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(RESEARCH ARTICLE)

Analysis of cytotoxic and antioxidant activities of robusta coffee bean extract-based toothpaste in vitro

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Abstract

The phenolic compounds in Robusta Coffee Bean Extract (RCBE) have strong antioxidant properties that provide a promising profile as natural ingredients in oral care formulation. However, there is limited evidence on the biological safety and functional performance of RCBE in toothpaste formulations. Therefore, this study investigates the cytotoxicity and antioxidant activity of toothpaste containing RCBE in vitro by using fibroblast cell cultures and DPPH radical-scavenging assays, respectively. Herein, both formulations showed concentration-dependent antioxidant activities, with significantly higher levels of free-radical inhibition obtained at higher RCBE levels. The cytotoxicity assay using MTT demonstrated that low to moderate extract concentrations maintained the viability of the fibroblasts above 70%, thereby indicating an acceptable biocompatibility, although higher concentrations reduced viabilities that were within tolerable biological limits. These results suggest that the balance between antioxidant potency and cellular safety is favorable for RCBE-based toothpaste; therefore, it may be used as a functional natural adjunct in oral health products.

Keywords: Robusta coffee bean extract; Cytotoxicity; Antioxidant activity; Toothpaste formulation; DPPH assay

1. Introduction

Periodontal disease and dental caries are still some of the most prevalent oral health issues around the world and affect all age groups of individuals, despite developments in preventive dentistry [1,2]. Control of dental plaque is widely recognized as the main strategy to prevent these diseases, and the most efficient mechanical means of removing plaque remains brushing [2,3,4]. Toothpaste forms an important adjunct to daily oral hygiene, and its formulation plays a key role in enhancing plaque control and oral tissue health [5,6,7].

Increased consumer interest in natural-based products has prompted research into the incorporation of herbal extracts into oral care products, toothpaste being one of them. In this context, Robusta coffee bean extract has been attracting attention as it is a rich source of bioactive components including phenolics, flavonoids, and alkaloids, which showed considerable antioxidant and antimicrobial activity. These bioactive compounds would have an ability to scavenge the free radicals that are responsible for tissue inflammation and cellular damage [8,9,10]. Since several oral pathologies, such as gingivitis and periodontitis, arise due to the accumulation of free radicals in the body, a toothpaste containing antioxidant-rich natural ingredients may exert therapeutic benefits beyond plaque control.

The ingredients of toothpaste should possess a proper safety profile, as this material comes in direct contact with oral tissues, especially gingival fibroblasts. Thus, formulations should present a nontoxic nature at effective concentrations so as not to provoke an adverse biological response [11,12,13]. Robusta coffee extract has shown promising biological activity, although the cytotoxicity evaluation along with its antioxidant capacity has been provided by limited evidence, especially after its formulation into toothpaste.

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This present study is important in providing empirical data on biocompatibility and antioxidant potency of RCBE-based toothpaste at different concentrations. The current research work is novel since it is a dual-assessment type of study: cytotoxic and antioxidant activities of RCBE-containing toothpaste were simultaneously assessed using standardized in vitro assays. This indeed provides basic scientific evidence for the safe development of natural-based oral care products enriched with bioactive from coffee beans.

2. Material and methods

This is an experimental study with a laboratory-based post-test only control group design. The study has received ethical clearance from the Health Research Ethics Commission, Faculty of Dentistry, University of Jember, No. 1999/UN25.8/KEPK/DL/2023. The experimental groups used in the study included RCBE-based toothpaste at concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5625%, 0.78125%, and a placebo control. Antioxidant testing was conducted four times for each group, while cytotoxicity testing was performed three times. Gingival fibroblast cultures were derived from the Molecular Medicine Laboratory (CDAST) - University of Jember.

2.1. Preparation of Robusta Coffee Bean Extract

Robusta coffee beans derived from Jember, Indonesia, were subjected to the maceration method at the Faculty of Pharmacy, University of Jember. The coffee beans weighing 500 g were crushed, soaked in a solvent of ethyl, stirred, and left standing for 24 hours. Filtrates were collected and the residues again treated in the same manner under maceration. Combined filtrates were dried by exposure to air until about 50 g of concentrated coffee bean extract was obtained.

2.2. Preparation of RCBE-Based Toothpaste

Toothpaste formulations were made with concentrations of RCBE from 0.78125% to 50%, combined with a placebo base containing magnesium carbonate, calcium carbonate, glycerin, propylene glycol, triethanolamine (TEA), distilled water, and mint oil.

