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ORIGINAL ARTICLE

Anti-inflammatory mechanisms of eucalyptol rich *Eucalyptus globulus* essential oil alone and in combination with flurbiprofen

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Abstract

Inflammation is the core contributor in the pathogenesis of various acute and chronic illness including appendicitis, bronchitis, arthritis, cancer and neurological diseases. NSAIDs, commonly used medications for inflammatory diseases, on prolonged use cause GI bleeding, ulcers and many more issues. Plant-based therapeutic agents including essential oils in combination with low-dose synthetic drugs have been shown to produce synergistic effects and reduce complications of synthetic drugs. This study was designed to evaluate the anti-inflammatory, analgesic and anti-pyretic properties of *Eucalyptus globulus* essential oil alone and in combination with flurbiprofen. GC–MS analysis was performed to screen chemical composition of oil. In vitro anti-inflammatory assay (membrane stabilization assay) and in vivo inflammatory acute (carrageenan and histamine-induced paw oedema) and chronic (cotton pellet-induced granuloma and Complete Freund's adjuvant-induced arthritis) models were performed to check anti-inflammatory properties. Acetic acid-induced algesia and yeast-induced pyrexia models were performed to check analgesic and anti-pyretic properties. qRT-PCR was performed to study the effect of treatments on the expression of inflammatory biomarkers. GC–MS analysis of *E. globulus* essential oil showed the presence of eucalyptol along with other active biomolecules. 500 + 10 mg/kg of oil-drug combination showed significantly ($p < 0.05$) better in vitro membrane stabilization effects as compared with groups treated with 500 mg/kg of *E. globulus* oil and 10 mg/kg of Flurbiprofen alone. 500 + 10 mg/kg of oil-drug combination showed significantly ($p < 0.05$) better anti-inflammatory, analgesic and antipyretic effects as compared to 500 mg/kg of *E. globulus* oil alone in all in vivo models. When comparison was done between 500 + 10 mg/kg of oil-drug combination-treated and 10 mg/kg Flurbiprofen-treated group, the former group showed significantly ($p < 0.05$) better anti-inflammatory and anti-pyretic effects, but there were non-significant differences in the analgesic model. Animal group treated with 10 mg/kg of Flurbiprofen showed significantly ($p < 0.05$) better anti-inflammatory and analgesic effects than group treated with 500 mg/kg of oil alone while, there were non-significant differences in anti-pyretic effects. qRT-PCR analysis showed significant ($p < 0.05$) down-regulation in the expression of IL-4 and TNF-α in serum samples of animals treated with 500 + 10 mg/kg of oil-drug combination as compared to the diseased control (arthritic) group. Overall, the current research demonstrates that *Eucalyptus globulus* essential oil in combination with flurbiprofen showed better anti-inflammatory, analgesic and anti-pyretic effects than oil and flurbiprofen alone which is attributed to the down-regulation of pro-inflammatory biomarkers (IL-4 and TNF-α). Further studies are required to formulate a stable dosage form and to check the anti-inflammatory efficacy in different inflammatory disorders.

Keywords Chemo-herbal · *Eucalyptol* · Flurbiprofen · Anti-inflammatory · IL-4 · TNF-α

Introduction

Inflammatory process involves series of complex cellular and molecular interactions, that can be triggered by variety of factors including infections, toxins, auto-immune reactions and damaged cells (Nathan 2002). It is a multi-step process in which various components of vascular tissue, blood vessels, circulating blood cells, and cell mediators

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take part. The net outcome of which is increased vascular permeability, recruitment of inflammatory and immune cells and release of plethora of inflammatory mediators (Fujiwara and Kobayashi 2005). Depending upon duration and severity of inflammatory reaction, it can be beneficial to the body or vice versa (Andleeb et al. 2022). Chronic inflammation-associated diseases are one of the most common causes of deaths worldwide. It is identified as a core issue in the pathogenesis of diseases like gastritis, chronic obstructive pulmonary disease, inflammatory bowel disease, diabetes, obesity and different types of cancers etc. (Furman et al. 2019; Ploeger et al. 2009).

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) block the production of various inflammatory mediators by inhibiting cyclooxygenases (COX-1 and -2). In chronic illness like arthritis, NSAIDs are prescribed for long duration. The prolonged use of these cause gastric bleeding and perforations, cardiovascular abnormalities including thrombus formation and acute renal failure (Marcum and Hanlon 2010). Plant-based drugs are known to mitigate the side effects of synthetic drugs when used in combination. A study conducted by Tanzeem el al. reported the gastroprotective effects of clove oil against flurbiprofen when used in combination (Tanzeem et al. 2019). Moreover, improvement in therapeutic efficacy of synthetic drugs is observed when given in combination with plant extracts. A study by Yehya et al. showed sensitization of gemcitabine resistant cells towards gemcitabine when used in combination with a standardised extract of *Orthosiphon stamineus* (C5EOSEW-5050ESA) (Yehya et al. 2023). An in vitro antimicrobial study conducted by Rosato et al. showed synergistic actions of Diclofenac sodium salt when used in combination with essential oils obtained from *Mentha piperita*, *Pelargonium graveolens* and *Melaleuca alternifolia* (Rosato et al. 2021). Essential oils obtained from various plant species are known to have anti-oxidant and anti-inflammatory effects. Reduction in the levels of reactive oxygen and reactive nitrogen species, elevation in the levels of anti-oxidant enzymes, inhibition of COX-2 enzyme, reduction in the levels of various pro-inflammatory cytokines including TFN-α, IL-6 and IL-1β and maintenance of balance between Th1 and Th2 cells population are among the few mechanisms responsible for anti-inflammatory attributes of essential oils (de Lavor et al. 2018; Zuo et al. 2020).

Eucalyptus is a large *Genera* comprising of almost 700 species. Various plant parts including bark and leaves of *Eucalyptus* species have been used as traditional remedies for the treatment of different human ailments since medieval times (Chandorkar et al. 2021). *Eucalyptus globulus* Labill. of family *Myrtaceae* is one of the most widely used medicinal plants globally due to its wide spectrum of pharmacological attributes (Shala and Gururani 2021). It is now widely cultivated throughout the world. Essential oil obtained from *E. globulus* have been shown to have anti-oxidant, analgesic, anti-inflammatory, antibacterial, antiviral, bronchodilator and CNS stimulant properties (Asif et al. 2020b; Chandorkar et al. 2021). *E. globulus* essential oil and its active component, eucalyptol (1,8–cineole) is the main ingredient of many marketed medicinal products. Soledum™ (100 mg of 1,8–cineole), a herbal product of *E. globulus* essential oil is used for the treatment of acute and chronic bronchitis, sinusitis, and other respiratory infections (Chandorkar et al. 2021). Vicks VapoRub™ containing 1,8-cineole (eucalyptol 1.33% w/w) as one of the active components is used to relieve nasal congestion (Asif et al. 2020b). Literature review at https://clinicaltrials.gov showed that multiple clinical trials are registered to explore the beneficial effects of *E. globulus* essential oil in muscle soreness, hypertension, migraine, COVID-19, tick bites, gingivitis and dental plague. This data highlights the high human acceptability and medicinal potential of *E. globulus* essential oil. Numerous pre-clinical studies have reported the anti-inflammatory and analgesic effects of *E. globulus* essential oil in in vitro and in vivo models (Chandorkar et al. 2021; Ho et al. 2020; Lin et al. 2018; Shao et al. 2020; Silva et al. 2003). However, to best of our knowledge there is no scientific study available which has reported the anti-inflammatory, analgesic and anti-pyretic effects of *E. globulus* essential oil in combination with Flurbiprofen (FB). Therefore, the current study was designed to explore the anti-inflammatory attributes of *E. globulus* essential oil alone and in combination with Flurbiprofen using series of in vivo models along with its possible molecular mechanisms.

