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Cytotoxicity assay of a combination of Aloe Vera, curcumin, Neem, sesame oil and olive oil: An in vitro study

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KEYWORDS

Aloe Vera, turmeric, sesame oil, neem, curcumin, cytotoxicity, cell viability, wound healing.

ABSTRACT:

Introduction: Wound healing is a crucial step in recovery post-surgical injury. Organically derived ingredients have been used for several years to boost wound healing. A combination of selected naturally sourced ingredients, each with their own unique property have the potential to form a potent wound healing solution.

Objectives: This study aims to study the cytotoxicity profile of the combination of Aloe Vera, Turmeric, Neem, Sesame oil and olive oil and determine its potential efficacy in post-surgical wound healing.

Methods: Cytotoxicity assay of the combination of Aloe Vera, Turmeric, Neem, Sesame oil and olive oil was conducted in order to determine cell viability. MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay was used in order to determine the cell viability.

Results: The control showed 100% cell viability, indicating no cytotoxicity under normal conditions. The combination of the ingredients at varying concentrations (50 µg - 200 µg) all show high cell viability, close to the control, suggesting that the combination at these concentrations does not negatively affect cell viability. Microscopy Images also revealed no significant cell death or morphological changes, indicating that the combination does not harm the cells adversely.

Conclusions: The combination maintains high cell viability across all tested concentrations, similar to the control, implying that these treatments are non-toxic to the cells. The absence of significant changes in cell morphology in the treated groups further supports that these treatments are non-cytotoxic. Overall, these results suggest that the combination of Aloe Vera-turmeric-sesame oil-neem- curcumin are safe for cells, showing no adverse effects on cell viability at the tested concentrations.

1. Introduction

Wound healing is a dynamic biological process involving inflammation, proliferation, and tissue remodelling, leading to the restoration of structural and functional integrity of the skin and underlying tissues(1) . The repair process depends on a balance between cellular proliferation, extracellular matrix synthesis, and angiogenesis, all of which can be modulated by biological or pharmacological agents(2). Traditional medicine has long employed natural compounds to accelerate wound repair due to their anti-inflammatory, antioxidant, and antimicrobial properties, which often complement the body's intrinsic healing mechanisms. Among natural products, Aloe vera, curcumin, neem, sesame oil, and olive oil have gained significant attention for their pharmacological benefits in wound management. Each of these components exerts distinct bioactive effects that target specific stages of wound repair. Aloe vera contains glucomannan, polysaccharides, and gibberellins, which enhance fibroblast proliferation and collagen deposition, while also reducing inflammation and bacterial colonization (3). Curcumin, a major constituent of Curcuma longa, modulates the inflammatory response by downregulating pro-inflammatory cytokines such as TNF-α and IL-6, while promoting fibroblast migration and angiogenesis (4). Neem (Azadirachta indica) offers potent

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antimicrobial and immunomodulatory activity due to compounds such as nimbidin and nimbin, which accelerate epithelialization and reduce oxidative stress (5). Sesame oil, rich in lignans and vitamin E, exhibits antioxidant and anti-inflammatory effects that mitigate tissue damage and enhance collagen maturation. Olive oil, abundant in phenolic compounds, further aids wound contraction through its antioxidant and barrier-protective effects on keratinocytes(6). Given that these compounds act through complementary mechanisms, their combined use may exert synergistic effects that enhance wound closure and tissue regeneration. However, before such a formulation can be applied for therapeutic purposes, establishing cytotoxic safety is critical to ensure that the combination does not adversely affect cell viability or morphology**.**

2. Objectives

This study aims to assess the cytotoxicity profile of a novel combination of Aloe vera, curcumin, neem, sesame oil, and olive oil using the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay, a colorimetric technique that evaluates mitochondrial activity as a measure of viable cells (7). Demonstrating non-cytotoxicity of this formulation would support its potential for future in-vitro and in-vivo evaluation as a biocompatible natural wound-healing agent**.**

3. Methods

Principle: Determination of cell viability by MTT assay is a colorimetric assay that measures the reduction of yellow coloured MTT by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes to the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with a solvent (DMSO-Dimethyl sulfoxide) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. Cell viability was assessed using MTT following the method described by Mosmann in 1983 (8) . 3T3 Cells (1x10^5 cells/ml) were cultured for 24 hours & 48 hours on 96-well micro plates. The cells were incubated for different time-points for different modes of studies with and without the combination of Aloe Vera, neem, curcumin, sesame oil and olive oil.

Materials for MTT.

(i) Freshly prepared MTT (5 mg/ml in serum-free media) kept in the dark, at 4 degrees Celsius.

(ii) DMSO- Dimethyl sulfoxide.

(iii) 96 wells flat bottom plate

(iv) DMEM (Dulbecco's modified Eagle's medium) (Complete media/Serum free media).

(v) 1X PBS (phosphate buffered saline)

Procedure.

(i) 3T3 Cells (1x10^5) were plated in 96-well plate supplemented with ~100µl cell culture media in each well.

(ii) After treatment with different concentrations (50 µg,100 µg, 150 µg, 200 µg)of the combination got over, 10µl of MTT solution was added to each well and mixed properly.

(iii) Cells were then incubated for 4 hours in the dark at 37 degrees Celsius.

(iv) After that incubation period, the media from each well was removed and the formazan crystals were dissolved with 100µl DMSO in each well by mixing properly.

(v) Then the mixture was again incubated in dark at 37 degrees Celsius for 30 minutes.

