Achyrocline satureioides (LAM) D.C. would improve sleep quality in patients with Obstructive Sleep Apnea Syndrome: a pilot study

Achyrocline satureioides (LAM) D.C. pode melhorar a qualidade do sono em pacientes com apneia obstrutiva do sono: um estudo piloto

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ABSTRACT

Achyrocline satureioides (As) (known as Marcela) is a plant belonging to the family Asteraceae, with a high content of flavonoids, which justifies its antioxidant and anti-inflammatory properties. Previous clinical studies confirm that compounds with these actions have beneficial effects on patients with Obstructive Sleep Apnea Syndrome (OSAS). This double-blind pilot study investigates the influence of chronic oral treatment (3 months duration) with a preparation of As on some sleep parameters. Eight patients selected as do not possess of other pathologies were diagnosed with OSAS by standard polysomnography (PSG) (5 treated with As and 3 with placebo). As extract used containing 3 mg/ml of the flavonoid quercetin, measured by HPLC, and a prepared without As was used as placebo. Another PSG was performed at the end of the study. The results showed a statistically significant increase of REM sleep (Stage R, 125% increase) and an increasing trend in N2 and N3 stages (slow wave sleep) in patients treated with As. Untreated patients showed random results. Epworth Sleepiness Scale decreased significantly, which means better quality of life and waking. There were no changes in rates of apnea/hypopnea or oximetry. Improving the quality of sleep and wakefulness, but not a direct influence on the parameters of OSAS, could be justified by the antioxidant and anti-inflammatory actions described for polyphenols, contents in preparations of As.

Keywords: achyrocline, flavonoids, obstructive sleep apnea.

RESUMO

Satureioides-Achyrocline (AS) (conhecido como Marcela) é uma planta pertencente à família das Asteraceae, com um elevado teor de flavonoídes, que justifica a sua propriedades antioxidantes e anti-inflamatórios. Estudos clínicos previos confirmam que os compostos com estas ações têm efeitos benéficos em pacientes com Síndrome da Apnéia Obstrutiva do Sono (SAOS). Este é um estudo piloto duplo-cego que objetiva investigar a influência do tratamento crónica oral (duração de 3 meses) de uma preparação de AS, com alguns parâmetros do sono. Oito pacientes selecionados que não possuem outras patologias foram diagnosticados com SAOS pelo padrão polissonografia (PSG) (5 tratados com AS e 3 com placebo). AS usado com extração contendo 3 mg/ml de flavonóide quercetina, medida por HPLC, e um preparado sem AS, usado como placebo. Outra PSG foi realizada no final do estudo. Os resultados mostraram um aumento estatisticamente significativo do sono REM (fase R, aumento de 125%) e uma tendência de aumento na N2 e N3 (estágios de sono de ondas lentas) em pacientes tratados com AS. Os pacientes não tratados apresentaram resultados aleatórios. A Escala de Sonolência de Epworth reduziu significativamente, o que significa melhor na qualidade de vida e estado de alerta. Não houve mudanças nas taxas de apnéia/hipopnéia ou oximetria. Melhorona qualidade de sono e vigília, mas não apresentou influência direta sobre os parâmetros da SAOS, pode ser justificada pela ação antioxidante e anti-inflamatórias descritas para os polifenóis, conteúdos em preparações de AS.

Descritores: achyrocline, apneia do sono tipo obstrutiva, flavonoídes.

INTRODUCTION

Obstructive Sleep Apnea Syndrome (OSAS) is a common disorder (affecting about 4% of middle-aged men and 2% of middle-aged women) where patient exhibits repetitive episodes of partial or complete obstruction (apnea) of the upper airway during sleep, producing oxyhemoglobin desaturation, sleep fragmentation, and excessive daytime sleepiness. Increasing evidence suggests that OSAS is associated with hypertension and other cardiovascular diseases, metabolic derangement, and impaired glucose tolerance(1). These episodes of hypoxia/re-oxygenation may induce the generation of oxygen free radicals(2). In fact,
several studies support that OSAS is associated with oxidative stress, and then patients with OSAS have alterations in antioxidant defenses. Simiakakis et al.\(^5\) showed that the obesity, smoking and sex are the most important determinants of oxidative stress in OSAS subjects. Sleep apnea might enhance oxidative stress through a reduction of antioxidant capacity of the blood due to hypoxia related to respiratory events.

