ANTI-INFLAMMATORY ACTIVITY OF STEM EXTRACT OF TRIDAX PROCUMBENS BASED CHITOSAN GEL

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Article History: **Received:** 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

Introduction: Tridax procumbens, often known as coat buttons or tridax daisy, is a species of blooming plant that belongs to the daisy family. It is well known for being a widespread weed and nuisance plant. Although it is native to the tropical Americas, it has since expanded to regions with tropical, subtropical, and mild temperate climates. T. procumbens has long been used in India to treat wounds and as an insect repellent, antifungal, and anticoagulant. The main aim of this study is to evaluate anti-inflammatory activity of stem extract of T. procumbens based chitosan gel.

Materials & methods: T. procumbens stem powder was prepared and the extract was collected. Medium molecular weight chitosan was added to the stem extract to prepare the wound healing gel. Anti- inflammatory activity of stem extract based chitosan gel at different concentrations (10 µL, 20 µL, 30 µL, 40 µL, 50 µL) was evaluated by Bovine serum albumin (BSA) & egg albumin assay (EA assay).

Results: In BSA and EA assay, as the concentration increases from 10 µL to 50 µL the % inhibition also increases. The results showed that an increase in concentration increases anti-inflammatory activity.

Conclusion: This study revealed that the stem extract of T. procumbens based chitosan gel had a potent anti-inflammatory activity and can be used as a wound healing agent for oral applications.

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DOI: 10.31838/ecb/2023.12.s2.056

1. Introduction

Inflammation is a mechanism of defense that enables the body to protect itself against infection, toxic chemical allergens, burns or any other harmful stimuli. Inflammation is characterized by redness, pain, heat, swelling, as well as loss of function in the affected area.

A species of blooming plant belonging to the daisy family is called Tridax procumbens, also referred to as coat buttons or Tridax daisy. It is most well-known for being a pervasive weed and pest plant. This species has been utilized in Indian Ayurveda since the beginning of time (Gadgil, 1998). This species has been used in the production of a variety of products, including oils, tea, and skin poultice. Although it is endemic to the tropical Americas, it has been spread throughout the world to tropical, subtropical, and mild temperate climates. T. procumbens possesses a wide range of pharmacological qualities, including anti-inflammatory, anti-oxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, and antibacterial effects (Cáceres et al., 1998). T. procumbens has historically been used in India as an anticoagulant, antifungal, and insect repellant in addition to being used to heal wounds. The leaf juice is used to treat wounds and stop bleeding (Cáceres et al., 1998). It is useful for dysentery and diarrhea.

In traditional treatments, its leaf extracts were known to treat infectious skin problems. Along with treating gastritis and heartburn, it is a well-known ayurvedic remedy for liver conditions or having a hepatoprotective nature (Ravikumar, Shivashangari and Devaki, 2005). To confirm the claims that tribal people in the Udaipur district of Rajasthan were using the herb, research was conducted. T. procumbens leaves powdered with other herbs has been traditionally consumed by the people to treat diabetes. Potassium, which is used to relieve cramps and is a safe source material for upcoming therapeutic uses, has proven to be an excellent source of the species. The potential uses of this plant are shown by these conventional uses. The different pharmacological properties of T. procumbens, including radical scavenging, wound healing, anti-diabetic, antibacterial activity (Sridevi et al., 2018) and blood pressure lowering effects, have been reported in recent years. However, T. procumbens has not yet been proven through any scientific studies. When a protein denatures, it means that its tertiary structure and secondary structure are disoriented by external variables like heat, a strong acid or base, an organic solvent, or a concentrated inorganic salt. This process is known as protein denaturation. Williams et al proposed for an assay to replace animals in the early stages of screening for non-steroidal anti-inflammatory drugs known for the stabilization of heat-treated BSA by NSAIDs (Jeevitha et al., 2022). Since then, the assay has been used by a number of research teams to validate substances with potential medicinal applications. According to other literature, a number of herbs are used to cure inflammation (Jeevitha and Rajeshkumar, 2019; Begum et al., 2020; Ganta et al., 2020) (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; Rajeshkumar and Jeevitha, 2021); (Rajeshkumar and Jeevitha, 2021; Santhakumar et al., 2021). The scientific data, however, is insufficient. The current study aims to evaluate the anti-inflammatory activity of stem extract of T. procumbens based chitosan gel.

