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# COMPARATIVE EVALUATION OF EMBRYONIC TOXICOLOGY IN COMMERCIAL MOUTHWASH AND MOUTHWASH FORMULATIONS ASSISTED BY *SALVADORA PERSICA* AND *ANDROGRAPHIS PANICULATA*

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**Abstract**

Maintaining good oral hygiene is essential, and mouthwashes play a crucial role in this regard. Herbal mouthwashes, particularly those containing *Salvadora persica and Andrographis paniculata* , offer notable antimicrobial benefits over commercial counterparts. This study aims to compare the embryotoxicity of commercial oral preparations with those containing S. persica and A. paniculata extracts. Using wild-type zebrafish (*Danio rerio*) embryos, we evaluated hatching rates, viability rates, and cytotoxicity through a brine shrimp lethality assay over 24 hours. The results demonstrated that increasing concentrations of both mouthwash types led to a decrease in live nauplii, hatching rate, and viability rate. Notably, the S. persica + A. paniculata mouthwash was more effective, showing significant reductions in hatching and viability at lower concentrations. These findings support the potential of herbal formulations in mitigating embryonic toxicological risks, positioning S. persica and A. paniculata as promising alternatives to conventional mouthwashes.

**Keywords:** Herbal Mouthwashes, Salvadora Persica, Andrographis Paniculata, Embryonic Toxicology, Commercial Mouthwashes.

## 1. INTRODUCTION

Maintaining optimal oral hygiene is fundamental for overall oral health. Regular brushing, flossing, a balanced diet, and adequate hydration form the cornerstone of a healthy oral cavity(Ambika, Manojkumar et al. 2019, Rudak and Andruškienė 2019). Complementary products such as rinses, tongue scrapers, and mouthwashes enhance the effectiveness of these basic practices(Pérez-Portilla, Ortíz-Benitez et al. 2023, Tayyeb, Priya et al. 2024). Mouthwashes, also known as oral washes or mouth rinses, are liquid aqueous solutions designed primarily to prevent, alleviate, and treat various oral diseases while promoting oral health. They aim to prevent dental caries, erosion, gingivitis, and periodontitis and manage issues such as mucositis, oral-dental injuries, and tooth loss. These products are widely popular as an additional element in oral hygiene routines and are available in numerous varieties worldwide. Despite their popularity, many mouthwashes have not undergone adequate testing, and there is insufficient information on their optimal use for maximum benefit(RUDAK , Sundaram, Bupesh et al. 2022). Ideally, any antimicrobial or antiseptic agents in these formulations should effectively target pathogens while maintaining the normal oral microflora(Kishore, Priya et al. 2020, Marunganathan, Kumar et al. 2024).

To meet these criteria, several herbal mouthwashes and extracts have been thoroughly evaluated both in vitro and in vivo, aiming to identify suitable additives for mechanical processing for long-term oral care. However, not all commercial mouthwashes effectively prevent caries, decay, and plaque buildup(Rieshy, PRIYA et

al. 2020, Velumani, Arasu et al. 2023). Many of those available in the market are alcohol-based and contain acidic ingredients, which can exacerbate issues such as dry mouth and ulcers. Therefore, the use of herbal mouthwashes is often recommended as a safer alternative. Herbal antibacterial agents are a cornerstone of traditional health care and possess well-documented antimicrobial properties. Unlike synthetic drugs, herbal drugs offer greater efficacy with minimal side effects(Murugesan 2016). Among these herbal agents, Salvadora persica L., an evergreen shrub from the Salvadoraceae family, stands out for its oral hygiene benefits, especially within the global Muslim community. This plant has numerous agro-pharmaceutical applications. Its roots and twigs are highly valued for their dental properties, often used as natural toothbrushes. In addition to its dental benefits, Salvadora persica possesses several medicinal properties, including antimicrobial, antipyretic, aphrodisiac, analgesic, anti-inflammatory, and astringent effects. Its range of medicinal applications includes the treatment of various ailments such as nasal problems, scabies, leukoderma, and toothache.

