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Research Article

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Inhibitory Effect of Garlic Extract on *Porphyromonas Gingivalis* Isolated from Sudanese Aggressive Periodontitis Patients Compared to Chlorhexidine Digluconate 0.2%

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ABSTRACT

Aggressive periodontitis is a destructive inflammatory condition affecting the investing and supporting tissues of the teeth. Adjunct use of antimicrobial agents was implemented for the treatment of the disease and also some antiseptic mouth wash like chlorhexidine digluconate 0.2%. With increase of bacterial resistant to antibiotics, now a day's medicinal plants are used as antimicrobial against bacteria. Garlic (Allium sativum) has antimicrobial antifungal and antiviral against oral pathogens. Since Porphyromonas gingivalis, is one of the causative microorganisms of aggressive periodontitis among Sudanese patients, the aim of the present study was to study the effect of water garlic extract on Porphyromonas gingivalis compared to chlorhexidine digluconate 0.2%. Materials and methods: Fresh garlic cloves (100 g) were blended in 20 ml distilled water filtered using cotton wool and ultrafilterd under reduced pressure by using a Buchner funnel and a side armed flask. The final Concentration was 16.1% (v/w). Serial dilutions from 100%, 50%, 25%, to 12.5% and 10% of garlic were used in Muller Hinton agar media with Porphryromonas gingivalis suspension to determine the minimum inhibitory concentration, using chlorhexidine digluconate 0.2% as positive control and distilled water as negative control (in-vitro study). Zones of inhibitions were measured and averaged .the mean diameter of the zone of inhibition in mm was compared to the zone of inhibition of chlorhexidine using Pearson correlation test to identify if there is a statically difference between garlic extract and chlorhexidine digluconate 0.2%. Correlation is significant at the 0.01 and 0.05 level (2 tailed). Results: There was growth inhibition of Porphyromonas gingivalis by water garlic extract. Compared to chlorhexidine digluconate 0.2% there was no statistical significance difference between the means.
***Correlation is significant at the 0.01 level (2 tailed). Pearson correlation of Garlic and chlorhexidine digluconate = 0.596; P-Value = 0.069. Conclusion: The present study indicates that garlic extract has the same effect of chlorhexidine digluconate 0.2% against Porphyromonas gingivalis, so it may have therapeutic value in prevention and treatment of aggressive periodontitis. So garlic extract can be used in well-designed clinical trial in form of mouth wash for prevention and treatment of aggressive periodontitis.*

Keywords: Garlic; Chlorhexidine digluconate 0.2%; Oral bacteria; *Porphyromonas gingivalis*

INTRODUCTION

Aggressive periodontitis is destructive inflammatory condition affecting the investing and supporting tissues of the teeth. It is classified into localized and generalized form [1]. Detectable levels *of Porphyromonas gingivalis, T.forthia, Prevotella inetrmedia, Campylobacter* and *Actinobacillus Actinomycetnsemcomita* are associated with disease progression and their elimination by therapy is associated with an improvement in the clinical signs of the disease [2,3]. Both *Porphyromonas gingivalis* and *Actinobacillus Actinomycetnsemcomita* are known to invade host tissue cells which may be significant in more aggressive forms of adult periodontitis [4]. One of the major virulence factors of *prophyrmonas gingivalis* is Lys-gingipain a lysine-specific cysteine proteinase, which interferes with both cell-matrix and cell-cell adhesion [5], fimbriae of *Porphyromonas gingivalis* plays an important role in its adhesion to and invasion of host cell [6]. Also *Porphyromonas gingivalis* diminishes the effect of EMD on Periodontal ligaments cells through a cooperative action of gingipains [7]. There are many trials to use vaccine against *prophyrmonas gingivalis* to aid in prevention of periodontitis in animals but it is still trials [8,9]. The treatment of aggressive periodontitis includes the use of systemic antibiotics e.g. tetracycline oral capsules or by tetracycline fiber therapy, monocycline and mitronidazole [10-13]. Some antiseptic mouth wash used after scalling, root planning and after surgical treatment, like Octenidine and Chlorhexidine gluconate 0.2% which is used as mouthwash or gel instead of toothpaste. Chlorhexidine has many disadvantages of staining the teeth, it has powerful taste, suppress normal sense of taste in few people, irritant to the oral tissues and may produce sore lips [14,15]. Also, ozonated water used as antimicrobials in the treatment of periodontitis patients [16]. With increase of bacterial resistant to antibiotics [17]. Nowadays the use of medicinal plants is applied widely for the treatment of many diseases; one of these plants is garlic which was used as an antimicrobial agent due to presence of allicin (sulpher compound) called thiosulphonate. The plant use allicin as antimicrobial against invaded bacteria or single cell pathogen [18]. Garlic used in different forms, powder, oil and heated garlic which has antmicrobial effect on *Staphylococcus aureus* B33 [19,20]. Garlic compound were used as antimicrobial, antiviral, antifungal and antiprotozoal. It has antibacterial effect on *Escherichia Salmonela,Staphylococcus,Streptococcus,Klebsiella,Proteus* and *Mycobaterium* [21] Allicin showed synergistic effect when combines with a antibiotics which may overcome the resistance of micro-organisms [22]. Also garlic used as antimicrobial against oral bacteria in the form of mouth wash which have inhibitory effect against Gram-positive, Gram-negative organisms and fungi by reducing bacterial counts [23]. In some studies tea tree oil, garlic, might be an alternative to chlorhexidine solutions against oral microorganisms [24,25]. Filter sterilized, aqueous extract of garlic has the ability to inhibit the growth of gram positive and gram negative micro-organisms and it has action against the arg and lys-gingipains activity of *P.gingivalis* and it has activity against multidrug-resistant (MDR) strains of *Streptococcus mutans* isolated from human carious teeth also Garlic extract inhibit *P. gingivalis* protease activity [26-29]. Garlic extracts in concentrations of 12.5% is cytotoxic to human gingival fibroblasts but it is safe at 6.25, 3.12, and 1.5%, the high concentration of garlic is less cytotoxic than chlorhexidine digluconate [30]. Since *porphyromonas gingivalis* is one of the major causative microorganisms isolated from plaque samples of aggressive periodontitis patients aged 18-35years old by culture and real time PCR [31], so the aim of the present study is to test the inhibitory effect of garlic water extract on *porphyromonas gingivalis* compared to chlorhexidine 0.2% (in *in-vitro* study).