Materials and methods

Collection of essential oils

Eucalyptus globulus oil was procured from local supplier (Go Natural), Lahore, Pakistan.

GC–MS analysis

GC–MS analysis of *Eucalyptus globulus* essential oil (*E.G*) was performed following reported protocols. The oil constituents were identified by comparing MS data with NIST mass spectral library and published mass spectra (Andleeb et al. 2022).

Animals

Albino Wistar rats (male and female) of weight 180–200 g and mice (male and female) of weight 20–30 g were obtained from the animal house of Department of Pharmacology, the Islamia University of Bahawalpur. The animals were kept in

the laboratory under standard conditions of humidity (50%), temperature (set at 25 ± 5 ℃) and 12 h light/dark cycles. All animals were given standard pellet diet and free access to tap water. One week acclimatization of animals was done prior to pharmacological activities. All the experimental procedures were approved by Pharmacy Ethics Committee (PAEC/22/86).

Anti-inflammatory studies

In vitro membrane stabilization assay (heat-induced haemolysis)

Human blood was collected in heparinized tubes followed by centrifugation at 3000 rpm for 5 min and then washed with equal volumes of 0.9% NaCl. Blood suspension (v/v) was prepared with isotonic buffer solution (pH: 7.4). Then, 0.05 ml of this suspension was mixed with 0.05 ml of different concentrations of *E. globulus* oil (*E.G*: 125, 250, 500 mg/ml), flurbiprofen (FB, 10 mg/ml) and *E. globulus* oil and flurbiprofen combination (*E.G* + FB: 125 + 10, 250 + 10, 500 + 10 mg/ml) respectively. In all these mixtures, 2.95 ml of buffer solution was added and incubated for 20 min in shaking water bath set at 54 ^{0}C, followed by centrifugation at 2500 rpm for 3 min. Later, supernatant was collected and absorbance was measured by UV spectrophotometer at 540 nm. Percentage of inhibition was calculated following reported formula (Gunathilake et al. 2018).

In vivo anti-inflammatory studies

For all in vivo models animals were treated orally with *E.G* (125, 250, 500 mg/kg), FB (10 mg/kg) and *E.G* + FB (125 + 10, 250 + 10, 500 + 10 mg/kg) respectively.

Carrageenan and Histamine-induced paw oedema models

After one hour of pre-treatment with *E.G* (125, 250, 500 mg/kg), FB (10 mg/kg) and *E.G* + FB (125 + 10, 250 + 10, 500 + 10 mg/kg), 0.1 ml of 1% w/v freshly prepared carrageenan and histamine solutions were injected subcutaneously into sub-plantar surface of left hind paw of each rat separately. Measurement of paw volumes was done at 0, 1, 2, 3, 4, 5 and 6 h. with the help of digital Vernier calliper. Anti-inflammatory effects are presented as percentage inhibition of paw oedema, calculated using reported formula (Shabbir et al. 2018).

Acetic acid-induced pain model

Acetic acid-induced writhing test was performed in mice following reported protocols. In brief, 10 ml/kg of 0.6% acetic acid was injected i.p in the abdominal area of mice and abdominal writhes were noted for 25 min starting 5 min after the administration of acetic acid. Animals were treated following the same scheme as mentioned in the above section and percent inhibition of writhing was calculated using reported formula (Naghizadeh et al. 2016).

Brewer's yeast-induced pyrexia model

In brief, digital thermometer was inserted 2 cm-deep into the rectum of each rat to record the initial rectal temperature (RT). Rats with starting RT between 37 and 37.5 ℃ were selected. Brewer's yeast suspension (20 mg/kg) was subcutaneously injected at the back of the neck to induce pyrexia. Animals having rectal temperature difference of 1 ℃ or above were given different treatments to test the anti-pyretic effects. Animals were treated following the same scheme as mentioned in the above section and rectal temperatures were noted at 1, 2, 3 and 4 h. (Kong et al. 2014).

Cotton pellet-induced granuloma model

In brief, rats were anaesthetised using mixture of ketamine and xylazine. Sterile cotton pellets (20 mg) were implanted subcutaneously in both shaved axilla regions of rats by creating small incisions. Treatments were given in the same manners as mentioned above for the consecutive 10 days. At day 11, animals were dissected and cotton pellets were removed, cleaned of extraneous tissue, weighed, and dried at 60 °C to a constant weight. Increase in the dry weight of pellets was calculated and percent inhibition of granuloma formation was calculated using reported formula (Sokeng et al. 2020).

Complete Freund's Adjuvant-induced arthritic model

In brief, 0.1 ml of Complete Freund's Adjuvant (CFA) was injected in sub-plantar region of left hind paw of each rat to induce arthritis. Rats were divided into eight groups as descried above and treatment was given for 21 days. Paw volumes were measured by digital Vernier calliper at day 3, 5,9,13 and 21 (Zhu et al. 2020). Anti-inflammatory effects are reported as percentage of inhibition of paw oedema which was calculated using reported formula (Nagarkar and Jagtap 2017).

Molecular Mechanistic studies

RT-PCR technique was employed to analyse effects of different treatments on the expressions of different inflammatory biomarkers following well-reported method (Andleeb et al. 2022). Expression of two major genes including TNF-α, and IL-4 was checked. Primer sequences of these genes are mentioned in Table 1. Data is presented as relative fold change values (n = 3) (De Cicco et al. 2020; Saleem et al. 2021).

Table 1 Gene sequencing of inflammatory biomarkers in RT-PCR

Inflammatory markers	Forward/reverse	Sequence	Gene accession	Tm	GC%
IL-4	Forward	5′GTACCGGGAACGGTATCCAC-3′	NM_201270.1	62.5	60
	Reverse	5′TGGTGTTCCTTGTTGCCGTA-3′		58.4	50
	Reverse	5′CTCCACCAGCTCTTTGATGGT-3′		63.1	52
TNF-α	Forward	5′ATGGGCTCCCTCTCATCAGT-3′	NM_012675.3	60.5	55
	Reverse	5′GCTTGGTGGTTTGCTACGAC-3′		60.5	55

Table 2 Compounds identified in *Eucalyptus globulus* essential oil

Name of compounds	Retention time (min)	Area %
Eucalyptol	5.340	78.16
Limonene	5.286	2.85
Cymene	5.254	2.17
Mycrene	4.932	0.48
Pinene	4.878	0.89
Phellandrene	4.824	1.65
Cyclohexene	6.435	0.95

Statistical analysis

Data is presented as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed using *ONE WAY* and *TWO WAY* ANOVA models. p values less than 0.05 were considered statistically significant.

