Original Article

Beneficial effect of aged garlic extract on periodontitis: a randomized controlled double-blind clinical study

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The purpose of this study is to assess the long-term efficacy of aged garlic extract to improve periodontitis. Two hundred and one participants were randomly stratified and assigned equally to the regimen group or the control group. At the start, 12 month, and 18 month subjects received dental examination and periodontal evaluation. Probing Pocket Depth and Gingival Recession were examined. For each efficacy parameter, the mean value of examination was calculated and assessed using paired-difference *t* tests. Statistical tests were two-sided using a 5% significance level. The mean value of pocket depth for the aged garlic extract group at 18 month was 1.06 ± 0.49 as compared to the baseline value of 1.89 ± 0.74 ($p<0.001$) and the corresponding value of 1.50 ± 0.46 for the placebo group ($p<0.001$), indicating the beneficial effect of aged garlic extract on periodontitis. According to a Multiple linear regression analysis the only three variables which reached statistical significance as predictors of PPD level were the baseline PPD scores ($p<0.001$), smoking ($p = 0.020$), and consumption of daily dose of aged garlic extract ($p<0.001$). These results demonstrated that aged garlic extract is an effective supplement for preventing or improving periodontal disease. The well demonstrated benefits of aged garlic extract for the oral disease may also be used as a means to improve general health because of the close relationship between periodontitis and some systemic diseases such as diabetes, hypertension, atherosclerosis, and others.

***Key Words*:** **aged garlic extract, periodontitis, prevention, oral health, general health**

Periodontitis is a chronic inflammatory disease resulting in progressive detachment of gum from tooth and alveolar bone loss and the major cause for tooth loss in adults. It is one of the most important oral diseases contributing to the global burden of chronic illness.[1] Its severe form affects 11% of adults, making periodontitis the sixth most prevalent disease of mankind and an important public health problem.[2,3] Many studies have suggested the possible association of periodontitis with various diseases such as diabetes, atherosclerotic vascular disease, rheumatoid arthritis, adverse pregnancy outcome, obesity, and Alzheimer's disease. Furthermore, periodontitis severely impairs oral health to affect the quality of life, self-esteem and general wellbeing.[1–3] The prevention and treatment of periodontal disease has been under the governance of the biomedical model which emphasizes the biological aspect of the disease and the professional therapy, while excluding psychological, environmental, and social influences.[3]

Garlic has a history of thousands of years of human consumption and use. It was used by ancient Egyptians for both food flavoring and a traditional medicine.[4–6] Many people consider garlic as a source of health both as a prevention and curative food additive. The aged garlic extract (AGE) is an odorless product resulting from prolonged extraction of fresh garlic at room temperature.[7] It is highly bioavailable and exerts biological activity in both animals and humans. AGE is prepared through extraction and aging in a water-ethanol mixture for over 10 months at room temperature. The process of aging reduces harsh and irritating compounds in raw garlic and generates unique and beneficial compounds through both enzymatic and chemical reactions. AGE is commercially available and has been widely studied for its high antioxidant activity and health-protective potential.[8–10] AGE exhibits a broad spectrum of beneficial effects against microbial infection as well as cardio-protective, anticancer, and anti-inflammatory activities.[11–15] A recent meta-analysis study demonstrated that it lowers blood pressure in hypertensive subjects, and also suggested the benefit of AGE on Alzheimer's disease.[16,17]

A recent review of preventive and therapeutic application of plant ingredients in the treatment of periodontal diseases highlights the effectiveness of both herbal extracts and polyphenols being applied as a mouthwash or dentifrice for the oral cavity.[18] Recently we have demonstrated that daily consumption of AGE benefits oral health by reducing gingival inflammation and bleeding.[19] It is the first study to show the effect of AGE on the oral cavity in general and specifically with regard to chronic gingivitis, the most common periodontal inflammation worldwide.[20] In view of the need to find an effective agent for periodontal diseases, the present clinical study was undertaken to assess the long-term efficacy of AGE for the treatment of periodontitis.