Additionally, it is beneficial for conditions like rheumatism, cough, asthma, and even helps in regulating cholesterol levels and maintaining gastric mucosal health. Another notable herbal agent is Andrographis paniculata, commonly known as the "king of bitters" or "Kalmegh." This annual herb, belonging to the Acanthaceae family, is native to India and Sri Lanka and is widely cultivated in regions of South and Southeast Asia, such as Bangladesh, China, Hong Kong, Indonesia, Malaysia, Myanmar, the Philippines, and Thailand(Balaji, Bhuvaneswari et al. 2022). A. paniculata, known for its bitter taste, is used to treat various diseases, including liver disorders, intestinal problems in children, colic, colds, and upper respiratory infections. The aerial parts of A. paniculata are often utilized in traditional Chinese medicine. According to Chinese medicinal theory, this herb has cooling properties and helps alleviate internal heat, inflammation, and pain, making it valuable for detoxification purposes. Embryonic toxicology, also known as developmental toxicology, is a specialized branch of toxicology that investigates the harmful effects of substances on the developing embryo or fetus during pregnancy.

This field aims to understand how exposure to various chemicals, drugs, or environmental factors can lead to congenital abnormalities, birth defects, or developmental disorders. Exceeding the threshold dose of toxicity during the sensitive embryonic and fetal periods can result in various developmental malformations, potentially having a fatal impact on exposed animals and their offspring. Therefore, understanding the potential effects on embryonic development is essential(Sivakumar, Geetha et al. 2021, Sundaram and Saravanan 2022). Plant materials and their extracts contain various toxic compounds synthesized by plants as defense mechanisms against diseases, insects, and other threats.

Assessing their toxicity is crucial for ensuring human safety. While many consider medicinal plants to be inherently safe, cases of poisoning can and do occur. Consumers may face potential health risks due to certain components or contaminants in botanical products, necessitating thorough risk assessments. Toxicity studies aim to quickly and accurately predict human toxicity using model systems(Nasim, Rajeshkumar et al. 2021, Ravikumar, Marunganathan et al. 2024).

Given the importance of assessing the safety of herbal products, this study aims to compare the embryonic toxicology of commercial oral preparations with those

containing *Salvadora persica and Andrographis paniculata* extracts. By evaluating these formulations' effects on embryonic development, this research seeks to support the potential of these herbal mouthwashes as safer and more effective alternatives to conventional mouthwashes available on the market(Nasim, Kumar et al. 2020).

## 2. MATERIALS AND METHOD

### 2.1 Zebrafish embryonic toxicology evaluation

### a) Fish maintenance

Wild-type zebrafish (*Danio rerio*) were acquired from local Indian vendors and were housed in individual tanks under controlled conditions of temperature ($28^0 \pm 2^0$C), light/dark cycle (14:10 h), and pH (6.8–8.5). The fishes were fed with commercially available dry blood worms or optimum food twice daily. Zebrafish embryos were obtained by crossing one female and three males per breeding tank, and viable eggs were collected and rinsed at least three times with freshly prepared E3 medium without methylene blue. The study involved the placement of fertilized eggs in culture plates of varying well sizes (6, 12, and 24 wells) with 20 embryos per 2 mL solution per well. The experimental treatment and control groups were replicated three times. To prepare the experimental treatment, a stock suspension of *Salvadora persica and Andrographis paniculata* plant extract with five different concentrations was freshly made and added directly to the E3 medium. The solution was sonicated for 15 minutes to disperse the while maintaining a pH range of 7.2-7.3. Healthy fertilized embryos were exposed to different concentrations of the plant extract ranging from (5, 10, 20, 40, and 80 µg/mL) for 24 to 96 hours post fertilization. The plant extracts were added to the E3 medium where the embryos were incubated. Control groups were also included in the experiment. Dead embryos were removed from the nanoparticles exposed groups every 12 hours. All experimental plates were wrapped in foil to exclude light and maintained at 28°C(Panzica-Kelly, Zhang et al. 2012, Anbarasu, Vinitha et al. 2024).

### b) Zebrafish embryo evaluation

Throughout the exposure period following fertilization, the developmental stages of Zebrafish embryos were monitored using a stereo microscope. The embryos were subjected to various concentrations of the plant extract (5, 10, 20, 40, and 80 µg/mL) for 24-78 hpf. Embryonic mortality and hatching rates were assessed at 24-hour intervals. The study endpoints included embryo/hatchling mortality, hatching rate, and the identification and documentation of any malformations among the embryos and larvae in both control and treatment groups(Umapathy, Pan et al. 2024). Photographs of malformed embryos were captured using a COSLAB - Model: HL-10A light microscope and the percentage of abnormal embryos was recorded every 24 hours(Vranic, Shimada et al. 2019, Baranikumar, Kumar et al. 2023).

