Anti-inflammatory activity of *Punica granatum* extract on oral microbes - In vitro

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**ABSTRACT**

To study the anti-inflammatory effect of *Punica granatum* extract against the oral microbes. Oral diseases continue to be a major health problem worldwide. Dental caries and periodontal diseases are among the most important global oral health problems, although conditions such as oral and pharyngeal cancers and oral tissue lesions are also significant health concerns. Pomegranate extracts have been used for centuries in traditional medicine to confer health benefits in a number of inflammatory diseases, microbial infections and cancer. The anti-inflammatory activity of pomegranate extract was evaluated by protein denaturation assay, and the results were read spectrophotometrically. Denaturation of proteins is a great documented cause of inflammation. As a part of the investigation on the mechanism of the anti-inflammatory activity, the ability to extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Table 1. Maximum inhibition, 70.12±1.12% was observed at 500µg/ml. IC50 value was found to be 105.35±1.99µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition, 77.12±1.42% at the concentration of 200µg/mL. Hence it can be concluded that pomegranate extract has anti-inflammatory property and also can be used in products such as toothpaste and mouthwash etc.

**INTRODUCTION**

Oral diseases continue to be a major health problem worldwide. Dental caries and periodontal diseases are among the most important global oral health problems, although conditions such as oral and pharyngeal cancers and oral tissue lesions are also significant health concerns (P. E. Petersen 2003). The association between oral diseases and oral microbiota is well established. Of the more than 750 species of bacteria that inhabit the oral cavity, a number are implicated in oral diseases. (*Punicagranatum* L. 2010). The development of dental caries involves acidogenic and aciduric Gram-positive bacteria (mutans streptococci, lactobacilli and actinomyces). Periodontal diseases have been linked to anaerobic Gram-negative bacteria (*Porphyromonas gingivalis, Actinobacillus Prevotella and Fusobacterium*). Given the incidence of oral disease, increased resistance by bacteria to antibiotics, adverse effects of some antibacterial agents currently used in dentistry and financial considerations in developing countries, there is a need for alternative prevention and treatment options that are safe, effective and economical. While several agents are commercially available, these chemicals can alter oral microbiota and have undesirable side-effects such as vomiting, diarrhea and tooth staining. (M. Dell’Agli, G. V. Galli, M. Bulgari et al.2010, S. D. Johannsmeier and G. K. Harris.)
Pomegranate extracts have been used for centuries in traditional medicine to confer health benefits in a number of inflammatory diseases, microbial infections and cancer (S. D. Johanningsmeier and G. K. Harris 2011). Peel fruit are rich in polyphenols that exhibit antioxidant and anti-inflammatory capacities in vitro (J.E.Crabtree, P.Peichl, J.I.Wyatt, et al., 1993). The pomegranate, an ancient, mystical, and highly distinctive fruit is the predominant member of two species comprising the Punicaceae family. It was lauded in ancient times in the Old Testament of the Bible, the Jewish Torah, and the Babylonian Talmud as a sacred fruit conferring powers of fertility, abundance, and good luck. It also features prominently in the ceremonies, art, and mythology of the Egyptians and Greeks and was the personal emblem of the Holy Roman Emperor, Maximilian. Pomegranate is the symbol and heraldic device of the ancient city of Granada in Spain–from which the city gets its name. The genus name, Punica, was the Roman name for Carthage, where the best pomegranates were known to grow. Pomegranate is known by the French as a grenade, the Spanish as Granada, and literally translates to seeded (“granatus”) apple (“pomum”). (J. E. Crabtree, Z. Xiang et al., 1995).