Results

GC–MS analysis of Eucalyptus globulus essential oil

GC–MS analysis of *Eucalyptus globulus* oil showed the presence of eucalyptol, limonene, cymene, mycrene, pinene, phellandrene and cyclohexene (Table 2 and Fig. 1).

Anti-inflammatory studies

In vitro membrane stabilization assay (heat-induced haemolysis)

Maximum membrane stabilization effects observed in groups treated with *E.G* (125, 250, 500 mg/ml), FB (10 mg/ml), *E.G* + FB (125 + 10, 250 + 10, 500 + 10 mg/ml) were 53.51 ± 4.31, 65.24 ± 1.22, 68.77 ± 1.08, 69.71 ± 3.07,

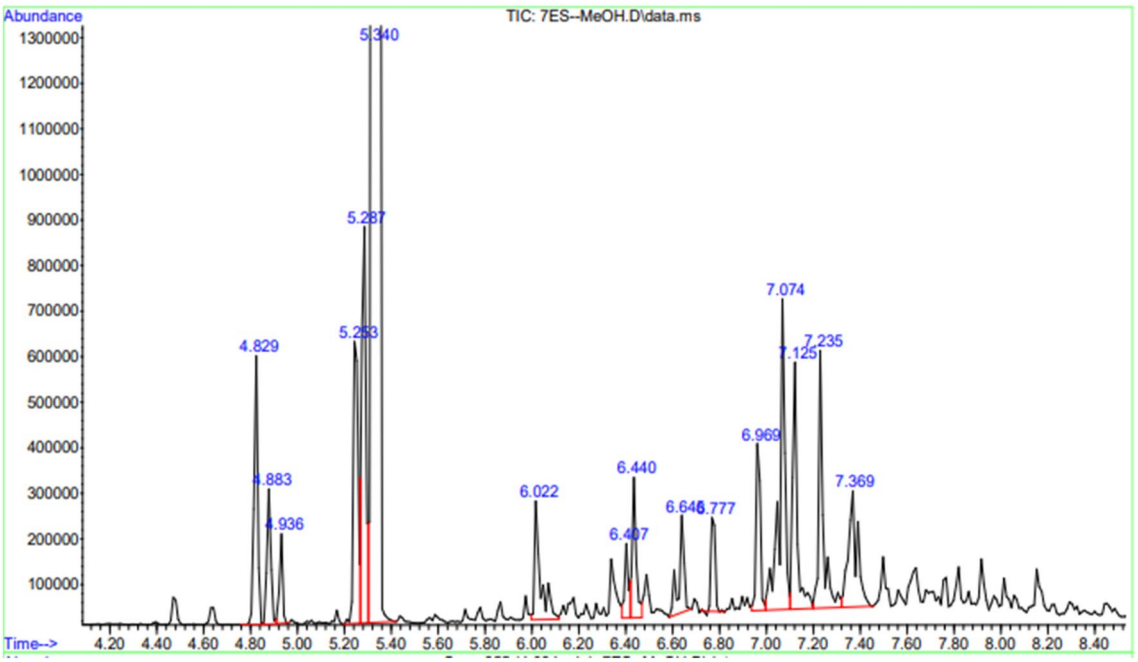

Fig. 1 Compounds detected in GC–MS of *Eucalyptus globulus* essential oil. Eucalyptol, limonene and cymene were the three most abundant compounds detected

70.97 ± 3.48, 75.02 ± 2.05, 80.18 ± 1.18% respectively. Better protective effects were observed in *E.G* + FB at the concentration of 500 + 10 mg/ml than other treatment groups. Flurbiprofen (10 mg/ml) and *E.G* 500 mg/ml treated samples showed almost same effects with no statistical difference ($p > 0.05$) (Table 3 and Fig. 2).

In vivo anti-inflammatory studies

Carrageenan-induced paw oedema model Maximum anti-inflammatory effects observed in groups treated with E.G (125, 250, 500 mg/kg), FB (10 mg/kg), E.G + FB (125 + 10, 250 + 10, 500 + 10 mg/kg) were 40.96 ± 1.00, 57.18 ± 4.03, 60.12 ± 3.89, 71.38 ± 8.10, 60.22 ± 13.61, 68.10 ± 7.94, 83.22 ± 11.09% respectively. During the first 2 h of study, no statistical differences in terms of percent inhibition of paw oedema was observed in E.G, FB and E.G + FB treatment groups when compared with each other. At 3rd hr. E.G + FB treated group showed significantly better anti-inflammatory ($p < 0.05$) activity in terms of percentage inhibition of paw oedema when compared with E.G 500 mg/kg treated group. From 4th hr onward, E.G + FB combination-treated groups showed significantly ($p < 0.05$) better anti-inflammatory effects than E.G alone treatment groups. No statistical difference in terms of percentage inhibition of paw oedema was observed in E.G (500 mg/kg) and flurbiprofen (10 mg/kg) treatment groups during first 4 h of study. At 6th hr flurbiprofen treated group showed significantly ($p < 0.01$) better anti-inflammatory effects when compared with E.G 500 mg/kg treated group (Table 4 and Fig. 3).

Histamine-induced paw oedema model Maximum anti-inflammatory effects observed in groups treated with E.G (125, 250, 500 mg/kg), FB (10 mg/kg), E.G + FB (125 + 10, 250 + 10, 500 + 10 mg/kg) were 49.64 ± 3.82, 56.81 ± 2.30, 60.30 ± 6.54, 68.01 ± 5.61, 69.14 ± 1.07, 77.22 ± 1.57, 85.93 ± 2.68% respectively. E.G + FB (500 + 10 mg/kg) treated group showed significantly ($p < 0.05$) better anti-inflammatory effects in terms of percentage inhibition of paw oedema than other treatment groups during the study. Flurbiprofen (FB 10 mg/kg) and E.G (500 mg/kg) treated groups showed almost same effects with no statistical differences ($p > 0.05$) from 1st to 5th hr. At 6th h. flurbiprofen treated group showed significantly ($p < 0.01$) better anti-inflammatory effects when compared with E.G (500 mg/kg) treated group (Fig. 4 and Table 5).

