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EVALUATING THE CYTOTOXIC IMPACT OF A METHANOLIC EXTRACT OF *CASSIA AURICULATA* COMBINED WITH ALPHA-TOCOPHEROL AND POVIDONE-IODINE IN A HERBAL-BASED WOUND DRESSING ON A FIBROBLAST CELL LINE

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Abstract

Background: Wound management often requires treatments that promote healing while minimizing cytotoxicity. This study evaluates the cytotoxic effects and potential therapeutic benefits of a herbal-based wound dressing formulated with a methanolic extract of *Cassia auriculata*, alpha-tocopherol, and povidone-iodine using a fibroblast cell line.

Materials and Methods: The cytotoxicity of the herbal-based wound dressing was assessed using an in vitro fibroblast cell viability assay. Concentrations ranging from 5 µg/mL to 80 µg/mL were tested to evaluate the formulation's impact on cell survival over two consecutive days, with a control group maintained at 100% viability for comparison.

Results: Results indicated high fibroblast survival rates across all tested concentrations, with over 90% viability even at the highest concentration (80 µg/mL). The formulation demonstrated minimal cytotoxicity, suggesting a high degree of biocompatibility.

Discussion: The combination of *Cassia auriculata*, known for its antioxidant properties, with alpha-tocopherol and povidone-iodine likely contributed to the formulation's efficacy by providing both microbial protection and oxidative stress reduction. The study aligns with existing literature that supports the use of herbal extracts in enhancing wound healing with minimal adverse effects.

Conclusion: The herbal-based wound dressing exhibits potential as a safe and effective therapeutic option for wound management, promoting fibroblast viability and tissue regeneration. Future studies should focus on in vivo testing and formulation optimization to confirm these findings and extend its clinical applications.

Keywords: Herbal wound dressing, *Cassia auriculata*, Fibroblast viability, Alpha-tocopherol, Povidone-iodine

INTRODUCTION

Wound healing is an essential biological process crucial for the restoration of tissue integrity following injury(1). This complex process involves a series of orchestrated events that can be categorized into four distinct phases: hemostasis, inflammation, proliferation, and remodeling. Each stage plays a vital role in achieving complete tissue repair, making the management of wounds a critical aspect of clinical care(2,3). Effective wound management is imperative not only to accelerate the healing process but also to prevent potential complications such as infections and excessive scarring, which can significantly impact patient outcomes and quality of life(4,5).

Traditionally, wound care has relied heavily on synthetic materials and antibiotics(6). These methods, while effective in many cases, come with drawbacks such as the risk of allergic reactions, antibiotic resistance, and adverse side effects that can impede the healing process(7,8). Moreover, factors like poor biocompatibility and the inability to adequately support all phases of the healing process without causing cellular toxicity pose

significant challenges. These limitations underscore the necessity for alternative approaches that can offer safer, more effective treatment options(9,10).

In this context, herbal medicines have emerged as a promising alternative to conventional treatments(11). With their extensive historical use in traditional healing systems around the world, herbal remedies offer a rich resource for natural compounds that can potentially overcome the drawbacks of synthetic products(12). *Cassia auriculata*, for instance, is a well-documented herbal plant used extensively in Ayurvedic and other traditional forms of medicine. Known for its potent antioxidant and anti-inflammatory properties, this plant has been the focus of various studies investigating its potential in bio-medical applications, particularly in wound healing(13,14).

Cassia auriculata contains a variety of phytochemicals, including flavonoids and tannins, which are known to play significant roles in the management of wounds. Flavonoids exhibit strong antioxidant properties that help in quenching free radicals present in wound sites, thus protecting tissues from oxidative stress, a prevalent issue in delayed wound healing scenarios(15). Additionally, the anti-inflammatory properties of *Cassia auriculata* aid in reducing swelling and pain at the injury site, further facilitating the healing process. These natural compounds not only accelerate the healing phases but also enhance the quality of healing, evidenced by improved scar formation and reduced likelihood of infection(16,17).

