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Experimental and Clinical Evaluation of *Plantago australis* Extract as an Anti-Inflammatory Agent to Treat Oral Pathologies

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Abstract

Background: *Plantago australis* is a native plant from Southern Brazil used to reduce inflammation. Interestingly, there are no previous studies evaluating its use to treat oral lesions.
Objectives: The study aimed to investigate in vivo the anti-inflammatory activity of 10% ethanol extract of *P. australis* in recurrent aphthous stomatitis (RAS), erosive lichen planus (ELP) and actinic cheilitis (AC).
Methods: Thirty patients with RAS, ELP and AC were treated topically with 10% *P. australis* solution-based or cream.
Results: In the comparison of in vivo data before and after the treatment and between different lesions, all P values were less than 0.05.
Conclusions: The pharmaceutical formulation of 10% *P. australis* was therapeutically effective in the subjects with inflammatory oral lesions of RAS, ELP and AC.

Keywords: *Plantago* spp., Anti-Inflammatory Agents, Oral Pathology, Clinical Study

1. Background

Plantago australis Lam. is a native plant found in Southern Brazil, other Latin American countries, and the western part of the United States (1). This species is used in folk medicine as antibiotic, anti-inflammatory and cicatrizing agent (1, 2) due to biological activities associated with a great number of active compounds, such as plantamajoside, baicalein, hispidulin, aucubin, ursolic acid and oleanolic acid (3-5). Nevertheless, clinical investigations about the anti-inflammatory potential of *P. australis* to treat oral pathologies were not previously mentioned in the English-literature.

Recurrent aphthous stomatitis (RAS), lichen planus (LP) and actinic cheilitis (AC) belong to different groups of oral pathology; however, they share a significant inflammatory process involved in the pathogenesis of the lesions (6-8).

Furthermore, there are few in vivo studies on the experimental clinical use of phytotherapeutic agents in dentistry (9-13) including the application for oral pathology treatment in public health programs.

2. Objectives

The current study aimed to investigate the in vivo anti-inflammatory activity of pharmaceutical formulation prepared with the ethanol extract of *P. australis* at a concentration of 10% in oral lesions of ELP, RAS, and AC.

3. Methods

3.1. Plant Material

The fresh whole specimens of *P. australis* were collected in September 2008 from Caraá in Southern Brazil. A voucher specimen of *P. australis* was deposited at Federal University of Pelotas Herbarium (Capão do Leão, Brazil) under the code: PEL23.993. The plants were collected, rolled up in papers and packed in cardboard pouches. Then, the plants were cleaned, and the leaves were separated from other aerial vegetable parts. Next, the leaves were dried in a stove with air circulation at 40°C for three days.

3.2. Plantago australis Extract

The leaves were harvested during the phase of flowering, washed and dried at 40°C for 72 hours. Then they were ground into powder using a pestle and mortar. Afterward, the powdered leaves were submitted to extraction with 70% v/v cereal alcohol (hydro-alcoholic extract). Initially, 200 g of this dry vegetable biomass of *P. australis* was added to 850 mL of ethanol (85% of the volume of 1000 mL of ethanol) and periodically agitated during 48 hours. The ethanol and *P. australis* mixture was stored in a sterile amber glass vial protected from light exposure and kept at room temperature. After 48 hours, the mixture was filtrated through a paper filter, and the liquid was stored in another sterile amber glass vial. The vegetable residue was then re-extracted with 150 mL of ethanol corresponding to 15% of the volume of 1000 mL of ethanol for another 48 hours. After the filtration, the *P. australis* crude extract was stored in a sterile amber glass vial in a refrigerator (4°C) until it was used. The protocol to obtain tinctures of medicinal plants was adapted from Brazilian and British pharmacopoeia guidelines (14, 15). A ratio of 10% v/v was used to dilute crude extract and obtain the final extract (19, 20) to be used in the experimental treatment of patients with intraoral lesions of RAS and ELP. *Plantago australis* cream 10% was prepared from oil/water emulsion base to treat patients with labial lesions of AC. The components of the emulsion cream base were stearic acid, acetyl alcohol, Vaseline, mineral oil, glycerol monostearate, glycerin, propylene glycol, triethanolamine, methylparaben, propylparaben and deionized water (16, 17). The *P. australis*/oil ratio in the cream was ~ 1/10. Both formulations (solution and cream) were tested in the topical form in the oral pathologies.

