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Effects of Sidamanik Black Tea on Salivary Flow Rate, Total Protein, and Albumin Levels in Young Adults: An in Vivo Study

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ABSTRACT

Saliva is crucial in maintaining oral health, performing functions such as antibacterial activity, buffering, and ensuring tooth integrity. In this regard, black tea, a medicinal plant with potential anti-cariogenic agents, has been suggested to exert preventive effects against caries. Therefore, this study aimed to determine effects of black tea consumption on salivary flow rate, pH, as well as total protein and albumin levels in young adults. A pre-experimental design was used with a one-group pretest-posttest approach. The samples selected were 18 healthy young adults aged 18-25 years, with DMF-T scores of 1-5. Salivary flow rate, total protein, and salivary albumin were measured using a digital scale after a 5-minute interval, the bicinchoninic acid assay method, and the bromocresol green method, respectively. The results showed a significant increase in salivary flow, a substantial decrease in albumin, and an insignificant elevation in total protein. In conclusion, black tea manifested the capability to prevent caries by enhancing salivary flow and total protein levels. Furthermore, the impact in inhibiting bacteria activity depended on the presence of salivary albumin.

Keywords: Black Tea; Flow Rate; Salivary pH; Salivary Total Protein; Salivary Albumin

ABSTRAK

Saliva berperan penting dalam menjaga kesehatan rongga mulut seperti sebagai antibakteri, *buffer*, dan menjaga integritas gigi. beberapa peneliti sepakat bahwa teh hitam sebagai tanaman herbal mampu mencegah aktivitas karies karena bersifat antikariogenik.tujuan dari penelitian ini adalah untuk mengetahui pengaruh minum teh hitam terhadap laju alir, total protein dan albumin pada saliva dewasa muda. metode penelitian ini adalah penelitian pra eksperimen yang dilakukan dengan pendekatan *one group pretest-posttest design*. pengambilan sampel dilakukan pada 18 orang dewasa muda sehat yaitu kelompok umur 18-25 tahun dengan dmf-t 1-5. laju alir diukur dengan timbangan digital setelah 5 menit, protein total saliva diestimasi dengan metode *bicinchoninic acid assay* dan albumin saliva diestimasi dengan metode *bromocresol green*. hasil menunjukkan peningkatan yang signifikan pada laju alir, penurunan signifikan pada albumin, peningkatan total protein tidak signifikan. dapat disimpulkan bahwa teh hitam mampu mencegah aktivitas karies melalui peningkatan aliran saliva, dan total protein. teh hitam mampu menghambat aktivitas bakteri yang peningkatannya bergantung pada albumin saliva.

Kata Kunci: Teh Hitam; Laju Alir; ph Saliva; Protein Total Saliva; Albumin Saliva

1. Introduction

Saliva is a fluid that plays an important role in regulating water balance, maintaining tooth integrity, exerting antibacterial activity, and functioning as a buffer to sustain oral cavity health. Changes in age, lifestyle, and diet can significantly impact saliva composition, influencing salivary flow rate, pH, volume, and buffer capacity [1]. Control over life quality directly affects saliva, impacting the availability of ions for remineralization and reducing sugar-containing foods, thereby triggering dilution effects that lower the flow rate and pH of saliva to buffer acids in plaque [2,3].

According to the Riskesdas report of North Sumatra Province in 2018, the prevalence of dental and oral problems was 54.56%, with tooth decay and cavities accounting for 43.07% and 36.35%, respectively, among 15-24-year-old individuals [4]. This age group comprises young adults (18–24 years) responsible for their oral and dental health. Furthermore, the prevalence showed an increase compared to a report from the past decade.

Black tea has beneficial effects on systemic and oral health due to antioxidant, anti-inflammatory, antibacterial, anti-cariogenic, and cancer-preventive properties [5]. Shetty et al. discovered that black tea could elevate salivary flow rate more effectively than green tea when administered through gargling in healthy young adults [5]. To prevent an increase in caries activity, each cup of black tea is recommended to be consumed without sugar addition [6].

2. Methods and Materials

This pre-experimental study used a one-group pretest-posttest design to assess effects of Sidamanik black tea consumption on salivary flow rate, pH, total protein, and salivary albumin. Before carrying out any major operation, ethical approval was obtained under the number 1334/kep/usu/2021. Salivary flow rate and pH measurements were performed at the Oral Biology Laboratory, Faculty of Dentistry, Universitas Sumatera Utara. Subsequently, saliva samples were frozen at -20ºc and transported to the Integrated Laboratory of the Faculty of Medicine for the analysis of total protein and salivary albumin concentrations. A total of 18 participants who were dental students at Universitas Sumatera Utara, were selected through purposive sampling based on specific inclusion and exclusion criteria. The total sample size was determined using the formula for testing the mean difference hypothesis with a simple random design.

