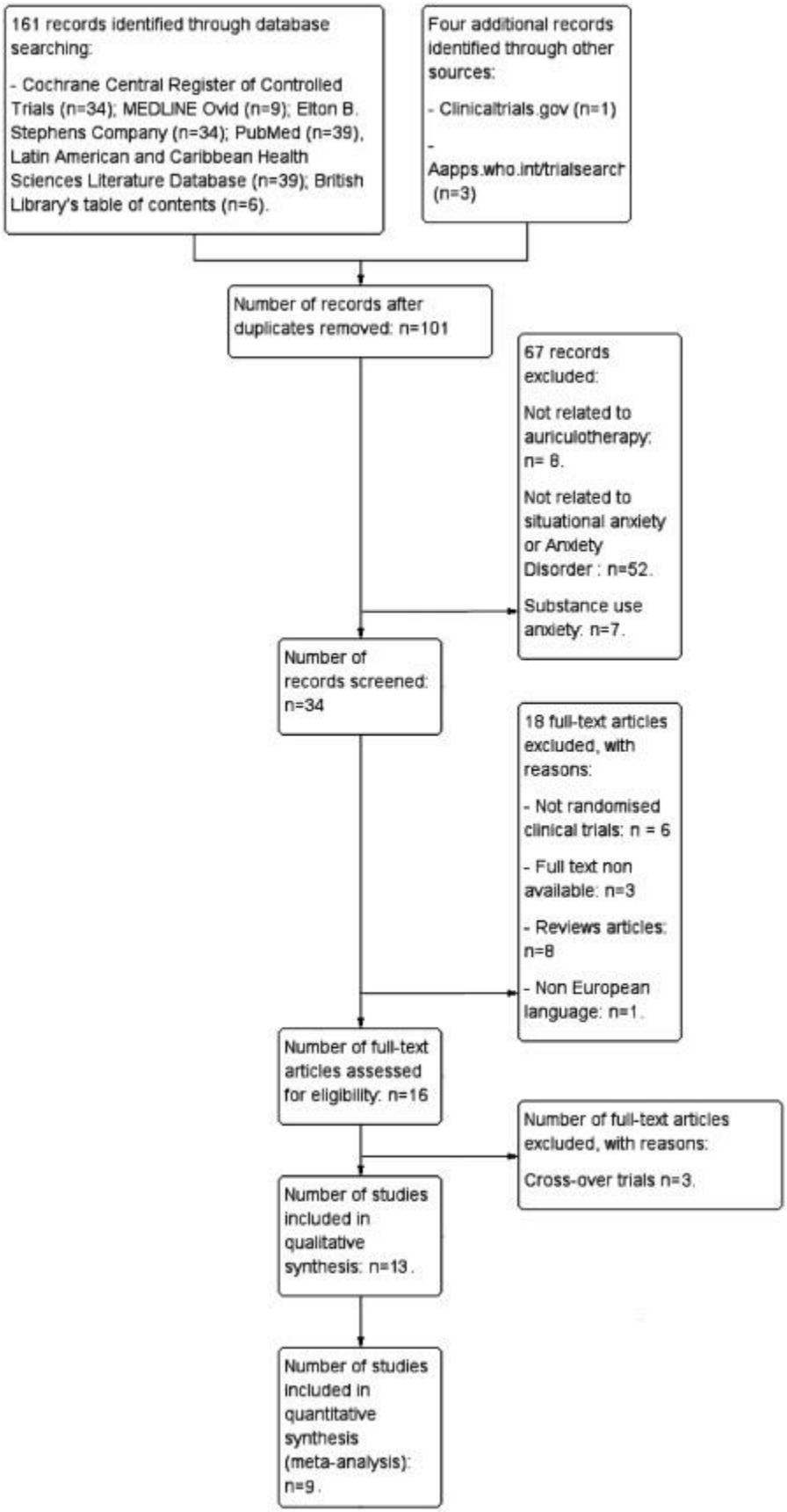
We conducted meta-analyses by carrying out separated analyses to identify whether AA was more effective post-treatment than waiting list, treatment as usual, or alternative therapies; and whether AA in combination with standard care was more effective than usual care alone or placebo.

Data was entered into Review Manager 5.4 (Collaboration, 2020) and presented graphically. The area to the left of the line of no effect indicates a favorable outcome for AA. Trials characteristics, and the excluded trials list can be found in supplementary file.

The five GRADE considerations (e.g., trial limitations, consistency of effect, imprecision, indirectness, and publication bias) were used to assess the quality of evidence following the Cochrane Handbook for Systematic Reviews of Interventions, employing GRADEpro GDT software (GRADEpro GDT: GRADEpro Guideline Development Tool [Software]. McMaster University).

3. Results

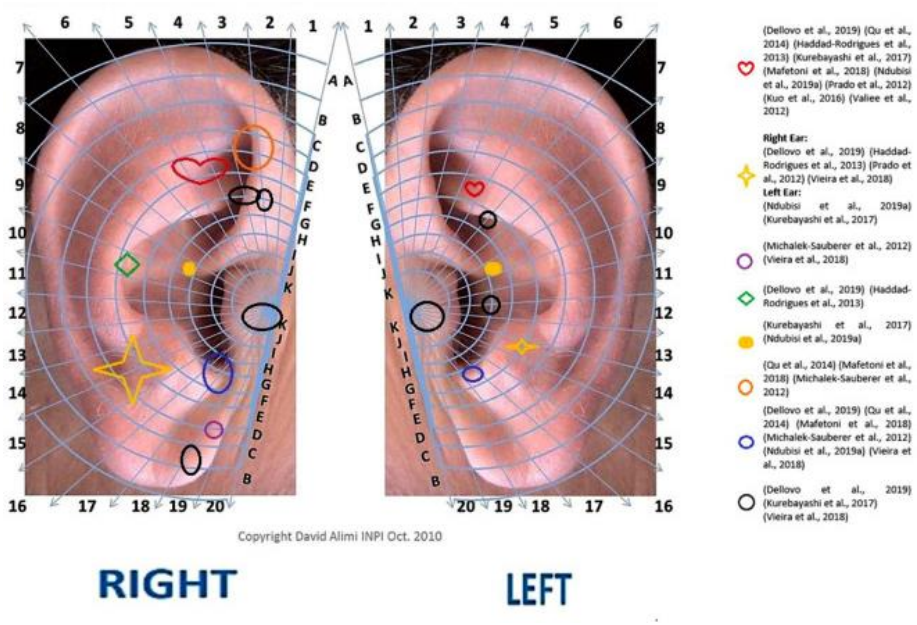
Our search identified 165 citations, and 34 full-text articles were reviewed, as shown in the flow diagram (figure 1). A total of 13 trials (1366 subjects) met our inclusion criteria (Table 1) and were included in the quality analysis. We attempted to contact the authors of three additional publications (Avisa, Kamatham, Vanjari, & Nuvvula, 2018; W. S. Li, Cui, Li, Zhao, & Wanlai, 2011; W. S. Li et al., 2013) that may have been eligible for inclusion. However, they did not reply. We also found some trials measuring one or more outcomes relevant to this review. But, those trials either did not report the outcomes in a format suitable for meta-analysis (Dellovo, Souza, de Oliveira, Amorim, & Groppo, 2019; Kuo, Tsai, Chen, & Tzeng, 2016; Prado, Kurebayashi, & Silva, 2012; S. Wu, Liang, Zhu, Liu, & Miao, 2011) or had a cross-over methodology (Klausenitz et al., 2016; Usichenko et al., 2020) or had a non-randomized arm (Wunsch et al., 2018a) and were therefore not included in the meta-analyses.

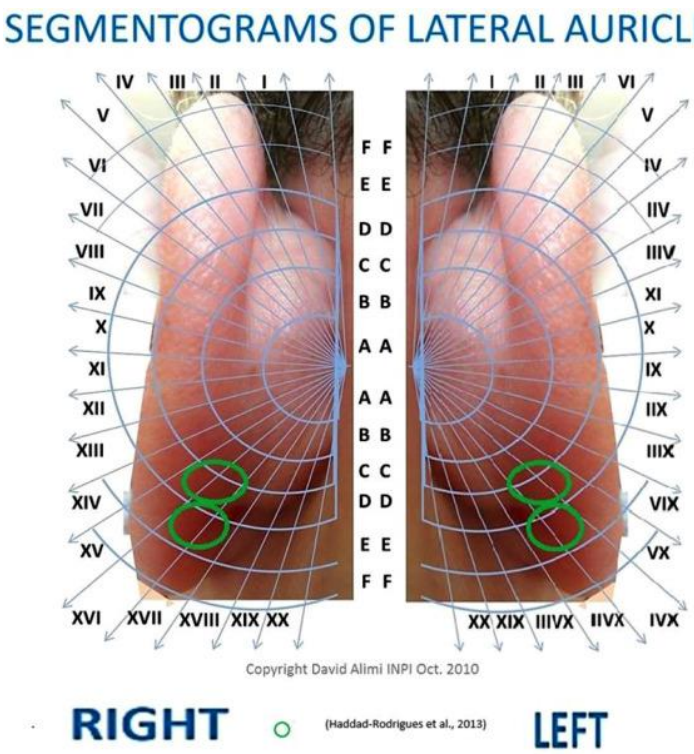


Chapter 4. Figure 1 - The systematic reviews and meta-analysis protocol flow chart.

The 13 included trials included a wide variety of AA interventions (Table 1). Auricular acupuncture trials were more frequent (eight trials) than acupressure (five trials). Regarding auricular acupuncture, ASP needles were more commonly used (Kurebayashi et al., 2017; Michalek-Sauberer et al., 2012; Prado et al., 2012; A. Vieira et al., 2018; S. Wu et al., 2011) than steel needles (Haddad-Rodrigues, Spano Nakano, Stefanello, & Campos Pereira Silveira, 2013; Ndubisi, Danvers, Gold, Morrow, & Westhoff, 2019). Concerning acupressure, only two trials had used semen vaccariae seeds (Kuo et al., 2016; Qu et al., 2014), while other trials used plastic beads (Valiee, Bassampour, Nasrabadi, Pouresmaeil, & Mehran, 2012); crystal microspheres (Mafetoni, Rodrigues, Jacob, & Shimo, 2018); mustard seeds (Dellovo et al., 2019) or Mexican Argemona seeds (Rivadeneira, Cifuentes, Hidalgo, Tejeda, & Sánchez, 2015). We found only one trial (Kurebayashi et al., 2017) comparing AA using seeds versus semi-permanent needles for exam anxiety in students. Authors have cited that AA produced the best result for reducing state anxiety with needles compared with seeds (Higgins JPT, 2020).

Following the International AA Nomenclature, the area covered from E3-5 and F5 (





Chapter 4. Figure10 and Chapter 4. Figure11) were the most frequent areas selected for treatment by the majority of the included trials (nine trials), followed by I17-16, H16-18, G18-17, F18 (6 trials) and F13-15, E14,15, D14-16 (4 trials). The most frequent areas chosen by most of the included trials (E3-5 and F5) referred to the “Shen-Men point” or “Cosmonaut point”, followed by “Hypophysis” (H17), “Hippocampus” (F14),” and “Sympathetic master point” (E14). Only two trials (Ndubisi et al., 2019; A. Vieira et al., 2018) reported using alternate ears bilaterally (e.g., right, or left ear) for selected points.

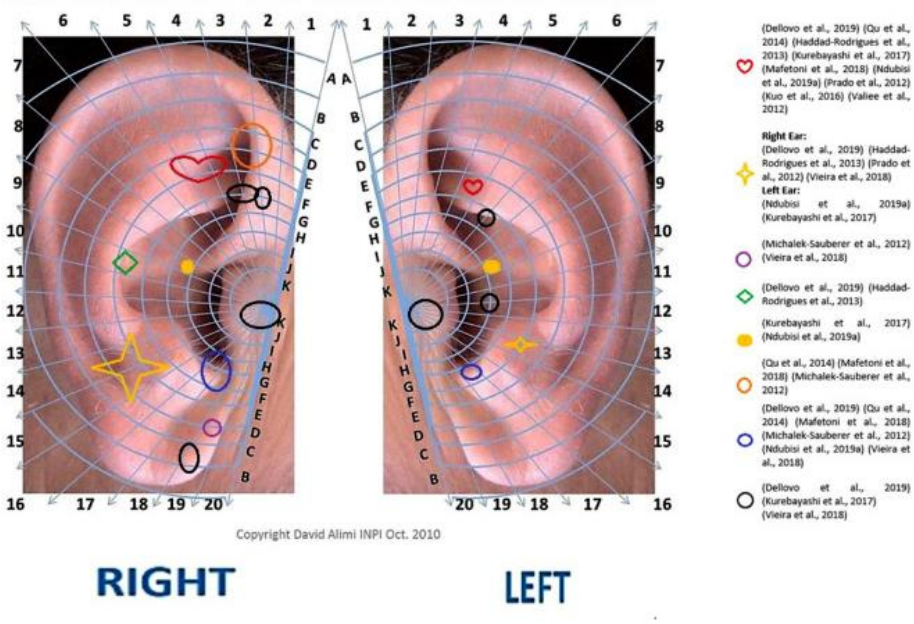
Regarding outcome measures (Table 1), most trials used the STAI (Haddad-Rodrigues et al., 2013; Kuo et al., 2016; Kurebayashi et al., 2017; Michalek-Sauberer et al., 2012; Prado et al., 2012; Qu et al., 2014; Rivadeneira et al., 2015; A. Vieira et al., 2018), followed by VAS for anxiety(Kurebayashi et al., 2017; Ndubisi et al., 2019; Valiee et al., 2012; A. Vieira et al., 2018), cortisol levels(Kuo et al., 2016). Other trials used vital signs (Dellovo et al., 2019; Kuo et al., 2016; Valiee et al., 2012) or SN-TCM (A. Vieira et al., 2018) as a secondary outcome. However, it was not possible to conduct a meta-analyses for those secondary outcomes. While Kuo et al (2016) have reported the cortisol levels and vital signs for post-caesarean section women comparing AA with control group, Valie et al (2012) have reported vital signs comparing the AA with placebo group and Dellovo et al (2019) but did not provide enough data to allow comparisons.

