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Pharmacological Study

An *in-vitro* evaluation of the efficacy of garlic extract as an antimicrobial agent on periodontal pathogens: A microbiological study

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Abstract

With the rise in bacterial resistance to antibiotics, there is considerable interest in the development of other classes of antimicrobials for the control of infection. Garlic (*Allium sativum* Linn.) has been used as medicine since ancient times and has long been known to have antibacterial, antifungal, and antiviral properties. This study was undertaken to assess the inhibitory effect of garlic on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans,* to assess the time-kill curve of *P. gingivalis* and *A. actinomycetemcomitans,* and to determine the antiproteolytic activity of garlic on *P. gingivalis*. Ethanolic garlic extract (EGE) and aqueous garlic extract (AGE) were prepared and the inhibitory effects of these extracts for two periodontal pathogens (*P. gingivalis* and *A. actinomycetemcomitans*) were tested. Antiproteolytic activity on protease of *P. gingivalis* was determined. 25 microliter (µl), 50 µl, and 75 µl of AGE showed 16 mm, 20 mm, and 25 mm zone of inhibition, respectively, on *P. gingivalis*. The AGE showed greater bacteriostatic activity against the *P. gingivalis* with minimum inhibitory concentration determined at 16.6 µl/ml. The time-kill assay of AGE and EGE were compared for *P. gingivalis* and *A. actinomycetemcomitans*. AGE showed better antiproteolytic activity on total protease of *P. gingivalis* compared to the EGE. Thus, the study concludes the antimicrobial activity of garlic extract against periodontal pathogens, *P. gingivalis, A. actinomycetemcomitans*. Its action against *P. gingivalis* includes inhibition of total protease activity, and this raises the possibility that garlic may have therapeutic use for periodontitis and possibly other oral infections.

Key words: *Aggregatibacter Actinomycetemcomitans*, antimicrobial, garlic, *Porphyromonas gingivalis*

Introduction

The accumulation and maturation of bacterial plaque at the gingival margin is widely recognized as the primary etiological factor in the development of chronic periodontitis.[1] Human oral cavity is inhabited by more than 500 species of bacteria at 10^8-10^9 bacteria per milligram of dental plaque. A distinct difference exists between composition of supragingival and subgingival plaque.[2] Supragingival plaque exhibits accumulation of predominantly Gram-positive cocci, whereas subgingival plaque is characterized by flora predominated by Gram-negative anaerobic bacilli such as *Porphyromonas gingivalis* (*P. gingivalis*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), and *Fusobacterium nucleatum* and have shown to be associated with the onset and progression of periodontal disease.[3]

The most important goal of periodontal therapy is to reduce or eliminate the subgingival microorganisms associated with periodontal disease and to maintain the periodontal health. The current treatment for gingivitis and periodontitis is directed at disruption of plaque maturation and/or reduction of the bacterial load which usually includes professional and homecare mechanical methods for the removal of plaque.[3] Scaling and root planning is a traditional method that has been shown to be an effective treatment for chronic periodontitis. Although mechanical treatment significantly decreases the prevalence and levels of subgingival microorganisms, it does not necessarily eliminate all pathogens. Also, as the probing depth increases, the effectiveness of scaling and root planning

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decreases, leaving subgingival plaque and calculus on root surfaces. It has been shown that only pockets of less than 3 mm in depth can be maintained plaque-free by home care. Regular professional non-surgical care is required to maintain the stability of deeper pockets over the years.[4] To overcome these problems, surgical procedure, local and systemic antibiotics have been employed to facilitate the elimination of subgingival micro flora.[3]

Systemic antibiotics can be used along or in conjunction with conventional therapy to retard bacterial proliferation, but their prolonged use can result in the development of resistant strains.

With the rise in bacterial resistance to antibiotics, there is considerable interest in the development of other classes of antimicrobials for the control of infection. Garlic (*Allium sativum* L.) has been used as medicine since ancient times and has long been known to have antibacterial, antifungal, and antiviral properties.[5] It has been suggested that development of resistance to allicin arises 1000-fold less easily than it does to certain antibiotics.[6] Garlic, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders. It is cultivated practically throughout the world, appears to have originated in Central Asia and then spread to China, and the Mediterranean region before moving west to Central and Southern Europe, Northern Africa (Egypt), and Mexico.[7]

