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Quercus Ilex Mouthwash Effect on Ph, Flow and Salivary Proteins of Geriatric Patients

KEYWORDS: Quercus ilex, salivary proteins, pH and salivary flow

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ABSTRACT *Tooth loss, denture defective mandibular mucosa atrophy, decreased salivary flow and alteration of its composition make the digestive process compromised, leading to the geriatric patient´s malnutrition1,2,3. The decrease in salivary flow promotes the growth of microorganisms and thus cavities. The Quercus ilex aqueous extract was used as a mouthwash to determine the effect on pH, flow, and total non-stimulated salivary protein in 90 geriatric patients in general good health conditions, both sexes, aged 60-75 years.*

Rinsing salivary flow increased by 125% (p <0.001), measured by gravimetric analysis. The pH in all samples was 7. Salivary total protein increased 40% (p> 0.001).

Introduction

Flavonoids are polyphenolic and phenolic compounds. They have antioxidant effects; they also prevent cell aging and degenerative processes. Its chemical structure is varied; at high doses its action is pro-oxidant [1-2]. For the study, we used the bark of Quercus Ilex (QI), perennial shrub of the Fagaceae family in aqueous extract.

Another effect is the wide pharmacological activity by binding to hormones protein carriers and DNA, chelate transient metal ions as Fe_2+, Cu_2+, Zn_2, to catalyze the transport of electrons and debug radicals accessing protective effects in diabetes mellitus, ulcers, inflammation, antithrombotic and anti-inflammatory properties. [4,5]

Furthermore QI aqueous extract has a high tannin content (15%-17%) used in wound healing by creating a dry environment, preventing bacterial growth [3,4,5] factor that is intended to prevent the geriatric patients´ (GP) oral cavity.

Nutritional and gastrointestinal processes are often altered In GP, as pharmacologically compromised patients. The lack of awareness of the population on prevention and oral hygiene promotes microbial growth, unbalanced eating can lead to malnutrition which is accompanied by muscle weakness and immune disorders which generates less resistance to infections and oral disease increases from GP.[6]

Since 1948, the United Nations raised the need to protect the rights of older adults as a guarantee of their well-being, the World Aging Assembly said that need of the GP whether is sick or healthy, avoid loss of dental organs, prevent or avoid hypo salivation and xerostomia because they limit the chewing function, promotes microbial growth plaque and dietary factors also intervene.[7,8]

Geriatric Stomatology confronts the complications related to dental treatment as changes in the GP´s[6] oral health of common own age chronic conditions, complicated by systemic diseases such as diabetes, hypertension, rheumatoid arthritis, Sjögren syndrome. On the contrary, having good oral health allows digestive functions, social interaction and communication not to be affected.

Therefore, the oral cavity deserves special attention for making up public health problems when they present degenerative alterations, tumor pathology, infectious diseases, malocclusion, temporomandibular joint arthrosis, caries and periodontal disease. It also shows deficiencies in the amount of saliva by age, sedentary lifestyle, minimal water consumption, and side effects from medications (analgesics, antidepressants, antihistamines, antihypertensives, anticholinergics) impacting speech, chewing, swallowing and patients with dental prostheses. The GP has deteriorating physical and mental disability, and disability associated with the management of personal attention, creating a relationship between skill and oral hygiene. The taste of food decreases, presenting hypo salivation, xerostomia, superior laryngeal dryness, little taste for food, loss of weight and muscle mass impacting when prosthesis is used and encouraging vulnerability to malnutrition.[9,11,12]

Saliva is a hypotonic complex fluid produced by the major and minor salivary glands, crevicular fluid, normal microflora bacteria and food debris, made up of 99% water and 1% organic and inorganic materials. It has antimicrobial, antiviral, lubrication, buffering capacity, remineralization functions and demineralization protection against tooth organs, clearance and taste.[12,13,14] Its composition is related to the amount of flow and influenced by factors such as lifestyle, health/disease process, dentures use risk, plaque playback, caries, periodontal diseases. [6,9,14]

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The salivary pH ranges from 6.6 to 7.4, when presented acidity and stays a while, the GP is prone to decay, demineralization of tooth neck, gum recession, enamel white spots, so it is necessary to reflect on the role of saliva in maintaining homeostasis of the oral cavity.

