Original Article

Efficacy of Drinking Green Tea in Antimicrobial Activity Following Surgical Removal of Impacted Lower Third Molars: A Randomized Controlled Trial

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Abstract

The aim of this article is to evaluate the effect of drinking green tea on antimicrobial activity and wound healing after the surgical removal of an impacted mandibular third molar. The study was carried out as a randomized controlled clinical trial. Twenty patients with impacted mandibular third molars were randomly divided into two equal groups (drinking green tea or drinking water) after lower third molar removal. The same conditions were applied to all the patients, including the interrupted sutures were given by 4-0 silk in both groups. The outcome variables were soft tissue healing and number of colony-forming units per milliliter. There were 3 males and 17 females, in the age range of between 19-31 years. The mean of soft tissue healing index was higher for the green tea group at all times but did not show statistical significance. The mean of microbial loads was lower in the green tea group on a postoperative day 7 (10^{-5} CFU/mL in concentration). The present study found that drinking green tea can decrease the bacterial count in the suture and may improve wound healing after the surgical removal of lower third molars.

Keywords : Bacterial count, Green tea, Surgical removal, Third molar, Wound healing

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Introduction

Surgical removal of an impacted third molar is one of the most common procedures carried out in oral and maxillofacial surgery. Pain, swelling, trismus, or anatomical variation can make the maintenance of oral hygiene difficult, which increases the risk of infection.[1] A previous article mentioned that microorganisms that were normally sequestered at the tissue surface could affect infection factors if the tissue surface was injured.[2] Since there has been continuous efforts to reduce these complications, various local antiseptic and antimicrobial medications like chlorhexidine, tetracycline, and metronidazole have been used.[3]

Currently, natural products have been significantly used in modern medicine worldwide. Naturally derived

substances are believed to have a low incidence of severe adverse reactions and are inexpensive compared to standard therapies.[4] Research has shown that natural active compounds have properties of modulating the host inflammatory response and of enhancing socket wound healing.[5] Drinking one cup of green tea every day is associated with decreasing the mean of the periodontal pocket, attachment loss, and bleeding on probing.[6] The flavonoids are the most important tea polyphenols, and the major components of flavonoids are the catechins. Catechins have antibacterial action and have shown utility in the treatment of oral and topical infection.[7] Studies have shown that the green tea polyphenolic catechins, in particular (–)-epigallocatechin gallate (EGCG) and (–)-epicatechin gallate (ECG), can inhibit the growth of a wide range of gram-positive and gram-negative bacterial species with moderate potency.[8]

Previous research revealed that green tea has an indirect antibacterial effect by the inhibition of glucosyl transferase, which leads to limiting the biosynthesis of sticky glucan. Human and bacterial amylase were inhibited by catechin in dried tea leaves.[9] Green tea has been considered to have the ability to modulate inflammation. Epigallocatechin-3-gallate (EGCG) influences multiple aspects of innate and adaptive immunity, including ameliorates mucosal inflammation by inhibiting a pro-inflammatory cytokine (TNF-α).[10] It has been reported that green tea possesses an anti-inflammatory effect against the denaturation of protein *in vitro*.[11]

Numerous studies have reported the beneficial properties of green tea. As far as it is known, there have been no clinical trials investigating the effects of drinking green tea on oral surgical wound healing. Therefore, this study aimed to evaluate whether the consumption of green tea can influence microbial loads and wound healing following surgical removal of the lower third molars.

Material and Methods

Subjects and study design

This study was a prospective randomized, controlled trial. Block randomization, 20 participants were randomly selected from the Oral and Maxillofacial Surgery Department between August 2019 and January 2020. The sample size was calculated based on the following formula from Nevas *et al.*[12]

$$n_1 = \frac{(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \left[\sigma_1^2 + \frac{\sigma_2^2}{r}\right]}{\Delta^2}$$

$$r = \frac{n_2}{n_1},\ \Delta = \mu_1 - \mu_2$$

The sample size was augmented 20% to compensate for possible loss.

The research protocol complied with the guidelines of the Declaration of Helsinki and the Consolidated Standards of Reporting Trials.[13] This project was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2020-005). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2010.

The inclusion criteria included healthy patients (ASA I), ages between 18 to 35 years old, either male and female who required surgical removal of impacted lower third molars under local anesthesia. The participants presented partial bony impaction in vertical, mesioangular or horizontal angulation.

The exclusion criteria were patients who had signs or symptoms of any infection, had used chewing nuts, tobacco, or consumed alcohol within a month before the start date of the study. Also, females on contraceptives, pregnant women, or breastfeeding or individuals whose surgical procedure lasted longer than 45 minutes (from the first incision until the last suturing) were also excluded. Patients who had an allergy or intolerance to drinking green tea or consumed any tea products and used mouthwash during the experiment, including those patients who had incomplete follow up.