Indeed, allicin and garlic extract have been shown to have a wide spectrum antibacterial activity, including effects on *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Clostridium*, *Mycobacterium*, and *Helicobacter* species.[8,9]

In addition, certain oral streptococci and lactobacilli have been shown to be sensitive to garlic extract and a mouth wash containing garlic extract was more effective at reducing the total salivary bacterial count and the mutans streptococcal count.[10,11] However, there are no specific data on the effect of garlic on oral gram negative species and none on its effect on their enzymes. Hence, the present study was undertaken to assess the inhibitory effect of garlic on *P. gingivalis* and A. *actinomycetemcomitans*, to assess the time-kill curve of on *P. gingivalis* and A. *actinomycetemcomitans*, and to determine the antiproteolytic activity of garlic on *P. gingivalis*.

Materials and Methods

Preparation of garlic extract

Uniform homogenized paste of fresh garlic was used for the preparation of the extract.

Cold maceration method

Aqueous garlic extract

Crushed garlic paste of 250 g was mixed with 500 ml of double standard distilled water in a glass container to obtain a homogenous mix by stirring it occasionally for 4 days at 3-5°C. The mixture was then filtered and further centrifuged at 10,000 rpm for 20 min. The supernatant was filtered through a 0.2-mm pore size Wattman filter paper grade 1 to remove any impurities. Aliquots were stored at –20°C until required. The filtrate thus obtained was the aqueous garlic extract (AGE). This liquid extract was used for antimicrobial assay.

Ethanolic garlic extract

Crushed garlic paste of 250 g was mixed with 500 ml of ethyl alcohol in a glass container to obtain a homogenous mix by stirring it occasionally for 4 days at 3-5°C. The mixture was then filtered and further centrifuged at 10,000 rpm for 20 min. The supernatant was filtered through a 0.2-mm pore size Wattman filter paper grade 1 to remove any impurities.

Thus, the obtained alcoholic extract was concentrated by heating to evaporate ethyl alcohol and to obtain a homogenized paste. Ethanolic garlic extract (EGE) was semisolid, brown to black in color with a pungent smell. Aliquots were stored at –20°C until required. This paste was further subjected to antimicrobial assay.

Bacteria and growth condition

Stock culture of periodontal pathogens (*P. gingivalis* and A. *actinomycetemcomitans*) used in this study were obtained from the Department of Microbiology, Maratha Mandal Dental College, Belgaum.

Kanamycin blood agar was used to isolate *P. gingivalis*. Major ingredients included trypticase blood agar base with 5% sheep blood, supplemented with yeast extract, hemin, vitamin k_1, L-cysteine, and in addition, contained 100 mg/L of kanamycin.

Dentaid agar was used to isolate A. *actinomycetemcomitans*. Dentaid was prepared using brain heart infusion (BHI) agar to which 5 g of yeast extract, 1.5 g of sodium fumarate (Sigma Chemical Co., St. Louis, Mo.), and 1 g of sodium formate (Sigma) per liter were added. The medium was autoclaved for 15 min at 121°C. The final pH was estimated to be 7.2 ± 0.2. Once the medium was cooled to 50°C, vancomycin (Sigma) was added to a final concentration of 9 μg/ml.

Subculturing of *P. gingivalis* and A. *actinomycetemcomitans* was done by incubating then at 35-37°C for 48-72 h. The agar plates were inoculated, placed in the anaerobic jars, and incubated for 48 h, and re-incubated for another 2-4 days, so as to allow those slow-growing organisms to form colonies. *P. gingivalis* was formed as black and mucoid colonies. A. *actinomycetemcomitans* was formed as tiny, translucent colonies.

Agar diffusion procedure

Inoculum preparations

The colonies were transferred from the plates to the BHI broth with a sterilized straight nichrome wire. The turbidity was visually adjusted with BHI broth to equal that of a 0.5 MacFarland unit turbidity standard that has been freshly prepared. Alternatively, the suspension was standardized with a photometric device.

Inoculation of agar plate

After adjusting the inoculum to a 0.5 MacFarland unit turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. Entire surface of kanamycin blood agar plate was swabbed three times, rotating plates approximately 60° between streaking to ensure even distribution. The inoculated plate was allowed to stand for at least 3 min but no longer than 15 min before punching the wells in the agar plate.

A hollow tube of 5 mm diameter was taken and heated. It was pressed on the inoculated agar plate and removed immediately

after making a well in the plate. Likewise, three wells were made on each plate.

