Efficacy of aged garlic extract on periodontal pockets: An 18-month dose response study

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Abstract. The present 18-month study assessed the long-term effects of aged garlic extract (AGE) on deep periodontal pockets in generally healthy adults with moderate-to-severe periodontal pockets. The present study was a randomized, controlled, examiner-blind trial, involving four parallel treatment groups, including three AGE dosage groups and a placebo (control) group, with 300 participants in total. Study participants were instructed to use the AGE products at home throughout the study, following both written and verbal guidelines provided at the start. Periodontal assessments, including periodontal measurements, were performed at baseline and at 6, 12 and 18 months. After 18 months, a significant reduction in pocket depth was observed in the AGE groups compared with that of the placebo group, indicating a dose-response trend. In a multiple linear regression analysis, the baseline pocket depth, smoking status and the amount of AGE consumed on a daily basis were all demonstrated to be predictors of pocket depth. The results of the present study indicate that AGE is effective in improving oral health and further emphasize the potential role of AGE in promoting overall health due to the link between periodontitis and systemic diseases such as diabetes, hypertension and atherosclerosis.

Introduction

Periodontal diseases present a notable challenge in oral health management, demanding continual exploration and assessment of potential therapeutic interventions. Periodontal disease is characterized by several different forms, with two of the more common forms being gingivitis and periodontitis (1), as well as a number of symptoms, including deep periodontal pockets (2). Increasing evidence indicates that systemic conditions, including cardiovascular disease and diabetes, are connected to periodontal diseases (3).

Several studies have assessed plant-based components, including garlic, for preventing and treating periodontal diseases, and they have reported that herbal extracts and polyphenols are effective when used as mouthwashes or toothpastes in oral care (4). Garlic is a plant in the Allium family, which has historically been used throughout the world for centuries as a remedy, including by the Ancient Greeks, Romans, Chinese, Egyptians and Japanese (5). Aged garlic extract (AGE) is an odorless compound derived from the prolonged extraction of fresh garlic at room temperature (6). It is available both biologically and commercially, and has exhibited substantial biological activity in both animals and humans, with numerous studies exploring its potent antioxidant capabilities and its possible role in promoting health-by reducing oxidative stress (7), prevention of cardiovascular disease (8), protection from oxidative injury and pathogenesis of some diseases (9).

The results of our previous study demonstrated that regular intake of AGE can improve oral health by markedly reducing gingival inflammation and bleeding (10). Furthermore, AGE is potentially effective in treating periodontitis in the long term by decrease measure of periodontitis such as probing pocket depth (PPD) (11). The present study aimed to evaluate the impact of AGE on periodontal pockets and assess the potential benefits of AGE in the treatment of periodontitis.

Patients and methods

Study design. The present study was a randomized, controlled, examiner-blind trial with four parallel treatment groups. The study was performed from April 2021 to September 2022 at the Faculty of Dental Medicine of the Hebrew University and Hadassah Ein Kerem Hospital (Jerusalem, Israel). A comprehensive screening process was employed to enroll 300 generally healthy adult volunteers presenting with moderate to severe periodontal pockets. Participants were classified and randomly assigned in equal numbers to three treatment groups with varying doses of AGE, or to a control group (placebo).

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The clinical protocol was reviewed and approved by the Helsinki committee of Hadassah Hospital (Jerusalem, Israel; approval no. HMO-0536-20). Prior to screening, participants were required to review and sign an informed consent form, after which they were provided with a signed copy.

Participants. Statistical power calculations were performed and a total sample size of 256 participants was computed for the completion of the study, with 64 participants assigned to each group. There was ≥90% power to detect a statistically significant difference between treatment groups, estimating a mean difference of 7 bleeding sites and a standard deviation of 14. These power calculations utilized a 2-sided 5% significance level. A suitable number of participants were screened to enroll ~300 generally healthy adult volunteers with mild to severe periodontitis with ≥3 eligible periodontal pockets sites and a PPD of 3.5-6 mm. The participants were divided into four groups of 75 each, to account for a projected dropout rate of ≤15%.

The key inclusion criteria were as follows: Aged 30-60 years old; overall favorable health (assessed through a health questionnaire); ≥16 natural teeth (excluding third molars); facial and lingual surfaces suitable for scoring; ≥20 bleeding sites [sum of sites with a score of 1 or 2 on the Gingival Bleeding Index (GBI) (12)]; ≥3 periodontal pocket sites with a PPD of 3.5-6 mm; and agreement to postpone any elective dental procedures until the conclusion of the study, adhere to the study protocols and attend all scheduled visits. The exclusion criteria were as follows: Signs of advanced periodontal disease, such as purulent discharge, widespread tooth mobility or severe gum recession; current active periodontitis treatment; medical conditions necessitating antibiotic prophylaxis before dental procedures; presence of fixed orthodontic appliances on the facial or lingual surfaces; use of removable partial dentures; use of antibiotics, chlorhexidine or anti-inflammatory drugs within 2 weeks prior to the initial visit; pregnancy; recent dental prophylaxis within 2 months before the screening visit; presence of any condition likely to compromise the ability of the participant to safely complete the study.