Methods

The present clinical study was reviewed and approved by the Helsinki committee of Hadassah Hospital, Jerusalem, Israel (Registration and approval number: HMO-114-17) and was conducted at the Hebrew University, Hadassah, Israel from March 2018 to September 2019 (ClinicalTrials. Gov: NCT03492723).

A sufficient number of participants was screened to obtain approximately 200 generally healthy adult volunteers with mild to moderate periodontitis. Prior to screening, participants were asked to read and sign an informed consent and received a signed copy.

The main inclusion criteria were 20–60 years of age; in a good general health, possess a minimum of 16 natural teeth (excluding third molars) with facial and lingual scorable surfaces, have at least 3 eligible periodontal sites [Probing Pocket Depth (PPD)

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3.5–6 mm], agree to delay any elective dentistry until completion of the study as well as to return for their scheduled visits, and follow study procedures. Participants were excluded from the study when there was an evidence of a severe periodontal disease such as purulent exudate, generalized mobility or severe recession, active treatment for periodontitis, any condition requiring for antibiotic pre-medication prior to dental procedures, fixed facial or lingual orthodontic appliances or removable partial dentures, use or antibiotic within two weeks prior to the initial visit, pregnancy, dental prophylaxis within two month prior to the screening visit, and any diseases or conditions that could be expected to interfere with the subject's safely completion of the study.

Participants were stratified and randomly assigned equally to either the regimen group or the control group based on gender, age, smoking, and baseline mean of PPD using covariate adaptive randomization.

The study and control groups were instructed to swallow eight tablets a day; four during morning and four in evening. These tablets were the AGE product, Kyolic® (Reserve formula; Wakunaga Pharmaceutical, Co., Ltd., Osaka, Japan) containing 300 mg of AGE powder per tablet or placebo tablet, respectively.

AGE was manufactured under a license issued by the Ministry of Health, Labor and Welfare of Japan and was prepared according to the following steps: organically grown raw garlic (*Allium sativum* L.) was cut into slices, immersed in aqueous ethanol, and aged at room temperature. The specific procedure and specifications are in compliance with the US Pharmacopeia/Natural Formula (USP/NF) for garlic fluid extract monograph.

Both groups received dental prophylaxes every 6 months consistent with local norms and standards. Tablets were supplied approximately every 6 months following the initial visit. Subjects that did not attend one examination (not first or last), were still considered to be part of the study group. At the start, 12 months, and 18 months subjects received dental examination, and periodontal evaluation was performed as described below. Prior to examinations subjects were asked to refrain from any oral hygiene procedures and to refrain from using medicated lozenges or breath mints, eating, drinking (except water), smoking, and chewing gum for 4 h prior to their visit.

Clinical findings were recorded excluding teeth with crowns, or large restorations, i.e., covering 50% or more of the tooth surface, bridges, orthodontic appliances, or implants. Statistical power calculations were conducted with $\alpha = 0.05$ with 85% power using a two-sided test and a sample size of 170 subjects completing the study was determined (85 subjects per group). Up to 200 subjects (100 subjects per group) were enrolled in the study.

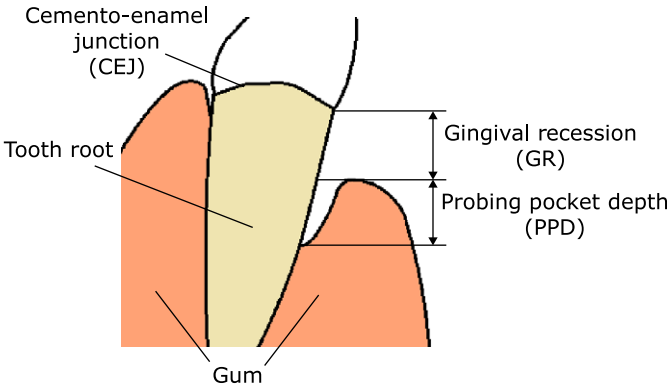

Fig. 1. Schematic description of probing pocket depth (PPD) and gingival recession (GR).