75 µl, 50 µl, and 25 µl of the 500 µl/ml of AGE and 500 µl/ml of EGE were added into the respective wells on each plate. The plates were incubated within 15 min of compound application for 18-24 h at 37°C anaerobically. The plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of the inhibition zone was measured to nearest whole millimeter by holding the calipers.

Minimum inhibitory concentration procedure

The minimum inhibitory concentration (MIC) of the AGE and EGE was determined by macrobroth dilution method.

One set of 1-10 tubes were labeled. 0.5 ml of BHI broth was added in tubes labeled from 2-10. One ml of the test material was taken in the first tube. 0.5 ml of the garlic extract from the first tube was transferred to second tube containing the BHI broth, mixed well, and 0.5 ml was serially transferred to the third tube, till the 9th tube. 0.5 ml was discarded from the 9th tube and the 10th tube served as a control.

Thus, garlic extract solutions were serially diluted and concentrations at 500, 250, 125, 62.5, 31.25, 16.6, 8.3, 4, and 2 mg/ml for EGE and µl/ml for AGE were obtained respectively. The tubes were then inoculated with 0.1 ml of cultures (10^7 cells). The lowest concentration of garlic extract that completely inhibited the growth of the organisms was considered as MIC.

Minimum bactericidal concentration procedure

To determine the minimum bactericidal concentration (MBC), the MIC dilution tubes, with no visible growth and the control tube were subcultured onto the respective media and incubated for 24 h anaerobically at 37°C and the colonies were counted on the next day.

The organisms grown from the control tube were then compared with the organism grown from the MIC test tubes.

The test was read as follows:

a. Similar number of colonies - indicating bacteriostatic activity only
b. Reduced number of the colonies - indicating a partial or slow bactericidal activity
c. No growth - if the whole inoculum has been killed.

MBC was carried out to observe the bactericidal effect of the garlic extract against the organism. If there is no growth of microorganisms, then the garlic extract is known to have bactericidal effect and if there is growth of microorganisms, then the garlic is known to have no bacteriostatic effect.

Growth kill curve

The dilutions were done in a manner similar to that of MIC. Then immediately it was plated and was noted at 0 h. The dilution tubes were kept in anaerobic jar for *P. gingivalis* and CO_2 jar for A. *actinomycetemcomitans*. At the end of 2 h again the first were plated. The same procedure was repeated after 4 h, 6 h, and 24 h. Then, the plates were incubated in either CO_2 jar as per the requirement. After 48 h of incubation at 37°C, the plates were removed and the colonies were counted.

Antiproteolytic activity of garlic on protease of *P. gingivalis*

Gelatin liquefaction test

Thioglycollate broth with 5% gelatin in distilled water was used for gelatin liquefaction test and this broth was inoculated with the test culture and garlic extract along with positive control (with microorganism) and negative control (without microorganism). It was incubated for 5 days at 37°C and the antiproteolytic activity of both AGE and EGE on protease of *P. gingivalis* were determined.

Statistical analysis

Mann–Whitney U-test was used to compare the time-kill assay of AGE and EGE for both the microorganisms.

Results

Antibacterial activity of garlic extracts on *P. gingivalis* and *A. actinomycetemcomitans* by Well diffusion method

The antibacterial activity of garlic on *P. gingivalis* and A. *actinomycetemcomitans* was done by well diffusion method. 25 microliter (µl), 50 µl, and 75 µl of AGE showed 16 mm, 20 mm, and 25 mm zone of inhibition respectively on *P. gingivalis*. However, there was no zone of inhibition seen for EGE on *P. gingivalis* [Table 1]. (25 µl, 50 µl, and 75 µl of) AGE and EGE did not show any zone of inhibition on A. *actinomycetemcomitans*.

Inhibitory effect of garlic extracts

Further, the antibacterial activity testing of the AGE and EGE of garlic by Macrobroth dilution revealed MIC and MBC at different concentrations of the garlic extract.

MIC and MBC of garlic extracts on P. gingivalis

The AGE exhibited MIC at 16.6 µl/ml. EGE did not exhibit the desired result in comparison to AGE, the MIC was higher at 62.5 mg/ml for EGE [Table 2]. In MBC, the AGE showed greater bacteriostatic activity against the *P. gingivalis*.