2.3. Cytotoxicity Test (MTT Assay)

Cytotoxicity was evaluated by MTT test. Eluates were prepared by incubation of the toothpaste samples in serum-free medium for 24 hours at 37°C. Gingival fibroblast cells (20×10^3 cells/well) were exposed to eluates for 24 hours; 100 µl of MTT solution was added at a concentration of 5 mg/ml. After incubation, the formazan crystals were dissolved by using DMSO, and the absorbance was measured at 595 nm. The viability of cells was expressed as a percentage of the control.

2.4. Antioxidant Assay (DPPH Method)

Antioxidant activity of RCBE-based toothpaste was measured by the DPPH radical scavenging assay at 517 nm. The % inhibition and IC50 value were calculated. Total phenolic content was determined using the Folin–Ciocalteu method and the results were expressed as mg gallic acid equivalent per gram of sample.

3. Results and discussion

3.1. Antioxidant Activity

Antioxidant levels of RCBE toothpaste concentrations of 50%; 25%; 12.5%: 6.25%; 3.125%; 1.5625%; 0.78125%; and placebo toothpaste control are shown in Figure 1. One-way ANOVA showed there were significant variations among the treatment groups at $p<0.05$. The LSD post hoc test was performed and showed most of the concentrations differ significantly from one another, with the exception of 1.5625%, 0.78125%, and placebo. Linear regression showed a positive significant relationship between RCBE concentration and antioxidant level at $p = 0.004$, which agreed with the idea that the higher the concentration of extract, the higher the antioxidant potency.

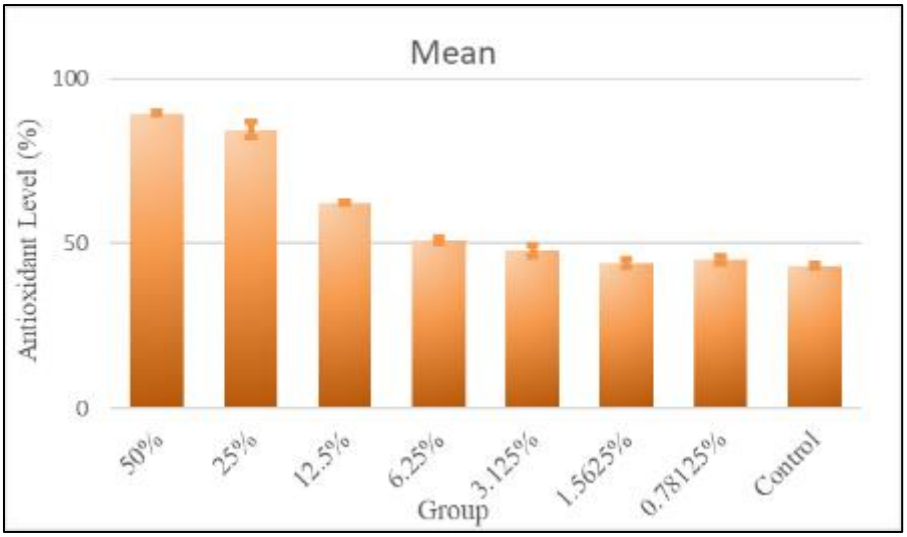

Figure 1 Antioxidant levels of RCBE -based toothpaste

3.2. Cytotoxicity Activity

The morphology of gingival fibroblast cells microscopically in robusta coffee bean extract toothpaste can be seen in Figure 2. The MTT test for cytotoxicity evaluation showed morphological changes in the gingival fibroblast cells after exposure to higher RCBE concentrations. Cytotoxicity of robusta coffee bean extract concentrations of 50%; 25%; 12.5%: 6.25%; 3.125%; 1.5625%; 0.78125%; and placebo toothpaste control (Figure 3), The Kruskal-Wallis analysis showed significant differences among the groups ($p<0.05$). Comparisons using the Mann-Whitney test revealed that concentrations of 50% and 25% showed moderate cytotoxicity, while 12.5% presented slight cytotoxicity. Concentrations ≤ 6.25% presented a non-toxic profile with cell viability >90%. Linear regression pointed to cytotoxicity increase with increased RCBE concentrations (Figure 4).

