IJCMBS

Original Article

doi.org/ 10.5281/zenodo.14583937 JCMBS 2025; 5(1):xxxxxx

In Vitro Investigation of the Effect of Mouthwashes on Fertility Tests

Ağız Çalkalama Solüsyonlarının Doğurganlık Testleri Üzerindeki Etkisinin İn Vitro Değerlendirilmesi

M. Ayse Tayman[1*], Ataman Gonel[2]

[1]Department of Periodontology, Faculty of Dentistry, Ankara Yıldırım Beyazit University, Ankara /Turkiye

[2]Sanko University, Faculty of Medicine, Department of Medical Biochemistry,Gaziantep/Turkiye

Abstract

Background: Mouthwashes are not devoid of side effects like other drugs and may affect the clinical outcomes of the patients. The aim of this study is to experimentally investigate the interference effect of mouthwashes containing different chemical components on fertility tests.

Materials and Methods: A (BisBiguanide), CH (QuaternaryAmmonium), L (EssentialOils), CO (QuaternaryAmmonium), and M (StannousFloride) were added (20 µL) to the N Protein Control SL/Low (180 µL) control solution. Samples were studied in immunoassay autoanalyzer. The same process was done by adding distilled water (20 µL) to the control solution.

Results: The bias formula was used to calculate how much the obtained results deviated from the target value. No test showed negative interference exceeding 10% in any mouthwash. The TESTO test had positive interference at a rate of 36.85% in CH. Interference was observed in the DHEAS test at a rate of 23.73% in A and 18.97% in CO. This was followed by Estradiol with 14.92%, Beta HCG with 12.82% in A and DHEAS with 11.45% in M.

Conclusions: This study has shown that the interaction potential of commercial kits with mouthwash solution contents may cause erroneous measurements in terms of hormone test results. Chlorhexidine should be prescribed with careful questioning. Essential oils seem to be safer than other mouthwashes in terms of interference.

Keywords: mouthwashes, interference, bias, fertility tests, immunoassay autoanalyzer, deviations

ÖZ

Amaç: Ağız gargaraları diğer ilaçlar gibi yan etkilerden yoksun değildir ve hastaların klinik sonuçlarını etkileyebilir. Bu çalışmanın amacı farklı kimyasal bileşenler içeren ağız gargaralarının doğurganlık testleri üzerindeki interferans etkisini deneysel olarak araştırmaktır.

Gereç ve Yöntem: A (BisBiguanide), CH (QuaternaryAmmonium), L (EssentialOils), CO (QuaternaryAmmonium), and M (StannousFloride) N Protein Control SL/Low (180 µL) kontrol solüsyonuna eklendi (20 µL). Örnekler immunoassay otoanalizöründe incelendi. Aynı işlem kontrol solüsyonuna distile su (20 µL) eklenerek yapıldı.

Bulgular: Elde edilen sonuçların hedef değerden ne kadar saptığını hesaplamak için bias formülü kullanıldı. Hiçbir test herhangi bir ağız gargarasında %10'u aşan negatif interferans göstermedi. TESTO testi CH'de %36,85 oranında pozitif interferansa sahipti. DHEAS testinde A'da %23,73 ve CO'da %18,97 oranında interferans gözlendi. Bunu A'da %14,92 ile Estradiol, %12,82 ile Beta HCG ve M'de %11,45 ile DHEAS izledi.

Sonuç: Bu çalışma, ticari kitlerin gargara solüsyonu içerikleriyle etkileşim potansiyelinin hormon test sonuçlarında hatalı ölçümlere neden olabileceğini göstermiştir. Klorheksidin dikkatli sorgulama ile reçete edilmelidir. Esansiyel yağlar, interferans açısından diğer gargaralardan daha güvenli görünmektedir.

Anahtar kelimeler: gargaralar, interferans, bias, doğurganlık testleri, immunoassay otoanalizörü, sapmalar.

Highlights

- The interference effect of mouthwashes should not be ignored in the evaluation of test results.
- The test that shows the most bias from the target value is DHEAS, and the mouthwash that causes the most bias is Bis Biguanide Antiseptic.
- Essential oil components seem to be safer than other mouthwashes in terms of interference.

