NOVEL DEVELOPMENT OF HYDROGEL LOADED WITH *DATURA STRAMONIUM* HERBAL EXTRACT FOR ANTI INFLAMMATORY ACTIVITY

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ABSTRACT

The present study focuses on the novel development of a hydrogel loaded with Datura stramonium herbal extract for enhanced anti-inflammatory activity. Datura stramonium, a medicinal plant traditionally recognized for its potent anti-inflammatory properties, was selected as the bioactive agent. The hydrogel matrix was formulated using biocompatible polymers to ensure optimal encapsulation, stability, and controlled release of the herbal extract. The Datura stramonium extract was obtained through solvent extraction and incorporated into a hydrogel base. The hydrogel was characterized for its physicochemical properties, including morphology, pH, viscosity, swelling index, and spread ability. In vitro anti-inflammatory activity was evaluated using protein denaturation and membrane stabilization assays.

INTRODUCTION:

Rheumatoid arthritis (RA) is characterized as a systemic autoimmune disorder marked by a persistent inflammatory response that can lead to damage in both the joints and various extra-articular organs, such as the heart, kidneys, lungs, digestive tract, eyes, skin, and nervous system. A variety of arthritic conditions have been studied and categorized, distinguishing between non-inflammatory arthritis, such as osteoarthritis, and inflammatory arthritis resulting from crystal deposits (including pseudogout, basic calcium phosphate disease, and gout), as well as those induced by bacterial and viral infections (such as Staphylococcus aureus, Neisseria gonorrhoeae, complications from Lyme disease, Parvovirus, and Enterovirus) or by autoimmune mechanisms.[1]

The diverse category of autoimmune rheumatic diseases encompasses conditions like systemic lupus erythematosus (SLE), Sjogren’s syndrome, adult-onset scleroderma, spondylarthritis (SpA), psoriatic arthritis (PsA), and polymyositis (PM), among others. Given their overlapping clinical manifestations, accurate differential diagnosis is crucial.

Despite numerous proposed biomolecular mechanisms, the precise etiology of RA remains incompletely understood, with current theories suggesting that dysregulated citrullination may lead to the formation of anti-citrullinated protein antibodies (ACPAs). The progression of RA is characterized by fluctuations, with episodic flare-ups; without appropriate treatment, symptoms tend to deteriorate progressively, resulting in irreversible joint damage and impairments in both physical and psychological well-being. Additionally, the complications and comorbidities associated with RA can significantly diminish patients' life expectancy.

Statistical analyses and quantitative data interpretations indicate that RA is not merely a clinical concern but also a significant public health challenge. Recent advancements in the pharmaceutical sector have introduced novel therapeutic strategies. Nevertheless, the challenge of identifying a curative treatment persists due to insufficient comprehension of the molecular mechanisms that dictate the behaviour of antibodies. The most effective therapeutic strategy hinges on early diagnosis, coupled with optimal pharmacological and non-pharmacological interventions, along with regular assessments of both therapeutic efficacy and safety. The primary objective of therapy is to achieve remission while minimizing adverse effects. Pharmacological agents that support the preservation of joint function are categorized into conventional synthetic disease-modifying antirheumatic drugs (DMARDs), biologic DMARDs, and targeted synthetic DMARDs, which the American College of Rheumatology (ACR) has classified as a new category of nonbiologic DMARDs. In cases where rheumatoid arthritis (RA) patients experience inadequate symptom management, the incorporation of nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids (GCs) as supplementary treatments is essential for alleviating inflammation. Common symptoms of RA include morning stiffness of the affected joints for >30 min, fatigue, fever, weight loss, joints that are tender, swollen and warm, and rheumatoid nodules under the skin. The onset of this disease is usually from the age of 35 to 60 years, with remission and exacerbation. It can also afflict young children even before the age of 16 years, referred to as juvenile RA (JRA), which is similar to RA except that rheumatoid factor is not found. In the West, the prevalence of RA is believed to be 1–2%, and 1% worldwide. Clinically, the diagnosis of RA can be differentiated from osteoarthritis (OA) as the affected areas in RA are the proximal interphalangeal (PIP) and metacarpophalangeal (MP) joints; OA typically affects the distal interphalangeal (DIP) joint (Fig. 1). OA is the most common type of arthritis and is caused by wear and tear rather than an autoimmune condition. It has no effects on the lungs, heart, or immune system. In addition, OA typically affects only one side of the body, as opposed to the symmetrical nature of RA.