Inflammation is one of the postulated links between OSAS and increased cardiovascular morbidity. Indeed, the pro-inflammatory transcription factor NF-kB is upregulated in OSAS. This is mediated by the alterations between hypoxia and reoxygenation, along with sleep deprivation. NF-kB plays a key role in inflammatory responses, regulating the expression of inflammatory genes\(^6\). Recent data demonstrate that OSAS is characterized by inflammatory response\(^8\). In this sense it is possible to assume that compounds with antioxidant and anti-inflammatory capacity may provide potential benefits against OSAS. Polyphenols are natural compounds with variable phe-nolic structures and are rich in vegetables, fruits, grains, bark, roots, tea, and wine. Most polyphenols have anti-oxidant, anti-inflammatory, and antiapoptotic properties and their protective effects on mitochondrial functioning, glutamate uptake, and regulating intracellular calcium levels in ischemic injury in vitro have been demonstrated\(^8\). Fruits and vegetables, foods rich in flavonoids and antioxidants, have been associated with lower risk of stroke, coronary heart disease, and markers of inflammation and oxidative stress in adults\(^7\). Especially flavonoids are a valuable source of natural compounds with antioxidant and anti-inflammatory actions\(^8,9\).

Burckhardt et al.\(^10\) showed to the oral Green Tea Catechin Polyphenols (GTP) administration attenuates the intermittent hypoxia (IH) that characterizes sleep disordered. The IH increases NADPH oxidase activity and oxidative stress in rodents. GTPs may attenuate IH-induced neurobehavioral deficits by reducing IH-induced NADPH oxidase expression, lipid peroxidation, and inflammation. However, the potential therapeutic role of GTP in sleep-disordered breathing deserves further.

The enormous variety of native plants of the South American region has been very poorly studied on their value in the prevention of nervous system diseases\(^11\). In a large Southern America region that covers Argentina, Uruguay, Brazil and Paraguay there are numerous plants with a great arsenal of molecules with antioxidant and anti-inflammatory capacity. In particular Achyrocline satureioides (Ar) (marcela) belongs to the Asteraceae family being a widely distributed South American native medicinal plant whose decoctions or infusions have been traditionally used for gastrointestinal disorders, as an antispasmodic and anti-inflammatory actions\(^11\). The antioxidant capacity and free radical scavenging of Ar has been demonstrated in diverse experimental models and it was reported that Ar protected cells in culture against an oxidative insult\(^13,14\). Ar is a plant widely consumed by South America population, during many years and dozens of toxicity have been reported. However a sub-chronic toxicity study in mice and rats and in two administration routes (oral and intraperitoneal way) with marcela was developed. This study showed that the Maximum Tolerated Dose of 5 g/kg, did not show evidence of toxicity in any of the animals organs, neither liver nor kidney toxicity was evidenced\(^15\).

The beneficial effect of Ar has been ascribed to its content in polyphenols and flavonoids that include: caffeic acid, 2 esters of calleryain (3,4-dihydroxybenzyl alcohol-4-glucoside), galangin, quercetin, quercetin-3-methyl ether\(^14,16\) luteolin, scoparol\(^14,17\) and a new chalcone: acyrobichalcone\(^18\), between other compounds. In special the quercetin a molecule that has been shown to be neuroprotective in several models in vitro\(^9,19\) and in vivo\(^10,20\), and has shown antioxidant\(^21\) and anti-inflammatory\(^22\) capacity. Also Luteolin showed antioxidant\(^23\) and anti-inflammatory\(^24\) effects in experimental models.

