Clinical Effectiveness of Green Tea Extracts as a Local Haemostatic Agent Following Mandibular Molar Extraction

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ABSTRACT

Objective: To determine the clinical effectiveness of different green tea extracts (GTEs) in reducing bleeding after extraction of mandibular molars.

Study Design: Randomised controlled trial.

Place and Duration of the Study: College of Dentistry, King Khalid University, Abha, Saudi Arabia, from October to December 2022.

Methodology: A total of 64 patients were selected from those who attended dental clinics at the College of Dentistry, King Khalid University for extraction of their mandibular molars. They were equally and randomly divided into a control and three test groups by asking the patient to choose a numbered piece of paper. In the first group, normal saline-soaked sterile gauze was used after the tooth extraction while in the three test groups, different GTEs (methanolic GTE, aqueous GTE, and tannin isolated from the green tea) were applied. Monitoring of the bleeding by observing the extraction socket was carried out at regular intervals of five minutes until the oozing subsided, and then once an hour after that.

Results: Each group had 16 patients. The mean of bleeding stop-minutes was significantly different among the groups (61.56 minutes for the control group, 7.50 minutes 8.44 minutes and 5.62 minutes for the test groups, $p < 0.001$). The median of bleeding stop-minutes of the control group was significantly higher than all test groups ($p < 0.001$). The number of patients in whom bleeding was continued one hour after surgery was significantly higher in the control group ($p = 0.005$). Moreover, tannin has the greatest haemostatic effect compared to aqueous and methanolic GTEs.

Conclusion: Significant haemostatic effect has been shown by all GTEs. Tannin isolated from green tea has shown a significantly higher haemostatic effect than to the aqueous and methanolic extracts.

Key Words: *Bleeding, Green tea extracts, Haemostasis, Tannin isolate, Molar extraction.*

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INTRODUCTION

Bleeding during and after the surgery, can be unpleasant for the surgeon and the patient. Intraoperatively, bleeding can affect accessibility and visibility at the site and if unmanaged, it can lead to catastrophic repercussions postoperatively. In major oral and maxillofacial surgical operations, several measures are utilised to control bleeding, including electrocautery and suture ligature. Molar teeth extraction is known to be a common minor oral surgery procedure in which many complications can arise postoperatively, e.g. bleeding from extraction sockets, infection, swelling, trismus, and postoperative pain.[1,2]

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Haemostasis can be particularly difficult to establish in cases involving bony surfaces, inflammatory or friable arteries, or tissues characterised by the presence of many and widespread capillaries. In such circumstances, achieving haemostasis with mechanical and thermal methods might be challenging, therefore, a frequently employed approach for managing intraoperative haemorrhage is the utilisation of a topical haemostatic agent. Local haemostatic agents such as Gelfoam, bone wax, absorbable collagen haemostat sponge, and oxidised regenerated cellulose (Surgicel), are utilised to manage external bleeding by facilitating or expediting the inherent clotting process by various mechanical and physical interactions between the agent and blood.[1,3]

It has been demonstrated that the application of topical haemostatic agents is linked to a range of undesirable adverse effects. For example, it was found that collagen-based substances are associated with allergic reactions. On the other hand, the use of oxidised regenerated cellulose has been linked to foreign body reactions with inflammatory reactions and a delay in wound healing. Moreover, the use of bone wax should be avoided due to some possible complications, such as

impaired bone healing and inflammatory reaction as a result of its non-absorbable characteristic. Consequently, green tea has emerged as a viable alternative haemostatic agent that offers the advantage of being devoid of the side effects when compared to other topical haemostatic agents.[4-7] Green tea is considered a valuable reservoir of tannins and has garnered significant interest in recent times for its potential use in topical treatments due to its notable attributes, including its haemostatic, antibacterial, and antiseptic qualities.[8,9]

Tannic acid is a commercial compound similar to the plant polyphenol tannin, which stops bleeding from mucous membranes *via* vasoconstriction and acceleration of clot formation.[10] Green tea is a rich source of tannins and can serve as a valuable adjunct to conventional local haemostatic techniques, such as, gel foam and suturing, particularly in situations when immediate access to medical intervention is limited or unavailable, owing to its widespread availability and affordable price.[9,11,12] In addition to this, the use of green tea as an adjuvant to non-surgical periodontal therapy has been shown to considerably reduce bleeding on probing as well as gingival inflammation.[13]

The objective of this study was to investigate the clinical effectiveness of different green tea extracts in reducing postoperative bleeding after the extraction of mandibular molars.

