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Long-term efficacy of Magnolia Bark Extract and Xylitol administered through chewing gums on caries in adults: A 2-year randomized controlled intervention trial

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ABSTRACT

Caries preventive effect of a sugar-free chewing-gum containing Magnolia Bark Extracts and Xylitol in high-risk adults was investigated (NCT02310308). Two-hundred seventy-one high-caries-risk subjects were assigned to three groups: Polyols (Pols), Xylitol (Xyl) and Xylitol plus Magnolia (Xyl + Mag). Caries lesions, gingival bleeding, mutans streptococci (MS), and plaque pH were re-evaluated after 2 years in 64 Pols, 66 Xyl and 64 Xyl + Mag subjects. Net caries increment was evaluated using the Mann–Whitney *U* test. Caries increment was significantly different among groups at the 2-year evaluation (t_3) ($p < 0.01$). MS concentration and plaque pH as mean Areas Under the Curves (AUC) differed among groups at t_2 (MS $p = 0.02$, $AUC_{5.7}$ $p = 0.03$ and $AUC_{6.2}$ $p = 0.04$,) and t_3 ($p = 0.03$, $p = 0.04$ and 0.05, respectively). Significantly lower gingival scores were observed in Xyl + Mag and Xyl groups ($p = 0.01$ and < 0.01, respectively). Chewing-gum containing Xylitol and Magnolia has a higher preventive effect compared to Xylitol alone.

1. Introduction

Dental caries continues to be one of the most prevalent human diseases, despite the availability of various preventive strategies (Bowen & Koo, 2011). Since it is difficult to control human behaviour, many caries-preventive measures have been proposed among which the use of fluoride toothpastes twice daily is the main and appreciated measure. However, the actual skewed caries figure suggests the need of developing new and effective preventive approaches, especially for high-risk groups (Bagramian, Garcia-Godoy, & Volpe, 2009; Marcenes et al., 2013). The use of sugar-free chewing gums may contribute to prevent dental caries (Deshpande & Jadad, 2008; nostro lavoro xylitolo). The increase of stimulated saliva flow rate promotes oral clearance and increases the buffering capacity, contributing to neutralise the plaque-pH (Dodds, 2012). It is widely known that the consumption of Xylitol contributes to reduce plaque amount, the numbers of mutans streptococci (MS) and the caries increment (Campus et al., 2013; Soderling, 2009).

Several phytochemicals have shown potential pharmacological or antimicrobial activity including oral bacteria (Abreu, McBain, & Simões, 2012; Ciric et al., 2011; Komarova et al., 2017). Magnolia Bark Extract (MBE) is a plant extract obtained from the bark of *Magnolia officinalis (Magnoliaceae)*, widely used in traditional Chinese medicine that is isolated by CO_2 super critical fluid extraction. Concentrated Magnolia Bark Extract is considered a novel food in Europe and it is listed in the EU Regulation 2017/2470. MBE was *in vitro* recently tested against multi-species oral biofilms (Fernández, Aspiras, Dodds, González-Cabezas, & Rickard, 2018; Komarova et al., 2017), showing a significantly reduction of biomass, thickness and viability of oral biofilms.

A variety of pharmacological properties are ascribed to the two main constituents (97%), magnolol [4-Allyl-2-(5-allyl-2-hydroxyphenyl)phe nol] and its isomer honokiol [2-(4-hydroxy-3-prop-2-enylphenyl)- 4-prop-2-enyl-phenol] (Lo, Teng, Chen, Chen, & Hong, 1994; Wang, Ho, Chang, & Chen, 1995), including the limitation of the growth of several oral pathogens (Campus et al., 2011; Chang, Lee, Ku, Bae, & Chung, 1998; Greenberg, Urnezis, & Tian, 2007; Ho, Tsai, Chen, Huang, & Lin, 2001; Li & Xu, 2004). Unfortunately, the mechanism of action of MBE against specific oral bacteria is still unknown as the majority of studies on MBE focused on the general antimicrobial effect

Abbreviations: MBE, Magnolia Bark Estract; Xyl, Xylitol; Pols, Polyols; Xyl+Mag, Xylitol plus Magnolia Bark Estract; MS, mutans streptococci

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of MBE against bacteria. Specific affinity of MBE for oral Gram-negative bacteria increasing their cell surface hydrophobicity was described (Wessel et al., 2017). *In vitro*, magnolol demonstrated an high bactericidal activity against *Streptococcus mutans,* while honokiol activity was weaker (Sakaue et al., 2016).

We have previously reported the effect of a daily-administered sugar-free chewing gum containing MBE in a sample of adults at high caries-risk (Campus et al., 2011). The short-term use of MBE chewing gum has demonstrated beneficial effects on oral health, including reduction of salivary MS, plaque formation (bleeding scores) and acidogenicity and bleeding on probing (Campus et al., 2011; Komarova et al., 2017).

In a previous paper (Cocco et al., 2017) the long-term effect of a low-dosage Xylitol chewing-gum was also described. In this study the caries preventive effect of the long-term use of a sugar-free chewing-gum containing MBE and Xylitol in a high-risk adult population was investigated through a three-arm randomised clinical trial.

2. Methods

This paper reports on findings obtained in a larger research project that examines the effect of several functional foods supplied through chewing gums on caries risk factors and caries lesion development in an adult population.