The duration and intensity of treatment specified in the trial protocol varied between trials. Most trials (n=10) in this review assessed outcomes after one session (Dellovo et al., 2019; Kuo et al., 2016; Kurebayashi et al., 2017; Mafetoni et al., 2018; Michalek-Sauberer et al., 2012; Ndubisi et

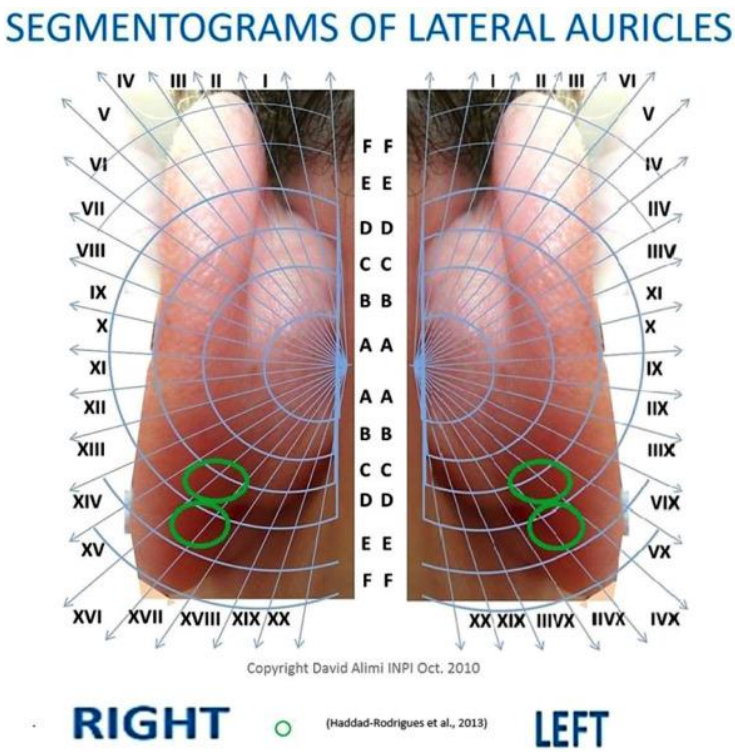
al., 2019; Qu et al., 2014; Rivadeneira et al., 2015; Valiee et al., 2012; A. Vieira et al., 2018), while one trial assessed after 2 treatments (Haddad-Rodrigues et al., 2013), only two trials assessed after 8 treatments (Rivadeneira et al., 2015; S. Wu et al., 2011), and one trial evaluated after 12 sessions of AA (one session per week) lasting 5 to 10 minutes per session (Prado et al., 2012). For the majority of included trials (6 trials) acupuncturists delivered the intervention (Michalek-Sauberer et al., 2012; Prado et al., 2012; Rivadeneira et al., 2015; A. Vieira et al., 2018) specifically, nurses who were also practicing acupuncture (Haddad-Rodrigues et al., 2013; Prado et al., 2012). In 3 trials, treatment was delivered by practitioners working in General Heath care with AA training (Kurebayashi et al., 2017; Mafetoni et al., 2018; Qu et al., 2014), only 1 trial used Traditional Chinese medicine physicians (Kuo et al., 2016). However, four trials did not report any details on the staff /practitioners providing the intervention(Dellovo et al., 2019; Ndubisi et al., 2019; Valiee et al., 2012; S. Wu et al., 2011).

Preoperative Anxiety was the most frequent type of anxiety (Dellovo et al., 2019; Kuo et al., 2016; Mafetoni et al., 2018; Michalek-Sauberer et al., 2012; Valiee et al., 2012; S. Wu et al., 2011; Wunsch et al., 2018b), followed by anxiety in students (Kurebayashi et al., 2017; Prado et al., 2012; A. Vieira et al., 2018) before examinations. We found only one trial on the use of AA for anxiety on the first-trimester abortion (Ndubisi et al., 2019), one trial for anxiety in lactating mothers (Haddad-Rodrigues et al., 2013), one trial for anxiety used after in vitro fertilization (Qu et al., 2014), and only one trial focused on the use of AA for generalized AD(Rivadeneira et al., 2015). Regarding follow-up, only Haddad-Rodrigues et al. (2013), Kurebayashi et al. (2017) mentioned high numbers of participants lost at follow-up, and Mafetoni et al. (2018) reported no losses at follow up. Of all the included trials, only Prado et al. (2012) cited the time frame for follow-up.

Unfortunately, most trials (10 studies) did not record or assess adverse effects (Dellovo et al., 2019; Haddad-Rodrigues et al., 2013; Kuo et al., 2016; Kurebayashi et al., 2017; Mafetoni et al., 2018; Michalek-Sauberer et al., 2012; Prado et al., 2012; Valiee et al., 2012; A. Vieira et al., 2018; S. Wu et al., 2011). Three trials did not identify or report any side effects (Ndubisi et al., 2019; Qu et al., 2014; Rivadeneira et al., 2015) after AA.



Chapter 4. Figure 2 – Points applied along the trials using Aimi D and Chelly J (2018) Cartography of French University scientific school of Paris (right and left medial auriculogram) from International AA Nomenclature. This cartography was used with permission (Alimi & Chelly, 2018).



Chapter 4. Figure 3- Points applied along the trials using Aimi D and Chelly J (2018) Cartography of French University scientific school of Paris (Medial auricular Segmentogram) from International AA Nomenclature. This cartography was used with permission (Alimi & Chelly, 2018).

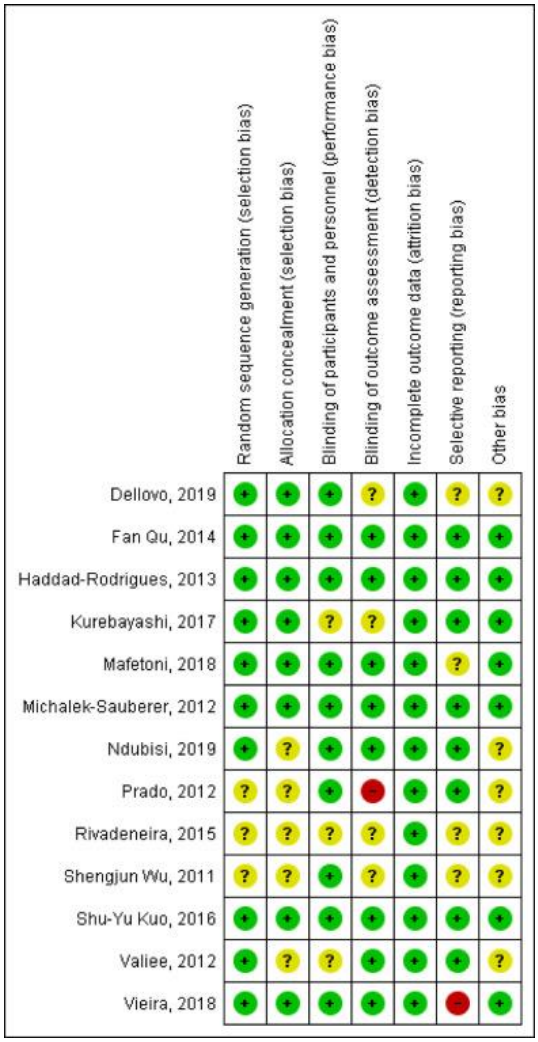
Risk of bias in included trials

• Randomization and allocation concealment

The summary of the risk of bias (Chapter 4. Figure) shows that three trials did not provide information on the methods of randomization (Prado et al., 2012; Rivadeneira et al., 2015; S. Wu et al., 2011) and five trials did not report the allocation concealment used (Ndubisi et al., 2019; Prado et al., 2012; Rivadeneira et al., 2015; Valiee et al., 2012; S. Wu et al., 2011). However, the risk of bias graph (Chapter 4. Figure) identified that around 75% of trials used a computer program to allocate participants to randomized groups, and the majority of these trials reported that allocations were concealed (Dellovo et al., 2019; Haddad-Rodrigues et al., 2013; Kuo et al., 2016; Kurebayashi et al., 2017; Mafetoni et al., 2018; Michalek-Sauberer et al., 2012; Qu et al., 2014)

• Attrition and reporting bias

As previously indicated, some trials presented their data and results statistically with p-values. However, most trials included complete data and had previously published their protocol (Haddad-Rodrigues et al., 2013; Kuo et al., 2016; Kurebayashi et al., 2017; Michalek-Sauberer et al., 2012; Ndubisi et al., 2019; Prado et al., 2012; Qu et al., 2014; Valiee et al., 2012). We did not find evidence of a previously published protocol for four trials (Dellovo et al., 2019; Mafetoni et al., 2018; Rivadeneira et al., 2015; S. Wu et al., 2011), so unclear risk was attributed. Only one trial was considered as having a high risk of attrition or reporting bias due to missing evidence of a previously published protocol (A. Vieira et al., 2018).



Chapter 4. Figure 4 - Risk of bias summary: review authors' judgements about each risk of bias item for each included trial.

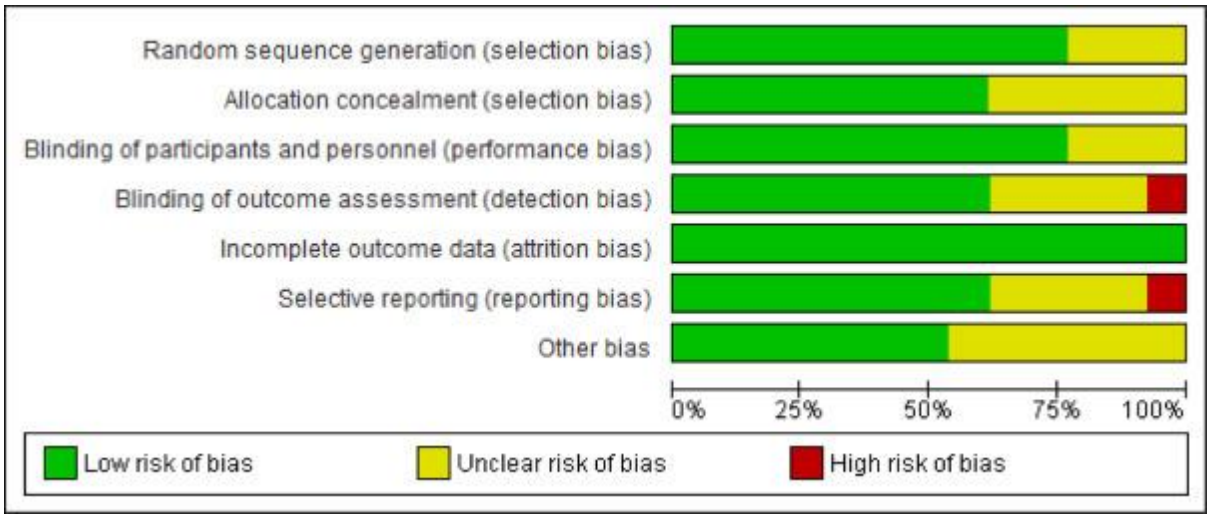
• Blinding

Due to the nature of the intervention, it was not possible to blind all the staff who delivered the intervention. Nevertheless, 75% of trials had ensured that participants and outcome assessment staff were "blinded", which was considered a low risk for personnel bias (Haddad-Rodrigues et al., 2013; Kuo et al., 2016; Mafetoni et al., 2018; Michalek-Sauberer et al., 2012; Ndubisi et al., 2019; Qu et al., 2014; A. Vieira et al., 2018; S. Wu et al., 2011).

Three trials were unclear regarding blinding of participants (Kurebayashi et al., 2017; Rivadeneira et al., 2015; Valiee et al., 2012) and, four trials were unclear about blinding outcome assessment (Dellovo et al., 2019; Kurebayashi et al., 2017; Rivadeneira et al., 2015; S. Wu et al., 2011). Although Prado et al. (2012) was the only trial reporting that the first author carried out data collection, we considered there was a high risk of detection bias.

• Other Bias

Some trials have not reported any therapist competence, and adherence to the treatment protocol, so unclear risk was given (Dellovo et al., 2019; Ndubisi et al., 2019; Valiee et al., 2012; S. Wu et al., 2011). Although more than half of the trials have appropriate or reasonable therapist competence and adherence to the treatment protocol, consequently, a low risk of other bias was attributed.



Chapter 4. Figure 5- Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included trials.

AA compared to Placebo:

Chapter 4. Table 0.1 - Grading of Recommendations Assessment, Development, and Evaluation summary of 9 randomized controlled trials.

Patient or population: anxiety disorders. Intervention: Auriculotherapy. Comparison: Placebo			
Outcomes	**№ of participants (studies) Follow-up**	**Certainty of the evidence (GRADE)**	**Anticipated absolute effects** **Risk difference with Auriculotherapy**
Auriculotherapy vs Placebo assessed with: State-Trait-Anxiety, Visual Analogic Scale for anxiety and Hamilton Anxiety Rating Scale	768 (8 RCTs)	⨁⨁⨁◯ Moderate[a]	SMD **0.44 lower** (0.6 lower to 0.28 lower)
Pre-operative anxiety assessed with: State-Trait-Anxiety, Visual Analogic Scale for anxiety and Hamilton Anxiety Rating Scale	259 (3 RCTs)	⨁⨁⨁◯ Moderate[a]	SMD **1.4 SD lower** (2.54 lower to 0.26 lower)
Exam anxiety in students assessed with: State-Trait-Anxiety Inventory	114 (2 RCTs)	⨁⨁◯◯ Low[c,d,e]	MD **2.44 lower** (5.67 lower to 0.79 higher)

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; **MD:** mean difference; **SMD:** standardized mean difference

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations

a. Different anxiety scales used

b. Some differences regarding the type of situational anxiety (students vs pre-operative).