### 2.2 Cytotoxic effect

### Brine shrimp lethality assay

### Saltwater preparation:

Two grams of iodine-free salt were weighed and dissolved in 200 ml of distilled water. Six well ELISA plates were taken, and each well was filled with 10-12 ml of saline water. Ten nauplii were then slowly added to each well, with plant extracts added

according to the concentration levels of 5, 10, 20, 40, and 80 µg/mL. The plates were incubated for 24 hours. After the incubation period, the ELISA plates were observed to note the number of live nauplii, and the percentage of dead nauplii was calculated using the formula:

(Number of dead nauplii / (Number of dead nauplii + Number of live nauplii)) × 100.

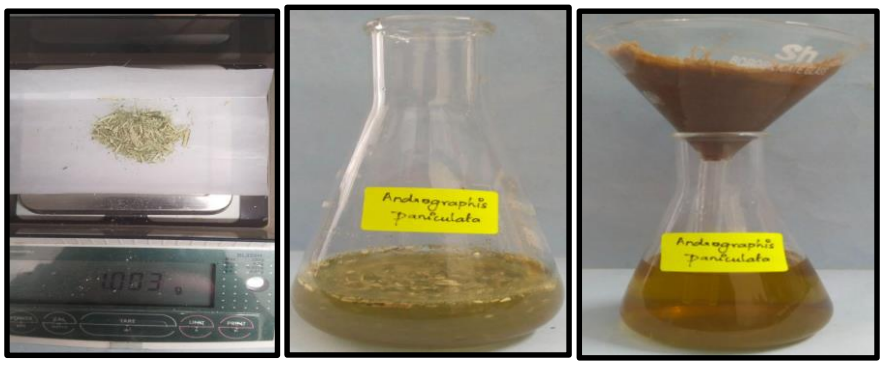


**Figure 1 : 0.003g of dried *Andrographis paniculata***

**Figure 2 : *Andrographis paniculata* + distilled water**

**Figure 3: *Andrographis paniculata* filtrate**

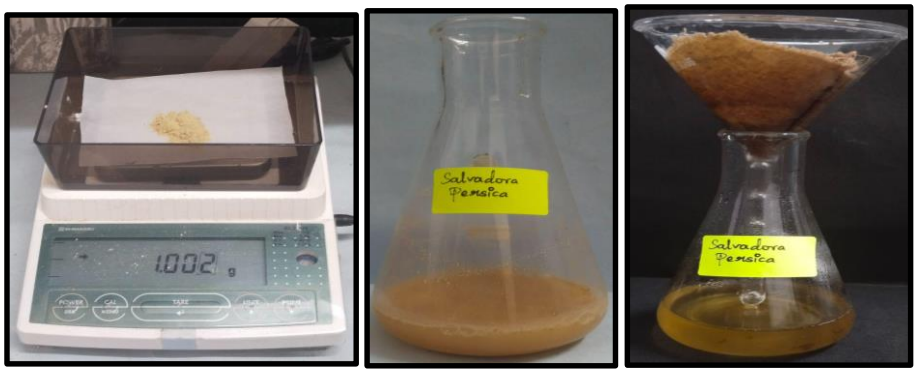