PPD at every site was assessed as the distance from the gingival margin to the apical end of the pocket using a WHO CPI probe (Fig. 1). Probe recordings were rounded off to the nearest millimeter mark. Gingival Recession (GR) at each site was measured as the distance between the Cemento-enamel Junction (CEJ) and the apical end of the pocket using a WHO CPI probe (Fig. 1). For both measurements, a score was given to six areas of the tooth. These are the disto-buccal, buccal, mesio-buccal, disto-lingual, lingual, and mesio-lingual area.

The value of the demographic characteristics as well as baseline and post-treatment scores were calculated for each treatment group and visit. Paired-difference *t* tests, repeated measures, and linear regression models were used. Statistical tests were two-sided using a 5% significance level.

Results

A total of 221 participants was screened, and following the inclusion criteria only 200 participants met all criteria. The final number of participants that completed the 18 months study was 182, which represents a 9.5% attrition rate (Fig. 2).

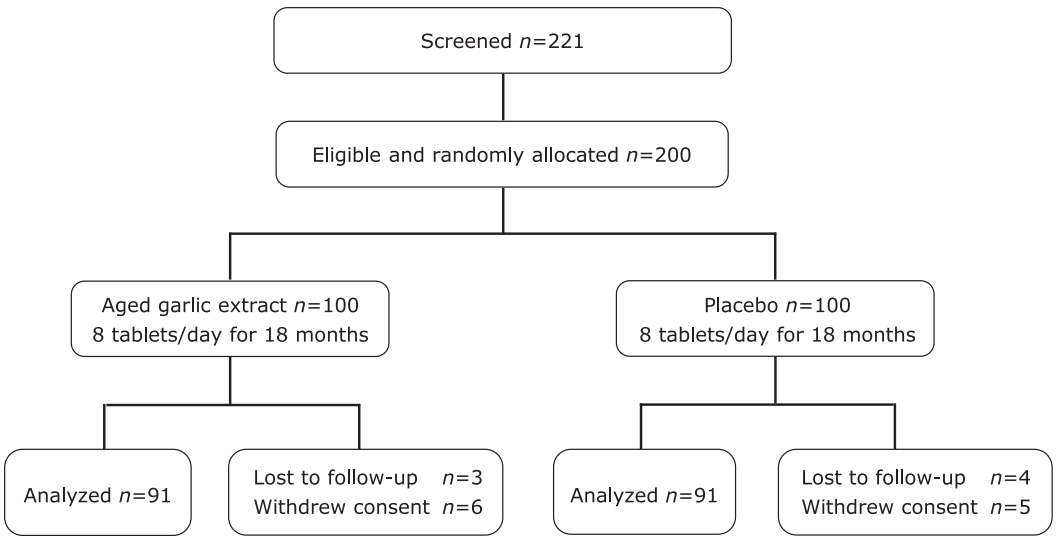

Fig. 2. Flow chart of the study population.

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The randomization of the treatment groups at the baseline is shown in Table 1. No significant difference between the regimen and the control groups was found with regard to gender, age, smoking, and mean of PPD. A total of 49.0% males participated, and the average age was 45.2 ± 8.1 years. Similar percentages of smokers were present in both groups (a total of 18.5%), and the baseline results of mean PPD in the two groups were not significantly different ($p = 0.502$).

Table 2 and Fig. 3A show the results of PPD during the 18 months study. A statistically significant difference of PPD scores was shown for the AGE and Placebo group at 12 and 18 months. The final mean of pocket depth for the AGE group at 18 months was 1.06 ± 0.49 as compared to the baseline value of 1.89 ± 0.74 ($p<0.001$) and the final value of 1.50 ± 0.46 for the placebo group ($p<0.001$).

Figure 3B represents the results of GR during the 18 months study. A statistically significant difference of the mean PPD scores was found within both groups ($p<0.001$), but no statistical difference was found between the AGE and Placebo groups ($p = 0.765$).

