



Sylwia Klewin-Steinböck¹, Agnieszka Nowak-Terpiłowska², Marzena Wyganowska-Świątkowska¹

The effect of chamomile solutions on primary gingival fibroblast *in vitro*

Wpływ naparu z rumianku na fibroblasty dziąsłowe w warunkach in vitro

¹ Chair and Department of Dental Surgery and Periodontology, Poznan University of Medical Sciences, Poland

² Department of Biochemistry and Biotechnology, Poznan University of Life Science, Poland

DOI: <http://dx.doi.org/10.20883/df.2020.8>

ABSTRACT

Oral health is an integral part of general health condition. Oral inflammatory diseases, such as gingivitis and periodontitis, are one of the most common problems in oral health apart from caries. Mechanical removal of dental plaque is considered to be the best option for inflammation reduction and as a consequence tissue injury protection. Prophylactic substances can facilitate and improve results of mechanical procedures. Among the most popular plant derived substances used in oral healing are extracts from *Chamomilla recutita*.

Keywords: chamomile, gingival fibroblasts, cell proliferation.

STRESZCZENIE

Zdrowa jama ustna jest integralnym elementem zdrowego organizmu. Choroby infekcyjne jamy ustnej, w tym zapalenie dziąseł i przyzębia, są obok choroby próchnicowej zębów najczęstszym problemem zdrowotnym. Mechaniczna kontrola płytki nazębnej, wspomagana chemicznymi środkami pielęgnacji jamy ustnej, jest najlepszą metodą prewencji chorób infekcyjnych prowadzących do destrukcji tkanek. Kwiat rumianku jest jednym z naturalnych środków stosowanych w procesie gojenia.

Słowa kluczowe: kwiat rumianku, fibroblasty dziąsłowe, proliferacja.

Introduction

Oral inflammatory diseases, such as gingivitis and periodontitis, are one of the most common problems in oral health apart from caries. Furthermore, oral health is integral to general health condition that extends beyond the functions of the oral cavity. Dental plaque is the source of inflammation and its mechanical removal is a basic procedure to limit the development of inflammation. The best results in reducing inflammation were obtained by combining mechanical plaque removal with prophylactic substances. The most popular are chlorhexidine, iodine and natural antiseptics (essential oils and plant extracts) [1].

From botany point of view, herbaceous plants are any plants that lack the woody tissue — characteristic of shrubs or trees. Herbs and their derivatives are used medicinally or for scent, or flavor in various forms for at least 5000 years [2]. Those with medicinal characteristics are source of several popular conventional drugs on the mar-

ket, like aspirin (from white willow bark), digitalis (from foxglove), and sudafed (created based on the ephedra plant component) [3]. The growing interest in homeopathic medicine on one hand, and simultaneously the search for new sources of drugs on the other, has led to increased interest in herbs. Some reasons that may explain popularity of herbs are their minimal toxic side effects. However greater interest in herbal remedies brings an increase in the risk and frequency of side effects and interactions with prescription drugs. It is important to remember that herbs should be used in accordance with their pharmacological activity and to avoid any potential negative interactions. Herbs that are useful in dentistry include chamomile, echinacea, peppermint, rosemary, sage and thyme.

One of the oldest and most widely used herbs is chamomile. Chamomile (*Chamomilla recutita*, *Matricaria recutita*) a member of daisy family (*Asteraceae*), is one of the well-known medicinal plant

used because of its anti-inflammatory, antimicrobial, sedative and antispasmodic effect [4]. The main part of the plant used to make medicines is a flower [5]. Chamomile flowers contain 1–2% of volatile oils. The main essential oil components extracted from the flowers are (E)- β -farnesene (4.9–8.1%), terpene alcohol (farnesol), chamazulene (2.3–10.9%), α -bisabolol (4.8–11.3%), α -bisabolol oxides A (25.5–28.7%) and α -bisabolol oxides B (12.2–30.9%). Other active chemical components include flavonoids, apigenin, luteolin, coumarins and quercetin [3, 5, 6].