**Figure 4 : 0.002g of dried *Salvadora persica***

**Figure 5 : *Salvadora persica* + distilled water**

**Figure 6 : *Salvadora persica* filtrate**

**Figure 7: Salvadora persica + Andrographis paniculata extract**

## 3. RESULTS

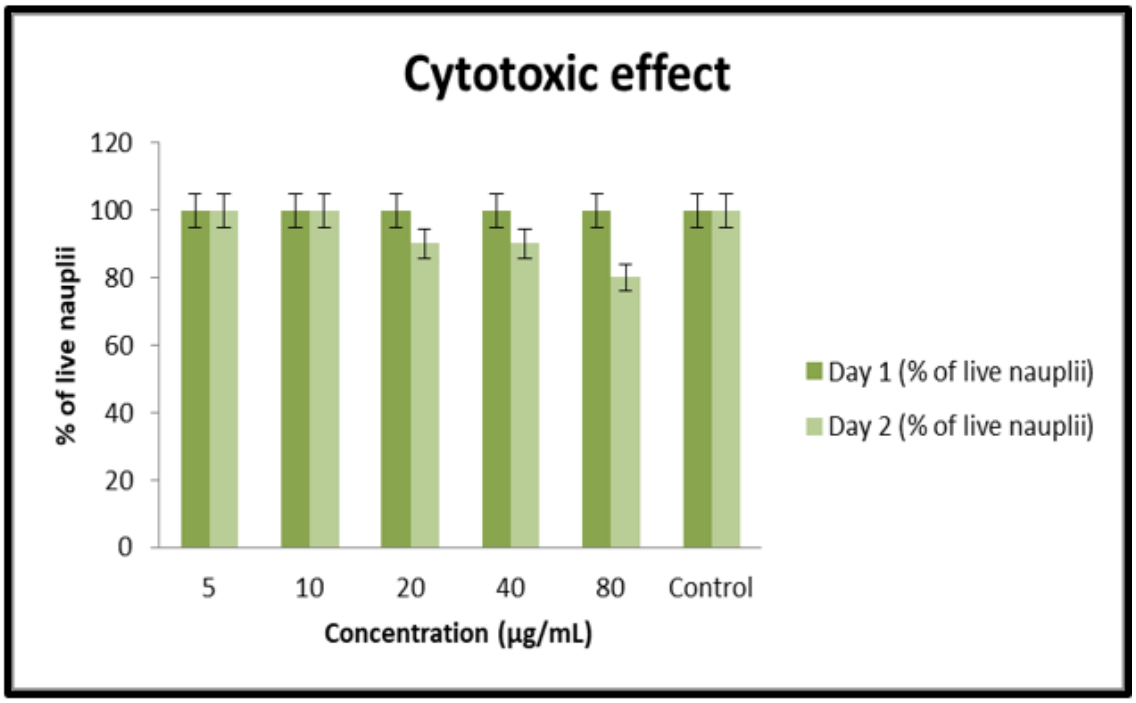


**Figure 8 : cytotoxic effect of *S.persica* + *A.paniculata* during mouthwash formulation**

The X axis represents concentration in **µ**g/ml and y axis represents % of live nauplii. The results showed that the percent of live nauplii decreases with the increase in concentration (Figure 8).

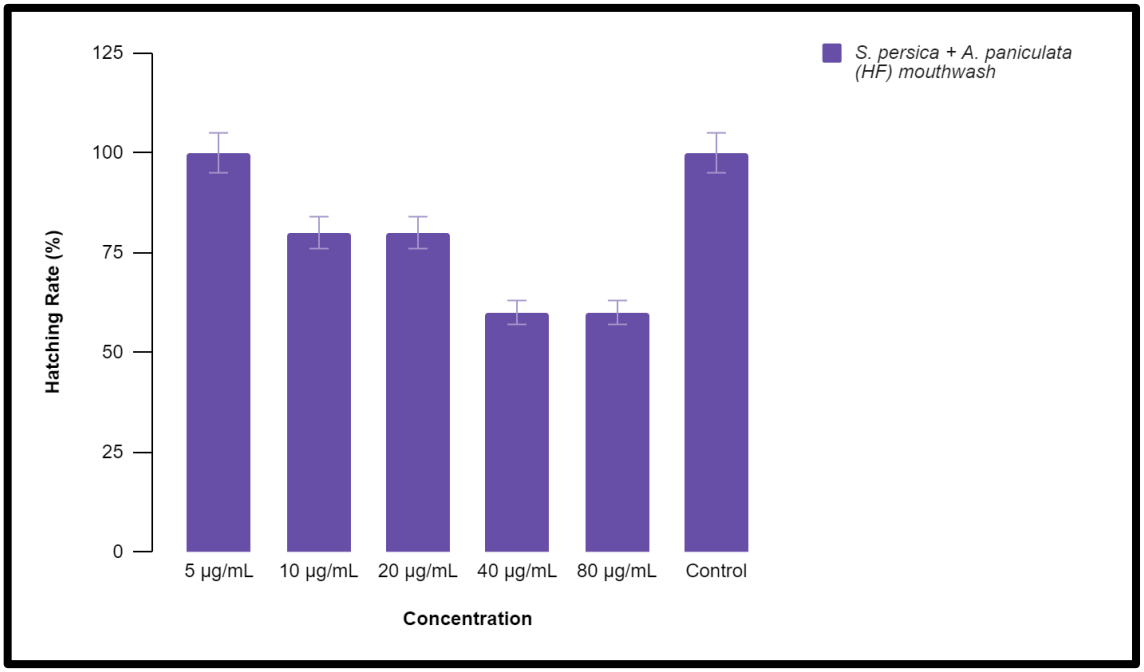


**Figure 9: Hatching rate % of *S.persica* + *A.paniculata* during mouthwash formulation**

X axis represents concentration in **µ**g/ml and y axis represents hatching rate in %