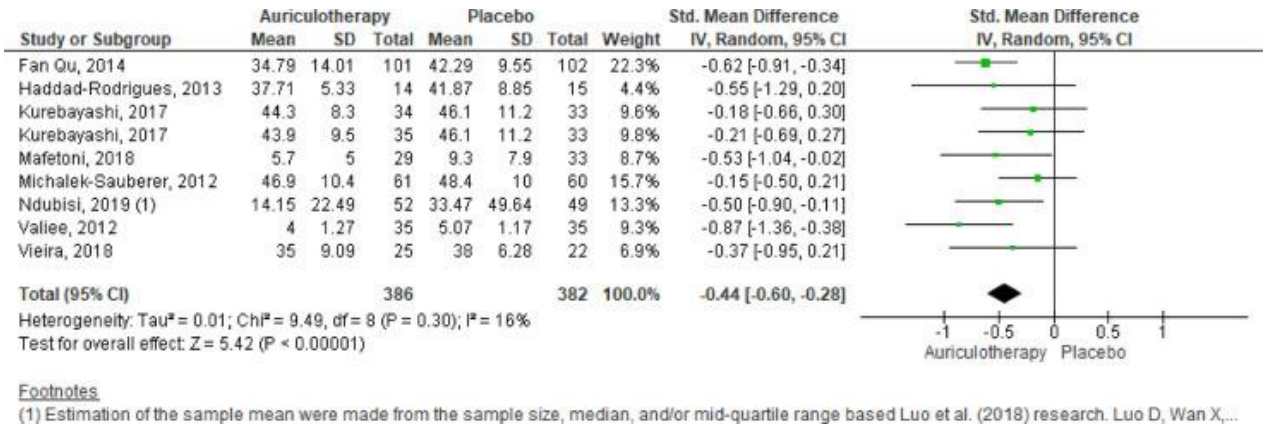
c. small sample size.

d. Possible selective reporting bias.

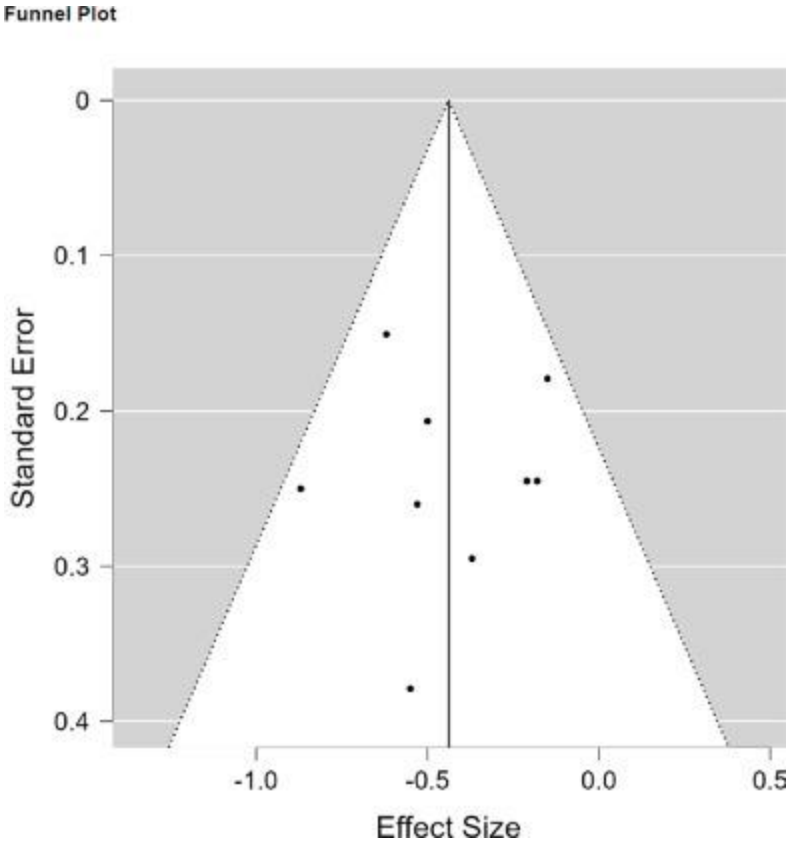
e. Wide confidence intervals along on the included trials.

Anxiety in general

Based on nine trials (Chapter 4. Figure) , the AA group (386 patients) and the placebo (group 382 patients) reported for all anxiety outcomes (STAI, VAS, Hamilton Anxiety Rating Scale) significant changes. Based on low Heterogeneity: Tau² = 0.01; Chi² = 9.49, df = 8 (P = 0.30); I² = 16% with a test for overall effect: Test for overall effect: Z = 5.42 (P < 0.00001), the pooled results showed a higher effect for AA group SMD -0.44 95% CI [-0.60, -0.28]. The estimation of the sample mean was optimally estimated from the sample size, median, and mid-quartile range based on Luo et al. (2018) recommendation in medical research (Luo, Wan, Liu, & Tong, 2018). Observing the funnel plot (Chapter 4. Figure) and by performing Egger's regression test, we considered the unlikely presence of publication bias p=0.961. Although we judged these as having moderate certainty of evidence (Table 1) due to different anxiety scales used in the analysis and there were some differences regarding the type of situational anxiety (students and pre-operative anxiety).



Chapter 4. Figure 6 - Forest plot of comparison: Auriculotherapy compared to placebo for all types of anxiety, outcome: Anxiety Scales.

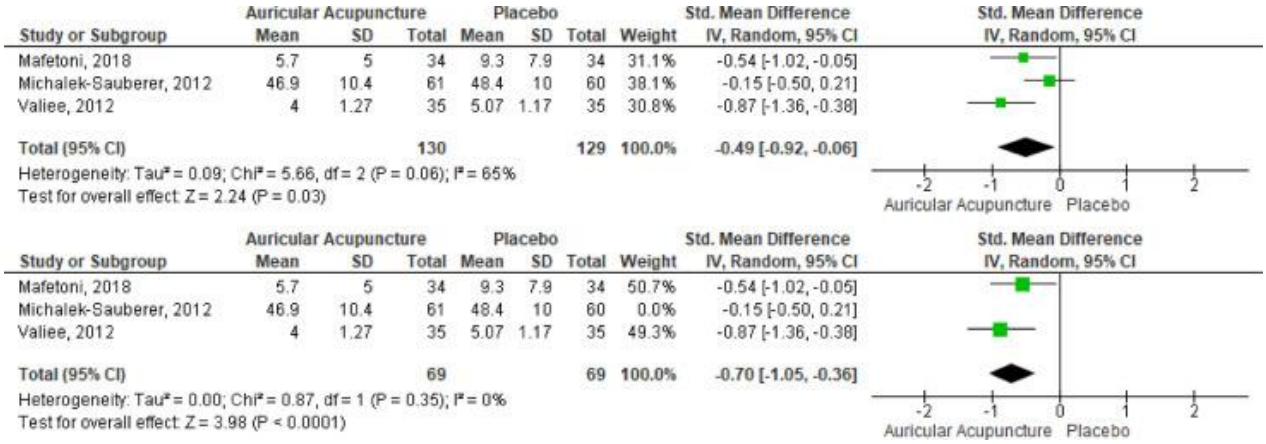


Chapter 4. Figure 7 - Funnel plot of comparison: Auriculotherapy compared with placebo for all types of situational anxiety using all anxiety scales.

- **Pre-operative anxiety**

The pooled results showed significant differences (Chapter 4. Figure) for outcomes STAI, Hamilton Anxiety Rating Scale, and VAS where the SMD was -0.49 [-0.92, -0.06], with high heterogeneity: $Tau^2 = 0.09$; $Chi^2 = 5.66$, df = 2 (P = 0.06); $I^2 = 65\%$, Test for overall effect: Z = 2.24 (P = 0.03).

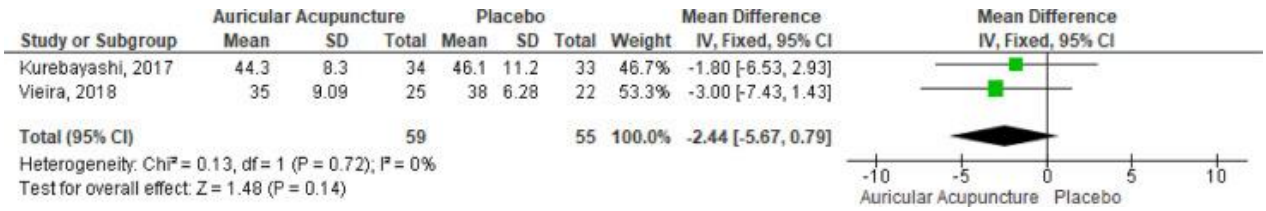
After conducting a sensitivity analysis, we realized that the heterogeneity vanished after removing Michalek-Sauberer (2012) study (Chapter 4. Figure) and the effect size turned out to be higher: SMD= -0.70 [-1.05, -0.36]. This result could be either due to STAI outcome used while the other RCT used VAS and Hamilton AnxietyRating Scale or due to different preoperative conditions between studies. Although, both studies included in the second analysis had used similar auricular points (F5-6) with seeds in only one treatment session. Therefore, we have graded moderate certainty of evidence (Table 1).



Chapter 4. Figure 8: Forest plot of comparison: AA compared to placebo for pre-operatory anxiety, outcome: State-Trait-Anxiety, Visual Analogic Scale for anxiety and Hamilton Anxiety Rating Scale.

- **Exam anxiety in students.**

Only two trials with 59 students in AA group and 55 students in the placebo group reported a decrease on STAI scale for AA, MD= -2.44; 95% CI: -5.67, 0.79, Heterogeneity: $Chi^2 = 0.13$, df = 1 (P = 0.72); $I^2 = 0\%$, Test for overall effect: Z = 1.48 (P = 0.14), however there was not strong evidence that the intervention had an effect (Chapter 4. Figure). We decided to use fixed effects because those two trials shared a similar effect size, and a random effect would not change the results. Due to the wide confidence intervals among the included trials, different auricular points used, different sessions (between one and 10 sessions) and the small sample, this was graded as having a low certainty of evidence (Table 1).

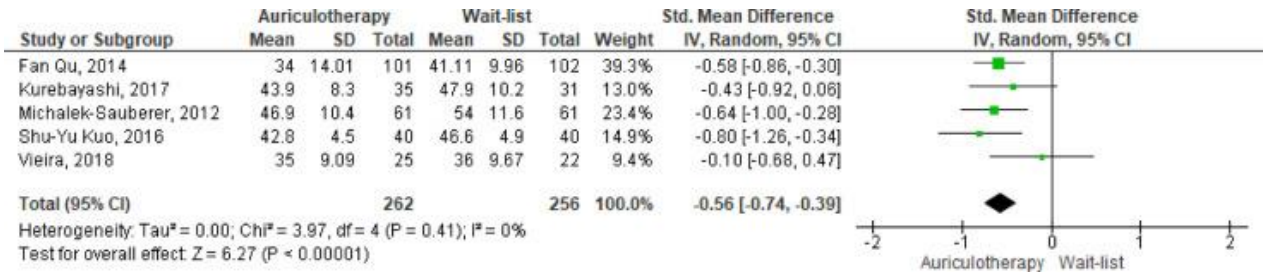


Chapter 4. Figure 9 - Forest plot of comparison: AA compared to placebo for exam anxiety in students, outcome: State-Trait-Anxiety Inventory.

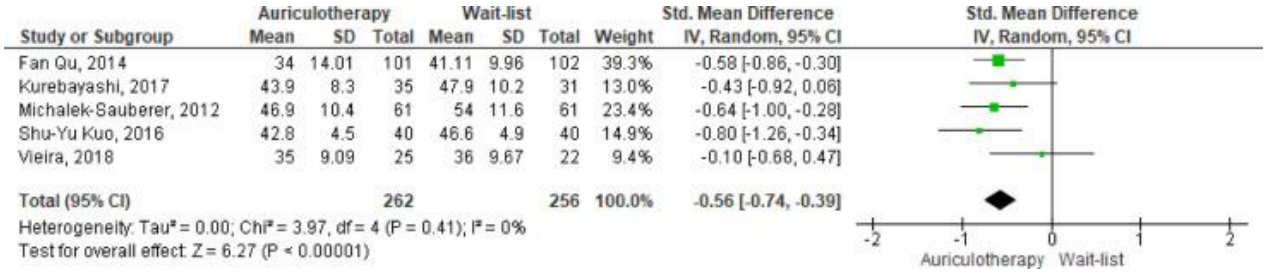
AA compared to waiting list:

- **Anxiety in general**

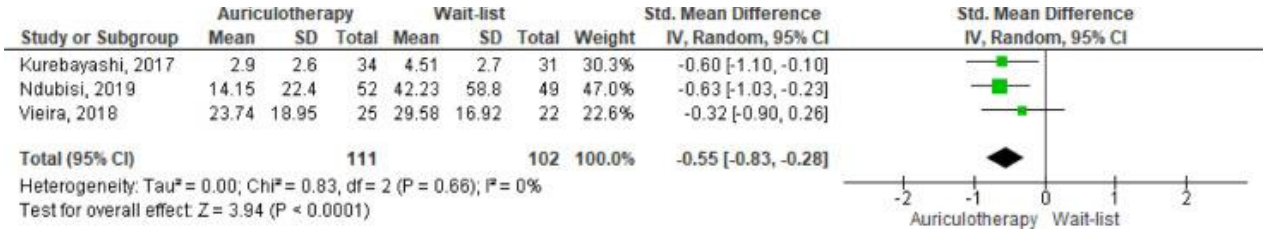
Linking the treatment effectiveness of AA versus waiting list when we compared all included trials, either, the outcome STAI (



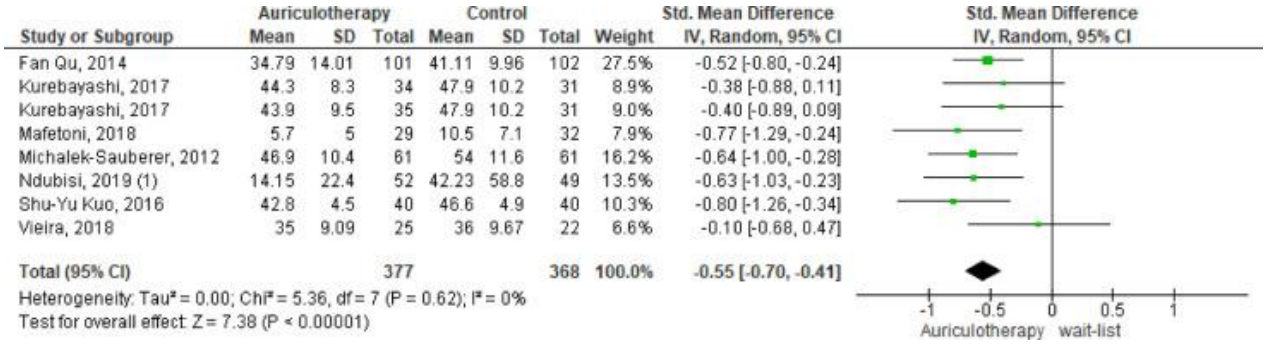
Chapter 4. Figure 18) and VAS (Chapter 4. Figure 19) has decreased in the AA group (SMD -0.56; 95% CI: -0.74, -0.39; in five trials, Heterogeneity was not found: $Tau^2 = 0.00$; $Chi^2 = 3.97$, df = 4 (P = 0.41); $I^2 = 0\%$; Test for overall effect: Z = 6.27 (P < 0.00001)), and SMD -0.55; 95% CI [-0.83, -0.28], 3 trials, Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.83$, df = 2 (P = 0.66); $I^2 = 0\%$; Test for overall effect: Z = 3.94 (P = 0.0001) respectively. Both outcomes STAI and VAS were stastistically significant, and therefore decreased with an AA intervention. To investigate the effect using both scales (Chapter 4. Figure 120), we compared the AA (377 participants) versus waitinlist (368 participants) with 8 trials in total, the preference was towards the AA group in all trials, where the SMD is -0.55; 95% CI [-0.70, -0.41], Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 5.36$, df = 7 (P = 0.62); $I^2 = 0\%$, Test for overall effect: Z = 7.38 (P < 0.00001), also showing statistical significance. By performing sensitivity analysis and removing one study assessed as having a high risk of selective reporting (Vieira et al, 2018), the results did not change but the effect was slighty higher (SMD -0.58 [-0.74, -0.43]). Observing the funnel plot (Chapter 4. Figure 21) and by performing Egger's regression test, we considered the unlikely presence of publication bias p=0.621. Although, due to different types of situational anxiety (pre-operatory and exam anxiety), selective reporting bias, unclear blinding of participants, we graded moderate certainty of evidence (Table 2).



