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Embryotoxicity evaluation of Salvadora persica- and fluoride-containing products in zebrafish model - Original research

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Abstract

AIM:

This research aimed to compare the toxicity effect of Dabur Meswak and Colgate Strong Teeth in different forms (as powder and toothpaste) by testing in zebrafish embryo/larvae model.

METHODOLOGY:

The powder and toothpaste form of Dabur Meswak and Colgate Strong teeth with a concentration of 1mg/ml was tested in zebrafish larvae to understand their toxicity effect. The developmental toxicity assay was performed to evaluate the malformation condition after the treatment. Also, different parameters like heart rate, survival rate, and hatching rate were investigated in the zebrafish larvae after the miswak & Colgate exposure.

RESULTS:

The results displayed that both in the powder and paste forms, miswak was noted with no toxic range. The developmental toxicity was not noted in the miswak group, but in the Colgate groups, malformation like yolk sac edema along with bent spine was observed. Also, it was observed that the parameters such as survival, heart, and hatching rate were reduced in the Colgate groups. Meanwhile, the miswak showed no sign of toxic level and was observed similar to the control group.

CONCLUSION:

From the results, we concluded that compared to the Colgate paste and powder, both forms of miswak in powder and paste showed the least toxic effect in larvae.

KEYWORDS: Salvadora persica, zebrafish, fluoride, toxicity, dental care

Introduction:

Maintaining good oral health significantly impacts the quality of life (QoL) of an individual [1]. There is a growing global requirement for the advancement of new, cost-effective, safe, and efficient preventive and treatment approaches as well as products [2]. The most frequently employed approach to enhance oral hygiene and gingival health is mechanical plaque removal. The purpose of tooth cleaning is to prevent the development of plaque, which is a precursor to conditions such as caries, gingivitis, and periodontitis [3].

Toothpaste is frequently utilized as a supplementary measure to support the effectiveness of mechanical plaque removal [4]. A review of relevant literature indicates that various dental products, including toothpastes with antimicrobial properties, play a vital role in eliminating dental biofilm along with gingivitis. These agents effectively reduce plaque-induced diseases and serve as alternative products in the control of plaque [5,6]. Nevertheless, these products contain additives that may have potential toxic effects on the oral mucosa [7].

Fluoride is incorporated into the toothpaste for its cariostatic properties, but it exhibits toxic impacts on different types of cells. The degree of toxicity depends on the duration and concentration of fluoride exposure. Elevated concentrations can lead to cell necrosis [8]. Additionally, prolonged exposure to higher concentrations of fluoride (10 parts / million or greater) often causes fluorosis, characterized by brownish enamel discoloration, resulting in a mottled appearance [9,10]. The sodium lauryl sulfate (SLS), which is used as a detergent in the majority of toothpastes, has shown significant toxic effects according to previous literature [11]. Research performed by Gerckens et al. and Herlofson et al. established the toxic SLS nature that may affect the oral mucosal cells, potentially responsible for epithelial desquamation [12,13].

In the rural regions of developing countries, herbal therapies serve as the primary form of medicine [14]. Derived from medicinal plants, natural products constitute the foundation for numerous active biological components, holding promise for the development of novel chemicals in medicine [15,16]. The antiviral, antibacterial, and anti-inflammatory properties inherent in herbal products have made inroads into the field of dentistry. Numerous researches have explored the impacts of plant extracts along with the products on particular oral pathogens. The exploration of essential oils, plant extracts, and phytochemicals has centered on their potential to prevent or treat bacterial adhesion which is responsible for dental plaque development [17], and demonstrate antibacterial efficacy against various bacteria, including Streptococcus mutans [18,19]. The extracts of Salvadora persica, (also known as Miswak), have demonstrated the ability to impede the development of cariogenic bacteria by their anti-plaque function [20], effectively reducing the colonization of certain streptococcal strains on the tooth surfaces [21]. The extract from this plant comprises several antimicrobial agents, notably benzyl isothiocyanate, that exhibit a potent and rapid bactericidal impact against oral microorganisms related to caries along with periodontal disease [22].

Toxicity tests for toothpaste and/or dental-related products are essential to guarantee consumer safety, assess potential risks associated with their ingestion or prolonged use, and comply with regulatory standards [23,24]. Various cytotoxicity along with cell viability assays find widespread use in the domains of toxicology and pharmacology. In vitro, cell cultures offer a valuable and cost-effective means to swiftly evaluate the cytotoxic and genotoxic impacts of chemicals and environmental pollutants [21,25]. However, embryotoxicity studies are more directly relevant to understanding the impact of a substance on human development. Zebrafish toxicity testing is a common approach utilized to analyze the potential toxic impacts of several substances [26], including dental materials, and provides valuable insights into the safety and potential risks associated. Zebrafish (scientifically known as Danio rerio) is presently recognized as a leading