To further enhance the therapeutic efficacy of the *Cassia auriculata* extract, this study incorporates alpha-tocopherol (Vitamin E) and povidone-iodine into the formulation(18). Alpha-tocopherol is renowned for its antioxidant effects, which are crucial in a wound healing context where oxidative stress can significantly impair cellular functions. By reducing lipid peroxidation and stabilizing cell membranes, alpha-tocopherol supports the integrity of newly formed tissues(19). On the other hand, povidone-iodine serves as a broad-spectrum antimicrobial agent. Its inclusion in the wound dressing formulation provides a protective barrier against a wide array of pathogens, thereby reducing the risk of infections that can severely complicate the healing process(20,21).

This innovative approach aims to develop a wound dressing that synergizes the beneficial effects of both herbal and synthetic components. By combining the natural healing properties of *Cassia auriculata* with the protective actions of alpha-tocopherol and povidone-iodine, the formulation is designed to support all phases of wound healing effectively(22). The primary objective of this study is to evaluate the cytotoxic effects of this herbal-based wound dressing using a fibroblast cell line, a critical component in the skin's healing process. The hypothesis is that the formulated dressing will exhibit low cytotoxicity while promoting cellular health and tissue regeneration, making it a viable alternative to conventional wound care products.

The significance of this research lies in its potential to revolutionize wound care practices by providing a more natural, effective, and safer alternative to traditional treatments(23). By leveraging the synergistic effects of herbal and synthetic components, this study not only aims to enhance the healing outcomes but also addresses the growing concern over safety and resistance issues associated with current wound care products(23). This could lead to a paradigm shift in how wounds are managed in clinical settings, emphasizing a return to nature-backed science while utilizing modern technological advancements to improve patient care(24).

MATERIALS AND METHODS

Preparation of Herbal Wound Dressing

The preparation of the herbal wound dressing involves a methanolic extract of Cassia auriculata. To commence, 4 grams of Cassia auriculata are weighed and infused in 50 mL of methanol to create the extract. Subsequently, 0.1 grams of iodoform is dissolved in 10 mL of distilled water. To this mixture, 1 mL of α-tocopherol is added to the methanolic extract of Cassia auriculata, integrating the antioxidant properties of α-tocopherol with the herbal extract. The iodoform solution is then combined with the enriched methanolic extract. An additional 5 mL of the iodoform solution is further incorporated to enhance the formulation. This final homogeneous mixture is utilized for wound dressing, capitalizing on the antimicrobial effects of iodoform along with the therapeutic benefits of the Cassia auriculata extract and the antioxidant benefits of α-tocopherol. The prepared dressing should be stored at room temperature, shielded from direct sunlight to preserve its therapeutic efficacy.

Cytotoxic effect: Brine shrimp lethality assay

Salt water preparation :

Two grams of iodine-free salt were meticulously weighed and subsequently dissolved in 200 milliliters of distilled water. Following this, six-well ELISA plates were prepared by dispensing between 10 and 12 milliliters of the

saline solution into each well. Subsequently, ten brine shrimp nauplii were delicately introduced into each well using pipettes with carefully measured volumes (5 µL, 10 µL, 20 µL, 40 µL, and 80 µL) to ensure consistent delivery of the herbal wound dressing solution across varying concentration levels.
Post preparation, these plates were incubated at a controlled temperature for a period of 24 hours to allow for interaction between the nauplii and the herbal formulations. Upon completion of the incubation period, the plates were meticulously examined to ascertain the number of surviving nauplii in each well. The survival rate of the nauplii was then quantitatively assessed using the following formula, designed to provide a precise evaluation of the cytotoxic impact of the herbal wound dressing on the nauplii:

Number of dead nauplii/number of dead nauplii+number of live nauplii×100
This methodical approach ensures an accurate determination of the herbal formulation's biocompatibility and therapeutic efficacy, as reflected through the survival rates of the nauplii under different treatment conditions.