Changes in pH are associated with salivary flow rates, so further increases in pH is for those individuals with high flow rates.[15]

There are several issues related to the functioning of the salivary glands, the normal non-stimulated salivary flow in GP is 0.3 to 0.4 ml/min, it is considered abnormal when values are less than 0.15 ml/min.[16,17]

Currently, more than 1,400 salivary proteins have been identified. Regarding salivary protein composition, 95% are proline-rich proteins, which adhere to the tooth enamel, promoting bacterial colonization on the surface of the tooth during the formation of dental plaque. Besides, proline, statherin and histatins influence the chemistry of calcium phosphate, and yeast infection. [14] Consequently the three proteins promote recalcification of dental organ by inhibiting the decalcification initiated by cariogenic bacteria. The salivary immunoglobulin can join salivary pellicle and join the dental biofilm, neutralizing bacterial virulence factors; mucins provide viscosity to saliva and helps the formation of a permeable barrier that protects the enamel, lysozyme have enzymatic activity, antibacterial activity and autolysis. Fibronectins are involved in tissue remodeling during embryogenesis and in the healing process of vascular injury after the formation of a fibrin clot, among others are agglutinins, cystatins, lactoferrin, histatins and statherins [10,13,18,19] whose protective role depend on the structure, they can also colonize some bacteria involved in mineralization of the oral tissues.

Outcome
To determine the effect of the QI aqueous extract mouthwashes on pH, total protein and salivary flow non-stimulated (TPSFns) in GP.

Methodology
This research is a clinical, observational, comparative, and longitudinal study.

Selection of patients: 90 GP in good general health (45 of each sex), who attended a dental clinic of the Faculty of Dentistry of the Autonomous University of Puebla (Facultad de Estomatología de la Benemérita Universidad Autónoma de Puebla) during 2011-2012. Grouped into three aged categories 60-65, 66-70, 71-75, participation was voluntary, following the guidelines of the Declaration of Helsinki and Health Act;[20] patients to retain 80% or more of the dental organs were evaluated.

Exclusion: patients with caries (higher rate of 80%), chronic periodontal disease (retaking the periodontal Russell Index),[21] under medical treatment or any chronic disease(s), type of food interfering with salivary flow, pH and protein concentration.

Clinical history was performed, panoramic radiograph, periodontal diagnosis, oral stomatological examination evaluating:

a. Presence of plaque,
b. Periodontal disease (including GP with clinically healthy gingiva, normal in relation to: color, appearance and consistency),
c. Presence of cavities.

Saliva Collection:
Saliva samples on the same features, following the rules of ALAIS were collected.

Patients rinsed their mouth with purified water, after five minutes the non-stimulated STH (STHne) was collected by the runoff method. For 5 minutes, it was placed in sterile disposable polypropylene tubes of 15 ml, previously weighed on a brand Radwag analytical scale Mod: As220/c2.

The flow was determined by gravimetric analysis to be expressed in ml/min. It was calculated by dividing the difference in weight between the time of collection.[19] After collecting the first sample, patients began treatment with QI aqueous extract (rinse to 40%) for 12 months.

Indications: Dissolve 20 drops in half a glass of water after brushing technique, to rinse three times a day. It is recommended not to use other rinses, do not use toothpaste containing triclosan, do not rinse with water after use of the extract. Again, it was performed the same technique to collect saliva in the 3, 6 and 12 upcoming months.

pH Measurement
After the salivary flow gravimetric analysis in order to clarify the saliva samples which were centrifuged. For the pH measurement, a Jenway 3540 potentiometer table was used. Temperature was verified from 25 to 27 degrees to avoid disturbing samples and results.

Protein Quantitation
The Coomassie blue colorimetric method, described by Bradford was used.[22] Following the manufacturer's instructions (PIERCE Chemco., Rockford III. U.S.) SIGMA bovine serum albumin was used as standard, supported by a Jasco-Ubest 30UV/LV spectrophotometer connected to the computer and printer having the results programmed which each saliva sample was read with an absorbance at 595 nm. With micropipettes, fifty microliters of each STHne sample were taken and suspended separately in 2.45 ml 0.80% NaCl. Coomassie solution was added (0.5 ml), stirred for 15 seconds and allowed to stand ready for two minutes at room temperature. The procedures in the handling of the samples were made using the criteria of the International Research Group in Saliva and the International Association for Dental Research (IADR).

Protein Analysis
After quantifying proteins, they were analyzed by polyacrylamide gel electrophoresis with 10% sodium dodecyl sulfate (SDS-PAGE) and Coomassie blue staining according to the method of Laemmli[23] et al., 1970.

Using this technique, proteins are denatured at 100°C in the presence of SDS, which is linked to the polypeptides making them in polyanions. Proteins to acquire the same charge density in the presence of an electric field on a porous gel caused an exclusive migration according to their molecular weight.

Statistical Analysis
An Excel database was created to carry out a statistical and figures analysis. According to the objectives and the comparative study, we calculated mean, standard deviation, standard error, variance, variation coefficient, maximum, minimum and range.

Protein concentration values were calculated by linear regression analysis of the derived standard curve at different concentrations and absorbance.