vertebrate model in developmental biology research. Despite anatomical along histological variations from mammals, lacking organs like the prostate, lungs, and mammary glands, zebrafish retain vertebrate body plan fundamental features at anatomical, molecular, and physiological levels [27,28]. Zebrafish, both in their adult and embryonic stages, serve as valuable laboratory models [29]. However, the embryo stages are preferred for toxicological assessments due to the transparent nature of the egg. This transparency enables direct observation of developmental stages and the evaluation of toxicity endpoints. Additionally, the rapid development of zebrafish's major organ primordia within 24 hours, ease of in vitro fertilization and development, and straightforward observation and manipulation contribute to its success as a highly suitable model for toxicity studies [30]. Moreover, the zebrafish genome encompasses homologues for approximately 70 percent of human genes, featuring over 80% representation of genes related to human diseases. This substantial genetic overlap enables a more direct extrapolation of research outcomes and justifies the considerable investment in utilizing this species across various translational biomedical research endeavors. The current research has aimed to assess the toxicity impacts of commercially available miswak- and fluoride-containing products on zebrafish larvae and embryos.

Materials and methods:

The research was performed in Saveetha Dental College and Hospital in February 2023. The Institutional Scientific Review Board has reviewed and approved the study (SRB/SDC/ENDO-2104/23/150).

1. Source and Management of Zebrafish

Adult zebrafish (Wild type – AB strain, 4 months old) have been procured, with males and females separated and housed in a 10L glass tank at 28.5˚C, following a 14/10 h lighter/darker cycle. The fish have been fed 3 times a day, with live brine shrimp (Artemia salina). Following a month of acclimatization, the zebrafish were used for breeding purposes. The resultant embryos obtained from the breeding process were

subjected to further examination under a microscope. The fertilized embryos were carefully placed in a six-well plate and subjected to incubation within an embryonic medium 3 (E3) for further experimental procedures.

2. Fabrication of samples

The assessed products containing Salvadora persica are Dabur Meswak, while those containing fluoride are Colgate Strong teeth. A total of 100 embryos were taken with 20 embryos in each group. The samples tested for toxicity are categorized into five groups.

Group 1: Control - E3 medium

Group 2: Meswak powder

Group 3: Meswak toothpaste

Group 4: Colgate tooth powder

Group 5: Colgate toothpaste

The composition of the evaluated materials (as per manufacturer) is enlisted in Table 1.

Table 1: Composition of evaluated groups

S.no	Groups	Manufacturer Details	Composition
1	Control (E3 medium)	-	0.17mM Potassium chloride, 5mM Sodium chloride, 0.33mM Magnesium sulfate, 0.33mM Calcium chloride
2	Miswak powder	MB Herbals, India	Salvadora persica St. powder 100% w/w
3	Dabur Meswak toothpaste	Dabur India Limited, India	Sorbitol, Calcium Carbonate, Silica, Sodium

			Lauryl Sulphate, Water, Miswak Extract, Flavor, Carrageenan, Cellulose Gum, Pvm/Ma Copolymer, Sodium Silicate, Zinc Gluconate, Sodium Saccharin, Benzyl Alcohol, Sodium Benzoate, Ci 77891, P-Thymolcalcium Carbonate
4	Colgate Strong Teeth tooth powder	Colgate-Palmolive Ltd, India	Sodium Lauryl Sulphate, Calcium Carbonate, Flavor, Sodium Saccharin, Sodium Monofluorophosphate
5	Colgate Strong Teeth toothpaste	Colgate-Palmolive Ltd, India	Sorbitol, Calcium Carbonate, Silica, Titanium Dioxide, Sodium Lauryl Sulphate, Flavor, Sodium Silicate, Sodium Bicarbonate, Benzyl Alcohol, Sodium Monofluorophosphate, Potassium Nitrate, Sodium Saccharin, Limonene

A stock solution of 1mg/ml was prepared for the respective group samples. The larvae were treated with the test group solutions (100µg/ml) for 5min.

3. Developmental embryotoxicity assessment

Embryos at the 4-hour post-fertilization (4 hpf) stage were employed to assess the incidence of developmental toxicity. The exposure was conducted within 6-well plates, with the larvae treated with their respective control and test solutions. Twenty embryos were placed in each well, with 3ml of E3 medium. The exposure was dynamic, with the exposure of the treatment solution for 5 minutes. The solutions were renewed every 24 hours continuously from 4 to 72 hpf. All experiments were performed in triplicate. The developmental stages of the embryos were monitored under an inverted microscope to examine potential malformations.

4. Assessment of heart rate

Assessing heart rate is an important aspect related to cardiac function. The embryos were immobilized in a small agarose volume and positioned beneath a microscope equipped with a high-speed camera [31]. This camera captured the heart movements (heartbeats), and dedicated software was employed to analyze the images and determine the heart rate. The heart rate assessment (in heartbeats/min) was performed at the end of 72 hpf interval.