***Corresponding author:** M Ayşe Tayman, DDS, Department of Periodontology, Faculty of Dentistry, Ankara Yıldırım Beyazıt University, Ankara / TÜRKİYE
E-mail: ayseatay06@hotmail.com
Received: 27 September 2024 **Accepted:** 31 December 2024

Introduction

Mouthwash are medicated liquid held in the mouth by the movements of the perioral muscles to eliminate oral pathogens (1). In the 1960s, Harald Loe showed that the chlorhexidine (CHX) compound could prevent the formation of dental plaque (2). Today, it is well documented that CHX is not devoid of side effects like other drugs such as increased staining of teeth and taste disturbance in long-term use (3). Recently, many herbal-containing mouthwashes on the market have been subjected to extensive research for their potential to prevent oral diseases. These mouthwashes have been claimed to be effective in reducing and preventing the formation of bacterial dental plaque, tooth decay and bad breath (1,4). The use of natural antimicrobials can contribute to controlling the erratic growth of the oral microbiota and overcoming the problems caused by strains resistant to conventional antimicrobial agents (5,6). The current situation supports the use of CHX, which still complies with the standards and can be labeled as the 'gold standard' (7).

Drug-drug interactions are very common during dental treatments. Drug interaction is defined as the change of the effect of one drug by another drug and may affect the clinical outcomes of the patients (8). This interaction is a situation that clinicians can predict and be aware of, and there are many studies on this subject (9). Also, low-level exposure to CHX may cause cross-resistances to antibiotics. Moreover, some mechanisms that allow CHX resistance in bacterial organisms include mutations in efflux pumps and cell membrane structure. It has been shown to have adverse effects on human tissues as well as multidrug resistance (6,7). Another condition that may indirectly affect the clinics of patients is 'interference'. Drug interference may develop due to metabolites or additives of a drug (10).

There is not enough data on how much mouthwashes and mouth rinse solutions prescribed during or after dental treatments interfere with laboratory parameters with diagnostic and prognostic importance and whether they affect the test results and cause false results. Interference is defined as “the effect of a substance in the sample that alters the true value of the result, usually expressed as concentration or activity for the analyte” (11) and there is no guidance on which drug affects which test. This lack of knowledge may even lead to incorrect test measurements and related malpractice in the therapeutic use of mouthwashes. Demonstrating and publishing possible errors before they occur will guide clinicians.

Most of the hormones evaluated in fertility tests are determined using immunoanalytical methods (12). Interference in analyzing using the immunoassay method is a serious problem that many clinicians are not aware of or even unknown (13). Since the kits in which the immunoassay method is used contain complex biological reagents (especially immunoglobulins), they are sensitive to different types of interference with other complex molecules during measurement. Immunoglobulins can also cause false positives or false negatives in test results by competing with enzyme-labeled immunoglobulins in the reagents of the immunoassay method or by forming an immunocomplex (14,15). Interference can thus obscure accurate test results, leading to unnecessary clinical investigations and inappropriate therapy for the patient. The focus of this study is to experimentally investigate the interference effect of mouthwashes containing different chemical components on fertility tests.

Material and Methods

Study design

“N Protein Control SL/Low (Siemens, Marburg, Germany, lot: 084654)” control solution was used in the study. The solutions obtained by adding BisBiguanide (A), QuaternaryAmmonium (CH and CO), EssentialOils (L), and StannousFloride (M) (20 µL) containing mouthwashes to 180 µL control solution were mixed with vortex for 5 seconds before the study. Obtained samples were studied in e601 (Roche, Germany) fully automatic immunoassay autoanalyzer. To determine the target value, the same process was done by adding distilled water (20 µL) to the control solution, and all measurements were repeated 3 times, and the average values were taken into account. The bias formula was used to calculate how much the obtained results deviated from the target value (16). Since this study was an experimental study that did not use any blood or tissue samples, it does not require ethics committee approval.

Statistical analyses

Statistical analysis was calculated with Microsoft Office Excel Program. In the Bias (%) formula used when calculating the deviation rates from the target value, C1 refers to the measurement result made from the distilled water mixture, and C2 refers to the measurement result prepared with the antibody. Bias (%) = ((C2-C1)/C1)x100. Negative deviation indicates false negative, positive deviation indicates false positive. The size of the percent

value indicates the amount of deviation and thus the severity of the interference.

