

**IN-VITRO STUDIES SUGGEST PROBABLE MECHANISM OF EUCALYPTUS OIL FOR ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY**

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**Abstract**

The *in-vitro* anti-inflammatory and anti-arthritis activity of *Eucalyptus globules* oil is performed to understand the basic mechanism for these activities. The anti-inflammatory activity of *Eucalyptus globules* oil at 100, 250, 500 mcg/ml were studied using human red blood cell membrane (HRBC) stabilization method and the anti-arthritis activity of *Eucalyptus globules* oil at the same concentration were performed using bovine serum albumin denaturation (BSA) method. Diclofenec and Aspirin at concentration of 100, 200 mcg/ml were assessed as standard anti-inflammatory and anti-arthritis drugs. Results showed significant (53.48%, 63.73% and 70.66% at 100, 250 and 500 mcg/ml of EGO) protection of HRBC and significant inhibition (16.98%, 58.49%, and 66.03% at 100, 250 and 500 mcg/ml of EGO) in BSA denaturation and which are comparable to standard drugs diclofenec and aspirin. This result suggests potential action as anti-inflammatory and anti-arthritis activity of *Eucalyptus globules oil* and sets a mechanism for therapeutic use.

**Keywords:** human red blood cell membrane (HRBC), bovine serum albumin (BSA), *Eucalyptus globules*, *in-vitro*, anti-inflammatory, anti-arthritis.

**1. Introduction**

Eucalyptus oil is obtained from fresh leaves of *Eucalyptus globulus* belongs to the family myrtaceae.<sup>1</sup> It mainly contains Volatile oil and till date forty-seven compounds were identified in the essential oils and the main constituents of the essential oils were 1, 8-eucalyptol (72.71 %),  $\alpha$ -pinene (9.22 %),  $\alpha$ -terpineol (2.54 %), (-)-globulol (2.77 %),  $\alpha$ -terpineol acetate (3.11 %), and alloaromadendrene (2.47 %).<sup>2</sup>

Eucalyptus oil has previously reported having different pharmacological activities like anti-bacterial<sup>3</sup>, anti-viral<sup>4</sup>, anthelmintic<sup>5</sup>, analgesic and anti-inflammatory<sup>6</sup> and anti-oxidant<sup>7</sup>. Though a few screening studies were performed for anti-inflammatory activity but those not suggest any mechanism.

Our present study for *in-vitro* anti-inflammatory on Human red blood cell membrane (HRBC) and anti-arthritis activity on bovine serum albumin (BSA) suggests about their mechanism for their therapeutic activity.

**Materials and Methods**

**2.1. Drugs and Chemicals:** Eucalyptus oil Pure (Kola products Ltd., Bhimavaram), Diclofenac (Symbel Pharm. Pvt. Ltd, Hyderabad) and Aspirin (Accord labs, Secunderabad) were used in the study. All the other chemicals were obtained from the store of the Institute.

**2.2. In-Vitro anti-inflammatory activity:** *In-vitro* anti-inflammatory activity of *Eucalyptus globulus* oil (EGO) was performed by using human red blood cell membrane stabilization method.<sup>8-9</sup> The blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of oil were prepared (100, 250, 500 mcg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of Human red blood cells (HRBC) suspension were added. It is incubated at 37°C for 30 min and Centrifuged at 3,000 rpm for 20 min. the hemoglobin content of the supernatant solution was

estimated spectrophotometrically at 560 nm. Diclofenac (50, 100 and 200 mcg/ml) were used as reference standard and a control was prepared omitting the EGO.

**2.3. In-Vitro Anti-Arthritic Activity:** *In-vitro* anti-arthritic activity of *Eucalyptus globulus* oil (EGO) was performed by using bovine serum albumin denaturation (BSA) method.<sup>10</sup> The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Eucalyptus globulus* oil (100, 250 and 500 mcg/ml of final volume) and aspirin (100 and 200 mcg/ml) in distilled water. pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Absorbance was measured spectrophotometrically at 660 nm for control test 0.05 ml distilled water was used instead of *Eucalyptus globulus* oil while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

### 3. Results and discussion

**3.1. In-vitro Anti-Inflammatory activity of *Eucalyptus globulus* oil (EGO):** The results of *in-vitro* anti-inflammatory activity of *Eucalyptus globulus* oil (EGO) on human red blood cell membrane were given in Table 1.

*In-vitro* anti-inflammatory activity of EGO was performed by using human red blood cell membrane stabilization method. EGO showed significant anti-inflammatory activity in a concentration dependent manner. EGO at concentration of 100, 250 and 500 mcg/ml showed 53.48%, 63.73% and 70.66% protection of HRBC in hypotonic solution respectably. All the results were compared with standard Diclofenac at 50, 100 and 200 mcg/ml which showed 43.74%, 63.93% and 86.73% protection of HRBC in hypotonic solution respectably.

**3.2. In-vitro Anti-arthritic activity of *Eucalyptus globulus* oil (EGO):** The results of *in-vitro* anti-arthritic activity of *Eucalyptus globulus* oil (EGO) on Inhibition of protein denaturation method were posted in Table 2.

*In-vitro* anti-arthritic activity of EGO was performed by using Inhibition of protein denaturation method. EGO showed significant anti-arthritic activity in a concentration dependent manner. EGO at concentration of 100, 250 and 500 mcg/ml showed 16.98%, 58.49%, 66.03% inhibition of protein denaturation respectably. All the results were compared with standard Acetyl Salicylic acid at 100 and 200mcg/ml which showed 79.24%, 88.67% inhibition of protein denaturation respectably.

**Table 1: *In-vitro* anti-inflammatory activity of *Eucalyptus globulus* oil (EGO) on human red blood cell membrane**

Drug	Concentration (mcg/ml)	Absorbance at 560 nm	Percentage inhibition
Control	---	2.526	-
EGO	100	1.175	53.48%
EGO	250	0.916	63.73%
EGO	500	0.743	70.66%
Diclofenac	50	1.421	43.74%
Diclofenac	100	0.911	63.93%
Diclofenac	200	0.335	86.73%

**Table 2: *In-vitro* anti-arthritic activity of *Eucalyptus globulus* oil (EGO) on Inhibition of protein denaturation**

Drug	Concentration (mcg/ml)	Absorbance at 660nm	Percentage Inhibition
Control	-	0.053	-
EGO	100	0.044	16.98
EGO	250	0.022	58.49
EGO	500	0.018	66.03
Aspirin	100	0.011	79.24
Aspirin	200	0.006	88.67

The *Eucalyptus globulus* oil exhibited membrane stabilization effect by inhibiting hypo tonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes.<sup>10</sup> Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release.<sup>11-12</sup>

Anti-arthritis effect of *Eucalyptus globulus* was studied significantly by using *in-vitro* inhibition of protein denaturation model. The *Eucalyptus globuloso* oil inhibited protein denaturation. *Eucalyptus globulus* at two different concentrations (dose levels) provided significant protection against denaturation of proteins. Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Obtained data stated that *Eucalyptus globulus* could be used as potent anti-arthritis agent.

### Conclusion

*Eucalyptus globulus* oil possesses potent anti-inflammatory activity by inhibiting the release of prostaglandins or other inflammatory mediators from cell membrane by stabilizing membrane by and anti-arthritis activity by inhibiting protein denaturation as protein denaturation is one of the possible pathway for pathogenesis of arthritis. By further studies it can be possible to formulate natural anti-inflammatory, anti-arthritis drugs of *Eucalyptus globulus* oil.

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