Examination data. Participants were categorized and randomly distributed in equal proportions between the regimen groups and the control group, considering factors such as sex, age, tobacco consumption, baseline mean of PPD of above or below 2 mm and mean GBI of above or below 0.44. The study coordinator performed the random allocation of subjects to one of the treatment groups using an encoded program. This allocation process, along with the distribution of test products, was performed in a controlled environment to ensure the blinding of the examiner to the identity of the test products.

Procedure. Participants were instructed to use the provided products at home throughout the study period, following both the written and verbal guidelines provided at the time of distribution. Oral soft tissue examinations and periodontal measurements were performed on participants at baseline and at months 6, 12 and 18, with the exception of PPD, which was not assessed at the 6-month visit.

The study and control groups were given the following instructions: Group A, two AGE tablets daily with a meal twice a day; Group B, three AGE tablets daily with a meal twice a day; Group C, four AGE tablets daily with a meal twice a day; and Group D, four placebo tablets daily with a meal twice a day.

Materials. The provided products were AGE tablets (Kyolic®; reserve formula; Wakunaga Pharmaceutical, Co., Ltd.) containing 300 mg AGE powder per tablet or placebo tablet (Microcrystalline Cellulose; Agar Powder; Hydroxypropyl Methylcellulose Carboxymethylcellulose Calcium). AGE was produced under a license granted by the Ministry of Health, Labor and Welfare of Japan, following a series of steps: Organically cultivated raw garlic (*Allium sativum* L.) was sliced, soaked in aqueous ethanol and aged at ambient temperature. The process and specifications adhered to the garlic fluid extract monograph outlined in the US Pharmacopeia/Natural Formula. S-allyl-cysteine (SAC) was used as the quality control standard of garlic extract. The content of SAC was defined as ≥0.05% per dried basis of the garlic extract.

All groups received a supra-gingival dental prophylaxis every 6 months in accordance with local guidelines and standards. Products were replenished approximately every 6 months after the baseline visit.

Participant and clinical guidelines. Throughout the study, participants showing signs of advancing periodontal disease (such as a ≥3 mm increase in pocket depth, attachment loss or recession) were withdrawn from the study and were provided treatment based on local protocols and standards. Participants who missed a single examination (excluding the first or final visit) were still considered as members of the study group. Subjects were instructed to avoid all oral hygiene activities for 4 h before each examination. Additionally, they were asked to abstain from consuming medicated lozenges, breath mints, food or beverages (except water), as well as from smoking or chewing gum for 4 h prior the visit.

Clinical observations excluded teeth that had crowns, extensive restorations covering ≥50% of the tooth surface, bridges, orthodontic devices or implants. In the end, the participant cohort consisted of 138 men (53%) and 123 women (47%) (median age, 46 years; age range, 29-61 years).

GBI. GBI was assessed using lightly air-dried gums and a periodontal probe with a 0.5 mm diameter tip, following the established GBI protocol as described by Saxton and Van Der Ouderaa (12). GBI full-mouth score was calculated by summing the sites scores and dividing by the total number of scorable sites. Bleeding sites were identified as those with a GBI score of either 1 or 2.

PPD. PPD at each site was measured in mm using a World Health Organization Community Periodontal Index probe as the distance from the gingival margin to the apical end of the pocket. Measurements were rounded to the nearest mm. The full-mouth PPD score was determined by summing all site measurements scores and dividing by the total number of scorable sites assessed.

Data analysis. Demographic data, along with baseline and post-treatment scores, were calculated for each treatment group

Table I. Randomization of the treatment groups.