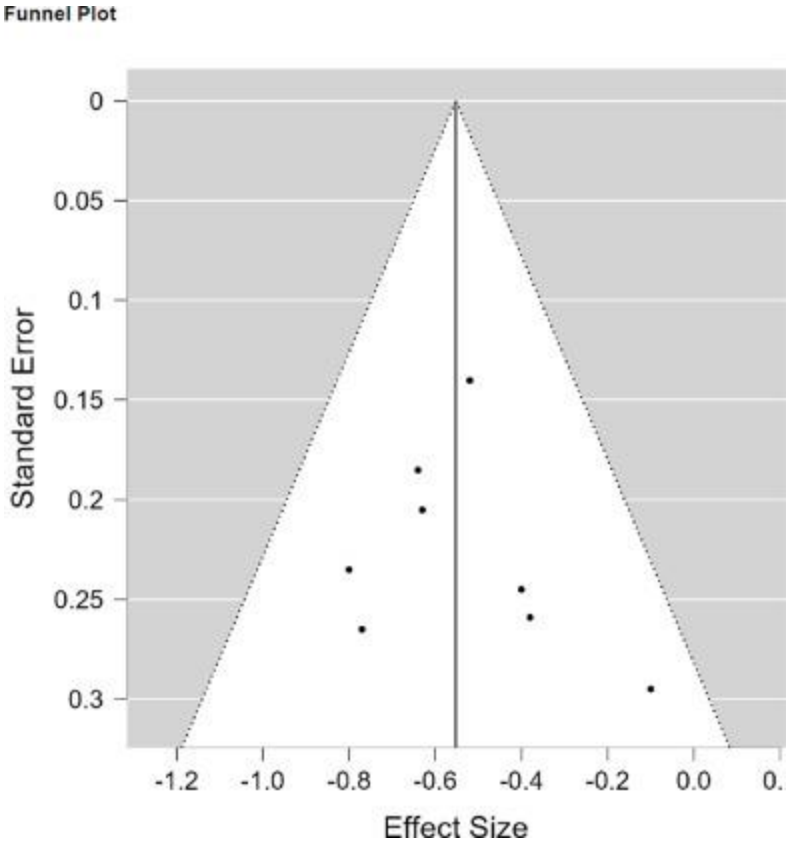
Chapter 4. Figure 18 – Forest plot of comparison: AA compared to waiting list for all types of anxiety, outcome: State-Trait-Anxiety Inventory.



Chapter 4. Figure 19 - Forest plot of comparison: AA compared to waiting list for all types of anxiety, outcome: Visual analogic scale for anxiety.



Chapter 4. Figure 120 - Forest plot of comparison: AA for all types of Anxiety, outcome: Anxiety scales.



Chapter 4. Figure 213 - Funnel plot of comparison: Auriculotherapy compared with waiting list for all types of situational anxiety using all anxiety scales.

Chapter 4. Table - Grading of Recommendations Assessment, Development, and Evaluation summary of Auriculotherapy compared to wait-list for situational anxiety.

Patient or population: situational anxiety. Intervention: Auriculotherapy. Comparison: Wait-list

Outcomes	№ of participants (studies) Follow-up	Certainty of the evidence (GRADE)	Anticipated absolute effects Risk difference with Auriculotherapy
Situational anxiety assessed with: State-Trait-Anxiety-Inventory	518 (5 RCTs)	⨁⨁⨁◯ Moderate[a,b]	SMD **0.56 SD lower** (0.74 lower to 0.39 lower)
Situational anxiety assessed with: State-Trait-Anxiety-Inventory and Visual Analogic Scale for anxiety	745 (8 RCTs)	⨁⨁⨁◯ Moderate[a,b]	SMD **0.55 lower** (0.7 lower to 0.41 lower)
Pre-operatory anxiety assessed with: State-Trait-Anxiety Inventory	198 (2 RCTs)	⨁⨁⨁◯ Moderate[cd]	MD **5.02 lower** (8.15 lower to 1.9 lower)
Students anxiety assessed with: State-Trait-Anxiety Inventory	113 (2 RCTs)	⨁⨁◯◯ Low[ce,f]	MD **2.53 SD higher** (5.99 higher to 0.94 higher)
Pre-operatory anxiety assessed with: Anxiety scales	266 (3 RCTs)	⨁⨁⨁◯ Moderate[c]	SMD **0.72 lower** (0.97 lower to 0.47 lower)
Exam anxiety assessed with: State-Trait-Anxiety Inventory	113 (2 RCTs)	⨁⨁◯◯ Llow[a,e]	MD **2.53 lower** (5.99 lower to 0.94 higher)

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention; MD: mean difference; SMD: standardised mean difference. Explanations:

a. Selective reporting bias.

b. Some differences regarding the type of situational anxiety (students vs pre-operative).

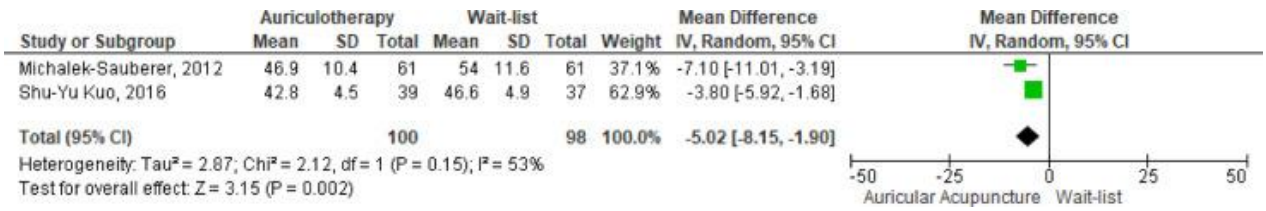
c. Wide confidence intervals along on the included studies.

d. Different preoperative anxiety post-caesarean population versus dental surgery patients. e. Unclear blinding of participants and outcomes. Possible selective reporting.

f. Small sample size. GRADE Working Group grades of evidence. High certainty: we are very confident that the true effect lies close to that of the estimate of the effect. Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect. Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

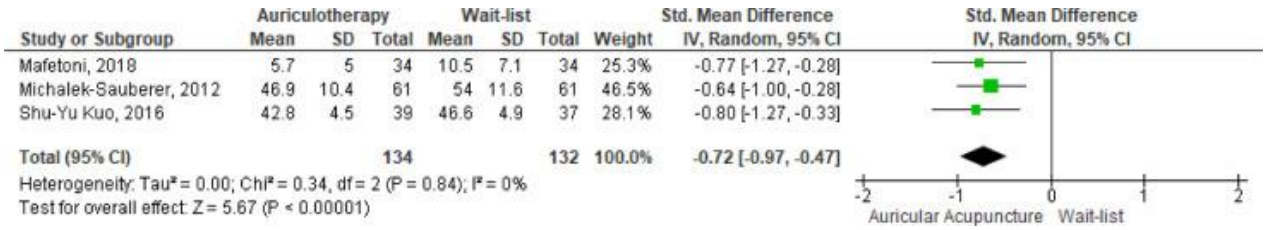
- **Pre-operatory anxiety**

There was only one study reporting the cortisol levels (Kuo et al., 2016) where the authors found differences for AA group (n=39) MD= -0.48 [-0.94, -0.03] compared with waiting list (n=37), however meta-analyses was not possible for this outcome. Regarding anxiety based on scales, two trials with 100 patients in the AA group and 98 patients in the waiting list group reported significant changes for the outcome STAI. Based on the I^2 test-value (Chapter 4. Figure), Heterogeneity was considered moderate : $Tau^2 = 2.87$; $Chi^2 = 2.12$, df = 1 (P = 0.15); $I^2 = 53\%$ with a test for overall effect: Z = 3.15 (P < 0.002), the pooled results showed a significant effect for AA group MD = -5.02, 95% CI [-8.152, -1.90]. In this case, we decided to use the random-effects model because trials did not share a common effect size. Sensitivity analysis was not possible as both studies were considered low risk of bias, but different preoperative anxiety populations in both trials could lead to a high percentage of heterogeneity as distinct auricular points choosen (F6-5 versus G17, D2,3,19).



Chapter 4. Figure 14 - Forest plot of comparison: AA compared to waiting list for pre-operatory anxiety, outcome: State-Trait-Anxiety Inventory.

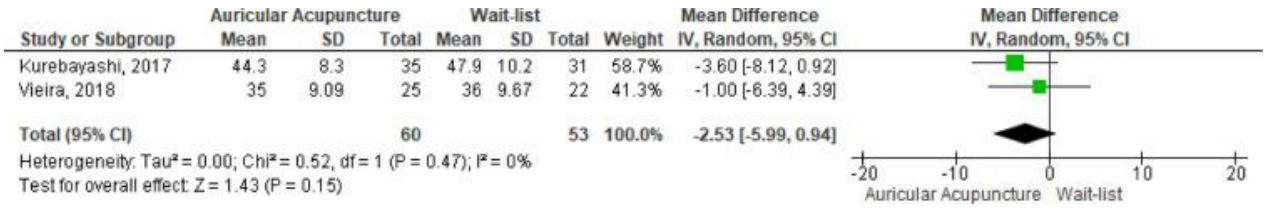
While Shu-yu Kuo (2016) trial participants were post-caesarean women, Michalek-Sauberer (2012) trial participants were dental surgery patients. Also, those results are corroborated without heterogeneity (Tau^2 = 0.00; Chi^2 = 0.34, df = 2,P = 0.84; I^2 = 0%), if we add one more trial (Mafetoni et al., 2018) to the analysis using the outcome Hamilton Rating Scale for Anxiety (Chapter 4. Figure). Therefore, with SMD= -0.72 95% CI [-0.97, -0.47], favorable for the AA group, and so, we consider moderate certainty of evidence (Table 2).



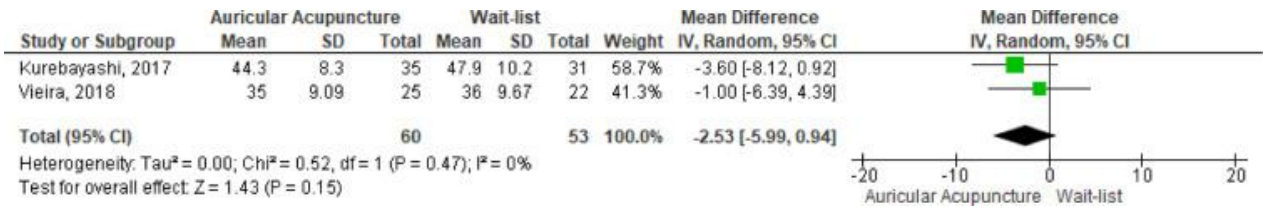
Chapter 4. Figure 15 – Forest plot of comparison: AA compared to waiting list for pre-operative anxiety.

- **<u>Exam anxiety in students.</u>**

Only two trials with 60 students in the AA group and 53 students in the waitinglist group reported a decrease (



Chapter 4. Figure) on the STAI scale for AA (MD -2.53; 95% CI: -5.99, -0.94), Heterogeneity: Chi^2 = 0.52, df = 1 (P = 0.47); I^2 = 0%, Test for overall effect: Z = 1.43 (P = 0.15). We decided to use fixed effects because those two trials shared a similar effect size, and a random effect would not change the results. In this case, due to small sample sizes, unclear blinding of participants/ outcomes, and possible selective reporting from Vieira et al. (2018), different auricular points used, different sessions (between one and 10 sessions) and due to the small sample, this was graded as low certainty of evidence (Table 2).



Chapter 4. Figure 16 - Forest plot of comparison: AA compared to waiting list for exam anxiety in students,

outcome: State-Trait-Anxiety Inventory.

Auriculotherapy alone or plus usual care versus usual care for Anxiety Disorder:

We found only one trial (Rivadeneira et al., 2015) comparing AA (n=30) with Mexican Argemona seeds versus conventional therapy (clorodiazepóxido, 10 mg and trifluoperazine, 1mg; n=30) for generalized AD treatment. Rivadeneira et al. (2015) performed a Self Assessment Inventory test (SAIT) and remission of anxiety symptoms after the 4th and 8th weeks. Those authors cited that "insomnia, irritability and memory symptoms decreased more remarkably in the group treated with AA". AA appeared to be more effective in treating anxiety which could potentially reduce the use of psychopharmacologic drugs. However, due to a lack of research in this area, comparison with other trials was impossible. Consequently, we have graded Low certainty of evidence (Table 3).

Chapter 4. Table 0.3 - Grading of Recommendations Assessment, Development, and Evaluation summary of 1 randomized controlled trial (RCT) comparing AA to usual care for generalized AD.