All the patients were informed about the study and written informed consent was obtained before enrollment. A panoramic radiograph was taken for the preoperative investigation to assess position (Pell and Gregory system) and angulation (Winter classification) versus the adjacent second molar. Participants were

randomly divided into two study groups. After the surgical removal of the impacted mandibular third molar, one group was given green tea to drink, another group was given drinking water.

Surgical procedure

A standardized operative procedure was performed similarly under strict aseptic precautions by one experienced oral and maxillofacial surgeon. After the anesthetic infiltration comprising 2% mepivacaine with 100,000 epinephrine, a mucoperiosteal flap was reflected, osteotomy and osteotomy using a tungsten carbide bur and micromotor were done. After surgical removal of the tooth, the socket was thoroughly irrigated with 0.9% normal saline, and then the flap was closed and sutured with 4-0 silk. Oral analgesics (Paracetamol 500 mg; one tablet every 4-6 hours as needed or Ibuprofen 400 mg; one tablet every 4-6 hours per day as needed) were prescribed, and post-extraction instructions (verbal and written) were given. The patients were advised not to take any drugs, mouthwash, or tea other than those prescribed and to seek help from the operator in the first instance if they had a problem.

Preparation of drinking green tea

The researchers prepared seven bottles of drinking water (500 ml) and 14 tea bags (2 tea bags per 1 bottle of water) for each patient. The 4-gram green tea bags (Royal project highland tea, Chiang Mai, Thailand) were steeped into 500 ml of drinking water at a temperature of 4°C. The green tea bag remained in the drinking water for 9 hours.[14] The daily tea preparation of 500 ml was repeated for seven consecutive days. The participants were advised to drink green tea or drink water 1/4 of the bottle continuously within 10 A.M., 1/2 of the bottle within noon, 3/4 of the bottle within 3 P.M., and finish within 5 P.M.

Soft tissue healing evaluation

All patients were recalled on postoperative days 3 and 7 for soft tissue healing assessment. The wound was assessed by a healing index (very poor =1, poor = 2, good = 3, very good = 4, excellence = 5) based on the criteria given by Landry *et al.*[15] The presence of dry socket or wound infection were also recorded. Two-blinded examiners had been calibrated before the study.

Measurement of antimicrobial activity

To determine whether drinking green tea alters plaque formation and the number of bacteria on black silk sutures in the mouth of volunteers. Five millimeters of black silk that was in contact with the patient's oral cavity was cut from the end-point of the suture and collected in Phosphate-buffered saline solution 10 milliliters for seven postoperative days. The sample was vortexed for five minutes, and sonicated at 500 Watt, 20 kHz, amp 20% for ten seconds. Then serial diluted to concentration 10−6, spread on Trypticase soy agar 1 milliliter at each concentration, and incubated at 37°C under 5% CO_2 atmosphere for 24 hours (Fig. 1). Subsequently, the number of colony-forming units (CFU) per milliliter was calculated by the number of colonies x dilution factor.

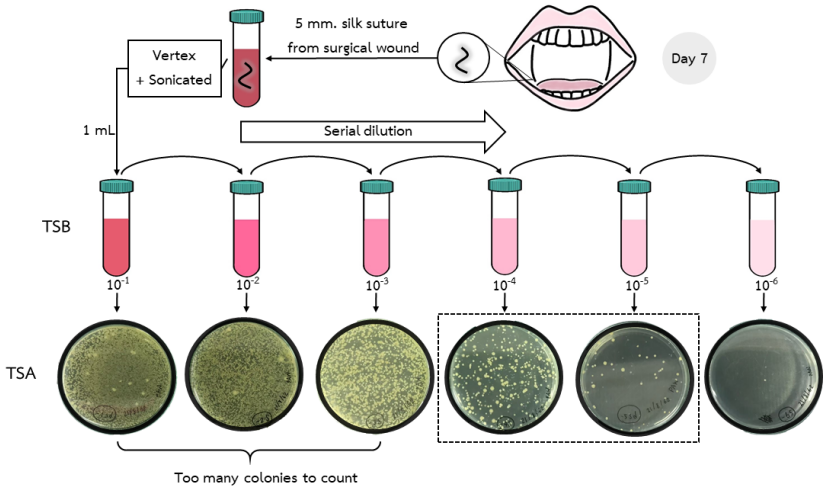

Figure 1 *Serial dilution and plate count of viable colony-forming bacteria. (TSA = Trypticase soy agar, TSB = Trypticase soy broth)*

Statistical analysis

Inter- and intra-observer reliability were evaluated using the intraclass correlation coefficient (ICC). Demographic data (age and gender) were analyzed using descriptive statistics. Quantitative variables were presented as mean ± standard deviation, then using Chi-square for statistical analysis. The Kolmogorov-Smirnov normality tests were used to assess the normality of the data. Meanwhile, nonparametric data for the comparison between groups were analyzed with the Mann-Whitney test. Spearman's correlation coefficient was used to assess the correlation among variables. Statistical analyses were conducted with SPSS software (IBM, SPSS Statistics version 21).

