Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 2265-2270 | ISSN:2251-6727

Cytotoxic Effects of Juglans Regia L. and Coffea Canephora on Human Gingival Fibroblasts

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(Received: 16 June 2025 Revised: 20 July 2025 Accepted: 07 August 2025)

KEYWORDS

Juglans regia L., Coffea canephora, cytotoxicity, human gingival fibroblasts, MTT assay, biocompatibility.

ABSTRACT:

BACKGROUND:

Juglans regia L. (English walnut) and Coffea canephora (robusta coffee) are widely recognized for their rich composition of bioactive compounds, including polyphenols, flavonoids, tannins, and alkaloids. These natural compounds exhibit various pharmacological properties, such as antioxidant, anti-inflammatory, and antimicrobial activities, which make them valuable candidates for therapeutic applications. Human gingival fibroblasts (HGF), crucial in maintaining gingival tissue integrity and oral health, are a focus of interest in evaluating the cytotoxicity and biocompatibility of these natural products. This study aimed to investigate the cytotoxic effects of walnut powder and coffee extracts on HGF cells, providing insights into their potential applications in dental formulations.

MATERIALS AND METHODS:

The cytotoxic effects of Juglans regia L. and Coffea canephora were assessed using the MTT assay. Human gingival fibroblasts were cultured in 96-well plates and treated with varying concentrations (0 µg/ml to 500 µg/ml) of the extracts for 24 hours. The MTT dye (10 mg/ml) was added to each well, and the plates were incubated at 37°C for 4 hours. After incubation, the medium was replaced with DMSO to dissolve the formazan crystals, and absorbance was measured at 570 nm using a Synergy Hybrid Multi-Mode Reader. Cell viability was calculated and analyzed using one-way ANOVA with Tukey's multiple comparisons. Morphological changes were assessed using phase-contrast microscopy at 20X magnification.

RESULTS:

The study revealed a concentration-dependent cytotoxic effect of the extracts on HGF cells. At lower concentrations (0–50 µg/ml), cell viability remained high, indicating minimal cytotoxicity. However, at higher concentrations (200 µg/ml and above), a significant reduction in cell viability was observed ($p < 0.001$). Morphological assessments showed clear cellular alterations, including changes in shape and density, particularly at higher extract concentrations. Control samples maintained normal morphology and viability, providing a baseline for comparison.

CONCLUSION:

This study demonstrated that Juglans regia L. and Coffea canephora exhibit concentration-dependent cytotoxic effects on human gingival fibroblasts. While lower concentrations were well-tolerated, higher concentrations significantly impacted cell viability and morphology. These findings underscore the need to optimize extract concentrations for safe and effective use in dental applications. Further research is recommended to explore the mechanisms underlying these effects and evaluate their potential therapeutic benefits in oral health.

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INTRODUCTION:

Juglans regia L., commonly known as English walnut, and Coffea canephora, commonly known as robusta coffee, have long been valued for their rich reservoir of bioactive compounds, which include polyphenols, flavonoids, tannins, and alkaloids. These compounds are associated with a range of pharmacological activities such as antioxidant, anti-inflammatory, and antimicrobial properties [1,2]. The potential of these natural substances to influence cellular mechanisms has made them a subject of interest in biomedical research. In particular, their cytotoxic effects on human gingival fibroblasts (HGF), a crucial component of the gingival connective tissue, hold significant implications for their application in dental formulations and therapies [3].

The gingival connective tissue is vital for maintaining oral health, supporting wound healing, and modulating inflammation. Gingival fibroblasts, as the primary cell type in this tissue, play an essential role in tissue repair and regeneration [4]. However, any therapeutic agents applied to this region must be carefully evaluated for their cytotoxic effects to ensure safety and efficacy. Previous studies on plant-derived compounds, including those from Juglans regia L. and Coffea canephora, have demonstrated their potential as therapeutic agents in dentistry due to their multifaceted properties [5,6]. For example, the antioxidant activity of walnuts has been associated with their ability to scavenge free radicals and reduce oxidative stress, while coffee extracts have shown antimicrobial properties that could be valuable in controlling oral infections [7,8].