5. Assessment of survival rate

The embryos were subjected to testing solutions and observed to evaluate the impact of these solutions on their viability. They were carefully observed to determine detrimental effects such as egg coagulation, absence of spontaneous mobility, and heartbeat [32]. Deceased embryos were removed immediately, and the surviving embryos were tallied. The survival rate (in %) was then evaluated at 72 hpf.

6. Assessment of the hatching rate

Analyzing the hatching rate in zebrafish embryos involves assessing the proportion of embryos successfully hatching from their chorions. Typically, zebrafish embryos hatch between 48 and 72 hpf, and a decrease in hatching rate may indicate potential

developmental abnormalities due to exposure to toxins. The embryo hatching rates were evaluated using a subset of embryos from each exposed group. The embryos were immersed in an embryonic medium for 24 hours to dissolve the chorion, facilitating hatching [33]. The deceased embryos, if any, were removed immediately. The number of hatched larvae in each treatment group was evaluated and the hatching rate (in %) was calculated as follows: hatched numbers/total exposed numbers × 100 [32].

7. Statistical analysis

The data have been depicted as the mean of the three repeated measurements along with their corresponding standard deviation. Statistical analysis has been conducted utilizing IBM SPSS Statistics version 23 software. A one-way ANOVA test has been performed, followed by Tukey's post-hoc test to establish the level of significance among the test and control groups. All the tests were performed at a 5% statistical significance level.

Results:

Morphological malformation

Figure 1 shows the microscopic image of embryos/larvae observed during the different exposure periods. The zebrafish embryos treated with Miswak powder and Meswak toothpaste exhibited normal morphological architecture when observed under the inverted microscope. No developmental malformations were formed in the larvae exposed to Miswak powder and Meswak toothpaste. Developmental malformations, i.e., YSE (Yolk Sac Edema) and BS (Bent Spine) have been seen in larvae exposed to Colgate powder and toothpaste, respectively.

Measurement of heart rate

Zebrafish embryo heart rates were evaluated at 72 hpf for the treatment toxicity. Atrial and ventricular contractions were observed under the microscope, and the average heart rate/min was documented. The tabulated mean values in Table 2 indicate that the Meswak groups had no significant impact on zebrafish embryo heart rates than to the control group. In contrast, the Colgate groups exhibited a major decrease in heart rate.

Measurement of survival rate

Table 3 illustrates the mean survival rate percentages. The Meswak groups did not result in significant mortality among zebrafish embryos. However, the Colgate groups showed a substantial reduction in the survival rate, with a mortality rate exceeding 10%.

Measurement of hatching rate

The hatching rate has been determined at 48 hpf and the mean values are presented in Table 4. In the control group, nearly all zebrafish embryos hatched from their chorions. The Meswak groups showed a similar hatching rate, while the Colgate groups exhibited a noteworthy decrease in hatching rate.

Figure 1: Microscopic assessment of zebrafish embryos/larvae upon exposure to the evaluated solutions at 24, 48, and 72 hpf.
(BS: Bent Spine, YSE: Yolk Sac Edema)