		N	Mean	Standard deviation	P-value
Age	Placebo	76	44.96	8.75	0.86
	AGE-4 tablets	79	45.47	8.78	
	AGE-6 tablets	76	45.51	8.81	
	AGE-8 tablets	80	46.12	7.58	
	Total	311	45.52	8.46	
Baseline GBI	Placebo	75	0.82	0.38	0.92
	AGE-4 tablets	76	0.78	0.38	
	AGE-6 tablets	75	0.80	0.42	
	AGE-8 tablets	76	0.81	0.43	
	Total	302	0.81	0.40	
Baseline PPD	Placebo	75	2.22	0.44	0.97
	AGE-4 tablets	76	2.19	0.48	
	AGE-6 tablets	75	2.21	0.37	
	AGE-8 tablets	76	2.22	0.47	
	Total	302	2.21	0.44	

AGE, aged garlic extract; GBI, gingival bleeding index; PPD, probing pocket depth.

and visit. For each efficacy variable and visit, paired t-tests were performed to compare mean values to those recorded at screening for each group. Linear regression analysis was used to identify predictors for improved periodontal status. All statistical tests were two-tailed and $P<0.05$ was considered to indicate a statistically significant difference. The analyses were performed using SPSS software (version 27.0; IBM Corp.).

Results

A total of 311 individuals were screened, of which 302 met the inclusion criteria. By the end of the 18-month study, 261 participants completed the trial, reflecting an attrition rate of 14%. The baseline randomization of the treatment groups is presented in Table I. No significant differences were observed between the AGE and control groups with regards to sex, age, tobacco usage, mean GBI or mean PPD (Figs. 1 and 2, respectively). Male participation was 49%, with a mean age of 45.6±8.5 years. Tobacco use was reported at 23.5%, baseline mean GBI was 0.81±0.40 (P=0.917) and baseline mean PPD was 2.21±0.44 (P=0.974).

The GBI results over the 18-month study period are presented in Table II and Fig. 3. There were no statistically significant differences in GBI scores between the AGE groups and the placebo group at the 6-, 12- and 18-month evaluations. A multiple linear regression analysis was performed to evaluate the final GBI scores adjusted by age, sex, tobacco use, baseline GBI score and study group (Table III). The regression model identified baseline GBI scores ($P<0.001$) and tobacco use (P=0.031) as the only variables significantly associated with the final GBI levels.

The PPD results over the 18-month study period are summarized in Table IV and Fig. 4. At 18 months, a statistically significant trend emerged, with the AGE groups showing significant differences in pocket depth scores compared with that of the placebo group. The final mean pocket depth was 1.6±0.33 (P=0.023 vs. placebo) for the group taking 4 AGE tablets per day (Group A), 1.59±0.33 (P=0.022 vs. placebo) for the 6-tablet group (Group B) and 1.55±0.34 (P=0.005 vs. placebo) for the 8-tablet group (Group C). These values were significantly lower than the final mean pocket depth of the placebo group (1.72±0.41; P=0.003 for the comparison of all AGE groups to placebo).

A multiple linear regression analysis was performed to assess the PPD, adjusted by age, sex, tobacco use, baseline PPD and study group (Table V). The regression model identified three variables as statistically significant predictors of the final PPD levels: Baseline PPD scores ($P<0.001$), tobacco use (P=0.044) and the daily dose of AGE consumed (P=0.012). Participants in the AGE groups demonstrated a marked reduction in PPD scores, with a mean decrease of ~0.15 units.

Discussion

Periodontitis, a chronic inflammatory condition, is the leading cause of tooth loss among adults. It represents a major oral health issue notably contributing to the global burden of chronic diseases (13). Severe periodontitis impacts ~11% of the adult population, ranking as the sixth most widespread disease worldwide and posing a substantial public health challenge (1,14). Numerous studies have suggested a potential link between periodontitis and several conditions, including diabetes, atherosclerotic vascular disease, rheumatoid arthritis, adverse pregnancy outcomes, obesity and Alzheimer's disease. Additionally, periodontitis markedly deteriorates oral health, impacting quality of life and self-esteem (11,13). AGE has been reported to offer a wide range of advantages, including antimicrobial properties and cardio-protective, anticancer

