Effect of Combination Electrolyzed Reduced Water and EGCG (Epigallocatechin-3-Gallate) on RANKL expression and Osteoclast Number in Orthodontic Teeth Relapse

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INTRODUCTION
Orthodontic relapse or returning of teeth to its initial position after orthodontic treatment, will then be treated by an active orthodontic device [1]. Relapse is a common problem that often occurs and reduces the success of orthodontic treatment in Indonesia [2]. There is increasing of osteoclast differentiation in the pressure region and decreasing of osteoclasts differentiation in the strain/pull region in the relapse and orthodontic tooth movement process. This process indicates remodeling of alveolar bone and being an important factor in the orthodontic relapse process [3]. The most common way to prevent relapse after completion of orthodontic tooth movement, is the presence of retention phase using a retainer [4]. Besides, administration of certain drugs also given to prevent relapse [5]. Consumption of ERW via tube approximately 2.5 ml/100 gr body weight every morning accelerates the process of bone remodeling [6]. Epigallocatechin-3-gallate (EGCG) is the most abundant and powerful green tea catechin. EGCG is beneficial for bone regeneration and stated as promising treatment strategy for bone repair therapy [7]. EGCG can inhibit osteoclast formation [8].

MATERIALS AND METHODS
This research is an experimental laboratory research with randomized post test only control group design. Samples from this study were rats (Rattus norvegicus) with criteria male rats weighed 200-250 grams, at least 3 months of age, and in healthy condition. Thirty six experimental animals Rattus norvegicus were divided into 6 groups with a total of 6 groups each and were assigned to one of three groups; control negative group, a control positive group and a treatment group. Each of these three groups was itself further sub-divided into two groups which were fed orally and subjected to observation on a daily basis between day 7 and day 14 after debonding. The six members of each group were fed from a small container twice a day; once in the morning and again in the afternoon. They drank up to 20 ml (4x5 ml) per day via a small pipe attached to a bottle of water. The negative control group was provided with 20 ml (4x5 ml) of distilled water per day, while the positive control group received daily 3mg/kg dose of simvastatin. The treatment group received daily 2.5 mg/L of ERW. All groups were sacrificed on day 14 after observation. Immunohistochemical results (IHC) were then observed with light microscope (Olympus, Japan) at 400x magnification.

RESULTS
The quantitative data obtained confirmed the RANKL expression and the number of osteoclast in each group. Combination of ERW and EGCG reduced RANKL expression (Fig. 1) and, therefore, also the number of osteoclast (Fig. 2). The results of Histopathology examination in terms of the RANKL expression can be seen in Fig. 3, and the number of osteoclast can be seen in Fig. 4.

EXPERIMENTAL
The application of 10 g/mm2 close coil spring to the between the right upper central incisor and the right maxillary first molar for seven days was intended to induce orthodontic tooth movement in the control and treatment groups. This represented the optimum force for promoting orthodontic tooth movement in Wistar rats (R. norvegicus) [10]. All samples were sacrificed on day 7 and day 14, anesthetized by means of rodent’s anesthesia. The maxillary bone tissue was subsequently removed and placed in a buffered formalin solution (10% buffer formalin solution with pH 7.0). 3,3’-Diaminobenzidine (DAB) and monoclonal antibody were used to examine the expression of RANKL. Meanwhile, Hematoxylin Eosin (HE) staining used to determine osteoclast number. Immunohistochemical results (IHC) were then observed with light microscope (Olympus, Japan) at 400x magnification.

CONCLUSION
Combination of ERW-EGCG significantly inhibits RANKL expression and the number of osteoclasts for relapse orthodontic tooth movement after 3, 7, and 14 days post debonding. Thus, combination ERW-EGCG is a potential therapy for enhancing bone remodeling in patients post orthodontic treatment.

REFERENCES