2.1. Ethics approval

The present study was carried out in Sassari (Italy), lasted from September 2012 to June 2015. The study was designed as a randomized clinical trial, approved by the Ethics Committee of the University of Sassari (n°1083/L 23/07/2012), and registered (Protocol Registration Receipt NCT02310308) at http://www.clinicaltrial.gov. All performed procedures were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments.

2.2. Physical, chemical characterization and extraction procedure of the Magnolia Bark extracts

The bark of Magnolia officinalis Rehder spp. Biloba has been collected from the trunk of trees grown in China. Harvesting time was from April to June. The external part of the bark is hard and grey-brown, while immediately below the surface it fades toward yellow and then even tending to red with slight longitudinal stripes. The gatherers take care to collect the bark complete with the three areas. The raw bark is cleaned from dirt and impurities through a triple water washing and dried in single or double rolls at 50 °C till residual moisture is below 9%. The dried bark is crushed and extracted in supercritical carbon dioxide (35 °C, 1200–1400 l/h, 3 h 30 min). The raw extract is dissolved in ethanol and crystallized therefrom under vacuum at 50 °C. The refined extract is dried, sterilized, and sieved below 180 μm. The outcome is a light brown powder. Magnolol and honokiol were determined by HPLC-DAD, as suggested in Tsai and Chen (1992), and accounted for 87.2% and 10.8% of the extract, respectively. All other components were present in traces (total Eudesmol: 0.0093%, Moisture: 0.70%, Arsenic: 0.010%; Lead: 0.086%).

2.3. Chewing gums characteristics

All chewing gums were produced and supplied by Perfetti Van Melle SpA (Lainate, Italy). The polyols chewing gum was a sugar-free gum containing 28% Isomalt, 31% Sorbitol, 9% Mannitol and 1% Maltitol syrup. Xylitol chewing gum contained 30% of Xylitol, 26% Sorbitol, 11% Mannitol and 1% Maltitol syrup.

Magnolia chewing gum contained the same ingredients and percentages of the Xylitol group plus 0.17% of MBE of the gum weight. All three types of chewing gums weighed 1.4 g each and were identical in colour, shape and taste. Chewing gums were supplied in plain white containers coded as 'green', 'yellow or 'blue' according to the group. The code was sealed by an independent monitor and not broken until the statistical analysis was finalized.

2.4. Analysis of Magnolia Bark Extract in chewing gum

The release of Magnolol and Honokiol from the sugar free chewing gum was analysed by HPLC equipped with a photodiode-array UV detector (DAD) as reported in the literature (Tsai & Chen, 1992), (Agilend 1200 Infinity), with a reference wavelength of 209 nm. A column C-18 was employed, type Lichrospher RP 100, 5 μm – Merck. The column temperature was set at 22 °C with a flow rate of mobile phase at 1.0 ml/min (CH3CN/H2O/H3PO4 85% 65/35/0,1 volume ratio). To determine the concentration of magnolol and honokiol in the chewing dragees of the present study prior to chewing, the coating of ten gum pieces was dissolved in 250 ml of distilled water. The solution was blended with 250 ml of ethanol, filetered and analysed by HPLC. To determine the release of magnolol and honokiol from the study chewing gum, ten subjects (5M, 5F) chewed one piece each of sugar free chewing gum dragee, containing MBE in the coating. The subjects were instructed to chew at their normal pace for 5 min. The gum bolus was then collected from the subjects, homogenized and dissolved in chloroform. The solution was analysed by HPLC as described. The amounts of magnolol and honokiol were determined according to calibration curves obtained through external starndards. The percentage of magnolol and honokiol released during mastication was calculated considering as 100% the respective amounts contained in the gum prior to chewing.

2.5. Study population

The sampling technique and methods are described in detail in the previous paper (Cocco et al., 2017). Briefly, sample size for preliminary screening was performed trough G*Power 3.1.3 for Apple, using logistic regression with an odds ratio of 1.8, an error probability of 0.04; the total sample was set at 312.

In order to get statistical comparable results, the number of subjects per group to be included in the analysis was calculated. Considering a 40% difference among groups to be significant, and a 95% probability of obtaining a significant difference among groups at the 5% level, the resulting number of subjects per group was set in 64.

With the collaboration of the Municipal Electoral Registry Office, a letter explaining the purpose of the study and the informed consent were distributed to 5% (1131 subjects) of the age group considered living in Sassari. A total of 577 subjects (51.0% acceptance rate), aged between 30 and 45 years, accepted to participate and were examined for conditions that would preclude participation. The flow-chart, displayed in Fig. 1, shows the design of the study.

The inclusion criteria were: age range between 30 and 45 years; presence of a minimum of 12 natural teeth; presence of at least one cavitated caries lesion (ICDAS score 5), but no more than three; a salivary concentration of mutans streptococci $\geq 10^5$ CFU/ml saliva; no current periodontitis (no sites of probing pocket depth ≥ 5 mm or attachment loss of ≥ 2 mm, apart from gingival recession); absence of dysfunction of temporo-mandibular joint; good general health as assessed by a medical questionnaire; no use of antibiotics or participation in a clinical study in the previous 30 days; no allergy to any of the ingredients of the study products; no orthodontic banding or removable prosthesis.

Those participants who referred to consume more than three pieces of sugar-free chewing gum a day were excluded. The elected participants agreed not to consume any other chewing gums than those supplied for the study.