MIC and MBC of garlic extracts on A. actinomycetemcomitans

A. *actinomycetemcomitans* showed greater resistance to both the extracts. The MIC for the AGE was determined at 62.5 µl/ml, whereas A. *actinomycetemcomitans* was completely resistant to all the concentrations of the EGE [Table 3]. In MBC, the AGE showed greater bacteriostatic activity than EGE.

In comparison of A. *actinomycetemcomitans* to *P. gingivalis*, more susceptibility was shown by *P. gingivalis* to the garlic extracts. The study showed that even though garlic extract exhibited bacteriostatic activity, it did not exhibit any bactericidal activity.

Table 1: The antibacterial activity of aqueous garlic extract on *Porphyromonas gingivalis*

AGE (µl)	ZOI (mm)
25	16
50	20
75	25

AGE: Aqueous garlic extract, ZOI: Zone of inhibition

Time-kill assay

The time-kill assay was also performed on the periodontal pathogens included in the study, namely *P. gingivalis* and A. *actinomycetemcomitans* in the presence of garlic extracts at their respective MICs.

Killing of *P. gingivalis* was not apparent over the first 2 h of incubation, but a greater bacteriostatic activity was observed over following 2-6 h. The bacteriostatic activity was better in AGE; the colony count was comparatively less with the EGE. After that there was a steady increase in the colony forming units and there was no bacteriostatic activity at 24 h. Control cell suspensions without garlic extract showed no drop in viability over the same period.

The A. *actinomycetemcomitans* exhibited resistance to bactericidal activity of the garlic extracts. Bacteriostatic activity was observed only between 0-2 h of incubation period for both extracts. Later on, a steady increase in the colony count was observed till 24 h [Table 4].

The time-kill assay of AGE and EGE were compared for both the microorganisms. The comparisons analyzed by Mann–Whitney U-test showed statistical significance with *P. gingivalis* at 2 h ($Z = 1.99$, $P = 0.04$), 4 h ($Z = 1.96$, $P = 0.05$), and 6 h ($Z = 1.96$, $P = 0.05$). However, no statistical significance was seen between AGE and EGE on A. *actinomycetemcomitans*.

Antiproteolytic activity of garlic extracts on *P. gingivalis*

The data related to the effects of garlic extracts on *P. gingivalis* protease activity are presented in the Table 5.

At 500 μl/ml concentration of the AGE, the gelatin liquefaction was completely inhibited. At 125 μl/ml concentration, moderate gelatin liquefaction inhibition was observed. At 250 μl/ml concentration of the AGE, the gelatin liquefaction was slightly inhibited suggesting the antiproteolytic activity of garlic on protease of *P. gingivalis*.

At 500 mg/ml concentration, of the EGE there was moderate gelatin liquefaction inhibition. At 250 mg/ml concentration,

Table 2: MIC and MBC of garlic extracts on *Porphyromonas gingivalis*

MIC on *Porphyromonas gingivalis*										
Garlic (μl)	500	250	125	62.5	31.25	16.6	8.3	4	2	Control
AGE	S	S	S	S	S	R	R	R	R	R
EGE	S	S	S	R	R	R	R	R	R	R
MBC on *porphyromonas gingivalis* (CFU/ml)										
AGE	150	>300	>350	>400	>450	>500	>500	>500	>500	>500
EGE	300	400	>500	>500	>500	>500	>500	>500	>500	>500

S: Susceptible, R: Resistant, MIC: Minimal inhibitory concentration, MBC: Minimal bactericidal concentration, AGE: Aqueous garlic extract, EGE: Ethanolic garlic extract

Table 3: MIC and MBC of garlic extracts on *Aggregatibacter actinomycetemcomitans*

MIC on *Aggregatibacter actinomycetemcomitans*										
Garlic (μl)	500	250	125	62.5	31.25	16.6	8.3	4	2	Control
AGE	S	S	S	R	R	R	R	R	R	R
EGE	R	R	R	R	R	R	R	R	R	R
MBC on *Aggregatibacter actinomycetemcomitans* (CFU/ml)										
AGE	300	350	M	M	M	M	M	M	M	M
EGE	M	M	M	M	M	M	M	M	M	M

S: Susceptible, R: Resistant, M: $>5\times10^5$, MIC: Minimal inhibitory concentration, MBC: Minimal bactericidal concentration, AGE: Aqueous garlic extract, EGE: Ethanolic garlic extract