METHODOLOGY

It was a randomised controlled double blind clinical trial performed at the dental clinics of the College of Dentistry, King Khalid University, Abha, Saudi Arabia, from October to December 2022. The study was conducted under the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of the College of Dentistry, King Khalid University (IRB/KKU-COD/ETH/2022-23/021). The trial is registered in the ISRCTN registry with study registration number ISRCTN60510450.

For the preparation of green tea extracts, leaves of China green tea (*Camellia sinensis*) G-401 containing dried leaves were procured at Aseer region, Saudi Arabia in October 2022. The total weight of one box of green tea was 100 gm. The chemicals and reagents used were Dichloromethane (DCM), methanol of analytical grades, absolute ethanol (CH_3CH_2OH) 96% pure and Calcium carbonate ($CaCO_3$) 98% extra pure. The distilled water (H_2O) used was generally regarded as safe (GRAS). Methanol and water extraction were performed by using the decoction method. The extraction process was performed at the Department of Pharmacognosy, Faculty of Pharmaceutical Science, King Khalid University using the method adapted from Abubakar and Haque, and Hmidani *et al*.[14,15] Course powdered plant material was kept in a clean glass container. Methanol and H_2O were then separately poured into two beakers, each beaker contained 600 gm of green tea and stirred. Throughout the extraction procedure, heat was subsequently applied to accelerate the process. The procedure lasted for a short duration typically around 15 - 20 minutes. In general, 1:4 was the ratio of unprocessed substance to solvent. After extraction, the extracts were filtered using a vacuum filter. The collected filtrates underwent rotary evaporation to completely remove the solvent and the residual extracts were dried. The percentage yield of the obtained dried extract (methanol and H_2O) was calculated. The dried extracts were stored in a refrigerator in a tight-fitted glass bottle for further analysis; the % yield being (Grams of dried extract obtained)/ (Grams of green tea) X 100.

Tannin was isolated using the calcium carbonate method with some modifications.[16-18] A total of 1500 mL of H_2O was added to a total of 600 gm of the green tea, which was divided into three portions; each portion was comprised of 200 gm of tea mixed with SS500 mL of H_2O to make three solutions. Each solution was treated separately, and their yields were combined. The solutions were brought to boiling. By boiling the green tea, H_2O soluble components which are mostly caffeine and tannins were extracted. Then, 70 gm of Ca_2CO_3 was added to each one of the three solutions. After 30 minutes of boiling, the solutions were transferred into separating funnels, an equal amount of DCM was added to the solutions and were shaken vigorously. Tannins are acidic and react with Ca_2CO_3 in the solutions to form salts that are dissolved in H_2O, while caffeine and other xanthene alkaloids are present in the DCM layer. Afterwards, the layers were allowed to settle to form two layers, and the bottom DCM layer was drained. This extraction process was carried out a total of three times using 200 mL of DCM each time for the removal of xanthene alkaloids. The H_2O layer was then collected into a round bottle flask (RBF). A curdy brown precipitate of calcium tannate was formed, separated from the solution, and dried by a rotatory evaporator. The obtained tannin salt was purified with 98% absolute ethanol and then dried again under vacuum. Tannin salt was packed and stored in a glass bottle for further analysis.[14,15,17-19]

After the extraction process, four solutions were prepared: Normal saline (0.9%), methanolic GTE (1mg/ml), aqueous GTE (1mg/ml), and tannins (1mg/ml). Sterile gauze was then soaked in each of the extract solutions. After one hour, they were removed from the solution using sterile forceps and a small amount of pressure was applied to extract any remaining solution. Subsequently, the pleated gauze was transferred to a glass container and subjected to 20 minutes of autoclaving at 121°C with moist heat. Finally, sterile pouches were used to enclose the 5x5 cm gauze that was used in the study.

Patients were selected from those who attended dental clinics at the College of Dentistry, King Khalid University for extraction of their mandibular molars. The inclusion criteria were the age of 18 years and above, with a consent of voluntary willingness. Whereas exclusion criteria were sensitivity to green tea, clotting disorder and liver disease, uncontrolled infection at the site of extraction, history of malignancy or radiation exposure at the site of extraction, use of antibiotics, corticosteroids, anticoagulants, and contraceptive medications during the preceding month, smoker, and impacted third molars. A statistician was consulted to estimate the sample size, and based on a previous study, an average of approximately 85% reduction of postoperative bleeding at 95% confidence level and study power of 80%

with expected dropout rate of 10% were considered and a sample of 64 patients was recommended for the study.[11]

Before the intervention, informed consent was obtained from all patients. The data collection sheet was used to document the demographic information (such as age and gender) of each patient after their inclusion in the study. Past medical / dental history and the presence of periodontal diseases were recorded as well. Randomisation was done by asking the patient to choose a numbered piece of paper (either 1,2,3, or 4) and accordingly, he/she received the corresponding intervention. Both patients and the principal investigator were blinded to the type of solution used.