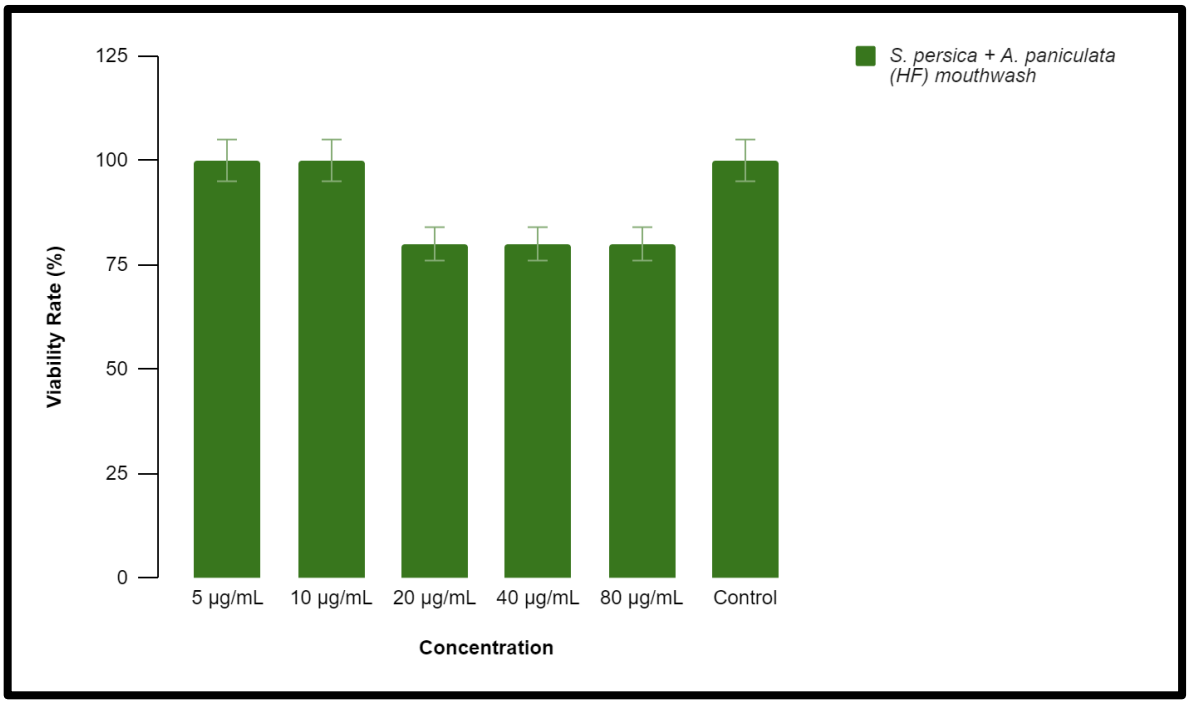


**Figure 10 : Viability rate % of *S.persica* + *A.paniculata* during mouthwash formulation**

X axis represents concentration in **µ**g/ml and y axis represents viability rate in %