MATERIALS AND METHODS

Cross-sectional analytical clinical and microbiologial hospital based study at Department of Periodontology Faculty of Dentistry, University of Khartoum. Thirty aggressive periodontitis patients both males and females Patients were within the age group 18-35 years, 25.4 ± 5.5 years newly diagnosed according to clinical and radiographic examination as aggressive Periodontits patients with pocket depth, >5 mm (mean pocket depth 5.3 ± 1.6 mm). Patients excluded if they had systemic diseases, those who were immunocompromised, pregnant ladies, patients used antibiotics 3 months before the examination and patients who had periodontal treatment 3 months before the start of the study.

Ethical Approval

Ethical clearance was given by the Research Committee (Faculty of Medicine/ University of Al-Neelain 2007) and approval from the director of faculty of dentistry, head department of periodontoloy for sample collection. Since the patient are adult, seeking periodontal treatment and capable of giving informed consent, voluntary and freely they agrees verbally to participate in the study after explanation of the aim of the study.

Bacterial Isolate

Clinical isolates of *P.gingivalis* from aggressive periodontitis Sudanese patients (ten pooled samples) identified by culture confirmed by biochemical test and real time PCR [31]. The isolates preserved at laboratory of microbiology department, faculty of science, El Neelain University under anaerobic a septic condition, one ml of 24 hours broth culture of the test bacteria was inoculated in bacterial preservers and incubated at 37°C.

Water Garlic Extract

Fresh garlic cloves (100 g) were blended in 20 ml distilled water filtered using cotton wool and ultrafilterd under reduced pressure by using a Buchner funnel and a side armed flask. By subtracting the weight of insoluble material from the weight of the original cloves, the final concentration of garlic extract in solution was determined to be 16.5% (w/v). The garlic extract was stored at -20°C and used for antibacterial testing.

***In vitro* Testing of Extract for Antimicrobial Activity**

Determination of minimum inhibitory concentration (MIC):

The antibacterial activity of *Allium sativum* was evaluated by cup-plate agar diffusion method for aqueous extract. The garlic extract used in present study was 16.5% (w/v). One ml of the standard bacteria suspension were thoroughly mixed with 60 ml of molten sterile Muller Hinton agar media and poured into the petridishes. The agars was left to set and in each of plate cups (10mm diameter) were cut using a sterile cork borer and agar discs were removed. Garlic extract were prepared in series of increasing concentrations. The bottom of each plat was marked off into 8 segments. Serial dilution from 100%, 50%, 25%, and 12.5% to 10% is used. 50 µl of extracted garlic introduced into the wells using automatic microlitre pipette and all plates were incubated at 37°C for 24 hours. Chlorhexidine 0.2% (Claradine, Mfd by MEDPHARMA, United Arab Emirates) used as a positive control, the negative control were distilled water. Sensitivity of *P.gingivalis* and control was determined by measuring the diameter of the zone of inhibition. The end point (MIC) is the least concentration of antimicrobial agent that completely inhibits the bacterial growth. The resultant growth inhibition zones were measured averaged and the mean values were tabulated (Table 1).