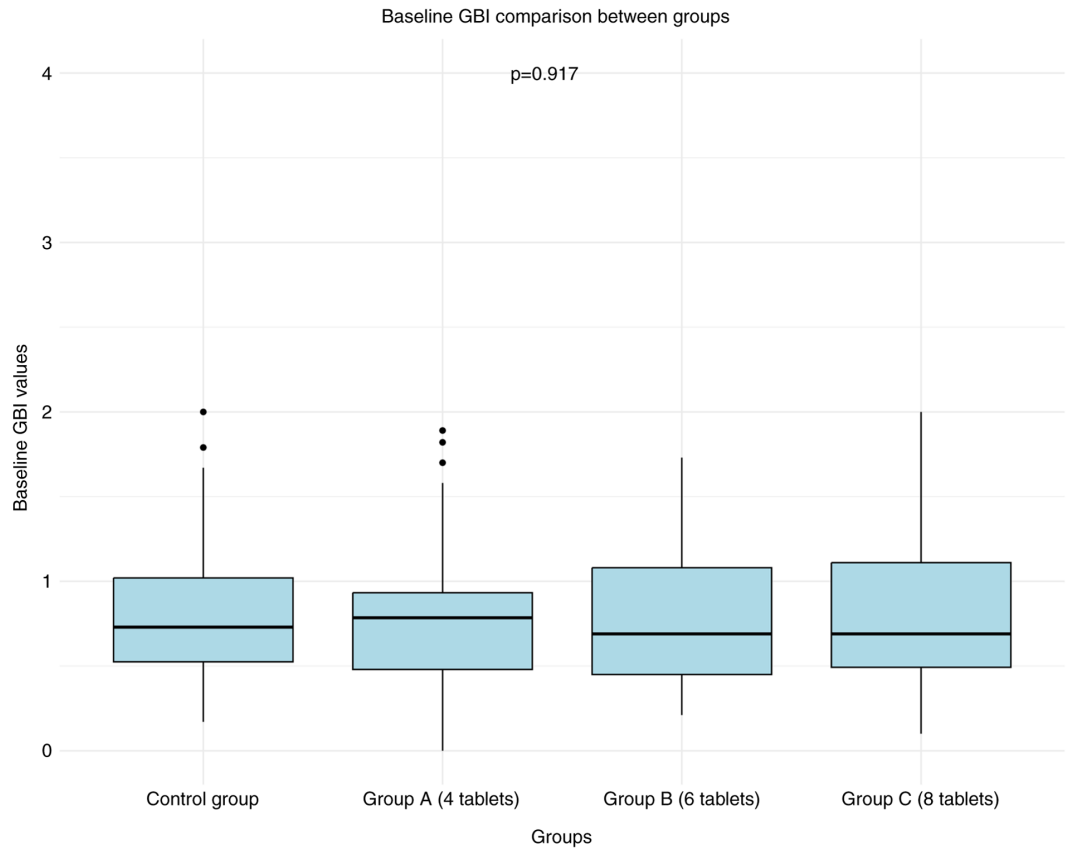

Figure 1. Baseline GBI comparison between groups. Box plots represent the GBI values for the control group and aged garlic extract groups (Group A: 4 tablets, Group B: 6 tablets, Group C: 8 tablets). P=0.917, indicating no significant differences between the groups at baseline. GBI, gingival bleeding index.