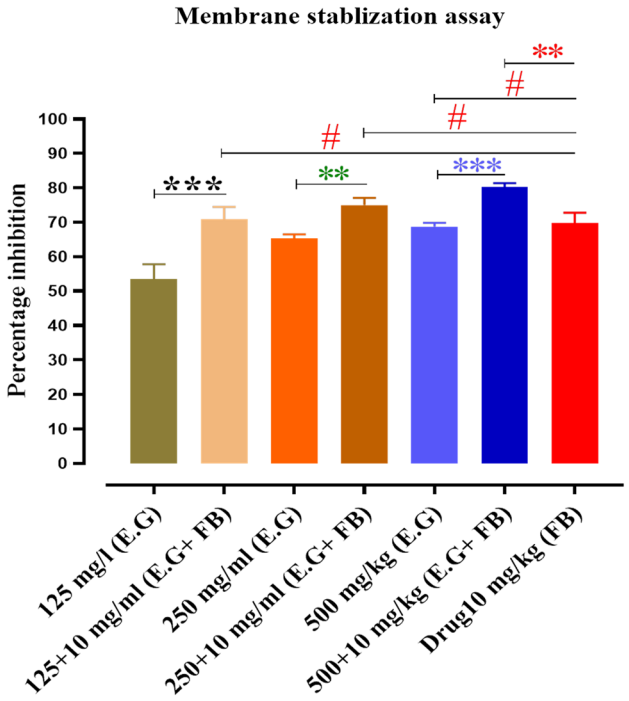

Fig. 2 Effect of different treatments on in vitro membrane stabilization. Values shown are mean ± SEM of percentage inhibition of RBC's haemolysis (n = 3). Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 vs 125 + 10 mg/ml and, 125 vs 250 mg/ml. Green colour sign indicates the comparison of 250 vs 250 + 10 mg/ml and, 250 vs 500 mg/ml. Blue colour sign indicates the comparison of 500 vs 500 + 10 mg/ml. Red colour sign indicates the comparison of FB with 500, 125 + 10, 250 + 10 and, 500 + 10 mg/ml

Acetic acid-induced pain model Maximum analgesic effects observed in groups treated with E.G (125, 250, 500 mg/kg), FB (10 mg/kg), E.G + FB (125 + 10, 250 + 10, 500 + 10 mg/

Table 3 Effect of E.G, FB, E.G + FB on heat-induced RBCs' haemolysis

E.G 125 mg/ml	E.G 250 mg/ml	E.G 500 mg/ml	E.G+FB 125+10 mg/ml	E.G+FB 250+10 mg/ml	E.C+FB 500+10 mg/ml	FB 10 mg/ml
53.51 ± 4.31	65.24 ± 1.22***	68.77 ± 1.08$^{\#/\#}$	70.97 ± 3.48$^{***/\#}$	75.02 ± 2.05$^{**/\#}$	80.18 ± 1.18$^{***/**}$	69.71 ± 3.07

Values shown are mean ± SEM of percentage inhibition of RBC, s haemolysis. Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen and E.G + FB = *Eucalyptus globulus* oil and Flurbiprofen combination. # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 *vs* 125 + 10 mg/ml, 125 *vs* 250 mg/ml. Green colour sign indicates the comparison of 250 *vs* 250 + 10 mg/ml, 250 *vs* 500 mg/ml. Blue colour sign indicates the comparison of 500 *vs* 500 + 10 mg/ml. Red colour sign indicates the comparison of FB with 500, 125 + 10, 250 + 10 and, 500 + 10 mg/ml

Table 4 Effect of different treatments on carrageenan-induced paw oedema

Time (hr)	E.G 125 mg/kg	E.G 250 mg/kg	E.G 500 mg/kg	E.G+FB 125+10 mg/kg	E.G+FB 250+10 mg/kg	E.G+FB 500+10 mg/kg	FB 10 mg/kg
1	4.50 ± 2.42	5.34 ± 2.07$^{\#}$	8.68 ± 3.19$^{\#/\#}$	5.22 ± 1.42$^{\#/\#}$	6.44 ± 1.27$^{\#/\#}$	9.23 ± 1.80$^{\#/\#}$	8.85 ± 1.70
2	9.19 ± 0.94	11.97 ± 1.55$^{\#}$	15.83 ± 3.18$^{\#/\#}$	11.30 ± 2.97$^{\#/\#}$	14.12 ± 5.05$^{\#/\#}$	23.21 ± 3.10$^{\#/\#}$	15.31 ± 4.56
3	16.87 ± 1.73	17.99 ± 0.99$^{\#}$	22.00 ± 2.15$^{\#/\#}$	17.44 ± 4.38$^{\#/\#}$	19.78 ± 5.24$^{\#/\#}$	35.18 ± 3.88$^{***/*}$	25.40 ± 5.50
4	26.28 ± 1.67	39.37 ± 4.67***	42.25 ± 5.66$^{\#/\#}$	39.26 ± 5.23$^{***/*}$	49.66 ± 11.58$^{*/\#}$	60.45 ± 9.95$^{***/**}$	48.85 ± 7.27
5	34.98 ± 2.44	42.65 ± 3.60$^{\#}$	48.92 ± 4.33$^{\#/***}$	50.13 ± 8.91$^{***/***}$	58.04 ± 10.11$^{***/\#}$	76.71 ± 6.63$^{***/**}$	64.52 ± 7.94
6	40.96 ± 1.00	57.18 ± 4.03***	60.12 ± 3.89$^{\#/**}$	60.22 ± 13.61$^{***/**}$	68.10 ± 7.94$^{**/\#}$	83.22 ± 11.09$^{***/**}$	71.38 ± 8.10

Values shown are mean ± SEM of percentage inhibition of paw oedema. Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 *vs* 125 + 10 mg/kg, 125 *vs* 250 mg/kg. Green colour sign indicates the comparison of 250 *vs* 250 + 10 mg/kg, 250 *vs* 500 mg/kg. Blue colour sign indicates the comparison of 500 *vs* 500 + 10 mg/kg. Red colour sign indicates the comparison of FB with 500, 125 + 10, 250 + 10 and, 500 + 10 mg/kg

kg) were 33.09 ± 4.81, 39.43 ± 3.63, 45.07 ± 3.63, 60.47 ± 2.65, 56.94 ± 3.58, 63.19 ± 4.74, 65.97 ± 4.74% respectively. E.G + FB combination-treated groups showed significantly ($p < 0.05$) better analgesic effects than E.G alone treatment groups and comparable effects with flurbiprofen. Flurbiprofen (10 mg/kg) showed significantly ($p < 0.05$) better analgesic effects than E.G (500 mg/kg) treated group (Fig. 5 and Table 6).

Yeast-induced pyrexia model Maximum antipyretics effects observed in groups treated with E.G (125, 250, 500 mg/kg), FB (10 mg/kg), E.G + FB (125 + 10, 250 + 10, 500 + 10 mg/kg) were 101.00 ± 0.34, 99.37 ± 1.35, 99.15 ± 1.04, 99.10 ± 0.54, 98.57 ± 0.42, 97.50 ± 0.60, 96.85 ± 0.52^{0}F respectively. No statistical differences between E.G (500 mg/kg) and E.G + FB (500 + 10 mg/kg) treatment groups were observed at 1st hr. From 2nd hr onwards E.G + FB (500 + 10 mg/kg) treated group showed significant ($p < 0.05$) antipyretic effects as compared with rest of the groups. Flurbiprofen (10 mg/kg) and E.G (500 mg/kg) treated groups showed almost same anti-pyretic effects ($p > 0.05$) (Table 7 and Fig. 6).