Table 1: Screening for antibacterial activity of garlic againist the clinical isolates *Prophyromonas gingivalis* compared to chlorhexidine (mean diameter of of inhibition zone)

Water garlic extraction (mm)	Concentration % negative control (distilled water)	Mean Diameter of zone of inhibition (mm)	Negative control (distilled water)
1	10%	13.6 ± 0.51	_
2	12.50%	14.2 ± 0.63	_
3	25%	15 .7 ± 1.4	_
4	50%	18.4 ± 1.26	_
5	100%	21.5 ± 1.77	_
Chlorhexidine	0.20%	25 ± 2.2	_

Values were expressed as mean ± S.D; (-) No growth

Data analysis:

Using SPSS version 16, Pearson Correlation and the value expressed as mean ± S.D. P value at 0.5.

RESULTS

The extract inhibited the growth of *P.gingivalis* at concentration of 100%, 50%, 25%, 12.5% and 10%. The end point (MIC) which is the least concentration of garlic that completely inhibits the growth of *P.gingivalis* was 10%. The sensitivity pattern as zone of inhibition (mm) of aqueous garlic extract concentration showed growth inhibition activities against *Proyhyromonas gingivalis* compared to sensitivity pattern in (mm) of positive control chlorhexidine 0.2%. The pearson correlation between water garlic extract and chlorhexidine 0.2% is 0.596 and, p value >0.069, so there was no significance difference between aqueous garlic extract and chlorhexidine 0.2%. No zone of inhibition around the negative controls, (distilled water) (Table 2).

Table 2: The antibacterial activity of aqueous garlic extract on *Porphyromonas gingivalis*

	Garlic water extract	Chlorhexidine
Pearson correlation	1	0.596
Sig.(2-talied) P. value		0.069
N	60	10
Pearson correlation	0.596	1
Sig.(2-talied) P. value	0.069	
N	10	10

*Correlation is significant at the 0.05 level (2 tailed); **Correlation is significant at the 0.01 level (2 tailed); Pearson correlation of Garlic and Chlorhexidine = 0.596; P-Value = 0.069

DISCUSSION

With the rise of bacterial resistance to antibiotics, there is need to search for other antimicrobial agents for treatment and control of oral infections. Garlic which is known to have antibacterial antiviral, anti-brotozoal and antifungal activity. It has an inhibitory effect against gram+ve and gram-ve bacteria in oral cavity [23,24] inhibition of *Streptococcus mutans* [25] in study of the inhibitory effect on putative periodontal pathogens and in study of inhibition of multidrug-resistant strain of *Streptoccocus mutans*, so in the present study, which is the first study in Sudan, we confirmed the marked growth inhibition activity of garlic (grows in northern Sudan) against *P. gingivalis* which has been described before [25]. Aqueous extract (concentration 16.5%) at serial dilution of 100%, 50%, 25%,12.5% and 10% showed activity. There was no significant difference (Table 2) between Chlorhexidine gluconate 0.2%, and the aqueous extract. The MIC of the aqueous extracts of garlic in the present study was 10%.

CONCLUSION

Since garlic water extract in the present study had *in-vitro* inhibitory effect on *P.gingivalis* isolated from the examined group of aggressive periodontitis patients compared to chlohexidine and due to the widespread use of antibiotic and the spread of antibiotic resistance ,we present evidence for the antimicrobial activity of garlic extract against *P.gingivalis* and this raises the possibility that garlic may has therapeutic use for prevention and treatment of the onset of aggressive periodontitis.

Recommendation

1. Further laboratory studies on the effectiveness of garlic extract on periodontal pathogens are needed.
2. Preparation of Garlic in dosage forms (mouth wash or local ointment) to be tested on volunteers patients with aggressive periodontitis in carefully designed clinical trials.

Limitation of Study

Since allicin is the main biologically active component part of garlic extract and garlic extract showed inhibition for growth of *P.gingivalis*, so the extracts should be subjected to GC-MS analysis or High Performance Thin Layer Chromatography to determine the amount of allicin.

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Competing interests

The authors declare that they have no competing interest.

Financial competing interest

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Authors' contribution

Professor Ghandour contributed in conception and study design, Professor Al Nima contributed in interpretation of the data.

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