Recent advances in biocompatibility assays, particularly those involving the MTT assay, have enabled researchers to quantify the cytotoxic effects of natural compounds on cellular viability [9]. The MTT assay measures the metabolic activity of cells by converting tetrazolium salt to formazan crystals, which are quantifiable by optical density. This assay has been widely used to evaluate the cytocompatibility of dental materials, including plant-derived substances [10,11]. In the context of Juglans regia L. and Coffea canephora, assessing their effects on HGF cells is pivotal to understanding their safety profile and potential therapeutic applications in oral health care.

Studies have reported the therapeutic properties of Juglans regia L., including its anti- inflammatory and antimicrobial activities, which could support the management of oral diseases such as periodontitis and gingivitis [12]. Similarly, Coffea canephora has shown promise in mitigating microbial growth and providing antioxidant protection, which could benefit oral tissues exposed to oxidative stress and bacterial challenges [13]. Despite their potential, the cytotoxic effects of these compounds, particularly at varying concentrations, remain underexplored.

This study aims to fill this gap by systematically evaluating the concentration- dependent cytotoxic effects of Juglans regia L. and Coffea canephora extracts on HGF cells using the MTT assay. By investigating the viability and morphological changes of gingival fibroblasts, this research seeks to provide insights into the safe and effective use of these natural compounds in dental applications. Furthermore, the study contributes to the broader understanding of plant-derived substances, emphasizing the importance of balancing therapeutic benefits with safety considerations [14,15].

In summary, this research endeavors to elucidate the biocompatibility of Juglans regia
L. and Coffea canephora extracts, offering a foundation for their potential integration into dental care formulations while addressing critical gaps in current knowledge regarding their cytotoxic effects [16,17].

MATERIALS AND METHODS:

This study aimed to evaluate the cytotoxic effects of Juglans regia L. and Coffea canephora extracts on human gingival fibroblast (HGF) cells using well-established laboratory techniques. The biocompatibility of these natural compounds was assessed using the MTT assay, which quantifies cellular viability by measuring metabolic activity. Morphological changes in the HGF cells were also observed to understand the cellular response to varying concentrations of the extracts. The experimental procedures adhered to standardized protocols to ensure reliability and reproducibility.

PREPARATION OF EXTRACT:

Juglans regia L. (walnut powder) and Coffea canephora extracts were prepared following a standardized extraction process. The walnut powder was finely ground, and an aqueous extract was prepared by dissolving a measured quantity of the powder in distilled water. Similarly, robusta coffee beans were ground, and their aqueous extract was prepared. Both extracts were filtered to remove particulate matter and stored at 4°C until further use. These preparations were diluted to

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(4), 2265-2270 | ISSN:2251-6727

achieve concentrations ranging from 0 µg/ml to 500 µg/ml for subsequent cytotoxicity testing.

CYTOTOXIC ACTIVITY:

The cytotoxic effects of the extracts were determined using the MTT assay. Human gingival fibroblast cells were seeded into 96-well plates and allowed to adhere for 24 hours. The cells were treated with different concentrations of the extracts (0 µg/ml to 500 µg/ml) and incubated at 37°C for 24 hours. Following incubation, 10 µl of MTT dye (10 mg/ml) was added to each well, and the plates were incubated for an additional 4 hours. After removing the medium, 100 µl of DMSO was added to dissolve the formazan crystals formed by metabolically active cells. The optical density (OD) was measured at 570 nm using a Synergy Hybrid Multi-Mode Reader. Cell viability was calculated using the formula:

$$\text{Cell Viability (\%)} = \frac{\text{OD (test sample)} - \text{OD (blank)}}{\text{OD (PC)} - \text{OD (blank)}} \times 100$$

Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons to determine the significance of differences between groups. Morphological changes were assessed under a phase-contrast microscope at 20X magnification to evaluate cellular responses to the extracts.