2. Materials & Method

Preparation of chitosan gel

0.5 mL of chitosan was dissolved in 49 mL of water and 1 mL of glacial acetic acid to make 50 mL of chitosan solution. The mixture was then maintained in a magnetic stirrer for 24 hours to ensure uniformity. T. procumbens stem extract was added and the magnetic stirrer was used once again for another 24 hours to prepare the gel. The anti-inflammatory activity of the prepared gel was then evaluated and compared with that of the commercial wound healing gel taken as standard.

Anti-inflammatory activity

Bovine Serum Anti-inflammatory assay

The following method, which Muzushima and Kabayashi proposed with particular modifications, was used to assess the anti-inflammatory effect of the gel (Pratik Das et al.,2019). Bovine serum albumin (1% aqueous solution) was mixed with 0.45 mL of chitosan gel of various concentrations (10 mL, 20 mL, 30 mL, 40 mL, and 50 mL), and a small amount of 1N hydrochloric acid was used to adjust the pH to 6.3. These samples were heated to 55 °C in a water bath for 30 minutes after being incubated at room temperature for 20 minutes. After the samples were cooled, a spectrophotometric calculation of the absorbance at 660 nm was made. The benchmark was diclofenac sodium. The control used is DMSO. Protein denaturation percentage was determined using the following equation,

% inhibition = (Absorbance of control – Absorbance of sample)/ Absorbance of control × 100

Egg albumin denaturation assay

A 5 mL solution was created using 2.8 mL of freshly manufactured, pH-6.3 phosphate buffered saline and 0.2 mL of hen's egg albumin extraction. For T. procumbens stem extract based chitosan gel, various preparations of specific concentrations (10 L, 20 L, 30 L, 40 L, and 50 L) were made. In this study, diclofenac sodium served as the positive control. The mixes were then heated for 15 minutes at 37ºC in a water bath. The samples were then allowed to cool to ambient temperature, and absorbance at 660 nm was measured.

3. Results

Anti-inflammatory activity of T. procumbens stem extract based chitosan gel was evaluated using denaturation of egg albumin and bovine serum albumin. In the present study, BSA assay & EA assay, T. procumbens stem extract based chitosan gel showed that the % inhibition was maximum at 50 µL concentration and lowest % inhibition at 10 µL concentration similar to the commercially available gel which is taken as the standard (Figure 1 and 2).

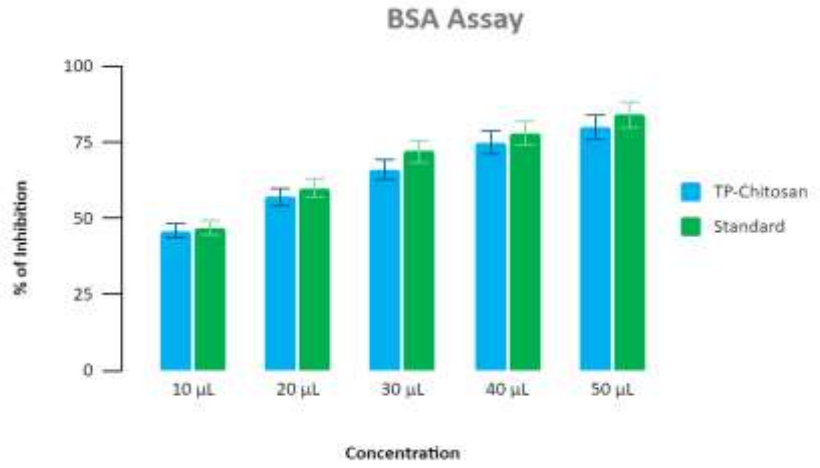

Figure 1: BSA assay, the graph depicts the comparison of % inhibition at various concentrations of T. procumbens stem based chitosan gel in comparison and the standard