Table 4: Time kill assay

Microorganisms	Time (h)	Mean±SD		Significance*	
		Age	Ege	*Z* value	*P* value
Porphyromonas gingivalis	0	130.6±40.5	140.0±26.4	0.21	0.82
	2	70.3±36.4	176.6±5.7	1.99	0.04*
	4	111.3±3.5	199.6±30.9	1.96	0.05*
	6	111.6±9.8	176.0±27.4	1.96	0.05*
	24	500.0±0.0	500±0.0	-	-
Aggregatibacter actinomycetemcomitans	0	72.0±50.4	215.3±138.8	1.52	0.12
	2	36.6±18.9	58.3±23.0	10.91	0.12
	4	47.3±35.9	60.3±11.9	0.65	0.51
	6	68.0±22.5	55.6±47.2	0.65	0.51
	24	500.0±0.0	500±0.0	-	-

*Significant. SD: Standard deviation, AGE: Aqueous garlic extract , EGE: Ethanolic garlic extract

Table 5: Protease activity of garlic extracts on *Porphyromonas gingivalis*

Rate of inhibition of liquefaction					
AGE(µl)			EGE (µl)		
500	250	125	500	250	125
Complete inhibition of liquefaction	Moderate inhibition of liquefaction	Slight inhibition of liquefaction	Moderate inhibition of liquefaction	Slight inhibition of liquefaction	No inhibition of liquefaction

AGE: Aqueous garlic extract, EGE: Ethanolic garlic extract

there was slight gelatin liquefaction inhibition observed. At 125 mg/ml concentration, the EGE did not show any inhibition of liquefaction. Therefore, AGE showed better antiproteolytic activity on total protease of *P. gingivalis* compared to the EGE in gel liquefaction test.

Discussion

Periodontal disease is an infectious process ranging in severity from mild gingivitis to advanced loss of connective tissue attachment and supporting bone.[12] The successful treatment of periodontitis requires suppression or elimination of the subgingival periodontopathogens. The efficacy of non-surgical mechanical procedures and conventional home care in controlling the pathogenic flora decreases as probing depth increases. Thereby, antimicrobial agents attempt to directly reduce the pocket microflora when applied as an adjunct to mechanical debridement.[13]

Garlic is known to have antibacterial, antifungal, and antiproteolytic activiies.[14] The present study assessed the activity of garlic, particularly against putative periodontal pathogens. The present study confirmed marked inhibitory effect of garlic against periodontal pathogens. Collectively, the putative periodontal pathogens tested were A. *actinomycetemcomitans* and *P. gingivalis*.

In the present study, the inhibitory effect of garlic extracts on *P. gingivalis* and A. *actinomycetemcomitans* was evaluated using agar well diffusion, MIC, MBC, and the time-kill profile. The antiproteolytic activity of garlic extracts on *P. gingivalis* was evaluated.

Role of garlic extracts on survival of periodontal pathogens

Garlic extracts employed in this study were 50% (w/v). The growth of *P. gingivalis* was inhibited showing 16 mm, 20 mm, and 25 mm zone of inhibition on *P. gingivalis* at 25, 50, 75 µl/ml respectively. EGE did not show any zone of inhibition for *P. gingivalis* at any concentrations. No zone of inhibition was observed by both AGE and EGE on A. *actinomycetemcomitans*. The *P. gingivalis* showed better inhibition to the AGE compared to EGE. However, both extracts did not show any inhibitory activity on A. *actinomycetemcomitans* in well diffusion method. Analysis of this data revealed that *P. gingivalis* was very much susceptible to AGE compared to EGE. These datas also revealed that A. *actinomycetemcomitans* is less susceptible to garlic extracts compared to *P. gingivalis*. This may be due to the structural differences between both the organisms.

Garlic extracts showed bacteriostatic activity on *P. gingivalis* and A. *actinomycetemcomitans*. MIC determined for AGE was at 16.6 µl/ml concentration for *P. gingivalis*, whereas EGE exhibited the activity at 62.5 mg/ml on *P. gingivalis*. MIC determined for AGE was at 125 µl/ml for A. *actinomycetemcomitans*. But A. *actinomycetemcomitans* was resistant to EGE.

AGE did not show any zone of inhibition for A. *actinomycetemcomitans* in Well diffusion method but AGE showed inhibitory activity on A. *actinomycetemcomitans*. This difference probably may be indicative of constituents of garlic binding to constituents in the agar medium limiting the diffusion. So MIC values obtained using the broth dilution method were considered more reliable.[15] Also, MIC value shown for A. *actinomycetemcomitans* was at 125 µl/ml, which was much higher than the concentration taken for Well diffusion that is 25, 50, 75 µl/ml. This must have influenced the results of inhibitory effect of garlic on A. *actinomycetemcomitans*.

