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Investigating The Phytochemical Composition and Anti-Inflammatory Activity of Martynia Annua

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ABSTRACT

Inflammation is a key factor in chronic diseases, and the search for safer therapeutic agents has led to increasing interest in plant-derived anti-inflammatory compounds. Martynia annua, traditionally used for inflammatory conditions, was investigated for its anti-inflammatory potential. To evaluate the in vitro anti-inflammatory activity of Martynia annua methanol extract (MAME) and develop hydrogel formulations for topical application. MAME was prepared by Soxhlet extraction and subjected to phytochemical estimation and TLC profiling. Anti-inflammatory activity was assessed using Anti-Lipoxygenase, Protein Denaturation, Proteinase Inhibition, Membrane Stabilization, and Hypotonicity-Induced Haemolysis assays. Hydrogels (MAME1–MAME5) were formulated using Carbopol-940 and Sodium CMC and evaluated for physicochemical properties and drug release. Dermal irritation studies were performed on Swiss albino rabbits. MAME exhibited significant in vitro anti-inflammatory activity, with 62% lipoxygenase inhibition and 75% inhibition of haemolysis at 500 µg/ml. The hydrogels showed acceptable pH (6.97–7.01), viscosity, spreadability, and sustained drug release. MAME3 demonstrated the highest release (66.07% at 240 min) and excellent topical characteristics. No dermal irritation was observed. MAME possesses promising anti-inflammatory potential, and its hydrogel formulation— particularly MAME3—offers an effective and safe approach for managing inflammatory conditions. Further in vivo studies and isolation of active constituents are recommended.

Keywords: Martynia annua; Methanol extract; Anti-inflammatory activity; Hydrogel formulation; In vitro assays; Lipoxygenase inhibition; Topical drug delivery

INTRODUCTION

Herbal medicine, one of the oldest forms of healthcare, has been used across cultures throughout history. Plants have been integral to human civilization, not only providing food, shelter, and clothing but also serving as a source of medicine (Patwardhan et al., 2005). Over time, the use of medicinal plants evolved from informal trial-and-error practices to well established pharmacopoeias, with each culture recording the healing properties of native herbs. Modern use of herbal medicine continues, particularly in the treatment of inflammatory conditions such as asthma, rheumatoid arthritis, colitis, and eczema (Chopra et al., 1956). While herbal supplements can be beneficial, it is crucial that they are used under the guidance of trained healthcare providers, especially considering the complexities involved in their use (Patwardhan et al., 2005). 1.2. Herbal Medicine and Inflammation Traditional medicine, passed down through generations, plays a central role in many communities, particularly in regions like India, China, and Africa. Well-known traditional systems such as Ayurveda, Siddha, and Unani have long utilized plants for their anti inflammatory properties (Ghasemian et al., 2016). Inflammatory disorders, characterized by the body's immune system attacking its own tissues, can result in pain, swelling, and other symptoms, ultimately leading to chronic conditions. Traditional medicine offers alternative treatments, many of which are based on plant-based compounds (Igbe et al., 2010). In recent years, increasing interest in alternative therapies has led to the examination of plant extracts for their anti-inflammatory effects. 1.3. Acute and Chronic Inflammation Page 1 Chapter-1 Introduction Inflammation, a natural response to injury or infection, can be classified as either acute or chronic. Acute inflammation occurs as an immediate response to harmful stimuli, such as injury or

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infection, and involves rapid immune system activation. It is characterized by the involvement of neutrophils, basophils, and eosinophils (Robbins and Cortran, 2004). Acute inflammation is typically short-lived, lasting a few days, and the outcome may involve resolution, the formation of an abscess, or progression to chronic inflammation. Chronic inflammation, on the other hand, is a prolonged response, lasting for months or even years. It is characterized by the persistent presence of macrophages, lymphocytes, and fibroblasts, which contribute to tissue destruction, fibrosis, and necrosis (Robbins and Cortran, 2004). Chronic inflammation can lead to several diseases, such as rheumatoid arthritis and inflammatory bowel disease, and is a significant focus of therapeutic research

