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ORIGINAL ARTICLE **OPEN ACCESS**

Exploring the *In Vitro* Anti-Inflammatory and Anti-Arthritic Potential of Swarna Bhasma, a Traditional Ayurvedic Preparation

[1]Mayuri Bapu Chavan*, [2]Apoorva Ranade, [3]Hrutuja Wagh, [4]Nivedita Gaikwad, [5]Atharva Tilak, [6]Mohini Kadam, [7]Saloni Shinde, [8]Meera Deshmukh, [9]Pranati Tilak
[1,4-7]Department of Pharmacology, TMV's Lokmanya Tilak Institute of Pharmaceutical Sciences, Pune
2Department of Pharmaceutics, TMV's Lokmanya Tilak Institute of Pharmaceutical Sciences, Pune
[3]Department of Pharmaceutical Chemistry, TMV's Lokmanya Tilak Institute of Pharmaceutical Sciences, Pune
[8-9]TMV's Lokmanya Tilak Institute of Pharmaceutical Sciences, Pune
Corresponding author:
Mayuri Bapu Chavan
E-mail address: cmayuri7282@gmail.com

ABSTRACT
Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder. It induces inflammation in the synovial tissue, leading to progressive joint degeneration and functional impairment. Conventional therapies can be effective, but they are associated with high costs, side effects, and limited ability to cure. Traditional Ayurvedic formulations, such as Swarna Bhasma, have long been valued for their anti-inflammatory and rejuvenating properties; however, their anti-arthritic potential still lacks comprehensive scientific validation. This study evaluated the in vitro anti-arthritic and anti-inflammatory effects of the ethanolic extract of Swarna Bhasma (EESB). A denaturation test for proteins was performed to evaluate the anti-arthritic activity. The anti-inflammatory potential of human red blood cells (HRBCs) was evaluated using a membrane stabilization assay, with diclofenac sodium serving as the standard reference drug.| EESB prevented protein denaturation in a concentration-dependent manner. The inhibition rates were 39.45% at 250 µg/mL, 51.37% at 500 µg/mL, and 61.01% at 1000 µg/mL. Similarly, in the HRBC membrane stabilization assay, EESB demonstrated 39.45%, 51.37%, and 61.01% inhibition at the same concentrations. Although diclofenac sodium consistently exhibited higher activity, EESB demonstrated significant protective effects against protein denaturation and membrane lysis, confirming its ability to stabilize proteins and cell membranes under inflammatory stress. These findings offer preliminary evidence supporting the traditional claims of Swarna Bhasma as an anti-arthritic and anti-inflammatory agent. Although less potent than diclofenac sodium, EESB demonstrated consistent dose-dependent effects, warranting further in vivo and mechanistic investigations.
Keywords: *Swarna Bhasma, Rheumatoid Arthritis, Anti-inflammatory, protein denaturation, Ayurvedic*

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INTRODUCTION

Rheumatoid arthritis is a long-lasting, immune-mediated condition marked by inflammation of the synovial membrane and gradual joint deterioration, which progressively damages the joints and results in significant pain, disability, and loss of function [1]. Epidemiological studies have consistently demonstrated a global prevalence of 0.5-1.0%[2]. Indian population data from 1990 to 2021 show a consistent increase in the occurrence, prevalence, and disability-adjusted life years of rheumatoid arthritis, with women having higher values. The peak incidence occurs in the 65-69 age group, and rates are expected to rise until 2036[3]. Inflammation is a fundamental protective mechanism of the body, characterized by the classic presentation of redness, swelling, pain, and warmth, which signals immune system activation and tissue repair processes[4]. However, in rheumatoid arthritis, this preventive response is chronically dysregulated, resulting in progressive damage to the tissue. The pathogenic process begins when immune cells migrate to the synovial region, resulting in a chronic inflammatory milieu. These cellular structures produce inflammatory cytokine proteins, including Interleukin-1β, Tumor Necrosis Factor-alpha, and IL-6, that play a role in progressing the disease. These inflammatory mediators activate transcription factors, thereby increasing the expression of inflammatory genes and maintaining a persistent state of inflammation. This environment promotes abnormal synovial tissue growth and pannus

