CYTOTOXIC EFFECT OF TRIDAX PROCUMBENS STEM BASED CHITOSAN GEL AND COMMERCIAL WOUND HEALING GEL- AN IN VITRO STUDY

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Abstract

Introduction: Chitosan is a glucosamine (2-amino-2-deoxy—D-glucose) polymer with trace amounts of N-acetylglucosamine. Tridax procumbens L. is a perennial plant native to tropical America. A plant steroid called beta-sitosterol promotes epithelialization and wound healing. Cytotoxicity assays are critical because they aid in determining the intended biological usage. This current study is focused to evaluate and compare the cytotoxic effect of T. procumbens stem based chitosan gel and commercial wound healing gel.
Materials and Method: The cytotoxic effect of T. procumbens stem based chitosan gel (test) and commercial wound healing gel (5 µL, 10 µL, 20 µL, 40 µL, 80 µL) at different concentrations was assessed by brine shrimp lethality assay.
Result: The viability of the shrimp was analyzed for different concentrations for T. procumbens stem based chitosan gel and commercial wound healing gel. The results proved that T. procumbens stem based chitosan gel showed no cytoxicity after 24 h and reduced cytotoxicity after 48 h similar to commercial wound healing gel..
Conclusion: This study proves that the cytotoxic effect of T. procumbens stem based chitosan gel was comparable to commercial wound healing gel.. Therefore, T. procumbens stem based chitosan gel can be used as an alternative to commercial wound healing gel as it is more eco-friendly and a form of traditional medicine with no side-effects.

Keywords: chitosan, innovative, sustainable, Tridax procumbens, wound healing

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1. Introduction

The biological process of tissue regeneration and growth is connected to the larger phenomenon of wound healing. When a wound heals, a variety of cellular and matrix components work together to restore the integrity of damaged tissue and replace lost tissue. These stages are interconnected and overlap (Cañedo-Dorantes and Cañedo-Ayala, 2019). A wound is a breakdown in the skin's barrier function and/or loss of the connective tissue beneath it, as well as a break in the continuity of the epithelium, brought on by surgery, ulceration, malignancy, or general wear and tear. Inflammation, proliferation, and remodelling are three overlapping and continuous processes in the complex process of wound healing.(Konop et al., 2016) .

Chitosan is a 2-amino-2-deoxy-D-glucose polymer that also contains very small levels of N-acetylglucosamine. It is a byproduct of chitin, the second most prevalent biopolymer after cellulose (poly-N-acetylglucosamine). There has been a great deal of research on the use of chitosan as a wound-healing accelerator, and there is convincing proof that chitosan may favourably affect each stage of wound healing (Ueno, Mori and Fujinaga, 2001). By increasing the functions of inflammatory cells such polymorphonuclear leukocytes (PMN), macrophages, fibroblasts, and osteoblasts, chitosan and its derivatives may speed up the healing of wounds.

Green synthesis is a comprehensive method to produce a product that avoids the development of detrimental byproducts (Zahoor et al., 2021) . It is a replicable, efficient, and long-term synthesis approach. Furthermore, biological approaches appear to be non-toxic, simple and quick alternatives (Akintelu, Bo and Folorunso, 2020). Plant-derived therapeutic substances have been used as alternative medicine since ancient times(Rajeshkumar and Jeevitha, 2021). Tridax procumbens L., a perennial weed that is native to tropical America and is taxonomically classified as a member of the Asteraceae family, is widespread in tropical and subtropical areas. (Jayasundera et al., 2021). A hispid, procumbent plant known as "Common button" or "Coat button" is used by traditional healers and tribal groups as a remedy for a number of illnesses and skin conditions (Kaushik et al., 2020). Phytoconstituents of T. procumbens include luteolin, sitosterol, carotenoids, and linolenic acid, among others. A plant steroid called beta-sitosterol promotes wound healing and epithelialization. T. procumbens has been shown to have antibacterial, antiviral, antifungal, antimalarial, anticandidal, anti-cancerous, analgesic, anti-inflammatory, antipyretic, antidiabetic, antioxidant, and insecticidal properties.(Sangeetha, Niranjan and Dhanalaksh, 2016) .