Results

Mouthwashes are evaluated as different groups such as their mechanism of action, ingredients, chemical structures and usage area. The content, category, active ingredient and letter code given to the mouthwash are shown in **Table 1.** The deviations of five different mouthwashes from the target value were calculated. As a result, interference rates below 10% were considered normal **(Table 2), (Figure 1)**.

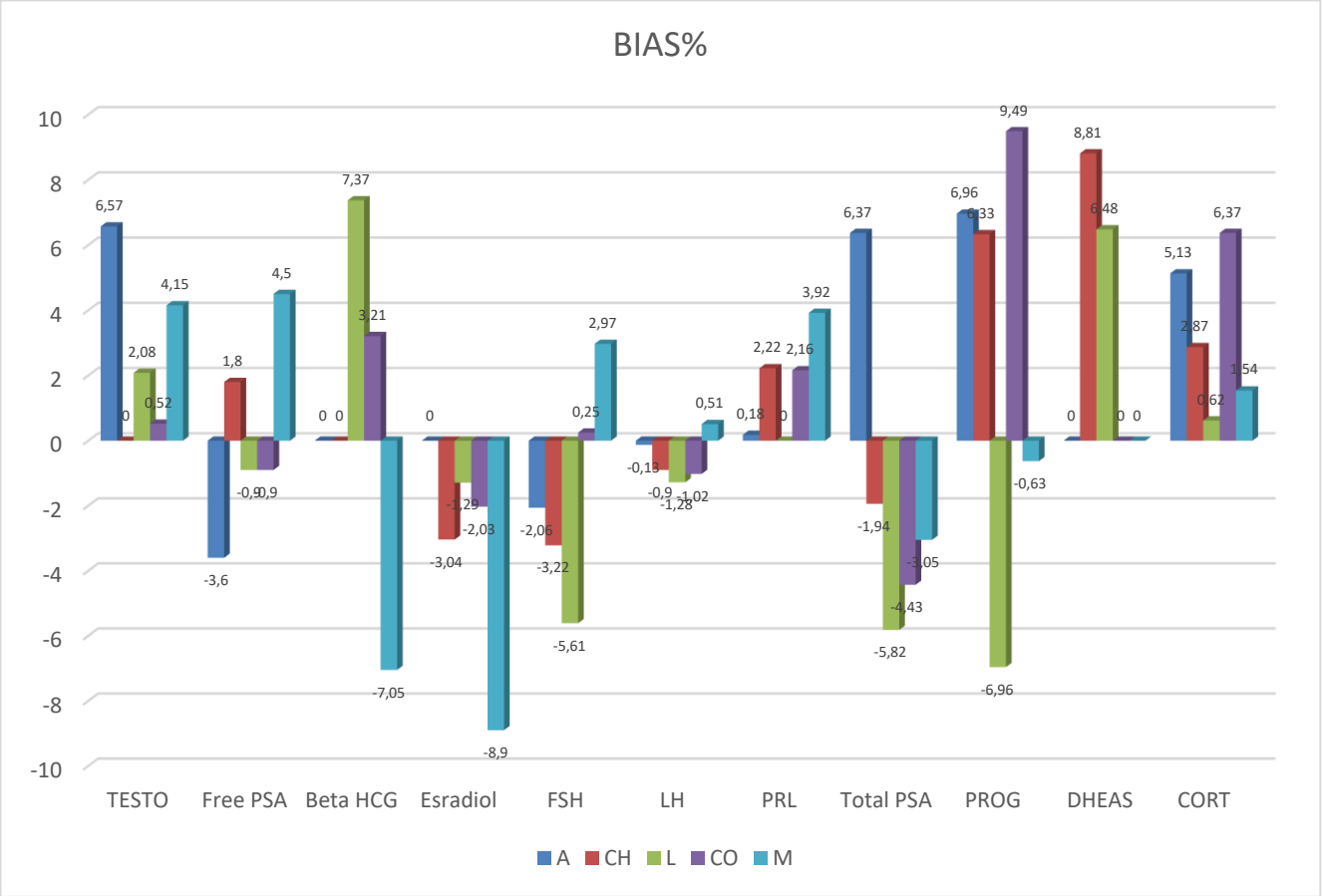

Figure 1. Percentage deviations from target value (%BIAS)