production, resulting in cartilage degradation and bone destruction[5]. Although disease-modifying antirheumatic drugs (DMARDs) have improved rheumatic disease management, these interventions are still palliative rather than curative, raising significant challenges such as severe side effects, high costs, and immunosuppressive complications[6]. Traditional medicine practices provide increased safety, cost-effectiveness, and comprehensive therapeutic solutions. The growing research base supports their efficacy, minimal side effects, and affordability. Integrative therapeutic strategies that combine conventional and traditional approaches can enhance patient outcomes, mitigate treatment-related risks and lower healthcare costs [7]. Swarna Bhasma, an Ayurvedic preparation derived from purified gold, has been esteemed for centuries as a potent therapeutic substance in classical Indian medicine[8]. Gold-derived bhasma is highlighted in ancient Ayurvedic texts as a bioactive formulation with rejuvenating and disease-modifying properties. In the past, it has been used to treat ailments such as accelerated aging, general debility, infertility, cognitive decline, and vision impairment. Ayurvedic practitioners have used Swarna Bhasma in therapeutic settings to treat conditions such as rheumatoid arthritis (RA), diabetes, neurological problems, and bronchial asthma[8,9]. Despite its historical use in autoimmune and inflammatory diseases, scientific data supporting its anti-inflammatory or anti-arthritic properties are still lacking. Membrane stabilization assays are frequently used in vitro to assess anti-inflammatory and anti-arthritic efficacy and are reliable models for preliminary screening. Lysosomal enzymes are released during inflammatory situations because of membrane destabilization, worsening tissue injury, and inflammation. Thus, maintaining a stable lysosomal membrane is a crucial therapeutic target for moderating inflammation [10]. Since human RBC membranes resemble lysosomal membranes, the RBC stabilization assay was used to assess the protective effect of the test samples against hypotonicity-induced hemolysis, suggesting their ability to prevent lysosomal enzyme release. Similarly, protein denaturation experiments, such as the suppression of heat-induced albumin denaturation, mimic arthritic surroundings, demonstrating the ability of drugs to interfere with inflammation[11]. Thus, the present study aimed to assess the *in vitro* anti-inflammatory and anti-arthritic effects of Swarna Bhasma.

MATERIAL AND METHODS

Procurement of Swarna Bhasma

For this study, the test compound, Swarna Bhasma (SB), was procured from a registered Ayurvedic pharmacy in Pune, Maharashtra, India. The formulation was manufactured by Unjha Pharmacy, a GMP-certified Ayurvedic pharmaceutical company based in Ahmedabad, Gujarat. The product was obtained under proper licensing and regulatory compliance, according to the guidelines prescribed by the licensing authority for Ayurvedic formulations. The product was stored in a sterile, airtight container under the recommended conditions until further use in experimental procedures.

Drugs and chemicals

Diclofenac sodium was obtained from a pharmacy in Pune, Maharashtra. All other chemicals used were of analytical quality and obtained from commercial sources. Throughout the study, double-distilled water was used.

In Vitro Anti Arthritic Activity

Protein Denaturation Assay

In certain patients with arthritis, the development of autologous antigens may result from damage to tissue proteins. This can be considered an indicator of arthritis and inflammation [12]. Therefore, a protein denaturation test was performed. To reach final concentrations of 250, 500, and 1000 µg/mL, a reaction mixture comprising 5.6 mL of phosphate-buffered normal saline (PBS, pH 6.4), 0.4 mL of fresh hen's egg albumin, and 4 mL of ethanolic extract of Swarna Bhasma (EESB) at different concentrations was prepared in a 10 mL volume[12]. The control was an equivalent volume of double-distilled water. After incubating the same for 15 min at 37 ± 2 °C, all mixtures were heated at 70 °C for five minutes. Absorbance was measured at 660 nm after cooling, using the vehicle as a blank. Diclofenac sodium at the same concentrations (250, 500 and 1000 µg/mL) was processed under the same conditions as the reference standard[13,14]. The following formula was used to determine the percentage of protein denaturation inhibition:

% inhibition = absorbance of control - absorbance of test/absorbance of control x 100 [15]

In Vitro Anti-Inflammatory Activity

HRBC Membrane Stabilization Assay

The human RBC stabilization technique was used to evaluate the anti-inflammatory effect of Swarna Bhasma. This technique is based on the idea that since the RBC membrane is similar to the lysosomal membrane, its stability suggests that the lysosomal membrane may also be stabilized during inflammation, preventing the release of enzymes that damage tissue [16]. Fresh blood (2 mL) from a healthy human

volunteer was drawn in a 1:1 ratio in Alsever's solution and centrifuged for 10 min at 3000 rpm. The RBC pellet was washed thrice with isotonic saline (0.9% NaCl), and a 10% RBC suspension was prepared in isotonic saline. Each test's reaction mixture included 1 milliliter of the test sample, which was either the standard reference drug diclofenac sodium or the ethanolic extract of Swarna Bhasma (EESB) at different concentrations (250, 500, and 1000 µg/mL), 1 milliliter of the 10% RBC suspension, and 1 milliliter of hypotonic solution (distilled water), which served as a hemolytic agent. After 30 min of incubation at 37 °C, the mixtures were centrifuged for 10 min at 3000 rpm. To ascertain the degree of hemolysis and the membrane-stabilizing effect, the absorbance of the supernatant was measured at 540 nm[13,14]. The following formula was used to calculate the percentage of HRBC membrane stabilization:

% inhibition of Hemolysis = absorbance of control - absorbance of test/absorbance of control x 100[17]

Statistical analysis

Statistical analyses were performed using two-way ANOVA followed by Tukey's multiple-comparisons test. Data are presented as mean ± SEM, and $p < 0.05$ was considered statistically significant.

RESULTS

***In Vitro* Anti Arthritic Activity by Protein Denaturation Assay**

The protein denaturation method was used to assess in vitro anti-arthritic activity, and the percent inhibition of denaturation at various concentrations (250, 500, and 1000 µg/ml) was calculated. The inhibitory effect of the ethanolic extract of ***Swarna Bhasma*** (EESB) on heat-induced protein denaturation was assessed and compared with that of diclofenac sodium, which was used as a reference standard. A concentration-dependent increase in inhibition was observed for the ethanolic extract of the sample (EESB). The extract exhibited 39.45 % inhibition at 250 µg/ml, which increased to 51.37 % at 500 µg/ml and reached a maximum of 61.01 % at 1000 µg/ml, as shown in **Error! Reference source not found.**. The highest EESB activity was recorded at 1000 µg/ml, indicating significant anti-arthritic potential through protein stabilization (**Error! Reference source not found.**).

***In Vitro* Anti-Inflammatory Activity by HRBC Membrane Stabilization Assay**

The HRBC membrane stabilization assay was used to assess the anti-inflammatory properties of the ethanolic extract of Swarna Bhasma (EESB), and the percentage inhibition was computed at various concentrations (250, 500, and 1000 µg/ml). The extract demonstrated a concentration-dependent increase in membrane-stabilizing activity. At 250 µg/ml, the extract showed 39.45% inhibition, which increased to 51.37% at 500 µg/ml, and reached a maximum of 61.01% inhibition at 1000 µg/ml. Although standard diclofenac sodium exhibited higher membrane stabilization across all tested concentrations, the extract showed significant activity in a dose-dependent manner, with the highest protection observed at 1000 µg/ml, as shown in (**Error! Reference source not found.**).