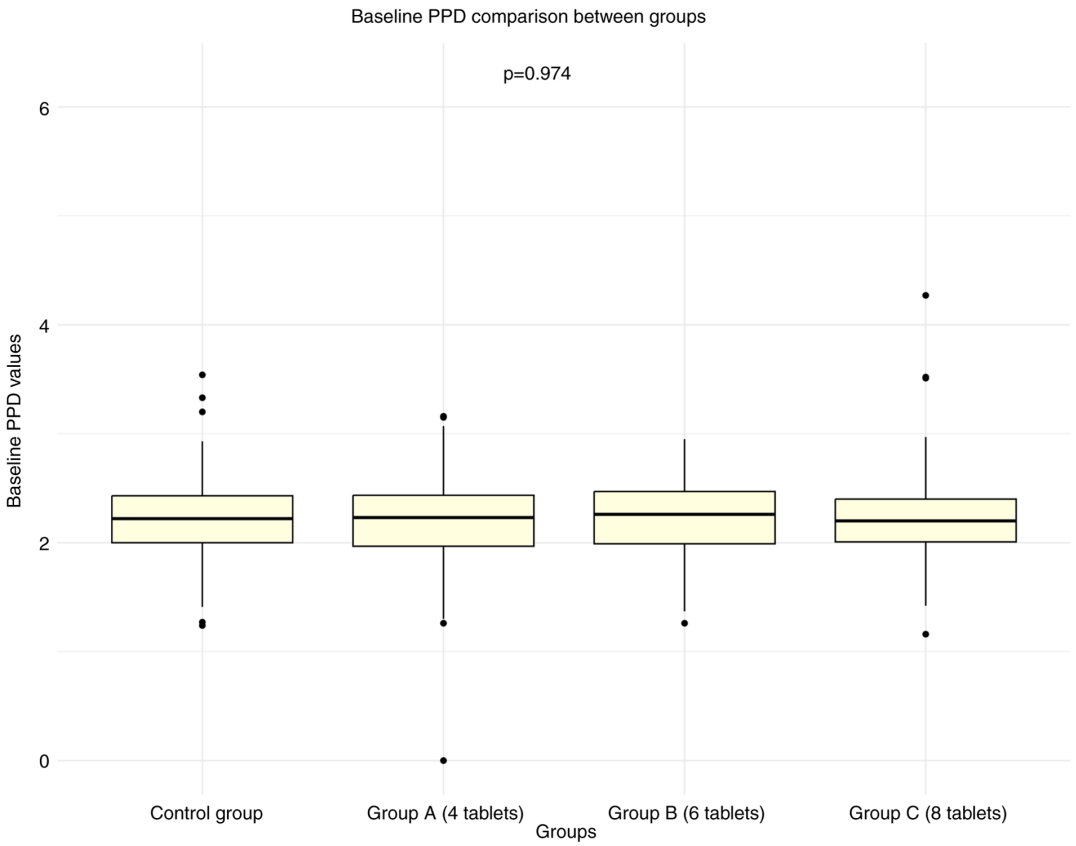

Figure 2. Baseline PPD comparison between groups. Box plots represent the PPD values for the control group and AGE groups (Group A: 4 tablets, Group B: 6 tablets, Group C: 8 tablets). P-value of 0.974, indicating no significant differences between the groups at baseline. PPD, probing pocket depth.

Table II. GBI data.

		N	Mean	Standard deviation	P-value
Baseline data	Placebo	75	0.83	0.38	0.92
	AGE-4 tablets	76	0.78	0.38	
	AGE-6 tablets	75	0.80	0.42	
	AGE-8 tablets	76	0.82	0.43	
	Total	302	0.81	0.40	
GBI 6 months data	Placebo	63	0.65	0.40	0.48
	AGE-4 tablets	63	0.63	0.36	
	AGE-6 tablets	62	0.67	0.42	
	AGE-8 tablets	63	0.65	0.40	
	Total	251	0.65	0.40	
GBI 12 months data	Placebo	62	0.51	0.39	0.13
	AGE-4 tablets	64	0.44	0.28	
	AGE-6 tablets	63	0.48	0.32	
	AGE-8 tablets	65	0.45	0.29	
	Total	254	0.47	0.32	
GBI 18 months data	Placebo	64	0.42	0.34	0.12
	AGE-4 tablets	65	0.33	0.30	
	AGE-6 tablets	66	0.41	0.30	
	AGE-8 tablets	66	0.34	0.26	
	Total	261	0.37	0.30	

AGE, aged garlic extract; GBI, gingival bleeding index.