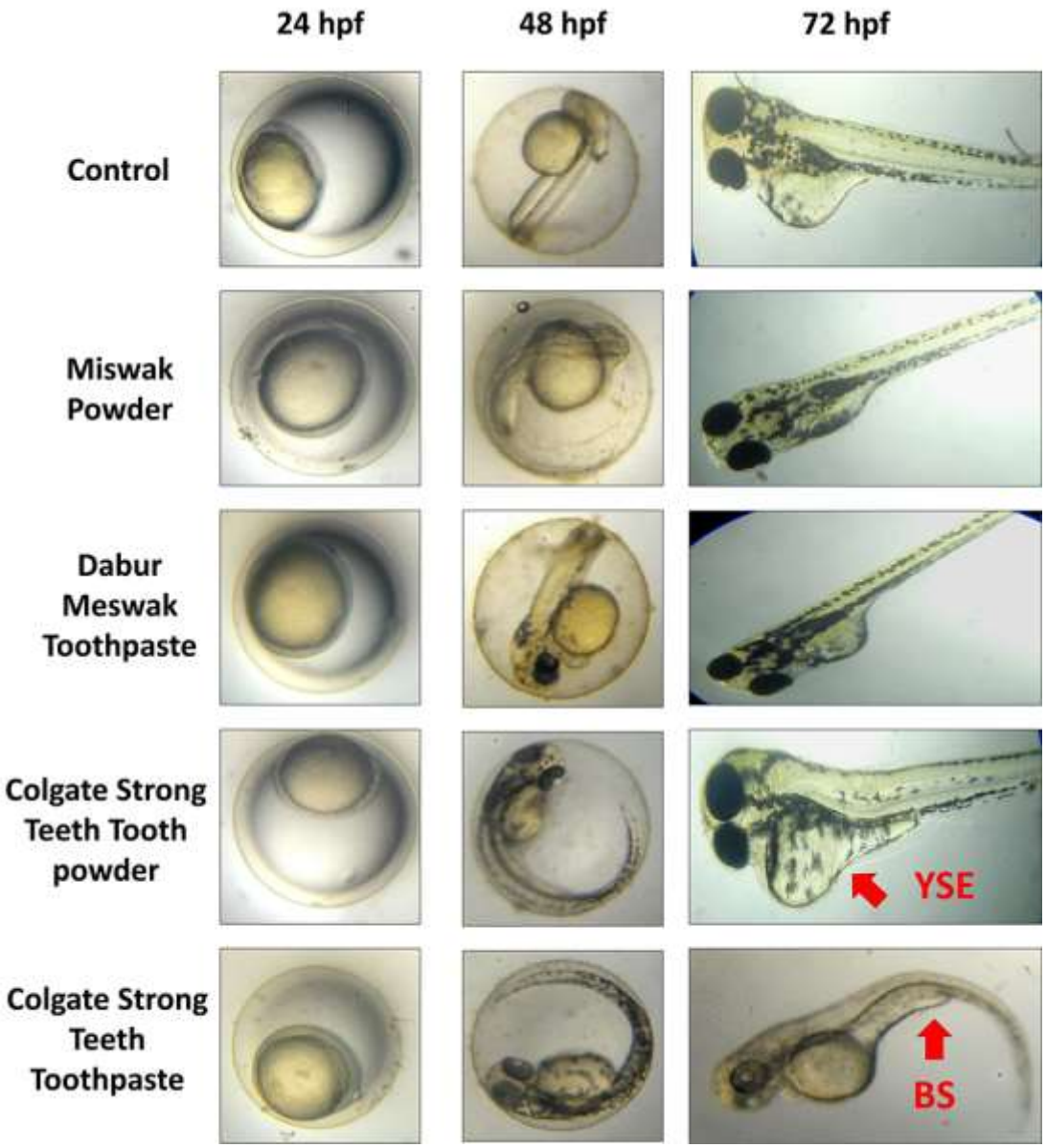


Table 2: Means and standard deviations of heart rate (per minute) values in each group

GROUPS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
HEART RATE (per minute)	170 ± 2.00	166 ± 1.00	166.67 ± 3.21	157 ± 1.00	150 ± 2.00

The table 3 shows the Means and standard deviations of survival rate (in percentage) values in each group

Table 3: Means and standard deviations of survival rate (in percentage) values in each group

GROUPS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
SURVIVAL RATE (in percentage)	100 ± 0.00	98 ± 2.65	96.67 ± 3.05	83 ± 2.65	76.67 ± 1.53

The table 4 shows the Means and standard deviations of hatching rate (in percentage) values in each group

Table 4: Means and standard deviations of hatching rate (in percentage) values in each group

GROUPS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
HATCHING RATE (in	99.33 ± 0.58	97 ± 1.73	96 ± 2.00	83.67 ± 3.21	79.33 ± 1.53

percentage)					

Discussion:

In the field of biomedical research, the study of biological processes often involves the use of model organisms. These organisms serve as valuable tools because they exhibit certain biological features and functions that are conserved or shared between humans and lower vertebrates. By utilizing model organisms, researchers can gain insights into fundamental biological mechanisms, disease processes, and potential therapeutic interventions. This approach allows scientists to extrapolate findings from these model systems to better understand similar processes in humans, providing a bridge between basic research and its application in human health [34]. Zebrafish are frequently employed as genetic modification-based models for studying human diseases [35]. They can serve as valuable tools to study the health impacts of environmental exposures [36]. This helps enhance our comprehension of the origins and mechanisms of diseases in humans that are linked to environmental factors [37].

It is essential to ensure the safe use of these regular utility products that come into direct contact with the oral cavity. The present study assesses the embryotoxicity of commercially available regular dental care products, specifically Miswak powder, Meswak toothpaste, Colgate powder, and Colgate toothpaste, using zebrafish embryos as a model system. The assessment involved multiple parameters, including morphological malformations, heart rate, survival rate, and hatching rate, to comprehensively understand the potential developmental impacts of these products.