The inclusion criteria comprised individuals aged 18 to 24 years, physically and mentally healthy, free from local and systemic diseases, non-allergic to tea, as well as had DMF-T scores ranging from 1-5 and low to moderate OHI-S scores. Meanwhile, the exclusion criteria consisted of those who were smokers or alcoholics, receiving radiation therapy, currently receiving orthodontic treatment, affected by periodontal disease and mucosal lesions, as well as had DMF-T scores <1 and >5 and high OHI-S scores. Participants were instructed to abstain from eating or drinking an hour before saliva collection. Salivary rapid tests were conducted before intraoral examinations to determine DMF-T and OHI-S scores.

The unstimulated salivary flow was obtained prior to Sidamanik black tea consumption using a draining method for 5 minutes. Subsequently, participants were offered tea that was steeped for 5 minutes at 100^0C without sugar addition before being ingested at 50^0C. At 30 minutes post-consumption, stimulated salivary flow was collected in 5 minutes. Both pre- and post-consumption salivary flow were measured in pre-weighed tubes using a digital scale immediately after collection. The calculated weights were subtracted and divided by 5 to convert the unit to ml/min. Salivary pH was estimated using the Hanna Instrument, and saliva was stored in a -20^0C fridge until analysis time [7].

Saliva samples removed from the -20^0C fridge were left to melt, then centrifuged at 1000 rpm for 30 minutes, and the supernatant was transferred to a new Eppendorf tube. Salivary total protein was evaluated with the bicinchoninic acid assay method using a PierceTM BCA Protein Assay Kit, while albumin levels were estimated through the bromocresol green method using a commercial kit from Biolabo, France.

3. Results

The results showed a significant increase in salivary flow rate ($p<0.05$) from 0.44 ml/min ± 0.23 to 0.80 ml/min ± 0.63, along with an insignificant elevation in total protein from 0.78 mg/ml ± 0.21 to 0.90 mg/ml ± 0.21. Meanwhile, salivary albumin substantially decreased from 0.43 mg/ml ± 0.6 to 0.26 mg/ml ± 0.31.

Table 1: Mean Values of Salivary Flow Rate, Total Protein, and Albumin Before and After Sidamanik Black Tea Consumption (n=18)

Variables	Groups	Mean	Standard Deviation	p-value
Flow Rate (ml/min)[(a)]	Before	0.45	0.23	0.00*
	After	0.80	0.63	
Total Protein (mg/ml)[(b)]	Before	0.78	0.21	0.235
	After	0.90	0.21	
Albumin (mg/ml)[(a)]	Before	0.43	0.26	0.048*
	After	0.26	0.31	

a. Wilcoxon Test with a significance level of $p < 0.05$
b. Paired T-Test with a significance level of $p < 0.05$
* Significant

Table 2: Salivary Flow Rate and Total Protein Correlation Before and After Sidamanik Black Tea Consumption

		Flow Rate (ml/min)	Total Protein (mg/ml)
Flow Rate (ml/min)	Correlation	1000	-1.106, weak correlation/no correlation
	Sig. (2-tailed)	-	0.538

Spearman's rho test with a correlation level of $p<0.05$

Table 3: Salivary Flow Rate and Albumin Correlation Before and After Sidamanik Black Tea Consumption

		Flow Rate (ml/min)	Total Protein (mg/ml)
Flow Rate (ml/min)	Correlation	-0.294, weak correlation/no correlation	1000
	Sig. (2-tailed)	0. 081	-

Spearman's rho test with a correlation level of $p<0.05$

4. Discussions

The effectiveness of Sidamanik black tea in increasing salivary flow rate among young adults was evident from the significant elevation of the mean value from 0.45 ml/min to 0.8 ml/min. This result was consistent with the study by Shalal which showed the ability of black tea to significantly enhance both salivary flow rate and pH [8]. Additionally, Shetty et al. observed a higher increase in salivary flow rate from 0.4 ml/min to 0.8 ml/min when black tea was ingested through the gargling method compared to green tea in healthy young adults [5].

In this study, black tea was administered at a reduced temperature of 50^{o}C following the approach described by Chong PH et al., leading to a significant flow rate increase after 30 minutes [9]. Elevated temperatures induce glandular vasodilation, resulting in increased blood flow, thereby stimulating secretory cells to produce more saliva [8]. Moreover, age, poor general health, environmental conditions, circadian rhythm, alcohol and drug consumption, smoking habits (influenced by nicotine levels in cigarettes), and psychological factors such as stress levels, may affect salivary flow rate [10,11].

Salivary secretion is mainly regulated by the sympathetic and parasympathetic nervous systems, which induce changes in the composition of saliva. The parasympathetic nervous system predominates during quiet "rest and digest" conditions, while the sympathetic nervous system drives the "fight or flight" response in stressful situations [12]. Participants in this study were mostly dental students engaged in thesis writing and dental co-assistant duties. These academic pressures might have contributed to the observed significant increase in salivary flow rate, which did not still reach the normal stimulated rate. Therefore, stress was suggested to potentially influence salivary flow rate, as supported by the report from Nair et al. showing a correlation between stress and salivary cortisol levels [10].