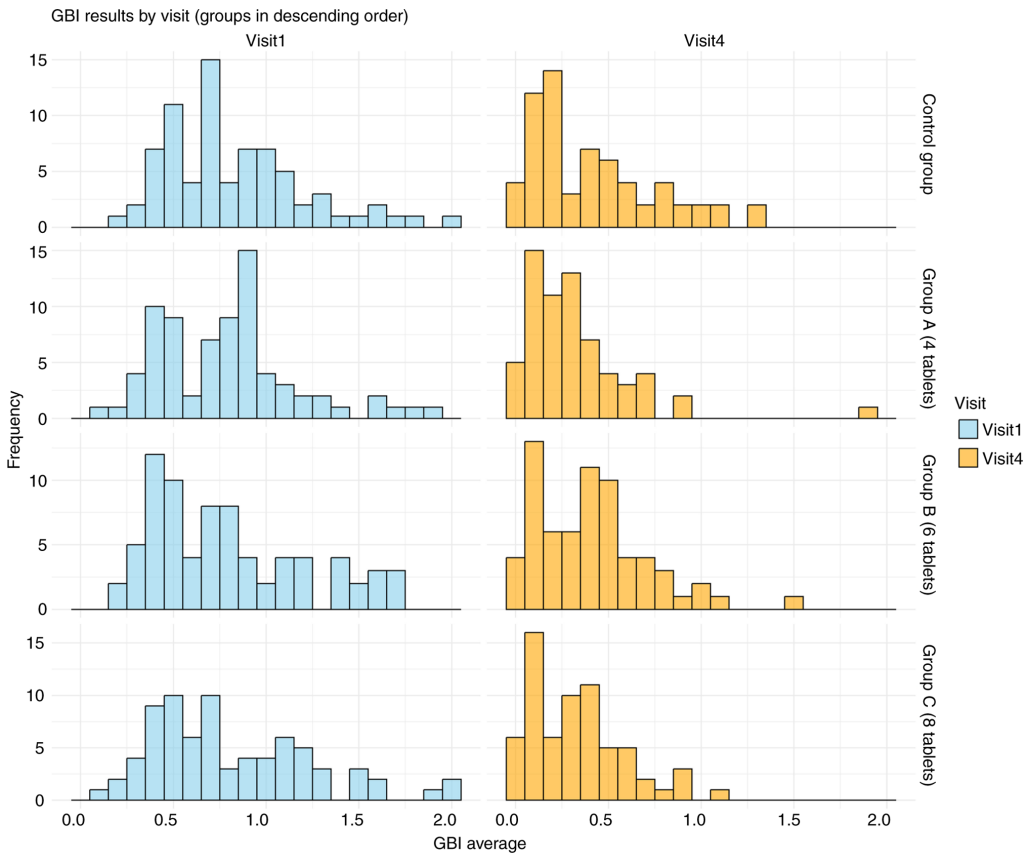

Figure 3. GBI data across visits. The histogram illustrates the effect of aged garlic extract tablets throughout the study period, showing a reduction in GBI values over time. GBI, gingival bleeding index.

Table III. GBI regression analysis.

	Coefficients unstandardized		Standardized coefficients		
	B	Standard error	Beta	t	Sig.
Constant	0.15	0.12	N/A	1.22	0.23
Groups	-0.02	0.02	-0.06	-0.99	0.32
Age	<0.01	<0.01	0.02	0.35	0.72
Smoking	-0.08	0.04	-0.12	-2.17	0.03
Sex	0.03	0.03	0.05	0.84	0.41
Baseline GBI	0.35	0.04	0.47	8.48	<0.01

GBI, gingival bleeding index; N/A, not applicable.