Tests with negative interference

Free prostate specific antigen (Free PSA) and follicle stimulating hormone (FSH) in An exposure; Estradiol, FSH, luteinizing hormone (LH), Total PSA in CH exposure; Free PSA, Estradiol, FSH, LH, Total PSA, Progesterone (PROG) in L exposure; Free PSA, Estradiol, LH, Total PSA in CO exposure; Beta human chorionic gonadotropin (Beta HCG), Estradiol, Total PSA, PROG showed negative interference in M exposure.

Among all tests, the most negative interference occurred in the Estradiol test with -8.90% in M exposure. This was followed by Beta-HCG with -7.05% in M exposure. No test showed negative interference exceeding 10% in any mouthwash.

Tests with positive interference

The Testosterone (TESTO) test had the highest bias (36.85%) with positive interference in CH exposure. The deviation of the Dehydroepiandrosterone sulfate (DHEAS) test, which showed positive interference was 23.73% in an exposure and 18.97% in CO exposure. This was followed by Estradiol with 14.92% and Beta HCG with 12.82% in an exposure. And DHEAS with 11.45% in M exposure.

Table 1. Product code, category and ingredients

Product Code (antiseptic, antiplaque mouthwashes)	Product Category	Content (active ingredients)	Content (Inactive Ingredients)
A	Bis Biguanide Antiseptics	0.15% Benzydamine Hydrochloride, 0.12% Chlorhexidine Gluconate	Mint Flavor, Sorbitol (E420), Patent Blue V, Glycerol, Polysorbate 20, Tartrazine (E102), Ethanol, Water
CH	Quaternary Ammonium Compounds	0.075% Cetylpyridinium Chloride	Aqua, Glycerin, Propylene Glycol, Sorbitol, Tetrapotassium Pyrophosphate, Polysorbate 20, Tetrasodium Pyrophosphate, Zinc Citrate, PVM/MA Copolymer, Aroma, Benzyl Alcohol, SodiumFluoride, Sodium Saccharin, Bambusa Vulgaris Shoot Extract, 15510, Charcoal Powder, CI 15510, CI 17200, CI 19140, CI 42051.
CO	Quaternary Ammonium Compounds	0.075% Cetylpyridinium Chloride	Aqua, Glycerin, Propylene Glycol, Sorbitol, Poloxamer 407, Aroma, Cetylpyridinium Chloride, Potassium Sorbate, Sodium Fluoride, Menthol, Sodium Saccharin, Cl 42051.
L	Phenol And Essential Oils	Essential Oils (Thymol, Eucalyptol, Menthol, Methyl Salicylate)	Zinc Chloride, Fluoride, Mint Flavor
M	Short-Acting Agents Containing Stannous Floride	Olaflur (Aminfluoride) and Stannous Floride, Fluoride Content 250 ppm	Aqua, Xylitol, Polyvinylpyrrolidone (PVP), Polyethylene Glycol (PEG-4) Hydrogenated Castor Oil, Olaflur, Aroma, Stannous Fluoride, Sodium Saccharin, CI 42051.

Tablo 2. Percentage of deviations from target value (BIAS) for tests with 180 µL of control solution added to 20 µL of mouthwash.

	Dis.Wat	A		CH		L		CO		M		
Test	**R**	**R**	**B%**	**R**	**B%**	**R**	**B%**	**R**	**B%**	**R**	**B%**	**Unit**
TESTO	0.58	0.62	6.57	0.79	36.85*	0.59	2.08	0.58	0.52	0.60	4.15	ng/mL
Free PSA	0.11	0.11	-3.60	0.11	1.80	0.11	-0.90	0.11	-0.90	0.12	4.50	ng/mL
Beta HCG	0.62	0.70	12.82*	0.70	12.50*	0.67	7.37	0.64	3.21	0.58	-7.05	mIU/mL
Esradiol	64.46	74.08	14.92*	62.50	-3.04	63.63	-1.29	63.15	-2.03	58.72	-8.90	pg/mL
FSH	12.13	11.88	-2.06	11.74	-3.22	11.45	-5.61	12.16	0.25	12.49	2.97	mIU/mL
LH	7.81	7.80	-0.13	7.74	-0.90	7.71	-1.28	7.73	-1.02	7.85	0.51	mIU/mL
PRL	17.11	17.14	0.18	17.49	2.22	17.11	0	17.48	2.16	17.78	3.92	ng/mL
Total PSA	0.36	0.38	6.37	0.35	-1.94	0.34	-5.82	0.35	-4.43	0.35	-3.05	ng/mL
PROG	1.58	1.69	6.96	1.68	6.33	1.47	-6.96	1.73	9.49	1.57	-0.63	ng/mL
DHEAS	94.48	116.90	23.73*	102.80	8.81	100.60	6.48	112.40	18.97*	105.30	11.45*	ug/dL
CORT	9.74	10.24	5.13	10.02	2.87	9.80	0.62	10.36	6.37	9.89	1.54	ug/dL