Table 1 shows an elevation in the average total salivary protein from 0.78 mg/ml before drinking Sidamanik black tea to 0.90 mg/ml subsequently. The paired T-test results with a p-value = 0.103 > 0.05 showed an insignificant difference in total salivary protein after drinking, with a marginal increase of 0.12 mg/ml. This observed increase was consistent with the report by Chong PH et al., but significantly higher salivary protein concentrations were found in the majority of participants examined post-tea consumption [9].

The insignificant elevation in total protein in this study could be due to a significant decrease in albumin. The relationship existing between total protein and albumin is generally associated with inflammatory processes. Total protein and albumin concentrations perform an anti-inflammatory function [13]. Proteins and peptides function biologically in saliva while the type and amount depend on diet, sex, age, circadian rhythm, and physiological status. Besides albumin, other factors affect total protein concentration of saliva [9].

Salivary proteins play a crucial role in various functions, including lubrication, protection, cleaning, support, maintenance of dental integrity, taste sensation, digestion, and antibacterial activity [13,14]. Higher total protein was identified in participants with low active caries compared to those with high active caries. The results were consistent with the study of Devi NR et al. which reported higher total protein concentrations in the caries-free group than in the affected group, thereby showing enhanced dental protection against caries activity [15].

Bhuptani et al. found a total protein ranging from 2.0-4.0 mg/ml in all age groups and 2.25 mg/ml in 13-25-year-old individuals, suggesting that total protein decreased with age and was not influenced by gender [13]. This observation was consistent with the results regarding gender reported by Ahmadi-Motamayel F et al. besides detecting higher total protein in caries-free group [7].

Diet is another factor impacting total protein levels since the interaction of food and saliva in the oral cavity is a complex process including the perception of sensory properties, food shape alteration, and various biochemical reactions [9]. Physiological stimulation through eating or drinking produces saliva that lasts up to 2 hours and the protein profile can be influenced by bitter taste [14]. The presence of bitter taste in Sidamanik black tea may contribute to variations in salivary flow rate and composition, such as total protein [12].

Participants refrained from consuming anything one hour before sampling according to the method adopted by Chong PH et al, Naritasari et al., and Bhuptani D et al. [13,16]. Food and drinks ingested 2 hours earlier might influence salivary secretions before and after the intake of Sidamanik black tea [9,17].

The average value of salivary albumin decreased from 0.43 mg/ml to 0.26 mg/ml after drinking Sidamanik black tea. The Wilcoxon test yielded a p-value = 0.048 < 0.05, showing a significant reduction in salivary albumin. Based on the study by Selvam and Willam, caffeine, a component present in higher amounts in coffee than in tea, diminishes salivary albumin concentrations. Salivary secretion, mainly regulated by the sympathetic and parasympathetic nervous system, experiences composition changes after consuming caffeinated beverages such as coffee. Loss of salivary enzyme and protein activity can result from various agents in coffee affecting enzymes through different mechanisms [18]. Differences in salivary proteins may vary depending on intra- and inter-individual factors that reflect general health status [18,19].

The condition previously described does not imply the ineffectiveness of caffeine content in Sidamanik black tea to enhance salivary albumin, which facilitates remineralization and enamel resistance. Salivary albumin is commonly found in the gingival crevicular fluid (GFC) [20]. GFC is a physiological fluid and inflammatory exudate originating from blood vessels of the gingival plexus in the lamina propria, located below the epithelial layer of the dentogingival space. Plaque accumulation triggers periodontal disruption, causing GCF to cross inflamed periodontal tissue during transport to the sulcus, thereby collecting biological molecular markers from the surrounding area for elution into saliva [21,22].

Treponema denticola, a bacterium present in supragingival plaque, increases salivary albumin and total protein levels because the proteins found in the periodontal pocket, including immunoglobulins and albumin, are often used as potential energy sources [21]. This expression corresponded to the observed decrease in salivary albumin following black tea consumption. Bedran TBL et al. reported that black tea extract and theaflavins could manifest antibacterial activity by mitigating interleukin-8 secretion and inducing human-defensin secretion in oral epithelial cells to reduce major periodontopathogens such as *T. denticola* and avert an increase in salivary albumin [23].

5. Conclusions

In conclusion, the results showed that Sidamanik black tea consumption exerted preventive effects against caries activity by elevating salivary flow and total protein levels. Additionally, the impact in inhibiting bacteria activity depended on the presence of salivary albumin.

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7. Conflicts of Interest

The authors declare that there are no conflicts of interest to disclose concerning this study.

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