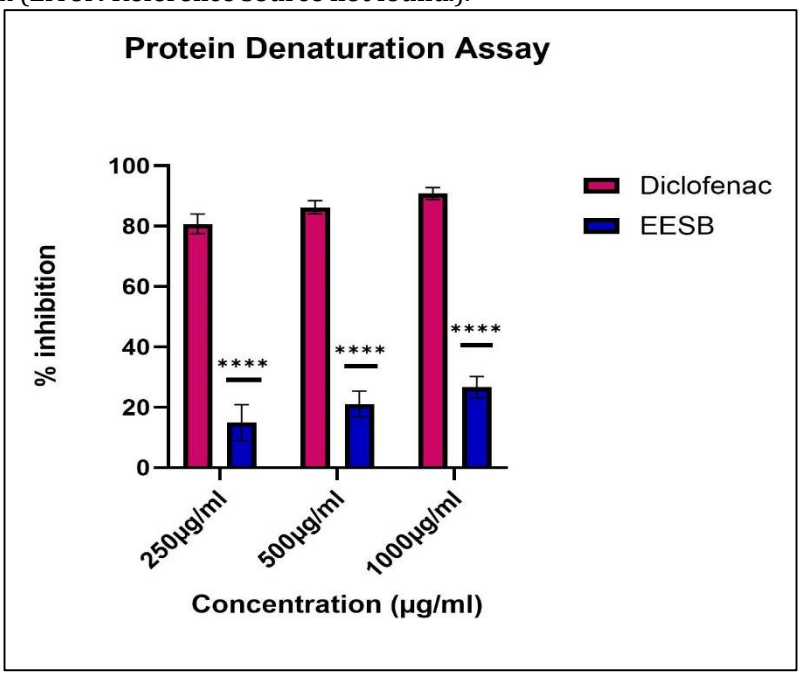

Figure 1: Protein denaturation-inhibitory activity of EESB and diclofenac at different concentrations. Data are presented as mean ± SEM (n = 3). ****P < 0.0001 compared to EESB (two-way ANOVA).

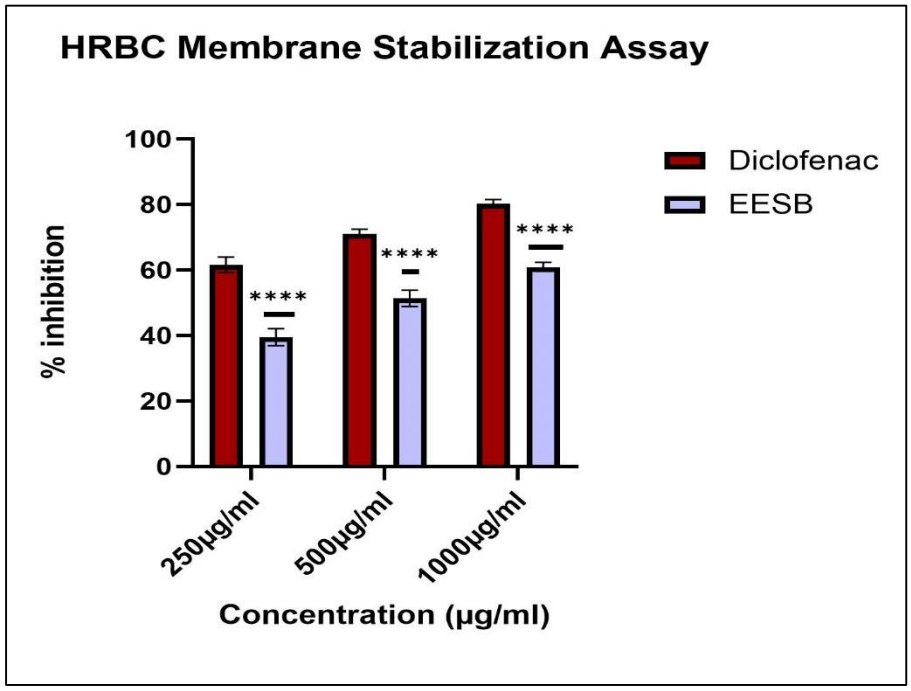

Figure 2.Effect of diclofenac and EESB on HRBC membrane stabilization at different concentrations. Values are expressed as mean ± SEM (n = 3).). ****P < 0.0001 compared to EESB (two-way ANOVA).