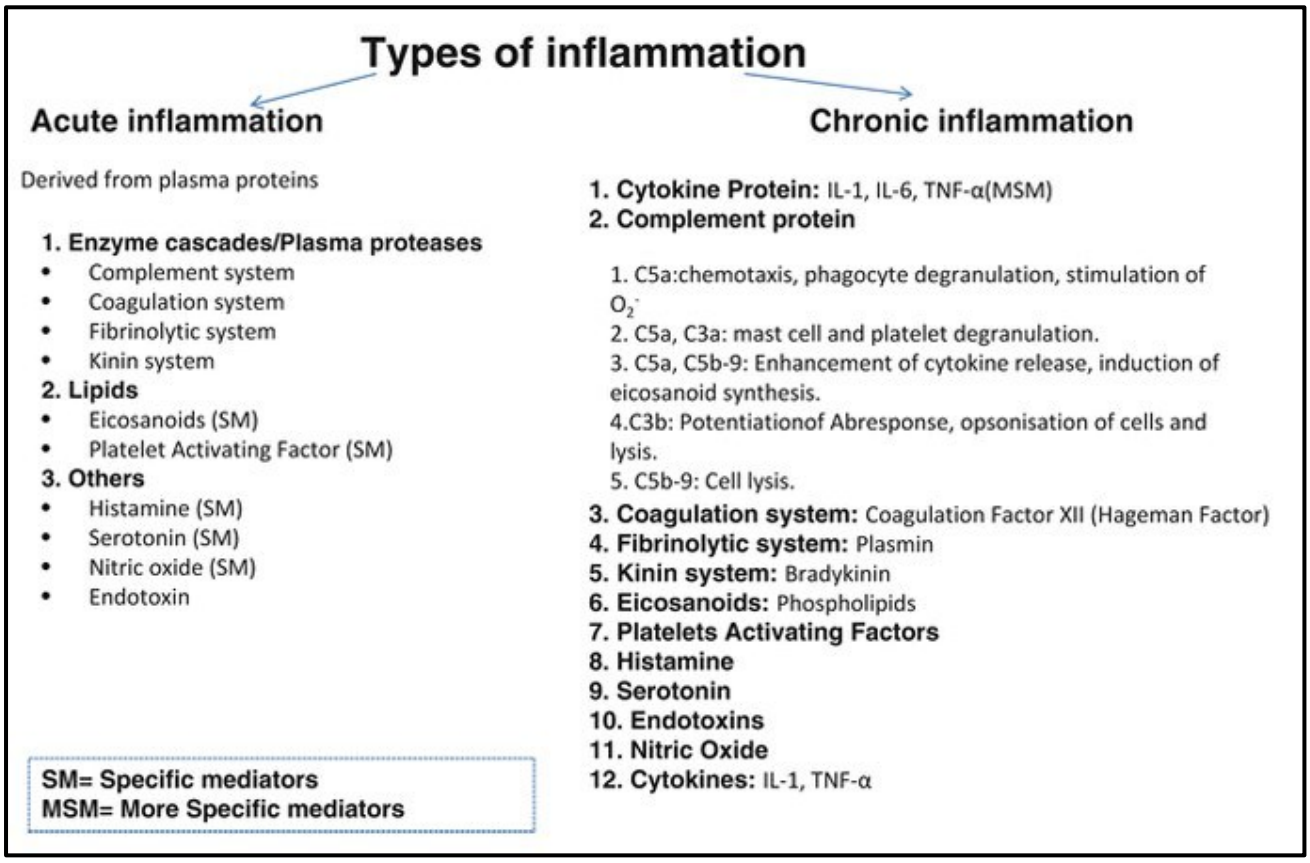


Figure1:Types of Inflammation

Bio-Mechanism of Inflammation

Inflammation is marked by a series of physiological events aimed at removing harmful stimuli and repairing tissue. The process begins with vasodilation, increasing blood flow to the affected area. Capillaries become more permeable, allowing fluid, proteins, and white blood cells (particularly neutrophils and monocytes) to enter the tissue (Rock and Kono, 2008). This increased vascular permeability leads to redness, heat, swelling, and pain at the site of inflammation. Redness and heat are caused by the increased blood supply, while swelling results from the accumulation of fluid and white blood cells. Pain arises from the release of chemical mediators and the compression of nearby nerves (Chen et al., 2018).