Our team has extensive knowledge and research experience that has translated into high quality publications (Ezhilarasan et al., no date; Sahu, Kannan and Vijayaraghavan, 2014; Bakshi et al., 2019; Babu and Jayaraman, 2020; Sivasamy, Venugopal and Mosquera, 2020; Thakur and Devaraj, 2020; Vikneshan et al., 2020; Ezhilarasan, 2021; Preethi and Sekar, 2021; Preethi, Lakshmanan and Sekar, 2021)(Ramesh Kumar et al., 2011; Jain, Kumar and Manjula, 2014; Krishnan, Pandian and Kumar S, 2015; Keerthana and Thenmozhi, 2016; Sivamurthy and Sundari, 2016; Felicita, 2017a, 2017b; Kumar, 2017; Sekar et al., 2019; Johnson et al., 2020)(Jeevitha et al., 2022)(Begum, Jeevitha and Preetha, 2020; Prathap et al., 2021; Santhakumar et al., 2021)(Rajeshkumar and Jeevitha, 2021; Santhakumar et al., 2021)(Jeevitha et al., 2022).

Cytotoxicity is defined as the toxicity induced by chemotherapeutic drugs acting on live cells. Cytotoxicity assays are critical because they aid in determining the intended biological usage. The current study is focused to compare the cytotoxic effect of T. procumbens stem based chitosan gel and commercial wound healing gel.

2. Materials and Methods

Preparation of 50 mL chitosan gel.
49mL of distilled water and 1mL of glacial acetic acid were used to dissolve the chitosan. To achieve homogeneous mixing, the solution was then agitated for 24 hours on a magnetic stirrer. The plant's stem, T. procumbens, was then crushed and ground into powder. In 100mL of distilled water, 1g of this powder was dissolved. A heating mantle was used to heat the solution. The solution was then filtered with filter paper. The filtered solution was heated again and condensed, until it was thick. Now, 9 mL of chitosan were added to 1 mL of the plant extract, which was then agitated for 24 hours in a magnetic stirrer.

Brine Shrimp Lethality Assay
200 mL of distilled water and 2g of iodine-free salt were combined to create 200 mL of salt water. Then, 10–12 ml of saline water were added to 6 well ELISA plates. To that end, 10 nauplii in each concentration (5µL, 10µL, 20µL, 40µL, 80µL) of the two gels and control were gently added to each well (Fig. 1) in two different plates for the two gels. The T. procumbens stem-based chitosan gel and commercial wound healing gel were then

applied in the appropriate concentration levels to two distinct ELISA plates. For 24 h, the plate was incubated. At 24 h and 48 h intervals, the ELISA plate was examined and its live nauplii content was recorded.

Figure 1: 6-well ELISA plates used for evaluation of cytotoxic effect of different concentrations of T. procumbens stem-based chitosan gel

3. Results

The ELISA plates were examined and counted for live nauplii at intervals of 24 h and 48 h. For a clear evaluation, the observed results were tabulated and graphically plotted. Table 1 depicts the number of nauplii present in each concentration level in comparison with commercial wound healing gel at a time interval of about 24 h and 48 h. Figure 2 shows the evaluation of cytotoxic effects at a concentration of 5 µL, 10 µL, 20 µL, 40 µL, 80 µL and control. After 24 h, it was found that at concentrations of 5µL, 10µL, 20µL, 40µL and 80µL, 100% of the brine shrimp were alive in both commercial wound healing gel and in T. procumbens stem based chitosan gel. After 48 h, it was found that at a minimal concentration of 5 µL, 90% of the brine shrimp were alive in commercial wound healing gel whereas only 80% of the brine shrimp were alive in T. procumbens stem based chitosan gel. At a concentration of 10 µL and 20 µL, 80% of the brine shrimp were alive in commercial wound healing gel whereas only 70% of the brine shrimp were alive in T. procumbens stem based chitosan gel. At a concentration of 40 µL, 70% of the brine shrimp were alive in commercial wound healing gel whereas only 60% of the brine shrimp were alive in T. procumbens stem based chitosan gel. At a concentration of 80 µL, 60% of the brine shrimp were alive in commercial wound healing gel whereas only 50% of the brine shrimp were alive in T. procumbens stem based chitosan gel. The control showed 100% of the brine shrimp to be alive at both 24 h and 48 h intervals. The results proved that increased concentration, increases the toxicity for both the gels. More extensive cytotoxic studies are needed to establish the precise mechanism of action of this combination.

