Investigation of nonclinical toxicity of cosmetic nail gel in
*Caenorhabditis elegans* model

Investigação de toxicidade não clínica de gel cosmético para unhas em
modelo *Caenorhabditis elegans*

Investigación noclínica de la toxicidad del gel cosmético para uñas en el
modelo *Caenorhabditis elegans*

DOI: 10.55905/oelv22n7-157

Receipt of originals: 06/08/2024
Acceptance for publication: 06/28/2024

*Ivancia Donato de Luna Sousa*
Master in Cellular and Molecular Biology
Institution: Universidade Federal da Paraíba (UFPB)
Address: João Pessoa, Paraíba, Brasil
E-mail: ivanciadr@gmail.com

*Fernanda Silva Galdino*
Master in Pharmacology
Institution: Universidade Federal da Paraíba (UFPB)
Address: João Pessoa, Paraíba, Brasil
E-mail: galdinofernanda02@gmail.com

*Igor Gabriel da Silva Ramalho*
Master in Pharmacology
Institution: Universidade Federal da Paraíba (UFPB)
Address: João Pessoa, Paraíba, Brasil
E-mail: igorgabriel0809@gmail.com

*Caio Fellipe Antunes Vieira*
Graduating in Pharmacy
Institution: Universidade Federal da Paraíba (UFPB)
Address: João Pessoa, Paraíba, Brasil
E-mail: caiofellipe01@gmail.com

*Pedro Vinícius da Silva*
Graduating in Pharmacy
Institution: Universidade Federal da Paraíba (UFPB)
Address: João Pessoa, Paraíba, Brasil
E-mail: pedro.vinicius@academico.ufpb.br
ABSTRACT
The study investigates the non-clinical toxicity of a cosmetic nail gel using the nematode model *Caenorhabditis elegans*. The research was motivated by the need for alternative methods to vertebrate animal testing due to ethical and legal considerations. The main objective was to evaluate the influence of cosmetic gel ingredients on the nematodes' behavioral and viability parameters. The N2 Bristol strain of *C. elegans* was used, exposed to different gel formulations. The methodology included analysis of egg hatching and L4 larval motility, comparing control and treated groups. The results showed no significant difference in egg hatching between groups, indicating that the gel components are not toxic to nematode reproduction. However, a decrease in the motility of L4 larvae exposed to the gel was observed, suggesting possible behavioral interference. The research concludes that while the formulation did not affect reproductive viability, the influence on motility requires further investigation to understand the nature of these behavioral changes. These findings underscore the relevance of using *C. elegans* as an alternative model for cosmetic safety evaluation.

Keywords: Toxicity, *Caenorhabditis elegans*, Cosmetic Gel, Alternative Methods, Non-Clinical Testing.
RESUMO
O estudo investiga a toxicidade não clínica de um gel cosmético para unhas utilizando o modelo de nematoide *Caenorhabditis elegans*. A pesquisa foi motivada pela necessidade de métodos alternativos aos testes em animais vertebrados, devido a questões éticas e legais. O objetivo principal foi avaliar a influência dos ingredientes do gel cosmético sobre parâmetros comportamentais e de viabilidade dos nematoídes. Utilizou-se a linhagem N2 Bristol de *C. elegans*, exposta a diferentes formulações de gel. A metodologia incluiu a análise da eclosão de ovos e da motilidade das larvas L4, comparando grupos controle e tratados. Os resultados mostraram que não houve diferença significativa na eclosão dos ovos entre os grupos, indicando que os componentes do gel não são tóxicos para a reprodução dos nematoídes. Contudo, observou-se uma diminuição na motilidade das larvas L4 expostas ao gel, sugerindo uma possível interferência comportamental. A pesquisa conclui que, enquanto a formulação não afetou a viabilidade reprodutiva, a influência sobre a motilidade requer investigações adicionais para entender a natureza dessas alterações comportamentais. Esses achados reforçam a relevância do uso de *C. elegans* como modelo alternativo para a avaliação de segurança de cosméticos.