Molar tooth extraction was performed after providing local anaesthesia of 2% Mepivacaine and 1% epinephrine solutions. The extraction was simple and non-surgical without raising a flap or bone removal. After extraction, the socket was cleaned and irrigated with normal saline, and the respective gauze piece was placed in the socket. Pressure was applied to the gauze area, every five minutes, the gauze was then removed from the patient's mouth in all groups, and any instances of bleeding or its cessation were observed and documented. Similar gauze was applied to the site under pressure in the event of bleeding; this process was repeated for up to one hour, or until the bleeding ceased. The findings were observed and documented. Furthermore, upon discharge (following the cessation of bleeding), every patient was provided with an additional piece of sterile gauze and instructed to apply pressure to the extraction site for one hour. Following this, they were contacted *via* telephone to inquire about the presence or absence of oozing at the site of extraction. The patients were followed up for the next three days and any postoperative complication was recorded. The mean time until bleeding ceased and the presence or absence of oozing were compared among the four groups.

Following collection and evaluation, data were entered into version 21 of the statistical package for the social sciences (SPSS by IBM). The statistical tests employed were two-tailed, with an alpha level of 0.05. Significance was determined if the p-value was less than or equal to 0.05. The descriptive analysis was obtained by determining the frequency distribution and percentage of the study variables, which included the demographic and clinical characteristics of the participants in each study group. Mean with standard deviation was calculated for post-extraction bleeding time among different groups while error bars with mean and 95% confidence interval were used. Chi-square test was reported for qualitative variables to compare groups. Mann-Whitney U test was used to compare the means of bleeding stop minutes among each of the two groups, and the Krushkal-Wallis test was used to compare bleeding stop minutes among four groups.

RESULTS

The mean age of patients in the study was 31.94 ± 10.207 years. The mean bleeding time in males was 20.31 ± 28.707 minutes and 21.25 ± 31.212 minutes in females (p = 0.303).

The mean bleeding time was 61.56 ± 36.273 minutes in Group 1, which was the highest followed by Group 2 (7.50 ± 3.651), Group 3 (8.44 ± 3.966), and Group 4 (5.62 ± 1.708). The median of bleeding stop minutes of Group 1 was 50.0 minutes while in Group 2 it was 5.0 minutes (p <0.001). When comparing the median of bleeding stop minutes in Group 1 (50.0 minutes) and Group 3 (7.5 minutes), the difference was similarly highly significant as z = -4.800, p <0.001. Moreover, the difference between the median bleeding stop minutes of Group 1 (50.0 minutes) and Group 4 (5.0 minutes) was highly significant as z = -5.047, p <0.001. Therefore, the bleeding stop minutes of the control group were more significant than all test groups.

When comparing the median of bleeding stop minutes among the three test groups, the authors found that the difference was not significant with regard to Group 2 and Group 3 (z = -0.718, p = 0.473, p >0.05). The same results were obtained when comparing the median of bleeding stop minutes of Group 2 and Group 4 (z = -1.695, p = 0.090, p >0.05). In contrast, the difference between the median bleeding stop minutes of Group 3 and Group 4 was statistically significant (z = -2.358, p = 0.018, p <0.05, Table I, Figure 1).

Table I: Comparison of means of stopping time of bleeding (Bleeding stop-minutes) among four groups by Krushkal-Wallis test and among each two groups by Mann-Whitney U test.