All participants were residents in an area with a low natural fluoride content in the tap water (0.04 mg/l) (http://www.abbanoa.it/distretto-6-sassari1), but they reported to use a fluoridated toothpaste on a

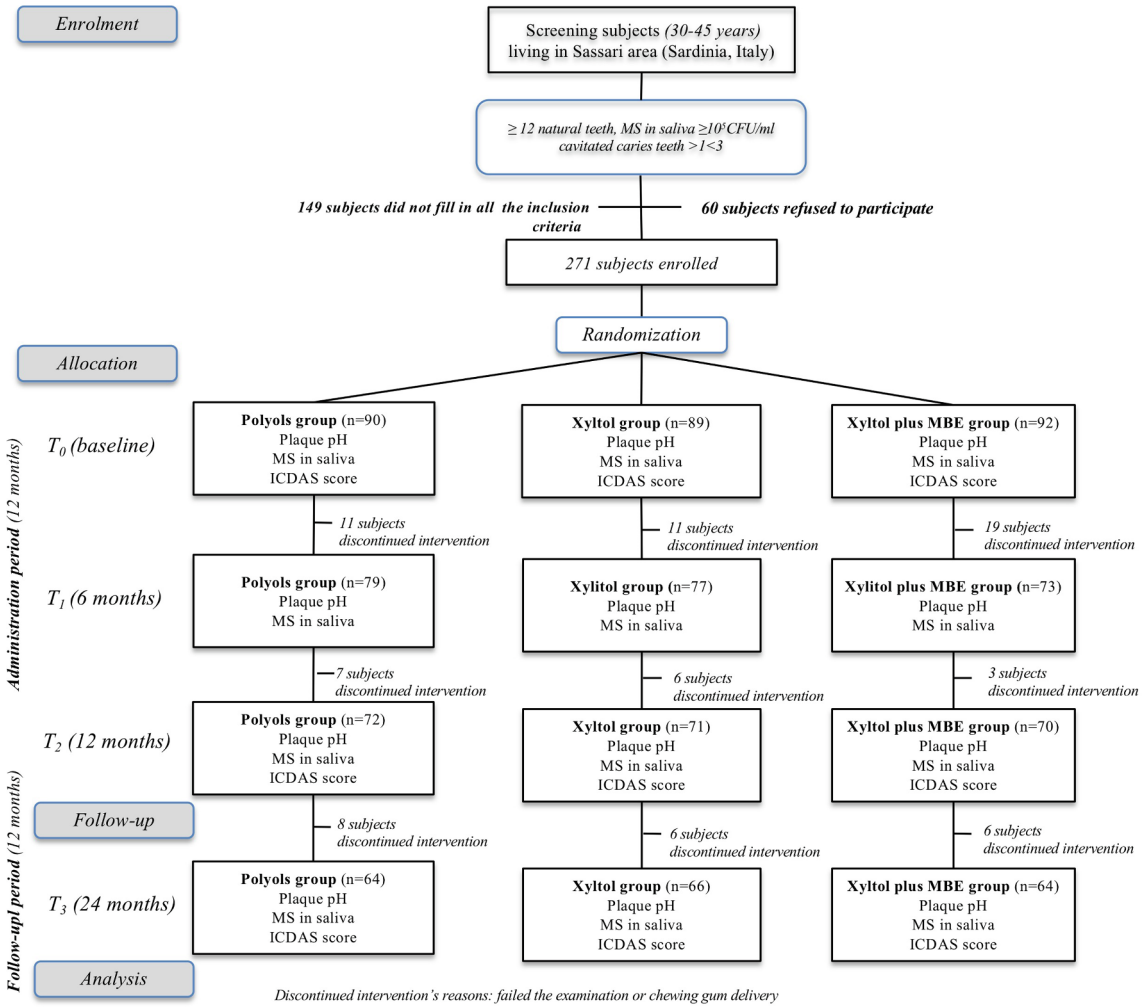

Fig. 1. Flow-chart of the study.

regular basis.

Randomization was performed (G. Campus) using Excel 2014 in permuted blocks of 2 or 4 with a random variation of the blocking number, and 3 groups were created: (1) the first group (Pols) received sugar-free gums containing several polyols except Xylitol; (2) the second group (Xyl) received gums containing the same polyols mixture plus a low amount of Xylitol and (3) the third group (Xyl + Mag) received the polyols mixture plus the low amount of Xylitol and magnolia. All personnel involved into the study were blinded to the participant assignment.

In total 480 subjects were examined and 331 fulfilled the inclusion criteria. Two-hundred seventy-one subjects accepted to be enrolled in the trial.

2.6. Clinical examination

The clinical examination of the enrolled sample was performed at baseline (t_0) and repeated at the end of the chewing-gum administration period (12 months, t_2) and at the end of the experimental period (24 months, t_3).

The subjects were examined using a mouth mirror, a ball ended probe and artificial light in a dental chair. The ICDAS index was used to register caries at tooth level as initial or moderate or severe lesions, the number of filled teeth and the missing teeth for caries (Ismail et al., 2007). The bleeding on probing score was registered in all subjects. At baseline, the examiner that performed all the dental screenings was trained and calibrated by a benchmark examiner. Baseline training consisted in one-day (6 h) theoretical course, followed by examination of 54 extracted teeth plus a session of 120 photographs of extracted teeth. Two days after the theoretical course a clinical training involving 55 adults was performed. The subjects were re-examined after 72 h. Inter-examiner reliability with the benchmarck examiner was evaluated using fixed-effects analysis of variance. Intra-examiner reproducibility was assessed as the percentage of agreement using Cohen's kappa statistic (Castiglia, Campus, Solinas, Maida, & Strohmenger, 2007). Good reliability was found between examiner and benchmark, ($p = 0.15$) with a low mean square of error (0.47). Intra-examiner reliability was also high (Cohen's Kappa statistic = 0.88). Interim and follow-up training was also performed.