DISCUSSION

Traditional Ayurvedic formulations, such as Swarna Bhasma, have been extensively used to manage chronic inflammatory disorders, including rheumatoid arthritis. Recent advances in nanomedicine suggest that metallic bhasmas contain ultra-fine or nanosized particles that can interact with biological macromolecules and modulate inflammatory pathways.[18]. The present investigation used protein denaturation and HRBC membrane stabilization assays to examine the ethanolic extract of Swarna Bhasma (EESB) for its in vitro anti-inflammatory and anti-arthritic properties[19].

Protein denaturation is a crucial pathogenic event in rheumatoid arthritis, as it generates altered autoantigens that trigger immune responses against host tissues[20]. At 250, 500, and 1000 µg/mL, EESB exhibited concentration-dependent reduction in protein denaturation, with 39.45%, 51.37%, and 61.01% inhibition, respectively. Although the standard exhibited comparatively higher inhibition at the same concentrations, the progressive response of EESB highlights its ability to stabilize protein structure under thermal stress. Previous reports have indicated that non-steroidal anti-inflammatory drugs (NSAIDs) exert anti-arthritic effects by interfering with hydrogen bonding, hydrophobic interactions, and electrostatic forces in denatured proteins. The comparable trends observed with EESB suggest that similar molecular interactions may underpin its stabilizing effect, thereby supporting its anti-arthritic relevance[21]. The HRBC membrane stabilization assay further substantiated the protective role of EESB in maintaining cellular integrity. Membrane rupture and hemolysis are associated with the release of lysosomal enzymes (e.g., proteases and phospholipases), which amplify inflammation through tissue degradation and cytokine release. In our study, EESB exhibited 39.45% protection at 250 µg/mL, increasing to 61.01% at 1000 µg/mL, indicating dose-dependent membrane stabilization comparable to that of standard anti-inflammatory agents. This activity may be attributed to the inhibition of lipid peroxidation and preservation of membrane phospholipids, mechanisms reported for other metallic and herbal anti-inflammatory preparations. Although diclofenac sodium exhibited stronger stabilization, the significant protection observed with EESB supports its potential role as a complementary anti-inflammatory agent[22].

Collectively, the dual inhibitory effects of EESB on protein denaturation and membrane destabilization confirm its anti-arthritic and anti-inflammatory potential. The dose-dependent pharmacological profile observed in both assays indicated reproducible and biologically relevant activity. Importantly, these results align with traditional claims regarding the therapeutic efficacy of Swarna Bhasma in inflammatory conditions[23].

This study provides preliminary *in vitro* evidence supporting the pharmacological basis of EESB; however, further investigations are required to validate its therapeutic potential. In vivo models should be used to study the effects of these compounds on cytokine regulation, including IL-1β, TNF-α, and IL-6. They should

also examine oxidative stress modulation and signaling pathways, such as NF-κB and MAPK. Additionally, physicochemical characterization of Swarna Bhasma, including particle size distribution, zeta potential, and surface chemistry, is critical for correlating its nanoscale properties with bioactivity. Such mechanistic and translational studies will help establish the scientific credibility of this traditional preparation and may provide insights into the development of safer metal-based adjunct therapies for arthritis[24].

CONCLUSION

The current findings validate the traditional use of Swarna Bhasma in treating inflammatory disorders and emphasize its potential as a natural complementary anti-arthritic agent. The observed dose-dependent effects in both assays, although less potent than those of diclofenac sodium, highlight the significance of further pharmacological investigation. With additional mechanistic studies and in vivo validation, EESB could serve as an effective option for managing rheumatoid arthritis and other inflammatory conditions through an integrative therapeutic strategy.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Mayuri Bapu Chavan conceived and designed the study and supervised the work. Apoorva Ranade and Hrutuja Wagh contributed to method development and data analysis. Nivedita Gaikwad and Atharva Tilak performed the experimental work and recorded observations. Mohini Kadam and Saloni Shinde interpreted the data and assisted in data collection and manuscript drafting. Meera Deshmukh and Pranati Tilak contributed to data interpretation, manuscript review, and overall guidance. All authors reviewed, edited, and approved the final version.

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