3.3. Subjects

This clinical study followed the declaration of Helsinki (revised in Tokyo) for human researches and was approved by the ethics committee of Pelotas dental school (protocol number 006/2008). All patients received information concerning the study and signed an informed consent. In the current study, 30 patients with diagnosis of RAS, ELP, and AC (aged 21 to 78 years; 17 females and 13 males) were selected among the patients assisted by the center of diagnoses of oral diseases, Pelotas dental school, Rio Grande do Sul, Brazil, from October 2008 to March 2010. Regarding the inclusion criteria in the sample, the patients should demonstrate such pathologies according to the following criteria: patients with RAS should present ulcerative lesions rounded of any size, single or multiple, found in any area of the oral mucosa and associated with painful symptoms and history of recurrent episode of oral ulcers during the preceding years. The diagnosis of RAS was based on the clinical features. Patients with ELP should show erosive lesions surrounded with white striae associated with variable degrees of pain symptoms and history of migrant lesions. Any area of the oral mucosa can be affected, but the presence of lesions in the bilateral cheek mucosa was considered a characteristic that significantly increases the correct diagnosis of ELP. Moreover, the conventional clinical presentation on the association of the microscopic aspects was the requirement for diagnosis. Patients with AC need to present focal or widespread ulceration in the lower lip that could be associated with rough and scaly areas, loss of the sharp border of the lip, atrophy of the vermilion border, and darkening at the border between the lip and the face skin. The painful symptoms could be either present or not. The diagnosis of AC was based on the clinical aspects, and the incisional biopsy was performed in the cases that there was a suggestive lesion to invasive carcinoma. All patients were assisted by Dr. A. Etges, an oral medicine and oral pathologist.

3.4. Experimental Treatment Protocol

Patients with each lesion group (10 RAS, 10 ELP, and 10 AC) were treated with the extract or cream for a minimum period of seven days and maximum of 30 days. All patients were instructed to suspend the treatment and contact the routine oral pathologist if developing excessive burning sensation and erythema. After the initial diagnosis, 2 mL of 10% solution-based *P. australis* was prescribed to be administered to patients as a topical mouthwash for two minutes, three to four times a day in the site(s) of occurrence of intraoral lesions in the subjects with RAS and ELP. The 10% *P. australis* cream was also prescribed for topical application in the lower lip, three to four times a day covering the ulcerated areas of AC lesions with a thin layer and no need to remove the cream after application. The patients received a weekly clinical monitoring to record clinical responses to experimental treatment with *P. australis*. The lenght of treatment depended on the type, the severity of the lesions, painful symptoms, and the individual response to the treatment. Compliance was monitored by asking the patients to use the drug correctly. Before the start of the therapy, all data, together with the treatment protocol, were recorded in the clinical charts, and the patients were examined by the same oral pathologist at each visit. After every 7^{th} day of examination, the treatment was stopped for patients who had shown complete improvement of the lesions, but continued the next 15 days for the patients with lesions and painful symptoms. In the subjects with RAS, the treatment success was considered when the pain complaint had ended and the ulcerative lesions showed complete healing with normal oral mucosa in the area affected previously. In the subjects with ELP,

the erosive component should disappear and the white striae could maintain being that with this clinical feature the lesions should be asymptomatic. The ulcerated area should present complete healing; however, the loss of the sharp border of the lip, atrophy of the vermilion border and darkening of the lip were irreversible aspects of AC lesions. The parameters used in the current study were based on the clinical aspects conventionally employed to routine monitoring of patients with RAS, ELP and AC under traditional anti-inflammatory therapy by specialists, and successful treatment was considered by a professional when these cited aspects were achieved. Then, the study used an open monitoring based on the clinical observation by an oral pathologist aspect such as size and complete healing of the RAS and AC lesions and resolution of the surface area of the erosions of ELP lesions. Moreover, the individual patient report about painful symptomatology disappearance associated to clinical resolution of the lesions was added to specialist's analyses.