Patient or population: Generalized anxiety disorder. Intervention: Auriculotherapy. Comparison: usual care

Outcomes	№ of participants (studies) Follow-up	Certainty of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects	
				Risk with usual care	Risk difference with Auriculotherapy
Nightmare	22 (1 RCT)	⨁⨁◯◯ Low[a,b]	**RR 0.64** (0.25 to 1.62)	667 per 1000	**240 fewer per 1000** (500 fewer to 413 more)
Nervousness	22 (1 RCT)	⨁⨁◯◯ Low[a,b]	**RR 0.62** (0.22 to 1.77)	538 per 1000	**205 fewer per 1000** (420 fewer to 415 more)
Sweating	10 (1 RCT)	⨁⨁◯◯ Low[a,b]	**OR 0.33** (0.01 to 8.18)	750 per 1000	**253 fewer per 1000** (721 fewer to 211 more)
Irritability	23 (1 RCT)	⨁⨁◯◯ Low[a,b]	**RR 0.28** (0.05 to 1.64)	722 per 1000	**520 fewer per 1000** (686 fewer to 462 more)
Memory difficulty	24 (1 RCT)	⨁⨁◯◯ Low[a,b]	**RR 0.23** (0.04 to 1.41)	722 per 1000	**556 fewer per 1000** (693 fewer to 296 more)

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** confidence interval; **OR:** odds ratio; **RR:** risk ratio. **Explanations:** a. Unclear selection, performance, detection and reporting biases. Small sample size and small number of events.

GRADE Working Group grades of evidence: High certainty: we are very confident that the true effect lies close to that of the estimate of the effect. **Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. **Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect. **Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

4. DISCUSSION

Recently, Nielsen et al. (2020) found that the most frequent risk of AA was infection, perichondritis, and chondritis from needles. We identified two trials that measured adverse events as a secondary outcome, and they did not find any serious adverse events. Nonetheless, using spheres in AA clinics will provide clinical benefits without the risks associated with needles (Nielsen, Gereau, & Tick, 2020). Consequently, we strongly recommend the adverse effects

reported in future research to establish if AA is safe for treatment of anxiety.

More than half trials included in this review appeared to report appropriate/reasonable therapist competence and adherence to the treatment protocol. In six included trials, the acupuncturists were responsible for the treatment. The lack of reporting important information like therapist experience, training, and competence in treatment protocols remains problematic. However, the literature has frequently described that acupuncture is considered safe in the hands of well-trained practitioners (McDonald & Janz, 2017).

We found only one trial (Rivadeneira et al., 2015) comparing Mexican Argemona seeds versus conventional therapy concerning generalized AD. Following that trial, AA was more effective in decreasing anxiety and reducing the use of psychopharmacologic drugs. Although, due to a lack of research in this field, comparisons with other trials were impossible. Our results follow Pilkington et al.'s (2007) research, which also identified the challenge of interpreting the findings of acupuncture for generalized AD. Pilkington et al. (2007) found two trials and these lacked methodological details. Moreover, when they compared acupuncture with drug therapy, no difference was observed because the trials were too weak to make a valid assessment and comparison (Pilkington et al., 2007). Regarding the type of AA, we found only one trial (Kurebayashi et al., 2017) comparing AA using seeds versus semi-permanent needles for exam anxiety in students. The outcomes VAS and STAI were performed after the 4th and 8th weeks. Authors have reported that AA produced the best results for reducing state anxiety using needles compared with seeds. However, due to the lack of randomized controlled trials, we were unable to make comparisons.

Generally, our results support the systematic review done on acupuncture by Pilkington et al. (2007), where all included trials reported positive findings, but lacked methodological detail. Similarly, they found that generally, the trials on perioperative anxiety were superior.

In our work, we did not find enough evidence to support the hypothesis that AA compared with placebo had a significant positive effect in reducing students' anxiety, but we did find that AA was better than a waiting list group. In fact, some researchers are against using placebo in AA trials (Zhang, Yang, Zhang, May, & Xue, 2014), as the ear is tiny organ and has more than 93 documented active acupoints (Jan et al., 2017). The stimulation of any point may produce physiological effects or affect the patient's belief (Zhang et al., 2014). Besides that, neurotransmitters such as endogenous opioids, dopamine and serotonin are also released, thereby modulating the individual's biological reactions (Miller & Miller, 2015).

On the other hand, there seems to be a continual tension between acknowledging the possible therapeutic utility of placebo prescription and the ethical issues surrounding its use (Finniss, 2018). The field of placebo research has accepted that a placebo might not be as distinctively

defined as it is necessary for conducting a clinical trial in the non-pharmacological area (Musial, 2019). Placebo effects are viewed as positive and valuable treatment factors, particularly in clinical practice, and are a part of every routine treatment (Evers et al., 2018).

Some results shown in our systematic review have clinically unimportant differences and low certainty, so we recommend different auricular approaches and more rigorous protocols to increase the certainty level of the results. The truth is that the type of auricular stimulation, type of method selected to choose the auricular points, number of sessions, and the treatment duration for anxiety varied between included studies has led to a lack of agreement on the optimal period of auricular therapy in the absence of consistency.

5. Limitations Conclusion

The number of patients and the quality of trials included limited this review. One of the strengths of our study was that only RCTs were used to minimize the amount of bias. However, we excluded other studies with interventions that could have changed the results.

Moreover, as AA is widely used in traditional medicine, the results of this systematic review may be affected by the exclusion of Chinese databases (32) and, any unpublished trials. Nevertheless, we strongly recommend further updated analysis in the future with the inclusion of Chinese databases to corroborate these results.

Based on this systematic review, there is evidence that AA may reduce anxiety levels as measured by psychometrically robust scales of anxiety symptoms. Clinicians may consider AA as an adjunct or alternative when concerns about anxiety drug side effects are severe, contraindicated, or previously ineffective. Auriculotherpy can decrease cortisol levels, however only one study was found, thus, more research would be ideal. There is still a gap of reporting the AA adverse events and lack of research in AD following appropriate diagnosis. We found the area covered from (E3-5 and F5) referred to as “Shenmen point” or “Cosmonaut point”, were the most frequent areas selected by most of the included trials. However the type of AA (e.g., needles vs seeds) and the methodology used behind the points chosen for anxiety treatment, also requires more research.

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contributed to drafting the article and revising it critically for important intellectual content. Andreia Vieira prepared figures and tables. All authors reviewed the manuscript and approved the version to be published.

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Data availability: The authors have supplied the characteristics of the included trials in supplementary file.

Additional Tables

1 Studies Characteristics of Auricular Acupuncture for Anxiety.

Author, year	Anxiety type;Sample size	Participants age range M (SD)	Number of sessions; Type of Auriculotherapy	Comparator	Important Outcomes	Experimental acupoints	Adverse Side effects
Shengjun Wu, 2011	Preoperative Anxiety, 35	No information provided.	8, ASP needles	Body acupuncture	SAS	Shenmen	No information provided.
Valie, 2012	Preoperative Anxiety, 70	44	1 + follow-up, acupressure	Placebo	VAS, vital signs	Plastic bead was placed on the Shen men point of the nonprevailing ear + craniopressure.	No information provided.
Prado, 2012	Exam Anxiety in Students; 71	28,7	12 + follow-up of 15 days; ASP needles performed by an nurse acupuncturist.	Placebo and Wait-list	STAI	Shenmen and Brainstem points	No information provided.
Michalek-Sauberer, 2012	Preoperative anxiety,182	37,5	1, ASP needles performed by an acupuncturist.	Placebo and wait-list	STAI	Relaxation, tranquillizer, and master cerebral points.	No information provided.
Haddad-Rodrigues, 2013	Anxiety in Lactating Mothers, 29	26,9	2 + follow-up, Stainless steel needle by licensed nurse acupuncturist	Placebo (Same treatment without perforate skin)	STAI + cortisol levels (ug/dL)	(a) shenmen; (b) muscle relaxation; (c) tension; (d) anxiety 1 and 2.	No information provided.
Fan Qu, 2014	Vitro Fertilization anxiety, 305	30.5	1, Semen vaccariae seeds performed by trained nurses.	Placebo + Wait-list	STAI + Amsterdam Preoperative Anxiety and Information Scale	Shenmen (TF4), Endocrine (CO18) and Internal Genitals (TF2)	No side-effect was reported

Rivadeneira, 2015	Generalized anxiety disorder, 60	No information provided.	8, Mexican Argemona seeds performed by acupuncturist.	Usual care (psychopharmacologic drugs)	Self Assessment Inventory test + Remission of anxiety simptoms related.	Auricular shenmen, occipital point, heart, liver, spleen and kidney.	No information provided.
Shu-Yu Kuo, 2016	Preoperative anxiety (Post-caesarean), 80	32.8	1, vaccariae seed performed by Traditional Chinese medicine physicians.	Wait-list/ usual care (nursing assessment, and postpartum nursing care)	STAI, vital signs, cortisol levels	shenmen acupoint preced twice/day for 3 minutes.	No information provided.
Kurebayashi, 2017	Exam Anxiety in Students; 180	35,7	10 sessions + follow-up; ASP needles and seeds performed by one psychologist and four nurses, trained in auriculotherapy	Placebo and Wait-list	VAS, STAI	Auricular Protocol for Pain & Anxiety - APPA: i) Sympathetic; ii) Shen - Men; iii) Point zero; iv) Tranquiliser; v) Thalamus.	No information provided.
Mafetoni, 2018	Preoperative Anxiety (during labor), 102	23.9	1 + follow-up, crystal microspheres performed by health care with auriculotherapy training	Placebo and Wait-list	Hamilton Anxiety Rating Scale	shenmen, uterus, neurasthenia area and endocrine points,	No information provided
Vieira, 2018	Exam Anxiety in Students; 69	20,8	1; ASP needles performed by an acupuncturist.	Placebo and no intervention group.	STAI, VAS, SN-TCM.	i) diazepam, II) lung parenchyma, III) anxiety, IV)psychosomatic V) joy	No information provided.
Wunsh, 2018	Preoperative anxiety; 62	36.5	1; ASP needles performed by an acupuncturist;	No intervention group.	STAI, VAS, cortisol levels.	MA-IC1 (Lung), MA-TF1 (ear Shenmen), MA-SC (Kidney), MA-AT1 (Subcortex) and MA-TC	One participant reacted with vasovagal syncope

						and MA-TG (Adrenal gland)	after AA,
Dellovo, 2018	Preoperative anxiety, 30	31.4	1 + Follow-up after 24hours, Mustard seeds + placebo medication.	placebo + midazolam	Corah Dental Anxiety Scale, Vital signs	Shen Men; Kidney; Sympathetic; Anxiety; Neurasthenia; Heart; Liver.	No information provided.
Ndubisi, 2019	Anxiety during first trimester abortion, 153	30.2	1, Pyonex™ press needles plus usual care	placebo plus usual care, or usual care alone	VAS.	Gold protocol acupoints [cingulate gyrus, thalamus, Point Zero, Shen Men, Cervix (only left ear), Uterus C (only right ear)]	No acupuncture-related adverse events

Footnotes

SAS: Self-Rating Anxiety Scale, VAS: visual analog scale for anxiety; STAI: StateTrait Anxiety Inventory; SN-TCM: scale on the neurovegetative status based on a scored analysis of the tongue according to the principles of Traditional Chinese Medicine.

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CHAPTER 5

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Open Access Article

The Effect of Auriculotherapy on Situational Anxiety Trigged by Examinations: A Randomized Pilot Trial

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The effect of Auriculotherapy on situational anxiety triggered by examinations: A Randomized pilot trial

Abstract

Background: Auriculotherapy may activate the parasympathetic nerve system and reduce anxiety levels. Short-term auriculotherapy's effects and safety on university students´ anxiety levels was assessed prior to exams. **Methods:** A randomized, controlled pilot trial was conducted. The day before the exam, university students were randomly allocated to the auriculotherapy group (AA, *n* = 13) or the waiting-list group (WG, *n* = 13). Baseline measures were taken 4 weeks before the exam at Time point (TP 0); at 7.30 a.m. on the day before the exam (TP I); at 11 a.m. before auriculotherapy (TP II); 30 min after AA (TP III); and at 7.30 a.m. before the exam (TP IV). The outcomes were the State-Trait-Anxiety Inventory (STAI); quality of night-sleep, Visual Analogue scale (VAS) for anxiety, and salivary cortisol. Adverse events were also recorded. **Results:** A total of 26 students participated in this study and became more anxious as assessed by STAI in TPII ($p = 0.002$) and TPIV ($p = 0.000$) than TP0. AA reduced the STAI in TPIII ($p = 0.045$) and PIV ($p = 0.001$) and the VAS ($p = 0.012$) in TPIV. Cortisol was reduced in TPIII ($p = 0.004$), and the AA slept better ($p = 0.014$) at TPIV. Discomfort at the auricular site was reported in only one AA participant. **Conclusions:** Auriculotherapy appeared safe and effective in reducing anxiety levels before university exams.

Keywords: auriculotherapy; anxiety; acupressure; auricular points; salivary cortisol; randomized controlled trial; parasympathetic nervous system; brain modulation

1. Introduction

Anxiety is one of the most natural body responses, and it is fundamental for humans to a given threat (Notebaert et al., 2020; World_Health_Organization, 2017). However, anxiety can become pathological due to hormonal imbalance during physical and emotional challenges over time (Dean, 2016).

Mental distress has been identified in undergraduates (Mboya et al., 2020). Young students are the most predisposed to stressful life events, especially those pursuing higher professional education in a competitive setting (Mofatteh, 2020). A high prevalence of anxiety among health care professional students has been identified globally (Fares, Al Tabosh, Saadeddin, El Mouhayyar, & Aridi, 2016; Mofatteh, 2020; Nia, Lehto, Ebadi, & Peyrovi, 2016). There are various factors that are responsible for the decline in the mental health of students with healthcare degrees, including academic pressure (Li, Li, & Li, 2019), an increased workload (Fares et al., 2016), financial issues (Li et al., 2019), sleep deprivation (Kalinin, Hocaoglu, & Mohamed, 2021; Wallace, Boynton, & Lytle, 2017), and being exposed to patient death (Nia et al., 2016).