RESULTS:

The cytotoxic effects of Juglans regia L. and Coffea canephora extracts on human gingival fibroblast (HGF) cells were evaluated using the MTT assay. The data revealed a clear concentration-dependent effect, with cell viability decreasing progressively as the concentration of the extracts increased. At lower concentrations (0–50 µg/ml), the cells exhibited high viability, maintaining a mean of approximately 94–97%. However, at concentrations exceeding 200 µg/ml, cell viability declined significantly, dropping to approximately 81% at the highest concentration of 500 µg/ml.

The raw data provided a detailed breakdown of cell viability percentages for Groups 1 (Juglans regia L.), 2 (Coffea canephora), and 3 (combined extracts). At 100 µg/ml, the mean cell viability across groups remained around 91%, but a more pronounced reduction was observed as the concentration increased to 200 µg/ml and beyond. Statistical analysis using one-way ANOVA with Tukey's multiple comparisons indicated significant differences between treated groups and controls at concentrations above 200 µg/ml ($p < 0.001$).

Morphological assessment using phase-contrast microscopy provided further insights. Cells treated with concentrations below 100 µg/ml exhibited normal morphology, appearing uniformly attached and well-spread. However, cells treated with higher concentrations, particularly 400 µg/ml and 500 µg/ml, showed signs of cytotoxic stress, including cellular shrinkage, loss of adhesion, and altered morphology. The control group consistently displayed healthy, intact cells throughout the study.

The graphical representation of the results clearly depicts the concentration-dependent reduction in cell viability. The bar graph illustrates a steady decline across all three groups, with minimal variability as indicated by the error bars. The trend confirms that higher concentrations of the extracts adversely affect cell viability, aligning with the observed morphological changes.

These findings highlight the significant impact of extract concentration on HGF cell viability, providing critical data for further evaluation of Juglans regia L. and Coffea canephora in biological applications. The results emphasize the need for careful concentration optimization in therapeutic contexts.

CONCLUSION:

This study demonstrated that Juglans regia L. and Coffea canephora extracts exhibit concentration-dependent cytotoxic effects on human gingival fibroblast cells. While lower concentrations were well-tolerated, higher concentrations significantly reduced cell viability and induced morphological changes. These findings highlight the potential of these natural products for therapeutic applications in dentistry, provided that their concentrations are carefully optimized to ensure safety. Future research should focus on elucidating the molecular mechanisms underlying these effects and exploring the therapeutic potential of these extracts in oral health. This study lays the foundation for the development of biocompatible dental formulations derived from Juglans regia L. and Coffea canephora.

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JCHR (2025) 15(4), 2265-2270 | ISSN:2251-6727

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JCHR (2025) 15(4), 2265-2270 | ISSN:2251-6727