Another differentiating factor is that RA patients suffer from persistent morning stiffness for at least ≥1 h. Patients with OA may have morning stiffness, but this typically resolves or decreases within 20–30 min. The goals of treatment for RA are to reduce joint inflammation and pain, maximize joint function, and prevent joint destruction and deformity. Treatment regimens consist of combinations of pharmaceuticals, weightbearing exercise, educating patients about the disease, and rest. Treatments are generally customized to a patient's needs and depend on their overall health. This includes factors such as disease progression, the joints involved, age, overall health, occupation, compliance, and education about the disease.[2]

2.DATURA STRAMONIUM:

Medicinal plants encompass a diverse array of bioactive compounds recognized for their pharmacological properties. Notably, a significant proportion of traditional medicines are derived from plant sources. Among these, Datura spp. stands out as a flowering herb belonging to the Solanaceae family, primarily utilized for its intoxicating and hallucinogenic effects. This species is extensively cultivated across Europe, Asia, the Americas, South Africa, and various tropical and subtropical regions.

Datura thrives in average soil conditions but shows a preference for nutrient-rich, moist, or alkaline soils. While the plant exhibits narcotic properties, it also possesses various health benefits, which can be attributed to its antimicrobial, antidiabetic, anti-asthmatic, anti-inflammatory, antioxidant, analgesic, insecticidal, cytotoxic, wound healing, and neurological effects. Furthermore, Datura is recognized for its larvicidal properties against the red flour beetle (Tribolium castaneum) and its efficacy as a mosquito repellent. Additionally, Datura spp. has been employed in treating animal bites, including snake bites, to alleviate pain. The species D. stramonium, in particular, is noted for its use in mystical and religious contexts, alongside its applications in herbal medicine. The seeds of D. stramonium are commonly smoked to induce hallucinogenic experiences.

However, it is crucial to acknowledge that the consumption of any part of the Datura plant can result in severe anticholinergic effects, leading to toxicity. The entire plant is toxic to varying degrees, with the seeds being the most hazardous; neither drying nor boiling mitigates these toxic properties. The Ayurvedic system of medicine recognizes D. stramonium as a valuable treatment for a range of ailments, including wounds, ulcers, rheumatism, fever, inflammation, asthma, and toothache.[4]

2.2 BOTANICAL DESCRIPTION OF DATURA:

The genus Datura is typically characterized by its annual or perennial herbaceous forms, which may exhibit glandular or predominantly simple trichomes. The leaves are petiolate, featuring a simple blade that may be sinuate or entirely dentate, measuring approximately 10–20 cm in length and 5–18 cm in width, and are covered with soft, short, greyish hairs. Flowers are solitary and are arranged in inflorescences located in the axils of leaves or at branch junctions. The larger flowers are predominantly actinomorphic and possess robust pedicels, while bracts, peduncles, and bracteoles are usually absent.

The seeds are dark, flat, and kidney-shaped, and the fruits resemble walnuts, being covered in spines, which has led to the common name "thorn apple."[4]

Table 1. Percentage compositions of chemical compositions in the seeds of d. metel, d. stramonium, d. innoxia.

