



## Assessment of the anti-inflammatory effect of a novel Chitosan-Sunflower seed gel – an in vitro study

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### ABSTRACT

**Objective:** This study aimed to evaluate the anti-inflammatory activity of a novel chitosan- sunflower seed gel.

**Materials and Methods:** A novel chitosan-sunflower seed gel was prepared. The antiinflammatory activity of the prepared gel, in five different concentrations (10, 20, 30, 40 and 50 $\mu$ l), was tested in a dual wavelength spectrophotometer.

**Result:** The 40 $\mu$ l dilution of the gel had the best antiinflammatory activity, followed by 50 $\mu$ l, 30 $\mu$ l, 20 $\mu$ l and 10 $\mu$ l dilutions in decreasing order.

**Conclusion:** The chitosan-sunflower seed gel is a new and potential alternative as a wound healing agent with excellent anti-inflammatory activity that requires further clinical assessment to better establish appropriate protocols for treatment.

**Keywords:** *anti-inflammatory activity, green synthesis, chitosan, sunflower seeds*

### INTRODUCTION

There are four phases of the wound healing process that are closely connected: hemostasis, inflammation, proliferation, and tissue remodeling. These phases are interdependent and occur in a coordinated manner (Guo and Dipietro, 2010)(Sridharan et al., 2019). Non-healing or delayed healing wounds can result when the wound-healing process fails, resulting in higher health-care expenses and a higher

chance of unfavourable side effects such as secondary infection, excessive use of nonsteroidal analgesics, or even opioid drugs. Drugs that are delivered locally have various advantages over those that are supplied systemically. Because the therapeutic drug does not need to be dispersed throughout the body before reaching the site, the dose or formulation concentration delivered to the site can be

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substantially higher (Frison, no date)(Ramesh et al., 2018).

Natural products have been employed in a variety of medicinal purposes, including the treatment of inflammations, infections, and skin conditions. The application of plant oils in dermatology has been studied (Daud et al., 2011; Vijayashree Priyadharsini, 2019). In 1929, research on the use of sunflowers to treat wounds began (Ramadurai et al., 2019; Ferreira et al., 2021). Their topical application has been documented in human medicine for treating pressure sores and burns. Sunflower seeds include oleic acid as well as a variety of unsaturated fatty acids, the most notable of which being linoleic acid, an arachidonate precursor (Manhezi, Bachion and Pereira, 2008). Arachidonic acid is a widely distributed polyunsaturated fatty acid found in the skin, and its activity is mostly dependent on the production of inflammatory mediators such as leukotrienes, prostaglandins and thromboxanes. These compounds promote migration of cells, new vessel formation, proliferation of fibroblasts and formation of extracellular matrix (Das, Behera and Pramanik, 2017)(Siddique et al., 2019).

Another natural compound of interest is chitosan. Chitosan is renowned for its strong biocompatibility (Ezhilarasan, Apoorva and Ashok Vardhan, 2019) and antibacterial (Kamjumhol, Chareonsudjai and Chareonsudjai, 2018; Mathew et al., 2020) and antifungal (Leuba and Stossel, 1986; Duraisamy et al., 2019) characteristics. Chitosan has been extensively studied in scientific literature and has various potential uses, including as an agent for wound healing, a means of releasing drugs in a controlled manner, for bleeding control, and as a biomaterial for regenerating cartilage and bone. It has also been shown to have immune modulation by reducing inflammation and promoting tissue granulation by recruiting fibroblasts and producing collagen. These properties suggest that chitosan could be used to accelerate wound healing and provide antimicrobial and anti-inflammatory benefits.

Chitosan is considered the ideal drug carrier with enhanced bioadhesion, because of which it causes sustained release of drugs.(J et al., 2018) However, the combination of chitosan and sunflower seed oil for wound healing has never been studied. Given the importance of discovering novel treatments for patients with wounds, the goal of this study was to assess the anti-inflammatory activity of a novel chitosan-sunflower seed gel in vitro using double wavelength spectrophotometer.