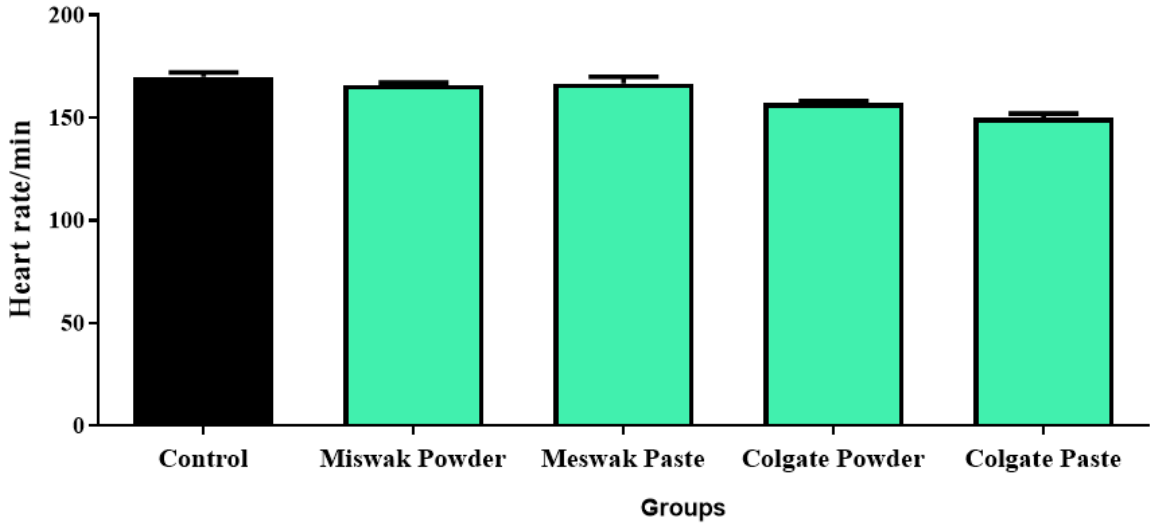
Bartlett et al., in zebrafish embryotoxicity studies, demonstrated the development of fluorosis on the enameloid surface of zebrafish teeth, characterized by a notable rise in organic content on surfaces exhibiting pits and roughness [38]. Meanwhile, Zhang and colleagues subjected zebrafish larvae to different fluoride doses for five days to induce dental fluorosis. They discovered a notable decline in RNA, DNA, and protein levels in the cerebellum, brain, and medulla oblongata of the larvae, corresponding to improved fluoride concentrations [39]. In the current study, the larvae exposed to fluoride-containing Colgate powder exhibited yolk sac edema, while those exposed to Colgate toothpaste displayed a bent spine. In contrast, the meswak powder and toothpaste groups displayed normal morphological architecture, with no observed developmental malformations. The microscopic examination of embryos and larvae during different exposure periods is shown in Figure 1. These morphological abnormalities highlight the adverse developmental effects associated with Colgate products, potentially impacting embryonic and larval integrity.

Evaluation of the heart rate at 72 hpf served as an indicator of cardiovascular toxicity. As shown in Graph 1, the Meswak groups showed no significant impact on the heart rate of zebrafish embryos than to the control group, suggesting a lack of adverse effects on cardiac development. In contrast, the Colgate groups exhibited a noteworthy decrease in heart rate, indicating potential cardiotoxicity associated with exposure to Colgate dental care products. In terms of survival rate, the findings reveal that the Meswak groups did not induce mortality in zebrafish embryos, indicating a relatively lower toxicity profile compared to the Colgate groups (Graph 2). Notably, the Colgate groups exhibited a significant decrease in the survival rate, with a mortality rate exceeding 10%. This suggests a potential adverse effect on the overall viability of zebrafish embryos when exposed to Colgate dental care products.

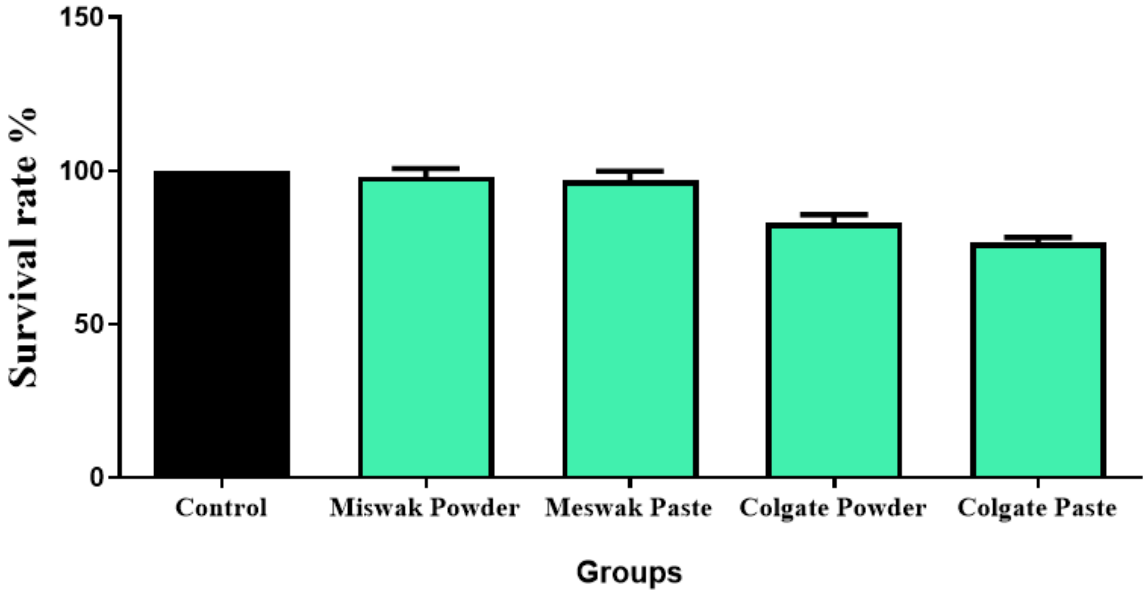
The hatching rate, assessed at 48 hpf, provides insights into the embryos' ability to emerge from their chorions. While the control group exhibited a 100% hatching rate, the