RESUMEN
El estudio investiga la toxicidad no clínica de un gel cosmético para uñas utilizando el modelo del nematodo *Caenorhabditis elegans*. La investigación fue motivada por la necesidad de métodos alternativos a las pruebas en animales vertebrados, debido a cuestiones éticas y legales. El objetivo principal fue evaluar la influencia de los ingredientes del gel cosmético sobre parámetros de comportamiento y viabilidad de los nematodos. Se utilizó la cepa N2 Bristol de *C. elegans*, expuesta a diferentes formulaciones de gel. La metodología incluyó el análisis de la eclosión de huevos y la motilidad de las larvas L4, comparando grupos control y tratados. Los resultados mostraron que no hubo diferencia significativa en la eclosión de los huevos entre los grupos, indicando que los componentes del gel no son tóxicos para la reproducción de los nematodos. Sin embargo, se observó una disminución en la motilidad de las larvas L4 expuestas al gel, lo que sugiere una posible interferencia en el comportamiento. La investigación concluye que, aunque la formulación no afectó la viabilidad reproductiva, la influencia sobre la motilidad requiere investigaciones adicionales para entender la naturaleza de estas alteraciones comportamentales. Estos hallazgos refuerzan la relevancia del uso de *C. elegans* como modelo alternativo para la evaluación de la seguridad de cosméticos.

Palabras clave: Toxicidad, *Caenorhabditis elegans*, Gel Cosmético, Métodos Alternativos, Pruebas No Clínicas.
1 INTRODUCTION

The integumentary system, composed of skin, hair, nails, and glands, is the first barrier of protection of the immune system due to its important mechanisms in controlling inflammation and infections. Some chemical compounds present in cosmetic formulations, when used, penetrate the skin and can develop immunotoxic reactions capable of initiating or exacerbating a severe immune response in some individuals (Welsch et al., 2017; Kimber et al., 2011). This process of skin irritation and/or sensitization can lead to an inflammatory reaction known as contact dermatitis (CD), a health problem representing 90 to 95% of occupational skin diseases (Zirwas et al., 2018; Peiser et al., 2012; Kimber et al., 2011).

CDs are classified as irritant (ICD) or allergic (ACD), where the difference between them is that ICD is an unspecific innate immune response and less severe (Usatine et al., 2010), and ACD is a delayed hypersensitivity provoking a potentially more severe adaptive immune response (Aquino and Rosner, 2019; Welsch et al., 2017; Hamann et al., 2017; Bonneville et al., 2007; Levin and Maibach, 2002).

To mitigate or prevent potential CDs, the cosmetic industry has conducted experimental tests on animals for product evaluation for many years. However, after numerous public demonstrations regarding the use of animals in experiments, legislative changes occurred, with Europe pioneering the adoption of new measures in 2003, generating a directive prohibiting animal testing in the evaluation of cosmetic products and their ingredients in the 7th Amendment of the Cosmetics Directive (Adler et al., 2011).

Currently, in Brazil, legislation prohibits the use of vertebrate animals for cosmetic product experiments and tests in many states and cities, where Normative Resolution CONCEA No. 58 of February 24, 2023, mandates the use of alternative methods recognized by the National Council for the Control of Animal Experimentation in scientific research in the development and quality control of personal hygiene products, cosmetics, or perfumes that use ingredients or compounds whose safety or efficacy have
not been scientifically proven (National Council for the Control of Animal Experimentation, 2023).

2 THEORETICAL FRAMEWORK

Alternative toxicity tests are increasingly employed for financial and ethical reasons (Xiong et al., 2017). Tests using nematodes are already a promising way to identify the biological effects of toxic substances in soil and aquatic environments (Hunt, 2017). Nematodes are abundant and highly diverse invertebrates, living in sediments, soils, and surface waters (Hodda et al., 2009; Traunspurger, 2000).

*Caenorhabditis elegans* is a free-living soil nematode that can also be found in aquatic environments (Politz and Philipp, 1992). It feeds on bacteria and occupies different trophic levels as well as different levels of environmental tolerance, and some species can be easily cultured (Monteiro et al., 2014). *C. elegans* has been widely used in ecotoxicological and nanotoxological studies due to its ease of culture, short reproduction time, body transparency, and known physiology, development, and genetics (Hoss et al., 2012).