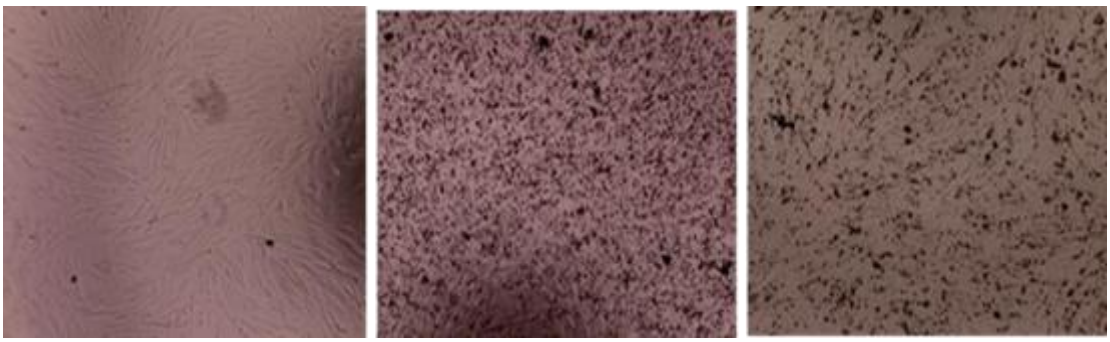

Figure 2 Morphology of gingival fibroblast cells before treatment using inverted microscope magnification 10x (a), after treatment with RCBE toothpaste (b), and after treatment with EBKR toothpaste and 24 hours incubation (c)

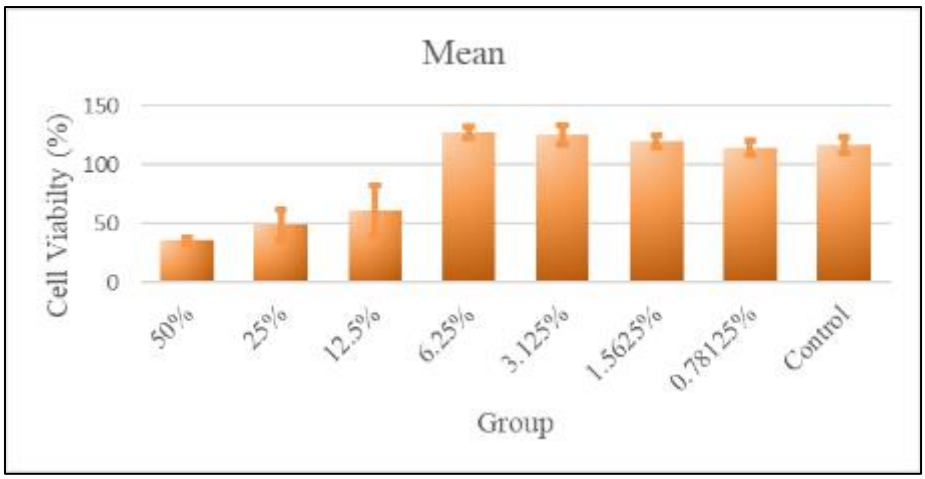

Figure 3 Cytoxicity activity bar chart of RCBE -based toothpaste

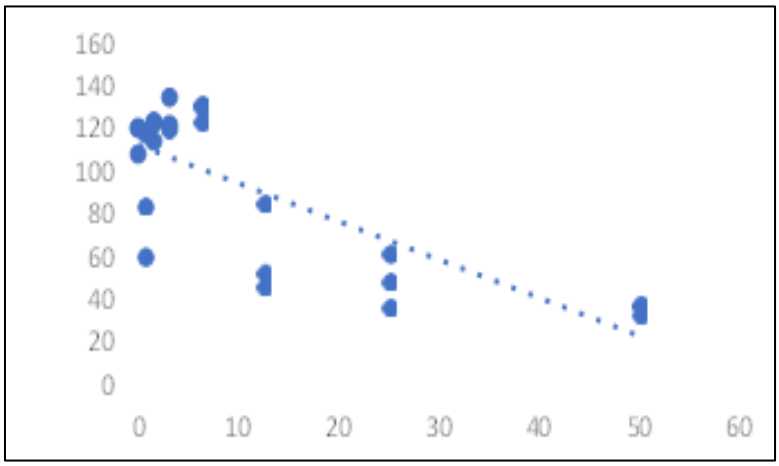

Figure 4 Linear regression curve between RCBE -based toothpaste concentration and cytotoxic activity

These findings of the present study reveal that a toothpaste formula containing robusta coffee bean extract exhibited significant antioxidant activity, which increased linearly with the concentration of extract. This is in agreement with previous literature showing that *Coffea canephora* (robusta) contains high levels of phenolic compounds, mainly chlorogenic acids, among major contributors to the antioxidant capacity of coffee [14,15]. The phenolic compounds usually act via hydrogen atom transfer and single-electron transfer mechanisms and are thus capable of neutralizing the free radicals DPPH by providing electrons or hydrogen atoms that stabilize the reactive species [16,17,18]. Further confirmation of the presence of potent radical-scavenging constituents in the toothpaste formulation was evidenced through the color change observed during the DPPH assay from purple to yellowish brown.