	N	Mean ± SD	Median	95% Confidence Interval for Mean		Chi-square	p-value
				Lower bound	Upper bound		
Group 1	16	61.56 ± 36.273	50.00	42.23	80.89	43.296	<0.001**
Group 2	16	7.50 ± 3.651	5.00	5.55	9.45		
Group 3	16	8.44 ± 3.966	7.50	6.32	10.55		
Group 4	16	5.62 ± 1.708	5.00	4.71	6.54		

Krushkal-Wallis test

Groups	Median	IQR	Z value	p-value
Group 1	50.00	69	-4.911	<0.001**
Group 2	5.00	5		
Group 1	50.00	69	-4.800	<0.001**
Group 3	7.50	5		
Group 1	50.00	69	-5.047	<0.001**
Group 4	5.00	0		
Group 2	5.00	5	-0.718	0.473^{NS}
Group 3	7.50	5		
Group 2	5.00	5	-1.695	0.090^{NS}
Group 4	5.00	0		
Group 3	7.50	5	-2.358	0.018*
Group 4	5.00	0		

Mann-Whitney U test. NS: Non-significant.

Table II: Association of patient's bleeding time with four groups.

	Bleeding time		Total	Pearson's Chi-square	p-value
	<= 60 min.	61-120 min.			
Group 1	12 (75.0%)	4 (25.0%)	16 (100.0%)	12.800	0.005**
Group 2	16 (100.0%)	0 (0.0%)	16 (100.0%)		
Group 3	16 (100.0%)	0 (0.0%)	16 (100.0%)		
Group 4	16 (100.0%)	0 (0.0%)	16 (100.0%)		

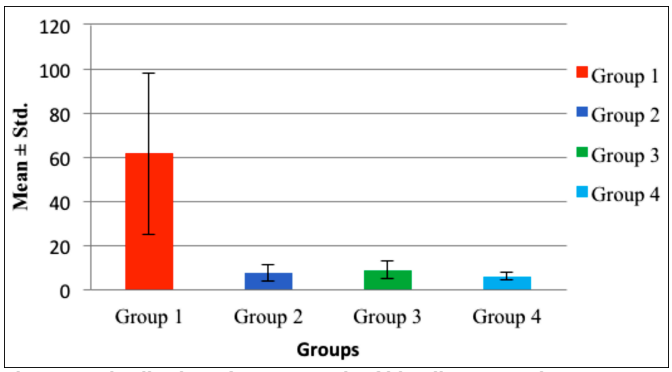

Figure 1: Distribution of mean ± Std. of bleeding stop minutes among four groups.

Regarding the distribution of patients according to their bleeding time in the four groups. The authors found that 25% patients in the control group had 61-120 min bleeding time and by applying Pearson's Chi-square test, p = 0.005, the result was significant. Therefore, there is a significant association between bleeding time and groups of patients, i.e., patients in the control group have more bleeding time than others (Table II).

DISCUSSION

In oral and maxillofacial surgical procedures and periodontal surgical procedures, electrocautery and suture ligatures are among the commonly performed methods to control bleeding from small and major vessels. However, in cases where there is generalised oozing and when the application of pressure proves ineffective and the use of electrosurgical equipment poses a risk to teeth or nearby nerves, the application of topical haemostatic agents may be necessary. Local haemostatic agents provide control of external bleeding by enhancing the natural clotting process through various physical reactions between the agent and blood or by various mechanical means. Tannic acid induces vasoconstriction and, hence, stops bleeding from mucous membranes.[16,20,21]

Tannin has been reported to exert many other physiological effects, such as reducing blood pressure, decreasing serum lipid level, and modulating immune responses, and at the same time, it has less side effects and is categorised and generally recognised as a safe (GRAS) food additive.[16]

This study evaluated the impact of green tea extract and tannin on gingival bleeding following the removal of mandibular molars. The control group had a longer average duration of bleeding cessation (61.56 ± 36.273 minutes) compared to the test groups, which had averages of (7.50 ± 3.651, 8.44 ± 3.966, and 5.62 ± 1.708 minutes. Moreover, patients in each test group had a significantly lower median bleeding stop minutes when compared to control group.

The group of patients who received gauze soaked with tannins exhibited the shortest duration of bleeding cessation (5.62 ± 1.708 minutes) compared to both the other experimental groups and the control group. This emphasises that the effect of green tea on the reduction of soft tissue bleeding observed in this study is probably due to its tannins, which causes the contraction of damaged blood vessels and accelerates the blood clotting.[1,16]

One study, conducted in Iran, examined the impact of green tea extract on the cessation of bleeding. The results revealed that a majority of the patients (83.8%) treated with gauze devoid of extract exhibited bleeding durations exceeding 5 minutes. Conversely, only 22.6% of the patients administered gauze impregnated with green tea ethanolic extract encountered such bleeding periods. Furthermore, a reduced proportion of patients in the green tea group experienced postoperative oozing in comparison to the control group (6 *versus* 29 persons, p = 0.001).[11]