3.5. Statistical Analysis

The McNemar test was employed to identify differences intra-group of the patients before and after the treatment with the *P. australis* extract and cream to 10%. A value of $P < 0.05$ was considered to be statistically significant.

4. Results

4.1. Demographic Aspects

The study sample consisted of 17 females (56.7%) and 13 males (43.3%). A significant difference detected was the predominance of males in the AC group (80%), compared with the RAS and ELP groups where females predominated, 80% and 70% of the subjects, respectively. The mean age was 47 years in the RAS and ELP groups and 58 years in the AC group (range, 21 - 78 years).

4.2. Clinical Diagnosis

A total of 30 patients (10 patients of each oral pathology group) were treated with 10% *P. australis* extract. Lower labial mucosa and the mouth floor were the most common sites for RAS affecting 80% of the subjects. Cheek mucosa (unilateral or bilateral) was the most common site for ELP affecting 80% of the patients, and lower lip vermilion was the unique site for AC affecting 100% of the patients. Regarding the clinical presentation, of the 10 patients with RAS, 80% showed minor RAS (0.3 - 1 cm) and 20% major RAS (1 - 3 cm). The clinical presentation of the 10 cases of ELP was erythema and whitish striae in 80% of subjects, and ulceration, erythema, and whitish striae in 20% of the subjects. In cases of AC, of 10 patients, 70% showed the acute form of the disease, and 30% presented the chronic form. Regarding the painful symptoms, 77% of patients had pain complaint: 100% with RAS and ELP and 30% with AC.

4.3. Clinical Evaluation

After the treatment period with 10% *P. australis* solution or cream, at least seven days and at most 30 days, the total number of patients did not reduce, demonstrating a patient withdrawal of 0% from the study. Therapeutic effectiveness was determined once the treatment was concluded: 73.3% of subjects; 90% with RAS, 70% with ELP, and 60% with AC presented no clinical manifestation and pain, and the success of the treatment was considered. $P = 0.023$ and $P < 0.001$, respectively, were found in the intragroup analysis before and after the treatment when the degree of severity of the lesions was analyzed (Table 1), whereas $P = 0.008$, $P = 0.023$ and $P = 0.041$, respectively, were found in the intragroup analysis of the effectiveness of the treatment to RAS, ELP and AC (Table 2). Regarding disappearance of painful symptoms among the subjects, 82.6% of the 23 patients with pain treated with 10% *P. australis* extract showed no pain after treatment. Analysis results considered the therapeutic effectiveness when the patient exhibited complete healing of the ulcerative lesions for the RAS and AC. In cases of ELP, the disappearance of the erosive aspect and healing of the ulcerated component showed the success of the treatment. In addition, the disappearance of the painful symptoms was considered an essential factor in the therapeutic effectiveness. Regarding treatment period, it was 7, 21 and 15 days for RAS, ELP and AC lesions, respectively. There was no significant variation of treatment period in different clinical presentations in the three pathologies (RAS, ELP and AC).

After treatment administration, 26.7% of the 30 subjects had no improvement regarding the clinical aspects and 21.7% of the 23 subjects showed no improvement in pain. Both treatment vehicles (liquid and cream) were tolerated satisfactorily because none of the subjects in the RAS, ELP and AC groups manifested episodes of any adverse effects during the study period.

