Republic of Iraq Ministry of Higher Education and Scientific Research University of Baghdad College of Dentistry





ANTIBACTERIAL EFFECTS OF POMEGRANATE EXTRACT (ELLAGIC ACID) ON SOME CLINICALLY ISOLATED PERIODONTAL PATHOGENS IN VITRO STUDY

A thesis Submitted to the College of Dentistry Baghdad University In Partial Fulfillment of the Requirements for The Degree of Master of Science In Periodontics



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Abstract

Background: Periodontal disease is considered as one of the most worldwide spread infectious diseases, that if it's not treated it may lead to functionally, aesthetically and systemically problems, hence the importance of treatment of this disease came from. And because the side effects of chemical antiseptic products that used with mechanical treatment of periodontal disease, the interesting with plant antimicrobial substances began. Pomegranate (Punica granatum Linne) is one of the oldest fruits that is used in all medical intervention because it has many biochemical constituents, from these, ellagic acid that has antioxidant and antibacterial effects.

Aims of the study: to test the antibacterial effect of pomegranate extract on *Aggregatibacter actinomycetemcomitans* (*A.a*) and *Porphyromonas gingivalis* (*P.g*) (as alone and in combination) in comparison with 0.2% chlorhexidine gluconate (CHX) and 10% Dimethyl Sulfoxide (DMSO) in vitro. And laboratory analysis of the active ingredients of pomegranate extract using high performance liquid chromatography (HPLC).

Materials and Methods: subgingival plaque samples were collected from 56 patients with no signs of any systemic disease, suffering from chronic periodontitis with probing pocket range from 5-6 mm in depth, *A.a* and *P.g* were isolated and diagnosed according to morphological characteristics and biochemical tests. The pomegranate white flesh was extracted by using a mixture 0f 200 ml triethylamine and 20% aqueous ethanol in a ratio 1: 9 (v/v). This study involved two experiments in vitro, the first experiments involved testing the sensitivity of *A.a* and *P.g* as alone and in combination to (0.0625mg/ml,

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0.125mg/ml, 0.25mg/ml, 0.5mg/ml, 1mg/ml, 2mg/ml, 4mg/ml, 8mg/ml, 16mg/ml and 32mg/ml) of the pomegranate extracts in addition to 0.2% CHX and 10% DMSO use agar well diffusion method, the second experiment involved determination of the minimum inhibitory concentration of the extracts that inhibits the bacterial growth and then the determination of the minimum bactericidal concentration of the extract that was required to kill the bacteria. The present study also involved laboratory analysis of pomegranate extract HPLC.

Results: pomegranate extract was effective in inhibition of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* as alone and in combination using the agar well diffusion method, CHX showed higher inhibition zones than all pomegranate extract concentrations.

The minimum inhibitory concentration (MIC) of pomegranate extract that inhibit *Aggregatibacter actinomycetemcomitans* growth was 8mg/ml and it was the same for *Porphyromonas gingivalis*, also it was the same for *A.a* and *P.g* in combination.

The minimum bactericidal concentration (MBC) of pomegranate extract that kills *Aggregatibacter actinomycetemcomitans* was 32mg/ml, the MBC of pomegranate extract that kills *Porphyromonas gingivalis* was 16mg/ml, the MBC of pomegranate extract that kills *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* in combination was 32mg/ml.

The HPLC analysis of pomegranate extract that was used in this study revealed that the extract contained higher concentrations of ellagic acid about 97%.

Conclusion: Pomegranate extract was effective against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* as alone and in combination, it showed bactericidal activity at 32mg/ml and 16mg/ml concentrations respectively.

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