CHEMICAL CONSTITUENTS	D.METEL (%)	D.STRAMONIUM (%)	D.INNOXIA (%)
FAT/LIPID	14.72	16.60	15.52
CARBOHYDRATES	51.22	26.20	-
PROTEIN	20.73	16.20	13.90
MOISTURE	4.63	8.50	10.00
ASH CONTENT	5.14	8.70	8.26
CRUDE FIBRE	17.35	23.70	6.55

Table 2. concentration (µg/mg) of atropine and scopolamine in different plant parts of d. stramonium

PLANT PARTS	ALKALOID	YOUNG PLANT	ADULT PLANT
STEMS	ATROPINE SCOPOLAMINE	0.915 ± 0.015 0.129 ± 0.014	0.001 ± 0.001
SEEDS	ATROPINE SCOPOLAMINE	0.670 ± 0.003 0.012 ± 0.001	0.387 ± 0.015 0.089 ± 0.010
FLOWERS	ATROPINE SCOPOLAMINE	0.299 ± 0.021 0.106 ± 0.031	0.270 ± 0.026 0.066 ± 0.004
ROOTS	ATROPINE	0.121 ± 0.015	-

	SCOPOLAMINE	0.014 ± 0.004	-
MEDIUM LEAVES	ATROPINE SCOPOLAMINE	0.831 ± 0.014 0.041 ± 0.005	0.150 ± 0.002 0.022 ± 0.005

Figure 1: Seed of *Datura stramonium*

EXCIPIENTS

1.GELLING AGENTS: CARBAPOL 940

Carbopol 940 is a synthetic polymer commonly used as a gelling agent in various pharmaceutical and cosmetic formulations. It is particularly effective in creating stable gels for topical applications due to its ability to form a network structure when neutralized with alkaline substances like triethanolamine.

USES: Gelling Agent: Carbopol 940 is widely used to create gels for topical drug delivery systems. It enhances the viscosity and stability of formulations, making them suitable for skin application.

Figure 2: Carbopol

2.PRESERVATIVES: METHYL PARABEN

Methylparaben is a widely used preservative in the cosmetic and pharmaceutical industries due to its antimicrobial properties.

Figure 3: Methyl paraben

3.TRIETHANOLAMINE:

Pharmaceuticals: Acts as a pH adjuster and stabilizer in drug formulations, ensuring the stability and efficacy of pharmaceutical products.

Gelling Agent Neutralizer: TEA is used to neutralize Carbopol 940, a common gelling agent in topical formulations, creating stable gel structures.

Figure 4: Triethanolamine

MATERIALS AND METHODOLOGY:

COLLECTION OF PLANTS:

The mature plant of Datura stramonium was collected and the seed part of the plants were isolated.

EXTRACTION PROCESS:

The seeds were cut into pieces and were grinded into slurry by using the water. The slurry was allowed to stand for a day for the particles to settled down. The supernatant fluid was removed and a thick slurry was formed.

Figure 5: Extract of *Datura stramonium*

PREPARATION OF THE POWDERED DRUG FROM THE EXTRACT:

The thick slurry was transferred to the petri dish and was dried by using hot air oven at 60°C and fine powder was obtained after sieving.

Figure 6: Powder of *Datura stramonium*

PREPARATION OF GEL:

Take 0.5g of sample and 0.5g Carbopol added with the 70ml of distilled water together with the suitable preservative and agitate them by using magnetic stirrer over 30 to 40 mins for the formation of the clear mixture without any clumps and finally added with the 3 to 5 drops of triethanolamine and a gel was formed.

Figure 7: Hydrogel loaded with *Datura stramonium*

FORMULATION OF DATURA STRAMONIUM GEL:

TABLE 1: List of ingredients

S.NO	INGRIDIENTS	ROLE	QUANTITY
1	Datura stramonium	Anti-inflammatory	0.5 gm
2	Carbopol 940	Gelling agent	0.5 gm
3	Triethanolamine	pH adjuster	3-5 Drops
4	Methyl Paraben	Preservative	0.2 gm

EXTRACTION OF DATURA STRAMONIUM:

- The fruit part of datura stramonium is collected
- The collected fruit is grinded into fine paste loke consistency
- Then obtained paste of datura stramonium is filtered in mesh cloth

PREPARATION OF CARBAPOL 945 GEL BASE:

- Add 1g of carbapol in 200 ml water.
- Then add triethanolamine of few ml.
- Add NaOH few drops to raise pH for thickening.