5. Discussion

It was the first study to show the potential anti-inflammatory properties of *P. australis*, a native species in South Brazil, along with the possibility of its inclusion in the list of plants currently used at healthy public service. Furthermore, this is the pioneering study demonstrating the potential efficacy of the topical use of *P. australis* extract to reduce signs and symptoms of inflammatory oral pathology lesions of RAS, ELP and AC (6, 9).

Table 1. Clinical Manifestations in Subjects With RAS, ELP and AC Before and After Treatment by *Plantago australis* Extract

Treatment 10% *P. australis*	Absolut Frequency		
	No Lesion and Pain	Lesion and Without Pain	Lesion and Pain
Before			
RAS (n = 10)	0	0	10
ELP (n = 10)	0	0	10
AC (n = 10)	0	7	3
Total (n = 30)	0	7	23
After			
RAS (n = 10)	9	0	1
ELP (n = 10)	7	0	3
AC (n = 10)	6	3	1
Total (n = 30)	22	3	5
The McNemar test	-	P = 0.023	P < 0.001

Abbreviations: RAS, recurrent aphthous stomatitis; ELP, erosive lichen planus; AC, actinic cheilitis.

Table 2. The Efficiency of Treatment by *Plantago australis* Extract in the Subjects With RAS, ELP and AC

Treatment by 10% *P. australis*	RAS	ELP	AC
Before	10	10	10
After			
No improvement	1	3	4
Total improvement	9	7	6
The McNemar test	P = 0.008	P = 0.023	P = 0.041

Abbreviations: RAS, recurrent aphthous stomatitis; ELP, erosive lichen planus; AC, actinic cheilitis.

The developed phytopharmaceutical (using *P. australis* species) was elaborated with the crude extract of the aerial parts of the plant in the experimental concentration of the active compounds such as polysaccharides, lipids, iridoid glycosides, flavonoids and terpenoids (1, 2). These compounds are active in reducing some chemical mediators of inflammation, such as leukotrienes and prostaglandins, and are frequently found in several species of *Plantago genus* recognized in traditional medicine (3, 5, 18). The 10% phytopharmaceutical elaborated on these crude extracts constituted an innovative medical alternative to treat oral pathologies of RAS, ELP and AC and had promising effectiveness and tolerability in the subjects groups. The 10% extract concentration was selected according to the recent articles and monographs in the British and Brazilian homeopathic pharmacopoeias and developed by expert pharmacists to prepare plant extracts derived from crude extracts (14-16). The current study also considered the previous preparation of 10% extract concentration from other species of *Plantago* (*P. major*) (14, 15, 18). Moreover, a *Calendula officinalis* ointment was also prepared in the 10% concentration according to American homeopathic pharmacopeia to treat exfoliative cheilitis, a reactive process chronically inflamed that affects the lips, and the treatment with this experimental concentration also showed success (16). The current study on *P. australis* was similar to the study on *Calendula officinalis* (16), and both formulations at 10% concentration showed positive results to heal lesions belonging to two distinct groups of oral pathologies. Then, 10% concentration of experimental phytomedicines can be a good concentration to treat oral lesions because it was recommended previously by different homeopathic pharmacopeias.

No side effects were exhibited by patients throughout the study period (at least seven days and at most one month), indicating 100% tolerability for both vehicles of treatments. It is considered a therapeutic success when the patient achieved therapeutic effectiveness with (a) the

disappearance of the painful symptoms, and (b) healing of lesions for RAS and AC or disappearance of the erosive aspect and/or healing of the ulcerated component for ELP cases. The current study was carried out as an open clinical assay, and some aspects were considered based on the study design, such as several ethnobotanical studies, including Brazilian studies that demonstrate the medicinal use of *Plantago* species based on active compounds (1-5).

5.1. Conclusions

The 10% extract of *P. australis* showed a potential therapeutic effect on the clinical resolution and the disappearance of painful symptoms. Moreover, both vehicles used (cream and solution) exhibited 100% tolerability.

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