Our aim was to explore the putative benefits of oral chronic treatment of Ar preparation in the sleep disorder in patients with OSAS.

**MATERIAL AND METHODS**

**Plant material**

The Ar was obtained from Institute of Agriculture Investigations (INIA, Uruguay), it was identified by Ing. Agr. P. Davies. A voucher specimen of Ar was kept in the College of Agronomy Republic University, Montevideo (MVFA32796).

**Hydro-alcoholic extract of Ar**

The maceration process of the extract was performed using a Soxhlet industrial size, a heater with a capacity of 50 liters of alcohol (ethanol/water to 70%) and a bed for the vegetal product of 50 liters too. This bed is filled with 5kg of dried marcela and 3 extractions were performed on each bed. The vegetal product content in the bed is changed 4 to 5-fold to obtain a quercetin content of 3 mg/ml, measured by HPLC. The yield obtained by extraction was approximately 1kg of extract per kg of marcela (1:1). The placebo was used inert colored liquid, similar to the color of the original extract. Preparations (Ar and placebo) differ in smell, but patients accessing only one of the preparations ignoring the other features. The extract and the placebo were packed in bottles for up to 25ml previously sterilized.

**Quercetin quantification in the extract**

Each lot was tested in the quercetin concentrations according to a standard procedure in a Waters modular HPLC system (Waters Associates, Milford, MA).\(^25\) Separation of constituents was achieved by reverse-phase HPLC using a C18 column (Phenomenex, USA) with 5 mm particle size. A binary HPLC pump (Waters 1525) with a 717 plus autosampler Waters and a photodiode array detector Waters 2998 linked to Empower 2 (Waters) chromatography data software was utilized. The temperature of the column was set at 30 °C. The mobile phase used was: (A) 100% MeOH, (B) 0.5% H3PO4 pH = 2.5% MeOH, at 0.7 ml/min. The gradient system consisted of (min/%B): 0/80, 40/0, 41/80, 47/80. The eluant was monitored by photodiode array detection at 375 nm and spectra of products obtained between 210-600 nm.
Patients extract dosage

The preparation has a Total Polyphenol content of 14.8 EAC (Equivalent of caffeic acid) mg/ml and a quercetin concentration of 3 mg/ml. When patients ingested 25 drops (approximately 2 ml) of the preparation, it will be incorporated EAC 30 mg/ml and 6 mg/ml of quercetin. Twice a day represent 60 EAC mg/ml of total polyphenols and 12 mg/ml of quercetin. The quercetin dose given by us in this study have been suggested in several studies performed in athletes undergoing intense exercise and where a preparation rich in polyphenols have shown decrease oxidative stress caused by the effort (25, 26). Preparation that was used as a placebo had similar color and appearance that the extract studied but did not contain Ar. The placebos experimental subjects were randomized and without knowledge of their status in the study, although this possibility was previously informed.

Experimental protocol

Patients recruitment

Forty one volunteers were interviewed, 23 did not meet the clinical inclusion criteria. Five out of the remaining 18 did not suffer OSAS underwent polysomnography (PSG). Thirteen patients started the study and 5 of them dropped-out (because abandoned or not properly complied with the established protocol), leaving 8 patients fulfilling the 3 months of treatment. This pilot study has been conducted with a double-blind protocol; patients selected a random number corresponding to Ar extract or placebo, were unaware of which group belonged (5 patients were treated with Ar and 3 with placebo). Three out of 5 of the treated group were women (Table 1), all of them were postmenopausic and without hormonal replacement. Two out of the placebo’s group were women, one of them postmenopausic and the other one (26 years old). Only one person of the clinical staff knew the patients’ group, those that performed the PSG, the scoring of sleep stages and statistical analyzes did not know in which group were the patients.