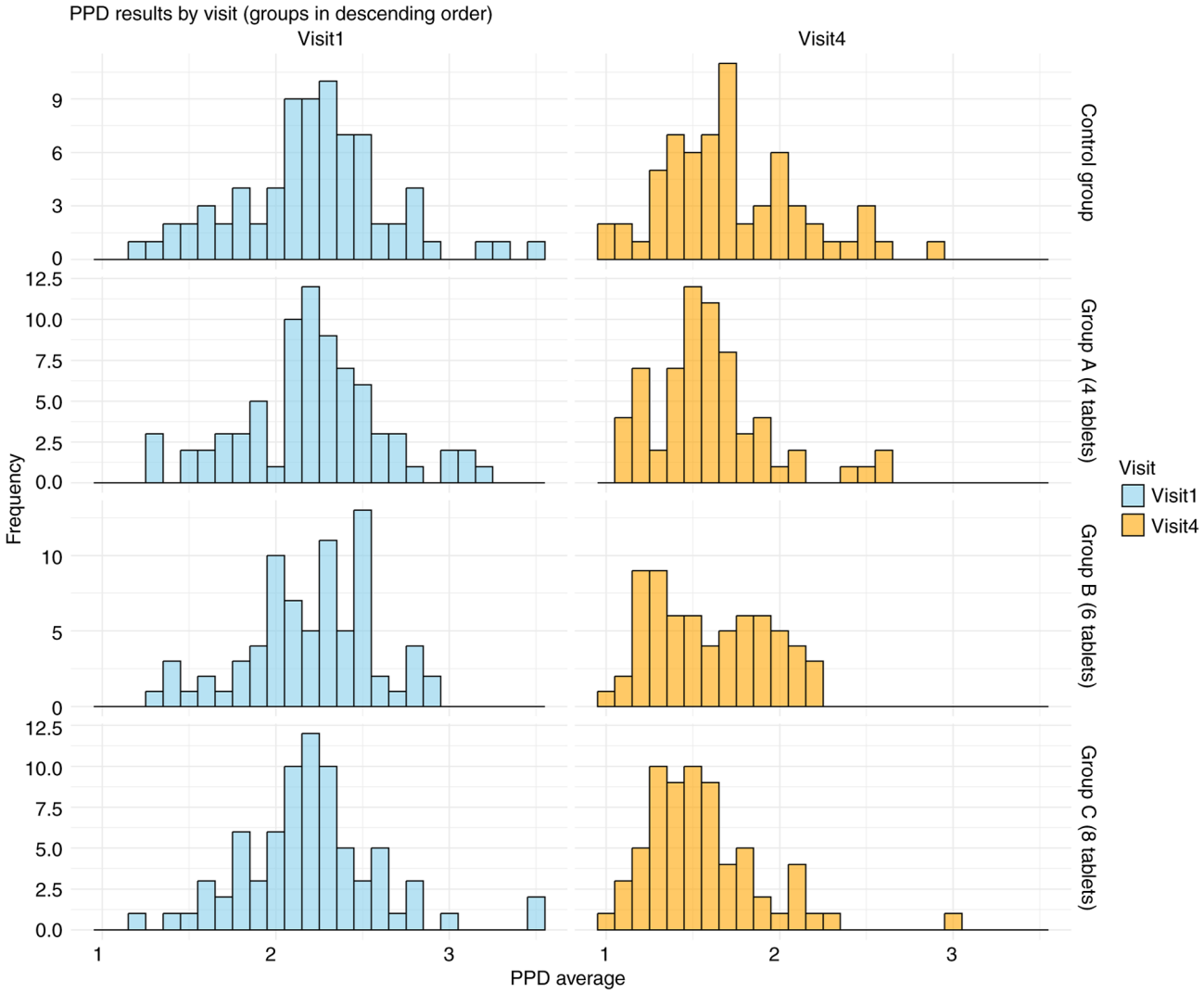

Figure 4. PPD data across visits. The histogram illustrates the effect of aged garlic extract tablets throughout the study period, showing a reduction in PPD values over time. PPD, probing pocket depth.

and anti-inflammatory effects (15). Previous meta-analysis findings demonstrated its ability to reduce blood pressure in individuals with hypertension and indicated its potential benefits for Alzheimer's disease (16). Moreover, a previous review on the preventive and therapeutic use of plant-based ingredients for treating periodontal diseases emphasized the efficacy of herbal extracts and polyphenols when used as mouthwashes or dentifrices for the oral cavity (4). In particular, two previous

Table IV. Probing Pocket Depth data.

		N	Mean	Standard deviation	P-value
Baseline data	Placebo	75	2.22	0.44	0.97
	AGE-4 tablets	76	2.19	0.48	
	AGE-6 tablets	75	2.21	0.37	
	AGE-8 tablets	76	2.22	0.47	
	Total	302	2.21	0.44	
12 months data	Placebo	62	1.60	0.44	0.73
	AGE-4 tablets	64	1.53	0.42	
	AGE-6 tablets	63	1.54	0.34	
	AGE-8 tablets	65	1.53	0.40	
	Total	254	1.55	0.40	
18 months data	Placebo	64	1.72	0.41	<0.01
	AGE-4 tablets	65	1.60	0.33	
	AGE-6 tablets	66	1.59	0.33	
	AGE-8 tablets	66	1.56	0.34	
	Total	261	1.62	0.36	

Table V. PPD regression analysis.

	Coefficients unstandardized		Standardized coefficients		
	B	Standard error	Beta	t	Sig.
Constant	0.97	0.18	N/A	5.38	<0.01
Groups	-0.05	0.02	-0.14	-2.54	0.01
Age	0.00	<0.01	-0.01	-0.18	0.86
Smoking	-0.10	0.05	-0.12	-2.03	0.04
Sex	0.01	0.04	0.01	0.21	0.84
Baseline PPD	0.38	0.05	0.44	7.64	<0.01

PPD, probing pocket depth; N/A, not applicable.

studies evaluated the effectiveness of AGE in treating periodontal diseases. The first reported that a daily intake of AGE improves oral health by reducing gingival inflammation and gingival bleeding (10), whilst the second reported that AGE is effective in preventing periodontitis (11).

AGE serves as a beneficial supplement for the prevention and management of periodontal disease. Based on evidence from previous studies, it is hypothesized that SAC, S-1-propenylcysteine (S1PC™; Wakunaga Holdings Co., Ltd., Japan) and S-allyl-mercapto-cysteine (SAMC) are the active components in AGE. SAC, S1PC and SAMC have been reported to suppress inflammatory responses induced by TNF-α in human gingival epithelial cells (17). Additionally, S1PC has been reported to inhibit the expression of matrix metalloproteinase-1 induced by lipopolysaccharides from *Porphyromonas gingivalis* in human gingival fibroblasts (18). AGE beneficial effect on periodontitis was also demonstrated in a previous randomized controlled trial in which the importance of performing further research to determine the exact duration and appropriate dosage for its use was emphasized (11). Therefore, the present 18-month dose response clinical study, which evaluated the efficacy of AGE on periodontal pockets, aimed to achieve this.

Following baseline clinical examination, the present study performed assessments after 12 and 18 months, and the results revealed that 18 months of daily consumption of the product was associated with a marked improvement in periodontal health in comparison with a placebo. Furthermore, regarding the daily dose of tablets, four regimens were utilized: i) Four AGE tablets per day; ii) six AGE tablets per day; iii) eight AGE tablets per day; and iv) four placebo tablets per day. The results of the present study revealed that the PPD of the three AGE groups was significantly reduced compared with that of the placebo group, with a dose response trend. Furthermore, a daily dose of only four tablets yielded a significant periodontal health improvement, compared with that of the placebo.