## MATERIALS AND METHODS

### *Gel Preparation*

0.25g chitosan was added to 50ml distilled water to obtain chitosan extract (5% m/v). 50 ml of sunflower seed extract (3% m/v) was prepared by adding 0.15g of sunflower seed powder to 50 ml distilled water. The two solutions were mixed together to obtain chitosan-sunflower seed extract. To this 2.5g carbopol was added. The mix was then kept on a stirrer for 48 hours until a homogenised mix of chitosan-sunflower seed gel was obtained.

### *Anti-inflammatory Activity Test*

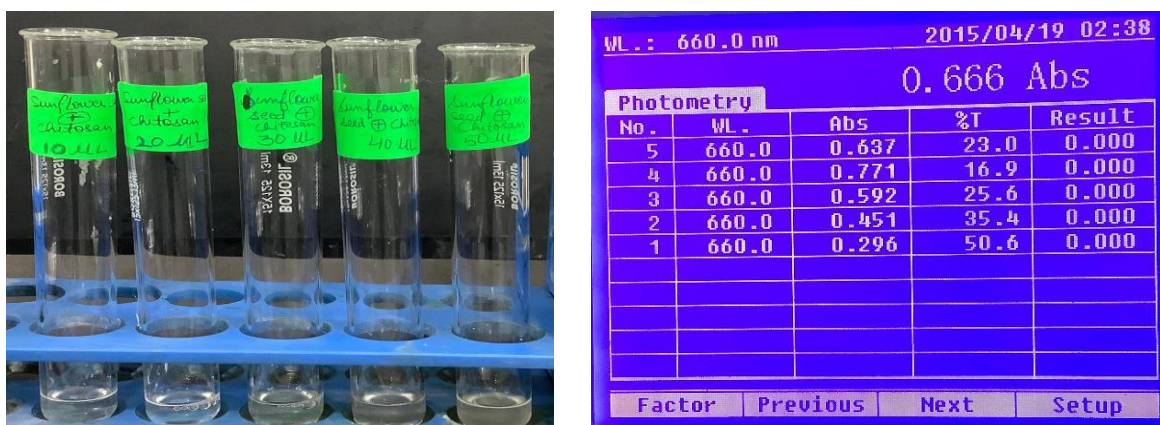
The antimicrobial activity of the prepared gel was done Different concentrations (10, 20, 30, 40 and 50µl) of chitosan-sunflower seed gel were tested under dual wavelength spectrophotometer at 660 nm wavelength. Absorbance and % transmittance values were noted.

## RESULTS

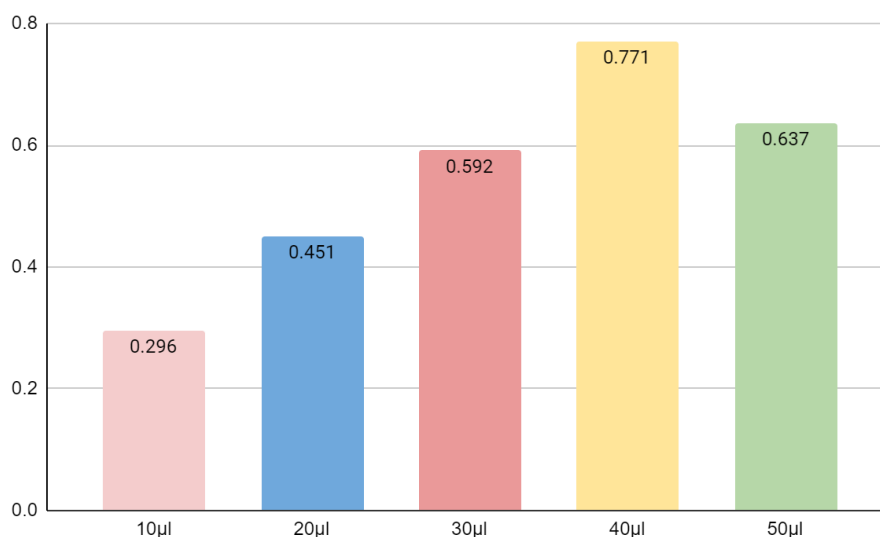
Results showed that the 40µl dilution of the gel had the highest value for absorbance (0.771), followed by 50µl (0.637), 30µl (0.592), 20µl (0.451) and 10µl (0.296) dilutions respectively. The %T value for the 40µl dilution (16.9%) was drastically lesser than that for all the other dilutions (23% - 50.6%).