Results

Twenty-one patients met the inclusion criteria, one of whom was excluded for not following the proper postoperative follow-up. Therefore, 20 patients were included in the study: 3 males and 17 females. The mean age of the patients was 22.25 ± 2.3 years old (range 19-31 years). Nine of the third molars were on the left side and 11 on the right side. The ICC for the inter-observers agreement was 0.98, an intra-observer agreement was 0.99, demonstrating excellent reliability.

Following random assignment between green tea and non-green tea groups, there were classification differences but no statistically significant differences in the side of surgery, tooth position, and mean of operating time (Table. 1). However, the operating time had a significantly positive correlation with classification (r_s = .542, p = .013).

In both groups, the mean of the healing index was the lowest at postoperative day 3 and gradually increased over the following 14 days (Fig. 2). The mean of the healing index was higher for the green tea group for all the time points when compared with the non-green tea group but not statistically significant.

Table 1 *Comparison of mandibular 3rd molars status and operating time between groups*

Variables	Categories	Non-green tea	Green tea	*p*-value
Side of surgery (N)	Left 3rd molar	5	4	.65 *
	Right 3rd molar	5	6	
Classification (N)	I	5	1	.004 *
	II	4	9	
	III	1	0	
Tooth position (N)	A	5	6	.65 *
	B	5	4	
Operating time (mean±SD)	Minutes	26.67 ± 9.5	25.56 ± 6.7	.76 **

*N = Number, SD = Standard deviation, * Chi-squares, ** one-way ANOVA*

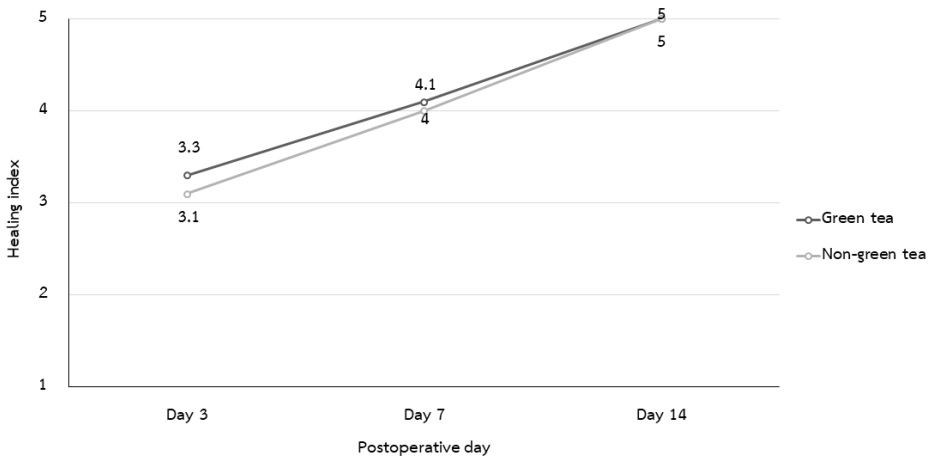

Figure 2 *Mean value of postoperative healing index in green tea versus non-green tea group*

According to the results, the mean of colony-forming units/mL on postoperative day 7 was considerably lower in the green tea group (34.5 x10^5) than the non-green tea group (73.7x10^5) (Fig. 3). Additionally, the colony-forming units/mL had a significant correlation to soft tissue healing assessed seven days after the removal of the third molar (r_s = -.649, p < .01).

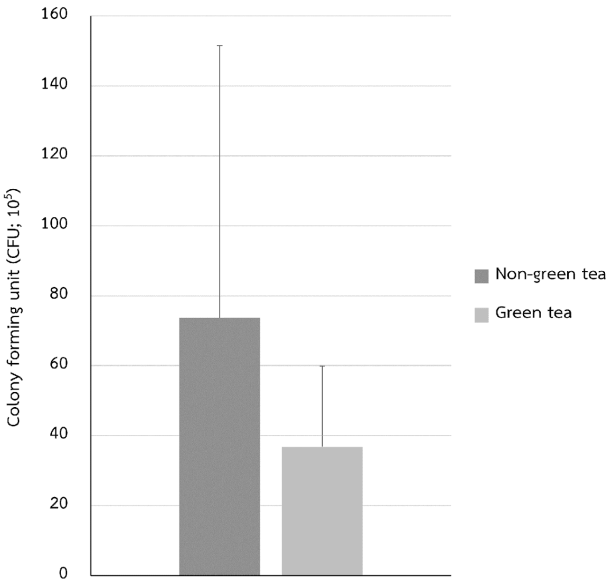

Figure 3 *Mean value of bacterial count from black silk suture on postoperative day 7; Colony-forming unit (CFU)/mL*