The subjects were instructed to chew a total of 5 pellets for 5 min divided into 3 intakes a day (2 in the morning, 2 after the midday meal and 1 in the afternoon). Thus, the total daily intake of Magnolol and Honokiol was 12 mg/day, and for Xylitol was 2.5 g/day. The subjects were asked to make no changes in their dietary and oral hygiene habits. Tooth brushing was not allowed for at least 1 h after the use of chewing gum. All subjects received a fluoridated toothpaste containing 1450 ppm NaF (Mentadent P; Unilever Italia, Milan, Italy) to be used during the experimental period. They were asked to avoid any other oral hygiene adjuvant and any commercial Xylitol or Sorbitol products throughout the study period. The body's tolerance of different polyols and Magnolia extract was assessed by means of a questionnaire administered to the participants shortly after the gum distribution had started and 6 months later, while the study was still proceeding. The questions focused on the potential side effects of using the gum. In

order to evaluate the success of the chewing gum intake, participants were given chewing gums necessary for a single month at a time and asked to return the empty packs when receiving those for the following month. At t_3 a new questionnaire was submitted to all the participants to investigate if during the no chewing gum period (from t_2 to t_3) a use of sugar-free chewing gums continued.

2.7. Plaque-pH measurements

Interproximal-plaque pH was evaluated using pH indicator strips (Carlen, Hassan, & Lingstrom, 2010), which measure a pH value in the range of 4.0–7.0 (Spezialindikator, pH range 4.0–7.0; Merck, Darmstadt, Germany). The strips determine changes in plaque pH, discriminating differences at the level of 0.2–0.5 pH units and they are easy to use. The strips were cut into 4 pieces (approx. 2 mm in width) in order to get a strip that could be easily inserted into the interproximal space and held *in situ* for 10 s, after which it was removed, and its colour compared to the colour index scheme supplied by the manufacturer.

For each subject, 3 measurements were carried out in 2 sites, between the 2nd premolar and the 1st molar right and left of the upper jaw. Measurements were performed before and at 2, 5, 10, 15, 20 and 30 min after a mouth rinse with 10% sucrose. All measurements were carried out by the one examiner. Plaque pH was assessed at baseline (t_0), after 6 (t_1), 12 months (t_2) of chewing gum use and 12 months after the cessation of the chewing gum use (t_3).

2.8. Microbiological evaluation

An evaluation of the number of mutans streptococci (MS) concentration in saliva was performed at baseline (t_0) and six (t_1), twelve (t_2) and twenty-four (t_3) months from baseline. No-stimulated whole saliva was collected over 150 s in sterile vials (Nunc, Kamstrup, Denmark). The samples were transported to the Department of Microbiology of the University of Sassari and processed within 45 min after collection.

Mutans streptococci counts in saliva were assessed and categorized using the dip-slide technique (CTR bacteria, Ivoclar Vivadent, Germany).

2.9. Statistical analysis

The Data on caries were based considered the tooth as the unit of analysis; the net caries increment for initial, moderate and severe caries level, using ICDAS (Δ-initial, Δ-moderate and Δ-severe), was calculated. Differences between groups in terms of the caries increment were evaluated using the nonparametric Mann–Whitney *U* test.

The data on inter-proximal plaque pH and gingival bleeding scores at t_0, t_1, t_2 and t_3 were analysed for statistically significant differences using repeated measures of ANOVA. Due to the non-symmetry, the log-linear regression, using robust SEs, was used to compare the data of the three interventions (Schafer, 1997, Chapter 8).

Differences in proportion relating to microbiological counts at baseline and follow-up were assessed using equality of proportion test.

The efficacy and consequences of treatment were also considered, calculating the event rate (ER) for each group and the number needed to treat (NNT) (Laupacis, Sackett, & Roberts, 1988). An event was defined as the change of status at tooth level, *i.e.* the development of a new lesion or the progression of an existing lesion to a more severe level or the change of a tooth previously affected in a filled or a missing tooth during the 2-year follow-up period. All data were analysed using the software STATA® (v13 for Macintosh). For all statistical analysis, the statistical significance level was set at $\alpha = 0.05$.

The number needed to treat (NNT) is the estimated number of patients who need to be treated with the new treatment rather than the standard treatment (or no treatment) for one additional patient to benefit (Altman, 1998). This method of analysis preserves the prognostic balance afforded by randomization.

3. Results

3.1. Magnolia Bark Extract in chewing gum

The release of the two neolignans was 86,86% with a $_{95\%}$Confidence Interval 75.43–96.82 and 60,10% ($_{95\%}$CI = 49.49–71.29) for magnolol and honokiol respectively (*data not in tables*).

3.2. Clinical examination

A total of 194 subjects (71.22% of the initial sample) completed the trial, 64 in the polyols group (Pols), 66 in the Xylitol group (Xyl) and 64 in the Xylitol plus Magnolia group (Xyl + Mag) (Fig. 1). The highest number of drop-out was reported at t_1 with the discontinued intervention as the main reason for dropping out. No side-effects were observed in any subjects. Moreover, the use of chewing gum after the experimental period was fairly insignificant; only 28 (10.9%) subjects reported the regular use (once a day or more) of sugar-free chewing gum (*data not in table*).