Pomegranate has great nutritional values and numerous health benefits. Pomegranates are used as a Treatment for Cancer, Osteoarthritis and Other Diseases. The pomegranate has been used in natural and holistic medicine to treat sore throats, coughs, urinary infections, digestive disorders, skin disorders, arthritis, and to expel tapeworms (K.Yasumoto, S.I.Okamoto, et al., 1992). However, modern research suggests that pomegranates might be useful in treating such serious conditions as prostate cancer, skin cancer, osteoarthritis, and diabetes. Studies also show that pomegranate seeds might help rid the digestive system of fats (R. Caruso, D. Fina, I. et al., 2007). Clinical research shows that pomegranates, when part of a healthy diet, might help prevent heart disease, heart attacks and strokes. This is because pomegranates have the potential to thin the blood, increase blood flow to the heart, reduce blood pressure, reduce plaque in the arteries, and reduce bad cholesterol while increasing good cholesterol (B. Romier, Y. J. Schneider et al., 2009). A decoction of seed is used to treat syphilis. Juice used to treat jaundice and diarrhoea. Juice of flower is used to treat nosebleeds. The fruit pulp and the seed are stomachic. Dried, pulverized flower buds are employed as a remedy for bronchitis (R. Al-Ashy, I. Chakroun et al., 2006). Thus the study is to check an anti-inflammatory effect of pomegranate extract on oral microbes.

MATERIALS AND METHOD

Punica granatum extract was obtained commercially and used for the study. Anti-inflammatory activity was tested using protein denaturation.

In vitro anti-inflammatory activity

Inhibition of protein denaturation

The denaturation of proteins is one of the causes of inflammation. Hence, protein denaturation can be employed as in vitro screening model for anti-inflammatory compounds. The reaction mixture consists of test extract at different concentration 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

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\text{Percentage inhibition} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100 \%
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RESULTS

Denaturation of proteins is a well-documented cause of inflammation. As a part of the investigation on the mechanism of the anti-inflammatory activity, the ability to extract to inhibit protein denaturation was studied. It was effective inhibiting heat induced albumin denaturation at different concentrations as shown in Table 1.

Maximum inhibition, 70.12±1.12% was observed at 500µg/ml. IC50 value was found to be 105.35±1.99µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition, 77.12±1.42% at the concentration of 200µg/ml.

DISCUSSION

In this study, Pomegranate has potent anti-inflammatory properties, which are largely mediated by the antioxidant properties of the punicalagins. Denaturation of proteins is a well-documented cause of inflammation. Denaturation of proteins involves the disruption and possible destruction of both the secondary and tertiary structures. (E. P. Lansky and R.A. Newman 2007, F. Afaq, V. M. Adhami et al., 2005, D. Heber 2011). Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure (sequence of amino acids) remains the same after a denaturation process. Denaturation disrupts the normal alpha-helix...
and beta sheets in a protein and uncoils it into a random shape (M.I. Gil, F.A. Tomas-Barberan et al., 2000). As a part of the investigation on the mechanism of the anti-inflammatory activity, the ability of extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation at different concentrations. Maximum inhibition, 70.12±1.12% was observed at 500µg/ml. IC50 value was found to be 105.35±1.99µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition, 77.12±1.42% at the concentration of 200µg/ml.

In 2006, a (B. Cerda, J. C. Espín, et al., 2004, S.Jitesh 2017.). IsoVasconcelos et al. investigated the antimicrobial effect of Punicagranatum L. (pomegranate) using a phytotherapeutic gel, pomegranate-based, and miconazole (Daktarin® oral gel) against the standard streptococci strains (mutans ATCC 25175, S. anguis ATCC 10577, and mitis ATCC 9811) and demonstrated the greater efficiency of pomegranate gel in inhibiting microbial adherence than miconazole (S. Quideau 2009, N. P. Seeram, R. Lee, and D. Heber, 2004, N. P. Seeram, Y. Zhang, R. McKeever et al., 2008). While the antibacterial activity of the pomegranate peel has been the subject of numerous researches few studies have investigated the anti-inflammatory off pomegranate juice against oral pathogens, and R. dentocariosa. Kote and Nagesh in 2011 conducted a clinical trial that showed the ability of pomegranate juice to reduce the micro-organisms of dental plaque (streptococci and lactobacilli).

CONCLUSION

The investigation conducted on pomegranate extract showed good anti-inflammatory activity. Maximum inhibition, 70.12±1.12% was observed at 500µg/ml. Hence through this study, it was found that pomegranate can be used to decrease anti-inflammatory activity on oral microbes, and can be used in products such as toothpaste and mouth wash etc.

REFERENCE


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