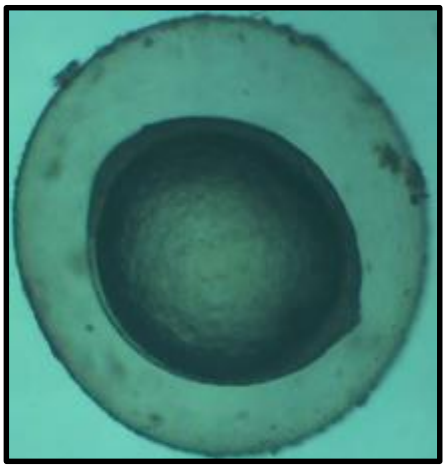

**Figure 11 : DAY 1 of the embryo**

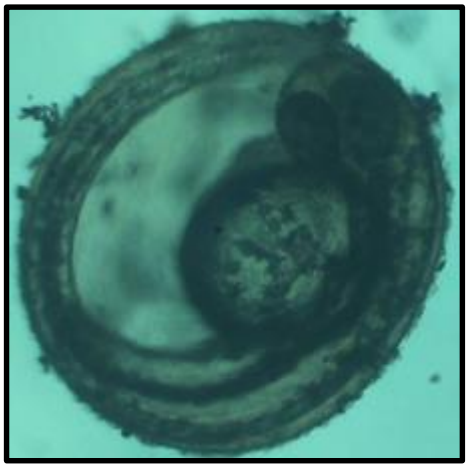

**Figure 12: DAY 2 of the embryo**

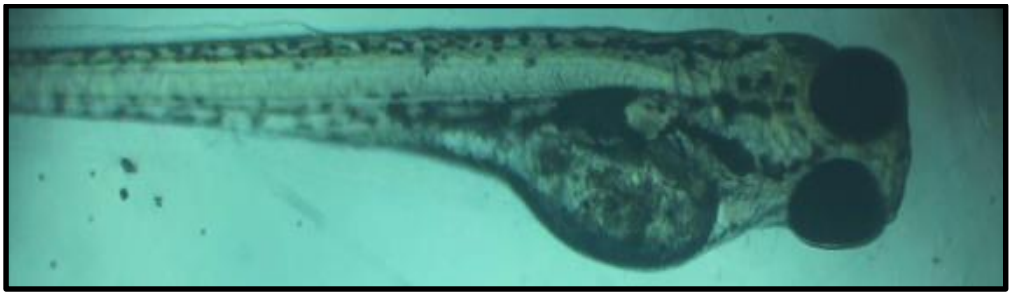

**Figure 13: DAY 3 of the embryo**

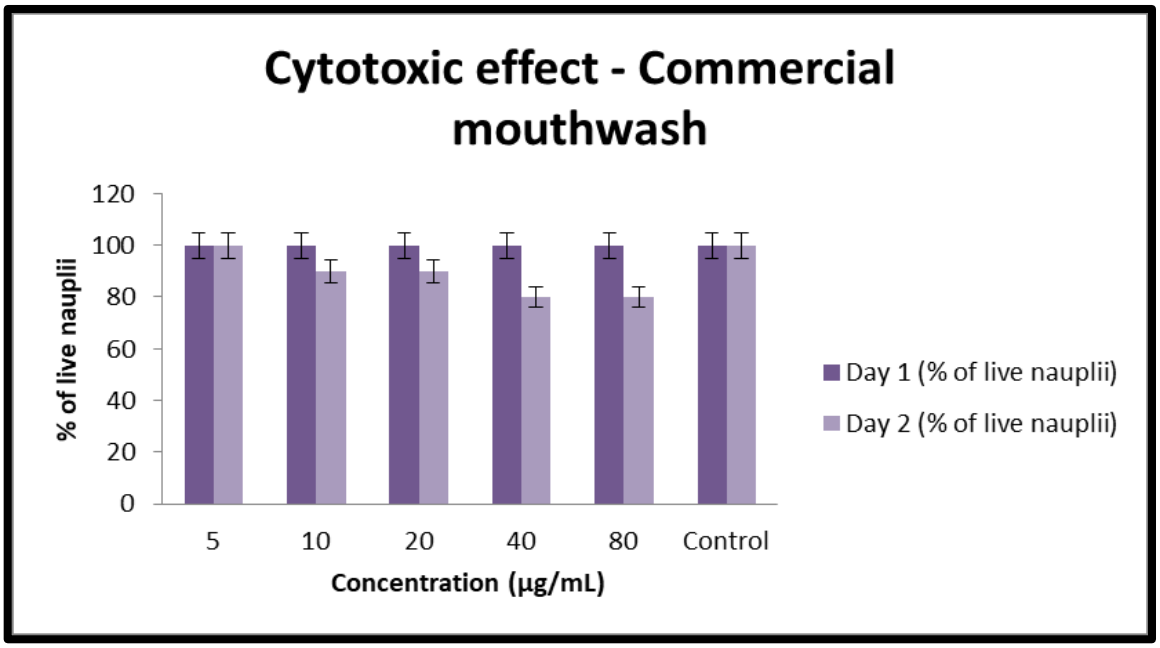


**Figure 14: Cytotoxic effect of commercial mouthwash**

X axis represents concentration in **µ**g/ml and y axis represents % of live nauplii. The results showed that the percent of live nauplii decreases as the concentration of the substance increases(Figure 14).