In the present study, MIC values of garlic extract were lower in *P. gingivalis* compared to A. *actinomycetemcomitans* observed in earlier study.[15] However, it is in contrast to another report who have shown lower MIC values for A. *actinomycetemcomitans* compared to *P. gingivalis*.[16]

A study reported that garlic extract exhibited bactericidal activity for both Gram-negative periodontal pathogens.[15] However, in the present study, both AGE and EGE did not exhibit any bactericidal activity.

The time-kill assay was performed on *P. gingivalis* and A. *actinomycetemcomitans* in presence of garlic extracts at their respective MBC's. Killing of *P. gingivalis* began almost immediately. The density of viable cells declined during the next 4 h. The viable cell count stayed roughly constant between 4 h and 6 h. Therefore, *P. gingivalis* exhibited a decrease in the colony count during 2-6 h interval. There were still substantial numbers of viable cells present in the majority of the cultures at 24 h reaching a density of 10^6 CFU/ml.

Even though killing of A. *actinomycetemcomitans* began almost immediately, the density of viable cells declined during the next 2 h and there was an increase in the viable cell counts from 4 h through 24 h. There were still substantial numbers of viable cells present in the majority of the cultures at 24 h reaching a density of 10^6 CFU/ml.

The time-kill assay of AGE and EGE were compared for both the microorganisms and the results were statistically analyzed using Mann–Whitney U-test. The comparisons showed statistical significance with *P. gingivalis* at 2 h ($Z = 1.99$, $P = 0.04$), 4 h ($Z = 1.96$, $P = 0.05$), and 6 h ($Z = 1.96$, $P = 0.05$). However, no statistical significance was seen between AGE and EGE on A. *actinomycetemcomitans*.

These observations suggested that the garlic extracts elicited its antimicrobial potency in a time-dependent manner producing distinct time-kill profiles suggesting variations in the growth

inhibitory responses of the tested isolates to garlic. Similar responses have been reported in earlier studies.[17,18] However, the uniqueness of time-kill profiles on Gram-negative microbes in this study may be connected with the structural difference between these two organisms.

In this study, aqueous and alcoholic garlic extracts showed antiproteolytic activity on *P. gingivalis* which was detected by gel liquefaction test. These results suggest AGE showed better antiproteolytic activity on total protease of *P. gingivalis* compared to the EGE.

In the study, AGE was found to be more potent than EGE, similar to observations made in few earlier studies,[19-21] but in contrast with others.[22] One of the possible explanations for this is when EGE was evaporated at 80°C, some the volatile constituents of garlic must have evaporated which possibly resulted in this difference in the inhibitory activity between AGE and EGE.

In this study, it has been shown that the protease activity of *P. gingivalis* is inhibited by garlic extract. Thus, garlic extract may be a potential therapeutic agent for periodontitis because it shows significant activity against both *P. gingiv*alis and A. *actinomycetemcomitans* and also on the proteases of *P. gingivalis*. However, to translate into effective *in vivo* therapies for periodontitis, therapeutic agents must ideally be active against biofilms rather than just planktonic cells.

In vivo studies showed good activity of that mouthwash containing garlic extract against salivary mutans streptococcal and total bacterial viable counts, although no direct assays have been performed on oral biofilms.[11] Garlic extract has, however, been reported to inhibit the formation of biofilms by *Staphylococcus epidermidis*, even at sub-MIC levels.[23]

One of the major disadvantages of the therapeutic usefulness of garlic extract is that, allicin (constituent of garlic) is unstable and breaks down within 16 h at 23°C.[24] However, the use of water-based extract of allicin stabilizes the allicin molecule. This may be due to two factors: The hydrogen bonding of water to the reactive oxygen atom in allicin which can reduce its instability; and/or there may be water-soluble components in crushed garlic that destabilize the molecule.[25] But one of the major disadvantages for this approach is that allicin can react with water to form diallyl sulfide which does not exhibit the same level of antibacterial activity as does allicin.[26,27]

A second limitation to therapeutic usefulness could be that constituents of garlic complexes with blood proteins and so its efficacy might be expected to be reduced in the presence of bleeding at periodontal sites. However, constituents of garlic administered systemically have been reported to retain sufficient biological activity in rabbits to be effective.[24,25]