Aim

The goal of this study was to observe changes in fibroblasts' viability and ability to proliferate under chamomile extract influence.

Material and Methods

Cells

The experiment was conducted on primary human gingival fibroblasts. The cell culture was established from the tissue samples collected in compliance with patients.

Herbs

The test used commercially available dried chamomile flower (Herbapol S.A.).

Cells culture method

Fibroblasts were grown in 25 cm² culture vessels containing 4 ml medium consisting of: DMEM (Dulbecco's Modified Eagle's Medium), 10% FBS (fetal bovine serum) and 1% mixture of: penicillin, streptomycin, amphotericin (Sigma-Aldrich) at 37°C and 5% CO₂ concentration. After reaching 80% confluency the growth medium was decanted and the adherent cells were covered with 6 ml of Hank's Balanced Salt Solution to remove residual FBS. Later on 750 ml of trypsin solution was added to the fibroblasts with a subsequent 10 min incubation under the standard culture conditions (37°C, 5%CO₂ atm). The digestive properties of trypsin were used to detach cells from the culture vessel medium. The effect of the enzyme on cell culture was checked by an inverted microscope (Carl Zeiss AG). After detaching cells from medium trypsin was inactivated by adding 4 ml medium. The cells-containing solution was centrifuged at 1000 rpm. for 10 minutes. The prepared suspension of 250 μ l human fibroblast and 1 ml DMEM was transferred to the PAN-sys3000 chambers where it was incubated for 24h.

Herbs preparation

Herbs have been prepared in three concentration variants: 10%, 50% and 100%. Herbal infusions were prepared as follows: water at 90°C temperature was added to 2 g of herbs, then boiled covered for 10 minutes. The solution was cooled and filtered through a 0.2 μ m filter afterwards. Three concentrations of chamomile extract prepared as following were used in the subsequent research: 10% (25 μ l herbal infusion with 225 μ l DMEM), 50% (125 μ l herbal infusion with 125 μ l DMEM) and 100% (250 μ l herbal infusion). The selected dilution in a volume of 250 μ l was added to the cells cultures in PAN-sys3000 wells. Control test was also used to determine the cells condition. Cell cultures were observed for 48 hours after the addition of the herbal preparations.

Results

Using PAN-sys3000 for visualization microscopic images were obtained in the conducted research. They were used as the basis for the assessment of the impact of herbal extracts on cells viability and ability to proliferate. PAN-sys3000 device allows to observe culture in real time and take pictures of the selected places (ROI — region of interest) in certain cycles.

Fibroblasts morphology

The morphology of control cells compared to fibroblasts cultured with 10% and 50% chamomile extract did not change significantly. The cells were considerably elongated, with small number of projections.

Fibroblasts proliferation

The addition of 10% and 50% chamomile extract to the cultured fibroblasts did not cause cell apoptosis (**Figure 1** and **2**) either. Fibroblasts retained their ability to proliferate throughout the test. Judging from the images it can be estimated that the addition of 50% chamomile extract resulted in higher proliferation (**Figure 2**).

The addition of 100% concentration of the test substance had a strong effect on the cell line. After the introduction of the chamomile extract to the cultured fibroblasts the cell growth has stopped which consequently led to the death of the cells (**Figure 3**).

Control sample confirmed the use of appropriate conditions for cell growth evidenced by the fibroblast growth on the surface of the test chamber (**Figure 4**).