Abbreviations: Dis.Wat: Distilled Water, **A:**BisBiguanide **Ch and CO:** QuaternaryAmmonium, **L**:EssentialOils, , **M:**StannousFloride, **R:**Result, **B**: Bias, **TESTO:** Testosterone, **PSA:** Prostate specific antigen, **HCG:** Human chorionic gonadotropin, **E2:** Estradiol, **FSH:** Follicle stimulating hormone, **LH:** Luteinizing hormone, **PRL:** Prolactin, **PROG:** Progesterone, **DHEAS:** Dehydroepiandrosterone sulfate, **CORT:** Cortisol, **PRG:** Progesterone, *Values deviating from the target value by more than 10%.

Discussion

This in-vitro study examining the interference effect of mouthwashes is the first pilot study. CHX. which is one of the bisbiguanides with wide pharmacological effects. breaks down the cell membrane of many microorganisms. stops their growth depending on its concentration and provides inhibition of proteolytic enzymes (17). In addition. due to its cationic structure. it can adhere to mucous membranes and tooth surface. and exhibits long-term release (18). In our study. the tests that had more than 10% positive interference in an exposure. containing 0.12% CHX as the active ingredient. were DHEAS (23.73%). Esradiol (14.92%) and Beta HCG (12.82%). respectively. DHEAS also showed highly positive interference in CO and M exposure (18.97% and 11.45%. respectively). It showed highly positive interference in three of the five mouthwashes and deviated the most from other fertility tests.

Moreover. A is the only mouthwash with positive interference of estradiol (14.92%). which has shown negative interference not exceeding 10% with other tested mouthwashes. Estradiol is the most potent estrogen. the level of which increases in hormone-producing tumors and ovarian cysts (12). Significant changes in test values. negative or positive. may lead to misdiagnosis and treatment. So, individuals should be questioned whether they use any mouthwash before being tested.

DHEAS with the most positive interference in exposure (23.73%) is the most abundant circulating steroid hormone in humans. Normal values in humans vary widely with age. gender and ethnicity and are affected by daily changes in corticosteroid production. alcohol intake. smoking. body mass index. medications. and thyroid function. While individual variables make interpretation of test results difficult (19); this study showed the DHEAS was least interfered with L exposure (6.48%).

EssentialOil is a combination product of essential oils such as thymol and eucalyptol. which acts non-specifically against bacteria and is widely used as a disinfectant and antiseptic. but can cause burning sensation and staining of oral tissue (20). It aimed to overcome the problems caused by strains resistant to traditional antimicrobials (21.22). In alternative medicine. most of the plants consist of flavonoids that have an antibacterial effect on bacterial cells by disrupting the cytoplasmic membrane and inhibiting enzymatic activity (22). In addition. it reduces bacterial load by decreasing plaque mass and prevents plaque maturation. thereby reducing pathogenicity (23). Mouthwashes, in which essential oils are active antiseptic substances, contain alcohol (e.g. ethanol) at rates exceeding 25% to dissolve the active ingredients. but the mouthwashes we chose in this study are alcohol-free. None of the hormone tests in our study showed positive or negative interference exceeding 10% in L exposure. According to this result. alcohol-free essential oils may be a safe mouthwash considering the interference of fertility tests. Clinical studies can be conducted to test the accuracy of the study design in real situations.

