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Editorial Address:
Faculty of Dental Medicine Universitas Airlangga
Jln. Mayjen. Prof. Dr. Moestopo No. 47 Surabaya 60132, INDONESIA
Telp. (062-31) 5039478/ 5030255. Fax. (031) 5039478/ 5020256
E-mail: dental_journal@yahoo.com; Website: www.e-journal.unair.ac.id/index.php/MKG

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CONTENTS

1. Xerostomia severity difference between elderly using alcohol and nonalcohol-containing mouthwash
   Hendri Susanto ................................................................................................................................ 109–112

2. Serum C-reactive protein and C-reactive gene (−717C>T) polymorphism were not associated with periodontitis in Indonesian male patients
   Suhartono AW, Sulijaya B, Djaman NZ, Masulili SLC, Talbot C, and Auerkari EI ................................. 113–118

3. Potential of Jatropha multifida sap against traumatic ulcer
   Basri A. Gani, Abdillah Imron Nasution, Nazaruddin, L. Sartika, and Rahmat K. Alam ............................. 119–125

4. Comparison between probiotic lozenges and drinks towards periodontal status improvement of orthodontic patients
   Natasia Melita Kohar, Victor Emmanuel, and Luki Astuti ................................................................. 126–129

5. Correlation between magnesium and alkaline phosphatase from gingival crevicular fluid on periodontal diseases
   Kasuma N. and Lipoeto I. ......................................................................................................................... 130–134

6. Apoptosis of Rattus novergicus gingival fibroblasts caused by silver nano-particles gel exposure
   Kharinna Widowati, Titiek Berniyanti, and Retno Pudji Rahayu ............................................................ 135–138

7. Autogenous tooth transplantation: an alternative to replace extracted tooth
   David B. Kamadjaja ............................................................................................................................... 139–143

8. The effect of ethyl acetate fraction of Citrus limon peel on mesenchymal cell proliferation and polybacterial growth
   Astrid Marinna, Priyo Hadi, and Desiana Radithia .................................................................................. 144–149

9. The effect of 25% Mauli banana extract gel to increase the epithel thickness of wound healing process in oral mucosa
   Maharani Laillyza Apriasari, Ariska Endariantari, and Ika Kustiyah Oktaviyanti ................................. 150–153

10. Experimental comparative study and fracture resistance simulation with irrigation solution of 0.2% chitosan, 2.5% NaOCl and 17% EDTA
    Ernani, Trimurni Abidin, and Indra ......................................................................................................... 154–158

11. VEGF expression and new blood vessel after dental X-ray irradiation on fractured tooth extraction wound
    Niluh Ringga Woroprobosari, Jenny Gunarani, and Eha Renwi Astuti .................................................. 159–164
Apoptosis of *Rattus novergicus* gingival fibroblasts caused by silver nano-particles gel exposure

Kharinna Widowati,¹ Titiek Berniyanti,² and Retno Pudji Rahayu³
¹ Department of Oral Medicine, Faculty of Dentistry, Universitas Hang Tuah
² Department of Dental Public Health, Faculty of Dental Medicine, Airlangga University
³ Department of Oral Pathology and Maxillofacial, Faculty of Dental Medicine, Airlangga University
Surabaya – Indonesia

**ABSTRACT**

**Background:** The use of silver nanoparticle are growing, especially in medical science, it’s used in many concentration. In dentistry, it’s used to decrease halitosis, and periodontal diseases, and wound healing. It can affect the viability of the cells, give bad effects to the human’s health and environment if used in a long duration and in certain concentration. **Purpose:** The purpose of this study was to learn the apoptosis of gingival fibroblasts in *Rattus novergicus* which is exposed with 15 µg/ml silver nano-particle gel by the expression of caspase-3. **Methods:** This study used 9 male wistar rats and were divided into 3 groups. Sample in group A were cut (hurt) in the oral gingiva and exposed to Ag-Np gel 15 µg/ml for 3 days. After 3 days, they were sacrificed and cut the gingival fibroblasts off 3 x 4 cm size with scalpel. Samples in group B were cut in the oral gingiva and exposed to Ag-Np Gel 15 µg/ml for 5 days. After 5 days they were sacrificed and the gingival fibroblasts off 3 x 4 cm with a scalpel. Samples in group C were cut in the oral gingiva and exposed to none for 3 days then cut the gingival fibroblasts off 3 x 4 cm size with scalpel. The expressions of caspase-3 in the apoptotic and wound healing process were analyzed by Immunohistochemical test. This data was analyzed by using the t-test method. **Results:** Mean expression numbers of caspase-3 in the group A = 5.67; group B = 11.33; and group C (control) = 18.67. T-test sign.number of group A and C = 0.009; group B & C = 0.000. **Conclusion:** The exposure of 15 µg/ml silver gel nanoparticle to gingival fibroblasts of *Rattus novergicus* reduces the expressions of caspase-3 in the day-3 and day-5 post exposure. The amounts of cell death through the apoptotic pathway which were analyzed by the expressions of caspase-3 will decrease too.