**FIGURE 1:** Chitosan- Sunflower seed gel preparation from Sunflower seed extract and Chitosan extract.



**FIGURE 2:** Results of different concentrations of the chitosan-sunflower seed gel under dual wavelength spectrophotometer for anti inflammatory activity.



**FIGURE 3:** Bar graph showing absorbance values for different concentrations of the chitosan-sunflower seed gel.

## DISCUSSION

The study was conducted to assess the anti-inflammatory effect of a novel chitosan-sunflower seed gel in various dilutions. The results showed that the gel had the best anti-inflammatory effect at 40 $\mu$ l dilution of the prepared gel. Since the concentration of each constituent can dramatically impact the behavior of the formulation, it was important to evaluate the same.

The anti-inflammatory effect of chitosan and sunflower oil individually is well documented. Sunflower oil contains many important antiinflammatory compounds like linoleic acid, oleic acid and phospholipids. Oleic acid present in sunflower oil can cause disruption of the barrier of skin and increase permeation of substances through it.(Danby et al., 2013) This can lead to enhanced permeation of chitosan, thus amplifying its action. Phospholipids present in sunflower oil show antiinflammatory activity by controlling the covalently attached  $\omega$ -hydroxy ceramides in the epidermal layer and reducing the production of two proteins, TSLP (thymic stromal lymphopoietin) and TARC (thymus activation-regulated chemokine).(Cardoso et al., 2004) The closure of skin wounds caused by surgery can be positively influenced by applying oleic and linoleic free fatty acids topically. Of the two, oleic acid is more efficient in doing so.(Cooke et al., 2016; Norlén, 2016) It also stops the formation of nitric oxide in the vicinity of the wound. Vitamin E and magnesium present in sunflower oil exert antioxidant and antiinflammatory activity. Sunflower seed oil contains peptides, enzymes, l-ascorbic acid, carotenoids and phenolic compounds which have anti-inflammatory properties.(Teja, Ramesh and Priya, 2018) Whereas linoleic acid is pro inflammatory, the combined effect of all the other constituents make sunflower oil an overall antiinflammatory agent The peroxisome PPAR- $\alpha$  (proliferator-activated receptor-alpha) is activated by linoleic acid, which increases the production of lipids and the proliferation of keratinocytes.(Kapadia et al., 2002) As a result, the repair of the skin barrier is improved.(Kuo et al., 2022) These factors make sunflower oil a powerful source for wound healing.

Chitosan has an immune modulation property. It can upregulate pro-inflammatory mediators PGE2 and IL-1 $\beta$  by inflammasome activation using the NLRP3 pathway. Whereas the cGAS-

STING pathway leads to downregulation of CXCL10/IP-10 chemokine and upregulation of anti-inflammatory factor, IL-1Ra.(Vargas Villanueva et al., 2019) There are several factors that can influence the specific cytokine responses to chitosan stimulation observed in experiments. These include the chitosan preparation's properties (like polydispersity, molecular weight and degree of deacetylation), the presence of other proinflammatory agents and the dose of chitosan used. Research has shown that chitosans with at least 18 consecutive GlcN residues induce a type 1 interferon reaction at low doses without inflammasome activation. However, the same chitosans at increased dosage cause inflammasome activation and no type 1 interferon reaction. Chitosan also shows anti-inflammatory activity by decreasing PGE2 production.(Fong and Hoemann, 2018) Similarly, PGE2 production has been shown to be inversely proportional to the dilution of chitosan. (Torres-Rosas et al., 2020) A combination drug gel containing chitosan was shown to have reduced facial swelling and pain after lower third molar extraction in patients owing to its antiinflammatory effect. (Lopez-Lopez et al., 2015)

### *Limitations and Future Scope*

In the current study, only a specific proportion of chitosan and sunflower seed oil was used. It would be interesting to see if different proportions of the gel constituents would yield different results or not. Also, the combination of chitosan with ozonized sunflower seed oil may give rise to superior properties. Further studies should also be done evaluating the antiinflammatory effect of the chitosan-sunflower seed gel in clinical scenarios after safety assessment.

## CONCLUSION

The chitosan-sunflower seed gel is a new and potential alternative as a wound healing agent with excellent anti-inflammatory activity that requires further clinical assessment to better establish appropriate protocols for treatment.

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