Information on circulating concentrations of more than a hundred steroid compounds can lead to problems in interpretation with current technology (12) TESTO (36.85%) and Beta HCG (12.50%) showed the highest positive interference in mouthwash exposure containing Charcoal. Testosterone is the main androgen that influences the development of primary and secondary sexual characteristics and forms the basis of spermatogenesis together with FSH activity in men (24). Determination of testosterone level is indicated in the differential diagnosis of testicular and ovarian endocrine function. Testosterone is a useful marker for diagnosis and monitoring of tumor-associated androgen production and androgenation disorders (25). Mouthwash containing charcoal is a quaternary ammonium compound whose active ingredient is 0.075% Cetylpyridinium Chloride. Like bisbiguanides. quaternary ammonium compounds have a positive charge and interact with the bacterial cell membrane and impair its permeability. They adhere to the mucosal surfaces, but this adhesion is weaker than bisbiguanides (17.26.27). It has been shown that mouthwashes containing cetylpyridinium chloride at 0.07% concentrations inhibit periodontopathogens (28). and in another study, they are effective in antibacterial. antiplaque and antigingivitis (29). Cetylpyridinium chloride has a history of safe and effective use in oral care (30.31). In addition to its limited side effects such as gingival irritation and mild tooth staining (32. 33). it is also known to be safe for use during pregnancy (34). However. because clinical and laboratory evidence to support the therapeutic efficacy and safety of the use of charcoal-based mouthwashes is insufficient. dentists should advise their patients to be cautious when using these non-prescription mouthwashes (35). The fact that a high testo-positive interference value of 36% was observed only in mouthwash containing charcoal in our study confirms the lack of clinical information regarding the mechanism of action of such mouthwashes. The different bias percentages of different mouthwashes may be due to the interaction of the chemicals in their content. Studies can be conducted on the effects of the active ingredients of bis biguanide. quarterner ammonium and essential oils. Considering the possibility that drug-test interactions may lead to clinical inconsistencies in test results and affect the risk of morbidity and mortality. commercial companies may need to update their measurement methods (36).

In addition. CO which is a mouthwash containing a quaternary ammonium compound. showed a high positive interference value (18.97%) on DHEAS. This was followed by DHEAS with 11.45% in exposure to M. whose active ingredient is fluoride components. Beta HCG. which showed positive interference in other mouthwashes. showed negative interference (-7.05%) only with M. Beta hCG can be used to detect pregnancy as early as 10 days following pregnancy and may cause false negative biases in the individual using a short-acting fluoride-containing mouthwash such as M. Or vice versa. it can cause false positive deviations with a bias of 7.37% in an individual using L. Stannous fluoride (SnF2). which is the active ingredient in M. is a broad-spectrum antimicrobial agent with effects on dental plaque and gingivitis (37) and is still considered superior to other fluoride compounds. SnF2 reduces the prevalence of bacteria in the biofilm composition when SnF2 toothpaste is combined with an essential oil-containing mouthwash (38). In this study. PSA. FSH. LH. PRL. PROG and CORT tests showed positive and negative interferences not exceeding 10%. The test that shows the most bias from the target value is DHEAS. and the mouthwash that causes the most bias is CHX. Chlorhexidine. the only agent that can be prescribed in our country. should be prescribed with careful questioning (39).

Study limitations

The study is experimental and needs to be supported by in vivo studies to increase its applicability in the clinical setting. Its limitation is that it was studied with control solutions similar to human blood. The density of the human blood matrix and the thermodynamic interactions it will show may reveal different bias values during interference. Further studies are needed using biological fluids such as human serum or plasma that better reflect mouthwashes clinically. Another limitation of the study is the lack of sufficient data on the relationship between the use of mouthwashes and the rate of passage from the mucosa (oral or gastrointestinal) to the systemic circulation.

Conclusion

Mouthwashes with essential oil components seem to be safer than other mouthwashes in terms of interference. This study has shown that the interaction potential of commercial kits with mouthwash solution contents may cause erroneous measurements in terms of hormone test results. Questioning the use of mouthwash in patients with suspicious hormone results may be a method to reach the correct result.