Meswak groups demonstrated a similar hatching rate, suggesting minimal impact on this developmental aspect (Graph 3). In contrast, the Colgate groups displayed a significant decrease in the hatching rate, indicating a potential interference with the normal hatching process. The reduction in the hatching rate may be due to the composition of the evaluated products. Oliveira et al. discovered that triclosan induces acute toxicity in zebrafish embryos, resulting in decreased hatching rates, pigmentation, and stature. Despite being added to toothpastes for its antibacterial properties, triclosan exhibited adverse effects on hatching rates [40]. The fluoride content in conventional toothpaste could potentially amplify toxicity by synergizing with the bacteriostatic effect of SLS. It is thought that SLS may adversely affect individuals with recurrent aphthous ulcers through potential peeling of the oral mucosa. SLS causes the removal of the protective mucin surface layer, thereby diminishing the oral mucosa resilience [41].

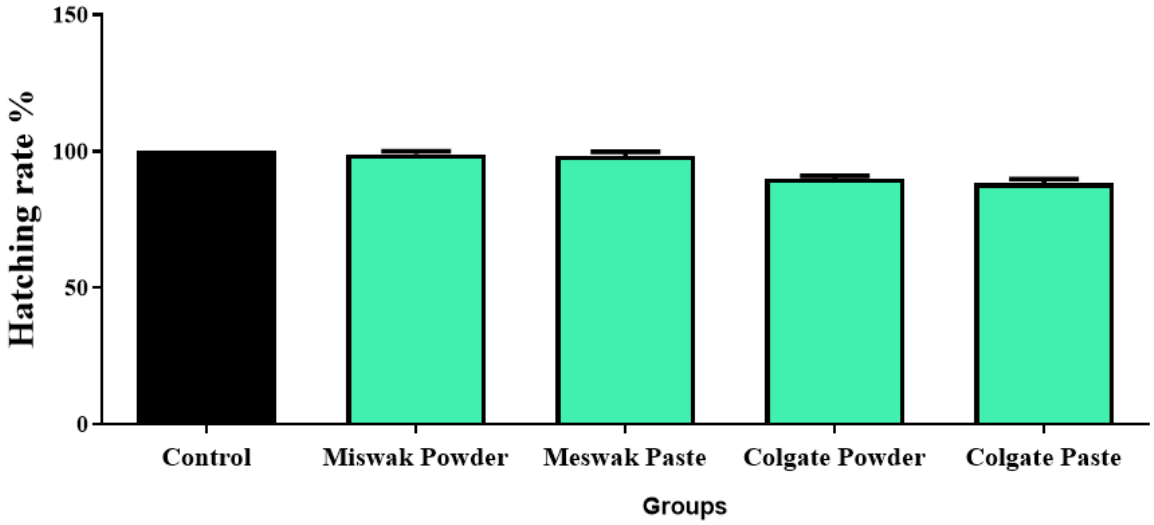
Our study indicates that Salvadora persica containing herbal dental care products exhibits greater biocompatibility on zebrafish embryos compared to conventional dental care products containing fluoride. While toothpaste offers various benefits such as caries prevention, remineralization, and whitening, it's crucial to recognize that certain ingredients may enter systemic circulation and accumulate in organs. Despite minimal concentrations in toothpaste, caution is warranted regarding potential toxicity risks on ingestion by water, food, and other personal care products.



Graph 1: Evaluation of heart rate of zebrafish larvae at 72hpf



Graph 2: Evaluation of survival rate of zebrafish larvae at 72hpf



Graph 3: Evaluation of hatching rate of embryos at 48hpf

Conclusion:

The study underscores the importance of considering embryotoxicity when assessing the safety of dental care products. The Meswak products demonstrated a more favorable profile, showing minimal impact on survival, hatching, heart rate, and morphology compared to the Colgate counterparts. These findings contribute valuable insights into the potential developmental risks associated with commonly used dental care products, emphasizing the need for further research and careful consideration of product safety in the interest of consumer health.