Studies investigating the properties of aqueous extract of green, black, and red tea showed that all three types of tea extract exhibited excellent and best antibacterial activity against both gram-positive and gram-negative bacteria and showed good inhibition zone in comparison to ciprofloxacin against different types of bacteria.[22]

Another *in vitro* study has proven that different green tea extracts have significant effects against certain bacterial species such as *Escherichia coli.* In addition to that, the study demonstrated that the antimicrobial effect varied among different extracts, i.e., the methanolic and ethanolic extracts have shown little antimicrobial activity against all microorganisms as compared to the aqueous extract, and all the active ingredients present in roasted green tea are better soluble in water than the other organic solvents such as ethanol and methanol. These findings could be compared to the findings of the present study in which no significant difference was found neither between the methanolic and aqueous extracts nor between the methanolic extract and tannin with regard to their ability to reduce gingival bleeding, while a significant difference was found between the aqueous extract and tannin. Thus, further extensive studies are recommended to explore these variations among different GTEs with regard to their antimicrobial and haemostatic effects.[23]

An animal study was conducted in Iran to assess the effect of green tea on bleeding control in the study sample (mice). Cuts were performed in the tails' ends, after which, the tails were placed inside tubes containing test solutions. The time taken to achieve haemostasis was then measured using a chronometer. The results showed that GTE significantly reduces bleeding and has a significant antimicrobial effect. It inhibited haemorrhage significantly more than the positive control groups, and was nearly at par with the most potent positive control group (50 mg/ml tranexamic acid). Additionally, the research findings suggest that the utilisation of a topical formulation consisting of green tea extract and PVA (Polyvinyl alcohol) results in a reduced duration of bleeding compared to the use of the extract alone. Notably, the application of a green tea/PVA 4% solution produced the shortest bleeding time.[9]

An *in-vivo* investigation revealed that intra-oral administration of green tea not only decreased haemorrhage time but also exhibited a significant anti-inflammatory effect, which may have a great effect on the prevention of surgical site infections. These findings were obtained from a randomised clinical trial aimed at evaluating the effect of green tea mouthwash on the formation of dental plaque and the process of healing following periodontal crown lengthening surgery. In addition, daily cleansing with green tea may be advantageous for managing postoperative complications, such as pain, associated with impacted molar surgery, according to another randomised trial. Furthermore, a notable reduction in the necessity for analgaesics was observed among patients who administered the green tea mouth rinsing. Hence, the study concluded that green tea mouthwash could be an appropriate and a safe choice to control postoperative pain following the third molar surgery.[24,25]

The strength of this study lies in the use of a randomised controlled-trial methodology to assess the efficacy of three distinct green tea extracts (GTEs) in reducing post-extraction haemorrhage. A potential constraint might arise from the absence of universally standardised techniques for categorising postoperative haemorrhage, thereby influencing the outcomes of the investigation. Another weakness of this study is its scope since it just investigated the effects of green tea on postoperative bleeding within the first one-hour time frame subsequent to tooth extraction. It is noteworthy to mention that oozing from the extraction socket may continue for a duration of up to three days subsequent to the surgical intervention. Therefore, it is crucial to do more experiments that include further green tea products, such as mouthwash, and to prolong the monitoring period in order to thoroughly evaluate its haemostatic effect.

CONCLUSION

Green tea methanolic, aqueous, and tannin extracts are clinically effective in reducing bleeding after extraction of mandibular molars. Tannin isolated from green tea has shown a significantly higher haemostatic effect than the aqueous and methanolic extracts. Tannin isolate being affordable and readily available is a suitable recommendation in individuals following the extraction of mandibular molars.

ETHICAL APPROVAL:

The protocol of this research was approved by the Institutional Review Board of the College of Dentistry, King Khalid University, Abha, Saudi Arabia.

PATIENTS' CONSENT:

Informed written consent was obtained from all the patients before the intervention.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

MH: Contributed to the conceptualisation of the study, project administration, and supervision. Involved in writing methodology, reviewing, and editing of the manuscript, as well as providing visualisation and managing data curation.
SSA: Contributed to the conceptualisation, visualisation, writing, formal analysis, and data curation.
JDA: Contributed to the conceptualisation, writing, investigation, and data curation.
BAA: Contributed to the conceptualisation, writing, resources, and data curation.
TAM, AAK: Contributed to writing and methodology.
All authors approved the final version of the manuscript to be published.

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