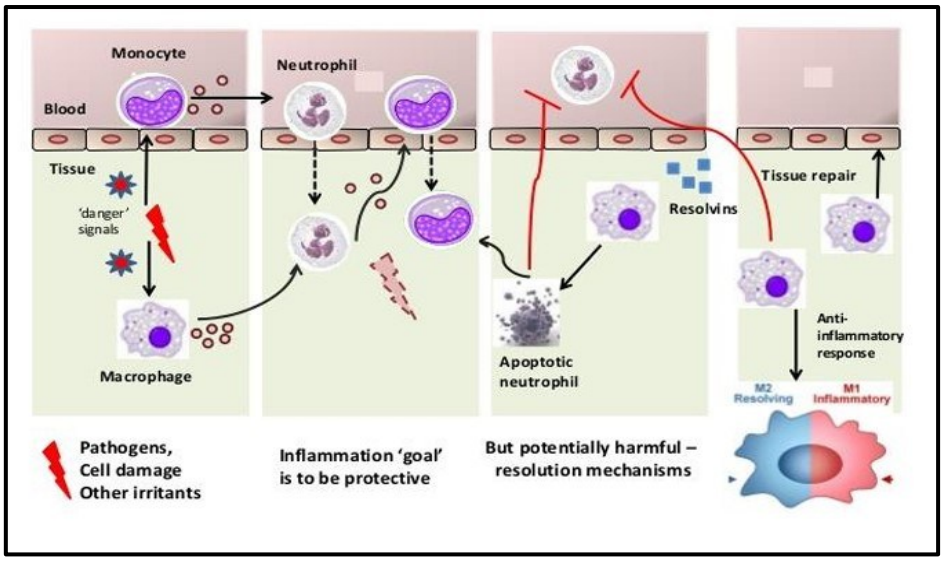


Figure 1.2: Inflammation and Body Response to Injury

Hydrogels in Drug Delivery

Hydrogels are swellable hydrophilic materials used in biomedical applications for controlled drug release. They are made from synthetic polymers such as polymethacrylate or polyvinylpyrrolidine, or natural polymers, offering biocompatibility and biodegradability (Tabata, 2009). In this study, hydrogels were formulated using Carbopol 940 and sodium carboxymethyl cellulose (CMC) to deliver M. annua extracts for topical anti-inflammatory therapy.

Current Treatments for Inflammation

While inflammation is a natural and protective response, it becomes problematic when it persists or is excessive. Chronic inflammation can lead to significant tissue damage and is associated with many diseases. Current treatment options primarily include anti-inflammatory drugs such as NSAIDs, steroids, and anti-histamines. NSAIDs (e.g., aspirin, ibuprofen) work by inhibiting cyclooxygenase (COX) enzymes, which are responsible for producing prostaglandins, key mediators of inflammation (Anderson, 2008). Despite their efficacy, long-term use of NSAIDs can result in serious side effects, including gastric ulcers, kidney damage, and increased risk of cardiovascular events (Birks et al., 2007). As a result, there is growing interest in alternative therapies, such as herbal medicine, which can offer anti-inflammatory effects without the

Hydrogels are swellable hydrophilic materials used in biomedical applications for controlled drug
adverse side effects associated with conventional drugs.

MATERIALS AND METHODS

Plant Material and Extraction

Martynia annua Linn leaves were collected from the GRKIST Pharmacy Campus in Jabalpur (M.P.), India, during August-September 2024. The specimens were authenticated at Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur. Dried powdered leaves (150 g) were subjected to Soxhlet extraction using petroleum ether for defatting, followed by ethanol extraction. The solvents were evaporated under reduced pressure, and extracts were stored in a desiccator.

Phytochemical Screening

Preliminary qualitative tests were performed for alkaloids, glycosides, saponins, sterols, carbohydrates, tannins, flavonoids, proteins, amino acids, fixed oils, gums, and mucilage using standard methods (Paech & Tracey, 1955; Kokate et al., 2002). Quantitative analysis included total phenolic content (Folin-Ciocalteu method) and total flavonoid content (aluminum chloride method), expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE), respectively. Thin-layer chromatography (TLC) was

conducted on silica gel plates with various solvent systems, visualized under UV light or iodine vapor.