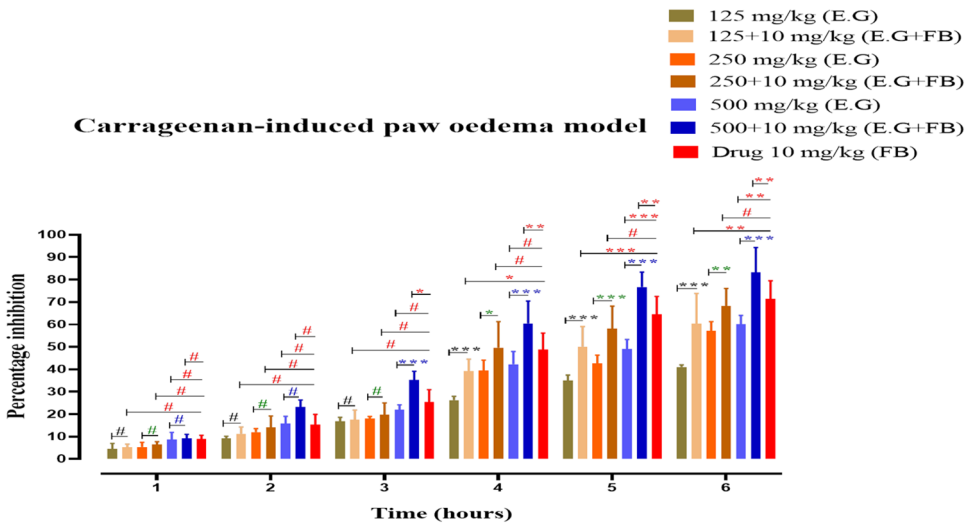

Fig. 3 Effect of different treatments on carrageenan-induced paw oedema. Values shown are mean ± SEM of percentage inhibition of paw oedema (n = 6). Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen. # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 vs 125 + 10 mg/kg, 125 vs 250 mg/kg. Green colour sign indicates the comparison of 250 vs 250 + 10 mg/kg, 250 vs 500 mg/kg. Blue colour sign indicates the comparison of 500 vs 500 + 10 mg/kg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10 and 500 + 10 mg/kg

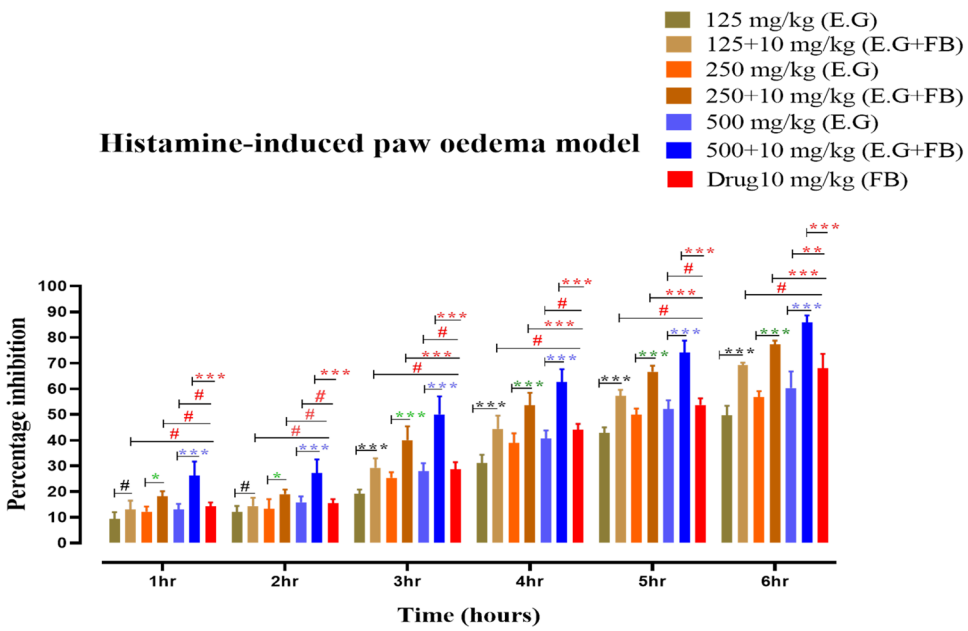

Fig. 4 Effect of different treatments on histamine-induced oedema. Values shown are mean ± SEM of percentage inhibition (n = 6). Where E.G = Eucalyptus *globulus* oil, FB = Flurbiprofen. # = non-significant, * = p < 0.05, ** = p < 0.01, *** = p < 0.001 respectively. Black colour sign indicates the comparison of 125 vs 125 + 10 mg/kg, 125 vs 250 mg/kg. Green colour sign indicates the comparison of 250 vs 250 + 10 mg/kg, 250 vs 500 mg/kg. Blue colour sign indicates the comparison of 500 vs 500 + 10 mg/kg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10, 500 + 10 mg/kg

Table 5 Effect of different treatments on histamine-induced oedema

Time (hr)	E.G 125 mg/kg	E.G 250 mg/kg	E.G 500 mg/kg	E.G+FB 125+10 mg/kg	E.G+FB 250+10 mg/kg	E.G+FB 500+10 mg/kg	FB 10 mg/kg
1	9.40 ± 2.64	12.24 ± 2.01$^{\#}$	13.00 ± 2.23$^{\#/\#}$	12.95 ± 3.52$^{\#/\#}$	18.30 ± 1.87$^{*/\#}$	26.30 ± 5.43$^{***/***}$	14.31 ± 1.52
2	12.01 ± 2.41	13.32 ± 3.77$^{\#}$	15.89 ± 2.23$^{\#/\#}$	14.44 ± 3.19$^{\#/\#}$	19.02 ± 1.81$^{*/\#}$	27.27 ± 5.25$^{***/***}$	15.48 ± 1.62
3	19.11 ± 1.66	25.40 ± 2.14*	27.86 ± 3.25$^{\#/\#}$	29.32 ± 3.64$^{***/\#}$	40.05 ± 5.34$^{***/***}$	50.10 ± 7.02$^{***/***}$	28.65 ± 2.78
4	31.21 ± 3.14	38.95 ± 3.73**	40.62 ± 3.23$^{\#/\#}$	44.45 ± 5.16$^{***/\#}$	53.55 ± 4.90$^{***/***}$	62.65 ± 5.00$^{***/***}$	44.09 ± 2.28
5	42.82 ± 2.24	50.00 ± 2.36**	52.12 ± 3.46$^{\#/\#}$	57.20 ± 2.42$^{***/\#}$	66.66 ± 2.33$^{***/***}$	74.06 ± 4.74$^{***/***}$	53.77 ± 2.56
6	49.64 ± 3.82	56.81 ± 2.30**	60.30 ± 6.54$^{\#/**}$	69.14 ± 1.07$^{***/\#}$	77.22 ± 1.57$^{***/***}$	85.93 ± 2.68$^{***/***}$	68.01 ± 5.61

Values shown are mean ± SEM of percentage inhibition of paw oedema. Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 *vs* 125 + 10 mg/kg, 125 *vs* 250 mg/kg. Green colour sign indicates the comparison of 250 *vs* 250 + 10 mg/kg, 250 *vs* 500 mg/kg. Blue colour sign indicates the comparison of 500 *vs* 500 + 10 mg/kg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10 and, 500 + 10 mg/kg

Cotton pellet-induced granuloma model Maximum anti-granulomatous effects in animals treated with E.G (125, 250, 500 mg/kg), FB (10 mg/kg), E.G + FB (125 + 10, 250 + 10, 500 + 10 mg/kg) were 44.54 ± 1.44, 51.26 ± 1.44, 57.98 ± 7.23, 65.54 ± 4.87, 66.94 ± 8.65, 71.99 ± 5.71, 76.47 ± 5.25% respectively. E.G + FB combination treated groups showed significantly ($p < 0.05$) better anti-granulomatous effects than E.G-treated groups and comparable effects with FB-treated group. Flurbiprofen (10 mg/kg) and E.G (500 mg/kg) treatment groups showed almost same anti-granulomatous effects when compared with each other (p > 0.05) (Table 8 and Fig. 7).