**Keywords:** Apoptotic; silver; nano-particles; caspase-3

**Correspondence:** Kharinna Widowati, c/o: Departemen Ilmu Penyakit Mulut, Fakultas Kedokteran Gigi Universitas Hang Tuah. Jl. Arif Rahman Hakim 150 Surabaya 60111, Indonesia. E-mail: kharinna.widowati@gmail.com

**INTRODUCTION**

The use of silver reminds us of the luxury culture in Greek, Roman, and Egyptian. Silver is well-known type of metal and ranks number third after gold and silver copper in those nations.¹ Silvers are generally used for water containers and the other liquid materials to keep the cleanliness and sterility.²

Silver use has developed very fast for health purpose. The Macedonians use silver to prevent infection after surgery. Aside from being antiseptic, Hippocrates used the preparation of silver nitrate for ulcer treatments, compound fractures, and a good wound healing supporting material.¹ Avicenna use silver nitrate to purify blood, to prevent heart palpitation, and to handle respiratory diseases. In 1880, Doctor Carl Siegmund Franz Crede, a German obstetrician, became the first person to use eye drops made from 1% silver nitrate to prevent ophthalmia neonatorum (gonorrheal ophthalmia) on babies.³ Anti-bacterial function of silver nano-particles are also utilized by the dentists as mouthwash therapy for periodontal diseases, to reduce bad breath, to help wound healing process and to prevent infection in tooth extraction and surgery.⁴

The use of silver nano-particles progresses rapidly in the field of nanobiotechnology; however, silver nano-particles also have negative consequences to human and environment
for prolonged use or in uncontrollable concentration. Silver nanoparticles have a nano size because they can easily fit into cells. Therefore, if silver nano-particles are used continuously with uncontrollable concentrations, they can lead to cells death and affect human biological system.

Several studies have been conducted to examine the effect of silver nano-particles use on rats liver cells. The result showed that the use of silver nano-particles with 5-10 µg/ml concentration can affect the decline of mitochondrial function and the integrity of liver cell membranes after 24 hours incubation. The other in vitro studies, 24-48 hours exposure of silver nano-particles with 10-25 µg/ml concentration on human lung cells fibroblasts can stimulate the release of pro-inflammatory cytokines as the oxidative stress level and reactive oxygen species (ROS) production that can potentially damage DNA cells. This implies that some information about the other effects caused by the use of silver nano-particles with concentration circulated in the market are needed so that people will be more vigilant in using silver nano-particles.

Based on the data above, we conducted a deeper research by using gingival fibroblasts cells of Rattus norvegicus as the subject test because their tissues are similar to human gingival fibroblasts cells that potentially get exposed directly when the application process of silver nano-particles was done topically. The purpose of this study was to examine the death of Rattus norvegicus gingival fibroblasts cells through apoptosis by analyzing caspase 3 expression.

**MATERIALS AND METHODS**

This study is experimental laboratory with post test only control group design. This study was conducted in the laboratory of Biochemistry and Pathology, Faculty of Medicine Universitas Airlangga, and Institute of Tropical Disease (ITD) Universitas Airlangga Surabaya. This study used male Rattus norvegicus, age 3–4 months, weight ± 200 grams, and declared healthy on physical examination by veterinarian. Materials tested were silver nano-particles from Aquasil brand produced by Nanonasb Pars Company in gel form with 15 µg/ml concentration. Nine Rattus norvegicus were divided into three groups. The incision was made on each group in the anterior mandibular gingiva, extending from interdental gingiva of the mandibular central incisiv downwardly along for ≥ 5 mm in length and 2 mm in depth. After the incision, 15 µg/ml silver nano-particles gel was topically applied into group A as much as ± 1 ml and then was followed by suturing process on the wound to prevent silver nano-particles gel leaked out from the wound area, and decapitation on the day 3 after exposure process of silver nano-particles gel application. The same steps including slicing process, gel application, and suturing process are also performed in group B, followed by decapitation on the day 5 after exposure process of silver nano-particles gel application. The incision of group C was made without followed by the application process of silver nano-particles gel 15 µg/ml, and then continued by suturing process and decapitation on the day 3 after the slicing process and suturing (control). Decapitation control group was only given on the day 3 because in the normal condition of healing process (without any supporting materials for healing process), is estimated that the cells death through apoptosis will appear 24-72 hours after the incident lesion. For the treatment groups, in addition to decapitation on day 3, they were also given decapitation on day 5 as the healing process could occur faster or slower than in normal condition due to the silver nano-particles exposure. The examination of caspase 3 was conducted by using immunohistochemistry test, and then was observed by using light microscope with 400x magnification. Comparative test independent t-test was used to examine the significant difference between treatment groups and control groups.