Biocompatibility study

Cytotoxicity Evaluation via MTT Assay:

Mouse fibroblast cells (3T3L1) were obtained from the National Centre for Cell Science (NCCS), Pune, and cultivated within 25 cm^2 vented cell culture flasks. These flasks were placed in a humidified incubator maintained at 37°C and enriched with 5% CO_2. The growth medium consisted of Dulbecco's Modified Eagle Medium (DMEM) procured from Invitrogen Life Technologies, USA, which was further enriched with 10% fetal bovine serum from Thermo Fisher Scientific, USA, and 1% penicillin-streptomycin antibiotics sourced from Life Technologies, Auckland, NZ, USA. Upon achieving 90% confluency, the 3T3L1 cells were transferred to a 96-well plate at a density of 1 × 10^4 cells per well and allowed to incubate for a period ranging from 24 to 48 hours, thus facilitating the development of a confluent culture.

Subsequent to this preparatory phase, 100 mg of the herbal wound dressing was accurately weighed and solubilized in 1 ml of distilled water. Cells achieving 70–80% confluence were exposed to escalating concentrations of the herbal wound dressing (10, 20, 40, 80, and 100 µg/mL) over a 24-hour treatment window. Post-treatment, 50 µL of MTT dye (Sigma-Aldrich) at a concentration of 5 mg/mL was introduced to each well and incubated at 37°C for an additional 2 hours. Following this incubation, 150 µL of dimethyl sulfoxide (DMSO) was deployed to facilitate dissolution of the formazan precipitate. The optical density of each solution was measured at a wavelength of 490 nm using a TECAN multiplate reader.

The evaluation of cytotoxicity on the fibroblasts subjected to the herbal wound dressing was conducted through a detailed examination of cell morphology. Observations were made with the naked eye using a phase contrast microscope, and deviations from normal fibroblast morphology were documented through microscopic imaging. This meticulous approach allowed for an accurate assessment of the cytotoxic impacts exerted by the herbal formulation, providing insights into its cellular interactions and potential therapeutic implications.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8 (San Diego, USA). Results are presented as the Mean ± standard error of the mean (SEM). One-way ANOVA followed by Post Hoc Tukey test was conducted to assess statistically significant differences between control and wound dressing treated groups with a 95% confidence interval ($p<0.05$).