Discussion

The purpose of this study was to evaluate the effectiveness of drinking green tea in antimicrobial activity and wound healing following the surgical removal of the lower third molars. The tea produced from the same plant, differ in terms of the manufacturing processes of the tea leaves and chemical compositions. Green tea leaves are heated rapidly by steaming or frying to prevent polyphenol oxidation (unfermented) while oolong tea (semi-fermented) and black tea (fermented) leaves are dried, rolled and crushed, which promotes oxidation.[16] Thereby, green tea has far more active catechins than other teas.[17]

The methods of preparing the beverage vary throughout the different parts of the world. Green tea manifested time dependence and temperature sensitivity. Prolong cold steeping of green tea exhibits better antioxidant activity compared to other methods.[18] For this research, green tea was also brewed by cold infusion. This innovative method achieved bioactive compounds, such as epigallocatechin gallate, with the highest values, about double in hot infusion.[14] Likewise, the cold brew is particularly appropriate for tropical countries and hot summer months due to a more simple preparation and easier consumption than water. All participants were regularly scheduled to consume 1/4 of a bottle to eliminate the bias due to additional antimicrobial effects from frequency and exposure time to green tea.

This study, performed the surgery under the same experienced operator, using the same surgical technique, instruments, and a controlled aseptic environment. Although the classification was a statistically significant difference, there was no statistically significant difference in the operating time between the green tea and the non-green tea group. It could have accounted for the similar condition of the surgical wounds.

The number of colony-forming units per milliliter was to indirectly evaluate the inflammatory reaction. The outcomes of this study demonstrated that the mean of microorganism count from silk suture on postoperative day 7 was lower in the green tea group. Although there were no statistically significant difference observed between the two groups (p = 0.156), the lower bacterial colonization for the green tea group validates its antimicrobial effects in the oral cavity, as founded by Thomas A *et al.*[19] A recent experiment found 0.5% green tea mouthwash against *S. mutans* was significantly better than 0.2% CHX mouthwash. Green tea catechins may help to maintain the normal plaque pH, which in turn is an unfavorable condition for *S. mutans* to colonize and multiply.[20]

During wound healing, it has been hypothesized that fibroblasts play an essential role in tissue healing. Although the difference in soft tissue healing in this study was not significant, it was shown that the soft tissue healing in the green tea group was more significant than the non-green tea group on both postoperative day 3 (p = 0.435) and day 7 (p = 0.69). These findings were supported by

the previous study that applied topical green tea extract over the wound. The histological results showed that the granulation tissue in the experiment group contained less inflammatory cells, more fibroblast growth, and more blood capillaries. It was concluded that green tea extract shortens the inflammatory period by increasing the rate of proliferation, angiogenesis, and collagen formation phases to enhance wound healing.[21]

Our finding that the mean value of bacterial count (CFU/mL) on a postoperative day 7 has shown high standard error. The microbial concentration might be affected by the initial inoculation and the length of the lag phase (the period between the introduction of a micro-organism into the culture medium in which the bacteria have not yet started dividing).[22] By this assumption, the growth rate variations were caused by a lack of control over these time conditions. Although our research methodology stipulated that the sutures were brought for serial dilution immediately after being collected from the patients, the exact length of the lag phase was not determined. Therefore, this might be the cause of this discrepancy.

In this study, dry sockets or other severe complications were not observed during the follow-up period. However, this experiment required a great deal of cooperation from all the participants, with not only the preparation of drinking green tea by themselves but consequently, several visits are needed during the follow-up period. Thus, the lack of participants was the limitation of this study. Because of the small sample size, it was challenging to identify significant relationships from data.

Conclusion

Within this limitation, we concluded that drinking green tea may display an advantage in clinical performance in oral wound healing. The anti-bacterial properties resulted in the remarkable depletion of bacterial loads in silk sutures. Green tea may reduce local oral mucosa inflammation and facilitate wound healing with no observed side effects. This data provides the first clinical scientific support for the effectiveness of drinking green tea in wound healing after third molar surgery. However, to better understand the effect of green tea, more clinical trials with larger samples are necessary.

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Conflict of interests

The authors declare that they do not have any conflicts of interest.

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