A third limitation is that glutathione reverses the action of some of the constituents of garlic (allicin) on cysteine proteases of *P. gingivalis*[26,27] and this is present in gingival crevicular fluid, although at significantly lower levels at diseased sites in periodontitis patients.[28]

Despite these potential problems, because the widespread use of antibiotics has led to the emergence and spread of antibiotic resistance, alternative agents that are effective against the pathogens of interest but which do not disturb the commensal flora unduly are of particular interest.[15]

In accord with a previous study that reported the inhibitory effect of garlic extract on oral bacteria,[15,17] the current study suggests that garlic extract might be a useful candidate for *in vivo* testing as an agent for the prevention and treatment of periodontitis and possibly for other dental diseases.

Future therapeutic considerations might include alternating drugs, using different antimicrobials from one treatment to the next, as more drugs become available. Targeted therapy based on rapid chair side assays of bacterial susceptibility would also be desirable to enhance the ability of the operator to select appropriate antimicrobials.

Conclusions

With the rise in bacterial resistance to antibiotics, there is considerable interest in the development of other classes of antimicrobials for the control of infection. Garlic has been used as a medicine since ancient times and has long been known to have antibacterial, antifungal, and antiviral properties.

From the current study, it is concluded that there is preliminary evidence for the antimicrobial activity of garlic extracts against periodontal pathogens, *P. gingivalis*, A. *actinomycetemcomitans*. Its action against *P. gingivalis* includes inhibition of total protease activity, and this raises the possibility that garlic may have therapeutic use for periodontitis and possibly other oral infections.

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हिन्दी सारांश

लहसुन सत्व का पेरिओडोन्टल पॅथोजेन्स पर प्रभाव – एक सूक्ष्मजैविक प्रायोगिक अध्ययन

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जीवाणु विरोधी औषधियों के जीवाणु प्रतिरोध में हुई वृद्धि के साथ, व्याधी संक्रमण के नियंत्रण हेतु अन्य सूक्ष्मजीवविरोध औषधियों की वर्गों के विकास में काफी रुचि है । लहसुन प्राचीन काल से औषध रूप में प्रयोग किया जा रहा है और इसमे जीवाणु विरोधी, कवक विरोधी और विषाणु विरोधी गुण है, यह ज्ञात किया गया है । इस अध्ययन मे, *Porphyromonas gingivalis* और *Aggregatibacter actinomycetemcomitans* जीवाणुओं पर लहसुन के निरोधात्मक प्रभाव का आकलन, दोनो जीवाणुओं के time–kill curve का आकलन, और *P. gingivalis* पर लहसुन की प्रोटीन अपघटक गतिविधि निर्धारित कि गई । लहसुन का इथेनॉलिक एवं जलीय सत्व तैयार किया गया और पेरिओडोन्टल रोगजनक जीवाणुओं पर निरोधात्मक प्रभाव का परीक्षण किया गया । लहसुन के जलीय सत्व कि २५, ५० और ७५ मायक्रोलीटर मात्रा से *P. gingivalis* जीवाणु पर क्रमशः १६, २० और २५ मि.मी. क्षेत्र में नियंत्रण देखा गया। लहसुन के जलीय सत्व कि अधिकतम जीवाणु विरोधी गतिविधि *P. gingivalis* जीवाणु के खिलाफ न्यूनतम निरोधात्मक मात्रा १६.६ मायक्रोलीटर पायी गयी । लहसुन के इथेनॉलिक एवं जलीय सत्व का दोनो जीवाणुओं पर time–kill assay का तुलनात्मक परीक्षण किया गया । इथेनॉलिक सत्व के तुलना मे जलीय सत्व से *P. gingivalis* जीवाणु के खिलाफ प्रोटीन अपघटक गतिविधि बेहतर पायी गयी । इसप्रकार अध्ययन से यह निष्कर्षित होता है कि लहसुन के सत्व मे पेरिओडोन्टल पॅथोजेन्स के खिलाफ जीवाणु विरोधी गतिविधि की क्षमता है । लहसुन की *P. gingivalis* जीवाणु के खिलाफ देखी गयी प्रोटीन अपघटक गतिविधि से यह संभावना है कि लहसुन का पेरिओडोन्टायटिस और अन्य मुख संक्रामक रोगों मे प्रयोग हो सकता है ।