**RESULTS**

Table 1 shows the results of 15 µg/ml silver nanoparticles gel exposure on caspase-3 expression of fibroblasts research (Table 1). The highest average of caspase-3 expressions were found in control group. The following bar chart shows the mean value of caspase-3 expressions of fibroblasts in treatment group on the day-3, day-5 (Figure 1).

Figure 2 shows the result of immunohistochemical examination. Thick and long brown colour lines pointed by the small arrows depict the form of fibroblast cells indicating caspase-3 expression. On the day-3, caspase-3

<table>
<thead>
<tr>
<th>Group</th>
<th>Group name</th>
<th>Mean (x)</th>
<th>Standard Deviation (SD)</th>
<th>p</th>
<th>Group name</th>
<th>Mean (x)</th>
<th>Standard Deviation (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day-3</td>
<td></td>
<td></td>
<td></td>
<td>Day-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>A</td>
<td>5.67 ± 0.577</td>
<td>0.009</td>
<td>B</td>
<td>11.33 ± 0.577</td>
<td>0.000</td>
<td></td>
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<tr>
<td></td>
<td>Control</td>
<td>C</td>
<td>18.67 ± 1.155</td>
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Notes: * There are significant difference (p<0.05).
expression on fibroblasts of control group (C) appeared to be denser than of treatment group (A). Caspase-3 expression on fibroblasts of treatment group B (day-5) appeared to be denser than of treatment group A (day-3).

T-test examination was performed after the data were normally distributed by using Kolmogorov-Smirnov test and homogeneous by using Levene test. The result of the comparative test by using t-test on A-C groups showed that the number of expressions of caspase-3 in fibroblasts have significant difference with significance value $p=0.009$. While the result of comparative test by using t-test on B-C groups also have significant difference with significance value $p=0.000$ ($p<0.05$).

**DISCUSSION**

The research of 15 µg/ml silver nano-particles gel exposure on the expression number of caspase-3 in fibroblasts showed the significant difference of the mean value between treatment group and control group. Caspase-3 expressions in fibroblasts of treatment group are lesser than of control group (0 µg/ml). The significant difference of caspase-3 expressions were also found between treatment group A (on the day-3) and B (on the day-5). This suggests that the exposure of 15 µg/ml silver nano-particles gel is able to suppress the expression of caspase-3 in gingival fibroblasts cells that suffered injury of incision. Typical sign of cells undergoing apoptosis is the expression of caspase-3 (caspase executioner) of the cells. The suppression of caspase-3 expression on gingival fibroblasts cells indicates the reduced number of cells undergoing apoptosis.

Exposure to 15 µg/ml silver nano-particles gel on injury (cuts) were able to suppress the activation of the innate immune response which is the beginning of an inflammatory mechanism in the immune cells activation, the complement system, the identification and the removal of foreign substances, as well as the activation of the adaptive immune system. Phagocytes cells such as polymorphonuclear neutrophils, monocytes, and macrophage are natural immune cells triggered the suppression of pro-inflammatory cytokines release and various systems such as the complement system and acute phase response as the increased oxidative. Macrophage cells as antigen presenting cell (APC) has MHC class II molecules. Through MHC class II, B cells will receive antigen, the antigen is presented to the cells surface to activate T helper cells which then will secrete pro-inflammatory cytokines. The declination of mitochondrial function resulted by cells stress also triggers the activation of gene p-53 as pro-apoptosis gene in mitochondria.\(^7\) The activation of gene p-53 followed by the inactivation of protein Bcl-2 and the increased production of Bax will affect the permeability of mitochondrial membrane that can release cytocrom c. The released cytocrom c will be bound by apoptosis activating factor (Apaf-1) and then will form apoptosom. Apoptosom will activate caspase 9 (initial caspase activated by the released cytocrom C), caspase-9 will activate caspase-3 which acts as apoptosis executioner.\(^12\) The number of expressed caspase-3 indicates the number of cells undergoing apoptosis. Caspase

**Figure 1.** Bar chart of mean value of caspase-3 expressions in fibroblasts on the day-3 and day-5.
Notes: A) treatment group on the day-3; B) treatment group on the day-5; C) control group.

**Figure 2.** Overview of caspase 3 expression in fibroblasts of each group. Fibroblasts are shown by the small arrows.
Notes: A) treatment group on the day-3; B) treatment group on the day-5; C) control group.
expression will appear in the next 6–9 hours so that the estimated death of cells will appear 24–72 hours after incident lesion.\(^9\)

It is concluded that the exposure of 15 µg/ml silver nanoparticles gel to gel *Rattus norvegicus* gingival fibroblasts decline caspase-3 expression. This suggests that apoptosis of the observed cells through caspase-3 expression is also decreasing.

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