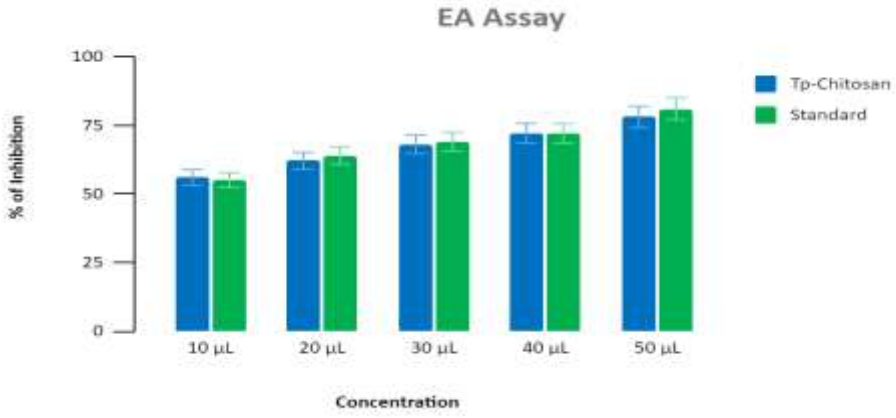

Figure 2: EA assay, the graph depicts the comparison of % inhibition at various concentrations of T. procumbens stem based chitosan gel in comparison and the standard

4. Discussion

The use of the BSA protein denaturation assay allowed researchers to avoid certain ethical issues associated with the use of animals for in vitro evaluation of anti-inflammatory activity, particularly in the early stages of screening for plant parts with potential anti-inflammatory compounds. Furthermore, protein denaturation has been described as a pathological process involving the loss of conformation and, as a result, functional loss (Sridevi et al., 2018). This causes the BSA protein denaturation assay perfect for determining anti-inflammatory compound's potential. It should be noted that the experiments were carried out at pH 6.3, which corresponds to the pathological pH

(6.2 - 6.5) at which heat treated BSA is apparently stabilized (denaturation is inhibited) by many NSAIDs (Oliveira et al., 2021), providing credence to the method of choice. As the standard drug, diclofenac sodium (at concentrations between 10 and 50 microL) also demonstrated concentration-dependent inhibition of protein denaturation. On the basis of prior reports describing its use for the same purpose, this was selected as the standard NSAID. Chitosan has shown to possess anti-inflammatory properties in toto (Rathinamoorthy and Sasikala, 2019; Chen et al., 2023). Due to chitosan's biocompatibility, non-toxicity, biodegradability, antibacterial activity, antioxidant activity, and muco-adhesive characteristics, many advantageous pharmacological qualities have been proposed. Also, it has been widely adopted in the pharmaceutical manufacturing of tablets as a controlled release dosage form, gel absorption enhancer, medication dissolving in wound-healing products, and the creation of micro/nanoparticles are only a few examples (El-banna et al., 2019). In addition to improving blood coagulation, it also stimulates the release of platelet-derived growth factor and transforming growth factor in vivo, all of which are essential for the wound healing process (Jain and Wairkar, 2019). The current inquiry examines stem extract in relation to the plant's potential use (Nwodo et al., 2011). The findings backed up the plant's traditional use for several painful and inflammatory conditions. It is hypothesized that one of the above mentioned constituents, or the combination of them all, is what causes the analgesic and anti-inflammatory actions because the same plant contains biologically active elements such flavonoids, tannins, phenolic compounds (Dahake and Kamble, 2014), and phytosterols. Further studies are being conducted to identify and describe the active ingredient in the stem extract of the T. procumbens. When investigating the anti-inflammatory effects of herbal medicines using the denaturation technique, the egg albumin method offers a less expensive option (Grant, Alburn and Kryzanauskas, 1970). Gel formulations have an advantage over solid products to have a better adherence to the mucosa and they can be applied all over the mouth. This study proves that the T. procumbens stem extract based chitosan gel can reduce inflammation in vitro comparable to commercial wound healing gel and thus can be used as an oral medication for wound healing.

5. Conclusion

T. procumbens stem extract based chitosan gel possesses anti-inflammatory activities in vitro which are shown in the present study and the results were comparable to commercial wound healing gel. The gel prepared was biosynthesized, economical and has potent biological properties. Further in vivo studies are needed to validate the current research.

Acknowledgement
We would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University for providing us support to carry out the study.

Conflict Of Interest
The authors declare that there were no conflicts of interest in the present study.

Source of Funding
The present project is supported by
- Saveetha Institute of Medical and Technical Sciences
- Saveetha Dental College and Hospitals, Saveetha University
- A R dental clinic

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