References:

1. Baiju RM, Peter E, Varghese NO, Sivaram R. Oral Health and Quality of Life: Current Concepts. J Clin Diagn Res. 2017 Jun;11(6):ZE21–6.

2. Listl S, Baltussen R, Carrasco-Labra A, Carrer FC, Lavis JN. Evidence-Informed Oral Health Policy Making: Opportunities and Challenges. J Dent Res. 2023

Nov;102(12):1293–302.

3. Figuero E, Nóbrega DF, García-Gargallo M, Tenuta LMA, Herrera D, Carvalho JC. Mechanical and chemical plaque control in the simultaneous management of gingivitis and caries: a systematic review. J Clin Periodontol. 2017 Mar 6;44:S116–34.

4. Binney A, Addy M, Newcombe RG. The plaque removal effects of single rinsings and brushings. J Periodontol. 1993 Mar;64(3):181–5.

5. Fine DH, Furgang D, Markowitz K, Sreenivasan PK, Klimpel K, De Vizio W. The antimicrobial effect of a triclosan/copolymer dentifrice on oral microorganisms in vivo. J Am Dent Assoc. 2006 Oct;137(10):1406–13.

6. Suresh S, Arumugham IM, Doraikannan S, Rathinavelu PK, Prabakar J, Balasubramaniam A. Comparing the Effectiveness of Herbal and Conventional Dentifrices in Reducing Dental Plaque and Gingivitis: A Systematic Review. J Int Soc Prev Community Dent. 2021 Nov 30;11(6):601–8.

7. Ghapanchi J, Kamali F, Moattari A, Poorshahidi S, Shahin E, Rezazadeh F, et al. In vitro comparison of cytotoxic and antibacterial effects of 16 commercial toothpastes. J Int Oral Health. 2015 Mar;7(3):39–43.

8. Barbier O, Arreola-Mendoza L, Del Razo LM. Molecular mechanisms of fluoride toxicity. Chem Biol Interact. 2010 Nov 5;188(2):319–33.

9. Heymann H, Swift EJ, Ritter AV, Sturdevant CM. Sturdevant's Art and Science of Operative Dentistry. Mosby; 2013.

10. Balasubramaniam A, Senior Lecturer, Department of Public Health Dentistry, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences. Prevalence of dental fluorosis amongst young adults and adolescents in

Chennai. Int J Dent Oral Sci. 2021 Aug 18;3990–4.

11. Cvikl B, Lussi A, Gruber R. The in vitro impact of toothpaste extracts on cell viability. Eur J Oral Sci. 2015 Jun;123(3):179–85.

12. Gerckens B, Eisinger G, Kaden P, Krüger W. [Comparative studies of toothpastes and toothpaste ingredients in biological systems: 1. Can various toothpastes be differentiated by relative biological effectiveness in cell culture studies?]. Oralprophylaxe. 1991 Jun;13(2):55–60.

13. Herlofson BB, Barkvoll P. Oral mucosal desquamation caused by two toothpaste detergents in an experimental model. Eur J Oral Sci. 1996 Feb;104(1):21–6.

14. Steinberg D, Feldman M, Ofek I, Weiss EI. Effect of a high-molecular-weight component of cranberry on constituents of dental biofilm. J Antimicrob Chemother. 2004 Jul 1;54(1):86–9.

15. Drug discovery from medicinal plants. Life Sci. 2005 Dec 22;78(5):431–41.

16. Nasim I, Jabin Z, Kumar SR, Vishnupriya V. Green synthesis of calcium hydroxide-coated silver nanoparticles using and Linn. leaf extracts: An antimicrobial and cytotoxic activity. J Conserv Dent. 2022 Aug 2;25(4):369–74.

17. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev [Internet]. 1986 Dec [cited 2023 Dec 14]; Available from: https://journals.asm.org/doi/10.1128/mr.50.4.353-380.1986

18. Botelho MA, Nogueira NAP, Bastos GM, Fonseca SGC, Lemos TLG, Matos FJA, et al. Antimicrobial activity of the essential oil from Lippia sidoides, carvacrol and thymol against oral pathogens. Braz J Med Biol Res. 2007 Mar;40(3):349–56.

19. Genotypic diversity of Streptococcus mutans in children with and without early childhood caries- A systematic review. Journal of Oral Biology and Craniofacial

Research. 2021 Apr 1;11(2):308–12.

20. Miswak and oral health: An evidence-based review. Saudi J Biol Sci. 2020 Jul 1;27(7):1801–10.

21. Kamath AK, Nasim I, Muralidharan NP, Kothuri RN. Anti-microbial efficacy of Vanilla planifolia leaf extract against common oral micro-biomes: A comparative study of two different antibiotic sensitivity tests. J Oral Maxillofac Pathol. 2022;26(3):330.

