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In Vitro Assessment Of The Anti-Inflammatory Activity Of Homoeopathic Eucalyptus Globulus Tincture Through Protein Denaturation Inhibition Model.

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ABSTRACT:

The present study evaluated the anti-inflammatory activity of Homoeopathic medicine Eucalyptus globulus tincture using the protein denaturation inhibition assay, a standard in vitro model for screening anti-inflammatory potential. Protein denaturation is a recognized cause of inflammation, and agents that inhibit this process are considered to possess anti-inflammatory properties. In this assay, bovine serum albumin (3% w/v) served as the substrate, and the sample was tested at concentrations ranging from 6.25 to 100 µL. The reaction mixture was incubated at 37°C for 20 minutes and subsequently heated at 80°C for 10 minutes. Absorbance was recorded at 660 nm using a UV spectrophotometer, and the percentage inhibition of protein denaturation was determined. Diclofenac sodium was used as the standard reference drug. Eucalyptus globulus tincture exhibited concentration-dependent inhibition of protein denaturation, showing a maximum inhibition of 50.76% at 100 µL and an IC_{50} value of 97.66 µL, compared with diclofenac's IC_{50} of 49.68 µg/mL. These findings indicate that Homoeopathic medicine Eucalyptus globulus possesses anti-inflammatory potential, possibly due to the presence of bioactive phytoconstituents capable of stabilizing protein structures and preventing denaturation. Further phytochemical and in vivo investigations are warranted to isolate and characterize the active compounds responsible for this activity.

Keywords: Anti-Inflammatory, Egg Albumin, Homoeopathy, Eucalyptus globulus

INTRODUCTION:

Inflammation is a complex biological response of vascular tissues to harmful stimuli such as physical injury, chemical agents, or microbial infections. It is characterized by the classical signs of heat, redness, swelling, pain, and loss of function.[1] This physiological process serves as a protective mechanism aimed at eliminating the injurious stimulus and initiating the healing process .At the onset of inflammation, activated cells release a variety of inflammatory mediators, including histamine, serotonin, slow-reacting substances of anaphylaxis (SRS-A), prostaglandins, and plasma enzyme systems such as the complement, clotting, fibrinolytic, and kinin cascades.[2] These mediators collectively induce vasodilation and increased vascular permeability, promoting the migration of plasma proteins, fluids, and leukocytes—predominantly neutrophils—into the affected tissue.[3] Inflammation may be classified as acute or chronic. Acute inflammation represents the body's immediate defensive response to injury, characterized by vascular and cellular changes mediated by pre-existing tissue cells. Chronic inflammation, on the other hand, is a prolonged process involving persistent tissue destruction and repair, with altered cellular composition at the inflammatory site. In recent years, there has been growing interest in natural and Homoeopathic agents with potential anti-inflammatory properties, owing to their safety and minimal side effects compared to conventional anti-inflammatory drugs such as NSAIDs. Among these, Eucalyptus globulus is a well-known medicinal plant traditionally recognized for its anti-inflammatory, analgesic, antimicrobial, and antioxidant effects.[4,5] The Homoeopathic mother tincture of Eucalyptus globulus is believed to possess bioactive phytoconstituents capable of modulating inflammatory pathways.[6,7] Therefore, the present study was designed to evaluate the anti-inflammatory activity of Eucalyptus globulus mother tincture using the protein denaturation inhibition assay, a well-established in vitro model for screening substances that can prevent heat-induced protein denaturation a major contributing factor in the inflammatory process.[8] This assay provides an effective and reproducible method to assess the potential of homeopathic preparations to stabilize proteins against denaturation, thereby reflecting their anti-inflammatory potential.

Principle of In vitro Egg Albumin Denaturation Method

The denaturation assay is designed to assess whether specific agents or compounds can prevent or reduce the denaturation of egg albumin under defined experimental conditions. Denaturation refers to the structural alteration of a protein, resulting in the loss of its native conformation and biological activity. [9] In this assay, egg albumin serves as a model protein, and denaturation is induced by exposing it to stressors such as high temperature, extreme pH, or chemical denaturants. These conditions disrupt the native structure of egg albumin, leading to physical and functional changes. The egg albumin denaturation assay therefore evaluates the potential anti-inflammatory activity of a substance by measuring its ability to

inhibit or minimize this denaturation process. [9,10]The underlying concept is that compounds with anti-inflammatory properties may help stabilize protein structures, thereby preventing denaturation—a process often associated with tissue damage and inflammatory responses [11]. Since protein denaturation is believed to play a role in the pathogenesis of inflammation, NSAIDs exhibit their effects partly by preventing protein denaturation in addition to inhibiting cyclooxygenase (COX) enzymes. In practice, different concentrations of the test compound are incubated with egg albumin under controlled laboratory conditions. After incubation, absorbance is measured to determine the percentage inhibition of denaturation, and the IC_{50} value is subsequently calculated using Graph Pad Prism software. Diclofenac sodium is commonly employed as the reference standard drug in this assay. [4]