No statistically significant differences between groups were recorded at baseline regarding caries severity and no gender difference was also observed (*data not in table*). At t_2 the Δ-values of the initial caries lesions measured in the three groups were statistically different ($p = 0.02$) and this difference increased at t_3 ($p < 0.01$). The moderate caries level did not differ among the three groups in any evaluations ($p = 0.08$ at t_2 and 0.10 at t_3, respectively). The analysis of the caries increment for the severe lesions level revealed a statistically significant difference among groups ($p = 0.02$ at t_2 and $p < 0.01$ at t_3). The caries increment was always lower in the Xylitol plus Magnolia

Table 1
Caries data (ICDAS) at t_0 (baseline) and caries increment (Δ) between t_0 and t_2 (12-month examination) and between t_0 and t_3 (24-month examination) at different threshold levels are shown. Differences between groups were evaluated using the nonparametric Mann–Whitney *U* test.

	Caries at baseline			**Caries increment (Δ)**						**Filled teeth (Δ)**
	mean (SD)			*mean (SD)*						*mean (SD)*
Chewing gums	t_0 (baseline)			t_2 (Δ0–12 months)			t_3 (Δ0–24 months)			t_3 (Δ0–24 months)
	Initial	Moderate	Severe	Initial	Moderate	Severe	Initial	Moderate	Severe	
Polyols	*6.92 (11.9)*	*3.83 (7.72)*	*2.11 (2.65)*	*0.09 (0.15)*	*0.10 (0.35)*	*0.18 (0.38)*	*0.20 (0.67)*	*0.18 (0.72)*	*0.44 (0.73)*	*1.12 (2.89)*
Xylitol	*7.10 (12.0)*	*3.78 (8.28)*	*2.20 (2.86)*	*0.05 (0.12)*	*0.07 (0.23)*	*0.11 (0.12)*	*0.14 (0.37)*	*0.16 (0.82)*	*0.30 (0.52)*	*0.73 (1.41)*
Magnolia	*7.21(11.73)*	*4.17 (7.49)*	*2.18 (3.01)*	*0.04 (0.14)*	*0.06 (0.42)*	*0.09 (0.44)*	*0.10 (0.34)*	*0.14 (0.51)*	*0.25 (0.38)*	*0.78 (1.34)*
p-value	*0.53*	*0.26*	*0.62*	**0.02**	0.08	**0.02**	< **0.01**	0.10	< **0.01**	**0.03**