Auriculotherapy (AA) has been employed for approximately 2500 years in Chinese civilization (Hou et al., 2015). It is a technique used to diagnose and treat physical and psychosomatic dysfunctions by stimulating a specific point in the ear (Gori & Firenzuoli, 2007) using needles (J. Z. Zhang, Wu, Chang, & Huang, 2021), seeds (Kurebayashi et al., 2012), magnetic stones and lasers (Suen, Molassiotis, Yueng, & Yeh, 2019), bloodletting, moxibustion, electric stimulations, or massaging the auricular points (Round, Litscher, & Bahr, 2013). In Europe, AA has been applied systematically and comprehensively since Doctor Nogier introduced the map of the inverted fetus in 1957 (Wirz-Ridolfi, 2019), and the idea that the whole body (e.g., the somatotopic/holography rule of points) is reflected by the ear (Nogier, 1972). The somatotopic perspective has received scientific support from double-blind studies investigating auricular diagnosis and treatment for several disorders (Ahlberg, Skårberg, Brus, & Kjellin, 2016; deMatos, Santos, Moreira, Machado, & Vieira, 2021; Mendonca, Coelho Dos Santos, Noll, Silveira, & Arruda, 2020; Moura et al., 2019; Stuyt & Voyles, 2016; T. I. Usichenko et al., 2022). The current understanding of the mechanisms underpinning auriculotherapy is based on the embryological hypothesis (Nogier, 1972; Rabischong & Terral, 2014) and the innervation of the ear (Rabischong & Terral, 2014). It is recognized that the AA technique might work in situational anxiety because the puncture of a reflex point in the ear elicits responses of the reticular formation and through the sympathetic and parasympathetic nervous systems activation (Badran et al., 2018; deMatos et al., 2021).

In recent randomized crossover trials (Andreia Vieira, Moreira, Machado, Robinson, & Hu, 2022),

AA with needle intervention reduced pre-exam anxiety and increased sleep duration in medical students, where the intervention was superior to a placebo procedure in treating anxiety. It seems that AA can have a critical role in treating situational anxiety. However, AA's effectiveness is subject to the acupuncturist´s professional qualification and assertiveness with which type of intervention and points are selected in their therapy.

In the past, we have shown a tendency to reduce anxiety levels after 30 min with AA, although the effect was significant after 48 h from the experimental session (A. Vieira, Hinzmann, Silva, Santos, & Machado, 2018; A Vieira et al., 2016). However, these results were related to AA being performed 2 weeks before students' examinations, so we designed a new study with new variables and outcomes. The question that guided this study was: Is AA effective and safe on anxiety levels provided the day before an exam compared to a waiting-list group (WG)? To address this question, anxiety levels were measured using STAI as the primary outcome, with the quality of night sleep, the VAS for anxiety, and levels of salivary cortisol as the secondary outcomes. Therefore, we expected a reduction in anxiety in the AA group but not in the WG. Our main aim was to assess the effectiveness of AA in reducing anxiety in students according to the STAI and report its safety. As secondary goals, we (i) evaluated AA effectiveness in decreasing secondary outcomes; and (ii) investigated which auricular side of the ear was more reactive with appropriate equipment before AA application.

2. Materials and Methods

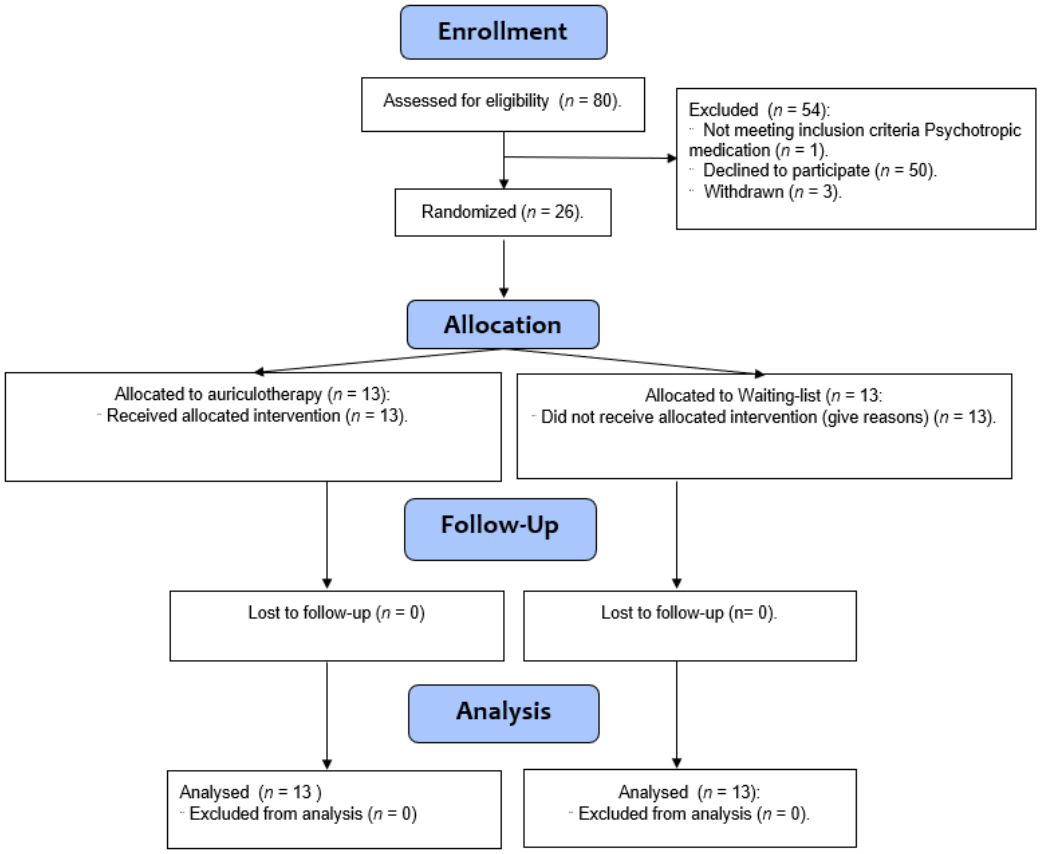
2.1. Study Design

An experimental, prospective, randomized, controlled, and single-blinded pilot study was conducted in December 2021 at the Private Superior Health School in Porto, Portugal. The Institutional Ethics Committee approved the research project on the 3 July 2021 (reference no. 2021-11). Before enrolling the participants, the authors registered the protocol at clinicaltrials.gov (registration number NCT05042778).

2.2. Participants and Setting

All participants agreed to provide written informed consent following the Declaration of Helsinki guidance and were made aware that they could withdraw from the study at any time. According

to the eligibility criteria, the participants were approached by one of the authors (CS), who informed them about the study before a routine lecture 10 weeks before their exam. The students interested in participating in this research provided their email addresses, and the information sheet was sent in September 2021. They were not given any incentives to engage in this trial. One month prior to the examination at the university, all students interested in taking part were accepted for the screening visit. The screening started by collecting their signed informed consent, completing questionnaires, such as demographic data, the trait and state anxiety questionnaire (STAI) and the inventory psychopathological symptom questionnaire (Canavarro, 1999) (Figure 1).



Chapter 5. Figure 1 - Study flow diagram based on Consolidated Standards of Reporting Trials (CONSORT) guidelines.

For inclusion in our study, the students were recruited if the following inclusion and exclusion criteria were met:

Inclusion Criteria:

- Able to sign the informed consent.
- Aged above or equal to 18 years or older.
- Unfamiliar with AA.

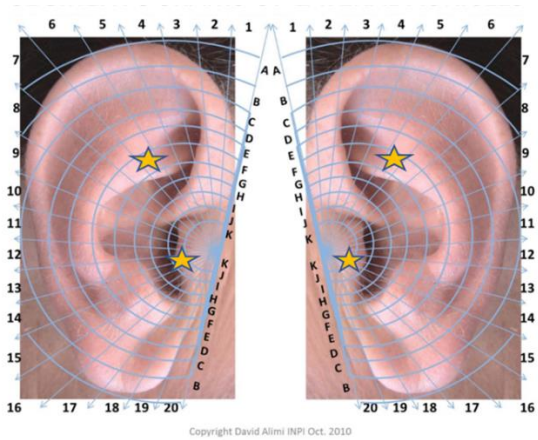
Exclusion criteria:

- Psychological disorders based on Brief Symptom Inventory scale.
- Students with any neurological disease, cardiovascular disease, renal disease, or chronic disease history.
- Known to be pregnant.
- Participants taking psychiatric medication.

The baseline study visit was conducted the day before the exam. It should be noted that before the formation of two groups and to ensure the highest homogeneity, the students screened were previously stratified into four groups according to their trait anxiety level as: first group were students with STAI from 20 to 35 points (6 students); the second group were subjects with STAI from 36 to 50 points (12 students); third group were students with STAI from 51 to 65 points (8 students); and the fourth group were subjects with STAI from 66 to 80 points (0 students). Subsequently, each stratified group was randomly allocated into two groups: (1) WG (participants who did not receive the intervention) and the AA group (participants who received AA 1 day before their exam). Thus, each student extracted at random out of a black bag a slip of paper that had the initials A or W. Each initial corresponded to: A = AA group, WG = no intervention. After the group allocation, the first investigator informed the acupuncturist of the participant's assignment before any intervention.

2.3. Study Intervention

A licensed acupuncturist(holder of civil liability insurance) with more than ten years of experience applied in the auricular area of the AA group, two steel balls (Ø 1.2 mm) covered by a transparent anti-allergic adhesive (Ø 7 mm). The points selected were F6 and J13 (Figure 2) based on the International Auricular Nomenclature guidance (Alimi & Chelly, 2018).



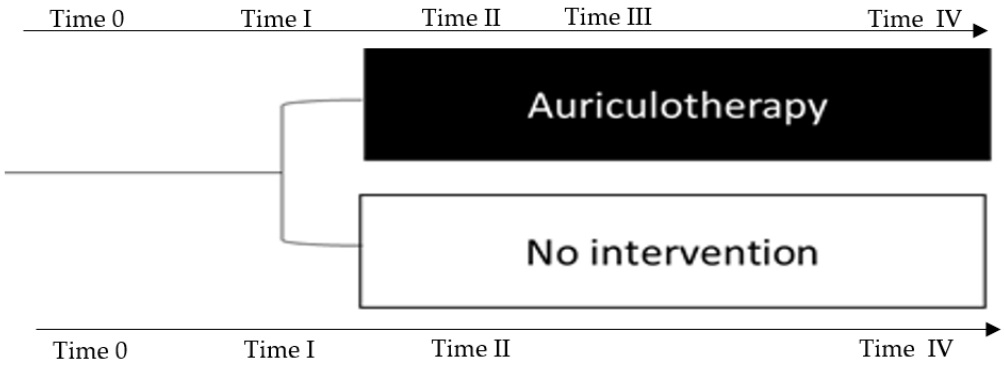
Chapter 5. Figure 26- Segmentogram based on French Cartography University/scientific school of Paris (right and left medial auriculogram) from International Auricular Nomenclature. Legend: stars indicate the points applied to the auriculotherapy group (F6 and J13). The laterality chosen was based on the most symptomatic point (right vs. left ear). This cartography was used with permission (Alimi & Chelly, 2018).

For the intervention group, we chose frequently used areas from most of the included studies cited in our recent systematic review (Andreia Vieira et al., 2022) and from the author's clinical experience. Both auricular points were chosen only from one ear (right or left ear) based on an assessment of the reactive site (e.g., painful or sensible by touching with appropriate material). The procedure took approximately 5 min, and the steel balls were left in the same place until the end of the exam. The students were instructed to manually manipulate the spheres during the day.

All WG participants were given the opportunity to receive the same treatment as the AA group after the exam at the end of this trial.

2.4. Types of Outcome Measures

Outcomes were measured at different times (Figure 3), where the outcome's assessor was unaware of the participants' allocation to the study group as a third party performed it. The screening visit occurred one month before the exam (TP 0) to assess whether participants met the inclusion criteria. The baseline visit started on the same day as the screening day (4 weeks before the exam), collecting STAI (state anxiety form) at TP 0. The TP I was at approximately 7.30 a.m. 1 day before the exam, and the TP II corresponded to before the AA was administered. The TP III was performed 30 min after AA, and the TP IV was completed at approximately 7.30 a.m. before the exam.



Chapter 5. Figure 3. Timeline of the study session. TP 0: Screening and baseline period (one month before exam) outcome: State Anxiety inventory. TP I: morning (7.30 a.m.) 1 day before exam (outcome: sleep quality and salivary cortisol). TP II: Before (11 a.m.) AA (outcome: State Anxiety inventory, visual analogue scale, sleep quality, and salivary cortisol). TP III: 30 min after AA (outcome: State Anxiety inventory, visual analogue scale, and salivary cortisol). TP IV: morning (7.30 a.m.) before examination (outcome: State Anxiety inventory, visual analogue scale, sleep quality, and salivary cortisol).

2.5. Primary Outcome

The anxiety levels were measured using the Portuguese version (Silva & Correia, 2006) of Spielberger's STAI form Y1, ranging from 20 (low anxiety) to 80 (highest level of anxiety). The score varies from 20 to 80 points, where 20 to 35 points mean not anxious, 36 to 50 points is considered a little anxious; 51 to 65 points means moderately anxious; and finally, 66 to 80 points means the participant is considered very anxious. This STAI form Y1 scale assesses the state of anxiety (situational anxiety), while the STAI-Y2 form assesses the trait anxiety (subjects' anxiety personality tendency). The STAI form Y2 was used only in TP 0, while the difference in STAI form Y1 for anxiety was compared during TPs 0, II, III, and IV.

2.6. Secondary Outcomes

The difference in VAS for anxiety was used during TPs II, III, and IV. It consists of a horizontal or vertical line, 100 mm long, which has marked the classification “totally calm and relaxed” at one end and at the other the category “Worst fear imaginable”. The respondent should mark the point representing the degree of intensity of his anxiety. The scale is reliable and correlated with STAI-Y1 ($p < 0.0001$) for the level of anxiety (Facco et al., 2013).

Saliva samples were taken at TPs I, II, III, and IV for the AA group and at TPs I, II, and IV for WG. The salivary collection was performed in a specific device called “salivette”. Both groups collected the samples at approximately 7:30 a.m. fasted before and 30 min after the AA. The saliva was extracted from the salivette cotton by centrifugation, and the cortisol measurement in saliva was performed through immunoassay by electrochemiluminescence, according to the manufacturer’s instructions (range 0.005–3 µg/dl, IBL International Cortisol Saliva ELISA, RE52611, Hamburg, Germany). The saliva samples collection was followed by Amorim, D (2022) research accepted protocol (Amorim et al., 2022).

We considered an adverse event “Any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and did not necessarily have a causal relationship with this treatment”. The acupuncturist documented all adverse events since the AA until Time point IV by using a questionnaire asking about the symptom, incidence, hour, and date of the event and if the subject could describe from zero to ten values the severity of the symptom.