Results
The concentration of flavonoids (cited in Martinez, Gonzalez and Snakes, 2002)[24] of the aqueous extract of QI used was at 1.086 ± 1 infusion.

The obtained results during the 12-month time period in the FSTHne of the GP in the three categories of study were: for the category of 60-65 y/o patients with rinse (c/e) shows an increase of 0.52 ml/min, compared to patients in the same

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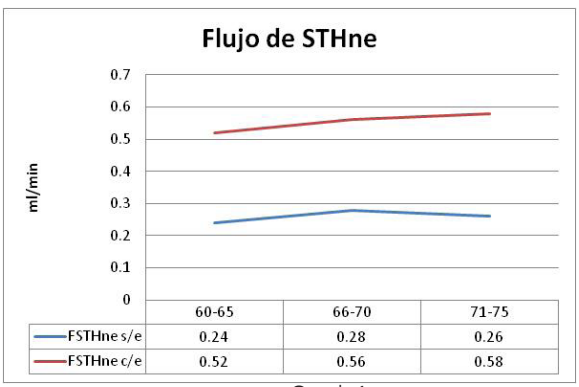
age range who had not received rinsing, who had an average flow of 0.24, making this difference statistically significant p <0.001.

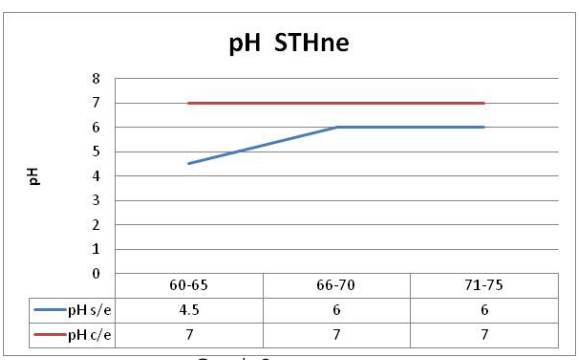
When comparing the results in the category of 66-70 y/o, c/e patients had a flow of 0.56 ml/min, and s/e of 0.28 ml/min, being equally significant p <0.001.

For the category of 71-75 y/o patients flows were 0.58 c/e and 0.26 ml/min respectively, being significant (p> 0.001). (Graph 1)



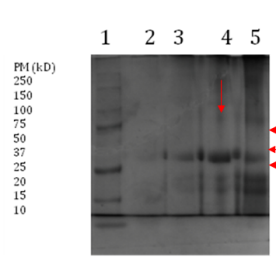
Graph 1

For the 12-month pH, the average of the samples was from 6.0 to 6.5 ±7 (Graph 2).



Graph 2

Figure 1. Polyacrylamide gel electrophoresis. Each lane contains 25 mg of protein.



Lane 1. Plus Protein Precision™ Dual Color Standards Biorad.

Lane 2. Total salivary protein before using the mouthwash.

Lane 3. Three months after using rinse.

Lane 4. Six months after using rinse.

Figure 1

Lane 5. Twelve months after using rinse, increased protein is observed between 20-37 kD, it is currently working with the identification of proteins with antibodies increased by Western blot hybridizations and will be determined if it is one or more proteins.

Discussion

One outcome of this work was to determine the FSTHne of 60-75-years-old GP. Considering the results obtained in this study, the FSTHne average in the study population is similar to the one obtained by the Morales LR. et al[25] and Ben-Aryeh[26] et al in older adults, with results are 0.31 ± 0.17.

Drawing on studies carried out by Sreebny LM[17], regarding the values of abnormal salivary flow (0.15 ml/min), in the present study, for the lowest FSTHne samples was 0.24 ml/m. Considering the effect of QI, an increase was obtained in the flow, allowing GP eat a proper diet and improve their digestion.

As a predisposing factor for the reduced inflow of FSTHne, it is observed in HC: Nutrient factors, high intake of bread is notorious, processed cereals, carbohydrates, low intake of vegetables and water. Besides, the older the patient is there is a considerable measure of decreased food consumption, more chewing and swallowing problems, coinciding with Susan Holmes (1999) and Michael WJ Dodds (2005).

Michael WJ Dodds (2005) mentions that there is an age-related decrease in non-stimulated saliva production, which we agree, so the proposal is to increase salivary production for further chewing, modify diet, water intake and the use of QI mouthwash to increase the GP´s FSTHne and PSTHne of what successfully modifies their prosthesis mismatch.

Christensen, M. C., Brand, G. J., Malamud, D., (1987) mention that for individuals with high flow rates, pH also increases, as we showed in our results.

Considering that in Mexico there is a high percentage of dental caries and periodontal disease in the studied patients, and it is related to the salivary quantification and the PTS concentration, we want to spread the use of the aqueous extract to improve the GP´s quality life.

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