Table 1: Table depicts the number of nauplii present in each concentration level in comparison with a commercial wound healing gel at a time interval of about 24 h and 48 h.

Concentration	Commercial gel day 1	TP gel day 1	Commercial gel day 2	TP gel day 2
5 µL	10	10	9	8

10 µL	10	10	8	7
20 µL	10	10	8	7
40 µL	10	10	7	6
80 µL	10	10	6	5
Control	10	10	10	10

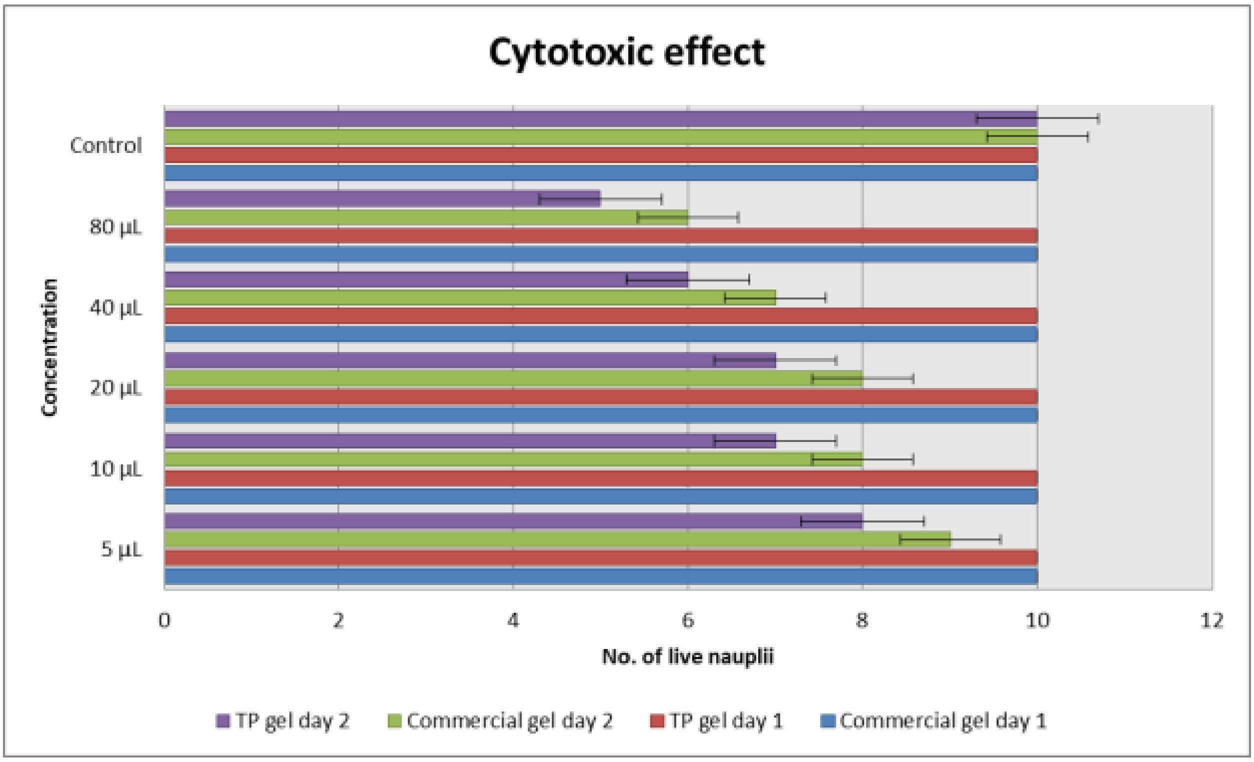

Figure 2: Bar chart depicts the comparison of the number of live nauplii present in T. procumbens stem based chitosan gel (TP gel) and commercial wound healing gel at a time interval of about 24 h and 48 h