In *C. elegans*, it is known that its cuticle has cells against invading pathogens or physical damage, but it is still unclear how the immune system and the entire epidermis recognize signals produced by physical injuries (Zhang et al., 2015). Mechanical injury to the worm's cuticle or the epidermis of a human can lead to an innate immune response, resulting in high production of antimicrobial peptides (AMPs) by epidermal cells (Patterson et al., 2013). Thus, the cuticle of *C. elegans* offers an excellent model of basic epithelial defense strategies, making it an excellent model for cutaneous toxicity.

Considering the safety issues involved, the complexity of classifying a chemical agent as toxic, the inability to perform tests with vertebrates, and the suggestion of alternative methodologies to evaluate the toxicity of a cosmetic product, we propose to use *C. elegans* in the detection of toxicity of a cosmetic gel formulation for nail extension. We proposed an approach using the wild-type strain N2 Bristol to verify if there is an alteration in measurable behavioral parameters of the worm *C. elegans* related to egg
hatching, survival rate, cutaneous injury after exposure, motility, and survival in the presence of the formulation compared to the control group.

3 OBJECTIVES

3.1 GENERAL

Evaluate the experimental model of the nematode *C. elegans* in detecting the toxicity of ingredients in the formulation of a cosmetic gel for nail extension.

3.2 SPECIFIC

Evaluate whether exposure of *C. elegans* to sensitizing or irritating cosmetic ingredients alters the worm's behavior by analyzing life expectancy, egg hatching and viability, body curvature, and motility.

4 METHODOLOGY

The commercial gel formulation analyzed was donated by Karla Beauty Cosméticos LTDA, and the test formulation was made by PhD student Ivancia Donato and provided for analysis.

The methodology used to evaluate toxicity was described by SANT'ANNA et al. (2013) and adapted for evaluating the toxicity of a cosmetic nail extension gel. The isolated strains of *Caenorhabditis elegans* wild-type N2 Bristol were acquired from the Caenorhabditis Genetics Center (CGC) and maintained under ideal storage and handling conditions described by Brenner (1974).
4.1 CULTURE AND TREATMENT CONDITIONS OF *C. elegans*

The strains of *C. elegans* used during the experiments were previously acquired through the Caenorhabditis Genetics Center (CGC) and stored on petri dishes filled with NGM (Nematode Growth Medium) containing the following composition: 15g of NaCl, 175g of peptone, 85g of bacteriological agar, 500μL of CaCl2 solutions (1M), MgSO4 (1M), cholesterol (5mg/mL), and streptomycin (100mg/mL). They were seeded with the OP50 strain of E. coli under controlled temperature of 20°C (Brenner 1974).

Forty larvae (*C. elegans*) were placed on each plate after the prior synchronization of the animals' age, which was obtained by treating pregnant adult hermaphrodites with lysis solution (50% sodium hypochlorite; 25 mM sodium hydroxide). After insertion into the treatment and control plates, the animals were exposed to the commercial gel (insert the name of the gels) from the L1 stage to adulthood (Sant’anna et al., 2013).

4.2 EGG HATCHING AND LOCOMOTION ASSAY

In the egg hatching and locomotion assay, pregnant adult larvae were subjected to a cuticular lysis process to obtain eggs and washed with M9 solution (3g of KH2PO4, 6g of Na2HPO4, 5g of NaCl, and 1M MgSO4). Thus, about 40 eggs immersed in M9 solution were placed on each Nematode Growth Medium (NGM) plate. Each plate received 500 microliters of E. coli OP50 seeded in Luria Bertani (LB) medium before the eggs were added.

The gels were uniformly deposited on the surface of the Petri dishes to receive the eggs obtained in the lysis procedure. Forty eggs were inserted into each plate containing the gel. After 24 hours of incubation at 20°C, unhatched eggs and L4 stage larvae were counted to calculate the hatching average. Once hatched, L4 stage larvae were individually placed on Petri dishes with NGM medium without OP50, and the number of curvatures was counted by an observer for 20 seconds using a Motic SMZ-168-TL stereoscopic magnifier (Sant’anna et al., 2013).
4.3 STATISTICAL ANALYSIS

The data were expressed as mean ± standard error of the mean (SEM) and analyzed by One-way Analysis of Variance (ANOVA) followed by Student’s T-test post-hoc test. Data were considered significant when p < 0.05. The statistical program used for data analysis and graph production was GraphPad Prism version 6.0 (GraphPad USA).