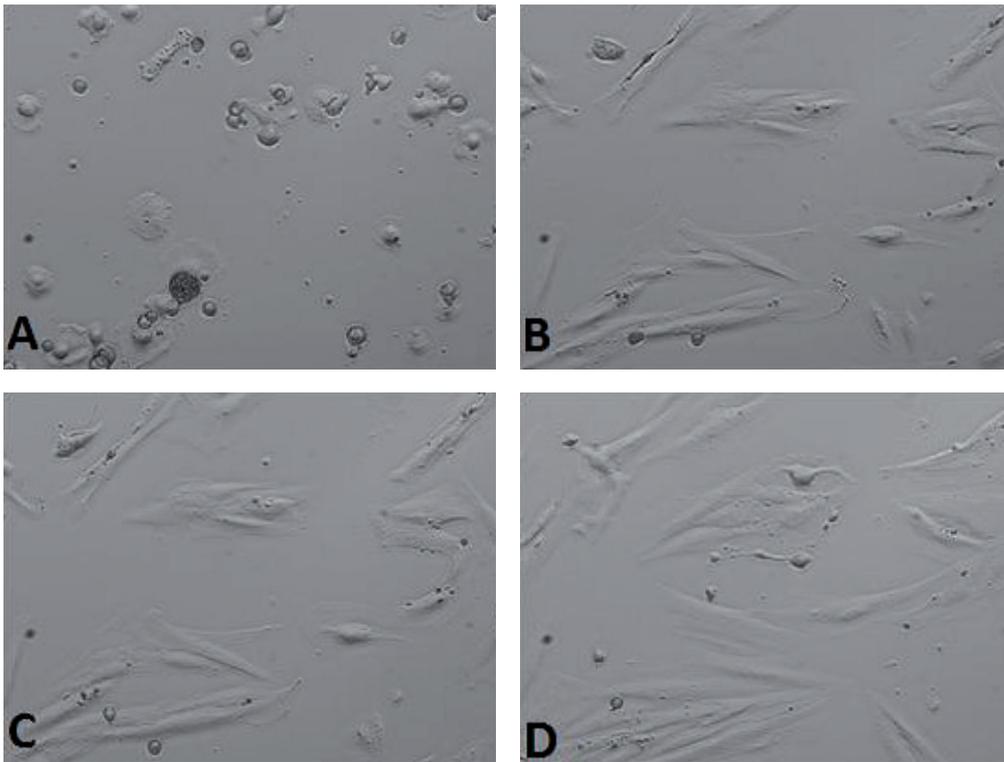


Figure 1. Fibroblasts cultured with 10% chamomile solution extract. The images are arranged in the following order: A — 0h, B — 24h, C — 36h, D — 48h

Rycina 1. Hodowla fibroblastów z 10% roztworu rumianku. Czas badania: A — 0h, B — 24h, C — 36h, D — 48h