MATERIALS AND METHODS:

Reagents and Chemicals:

- Bovine serum albumin (BSA) – 3% aqueous solution
- Phosphate-buffered saline (PBS, pH 6.3)
- Diclofenac sodium (standard drug)
- Eucalyptus globulus tincture (test sample)

Equipment:

- Clean pipettes and puppet tips
- Khan tubes or test tubes
- Incubator
- Spectrophotometer

Egg Albumin Denaturation Assay: Methodology

The anti-inflammatory activity of the homoeopathic medicine Eucalyptus globulus was evaluated using the egg albumin (BSA) denaturation assay, which assesses the ability of compounds to inhibit heat-induced protein denaturation. Protein denaturation is a well-recognized cause of inflammation, and substances capable of stabilizing proteins against denaturation are known to exhibit anti-inflammatory properties. Hence, this method provides a simple, reliable, and reproducible in vitro model for screening the anti-inflammatory potential of natural and homeopathic preparations. For this experiment, a reaction mixture with a total volume of 0.5 mL was prepared by combining 0.4 mL of 3% bovine serum albumin (BSA) solution with varying volumes (6.25–100 µL) of the test sample — Eucalyptus globulus in different concentrations. The control tube contained only the BSA solution without the test sample. All mixtures were gently vortexed to ensure proper homogenization and were then incubated at 37 °C for 20 minutes to facilitate interaction

between the protein and the sample components. Following incubation, the mixtures were heated at 80 °C for 10 minutes to induce protein denaturation. After the heating phase, the samples were allowed to cool to room temperature, and 2.5 mL of phosphate buffer (pH 6.3) was added to each tube to stabilize the solutions and maintain a uniform optical density. The absorbance of each reaction mixture was measured at 660 nm using a Thermo Scientific Orion Aquamate 8000 UV–Visible spectrophotometer. An increase in absorbance indicated greater turbidity due to protein denaturation, whereas a reduction in absorbance reflected inhibition of denaturation by the test compound. The percentage inhibition of protein denaturation was calculated using the formula:

Inhibition (%) = $[(A_{(c)} - A_{(t)}) / A_{(c)}] \times 100$

Where,

Ac=Absorbance of control

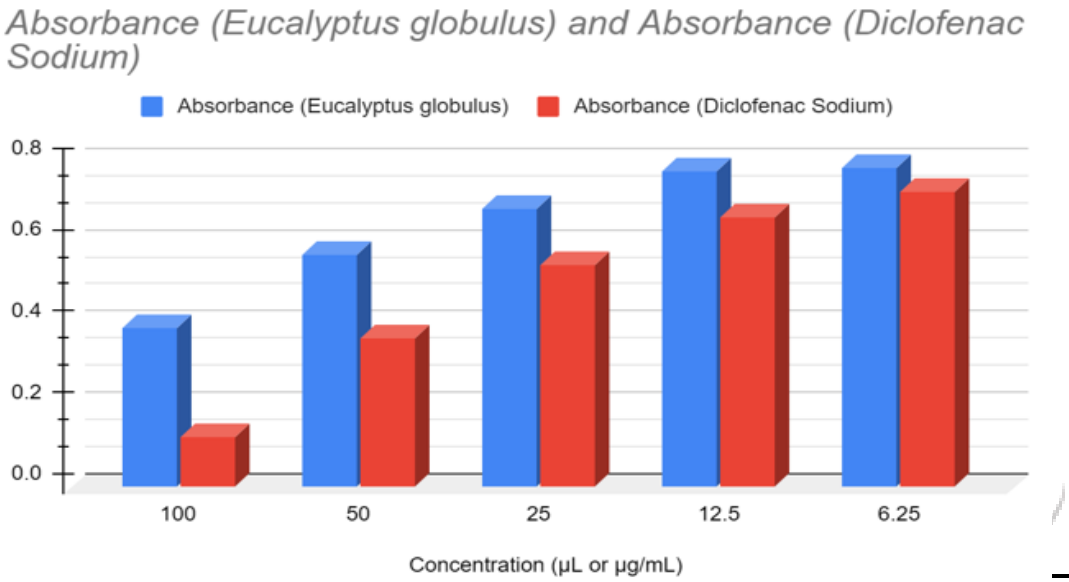
At= Absorbance of test sample

For comparative evaluation, Diclofenac sodium, a well-established nonsteroidal anti-inflammatory

drug (NSAID), was used as the reference standard under identical experimental conditions. All reactions were conducted in triplicate to ensure the accuracy and reproducibility of results. The percentage inhibition values obtained for various concentrations of Eucalyptus globulus were plotted against their respective concentrations to generate a dose–response curve.