Formulation of Hydrogels

Five hydrogel formulations (MAMFH1-MAMFH5) were prepared using Carbopol 940, sodium CMC, methanol extract of M. annua (MAME, 1% w/w), propylene glycol, methyl paraben, propyl paraben, triethanolamine, and distilled water. Polymers were dispersed in water, preservatives added, glycerin incorporated, extract mixed, and pH adjusted to 6.8-7.0 with triethanolamine.

Characterization of Formulations

Physical evaluation included appearance, color, odor, and homogeneity. pH was measured using a digital pH meter. Spreadability was calculated as M × L / T, where M is weight (80 g), L is distance (7.5 cm), and T is time. Viscosity was determined using a Brookfield viscometer. Drug content was quantified spectrophotometrically at 253 nm for luteolin. In vitro drug release was assessed using Franz diffusion cells over 240 minutes, with samples analyzed at 253 nm.

Pharmacological Studies

Anti-Lipoxygenase Activity

MAME (100-500 µg/mL) was incubated with lipoxidase enzyme and linoleic acid. Absorbance was measured at 234 nm, and inhibition calculated relative to control.

Protein Denaturation Inhibition

BSA solutions with MAME (100-500 µg/mL) were heated to 70°C, cooled, and turbidity measured at 660 nm. Inhibition was calculated as $100 \times (1 - A_2/A_1)$.

Proteinase Inhibition

MAME (100-500 µg/mL) was tested for inhibition of proteinase activity, with absorbance at 660 nm.

Membrane Stabilization

Human RBCs were exposed to hypotonic stress with MAME (100-500 µg/mL), and hemolysis inhibition measured at 660 nm.

Hypotonicity-Induced Hemolysis

RBCs in hypotonic solution with MAME (100-500 µg/mL) were assessed for hemolysis inhibition.

Dermal Irritation and Stability Studies

Primary dermal irritation was evaluated on albino rabbits, with Primary Dermal Irritation Index (PDII) calculated. Stability was assessed at 40°C ± 2°C / 75% RH ± 5% RH for 30 days.

RESULTS AND DISCUSSION

Phytochemical Composition

Petroleum ether extract yielded 2.6% (greenish-brown), ethanol extract 3.4% (brown). Qualitative screening showed alkaloids, glycosides, saponins, sterols, carbohydrates, phenolic compounds, flavonoids, proteins, amino acids, tannins, resins, and fixed oils in ethanol extract. Quantitative analysis revealed total phenolics (107.25 ± 3.66 mg GAE/g) and flavonoids (136.24 ± 4.08 mg QE/100 g). TLC profiles indicated multiple phytoconstituents with Rf values 0.36-0.74.

Formulation Characterization

All hydrogels were homogeneous, brownish, odorless, with pH 6.97-7.01, viscosity 193,600-197,500 cps, spreadability 14.27-17.64 g.cm/s, and drug content 0.084 µg/mL. MAMFH3 (1.5% Carbopol 940, 1% CMC) showed highest drug release (66.07% at 240 min).

Pharmacological Activity

MAME inhibited lipoxygenase (62% at 500 µg/mL), protein denaturation (71% at 500 µg/mL), proteinase (53% at 500 µg/mL), membrane stabilization (51% at 500 µg/mL), and hypotonic hemolysis (75% at 500 µg/mL), comparable to standards like indomethacin and aspirin.

Dermal Irritation and Stability

PDII was 0.25 (non-irritant). Formulations remained stable with no changes in appearance, odor, or pH.

These results highlight M. annua's potential as a source of anti-inflammatory agents, with hydrogel formulations offering safe topical delivery.

CONCLUSION

This study demonstrates that Martynia annua methanol extract possesses significant anti-inflammatory activity through multiple mechanisms, supported by its rich phytochemical profile. Hydrogel formulations, particularly MAMFH3, provide effective, controlled release for topical applications. The findings underscore the potential of M. annua as a natural alternative to synthetic drugs, warranting further in vivo and clinical studies for therapeutic development.

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