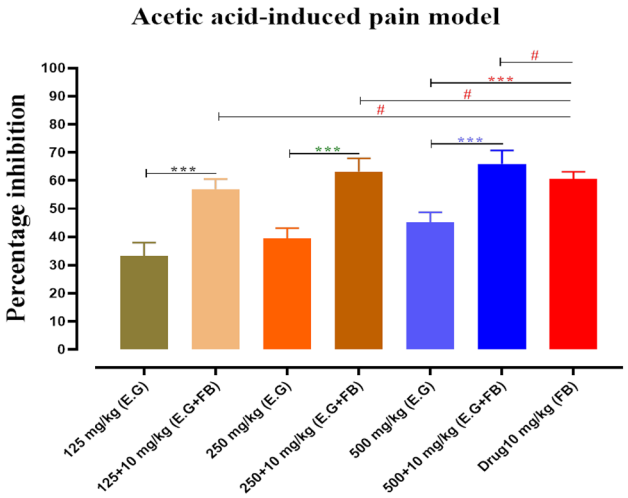

Fig. 5 Effect of different treatments on acetic acid-induced algesia. Values shown are mean ± SEM (n = 6). Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 vs 125 + 10 mg/kg and, 125 vs 250 mg/kg. Green colour sign indicates the comparison of 250 vs 250 + 10 mg/kg and, 250 vs 500 mg/kg. Blue colour sign indicates the comparison of 500 vs 500 + 10 mg/kg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10, and 500 + 10 mg/kg

CFA-induced arthritic model Maximum anti-arthritic effects as reported in terms of percent inhibition of inflammation observed in groups treated with E.G (125, 250, 500 mg/kg), FB (10 mg/kg), E.G + FB (125 + 10, 250 + 10, 500 + 10 mg/kg) were 39.69 ± 2.25, 40.53 ± 1.65, 46.06 ± 5.26, 57.62 ± 18.58, 50.39 ± 5.05, 61.23 ± 2.14, 78.95 ± 2.36% respectively. No statistical differences in terms of percent inhibition of paw oedema were observed in E.G (500 mg/kg) and E.G + FB (500 + 10 mg/kg) treatment groups from day 1 to 5. At day 9, 13 and 21, E.G + FB (500 + 10 mg/kg) combination treated group showed significant ($p < 0.05$) anti-inflammatory effects than E.G 500 mg/kg treated group. Flurbiprofen (10 mg/kg) and E.G (500 mg/kg) treated groups showed same anti-inflammatory effects from day 1st to 13th. At day 21, flurbiprofen (10 mg/kg) treated group showed significant ($p < 0.05$) anti-inflammatory effects than E.G 500 mg/kg group (Table 9 and Fig. 8).

Relative fold change values of TNF-α and IL-4 in different treatment groups are presented in Fig. 9. Relative fold change values of TNF- α were 0.96 ± 0.05, 0.82 ± 0.00 and 0.80 ± 0.05 in the diseased control, E.G (500 mg/kg) and E.G + FB (500 + 10 mg/kg) treated group respectively. Relative fold change values of IL-4 in E.G (500 mg/kg), E.G + FB (500 + 10 mg/kg) and diseased control were calculated to be 0.33 ± 0.00, 0.25 ± 0.00 and 0.96 ± 0.03 respectively.

Discussion

Chemo-herbal combinations have shown remarkable effects in the management of various types of cancers. These combinations are known to enhance the efficacy, reduce the side effects and increase the sensitivity of cancer cells towards the standard drugs (Hu et al. 2016; Yehya et al. 2023). *Eucalyptus globulus* essential oil has been in clinical use since long. Soledum™ (100 mg of 1,8–cineole), a herbal product of *E. globulus* essential oil is clinically used for the treatment of acute and chronic bronchitis, sinusitis, and other respiratory infections (Chandorkar et al. 2021). Vicks VapoRub™ containing 1,8-cineole (eucalyptol 1.33% w/w) as one of the active components is clinically used to relieve nasal congestion (Asif et al. 2020b). Based on the concept of mitigation of adverse effects of synthetic drugs *via* combination with natural products, the current study was designed to evaluate the anti-inflammatory, analgesic and anti-pyretic properties of *Eucalyptus globulus* essential oil in combination with flurbiprofen. Moreover, same doses of *E. globulus* essential oil and flurbiprofen were tested alone to establish synergism.

Rupture of lysosomal membranes leading to the release of degrading enzymes is among the variety of events which take place during inflammation. These leaked enzymes cause injury to neighbouring cells. Heat-induced haemolysis of human RBC's is one of the most commonly employed in vitro methods to test the efficacy of drugs to prevent lysosomal membrane rupture. This membrane is considered analogous to the lysosomal membrane and its stabilization suggests that test drug may also stabilise lysosomal membranes (Boukhatem et al. 2020). Eucalyptol rich essential oil

Table 6 Effect of different treatments on acetic acid-induced pain

Time (min)	E.G 125 mg/kg	E.G 250 mg/kg	E.G 500 mg/kg	E.G+FB 125+10 mg/kg	E.G+FB 250+10 mg/kg	E G+FB 500+10 mg/kg	FB 10 mg/kg
30	33.09 ± 4.81	39.43 ± 3.63$^{\#}$	45.07 ± 3.63$^{\#/***}$	56.94 ± 3.58$^{***/\#}$	63.19 ± 4.74$^{***/\#}$	65.97 ± 4.74$^{***/\#}$	60.47 ± 2.65

Values shown are mean ± SEM of percentage inhibition of writhing. Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 *vs* 125 + 10 mg, 125 *vs* 250 mg. Green colour sign indicates the comparison of 250 *vs* 250 + 10 mg, 250 *vs* 500 mg. Blue colour sign indicates the comparison of 500 *vs* 500 + 10 mg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10, 500 + 10 mg