PREPARATION OF DATURA STRAMONIUM GEL:

- The required amount of extract powder is then incorporated with Carbopol 945 i.e., a gelling agent.
- It is then finely mixed with the gelling agent using mechanical stirrer.
- The ideal preservatives are then added and incorporated.
- pH regulators are then added.
- The extract is then stored in a container.

RESULTS AND DISCUSSION:

Physical Examination:

Physical parameters like colour, odour, taste and appearance were examined by visual examination.

pH:

The pH test was conducted using a pH meter and repeated three times. The pH values that meet the criteria are 4-6.

Drying Time

The drying time test on the formed film was carried out by applying the preparation on a glass object that had previously been heated at 370 C. The test parameters for drying time are less than 5 minutes.

Viscosity:

Apparent Viscosity Apparent viscosity was obtained with a rheometer. All rheological measurements were conducted by a parallel plate geometry (40 mm diameter) at 32 ◦C. Samples were analyzed monitoring the viscosity (η, Pa·s) as a function of the shear rate in the

range of 10–1000 s−1. The gap of the assay was 400 µm. Apparent viscosity was selected at 61.55 s

pH STABILITY:

A calibrated pH meter was used to measure pH stability. To assess pH changes, gel formulations were stored on a shelf at 37°C and 40°C. The results were compared over a six-week period.

DSC

The thermal stability of the hydrogel was evaluated by performing thermogravimetric analysis. The thermal degradation temperature refers to the temperature of the first weight loss. Figure shows the thermogravimetric curves for hydrogel. Moreover, with the increase of the degree of Hydrogel, the T_m value gradually increased, completely consistent with the results of the thermogravimetric analysis. The glass transition temperature (T_g) did not much vary from 120 °C as the PLA polymerization degree was increased, indicating little effect of the stereocomplex on the movement of the segments in the blend.

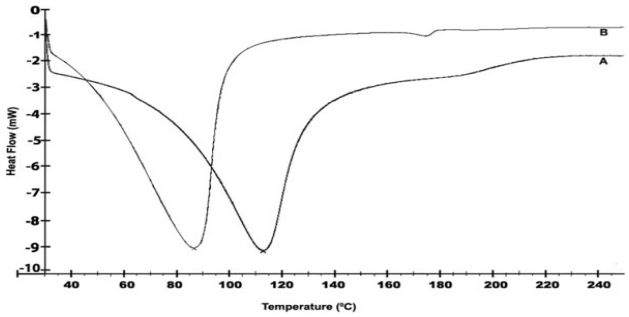

Figure 8: DSC for *Datura Stramonium* hydrogel

XRD

An X-ray diffractometer operating at a voltage of 40 kV and current of 40 mA and with Cu Kα radiation (λ = 0.15418 nm) was used to determine the formation of the stereo complex. The powdery macromonomer and the gel were fully dried, and evenly placed on the glass slide. The X-ray data were recorded using a scanning angle ranging from 10° to 50° and a measuring speed of 3° min^{-1}.

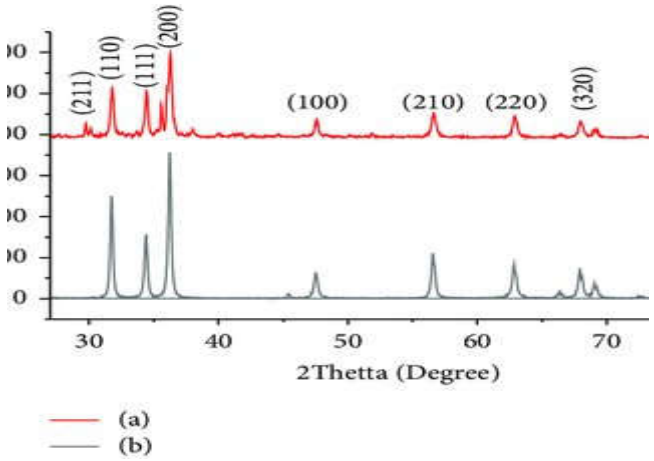

Figure 9: XRD for *Datura Stramonium* hydrogel

In Vitro Release Studies

The release behavior of Curcumin from the hydrogel was evaluated by dialysis, as other studies have already performed. Briefly, 1 g of each formulation was placed inside a dialysis bag, previously rinsed with PBS pH 7.4/ethanol/Tween® 80 mixture in ratio 74.5:25:0.5 (% v/v). The system was placed in 100 mL of the same medium under gentle stirring at room temperature. Samples (1 mL) were taken at scheduled times (0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 24, 48 and 72 h) and the same amount of fresh medium was added.