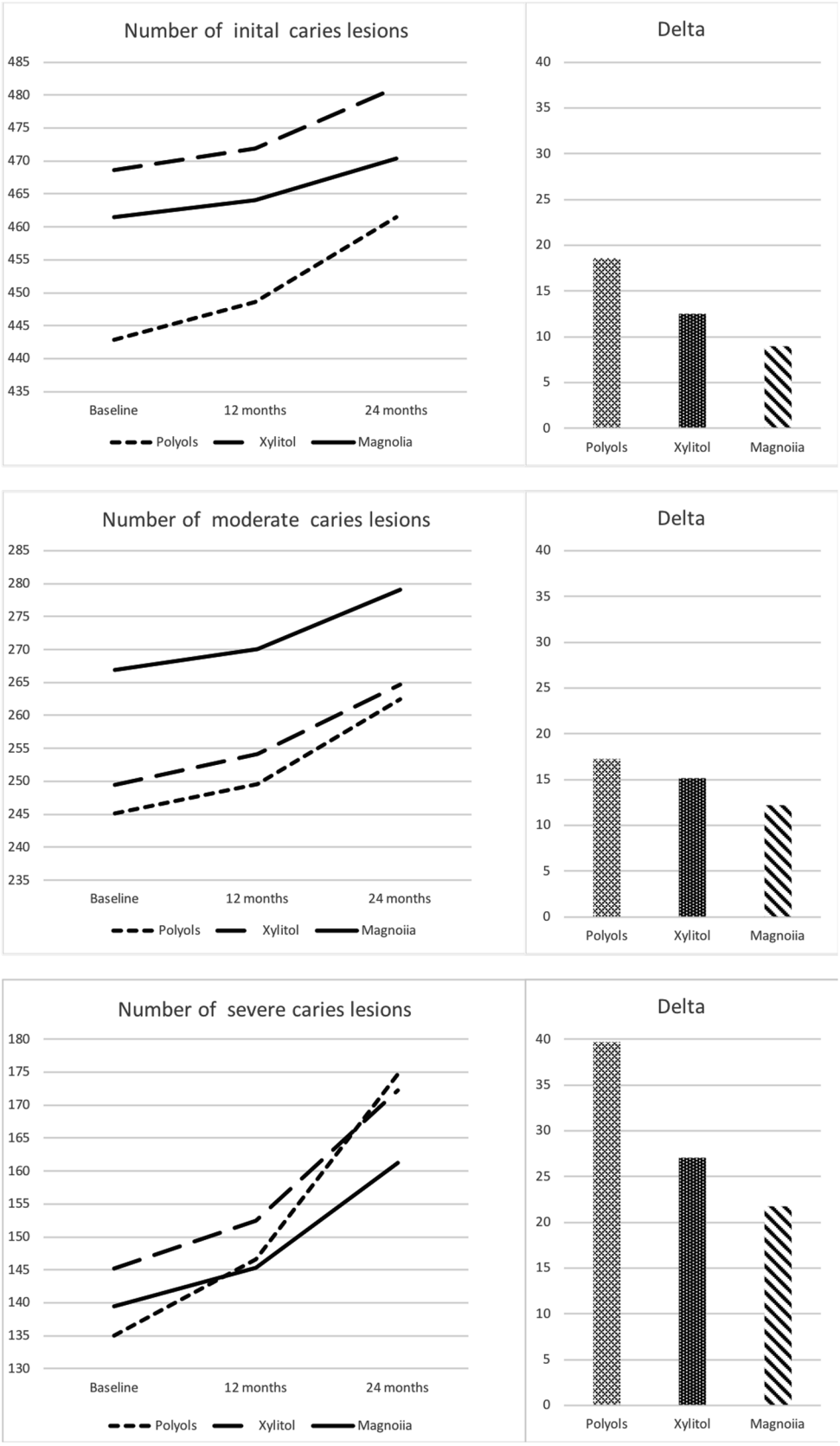

Fig. 2. Number of caries lesions (Initial, Moderate and Severe) recorded during the trial.

group compared to the Xylitol and the Polyol groups in any evaluation. Finally, fillings increment does not significantly differ among groups (Table 1).

The number of caries lesions recorded at baseline, at 12 months and 24 months follow-up evaluations and the difference (Δ) between baseline and 24 months follow-up evaluation was compared among

Table 2
Effectiveness of the treatment. An event was defined as the change of status at tooth level.

	Events	Non Events	Event Rate
Polyols	*112*	*1290*	*0.087*
Magnolia	*66*	*1298*	*0.052*
*z = 3.33**p* < **0.01** RR = 0.61 $_{95\%}$CI = 0.45/0.81 NNT = 32 $_{95\%}$CI = 20.10/75.50*			
Polyols	*112*	*1290*	*0.087*
Xylitol	*85*	*1291*	*0.066*
z = 1.85p = 0.06 RR = 0.77 $_{95\%}$CI = 0.59/1.01 NNT = 55 $_{95\%}$CI = 1034.43/26.89			
Xylitol	*85*	*1291*	*0.066*
Magnolia	*66*	*1298*	*0.052*
z = 1.53p = 0.13 RR = 0.78 $_{95\%}$CI = 0.57/1.07 NNT = 75 $_{95\%}$CI = 270.59/32.82			

groups considering the subjects who completed the trial. The difference in the number of severe caries lesions between baseline and the 24 months follow-up examination was statistically significant lower in Xyl + Mag group (χ^2 = 4.79 p < 0.02) compared to the other groups (Fig. 2).

Subjects treated with Magnolia chewing-gums had a Reduction of Risk Rate (RRR) of 39% respect to those treated with Polyols with a Number Needed to Treat (NNT) of 32; subjects treated with Xylitol chewing-gums had a RRR of 23% respect to those treated with polyols with a Number Needed to Treat (NNT) of 55. Finally, subjects treated with Xylitol plus Magnolia chewing gum had an RRR of 22% respect to those treated with Xylitol only with a NNT of 75. The event rate was 0.087 in the Pols group, 0.052 in the Xyl + Mag group and 0.066 in the Xyl group (Table 2).

Subjects from the magnolia and xylitol groups showed a significantly lower gingival bleeding scores at the end of the chewing period (t_2) compared to baseline (p = 0.01 and < 0.01, respectively). A reduction in the bleeding score was also noted for the polyols group even if not statistically significant (Table 3).

3.3. Plaque-pH measurements

At baseline (t_0), plaque pH expressed as the areas under the curve (AUC) at pH 5.7 and 6.2 and the salivary MS concentration were similar in the three groups, with no statistically significant differences (Table 4). Mean AUCs were statistically significantly different at t_2 and t_3 in the three groups ($AUC_{5.7}$ p = 0.03 and $AUC_{6.2}$ p = 0.04 at t_2 and p = 0.04 and 0.05 respectively at t_3). During the trial, intra-group differences were also observed for Xyl and Xyl + Mag group. $AUC_{5.7}$ and $AUC_{6.2}$ decreased in both groups, with a wider reduction in Xyl + Mag group ($AUC_{5.7}$ p = 0.02 for Xyl group and p < 0.01 for Xyl + Mag group and $AUC_{6.2}$ p < 0.01 for both).

3.4. Microbiological evaluation

At baseline, salivary mutans streptococci in all the subjects had level of $\geq 10^5$ CFU/ml as an inclusion criterion for the trial. A decrease of MS concentration was noted in each group during the experimental period, but the differences reached a statistically significant value for Xyl + Mag and Xyl group only (p < 0.01 for both). The comparison among the three groups showed a statistically significant difference at t_2 (p = 0.02) and t_3 (p = 0.03) (data not in Tables).

4. Discussion

The purpose of this RCT was to assess the caries preventive effect of the long-term use of sugar-free chewing-gums in a high-risk adult population. To elucidate this hypothesis, a three-arm randomised clinical trial was designed and carried out. Three types of chewing gums were included into the trial: a polyol chewing gum, a low-dosage Xylitol chewing gum and a chewing gum contains Xylitol plus Magnolia Bark Extract (MBE). The MBE was found to be effectively released in a 5 min chewing time. In a previous study (Greenberg et al., 2007) MBE release was tested at 6, 12 and 20 min of chewing and it was found to be about 50–60% at all time points when included in the gum coating, while the release was negligible, when the extract was included in the center. The chewing gum dragees used in one arm of the present clinical study contained MBE, which was included in the gum coating. The release was determined according to literature, however it was found to be slightly higher. We hypothesize that this difference may be due to the formulations of gum base used in the present and int the previous study. However the main point of the effective release of active neolignans during gum chewing, when they are included in the gum coating, was confirmed.