22. Darmani H, Nusayr T, Al-Hiyasat AS. Effects of extracts of miswak and derum on proliferation of Balb/C 3T3 fibroblasts and viability of cariogenic bacteria. Int J Dent Hyg. 2006 May 1;4(2):62–6.

23. Bauer B, Mally A, Liedtke D. Zebrafish Embryos and Larvae as Alternative Animal Models for Toxicity Testing. Int J Mol Sci. 2021 Dec 14;22(24):13417.

24. Nasim I, Rajesh Kumar S, Vishnupriya V, Jabin Z. Cytotoxicity and anti-microbial analysis of silver and graphene oxide bio nanoparticles. Bioinformation. 2020 Nov 30;16(11):831–6.

25. Chapter 2 Use of fish cell lines in the toxicology and ecotoxicology of fish. Piscine cell lines in environmental toxicology. In: Biochemistry and Molecular Biology of Fishes. Elsevier; 2005. p. 43–84.

26. Issac PK, Guru A, Chandrakumar SS, Lite C, Saraswathi NT, Arasu MV, et al. Molecular process of glucose uptake and glycogen storage due to hamamelitannin via insulin signalling cascade in glucose metabolism. Mol Biol Rep. 2020 Aug 18;47(9):6727–40.

27. Lewis KE, Eisen JS. From cells to circuits: development of the zebrafish spinal cord. Prog Neurobiol. 2003 Apr;69(6):419–49.

28. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013 Apr 25;496(7446):498–503.

29. Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. Nat Rev Genet. 2007 May;8(5):353–67.

30. Mendis JC, Tennakoon TK, Jayasinghe CD. Zebrafish Embryo Toxicity of a Binary Mixture of Pyrethroid Insecticides: d-Tetramethrin and Cyphenothrin. J Toxicol [Internet]. 2018 Dec 26 [cited 2023 Dec 19];2018. Available from: https://doi.org/10.1155/2018/4182694

31. AlshutRüdiger. Automatic Zebrafish Heartbeat Detection and Analysis for Zebrafish Embryos. Zebrafish [Internet]. 2014 Jul 28 [cited 2024 Apr 14]; Available from: https://www.liebertpub.com/doi/10.1089/zeb.2014.1002

32. Curcio V, Macirella R, Sesti S, Pellegrino D, Ahmed AIM, Brunelli E. Morphological and Molecular Alterations Induced by Lead in Embryos and Larvae of Danio rerio. NATO Adv Sci Inst Ser E Appl Sci. 2021 Aug 13;11(16):7464.

33. Valan AS, Krithikadatta J, Guru A. Exploring periostracum as an alternative root canal irrigant: Insights from zebrafish embryo experiments. Cureus [Internet]. 2024 Mar 21; Available from: https://www.cureus.com/articles/232960-exploring-periostracum-as-an-alternative-root-canal-irrigant-insights-from-zebrafish-embryo-experiments

34. Veldman MB, Lin S. Zebrafish as a developmental model organism for pediatric research. Pediatr Res. 2008 Nov;64(5):470–6.

35. Chokkattu JJ, Mary DJ, Shanmugam R, Neeharika S. Embryonic Toxicology Evaluation of Ginger- and Clove-mediated Titanium Oxide Nanoparticles-based Dental Varnish with Zebrafish. J Contemp Dent Pract. 2022 Nov 1;23(11):1157–62.

36. Garapati B, Malaiappan S, Rajeshkumar S, Murthykumar K. Cytotoxicity of lycopene-mediated silver nanoparticles in the embryonic development of zebrafish—An animal study. J Biochem Mol Toxicol. 2022 Oct 1;36(10):e23173.

37. Bambino K, Chu J. Zebrafish in Toxicology and Environmental Health. Curr Top Dev Biol. 2017;124:331–67.

38. Bartlett JD, Dwyer SE, Beniash E, Skobe Z, Payne-Ferreira TL. Fluorosis: A New Model and New Insights. J Dent Res [Internet]. 2005 Sep 1 [cited 2023 Dec 15]; Available from: https://journals.sagepub.com/doi/10.1177/154405910508400910

39. Grading and quantification of dental fluorosis in zebrafish larva. Arch Oral Biol. 2016 Oct 1;70:16–23.

40. Oliveira R, Domingues I, Koppe Grisolia C, Soares AMVM. Effects of triclosan on zebrafish early-life stages and adults. Environ Sci Pollut Res. 2009 Mar 13;16(6):679–88.

41. Shim YJ, Choi JH, Ahn HJ, Kwon JS. Effect of sodium lauryl sulfate on recurrent aphthous stomatitis: a randomized controlled clinical trial. Oral Dis. 2012 Oct 1;18(7):655–60.