4. Discussion

An essential parameter in the research of toxicity is the brine shrimp lethality assay which provides data on an organism's susceptibility to toxicity. The effectiveness of commercial wound healing gel and chitosan gel derived from T. procumbens stem extract at various concentrations on brine shrimp viability was examined. Previous literature have demonstrated decreased cytotoxic effects of Tridax procumbens flower mediated silver nanoparticles (J. Soni et al., 2022). T. procumbens (leaves) extract is not only useful in healing but also as an antidepressant therapy which was experimented in mice (Uppala et al., 2016). The increase in lysyl oxidase activity caused by repair has been studied to determine the role of wound healing. Increased nucleic acid levels suggest cellular activity. T. procumbens leaf juice has been proven to inhibit wound penetration in experimental animals (Pandurangan, 2022). Other studies were conducted on the effectiveness of T. procumbens as potential solution for wound healing depending on its properties based on the derived nanoparticles from the plant source. Synthesis of silver NPs through non - hazardous methods derived from this plant proved to possess potent antibacterial and antioxidant properties (Rani et al., 2020) . By increasing the concentration of T. procumbens in the poly-vinyl alcohol membrane, the percentage of wound healing increased, and therefore the curative length period was decreased. In-vitro cytotoxicity studies employing L929 cells in the MTT test revealed good cell viability for T. procumbens blended PVA thin membrane (Ayyanar et al., 2022). Biopolymer hydrogels based on cellulose and chitosan exhibited remarkable wound healing properties. Increased porosity, rapid water absorption, non-immunogenic effects, and prolonged and regulated medication release were all characteristics of biopolymer-based hydrogels used in wound dressings. In vitro and in vivo, the aforementioned features facilitated rapid re-epithelialization, granulation tissue formation, and wound healing. The integration of nanotechnology and medicine opens up new possibilities for wound dressings that are now accessible (Alven and Aderibigbe, 2020) . Chitosan has outstanding physicochemical properties, as well as strong biocompatibility and interactions with human proteins, cells, and organs (Andriana et al., 2019). Tridax procumbens leaves based chitosan gel have shown to have potent antimicrobial activity against common orl pathogens (Rieshy et al., 2022). When the amine groups in its skeleton undergo protonation, the terminal amino groups present in its skeleton encourage the development of polycations in an acidic media, allowing interactions with anionic polymers of diverse forms and geometries (Niño et al., 2006) . Chitosan and chitosan-based derivatives are predicted to be utilized to create tissue repair scaffolds with acceptable qualities for a variety of tissues such as bone, cartilage, skin, cornea, blood vessels, and so on. Such matrices are advantageous due to their intrinsic similarity to host tissues and structural and functional similarities to biological components (Ravikumar, Shivashangari and Devaki, 2005) The limitations of this study is that the study was done in an in-vitro environment and so the cytotoxic effect cannot be assured to be clinically effective. However, the present study has led to a future scope of more in- vivo based research on this traditional plant based gel and clinical trials in order to determine the cytotoxic effect that can lead to the development of an eco-friendly and non-toxic wound healing gel as an alternative to synthetic gels.

5. Conclusion

To ensure an effective and safe alternative dosage form for smart, eco-friendly, highly effective, and multidimensional wound healing gel, topical T. procumbens stem-based chitosan-gels require accurate and comprehensive characterizations. The current work reveals the green synthesis of chitosan gel based on T. procumbens stems and its reduced ytotoxic activity against brine shrimp. When compared to conventional wound healing gel, the cytotoxic effect of T. procumbens stem-based chitosan gel was shown to be less harmful. Therefore, T. procumbens stem based chitosan gel can serve as an excellent alternative to commercial wound healing gel.

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Conflict of Interest:
The author declares that there were no conflicts of interests in the present study.

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