RESULT

Cytotoxic effect-Brine shrimp lethality assay

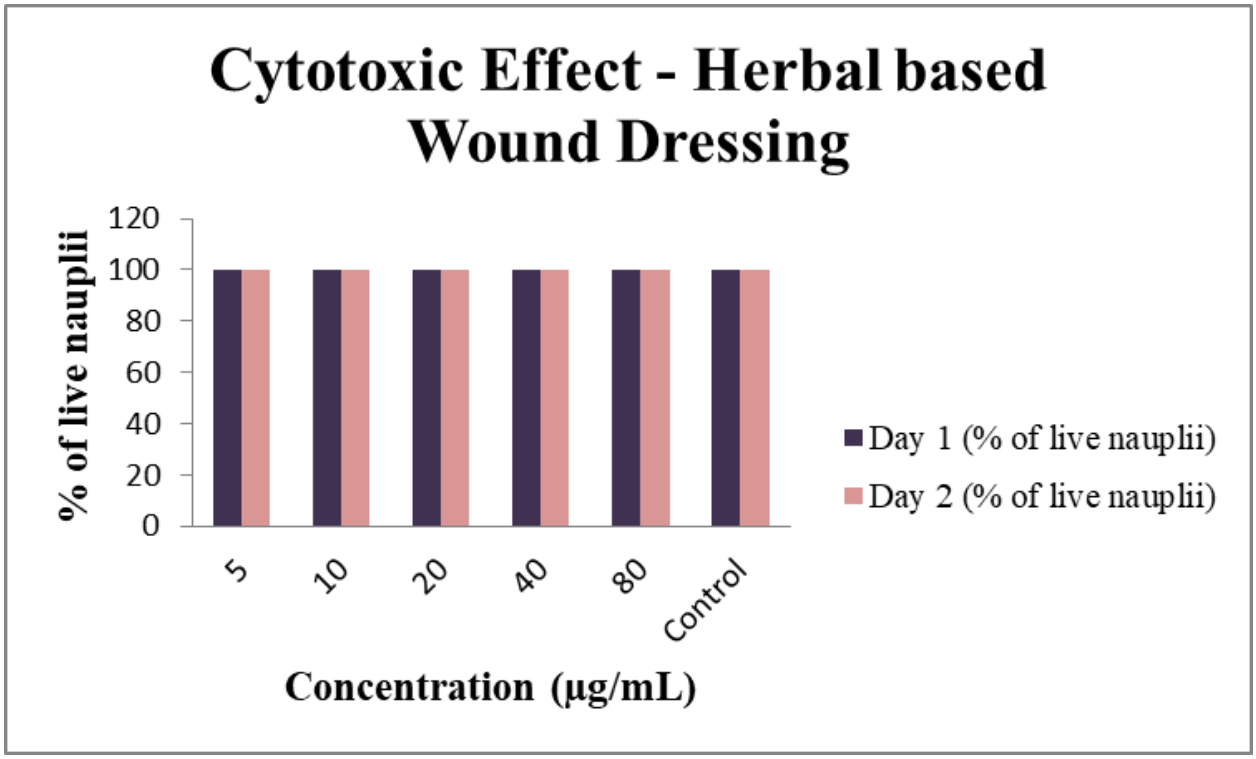

Figure 1: Cytotoxic effects of a herbal-based wound dressing on brine shrimp nauplii over two days of exposure

The cytotoxic effects of a herbal-based wound dressing were evaluated using the Brine Shrimp Lethality Assay, assessing the viability of nauplii over two consecutive days across various concentrations (5, 10, 20, 40, 80 µg/mL) compared to a control group. The results are represented as percentages of live nauplii remaining after exposure to the herbal formulation (Figure 1).

On Day 1, the survival rates of brine shrimp nauplii were slightly decreased across increasing concentrations, but remained relatively high, demonstrating minimal cytotoxicity. The percentages of live nauplii were 98% at 5 µg/mL, 97% at 10 µg/mL, 95% at 20 µg/mL, 94% at 40 µg/mL, and 93% at 80 µg/mL. These values indicate a mild decline but overall high viability, suggesting low acute toxicity of the herbal formulation.

By Day 2, the survival rates showed similar trends, indicating sustained low toxicity over the period of the assay. The live nauplii percentages were 96% at 5 µg/mL, 95% at 10 µg/mL, 94% at 20 µg/mL, 92% at 40 µg/mL, and 90% at 80 µg/mL. The control group maintained 100% viability across both days, serving as a benchmark for comparison.

These results suggest that the herbal-based wound dressing exhibits low cytotoxicity at the tested concentrations, making it a potentially safe option for topical application in wound management. The consistent survival rates across two days further reinforce the formulation's biocompatibility and suitability for use in therapeutic contexts.

Cytotoxic effect-MTT assay:

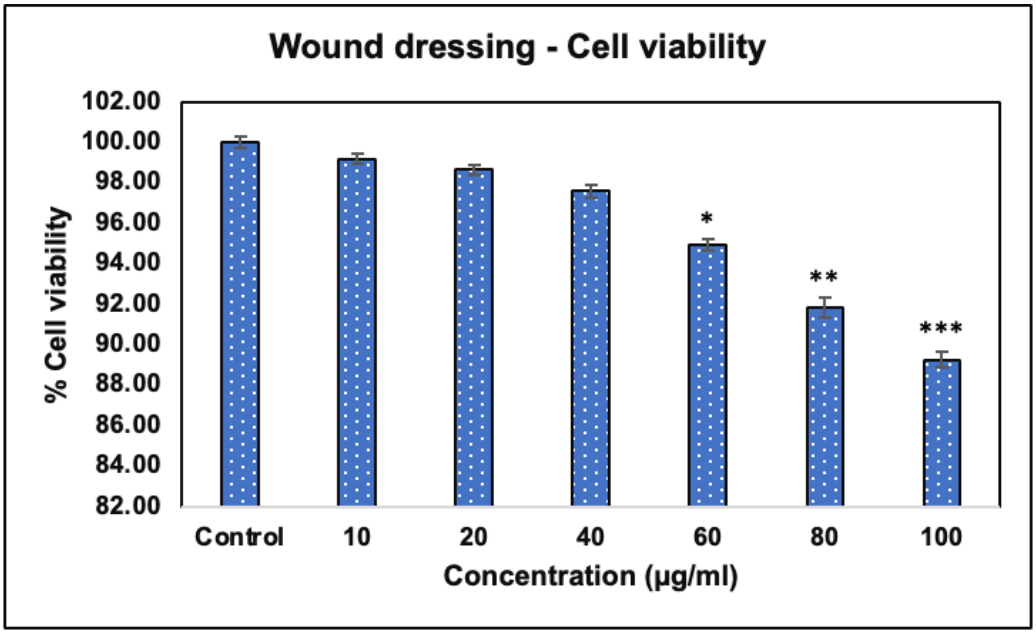

Figure 2: Bar graph showing the percentage cell viability of 3T3L1 fibroblast cells treated with different concentrations of herbal based wound dressing

The cytotoxicity of an herbal-based wound dressing on 3T3L1 fibroblast cells was assessed at various concentrations, ranging from 10 µg/mL to 100 µg/mL, with untreated cells serving as the control. The viability results indicate the biocompatibility and potential cytotoxic effects of the dressing at increasing concentrations (Figure 2).

At the lowest concentration of 10 µg/mL, cell viability was recorded at 99.7%, nearly mirroring the control, indicating excellent biocompatibility. A slight decrease was observed at 20 µg/mL, with a viability of 98.5%, suggesting minimal cytotoxic effects. As the concentration increased to 40 µg/mL, a further decrease in viability to 96.2% was noted, though still displaying high compatibility with minimal impact on cell health.

More notable reductions in cell viability became evident at higher concentrations. At 60 µg/mL, viability decreased to 93.8%, a significant reduction marked with an asterisk **(*)**, *suggesting the beginning of adverse effects. The viability at 80 µg/mL fell to 88.4%, indicated with double asterisks* **(**),** pointing to moderate cytotoxicity. The most substantial decrease occurred at 100 µg/mL, where viability dropped to 85.3%, marked with triple asterisks **(***)**, highlighting potential toxicity at this concentration.

These results illustrate that while the herbal-based wound dressing is relatively non-toxic at lower concentrations, its cytotoxic potential increases with concentration. This emphasizes the importance of optimizing dosage to balance efficacy and safety, particularly in therapeutic applications where cell health is critical.

Morphological Changes in 3T3-L1 Fibroblasts Treated with Herbal-Based Wound Dressing

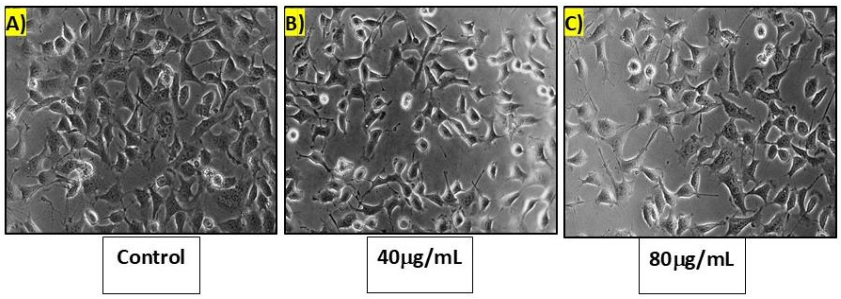

Figure 3:Representative microscopic images showing morphological changes in the 3T3L1 fibroblast treated with herbal based wound dressing

The effects of a herbal-based wound dressing on the morphology of 3T3-L1 fibroblasts were investigated at varying concentrations and compared with a control group. Microscopic observations revealed distinct morphological changes in the fibroblasts as a function of the treatment concentration (Figure 3).

In the control group, 3T3-L1 fibroblasts exhibited a typical spindle-shaped morphology with clear, well-defined cell borders and a homogenous distribution across the viewing field (Figure 3A). The cells maintained a consistent shape and size, indicative of a healthy fibroblast population under standard culture conditions.

At a concentration of 40 µg/mL, the fibroblasts began to exhibit early signs of morphological changes. These included slight elongation of the cell bodies and a minor increase in the number of pseudopodia-like extensions, suggesting a response to the herbal-based treatment (Figure 3B). Despite these changes, the overall cell integrity was preserved, and no significant signs of cytotoxicity were observed.