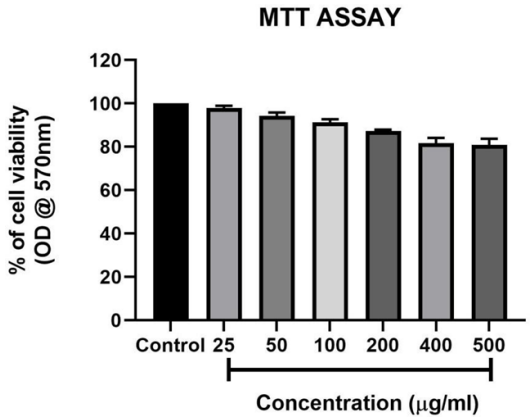

Figure 1 : MTT Assay Results

Walnut + Coffee bean powder

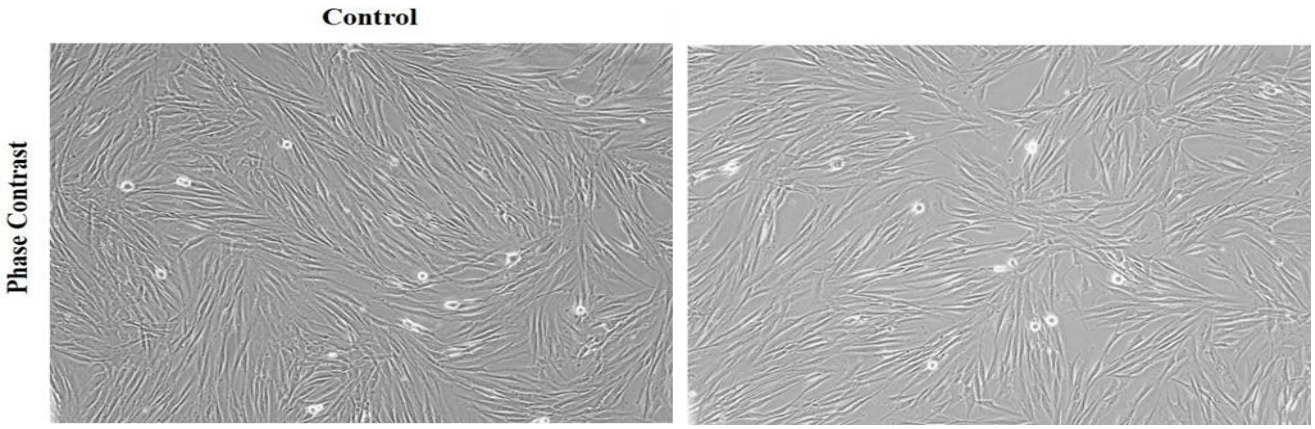

Figure. 2: The biocompatibility of Walnut + Coffee bean powder along with control group treated on human gingival fibroblast cells was evaluated by morphological assessment using Phase, Magnification 20X.

	0	25	50	100	200	400	500
Group 1	0.842	0.813	0.779	0.754	0.729	0.711	0.709
Group 2	0.901	0.889	0.856	0.828	0.791	0.728	0.715
Group 3	0.894	0.878	0.853	0.825	0.783	0.713	0.709
%	100	96.55582	92.51781	89.54869	86.57957	84.44181	84.20427553
	100	98.66815	95.00555	91.89789	87.79134	80.79911	79.35627081
	100	98.21029	95.41387	92.28188	87.58389	79.75391	79.3064877
Mean	100	97.81142	94.31241	91.24282	87.31827	81.66494	80.95567801
SE	0	0.907306	1.279874	1.208143	0.529159	2.009366	2.297195243
p-Value		0.032912	0.009028	0.002821	3.14E-05	0.004537	0.005340041

Table 1: MTT Assay Results of Walnut + Coffee Bean Powder on HGF Cells

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JCHR (2025) 15(4), 2265-2270 | ISSN:2251-6727

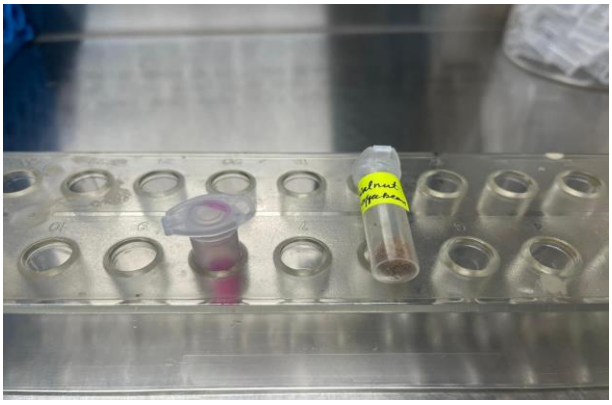

Figure 3: Experimental Analysis and Morphological Assessment of Cytotoxicity in Human Gingival Fibroblasts Treated with Juglans regia L. and Coffea canephora Extracts