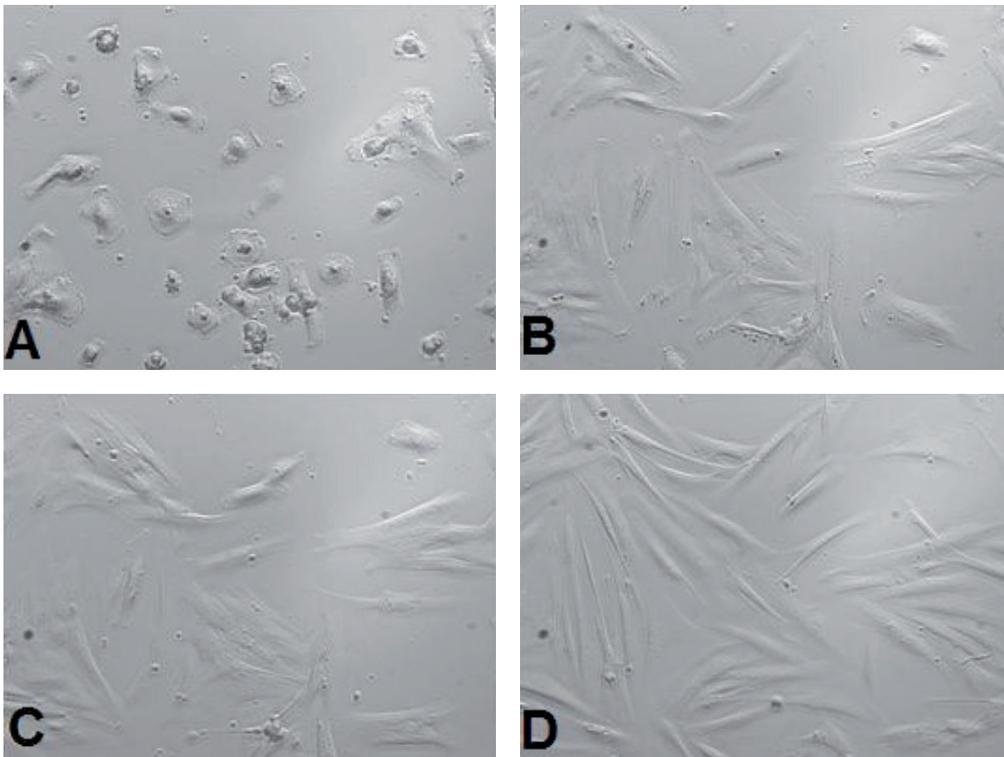


Figure 2. Fibroblasts cultured with 50% chamomile solution extract. The images are arranged in the following order: A — 0h, B — 24h, C — 36h, D — 48h

Rycina 2. Hodowla fibroblastów z 50% roztworu rumianku. Czas badania: A — 0h, B — 24h, C — 36h, D — 48h

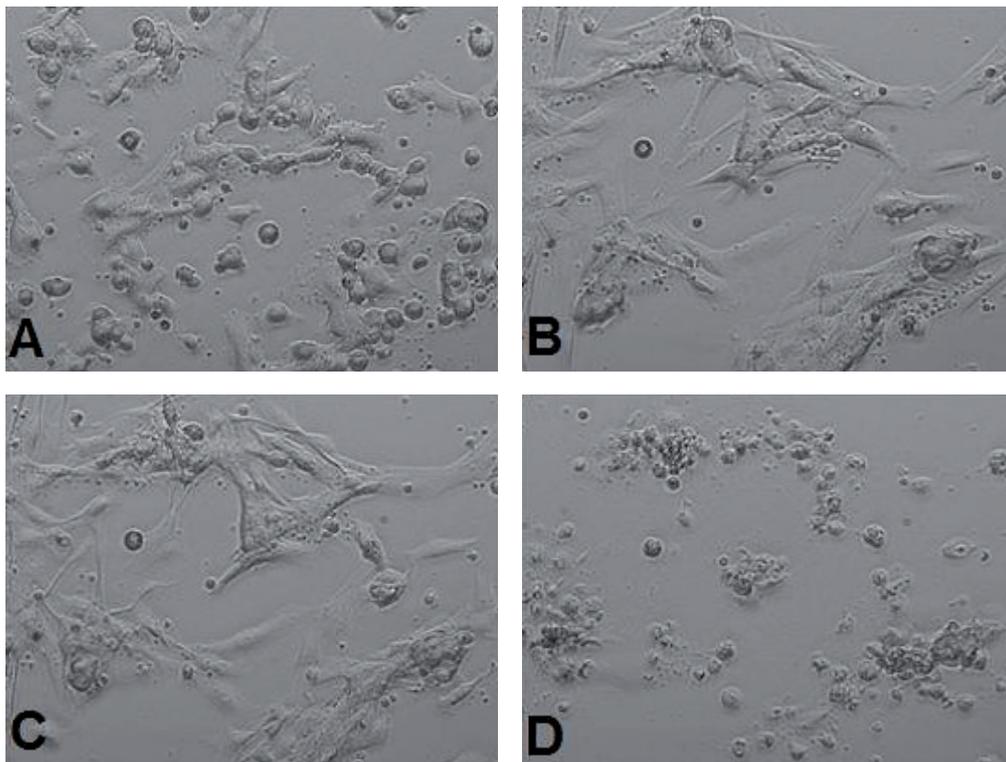


Figure 3. Fibroblasts cultured with 100% chamomile solution extract. The images are arranged in the following order; A - 0h, B — 24h, C — 36h, D — 48h