Inclusion criteria were: (1) OSAS diagnosis without previous treatment and non-smoking status; (2) patients should not take any medication or suffer any additional disease (hypertension and obesity were the only ones tolerated). The age range was between 26 and 62 years old.

At the beginning each patient was evaluated with the PSG and the sleepiness at daytime with the Epworth Sleepiness Scale (ESS). The PSG and ESS were repeated at the end of the protocol (3 months later). At the end also were applied a questionnaire (20 questions) that explored the subjective perception of sleep quality from the own patient perception and from the room partner and changes in the subjective perception of the quality of life. Patients were asked if each situation presented had improved, worsened or remained unchanged.

Polysonomographic recording

The PSG was carried out as any regular PSG clinical test. A Polysomnograph (Akonic SA, Argentina) was used recording six electroencephalographic channels, electrocardiogram, electromyograms, eye movements, oxygen saturation and respiration (nasobuccal flow, abdominal and thoracic movements).

Data processing

Each patient was analyzed as its own control. The off-line sleep recording analysis was performed manually by a trained judge. The changes were statistically anaylized by the Mann Whitney’s Test, using a 95% confidence intervals.

The terminology recommended by the Manual for scoring sleep (2007) of the American Academy of Sleep Medicine were used: Stages N1, N2, N3 and R, corresponding to classical Stages 1-2, 3-4 slow wave sleep, and Paradoxical Sleep or REM (Rapid Eye Movements) respectively (27).

All participants signed their consent after being fully informed of the goal and characteristics of the study. This research was approved by the Medical School Ethical Committee for Human Research of the CLAEH Faculty of Medicine, accordance with international guidelines for human research.

RESULTS

Changes in sleep parameters were found in patients treated with Ar, the most evident was the increment in the Stage R or REM (125% of increment in the averaged 5 patients, statistically significant) comparing the first PSG (control) with the last one, after Ar treatment (Figure 1). Stages N2 and N3 also shifted, showing a tendency to increase (Table 1). These increases occur at the expense of a decrement of the awakening periods that occur during the night as well as the decrease in light sleep, stage 1 (N1). The Apnea/Hypopnea Index (AHI) increased in 3 of the 5 patients treated with Ar, decreasing in the remaining 2. Oximetry showed no improvement in the mean values or minimum values, the average values dropped in 3 patients and kept in two, while the minimum values lowered in 2 and rose in 3 following administration of Ar.

The subjective perception of sleep quality also improved not only for the patient but for the room partner (less movement, less snoring, less awakes). These changes were accompanied by the subjective perception of better quality of life (decrease in headaches, more alert, improved memory, and better job performance), and the objective decrement in the ESS (Figure 2 and Table 1). Some patients refer increment in the sleep time, it was not demonstrated statistically. The sleep time average was about 8 hours, since 11:00 pm to 7:00 am.

The placebo group showed random results in polysomnographic parameters. Patients do not report improvement in either the ESS or the subjective perception of sleep. However, no changes either in the oximetry or in Apnea Hipopnea Index appeared; no differences between Ar treated and placebo’s group were found (Table 1).

DISCUSSION

Based on the results showed in this pilot study we can speculate that the improvements in sleep quality could be related to the polyphenolic actions present in the Ar preparation; nevertheless, these changes are not clearly dependent on improvement in OSAS.
Table 1. Patients treated with Achyrocline satureioides (As).

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<tr>
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Statistical analysis

- p < 0.05

Table 1. Patients treated with Placebo (Pl).

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OSAS is a medical disorder strongly associated with multiple co-morbidities. Current evidence suggests that OSAS disturbs fundamental biochemical processes, leading to low-grade systemic inflammation and oxidative stress. Animal models have shown that OSA may lead to apoptosis of central neurons. In clinical studies, oxygen desaturation index and sleep fragmentation have been shown to be independently associated with cognitive dysfunction\(^{29}\).