However, a notable limitation of the present study was the inability to monitor participant compliance to the assigned

regimens or their consumption patterns at home. Whilst participants were instructed on proper usage, individual variability in compliance may have influenced the outcomes.

In conclusion, in addition to strengthening the already well-established benefits of AGE for oral health, the present research provides research-based evidence for the use of AGE as a mean of promoting public health. Furthermore, the present study provides practical, valid and important data concerning the effective duration of usage and dosage of AGE, thereby tackling the challenge of dealing with chronic diseases, which is a common practice and interest of dentistry, medicine and public health.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

AZ and YV initiated and designed the study concept, and confirm the authenticity of all the raw data. AZ, LZ, HG and YV participated in data collection and manuscript writing. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The clinical protocol was reviewed and approved by the Helsinki committee of Hadassah Hospital (Jerusalem, Israel; approval no. HMO-0536-20). Prior to screening, participants were required to review and sign an informed consent form.

Patient consent for publication

Not applicable.

Competing interests

Financial support was received from Wakunaga Pharmaceutical Co., Ltd.

References

1. Mann J, Bernstein Y and Findler M: Periodontal disease and its prevention, by traditional and new avenues. Exp Ther Med 19: 1504-1506, 2020.
2. Kim J and Amar S: Periodontal disease and systemic conditions: A bidirectional relationship. Odontology 94: 10-21, 2006.
3. Bui FQ, Almeida-da-Silva CLC, Huynh B, Trinh A, Liu J, Woodward J, Asadi H and Ojcius DM: Association between periodontal pathogens and systemic disease. Biomed J 27-35, 2019.
4. Ohtani M and Nishimura T: The preventive and therapeutic application of garlic and other plant ingredients in the treatment of periodontal diseases. Exp Ther Med 19: 1507-1510, 2020.
5. Petrovska BB and Cekovska S: Extracts from the history and medical properties of garlic. Pharmacogn Rev 4: 106-110, 2010.
6. Amagase H, Petesch BL, Matsuura H, Kasuga S and Itakura Y: Intake of garlic and its bioactive components. J Nutr 131: 955S-962S, 2001.
7. Ide N and Lau BH: Garlic compounds minimize intracellular oxidative stress and inhibit nuclear factor-kappa B activation. J Nutr 131: 1020S-1026S, 2001.
8. Morihara N, Sumioka I, Moriguchi T, Uda N and Kyo E: Aged garlic extract enhances production of nitric oxide. Life Sci 71: 509-517, 2002.
9. Kim KM, Chun SB, Koo MS, Choi WJ, Kim TW, Kwon YG, Chung HT, Billiar TR and Kim YM: Differential regulation of NO availability from macrophages and endothelial cells by the garlic component S-allyl cysteine. Free Radic Biol Med 30: 747-756, 2001.
10. Zini A, Mann J, Mazor S and Vered Y: The efficacy of aged garlic extract on gingivitis-a randomized clinical trial. J Clin Dent 29: 52-56, 2018.
11. Zini A, Mann J, Mazor S and Vered Y: Beneficial effect of aged garlic extract on periodontitis: A randomized controlled double-blind clinical study. J Clin Biochem Nutr 67: 297-301, 2020.
12. Saxton CA and van der Ouderaa FJ: The effect of a dentifrice containing zinc citrate and Triclosan on developing gingivitis. J Periodontal Res 24: 75-80, 1989.
13. Eke PL, Borgnakke WS and Genco RJ: Recent epidemiologic trends in periodontitis in the USA. Periodontol 2000 82: 257-267, 2020.
14. Dumitrescu AL: Editorial: Periodontal disease-a public health problem. Front Public Health 3: 278, 2016.
15. Wang HP, Yang J, Qin LQ and Yang XJ: Effect of garlic on blood pressure: A meta-analysis. J Clin Hypertens (Greenwich) 17: 223-231, 2015.
16. Rohner A, Ried K, Sobenin IA, Bucher HC and Nordmann AJ: A systematic review and metaanalysis on the effects of garlic preparations on blood pressure in individuals with hypertension. Am J Hypertens 28: 414-423, 2015.
17. Ohtani M and Nishimura T: Sulfur-containing amino acids in aged garlic extract inhibit inflammation in human gingival epithelial cells by suppressing intercellular adhesion molecule-1 expression and IL-6 secretion. Biomed Rep 12: 99-108, 2020.
18. Nango H and Ohtani M: S-1-propenyl-L-cysteine suppresses lipopolysaccharide-induced expression of matrix metalloproteinase-1 through inhibition of tumor necrosis factor-α converting enzyme-epidermal growth factor receptor axis in human gingival fibroblasts. PLoS One 18: e0284713, 2023.