Table 7 Effect of E.G, FB, E.G + FB on yeast-induced pyrexia

Time (hr)	E.G 125 mg/kg	E.G 250 mg/kg	E.G 500 mg/kg	E.G+FB 125+10 mg/kg	E.G+FB 250+10 mg/kg	E.G+FB 500+10 mg/kg	FB 10 mg/kg
BRT	98.00 ± 1.15	97.82 ± 1.29	97.22 ± 0.90	97.42 ± 0.59	96.75 ± 0.52	96.42 ± 0.45	97.82 ± 1.20
0	102.17 ± 0.22	101.55 ± 1.23	100.82 ± 1.15	101.40 ± 0.62	100.42 ± 0.58	100.42 ± 0.51	101.90 ± 0.70
1	101.90 ± 0.24	100.90 ± 1.21$^{\#}$	100.40 ± 1.19$^{\#/\#}$	101.05 ± 0.59$^{\#/\#}$	99.77 ± 0.75$^{\#/\#}$	99.37 ± 0.27$^{\#/*}$	101.25 ± 0.80
2	101.62 ± 0.29	100.52 ± 1.49$^{\#}$	100.05 ± 1.21$^{\#/\#}$	100.20 ± 0.43$^{\#/\#}$	98.92 ± 0.81$^{*/\#}$	98.25 ± 0.12$^{*/**}$	100.20 ± 0.66
3	101.35 ± 0.342	99.87 ± 1.37$^{\#}$	99.55 ± 1.21$^{\#/\#}$	99.45 ± 0.52$^{**/\#}$	98.22 ± 0.69$^{*/\#}$	97.65 ± 0.42$^{**/**}$	99.62 ± 0.85
4	101.00 ± 0.34	99.37 ± 1.35*	99.15 ± 1.04$^{\#/\#}$	98.57 ± 0.42$^{**/*\#}$	97.50 ± 0.60$^{*/*}$	96.85 ± 0.52$^{***/**}$	99.10 ± 0.54

Values shown are mean ± SEM. Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 *vs* 125 + 10 mg/kg, 125 *vs* 250 mg/kg. Green colour sign indicates the comparison of 250 *vs* 250 + 10 mg/kg, 250 *vs* 500 mg/kg. Blue colour sign indicates the comparison of 500 *vs* 500 + 10 mg/kg. Red colour sign indicates the comparison of FB with 500, 125 + 10, 250 + 10 and, 500 + 10 mg/kg

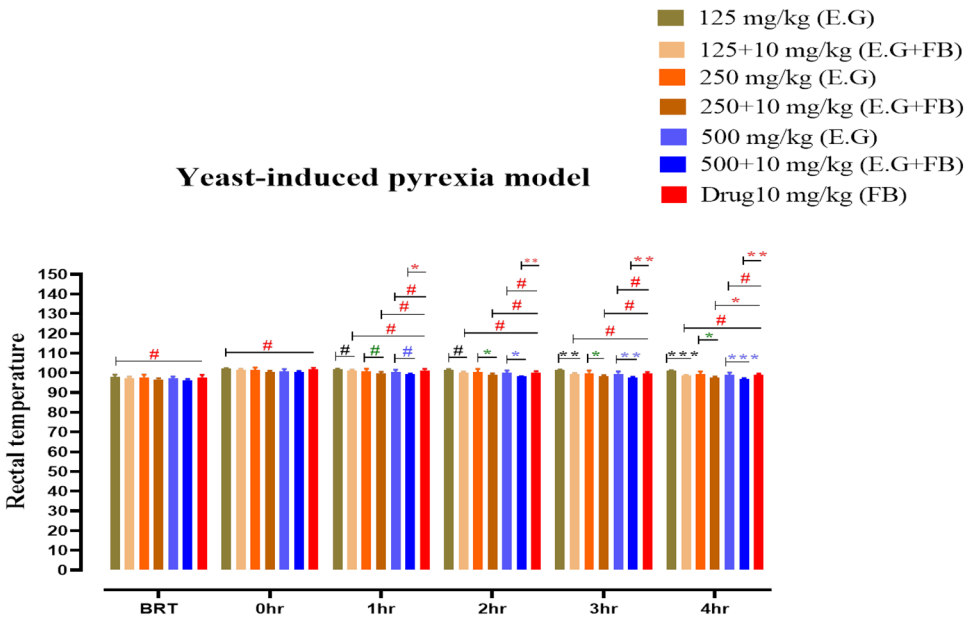

Fig. 6 Effect of E.G, FB, E.G + FB treatments on yeast-induced pyrexia. Values shown are mean ± SEM (n = 6). Where E.G = *Eucalyptus globulus* essential oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 vs 125 + 10 mg/kg and, 125 vs 250 mg/kg. Green colour sign indicates the comparison of 250 vs 250 + 10 mg/kg and, 250 vs 500 mg/kg. Blue colour sign indicates the comparison of 500 vs 500 + 10 mg/kg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10, and 500 + 10 mg/kg

Table 8 Effect of different treatments on cotton pellet-induced granuloma

E.G 125 mg/kg	E.G 250 mg/kg	E.G 500 mg/kg	E.G+FB 125+10 mg/kg	E.G+FB 250+10 mg/kg	E.G+FB 500+10 mg/kg	FB 10 mg/kg
44.54 ± 1.44	51.26 ± 1.44$^{\#}$	57.98 ± 7.23$^{\#/\#}$	66.94 ± 8.65$^{***/\#}$	71.99 ± 5.71$^{***/\#}$	76.47 ± 5.25$^{***/\#}$	65.54 ± 4.87

Values shown are mean ± SEM of percentage inhibition of granuloma. Where E.G = *Eucalyptus globulus* essential oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 *vs* 125 + 10 mg/kg, 125 *vs* 250 mg/kg. Green colour sign indicates the comparison of 250 *vs* 250 + 10 mg/kg, 250 *vs* 500 mg/kg. Blue colour sign indicates the comparison of 500 *vs* 500 + 10 mg/kg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10 and, 500 + 10 mg/kg

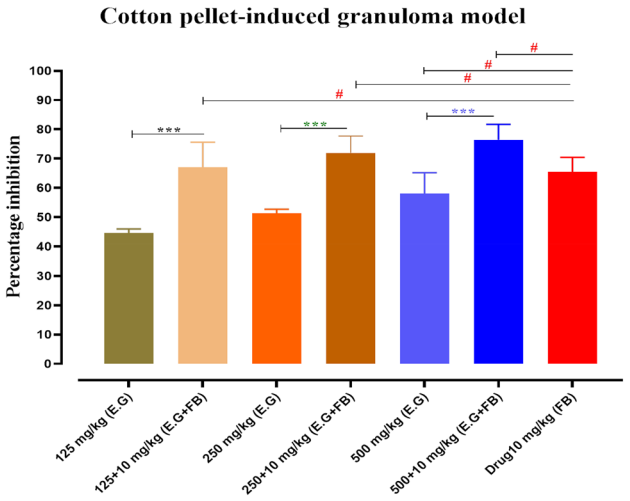

Fig. 7 Effect of different treatments on cotton pellet-induced granuloma. Values shown are mean ± SEM of percentage inhibition of granuloma (n = 6). Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 *vs* 125 + 10 mg/kg and, 125 *vs* 250 mg/kg. Green colour sign indicates the comparison of 250 *vs* 250 + 10 mg/kg and, 250 *vs* 500 mg/kg. Blue colour sign indicates the comparison of 500 *vs* 500 + 10 mg/kg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10, and 500 + 10 mg/kg

of Lavender has been shown to prevent hypotonicity-induced lysis of RBC's membrane. In the current study, combination of *Eucalyptus globulus* oil and flurbiprofen (500 + 10 mg/ml) showed maximum protection against heat-induced haemolysis. Eucalyptol is suggested to play a major role in this protection as reported by Boukhatem and colleagues (Boukhatem et al. 2020).