Multiple linear regression analysis was performed for PPD adjusted for gender, smoking, baseline PPD and study groups (Table 3). According to the regression model, the only three variables that reached statistical significance as predictors of PPD level were the baseline PPD scores ($p<0.001$), smoking ($p = 0.020$), and consumption of daily dose of AGE ($p<0.001$).

Discussion

The previous clinical study demonstrated that the daily consumption of AGE for four months benefits oral health by reducing gingival inflammation and gingival bleeding.[19] This findings prompted us to further explore the potential benefit of AGE for oral health by carrying out a long-term study to investigate its effect on periodontal disease. Thus, we initiated the present 18 months clinical study with the aim to assess the long-term efficacy

Table 1. Distribution of the study groups at baseline by gender, age, smoking, and mean Probing Pocket Depth

	Total (n = 182)	AGE (n = 91)	Placebo (n = 91)	p value
Gender – male vs female (%)	49.0	49.0	49.0	1.000
Age	45.2 ± 8.1	45.3 ± 8.1	45.2 ± 8.0	0.916
Smoking – yes (%)	18.5	18	19	0.856
Mean probing pocket depth (mm)	1.93 ± 0.73	1.89 ± 0.74	1.96 ± 0.72	0.502

Table 2. Descriptive statistics of the comparisons of post-treatment Probing Pocket Depth (PPD) results to baseline for each treatment (between and within groups analysis)

	Group	Mean (mm)	SD	p value[†]
PPD at baseline	AGE (n = 91)	1.8905	0.7356	0.502
	Placebo (n = 91)	1.9596	0.716	
	Total (n = 182)	1.9321	0.7248	
PPD at 12-month	AGE (n = 91)	1.092	0.5591	<0.001
	Placebo (n = 91)	1.6029	0.5621	
	Total (n = 182)	1.346	0.615	
PPD at 18-month	AGE (n = 91)	1.0656	0.4944	<0.001
	Placebo (n = 91)	1.5005	0.5155	
	Total (n = 182)	1.2818	0.4587	
p value	<0.001 for AGE group			
Within group	Not significant for placebo group			

[†]Between groups.

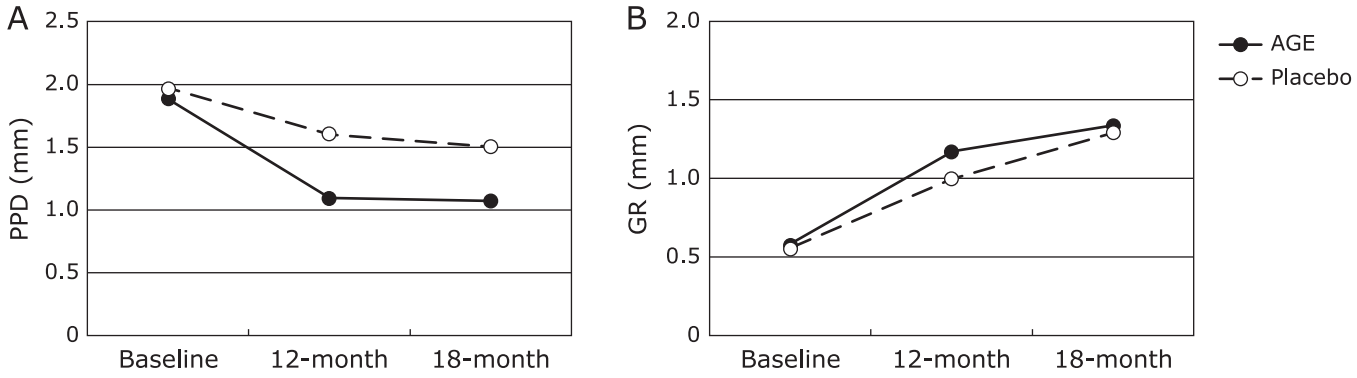

Fig. 3. A comparison of post-treatment scores. The value were adjusted for baseline of Probing Pocket Depth Scores (A), and Gingival Recession Scores (B) of AGE group versus Placebo group from baseline to the final examination at 18 month.