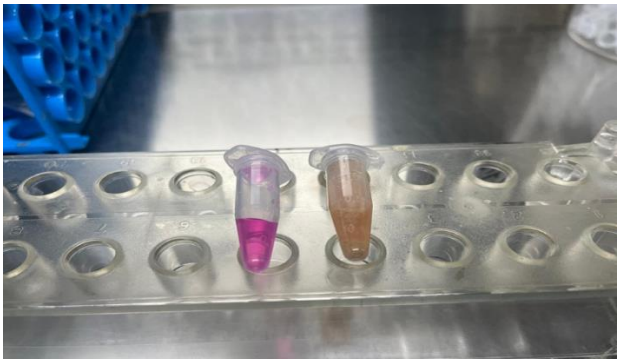

Figure 4: Experimental Analysis and Morphological Assessment of Cytotoxicity in Human Gingival Fibroblasts Treated with Juglans regia L. and Coffea canephora Extracts

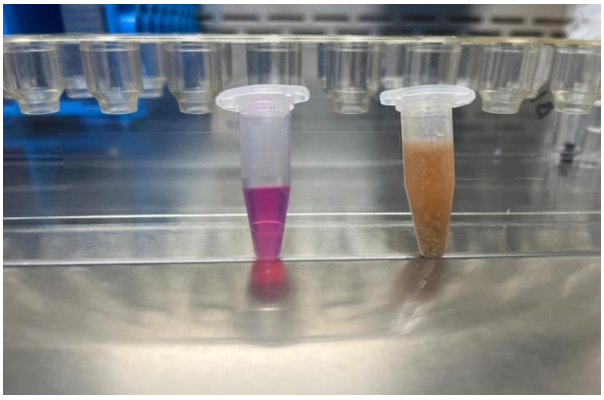

Figure 5: Experimental Analysis and Morphological Assessment of Cytotoxicity in Human Gingival Fibroblasts Treated with Juglans regia L. and Coffea canephora Extracts

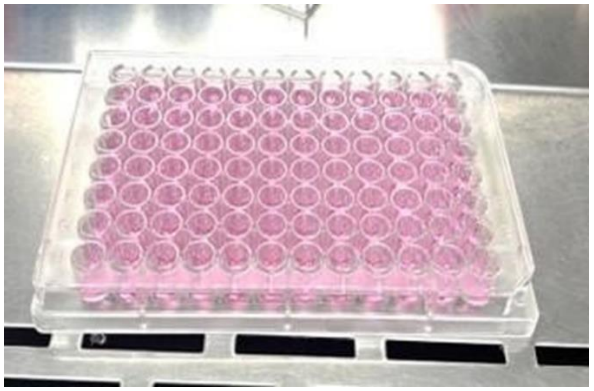

Figure 6: Experimental Analysis and Morphological Assessment of Cytotoxicity in Human Gingival Fibroblasts Treated with Juglans regia L. and Coffea canephora Extracts

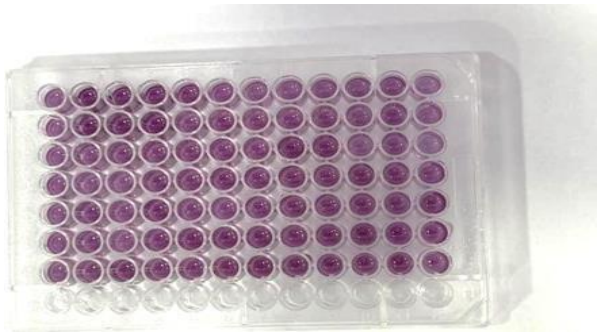

Figure 7: Experimental Analysis and Morphological Assessment of Cytotoxicity in Human Gingival Fibroblasts Treated with Juglans regia L. and Coffea canephora Extracts