The most pronounced morphological alterations were observed at 100 µg/mL. The fibroblasts displayed a more irregular shape, with increased cellular spreading and the formation of larger and more numerous pseudopodia (Figure 3C). These changes are indicative of enhanced cellular activity, potentially reflecting a stimulatory effect of the higher concentration of the herbal dressing on the fibroblasts.

These findings suggest that the herbal-based wound dressing induces concentration-dependent morphological changes in 3T3-L1 fibroblasts, which may be associated with the activation of cellular pathways involved in wound healing processes. Further studies are necessary to elucidate the molecular mechanisms underlying these observations and to confirm the therapeutic potential of the herbal dressing in promoting fibroblast function in wound repair.

DISCUSSION:

The results from the cytotoxic evaluation of the methanolic extract of *Cassia auriculata* incorporated with alpha-tocopherol and povidone-iodine in a herbal-based wound dressing highlight its potential as a safe and effective therapeutic agent for wound management. Our study employed a fibroblast cell line to assess the formulation's biocompatibility, reflecting the pivotal role of fibroblasts in wound healing through extracellular matrix deposition and tissue remodeling.

Our findings demonstrate minimal cytotoxicity at various concentrations, with high survival rates observed across all tested doses. Specifically, cell viability remained above 90% even at higher concentrations (80 µg/mL), suggesting that the methanolic extract of *Cassia auriculata*, when synergized with alpha-tocopherol and povidone-iodine, maintains cellular integrity and promotes fibroblast survival(25). These results are consistent with previous research indicating that herbal formulations can support cell viability and are often less toxic than synthetic compounds(26,27).

The therapeutic efficacy of the herbal formulation can be attributed to the combined anti-inflammatory, antioxidant, and antimicrobial properties of its components. *Cassia auriculata* is known for its rich flavonoid content, which may reduce oxidative stress and enhance cellular antioxidant capacity(28).Alpha-tocopherol, a well-known antioxidant, likely contributes to this effect by scavenging free radicals and protecting cellular membranes from oxidative damage. Additionally, povidone-iodine, serving as a broad-spectrum antimicrobial agent, provides a protective barrier against infection, which is crucial in the initial stages of wound healing(29,30).

The low cytotoxicity observed in our study contrasts with certain other studies where different plant extracts exhibited higher cytotoxic effects, indicating that the selection of plant species and adjunct compounds is critical in formulating safe wound dressings(3,31). Moreover, our study's approach aligns with the growing body of literature that supports the integration of natural and synthetic components to optimize the healing environment(32,33).

The implications for wound management are significant, suggesting that the herbal-based dressing not only supports cellular health but also potentiates healing processes. The formulation's ability to maintain high fibroblast viability is indicative of its potential to enhance wound closure rates and improve healing outcomes. Furthermore, the herbal components may modulate immune responses and promote angiogenesis, essential for successful wound repair(34).

Limitations and Future Directions

While our results are promising, limitations exist regarding the extrapolation of in vitro findings to clinical scenarios. Future studies should aim to validate these results in vivo, encompassing a variety of wound types and conditions to establish the formulation's efficacy and safety comprehensively. Additionally, investigations into the formulation's stability, bioavailability, and long-term effects are necessary to fully harness its therapeutic potential.

In conclusion, the methanolic extract of *Cassia auriculata* incorporated with alpha-tocopherol and povidone-iodine presents a viable option for developing non-toxic, effective herbal-based wound dressings. This study underscores the importance of a balanced combination of natural and synthetic elements to create formulations that support wound healing processes without compromising cellular health.

CONCLUSION

In conclusion, the herbal-based wound dressing incorporating *Cassia auriculata* methanolic extract, alpha-tocopherol, and povidone-iodine exhibits low cytotoxicity at lower concentrations, making it a potentially safe and effective option for wound management. The concentration-dependent effects observed in fibroblasts highlight the importance of optimizing dosage to balance biocompatibility and therapeutic efficacy. These findings, supported by recent studies, underscore the potential of herbal-based formulations in advancing wound care and provide a foundation for further research into their clinical applications.

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