Acknowledgements: *None.*

Ethical Approval: *Ethical Approval: Since our study does not involve human or animal material, it does not require any ethics committee approval; we accept and declare that no ethical rule violations were made during the preparation and publication of the study.*

Author Contributions: *Concept: M.A.T. A.G. Literature Review: M.A.T. A.G. Design: M.A.T. A.G. Data acquisition: M.A.T. A.G. Analysis and interpretation: A.G. Writing manuscript: M.A.T. Critical revision of manuscript: M.A.T. A.G.*

Conflict of Interest: *The author(s) do not have any potential conflict of interest regarding the research. authorship and/or publication of this article.*

Financial Disclosure: *No financial support was received for this study.*

References

1. Yamaguti-Sasaki E. Ito LA. Canteli VC. et al. Antioxidant capacity and in vitro prevention of dental plaque formation by extracts and condensed tannins of Paullinia cupana. Molecules. 2007;12(8):1950-63.
2. Budtz-Jörgensen E. Löe H. Chlorhexidine as a denture disinfectant in the treatment of denture stomatitis. Scand J Dent Res. 1972;80(6):457-64.
3. Rath SK. Singh M. Comparative clinical and microbiological efficacy of mouthwashes containing 0.2% and 0.12% chlorhexidine. Dent Res J (Isfahan). 2013;10(3):364-9.
4. Smullen J. Koutsou GA. Foster HA. et al. The antibacterial activity of plant extracts containing polyphenols against Streptococcus mutans. Caries Res. 2007;41(5):342-9.
5. Betoni JE. Mantovani RP. Barbosa LN. et al. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Mem Inst Oswaldo Cruz. 2006;101(4):387-90.
6. Nascimento GG. Locatelli J. Freitas PC. et al. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000; 31:247-56.
7. Manipal S. Hussain S. Wadgave U et al. The Mouthwash War - Chlorhexidine vs. Herbal Mouth Rinses: A Meta-Analysis. J Clin Diagn Res. 2016;10(5): ZC81-3.
8. Moreira MB. Mesquita MGDR. Stipp MAC. et al. Potential intravenous drug interactions in intensive care. Rev Esc Enferm USP. 2017; 20:51: e03233.
9. Kovačević M. Vezmar Kovačević S. Radovanović S. et al. Potential drug-drug interactions associated with clinical and laboratory findings at hospital admission. Int J Clin Pharm. 2020;42(1):150-7.
10. Dimeski G. Interference testing. Clin Biochem Rev. 2008;29 Suppl 1: S43-8.
11. Thompson M. Ellison SLR. A review of interference effects and their correction in chemical analysis with special reference to uncertainty. Accred Qual Assur. 2005;10: 82–97.
12. Dušková M. Kolátorová L. Šimková M. et al. Steroid diagnostics of 21st century in the light of their new roles and