It is for this reason that the polyphenolic compounds, especially flavonoids for their antioxidant and anti-inflammatory may be a natural alternative to explore against the pathologies associated with inflammation and oxidative imbalance as the OSAS. The use of plant-derived supplements for health benefits, is gaining popularity because most people consider safe and natural products can produce less side effects than synthetic drugs. Research on the health benefits provided by the natural products, is considered a major challenge for modern medicine\(^{29}\).

As such we decided to explore the benefits of As in the OSAS. The effects antioxidant and anti-inflammatory of As has been ascribed to its content in polyphenols and flavonoids as: caffeic acid, galangin, quercetin, quercetin-3-methyl ether\(^{14,16}\) luteolin, scoparol\(^{18,17}\) between other compounds.

Figure 1. Effect of Achyrocline satureioides (As) in five patients suffering Obstructive Sleep Apnea Syndrome is shown (mean and standard deviation). Percentages of Stages N2, N3 en R (or REM) were compared previous (control, C) and after the As treatment (As). The three stages showed increment being statistically significant in Stage R (p < 0.05, Mann-Whitney’s test, 95% confidence intervals).

Figure 2. Epworth Sleepiness Scale before (Control) and after treatment with Achyrocline satureioides (Post As) is shown (mean and standard deviation). Changes are statistically significant (p < 0.05, Mann-Whitney’s test, 95% confidence intervals).
The Polyphenol content (14.8EAC) (Equivalent of caffeic acid) mg/ml and the quercetin concentration (3 mg/ml) del hidro-alcoholic extract of As was quantified with the purpose of the administration dose of the preparation. In this sense it is interesting to note that to produce their beneficial effects, other than on the gastrointestinal (GI) tract itself, the polyphenols must be absorbed into the body after oral ingestion and be carried by the blood stream from the absorption site to target tissues and organs. Biological activity has been demonstrated for many of these polyphenols in numerous in vitro systems(30), but it is apparent that the effective concentrations ([IIM] levels) in vitro are at least an order of magnitude higher than those (\(1\) IM) normally achieved in human plasma(31).

However in relation to plasmabio availability of quercetin during a chronic oral treatment, Egert et al.(32) showed that the daily supplementation to healthy humans with graded concentrations of quercetin for 2 week dose-dependently increased plasma quercetin. This and other evidence of the bioavailability of the polyphenolic compounds could explain that the polyphenolic content in As could be responsible for the observed effects(29).

Although this pilot study was intended to have a first approach to the problem, there are many variables that must be controlled more strictly in future studies: mild, moderate and severe apneas should be considered separately, the Body Mass Index must be considered (in this study the range is from 27-43), men and women should also be considered separately, paying attention primarily to women who submitted yet infradian rhythms led by menstruation (1 of the 5 women included in this study), for all physiological and pathophysiological implications that these characteristics determine.

There is a strong relationship between sleep quality, mainly N3 stages and R, and the quality of wakefulness since these stages have important physiological functions including general cognitive aspects, learning and memory(33)(34). Furthermore, Stage R deprivation is a feature in apneic patients since physiologically it is a period of great homeostatic vulnerability(35).

The daytime sleepiness and decreased REM sleep would be dependent of oxidative stress produced by the OSAS. These changes would not depend directly from the disturbance produced by the cessation of breathing - with its consequent arousal and sleep disruption in general - since the improvement in patients taking As was not accompanied by an improvement in neither the AHI nor the oximetry. As could share the same metabolic mechanisms than the melatonin - cytoprotective and antioxidant - which also improves the quality of sleep(36). However, it will be necessary to perform the study to a larger number of patients, limiting different populations. It is also necessary validate the antioxidant and anti-inflammatory status in plasma of patients treated with the As preparation through the quantification of plasma antioxidant capacity and systemic inflammatory markers such as glutathione levels and interleukin 6 (IL-6) respectively.

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REFERENCES