The trial focused on the concentration of cariogenic bacteria in saliva, plaque acidogenicity and caries increment over a two-year period, during which no other community-based caries prevention strategies were carried out.

At the end of the follow-up, a statistically significant difference in caries lesions increment and severity compared to baseline was observed among groups. Subjects using the Xylitol plus Magnolia chewing gum showed the lowest caries increment of new lesions, the lowest caries progression and the lowest number of restorative treatments compared to the Xylitol and the Polyol groups.

Today, non-fermentable sweeteners are incorporated into many products, such as chewing-gums. The main sugar substitutes used in chewing-gum are polyols, especially Xylitol (Bar, 1988). Although the mechanisms of Xylitol are not fully known, several studies have demonstrated its benefits both as non-cariogenic and even cariostatic agent (Campus et al., 2013; Cocco et al., 2017). Magnolia Bark Extract is a traditional Chinese medicine that has demonstrated an antibacterial effect against oral microorganisms (Brambilla, Cagetti, Ionescu, Campus, & Lingstrom, 2014; Chang, Lee, Ku, Bae, & Chung, 1998; Greenberg, Urnezis, & Tian, 2007; Ho, Tsai, Chen, Huang, & Lin, 2001; Namba, Tsunezuka, & Hattori, 1982). MBE showed promising results reducing oral bacteria, including mutans steptococci by 43.0% at 40 min (Greenberg et al., 2007; Greenberg, Dodds, & Tian, 2008). Even if there is only one paper (Sakaue et al., 2016) that describes on the different actions of two main constituents magnolol and honokiol; the

Table 3
Gingival bleeding scores in the three groups.

Gingival bleeding score					
		Gum use		No-Gum use	
	t_0 (Baseline)	**t_1 (6 months)**	**t_2 (12 months)**	**t_3 (24 months)**	*p-value*
	% ($_{95\%}$CI)	*% ($_{95\%}$CI)*	*% ($_{95\%}$CI)*	*% ($_{95\%}$CI))*	*(repeated Anova)*
Polyols	32.14 (25.14–38.94)	29.45 (21.54–38.27)	27.32 (20.49–34.45)	29.48 (21.20–37.21)	0.07
Xylitol	32.81 (25.78–39.25)	27.03 (21.74–33.57)	24.65 (15.74–33.37)	26.85 (18.98–34.21)	**0.01**
Xylitol + Magnolia	33.05 (26.14–36.24)	25.86 (14.73–32.80)	20.87 (11.44–30.31)	22.24 (17.41–30.55)	< **0.01**
p-value (One-way Anova)	*0.32*	***0.03***	***< 0.01***	***0.02***	

Table 4
Plaque pH. Representation of $AUC_{5.7}$ and $AUC_{6.2}$ expressed as mean (SE). Salivary mutans streptococci. Concentrations as Log_{10} CFU/ml in saliva mean (SE) at the different time intervals (t_0, t_1, t_2 and t_3) in the three groups.

$AUC_{5.7}$		Gum use		No-Gum use	
	t_0 (Baseline) *mean (SD)*	**t_1 (6 months)** *mean (SD)*	**t_2 (12 months)** *mean (SD)*	**t_3 (24 months)** *mean (SD)*	*p-value (One-way Anova)*
Polyols	11.68 (0.59)	11.44 (0.54)	9.53 (0.35)	11.45 (0.20)	0.08
Xylitol	11.45 (0.46)	11.27 (0.33)	9.65 (0.38)	9.85 (0.34)	**0.02**
Magnolia	11.32 (0.24)	10.66 (0.73)	7.87 (0.31)	8.06 (0.41)	< **0.01**
p-value (One-way Anova)	*0.32*	*0.27*	***0.03***	***0.04***	
$AUC_{6.2}$					
Polyols	21.56 (0.24)	18.94 (0.81)	18.98 (0.77)	19.75 (0.16)	0.12
Xylitol	22.78 (0.57)	20.11 (0.48)	18.61 (0.71)	18.67 (0.56)	< **0.01**
Magnolia	23.97 (0.53)	21.08 (1.01)	15.89 (0.88)	16.26 (0.78)	< **0.01**
p-value One-way Anova	*0.32*	*0.27*	***0.04***	***0.05***	
Salivary mutans streptococci					
Polyols	*5.32 (0.41)*	*5.22 (0.21)*	*5.33 (0.37)*	*5.33 (0.42)*	0.42
Xylitol	*5.41 (0.26)*	*5.33 (0.43)*	*5.16 (0.44)*	*5.15 (0.56)*	< **0.01**
Magnolia	*5.40 (0.12)*	*5.33 (0.42)*	*5.12 (0.30)*	*5.14 (0.31)*	< **0.01**
p-value (One-way Anova)	*0.20*	*0.47*	***0.03***	***0.02***	

different concentration of the two products on the chewing-gums used in the trial might explain the highr antibacterial and caries preventive effect of the chewing-gum enriched by MBE plus Xylitol respect to chewing enriched by Xylitol.