5 RESULTS AND DISCUSSIONS

5.1 EFFECT OF COSMETIC NAIL GEL ON L1 LARVAE HATCHING OF Caenorhabditis elegans

Graph 1 shows the hatching percentage of larvae in different experimental groups. It is possible to observe that there were no statistically significant changes between the control group, where no gel formulation was added, and the groups in contact with gel formulation 1 and gel formulation 2, demonstrating no interference in egg hatching with 100% hatched eggs.

Graph 1 – Hatching percentage of L1 Caenorhabditis elegans not treated and treated with control, commercial, and test gel formulations. Result of 24-hour incubation. Values are mean ± standard error of the mean (SEM) of three experiments in triplicate. Different letters indicate statistically significant differences (P < 0.05)
The absence of changes in the egg viability rate of *C. elegans* in contact with the formulations compared to the control group demonstrates that the components present in the Commercial and Test gels do not act as aggressive or toxic agents to egg reproduction, which according to previous studies, have high sensitivity to toxic agents present in the hatching environment (Kilen, 2016).

5.2 EFFECT OF COSMETIC NAIL GEL ON L4 LARVAE MOVEMENT OF *Caenorhabditis elegans*

Graph 2 represents the results obtained in the evaluation assay of L4 larvae curvatures after 48 hours of contact with the two tested gels. In this experiment, it was possible to observe a significant difference in the decrease in curvatures performed by the worms in the groups in contact with the gel compared to the control group.

The curvature quantification assay performed by L4 larvae is used to identify physiological alterations caused by the tested substances that may generate changes in worm locomotion and/or reactivity; however, due to the high viscosity characteristics present in the gel formulations tested in this experiment, it is not possible to determine the nature of this behavioral alteration.

Graph 2 – Curvature percentage of L4 *Caenorhabditis elegans* not treated and treated with control, commercial, and test gel formulations. Result of 48-hour incubation. Values are mean ± standard error of the mean (SEM) of three experiments in triplicate. Different letters indicate statistically significant differences (P < 0.05)

Source: Prepared by the authors
Toxicity tests for cosmetics using the *C. elegans* evaluation model are not yet consolidated in the literature; however, some studies have demonstrated the feasibility of using this technique to investigate the toxic effects of substances of cosmetic interest. For example, the study conducted by De la Parra-Guerra et al. (2020) in evaluating the cosmetic ingredient Nonylphenol (NF9), where viability analysis methods on egg hatching, survival rate, and behavioral analysis as well as physiological and antioxidant markers were executed.

Another study developed by Richaud et al. (2024) investigated the water quality used in cosmetic formulation preparation by evaluating the effects generated by these on the life span of *C. elegans*. In this study, researchers identified that different types of water used in cosmetic preparations had different effects on the larvae's life cycle, which can be used as an indicator of the quality of substances employed in cosmetic formulation.

Additionally, another study by Wang et al. (2020) also used the *C. elegans* model to observe the effects generated by Paeonia suffruticosa Andrews extract, a species widely used in cosmetic formulations to investigate its antioxidant and anti-aging effects generated in *C. elegans* strains and its property in increasing or decreasing larvae life span.

6 CONCLUSION

Based on the presented results, we can conclude that the tested gel formulations did not exert significant adverse effects on *Caenorhabditis elegans* egg hatching, indicating their potential safety for cosmetic use. The absence of statistical differences between the experimental and control groups suggests that the gel components did not interfere with egg viability.

However, regarding the locomotion of L4 larvae, a significant decrease in the curvature of worms exposed to the gels was observed, suggesting a possible behavioral influence of the formulations. Although the nature of this behavioral alteration was not determined due to the high viscosity of the gels, previous studies highlighted the usefulness of the *C. elegans* model in evaluating the effects of cosmetic substances.
Therefore, these results reinforce the importance of assessing the safety and behavioral effects of cosmetic ingredients using models such as *C. elegans*, contributing to the development of safer and more effective products.
REFERENCES