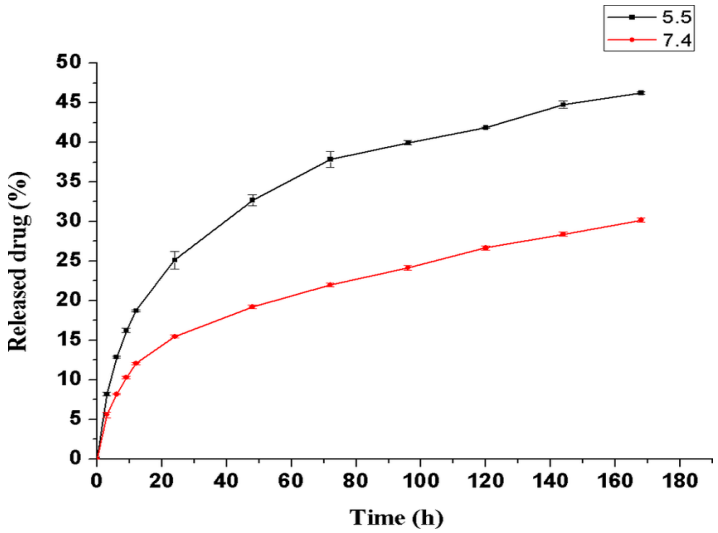

Figure 10: Release profile for *Datura Stramonium* hydrogel

ANTIMICROBIAL STUDY:

The formulated gel was inoculated in the plates of agar media by streak plate method and a control was prepared. The plates were placed in the incubator and are incubated at 37°C for 24 hours. After the incubation period plates were taken out and checked for microbial growth by comparing it with the control.

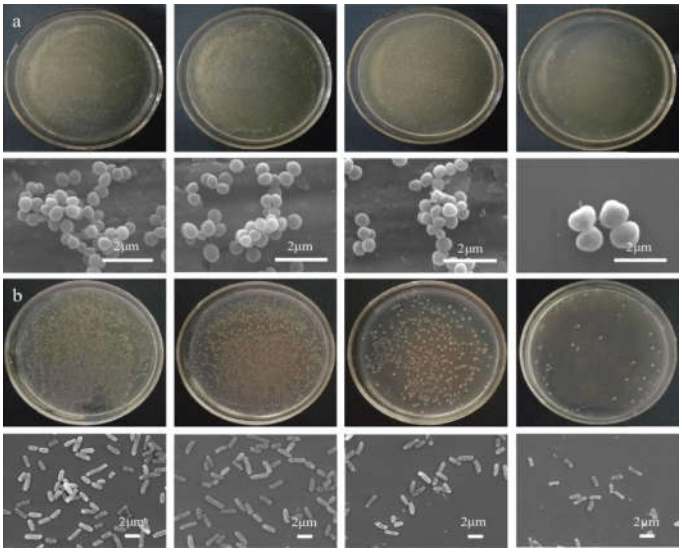

Figure 11: Antimicrobial activity for *Datura Stramonium* hydrogel

ANTI-INFLAMMATORY ACTIVITY

The in vitro anti-inflammatory activity of the sample was determined by the inhibition of protein denaturation method. The different concentrations of samples, such as 6.25 µl/mL, 12.5 µl/mL, 25 µl/mL, and 50 µl/mL, were made from stock solution. Bovine serum albumin (BSA) and distilled water were made up of the test control; the test solution consists of 0.45 ml of distilled water and different concentrations of the sample. The diclofenac sodium was used as a standard solution. All the above solutions were adjusted to pH 6.3 using 1N hydrochloric acid. The samples were incubated at 37°C for 20 minutes, and the temperature was increased to 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer was added to the solutions. The absorbance was measured using a UV-visible spectrophotometer at 416 nm.