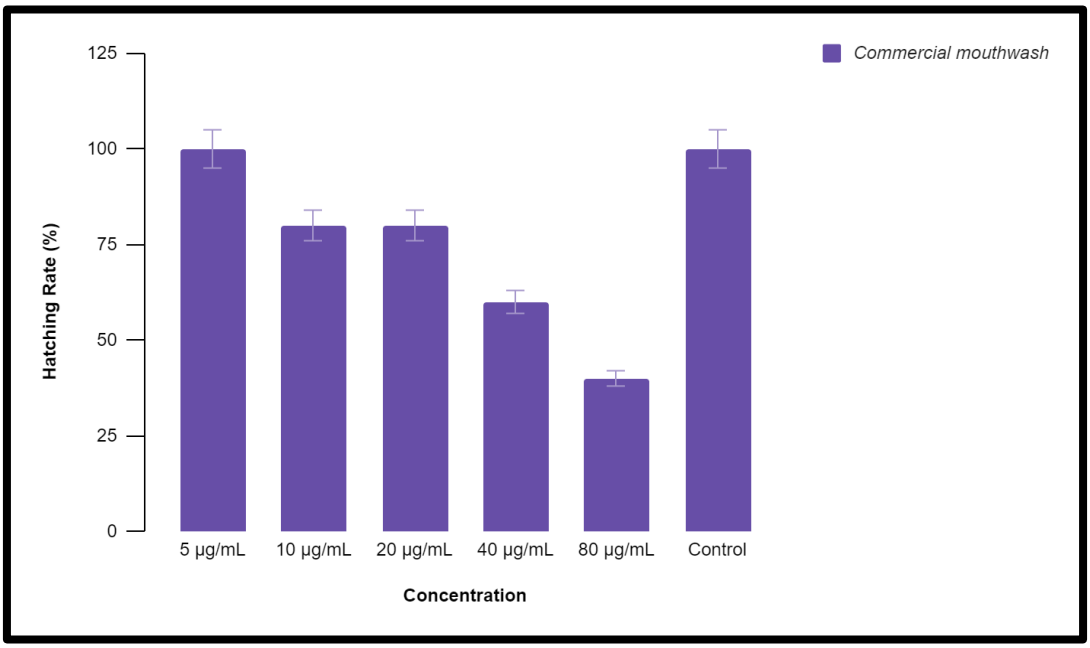


**Figure 15: Hatching rate % of commercial mouthwash**

X axis represents concentration in **µ**g/ml and y axis represents hatching rate in %

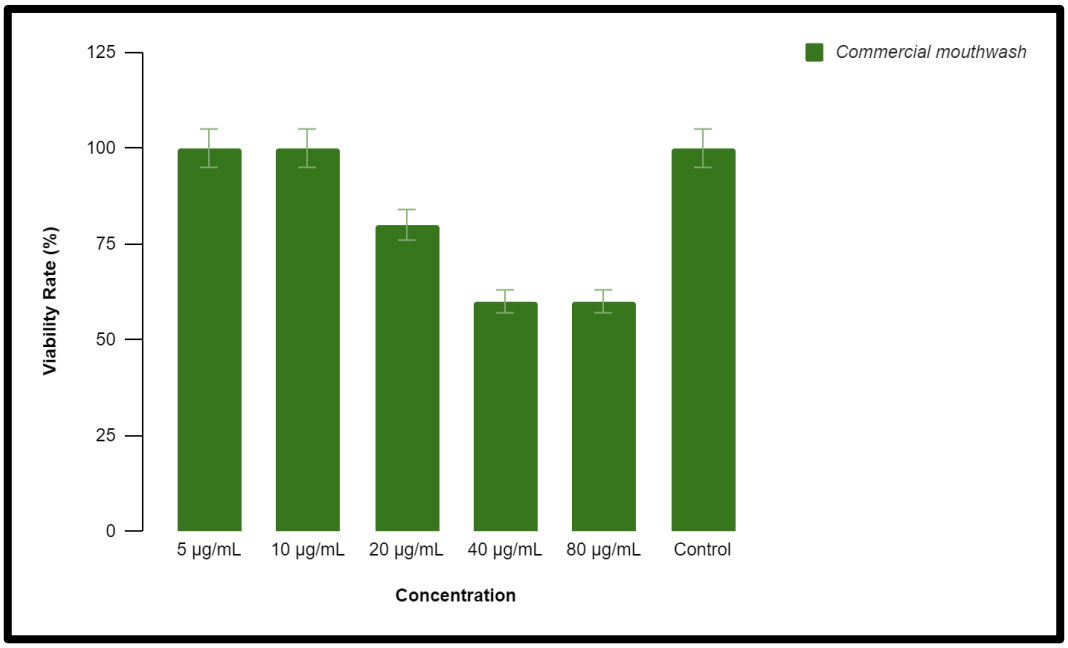


**Figure 16: Viability rate % of commercial mouthwash**

X axis represents concentration in **µ**g/ml and y axis represents viability rate in %