Rycina 3. Hodowla fibroblastów z 100% roztworu rumianku. Czas badania: A — 0h, B — 24h, C — 36h, D — 48h

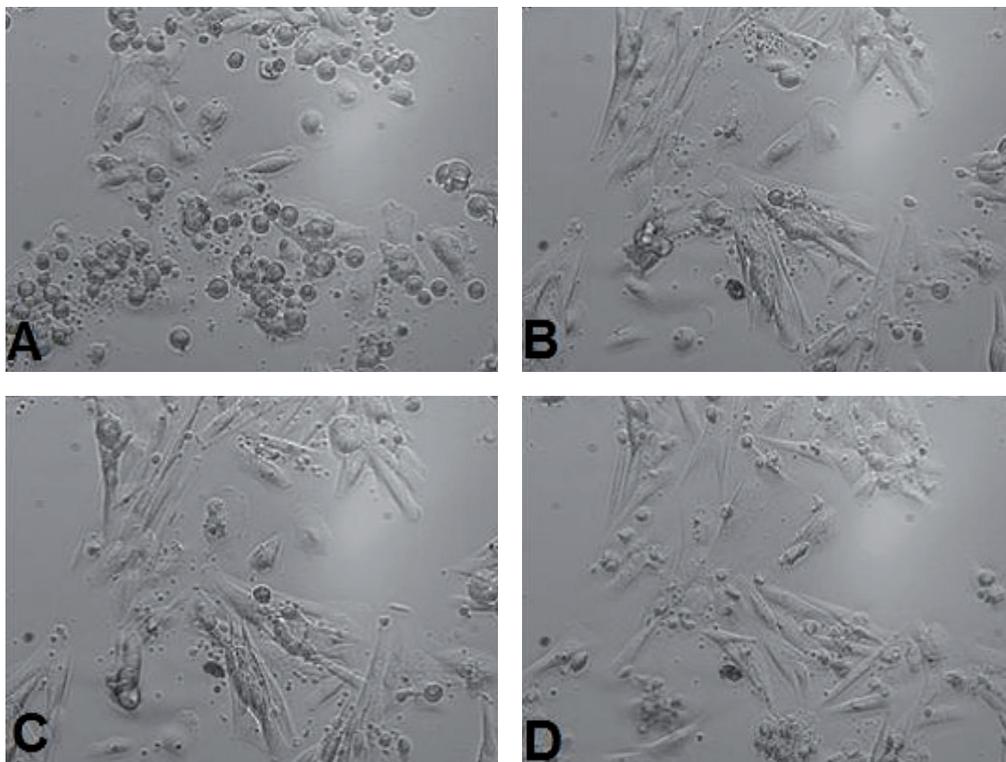


Figure 4. Fibroblasts control culture. The images are arranged in the following order; A - 0h, B — 24h, C — 36h, D — 48h

Rycina 4. Hodowla fibroblastów, próba kontrolna. Czas badania: A — 0h, B — 24h, C — 36h, D — 48h

Discussion

Fibroblasts, the main cells in the connective tissue, were previously believed to be static cells, responsible for maintaining structural tissue integrity. Currently we have the knowledge that they are actually active cells that play an important role in inflammatory processes. They can synthesize a number of cytokines through IL-1 and TNF- α stimulation, which are produced early during the inflammatory reaction. Fibroblast-derived cytokines may play a significant role in strengthening of the inflammatory response [7].

Inflammation belongs to one of the most common groups of oral cavity disorders and it is caused mainly by inadequate oral hygiene, which leads to the accumulation of bacterial biofilm around gingiva. Herbal extracts have been used for a long time to control and prevent diseases. They are effective due to interaction with specific chemical receptors within the body and are in a pharmacodynamic sense, drugs themselves. Given their antibacterial qualities, they are commonly used as an additive to toothpastes and oral hygiene liquids. In many cases, herbal substances are used as the main drug, not just as one of the ingredients. This is due to the general availability of these agents, their natural origin and competitive price compared to synthetic drugs. It is very important that they have fewer side effects compared to conventional medications.