analytical tools. Physiol Res. 2020; 30:69(Suppl 2):S193-S203.
13. Ismail AA. Walker PL. Barth JH. et al. Wrong biochemistry results: two case reports and observational study in 5310 patients on potentially misleading thyroid-stimulating hormone and gonadotropin immunoassay results. Clin Chem. 2002;48(11):2023-9.
14. García-González E. Aramendía M. Álvarez-Ballano D. et al. Serum sample containing endogenous antibodies interfering with multiple hormone immunoassays. Laboratory strategies to detect interference. Pract Lab Med. 2015;27:4:1-10.
15. Sturgeon CM. Viljoen A. Analytical error and interference in immunoassay: minimizing risk. Ann Clin Biochem. 2011;48(Pt 5):418-32.
16. Caglayan. M. Gonel. A. Experimental investigation of immunoglobulin and complement concentrations in exposure to ivig. hbig. rituximab. tocilizumab. and bevacizumab. Letters In Drug Desıgn & Dıscovery. 2023;20(6):713-7.
17. Dumitrescu AL. The Use of Chemical Supragingival Plaque Control in Periodontal Therapy. In Antibiotics and Antiseptics in Periodontal Therapy. 2011;205-39.
18. Aktaş A, Giray B. Diş hekimliğinde klorheksidin: özellikleri ve güncel kullanım alanları. Türkiye Klinikleri J Dental Sci 2010;16(1):51-8.
19. Rutkowski K. Sowa P. Rutkowska-Talipska J. et al. Dehydroepiandrosterone (DHEA): hypes and hopes. Drugs. 2014;74(11):1195-207
20. Siegrist BE. Gusberti FA. Brecx MC. et al. Efficacy of supervised rinsing with chlorhexidinedigluconate in comparison to phenolic and plant alkaloid compounds. J Periodont Res. 1986; 21:60-73.
21. Betoni JE. Mantovani RP. Barbosa LN. et al. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Mem Inst Oswaldo Cruz. 2006;101(4):387-90.
22. Nascimento GG. Locatelli J. Freitas PC. et al. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000; 31:247-56.
23. Araujo MWB. Charles CA. Weinstein RB et al. Meta-analysis of the effect of an essential oil-containing mouthrinse on gingivitis and plaque. J Am Dent Assoc. 2015;146(8):610-22.
24. Dušková M. Pospíšilová H. The role of non-aromatizable testosterone metabolite in metabolic pathways. Physiol Res. 2011;60(2):253-61.
25. Dušková M. Kolátorová L. Stárka L. Androgens in women - critical evaluation of the methods for their determination in diagnostics of endocrine disorders. Physiol Res. 2018; 28:67(Suppl 3): S379-S90.
26. Brecx M. Strategies and agents in supragingival chemical plaque control. Periodontol 2000. 1997; 15:100-8.
27. Hegstad K. Langsrud S. Lunestad BT. et al. Does the wide use of quaternary ammonium compounds enhance the selection and spread of antimicrobial resistance and thus threaten our health? Microb Drug Resist. 2010;16(2):91-104.
28. Sreenivasan PK. Haraszthy VI. Zambon JJ. Antimicrobial efficacy of 0·05% cetylpyridinium chloride mouthrinses. Lett Appl Microbiol. 2013;56(1):14-20.
29. He S. Wei Y. Fan X. et al. A clinical study assessed the 12-hour antimicrobial effects of cetylpyridinium chloride mouthwashes on supragingival plaque bacteria. J Clin Dent. 2011;22(6):195-9.
30. Haps S. Slot DE. Berchier CE. et al. The effect of cetylpyridinium chloride-containing mouth rinses as adjuncts to toothbrushing on plaque and parameters of gingival inflammation: a systematic review. Int J Dent Hyg. 2008;6(4):290-303.
31. Gunsolley JC. Clinical efficacy of antimicrobial mouthrinses. J Dent. 2010;38 Suppl 1: S6-10.
32. Rajendiran M. Trivedi HM. Chen D. et al. Recent Development of Active Ingredients in Mouthwashes and Toothpastes for Periodontal Diseases. Molecules. 2021;26(7):2001.
33. Witt JJ. Walters P. Bsoul S. et al. Comparative clinical trial of two antigingivitis mouthrinses. Am J Dent. 2005;18 Spec No:15A-17A.
34. Jeffcoat M. Parry S. Gerlach RW. et al. Use of alcohol-free antimicrobial mouth rinse is associated with decreased incidence of preterm birth in a high-risk population. Am J Obstet Gynecol. 2011;205(4): 382.e1-6.
35. Brooks JK. Bashirelahi N. Hsia RC. et al. Charcoal-based mouthwashes: a literature review. Br Dent J. 2020;228(4):290-4.
36. Tascanov MB. Gönel A. How Do Contrast Agents Affect Cardiac Markers and Coagulation Tests? Experimental Study. Comb Chem High Throughput Screen. 2019;22(5):355-60.
37. Tinanoff N. Progress regarding the use of stannous fluoride in clinical dentistry. J Clin Dent. 1995;6 Spec No:37-40.
38. Jongsma MA. van der Mei HC. Atema-Smit J. et al. In vivo biofilm formation on stainless steel bonded retainers during different oral health-care regimens. Int J Oral Sci. 2015;7(1):42-8.
39. Abbood HM. Hijazi K. Gould IM. Chlorhexidine Resistance or Cross-Resistance. That Is the Question. Antibiotics (Basel). 2023;12(5):798.

***Cite as*:** TAYMAN. MA et al. In Vitro Investigation of the Effect of Mouthwashes on Fertility Tests. JCMBS *2025; 5(1):xxxxxx doi.org/ 10.5281/zenodo.14583937*