On the morning of the exam (TP IV), the participants were asked to classify the quality of sleep the night before as (1) worse, (2) no change, or (3) better than the quality of sleep during the previous week.

2.7. Statistical Analyses

The pilot sample size was calculated based on the confidence level of 95%, margin of error 5%, population proportion of 50%, and population size of 26. So, the minimum total sample size of 25 participants needed to have a confidence of 95% that the real value was within ±5% of the measured value.

Data analysis was performed using IBM SPSS Statistics Software for Windows (Version 28.0.1.1, IBM Corp., New York, NY, USA). Normality of distribution was evaluated in the groups before the

analyses assessed by the Shapiro–Wilk test. Parametric analyses were carried out in cases where normal distribution assumptions were met, and nonparametric tests were used in cases where normal distribution assumptions were not met. Continuous variables were presented using mean, standard deviation, median, minimum, and maximum values. A comparison of two independent groups was carried out with an independent sample *t*-test/Mann–Whitney U test. The McNemar test was used to analyze sleep quality the night before the anatomy exam. Missing values >5% were inferred with multiple imputation analysis by the regression method. Statistical significance was considered unilateral at the level of $p < 0.05$.

3. Results

A total of 80 students were pre-screened, but only 30 were interested in participating in the research. Table 1 shows the demographic data through each stage of the study. In the end, a total of 26 students (*n* = 26, where 20 were women and six men, the age 20.2 ± 1.78, the weight 67,6 + 18 kg, the height 1.66 + 0.07 m, and the coffee consumption of 1 + 1.2 coffees per day) were recruited and completed the study.

Chapter 5. Table 1. Demographic data.

Variables	Categories	Auriculotherapy	Waiting List	Significance
Alcohol Intake	Yes	0%	0%	
	No	100%	100%	
Coffee	*n*	13	13	0.998
	M (SD)	1.38 (1.26)	1.6 (1.3)	
Smoking Habits	Yes	66.7%	33.3%	
	No)	47.8%	52.2%	
Height(cm)	*n*	13	13	0.458
	M (SD)	1.65 (0.08)	1.67 (0.76)	
Weight(kg)	*n*	11	13	0.892
	M (SD)	68.18 (23.5)	67.15 (12.73)	

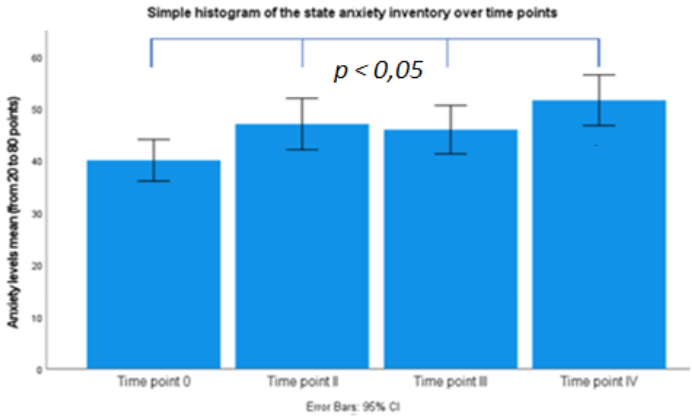
Gender	Female	10	10	
	Male	3	3	
Age	*n*	12	12	0.683
	M (SD)	20.08 (1.72)	20.38 (1.89)	
STAI-Trace	M (SD)	44.8 (7.9)	42.8 (8.4)	0.269
	n	13	13	13

Footnote: n = participant's number; M = mean; SD = Standardized mean.

The mean age difference between each study group did not reach statistical significance. In addition, there were no statistically significant differences in gender, height, weight, alcohol intake, smoking, and coffee habits between the two groups ($p > 0.05$) (see Table 1). Based on trait anxiety assessed using the STAI form Y2, 53.8% of participants were considered not anxious, 26.9% were judged as moderately anxious, and 19.2% showed little trace of anxiety. There were no significant differences between AA and WG regardless of demographic data analyzed by the independent samples *t*-test. All the participants were unfamiliar with acupuncture and with no psychiatric or thyroid disorders. Only one participant was excluded due to psychotropic medication ($n = 1$), and three did not attend. Regarding the reactive points, 88% of the participants reported the J13 point from the right side as more sensitive to touch than the left side of the ear, and 58% reported the F6 point from the left side as more sensitive than the right side.

3.1. Anxiety Levels One Month, 24 h, and Immediately Before Exam

Analyzing the state anxiety levels based on paired-samples *t*-test, assessed with STAI, the participants were more anxious 24 h (Figure 4) before the auriculotherapy ($M = -6.96$, $SD = 10.4$; $t(25) = -3.40$ $p = 0.002$) and immediately before the examination compared to one month previous ($M = -11.54$, $SD = 10.88$; $t(25) = -5.40$ $p = 0.000$).



Chapter 5. Figure 4. Simple Bar chart summaries of separate variables from anxiety level assessed with state anxiety inventory. Time point 0: 4 weeks prior exam; Time point II: before auriculotherapy; Time point III: 30 min after auriculotherapy and Time point IV: morning before examination, CI: confidence interval.

3.2. Anxiety Levels Using State-Trait-Anxiety-Inventory

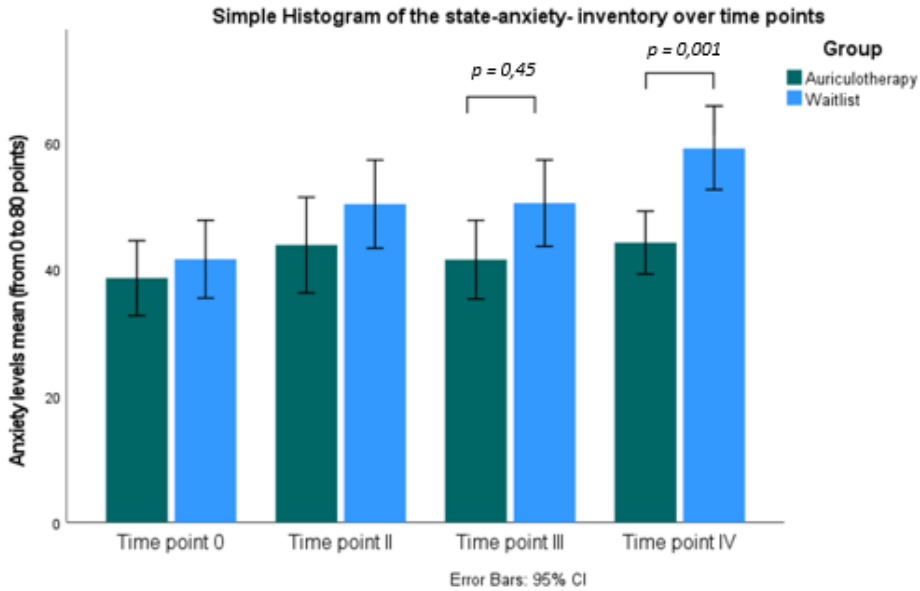
Table 2 and Figure 5 shows the mean and standard deviation of the STAI anxiety score for each group. The state significantly decreased in the AA group compared with the WG after 30 min $t(24) = 2.11$ $p = 0.045$ and in the morning before examination $t(24) = 3.96$ $p < 0.001$.

Chapter 5. Table 2. Outcome of the investigation data by independent samples t-test given as mean ± standard deviations of anxiety levels measured by the Staite Anxiety Inventory Visual Analogue Scale.

Outcome	**Time Points**	**Auriculotherapy Group M(SD)**	**Significance**	**Waiting List Group M(SD)**
n		**13**		**13**
State Anxiety Inventory Visual	I	38.5 (9.7)	0.451	41.5 (10.1)
	II	43.7 (12.4)	0.183	50.23 (11.5)
	III	41.4 (10.2)	0.046	50.3 (11.2)
	IV	44.1 (8.2)	0.001	59.0 (10.7)

Analogue Scale	II	5.5 (1.85)	0.840	5.8 (1.1)
	III	5.3 (1.7)	0.479	5.8 (1.1)
	IV	5.3 (1.6)	0.016	6.9 (1.1)

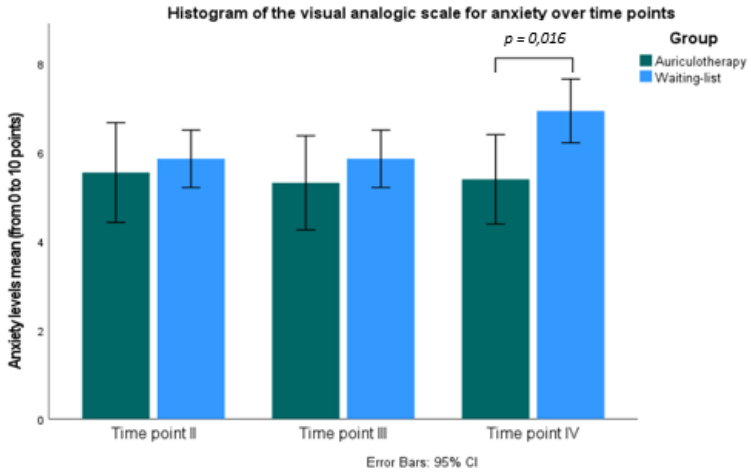
Footnotes: n = participant's number M = Mean; SD = Standard deviations; Time point 0 = 4 weeks before exam; Time point II = before auriculotherapy; Time point III = 30 min after auriculotherapy, and Time point IV = morning before examination.



Chapter 5. Figure 5. Histogram of separate anxiety levels measured using state anxiety inventory for both groups by independent samples t-test Time point 0: 4 weeks prior exam; Time point II: before auriculotherapy; Time point III: 30 min after auriculotherapy and Time point IV: morning before examination, CI: confidence interval.

3.3. Anxiety Levels Based on Visual Analogue Scale

Table 2 and Figure 6 reveal the mean and standard deviation of the VAS anxiety score for each group. The state anxiety levels significantly decreased in the AA group compared with the WG before examination U = 100,500; p = 0.016.



Chapter 5. Figure 6. Histogram of separate anxiety levels measured using visual analogue scale for both groups by independent samples t-test. Time point II: before auriculotherapy; Time point III: 30 min after auriculotherapy and Time point IV: morning before examination, CI: confidence interval.

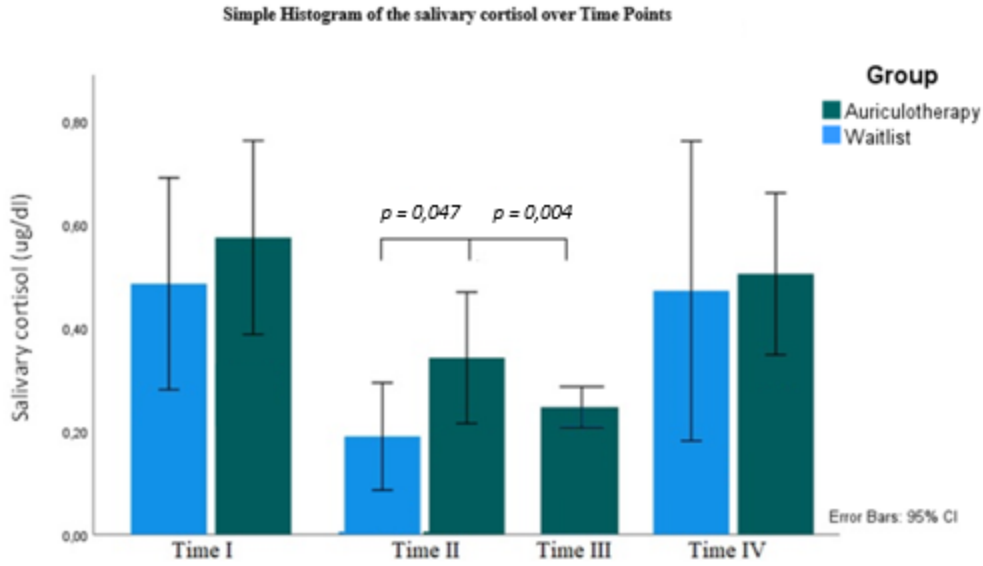
3.4. Salivary Cortisol

Table 3 and Figure 7, demonstrate a decrease in salivary cortisol after AA, although we did not find a significant difference between groups except immediately before AA where the salivary cortisol was significantly higher T(16) = −1.775, p = 0.047 in the AA group compared with WG.

Chapter 5. Table 3. Outcome of the investigation data by independent samples t-test given as mean and standard deviations of anxiety levels measured by salivary cortisol.

Time Points									
Outcome	**Comparation**	**TP I**	***p***	**TPII**	***p***	**TP III**	***p***	**TP IV**	***p***
Salivary cortisol from original data (ug/dl)	Auriculotherapy Group M(SD)	0.51 (0.28) *n* = 12	0.374	0.34 (0.17) *n* = 10	0.047	0.25 (0.06) *n* = 11	N.A	0.49 0.21 *n* = 12	+ 0.431
	Waiting list group M(SD)	0.47 (0.20) *n* = 8		0.21 (0.12) *n* = 8		N.A		0.47 (0.31) *n* = 12	

Footnotes: *n* = participant's number; M = Mean; SD = Standard deviations; Time point I = morning before auriculotherapy; Time point II = before auriculotherapy; Time point III = 30 min after auriculotherapy and Time point IV = morning before examination; *p* = significance level.



Chapter 5.Figure 7. Mean of salivary cortisol by group, collected over four time periods, Time point I: morning before auriculotherapy; Time point II: before auriculotherapy; Time point III: 30 min after auriculotherapy and Time point IV: morning before examination, CI: confidence interval.

Besides that, the salivary cortisol significantly decreased T(8) = 2.291 *p* = 0.026 immediately 30 min after AA in the AA group (Table 4).

Chapter 5. Table 4. Outcome of the investigation data by pared samples t-test given as mean and standard deviations of anxiety levels measured by salivary cortisol.