In addition, the flavonoids from the extract could contribute to its antioxidant activity. These compounds have established roles in modulating oxidative stress by chelating metals and scavenging ROS [19,20,21]. Oxidative stress in the oral cavity plays a part in gingival inflammation and periodontal tissue destruction through overproduction of ROS, which results in cellular injury, degradation of the extracellular matrix, and disturbance in wound healing [22,23,24]. The addition of an antioxidant-rich natural extract in dentifrice formulations may provide further benefit for protection against normal plaque formation and thus reduce inflammation and maintain periodontal health [25,26,27].

The current study reported that higher concentrations of robusta coffee bean extract (≥12.5%) elicit a moderate to significant cytotoxic effect against gingival fibroblasts. This is in agreement with the widely established dose-response relationship in phytochemical research, where the polyphenols and alkaloids from plants exert beneficial bioactivity at low doses but become cytotoxic or even pro-oxidant at high levels due to mechanisms such as redox cycling and mitochondrial dysfunction [10,28]. Besides, caffeine, which was detected as the most abundant compound in the extract from this study, may also be responsible for the increased cytotoxicity.

According to this perspective, previous evidence has demonstrated that caffeine, while capable of exerting antioxidant actions at low concentrations, promotes reduced viability and apoptosis in fibroblasts at higher concentrations. These cytotoxic effects are generally believed to be mediated by disruptions of intracellular calcium homeostasis and the activation of caspase-dependent pathways of apoptosis [29,30]. In summary, the present findings further emphasize the need to establish nontoxic concentration ranges while exploiting natural extracts in toothpaste products to balance therapeutic efficacy with biocompatibility.

In contrast, the extract showed very good biocompatibility over the narrower concentration window of 0.78125%–6.25%, remaining nontoxic while offering detectable levels of antioxidant activity. This invites comparison with several other studies on herbal dentifrices that all point toward the same conclusion, namely, that optimum biological behavior from plant-based extracts is usually expressed only within tightly constrained dosage windows [5,31,32]. These comparisons support the view that natural bioactive have the potential to serve effectively in oral care formulations but that their efficacy depends not just on the selection of an appropriate ingredient, but also on careful dose optimization.

Beyond the question of dosing, formulation interactions provide another essential focus that impacts not only efficacy but also safety. Natural extracts are likely to interact with common excipients in toothpaste, such as surfactants, humectants, and abrasives, possibly altering the stability of compounds, their bioavailability, or cytotoxic responses. For instance, triethanolamine and some carbon-based materials could modify pH or control the kinetics of active components, thus affecting their biological behavior [33,34]. These formulation complications therefore suggest that further optimization is required to assure constant performance with the RCBE-based toothpaste.

Importantly, the novelty of this study is in the examination of robusta coffee bean extract directly within an actual toothpaste matrix, an area still unexplored in dental biomaterial science. Most research previously conducted, related to either systemic antioxidant or antimicrobial properties from compounds derived from coffee, has not strongly taken into consideration their incorporation into oral care formulations. This study provides key foundational in vitro evidence regarding antioxidant capacity and cytotoxic safety that significantly contributes to the evolving understanding of the feasibility of RCBE as a functional dentifrice ingredient when formulated within appropriate concentration limits.

The overall findings suggest that RCBE-based toothpaste might serve as a natural adjunct in preventive dentistry, particularly for individuals who are prone to oral conditions associated with oxidative stress. These preliminary results will need more studies for translation into clinical practice, including in vivo biocompatibility, long-term exposure, microbiological, and formulation stability tests. This will provide a better understanding of whether RCBE could be reliably and safely used in dental products in the future.

4. Conclusion

Robusta coffee bean extract-based toothpaste has shown high antioxidant activity that is directly proportional to the concentration of the extracts. The cytotoxic effect, however, has also been registered with higher concentrations, meaning the biocompatibility decreases proportionately with increased extract levels. RCBE-based toothpaste is defined as nontoxic at a concentration of 6.25% and below, which indicates that these levels can safely be applied to gingival fibroblast cells.

Compliance with ethical standards

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Disclosure of conflict of interest

All the authors at this moment declare that there is no conflict of interest.

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