The low number of new caries lesions recorded twelve months after the end of the chewing gum use in Xylitol and in Xylitol plus Magnolia group in particular is probably related to the long-term effect of Xylitol that persists over time. In a recent placebo-controlled RCT (Bader et al., 2013), adults at high caries risk consumed 5 g/day of Xylitol through lozenges during a 33-month period. No significant effect was observed regarding caries increment even if a quite high dose was used. The different Xylitol administration modalities, lozenges *versus* chewing gum and the no specific administration time *versus* far from the main meals, might explained this apparent contradiction. The addition of Magnolia Bark extracts to the Xylitol chewing seems to increase the lowering effect on caries increment, both on the development of new lesions and on the progression of the existing lesions. This result is totally new since no RCT are available in literature aiming to verify the effect of Magnolia extracts on a clinical outcome related to caries, so it is not possible to compare the results of the present investigation to those of previous studies. A combined effect of Xylitol and Magnolia might be speculated, since both ingredients have shown to be effective in reducing cariogenic bacteria both *in vitro* and *in vivo* (Campus et al., 2011).

Moreover, this study investigated the effects of the three sugar-free chewing gums on two risk factors of caries, salivary mutans streptococci and interproximal plaque acidogenicity. Considering the effect on mutans streptococci, the one-year use of the chewing-gum containing MBE and Xylitol showed to be more efficient in the reduction of the concentration of the cariogenic bacteria in saliva than Xylitol and Polyol chewing gum. Nevertheless, twelve months later the end of the chewing gum use, the effect on MS of Xylitol chewing and MBE plus Xylitol tends to be similar in both groups, assuming that the effect of MBE is lost overtime while that of Xylitol persists overtime.

Regarding plaque pH, comparisons among groups revealed statistically significantly differences, showing that the areas under the curve both for enamel and dentine dissolution were less pronounced in the Xylitol plus Magnolia group and in Xylitol group both at the end of the chewing gum use and one-year after compared to Polyol group. These pH figures are related to the trend of cariogenic bacteria during the two-year period described above. Xylitol plus Magnolia chewing gum also showed an effect on gingival tissue, measured through bleeding on probing, indicating that the use of chewing-gums might improve gingival health by reducing gingival bleeding. The affinity of MBE for oral Gram-negative bacteria increasing their cell surface hydrophobicity might be linked to this outcome resulting in a decreased accumulation of dental plaque and reduction of gingival inflammation (Walker et al., 2013).

This study holds almost unique characteristics like the adult population considered, the length of the administration, the quite long follow-up period, but above all the evaluation for the first time the effect of a chewing gum containing MBE on the incidence of caries as well as on caries risk factors within the same study. On one end this study reinforces the hypothesis that chewing a gum containing Xylitol and MBE is able to reduce caries risk, decreasing the number of mutans streptococci and the production of acids and caries increment. On the other hand, as it was described in the previous paper (Cocco et al., 2017), the population enrolled for this trial was selected at high risk of caries and belonged to an age range in which the regular use of chewing gums is not common, and this might explain the quite high drop-out rate recorded at the 12 months interim examination. Therefore, it is questionable whether or not the selected sample is representative for the general adult population of that age range.

Caries still remains a social problem even in industrialized country as Italy where socioeconomic inequalities across a broad spectrum of oral indicators reflecting unmet needs are described (Arrica et al., 2017). Moreover, since dental care is not covered by the National Health System, it has a great importance to promote effective low-cost caries prevention strategies and the administration of a chewing gum even if for a long period, might be an interesting possibility. It was recently postulated that the elevation of the consumption of sugar-free chewing gum in Germany to the level of Finland would lead to a considerable benefit for cost saving and oral health (Zimmer, Spyra, Kreimendahl, Blaich, & Rychlik, 2018). The present RCT contributes to promote this kind of low-cost intervention.

In conclusion, the present study provides robust but still non-conclusive evidence on the efficacy of Xylitol plus Magnolia chewing gum compared to a Xylitol and Polyols gum to reduce caries risk factors and to prevent caries lesions, therefore being possible to accept the study's hypothesis. A clinical suggestion deriving from the results of this trial is the opportunity to administer daily Xylitol plus Magnolia gum to high caries risk subjects. To attest the superiority or equivalence of Xylitol plus Magnolia gums according to the EFSA guidelines, however, at least two independent clinical studies are needed. Thus, future studies are

still necessary, aiming at assessing the effects of the products not only in caries progression, but also in the reversal of non-cavitated lesions.

5. Ethics statements

The study was approved by the Ethics Committee of the University of Sassari (n°1083/L 23/ 07/2012), and registered (Protocol Registration Receipt NCT02310308) at http://www.clinicaltrial.gov. All performed procedures were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments.

A letter explaining the purpose of the study and the informed consent were distributed to 1131 subjects of the age group considered. A total of 577 subjects (51.0% acceptance rate), accepted to participate.

CRediT authorship contribution statement

Maria Grazia Cagetti: Conceptualization, Writing - original draft, Methodology. **Fabio Cocco:** Data curation, Formal analysis, Writing - original draft, Supervision. **Giovanna Carta:** Visualization, Investigation, Supervision. **Cinzia Maspero:** Writing - original draft. **Guglielmo Campus:** Conceptualization, Formal analysis, Writing - original draft, Methodology, Writing - review & editing.

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Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

F.C. study design, statistical analysis, review the literature, manuscript writing. M.G.C. study design, manuscript writing. G.C. examination and data collection, manuscript writing. C.M. review the literature, review final revision of the manuscript. L.O. review final revision of the manuscript. G.C. patient qualification for the study, data and statistical analysis, manuscript writing.

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