% Denaturation inhibition,

$$= \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

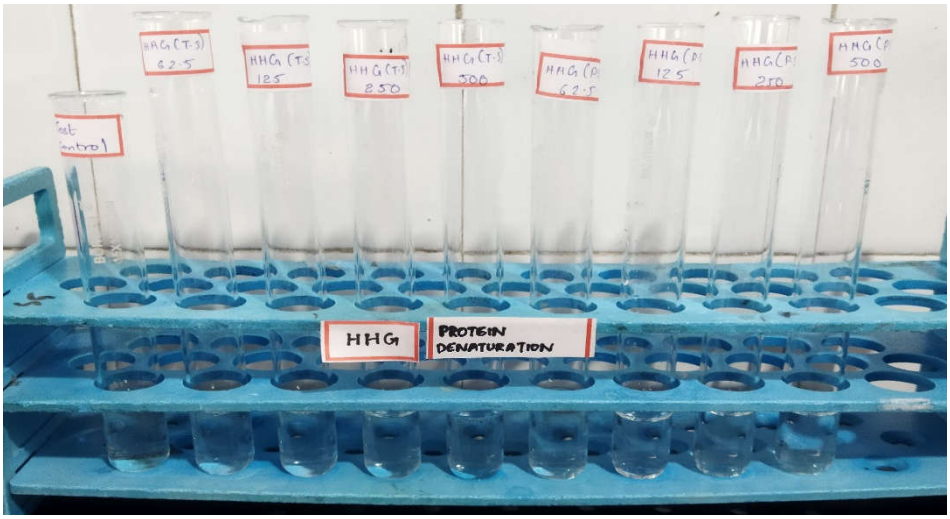

Figure 12 : Antiinflammatory activity for *Datura Stramonium* hydrogel

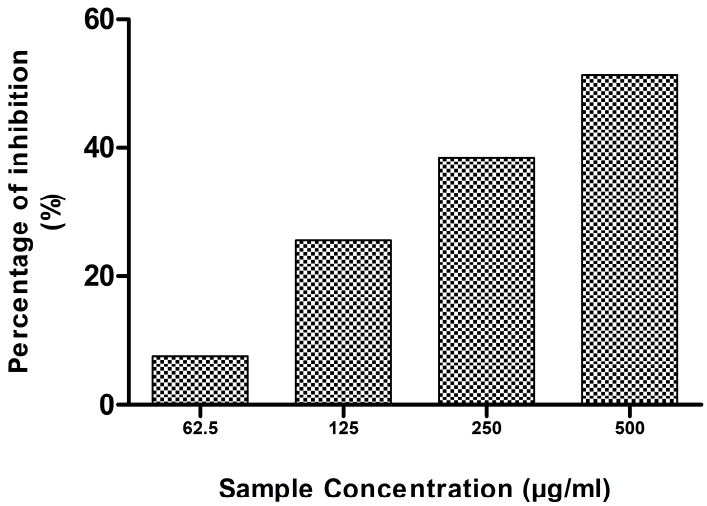

Figure 13 : Antiinflammatory activity for *Datura Stramonium* hydrogel

CONCLUSION

The developed hydrogel loaded with Datura stramonium extract exhibits **promising anti-inflammatory activity**, making it a potential candidate for topical therapeutic applications in the management of inflammatory disorders. This approach highlights the synergy between herbal medicine and advanced drug delivery systems, paving the way for further pharmacological and clinical investigations. The hydrogel formulation demonstrated favourable physical characteristics and stability. In vitro assays revealed significant inhibition of protein denaturation and membrane stabilization, indicating strong anti-inflammatory potential. No adverse effects were observed, supporting the safety of the formulation.

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