Chamomile is a common flavoring agent in foods and beverages, and other products such as mouthwash, soaps, and cosmetics. When used as a food ingredient, chamomile is unlikely to produce health benefits or side effects. On the contrary when used as a medicinal product, chamomile may produce both desired and unwanted effects.

Chamomile contains many different substances, like α -bisabolol, spiroethers, flavonoids, chamazulene. Clinical and histological evaluation has showed positive influence of topical chamomile on wound healing. This effect could be explained by the strong anti-inflammatory, antibacterial and antifungal attributes of previously mentioned substances.

The component related to anti-inflammatory effects of chamomile is apigenin, a flavonoid that is mostly found in its glycosylated form, apigenin-7-O- β -glucoside (APG). Apigenin has become a popular research subject due to antitumor properties [8]. The efficacy of chamomile extracts as mouth rinse was examined in a clinical study, and showed antimicrobial and anti-inflammatory qualities [9]. Research by *Cvetanovic et al.* checked antimicrobial activity for various microbial strains like *Staphylococcus aureus*,

Klebsiella pneumoniae, *Proteus vulgaris*, *Candida albicans*, *Aspergillus niger*. Results show good antimicrobial activity of both water and ethanol chamomile extracts compared to amracin and nystatin [10]. What should be taken into consideration as well, is that the content of pure apigenin (0–0,5%) is much lower than bound form of apigenin — apigenin glucosides (3–9%) encapsulated in chamomile flowers. Many studies confirm higher biological activity of apigenin compared to its bound form [11]. Some reports show that the hydrolysis of the main glucoside in chamomile apigenin-7-O- β -glucoside to apigenin can be processed before extraction [12, 13]. B-glucosidase, enzyme that breaks glycoside bond between aglycone and sugar component in APG is naturally presents in chamomile flowers and can be activated by fermentation process [10]. In vitro studies on dermal fibroblasts indicate that apigenin can inhibit the migration of fibroblasts through the TGF- β 1 pathway, regulate fibroblast growth, apoptosis and migration. It has been shown that apigenin inhibits fibroblasts survival by blocking progression from G1 to S phase [14, 15].

Other research [4] evaluated oral administration of chamomile water extract. Rapid epithelization and collagen growth percentage were shown after 10 days of treatment, but no significant changes in fibroblasts count were observed. Some authors [16] reported accelerated healing after 20 days of topical treatment with chamomile.

Other research evaluated fractions of chamomile against two gram-negative bacteria. Results of study confirmed chamomile antibacterial effect through its main essential oil components, including coumarin, flavonoids, phenolic acids, and fatty acid [17].

Study [18] on human lung fibroblasts (cell line MRC5 and A375) has shown cytotoxic effect of chamomile extract compounds. Research confirmed a connection between cytotoxicity and solution concentration. Anti-proliferative activity of lower concentration extracts was not so prominent.

Conclusion

The study demonstrated that depending on extract concentration, chamomile can negatively affect gingival fibroblast proliferation. But no changes in cells morphology were observed. Herbs may be good alternatives to current treatments for oral health problems but it is clear that more research is needed. The use of herbs can limit the amount of side effects that generally come with traditional medicines, but this does not mean that side effects will not occur. Chamomile is usu-

ally administered as a tea or a liquid extract commonly used in gingivitis, periodontal disease and ulcers as a mouth wash. Chamomile is considered to be safe during pregnancy and breast-feeding [3], but some side effects like skin rashes or nausea have been reported.

Oświadczenia

Oświadczenie dotyczące konfliktu interesów

Autorzy deklarują brak konfliktu interesów w autorstwie oraz publikacji pracy.

Źródła finansowania

Autorzy deklarują brak źródeł finansowania.

Piśmiennictwo

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Zaakceptowano do edycji: 2020-06-01
Zaakceptowano do publikacji: 2020-06-15

Adres do korespondencji:

Sylwia Klewin-Steinböck
ul. Bukowska 70, 60-812 Poznań