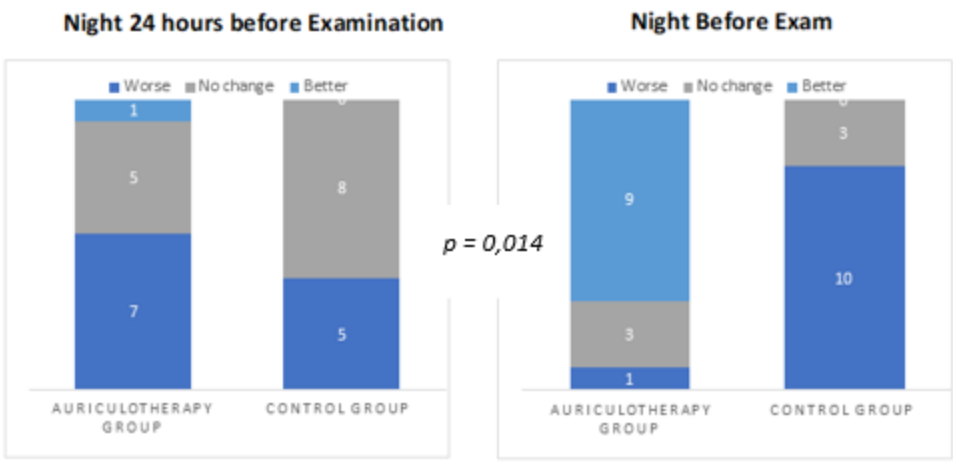
Outcome	Comparation	*n*	M (SD)	*t*	*df*	*p*
Salivary cortisol from original data (ug/dl)	Par 1: TP I—TP IV(auriculotherapy group)	12	0.19 (0.31)	0.215	11	0.417
	Par 1: TP I—TP IV(waiting list group)	7	0.01 (0.18)	0.197	6	0.425
	Par 2: TP II—TP III (auriculotherapy group)	9	0.10 (0.13)	2.291	8	0.026

Footnotes: *n* = participant's number; M = Mean; SD = Standard deviations; Time point I = morning before auriculotherapy; Time point II = before auriculotherapy; Time point III = 30 min after auriculotherapy, and Time point IV = morning before examination; *t* = distribution values for *t*-test; *df* = degrees of freedom *p* = significance level.

To minimize the missing data and bias in the salivary cortisol sample (between 38.5%–46.2% of missing data), we performed multiple imputations by regression method (five imputations). Surprisingly, the Mann–Whitney test for independent samples corroborated the results obtained previously, therefore we decided to use the original data (Tables 3 and 4).

3.5. Sleep Quality

AA group increased sleep quality compared to the WG confirmed by McNemar–Bowker, Test value = 10.667 df = 3, $p = 0.014$. As shown in Figure 8, the AA group increased sleep quality compared to the WG.



Chapter 5. Figure 8. Simple bar chart summaries of separate variables by sleep quality.

3.6. Adverse Events

Of the 13 participants in the AA group, only one adverse event was described by one participant. The student reported discomfort during the night caused by the pressure of the ear on the pillow when lying down on their side. The discomfort was rated as 4/10 through a VAS. This event was considered non-severe as the discomfort disappeared after removing the AA as previously instructed.

4. Discussion

This trial aimed to assess the clinical effect and safety after the application of one AA session provided to university students before their school examinations in order to reduce their anxiety levels.

The results show that the participants became more anxious 24 h before the exam and even more anxious immediately before the exam than one month earlier. According to STAI and VAS outcomes, AA reduced anxiety levels 30 min after the AA (also shown by salivary cortisol) and immediately before the examination, similar to several other studies (Chueh, Chang, & Yeh, 2018; Klausenitz et al., 2016; Prado, Kurebayashi, & Silva, 2012; T. Usichenko et al., 2020; A Vieira et al., 2016). However, during our previous trial where we assessed the effects of AA on anxiety levels in students as well, we did not find strong evidence for anxiety reduction after 30 min, maybe because we performed the AA 2 weeks before the examination period or the intervention selected was not following an individualized assessment (A. Vieira et al., 2018; A Vieira et al., 2016).

Salivary cortisol as a biomarker of anxiety and the hypothalamic–pituitary–adrenocortical axis (HPA) role is a well-established approach in psychological research, dating back at least 20 years (Hellhammer, Wüst, & Kudielka, 2009; Kirschbaum & Hellhammer, 1994; Ryan, Booth, Spathis, Mollart, & Clow, 2016). For this study, we had in mind the stability of measuring salivary cortisol as an outcome once the proportion of salivary cortisol to total cortisol (blood cortisol levels) was approximately 1–2% in the lower range, and 8–9% in the upper range (Hellhammer et al., 2009; Kalinin et al., 2021). Thus, the researcher should treat the salivary cortisol levels with caution since they will not behave linearly to serum levels in response to a challenge or under conditions that affect cortisol binding globulin levels, such as oral contraceptives (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999) and menstrual cycle (Peng et al., 2018). Several other factors could also affect the salivary cortisol response, including gender and smoking (Kirschbaum et al., 1999), age (Salvolini et al., 1999), exercise (Hackney & Anderson, 2016), and awakening time (Kuhlman, Robles, Dickenson, Reynolds, & Repetti, 2019). Although the groups in our study were identical, the sample population was mainly female; consequently, menstrual cycle and oral contraceptive use could have also influenced the cortisol levels. Other researchers have reported the same issue where changes in the cortisol awakening response varied during the treatment period (Huang, Taylor, Howie, & Robinson, 2012). In our study, we tried to minimize those biases by introducing salivary cortisol collection in all groups at the same TP (the participants were all instructed to take their samples first thing in the morning) and measuring it immediately before and after the intervention. Recently, Usichenko and Wenzel (2020) found that salivary cortisol activity was lower before the anatomy exam than in the

evening before the exam after AA compared with the WG (T. Usichenko et al., 2020). Our pilot study also found a significant decrease in the AA group's salivary cortisol levels immediately 30 min after AA.

Interestingly, we also found that the salivary cortisol measured in the morning before the treatment in the AA group showed a fall in the morning before the exam compared with WG. However, the relevance of these results carries no statistical significance due to the sample size. Perhaps a more comprehensive sample would have been crucial to obtaining the statistically significant effects of the present investigation and, therefore, it would be necessary to increase the number of participants to increase the magnitude of our results. Curiously, we also noticed that immediately before the intervention, the salivary cortisol showed a trend toward a steeper rise in the AA group compared with WG. From the author's point of view, this finding might suggest that AA group participants also became slightly more distressed than WG before AA, maybe because the participants saw AA stimulation as a challenge. Research evidence indicates that psychological variables, including other conditions, such as novelty and unpredictability, are associated with increased HPA activity and cortisol release (Kuhlman et al., 2019; Wirz-Ridolfi, 2019). Nevertheless, in many of these studies, a considerable percentage of subjects did not respond (i.e., did not show elevated cortisol levels) after exposure to a stressor, perhaps due to anticipatory effects (Huang et al., 2012). The sensitivity of the HPA to a combination of different situations may lead to less valid cortisol assessments as a specific indicator of anxiety. The interpretation issues presented by non-responders or those who demonstrate inverted responses make the cortisol measurements difficult to interpret (Huang et al., 2012).

To support the results obtained in Chueh et al. (2018) and Usichenko Wenzel's (2020) study, we also found AA improved sleep quality compared with the night before. Surprisingly, we could not find any research about the effects of AA on melatonin levels, so perhaps it would be interesting to investigate melatonin assays in future research.

Regarding adverse events related to AA, Correa et al. (2020) reported that AA with needles could cause bleeding and headaches (Correa et al., 2020) and local pain (Kurebayashi et al., 2017). For Tan Jing et al. (2014), the most often adverse events for AA with seeds are local skin irritation, discomfort, mild tenderness or pain, and dizziness (Tan, Molassiotis, Wang, & Suen, 2014). In our systematic review of AA for anxiety (Andreia Vieira et al., 2022), from 13 included studies, most trials (11 studies) did not assess adverse effects properly (Dellovo, Souza, de Oliveira, Amorim, & Groppo, 2019; Haddad-Rodrigues, Spano Nakano, Stefanello, & Campos Pereira Silveira, 2013; Kuo, Tsai, Chen, & Tzeng, 2016; Kurebayashi et al., 2017; Mafetoni, Rodrigues, Jacob, & Shimo, 2018; Michalek-Sauberer, Gusenleitner, Gleiss, Tepper, & Deusch, 2012; Prado et al., 2012; Rivadeneira, Cifuentes, Hidalgo, Tejeda, & Sánchez, 2015; Valiee, Bassampour, Nasrabadi, Pouresmaeil, & Mehran, 2012; A. Vieira et al., 2018; Wu, Liang, Zhu,

Liu, & Miao, 2011), and two studies did not report any side effects (Ndubisi, Danvers, Gold, Morrow, & Westhoff, 2019; Qu et al., 2014) after AA. In this pilot study, we only found one participant reporting discomfort. However, the symptoms disappeared immediately after the auricular steel balls were removed as previously instructed to the participant. In this research, 88% of the participants reported the J13 point from the right ear side as more sensitive/painful than the left side, and 58% stated the F6 point from the left side as more sensitive/painful than the right side. We decided to personalize the treatment based on patient needs as AA literature and acupuncture schools recommended. The rationale behind this type of AA assessment is that some auricular areas have lower levels of electrical skin resistance than involving tissue (Oleson, 2002), and that electrodermal differences are linked to autonomic control of blood vessels rather than increased sweat gland activity (Oleson, 2002). Therefore, the heightened tenderness of reactive AA points may be clarified by the accumulation of toxic, subdermal substances. So, the activation of specific auricular points could lead to site-specific neural responses in distinct brain regions (Oleson, 2002). Recently, the stimulation of the vagus nerve via the tragus region (J13 point) and triangular fossa (F6 point) region (Peuker & Filler, 2002) in the ear has been demonstrated by functional magnetic resonance studies[26,64,65]. These studies have shown that the intrinsic and extrinsic brain feedback from auricular stimulations is the best way to neuromodulate brain plasticity for therapeutic purposes (Kurebayashi et al., 2017; Mercante, Deriu, & Rangon, 2018). It is noteworthy that both above-described auricular points have been shown to activate the left amygdala, anterior cingulate cortex, and cerebellum (Facco et al., 2013) and can instantly and effectively generate changes in the prefrontal auditory cortex and limbic cortices (Peng et al., 2018).

Interestingly, in 2001, Wang and Kain observed that the anxiolytic effect at the F6 point was not as profound as the “relaxation” point, also localized in the auricular triangular fossa. The previous authors also agree that in general AA practice, there is always more than one auricular point selected for the treatment of anxiety (Wang & Kain, 2001). As we have shown in our results, only 58% of the participants have referred to the F6 point from the left ear as sensitive/ painful with pressure compared with the right side. This means that 48% of the participants have chosen the F6 from the right ear. Because there are still authors insisting on doing AA research with pre-prepared protocols [19] instead of first following the basic AA principles with the treatment based on Chinese Medicine Criteria diagnosis (Huang et al., 2012), perhaps we must instead establish experimental models in AA in anxiety and then select frequent auricular points used that might not be the most appropriate for all the patients. We agree with the study of Huang W., et al. (2012), where possible, pragmatic approaches used in acupuncture trials (in this case, AA trials) could lead to more successful evidence (Huang et al., 2012).

5. Limitations and Conclusions

There are several limitations to this study. Firstly, the samples used in the current study were small, so other tests would need much larger sample sizes to provide reliable results. Our observations exclusively reflected the acute changes in anxiety levels after one AA session. Similar studies about AA have included long-term effects analyses, which we will address in future studies.

Secondly, our study did not assess the WG salivary cortisol 30 min after TP II, as we assumed the salivary levels would not be different. Additionally, we faced more than 30% missing data from WG's salivary cortisol samples. Because the WG was instructed to collect their samples from home and did not need to wait for the AA approach, it is understandable that those participants forgot to collect the samples. Therefore, we strongly recommend a more extensive study to confirm our observations. Nonetheless, we remember future considerations regarding cortisol concentrations that are different throughout the day (Ross, Murphy, Adam, Chen, & Miller, 2014), which also has variability in demographic participants (Kuhlman et al., 2019).

Moreover, we recognize the lack of a placebo group; however, in our previous study (A. Vieira et al., 2018), the placebo group did not produce significant changes in the students anxiety 'levels (either 30 min or 48 h after the auriculotherapy). There is a persistent controversy over the results regarding the placebo evaluation and non-pharmacological therapies research. On the one hand, there seems to be a constant conflict between recognizing the potential therapeutic benefit of the placebo effect and the ethical questions surrounding its use (Finniss, 2018). On the other hand, some researchers are against using a placebo in AA trials (C. S. Zhang, Yang, Zhang, May, & Xue, 2014) as the external ear is a tiny organ with more than 93 recognized active stimulation points (Jan et al., 2017). Following Appleyard et al. (2014), the word sham in acupuncture research has become confused with the term placebo. The authors explain in their work that there have been no actual placebo-controlled trials in acupuncture because the sham-controlled trials compared different acupuncture concepts. From this point of view, as these acupuncture concepts have led to different outcomes (Round et al., 2013), it seems that there is a physiological basis for acupuncture (Appleyard, Lundeberg, & Robinson, 2014; C. S. Zhang et al., 2014). Consequently, the notion of a sham–placebo acupuncture remains an aspect of research that needs more rigorous evaluation (Appleyard et al., 2014).

As we have already mentioned, the participants reported the left F6 point and the right J13 points were more frequently reactive in this trial. So, it would be interesting to assess in future research

if the conjugation of the points would affect the results (e.g., stimulation of right F6 and left J13 is more effective than stimulation of left F6 and right side J13) or if making both points at the same time (e.g., stimulation of bilateral points, F6 and J13) was more effective than using just two points.

Finally, AA applied to the auricular areas innervated mainly by the auricular branch of the vagal nerve seems to show a reduction in situational anxiety triggered by school examinations and improved the quality of sleep in students compared with the WG.

Potentially, AA with seeds can be used as a complementary or alternative treatment for anxiety for the period before school exams as it appears safe and effective, with minimal adverse effects, rare contraindications, and a theoretically reduced cost.

Supplementary Materials: Not applicable.

Author Contributions: Conceptualization, A.V., J.M. and A.M. (António Moreira); methodology, A.V., P.S. and A.M. (António Moreira); validation, A.V., A.M. (António Moreira) and L.L.; formal analysis, A.V., P.S. and A.M. (António Moreira); investigation, A.M. (Alexandra Moura), C.S. and J.M.; resources, C.S. and J.M.; data curation, A.V. and A.M. (António Moreira); writing—original draft preparation, A.V. and N.R.; writing—review and editing, all authors.; visualization, N.R.; supervision, J.M., A.M. (António Moreira) and N.R. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

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