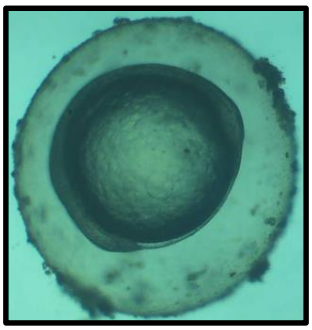

**Figure 17: DAY 1 of the embryo**

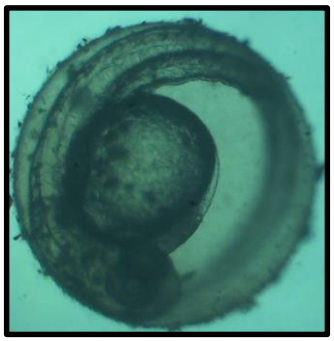

**Figure 18: DAY 2 of the embryo**

**Figure 19: DAY 3 of the embryo**

## 4. DISCUSSION

The comparative study between the *Salvadora persica and Andrographis paniculata* formulated mouthwash and commercially available mouthwash revealed critical insights into their embryonic toxicology profiles(Joseph, Sabarish et al. 2023). The findings underscored that the percentage of live nauplii diminished with increasing concentrations of both mouthwashes. Notably, for the *S. persica* + *A. paniculata* formulation, the decline in live nauplii began at a concentration of 20 µg/mL on the second day. This pattern was consistent in the hatching and viability rates, demonstrating a clear relationship between higher concentrations and lower survival rates of the embryos. On the other hand, the commercial mouthwash exhibited a similar trend, with the percentage of live nauplii starting to decrease at a lower concentration of 10 µg/mL on the second day. This early onset of toxicity at lower concentrations highlights the relatively higher embryotoxicity of the commercial mouthwash compared to the *S. persica* + *A. paniculata* formulation. Both the hatching rate and viability rate of the nauplii were adversely affected by increasing concentrations, further confirming the toxic effects(Rajeshkumar, Lakshmi et al. 2021). These results suggest that the *S. persica* + *A. paniculata* mouthwash is more effective at higher concentrations, as it demonstrated toxicity at a higher threshold compared to the commercial mouthwash.

This finding is significant because it supports the notion that herbal formulations may be less harmful to embryonic development than some commercial alternatives(Khalid, Martin et al. 2024). The lower toxicity levels observed in the herbal mouthwash imply that it could be a safer option, particularly for populations that may be more sensitive to chemical exposures, such as pregnant women. Herbal mouthwashes offer several benefits due to their natural antimicrobial properties, which have been well-documented in traditional medicine. Salvadora persica, commonly known as the toothbrush tree or miswak, has been used for centuries for oral hygiene and is known for its antimicrobial, anti-inflammatory, and analgesic properties. *Andrographis paniculata*, often referred to as the king of bitters, is renowned for its anti-inflammatory and immune-boosting effects(Raj, Martin et al. 2024). The combination of these two herbs in a mouthwash formulation leverages their synergistic effects to enhance oral health while potentially reducing harmful side effects.

The lower embryotoxicity of the herbal mouthwash formulation could be attributed to the natural bioactive compounds present in *S. persica* and *A. paniculata*, which may be more biocompatible and less harmful than synthetic chemicals used in commercial mouthwashes. These natural compounds might interact more gently with biological systems, thereby reducing the likelihood of causing developmental toxicity. Moreover, the growing consumer preference for natural and sustainable products underscores the relevance of developing effective herbal mouthwashes. This shift is driven by increasing awareness of the potential health risks associated with synthetic chemicals and a desire for safer, eco-friendly alternatives. Herbal mouthwashes not only meet these consumer demands but also align with broader public health goals of minimizing exposure to potentially harmful substances(Prathap).

The study's findings highlight the potential of *Salvadora persica and Andrographis paniculata* formulations as safer alternatives to conventional mouthwashes. The lower embryotoxicity observed in the herbal mouthwash suggests that it may be a viable option for reducing the risk of developmental toxicity. Further research and

development are warranted to optimize these formulations for safety and efficacy. Additionally, exploring the broader applications of these and other plant extracts in oral care products could contribute to the development of non-toxic, sustainable healthcare solutions, benefiting both consumers and the environment(Ranjan and Hemmanur 2021).

## 5. CONCLUSION

The study concluded that the *Salvadora persica* and *Andrographis paniculata* formulated mouthwash exhibited lower embryotoxicity compared to commercial mouthwash. This finding suggests that the herbal formulation is a safer alternative, particularly for sensitive populations. The results support the potential of these herbal agents in reducing embryonic toxicological risks, promoting them as viable options for oral care. Further research is recommended to optimize the safety and efficacy of these formulations, paving the way for natural, non-toxic, and sustainable healthcare solutions.

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