(vi) Finally, the absorbance of the dissolved formazan reagent was measured by microplate reader at 595nm. (vii) Calculation of cell viability was done as follows:

(OD= optical density)

Viable cells(%)= (OD of treated sample/OD of control sample) x100

4. Results

The control showed 100% cell viability, indicating no cytotoxicity under normal conditions. The combination of the ingredients at varying concentrations (50 µg - 200 µg) all show high cell viability, close to the control, suggesting that the combination at these concentrations does not negatively affect cell viability. Microscopy Images also revealed that the control image shows a healthy cell monolayer, typical of viable cells. The microscopy images of cells treated with the combination at different concentrations (50 µg, 100 µg, 150 µg, 200

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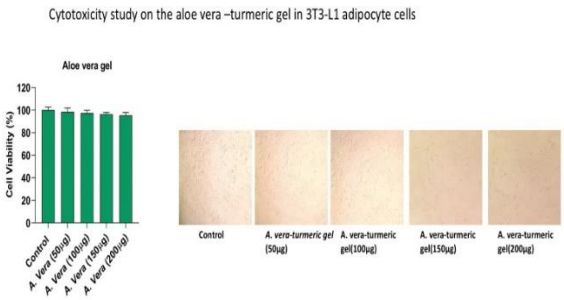
µg) appear similar to the control. There seems to be no significant cell death or morphological changes, indicating that the combination does not harm the cells adversely.



5. Discussion

Aloe vera, a medicinal plant that has been traditionally used, contains 20 minerals, 20 amino acids, vitamins and water. In vitro studies and studies conducted on living organisms have shown that Aloe vera can inhibit thromboxane (an inhibitor of wound healing), improve the wound healing process and reduce inflammation. Magnesium lactate available in the gel can prevent the production of histamine that causes itching and irritation of the skin(3). It also enhances the immune system and the synthesis of cytokines. Its regenerative properties are due to the compound glucomannan, which is rich in the polysaccharide mannose. Glucomannan affects fibroblast growth factor receptors and stimulates their activity and proliferation, which in turn increases the production of collagen. Aloe vera gel can not only increase the amount of collagen in wounds but also change the composition of collagen, increase collagen cross-linking and thereby promote wound healing(9). Curcumin is at the top in the wound healing domain. Curcumin is a naturally occurring low-molecular-weight polyphenolic constituent present in the rhizome of Curcuma longa and Curcuma aromatic. The topical application of curcumin is documented to have an effective role in wound healing mechanisms. Curcumin acts in different stages, such as the inflammatory, maturation and proliferative phases and thus enhances the overall process of wound healing (10). Recent studies and research show that neem contains many therapeutic effects such as anti-inflammatory, anti-diabetic, antifungal, antiviral, antibacterial, and anti-malarial. Neem contains many active ingredients such as nimbidin, nimbin, and nimbidol with anti-inflammatory, antibacterial, antifungal and antiviral properties that may help it accelerating the wound healing process. In addition, neem contains an excellent amount of amino acids, vitamins and minerals which are crucial in the proliferative phase of wound healing (11). Hydrophilic phenols are the most abundant antioxidants of olive oil. The phenolic contents have antioxidant properties higher than those of vitamin E. Studies on mice have shown that topical application of olive oil on pressure ulcers improves wound healing through the effects of anti-inflammation, reducing oxidative damage and promoting dermal reconstruction (12). In a clinical study by Shamloo et al., topical application of sesame oil was shown to lower the severity of pain and reduce the frequency of nonsteroidal anti-inflammatory drug use in patients with limb trauma. Sesame seeds contain significant amounts of lignans such as sesamin, sesamolin and sesaminol, all of which exhibit antioxidative activity which play a crucial role in wound healing. The combination of all the aforementioned ingredients with their rich properties have the potential to form a potent combination which will have superior healing properties.(13, 14)

The present in-vitro study assessed the cytotoxicity of a novel polyherbal combination comprising Aloe vera, curcumin, neem, sesame oil, and olive oil using the MTT assay to determine cell viability. Across all tested concentrations (50–200 µg/ml), the results revealed high levels of cell viability, comparable to control samples, with no significant morphological alterations or cell death under microscopic evaluation. These findings clearly indicate that the formulation is non-cytotoxic and biocompatible, validating its safety for potential therapeutic use in wound-healing applications. The non-toxic behaviour of the tested formulation is particularly relevant because maintaining cellular integrity is a prerequisite for any topical wound-healing agent. Compromised cell viability could impede fibroblast proliferation, epithelialization, or collagen remodelling—all of which are crucial for effective tissue regeneration. The components of this formulation act synergistically: Aloe vera promotes fibroblast proliferation and collagen crosslinking; curcumin modulates inflammatory mediators and oxidative stress; neem contributes antimicrobial and immunomodulatory effects; sesame oil offers antioxidant lignans that support tissue remodelling; and olive oil provides phenolic

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antioxidants that maintain the lipid barrier and promote repair. Their combined activity targets multiple stages of wound healing, including inflammation, proliferation, and remodelling, suggesting an integrated biological benefit. The encouraging cytotoxicity results serve as a foundation for further exploration of this combination in preclinical wound models. Future work should include in-vivo wound closure studies, histopathological evaluation, and quantification of collagen deposition and angiogenesis markers. Furthermore, assessing gene expression profiles of cytokines, growth factors, and extracellular matrix proteins will help elucidate the molecular pathways involved. Advanced formulation strategies, such as incorporating the blend into nanogels, emulsions, or hydrocolloid dressings, could further enhance bioavailability, stability, and sustained therapeutic release. In conclusion, the studied combination exhibits excellent cellular safety and high biocompatibility, positioning it as a promising candidate for the development of natural, plant-based wound-healing formulations. The findings reinforce the potential of combining traditional herbal agents to achieve enhanced regenerative outcomes, supporting a sustainable, biologically safe approach to modern wound management.

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