Table 3. Multiple logistic regression analysis for Probing Pocket Depth (PPD) adjusted for gender, smoking, baseline PPD and study groups

	Unstandardized coefficients		Standardized coefficients	*t*	Significance	95.0% confidence interval for B	
	B	SE	Beta			Lower bound	Upper bound
(Constant)	0.673	0.13		5.162	<0.001	0.416	0.931
Mean PPD1*	0.451	0.046	0.551	9.846	<0.001	0.36	0.541
Gender	–0.044	0.061	–0.04	–0.717	0.475	–0.165	0.077
Smokes	0.194	0.083	0.132	2.352	0.02	0.031	0.358
Groups	–0.438	0.061	–0.4	–7.174	<0.001	–0.559	–0.318

*PPD at baseline.

of AGE to inhibit the progression of periodontitis at its early to mild stages.

Periodontal disease is an infection of the oral gum tissue that holds one's teeth in place. It is typically caused by poor oral hygiene that allows bacteria to form the sticky film, known as dental plaque, to build on the teeth and to later develop into calculus.[21] Periodontitis affects the bone supporting gum tissue, and is characterized by the formation of pockets or "spaces" between the tooth and the gums. Periodontal disease is thought to be often associated with various systemic diseases such as diabetes, hypertension, atherosclerosis, and others.[22] The growing evidence for the correlation between periodontal disease and some systemic diseases points the need for the preventive measures for periodontitis. It is well known that there are some mechanical or chemical means that are easy to take and effective to a certain extent. These include tooth brushing, use of various toothpastes, rinses such as chlorhexidine and ozone, and other primary prevention measures.[23] However, no effective supplements taken orally have been found for preventing periodontitis up to now.

In this study, the PPD of the AGE group was found to be significantly lower in comparison to the placebo group. The results demonstrated that AGE is effective in preventing the progression of periodontal disease. We also found some improvement of PPD resulting from consumption of placebo. This has been often the case in clinical studies because the subjects take better care of their teeth daily by receiving toothpastes and toothbrushes in addition to the regular checkup and session with a dental hygienist.

We demonstrated the oral intake of AGE tablets to be effective as a preventive measure of periodontitis. There have been an increasing amount of data indicating the beneficial effect of AGE on many systemic diseases such as diabetes, hypertension, atherosclerosis, arterial elasticity, endothelial dysfunction, and etc.[16,24–26] The present study adds another important piece of information to the list of AGE effect, i.e., an effective prevention of the oral cavity disease that may also help reduce the global burden of chronic diseases. However, it is important to carry out some additional studies to determine its accurate dosage and duration of usage and above all to investigate the basic mode of its action.

With regard to the mechanism of AGE action to improve the periodontal diseases, it is noteworthy that AGE contains various sulfur compounds such as *S*-allylcysteine (SAC), *S*-1-propenylcysteine (S1PC) and *S*-allylmercaptocysteine (SAMC) that possess antioxidant and biological activities. The antioxidant property of these substances may partly contribute to the effect of AGE on periodontitis as the importance of salivary protection for oxidative stress has been shown to keep the oral health conditions.[27,28] In addition to the antioxidant property, SAC, S1PC and SAMC have been shown to help improve the peripheral circulation, reduce inflammation, and enhance immunological potency, that may all contribute to the beneficial effect of AGE on periodontitis.[29–33] Most recently, Ohtani and Nishimura[34] showed that S1PC, SAC and SAMC act together to inhibit LPS-induced inflammation in human gingival epithelial cells in culture by suppressing intercellular adhesion molecule-1 expression and IL-6 secretion. In view of the growing knowledge concerning with the causal association of periodontal diseases with some important systemic diseases, the oral benefit of AGE demonstrated in this study could be extended for general health promotion through enhancement of the bridge between density and medicine.

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Conflict of Interest

The authors declare that they were independent and with no conflict of interest in all stages of the research, including the protocol preparation, data collection, and data analysis.

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