STATISTICAL ANALYSIS:

As a statistical analysis tool, GraphPad Prism software for Windows versions up to 9 (GraphPad Software, San Diego, CA, USA) can be used. To calculate the mean and the standard error of the mean, all results (absorbance) must be triplicated. Nonlinear regression was applied using the GraphPad Pris.



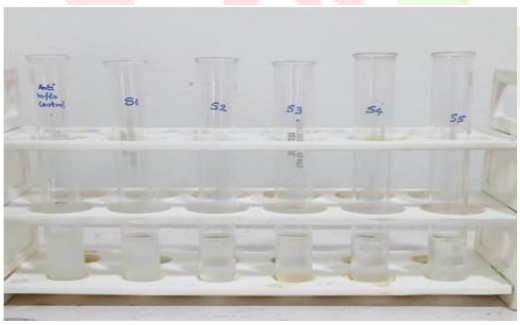


FIG 1: Control Test Sample

FIG 2: Testing Sample

Sample	Concentration / Volume	Absorbance (660 nm)	% Inhibition
Control (EG)	–	0.790	–
EG	6.25 µl	0.784	0.76%
EG	12.5 µl	0.775	1.90%
EG	25 µl	0.686	13.16%
EG	50 µl	0.572	27.59%
EG	100 µl	0.389	50.76%
IC_{50} (EG)	–	–	**97.66 µl**
Diclofenac (Std)	6.25 µg/ml	0.725	7.88%
Diclofenac	12.5 µg/ml	0.661	16.01%
Diclofenac	25 µg/ml	0.545	30.75%
Diclofenac	50 µg/ml	0.368	53.24%
Diclofenac	100 µg/ml	0.125	84.12%
IC_{50} (Std)	–	–	**49.68 µg/ml**

RESULT: Protein Denaturation by Eucalyptus Globulus and Standard

DISCUSSION:

The present study demonstrated that Eucalyptus globulus mother tincture inhibited protein denaturation in a dose-dependent manner, with a gradual increase in inhibition percentage corresponding to rising concentrations. This indicates the presence of bioactive constituents capable of stabilizing protein structures and preventing thermal denaturation. Although the inhibitory potential of Eucalyptus globulus was comparatively lower than that of the standard diclofenac sodium, it exhibited significant anti-inflammatory activity, with an IC_{50} value of 97.66 µL, suggesting moderate efficacy relative to synthetic agents .The observed effects are consistent with earlier findings that Eucalyptus globulus contains secondary metabolites such as flavonoids, tannins, and phenolic compounds, which contribute to its anti-inflammatory and antioxidant properties. These compounds likely act by maintaining protein integrity through hydrogen bonding and reduction of conformational alterations. Overall, the results substantiate the traditional use of Eucalyptus globulus in inflammatory conditions. Further studies involving isolation of active constituents, in vivo evaluations, and molecular docking analyses are recommended to elucidate its mechanism of action and therapeutic potential.

CONCULSION:

In summary, the present investigation establishes that Eucalyptus globulus mother tincture exhibits notable anti-inflammatory potential, as demonstrated by its capacity to inhibit protein denaturation in a concentration-dependent manner. Although the inhibitory activity was comparatively lower than that of the reference standard, diclofenac sodium, the results clearly indicate that Eucalyptus globulus contains pharmacologically active phytoconstituents capable of maintaining protein structural integrity under denaturing conditions. The observed activity may be attributed to the presence of flavonoids, tannins, and phenolic compounds, which are known for their protein-stabilizing and antioxidant properties. These findings provide experimental evidence supporting the traditional therapeutic applications of Eucalyptus globulus in inflammatory disorders. Nonetheless, further studies involving the isolation and characterization of active constituents, along with in vivo and clinical evaluations, are essential to elucidate the precise mechanisms underlying its

anti-inflammatory effects and to validate its potential as a natural alternative or adjunct to conventional anti-inflammatory drugs.

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