Carrageenan-induced inflammation is a two phase process as reported by various scientists. The early phase involves release of serotonin, histamine and other mediators followed by the release and subsequent degrading actions of lysosomes, prostaglandins and proteases on tissue cells (Cronstein et al. 1999; Mworia et al. 2021; Pillinger et al. 1998). Histamine released during an acute inflammatory phase increases vascular permeability, draws neutrophils at the site of inflammation where exudate forms, and works in conjunction with prostaglandins to promote oedema. This inflammatory phase lasts only for a brief period (de Morais Oliveira-Tintino et al. 2018). 1,8- Cineole has been shown to inhibit carrageenan-induced oedema by blocking the increase in capillary permeability (Takaki et al. 2008). Moreover, it is known to suppress mast cells degranulation (Nakamura et al. 2020). Flurbiprofen is known to inhibit carrageenan-induced paw oedema through COX inhibition (Sugimoto et al. 2016). Combination treatment of *E. globulus* oil and flurbiprofen (500 + 10 mg/kg) showed significantly better results in both acute model of inflammation as compared with other treatments. This could be due to multi-targeted actions of bioactive molecules of *Eucalyptus globulus* oil and flurbiprofen.

Acetic acid serves as a stimulus for acute nociception in an inflammatory pain model. Acetic acid damages tissues and releases chemicals that cause pain. It also activates peripheral nociceptors on sensory nerve fibres (Naghizadeh et al. 2016). Flurbiprofen is known to rapidly decrease PGE_2 levels in paw exudate and inflammatory pain in the

Table 9 Effect of different treatments on CFA-induced arthritis

Days	E.G 125 mg/kg	E.G 250 mg/kg	E.G 500 mg/kg	E.G+FB 125+10 mg/kg	E.G+FB 250+10 mg/kg	E.G+FB 500+10 mg/kg	FB 10 mg/kg
1	4.97 ± 1.16	6.65 ± 0.27$^{\#}$	9.26 ± 0.75$^{\#/\#}$	9.68 ± 0.55$^{\#/\#}$	10.95 ± 1.46$^{\#/\#}$	12.72 ± 1.29$^{\#/\#}$	10.70 ± 1.23
3	8.96 ± 0.83	10.89 ± 0.94$^{\#}$	11.54 ± 0.94$^{\#/\#}$	13.47 ± 2.36$^{\#/\#}$	14.19 ± 1.03$^{\#/\#}$	17.06 ± 1.77$^{\#/\#}$	13.04 ± 2.25
5	11.34 ± 0.48	13.49 ± 1.14$^{\#}$	16.96 ± 1.13$^{\#/\#}$	19.11 ± 2.70$^{*/\#}$	21.17 ± 0.74$^{*/\#}$	22.39 ± 3.53$^{\#/\#}$	18.53 ± 3.10
9	21.00 ± 1.64	23.68 ± 0.59$^{\#}$	24.14 ± 2.16$^{\#/\#}$	28.34 ± 2.43$^{*/\#}$	28.69 ± 0.44$^{\#/\#}$	35.32 ± 2.34$^{***/***}$	24.34 ± 2.35
13	23.81 ± 1.25	26.02 ± 0.52$^{\#}$	26.95 ± 1.45$^{\#/\#}$	34.45 ± 4.32$^{***/\#}$	37.87 ± 1.75$^{***/**}$	45.53 ± 2.29$^{***/***}$	28.77 ± 2.18
21	39.69 ± 2.25	40.53 ± 1.65$^{\#}$	46.06 ± 5.26$^{\#/***}$	50.39 ± 5.05$^{***/*}$	61.23 ± 3.14$^{***/\#}$	78.95 ± 2.36$^{***/***}$	57.62 ± 18.58

Values shown are mean ± SEM of percentage inhibition of arthritis. Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 vs 125 + 10 mg/kg, 125 vs 250 mg/kg. Green colour sign indicates the comparison of 250 vs 250 + 10 mg/kg, 250 vs 500 mg/kg. Blue colour sign indicates the comparison of 500 vs 500 + 10 mg/kg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10, and 500 + 10 mg/kg

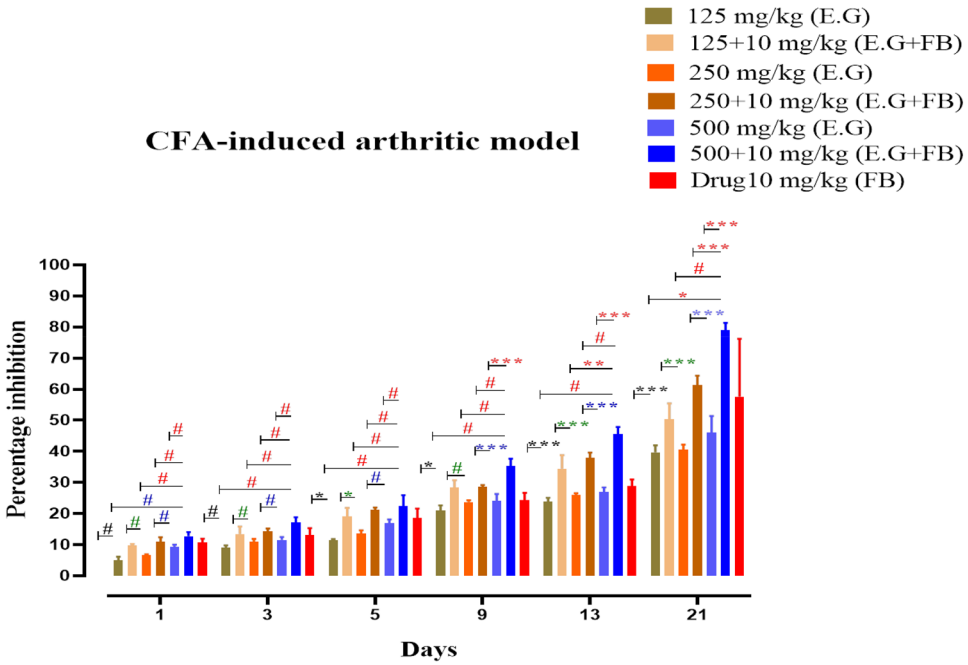

Fig. 8 Effect of different treatments on CFA-induced arthritis. Values shown are mean ± SEM of percentage inhibition (n = 6). Where E.G = *Eucalyptus globulus* oil, FB = Flurbiprofen, # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Black colour sign indicates the comparison of 125 *vs* 125 + 10 mg/kg and, 125 *vs* 250 mg/kg. Green colour sign indicates the comparison of 250 *vs* 250 + 10 mg/kg and, 250 *vs* 500 mg/kg. Blue colour sign indicates the comparison of 500 *vs* 500 + 10 mg/kg. Red colour sign indicates the comparison of drug with 500, 125 + 10, 250 + 10, and 500 + 10 mg/kg

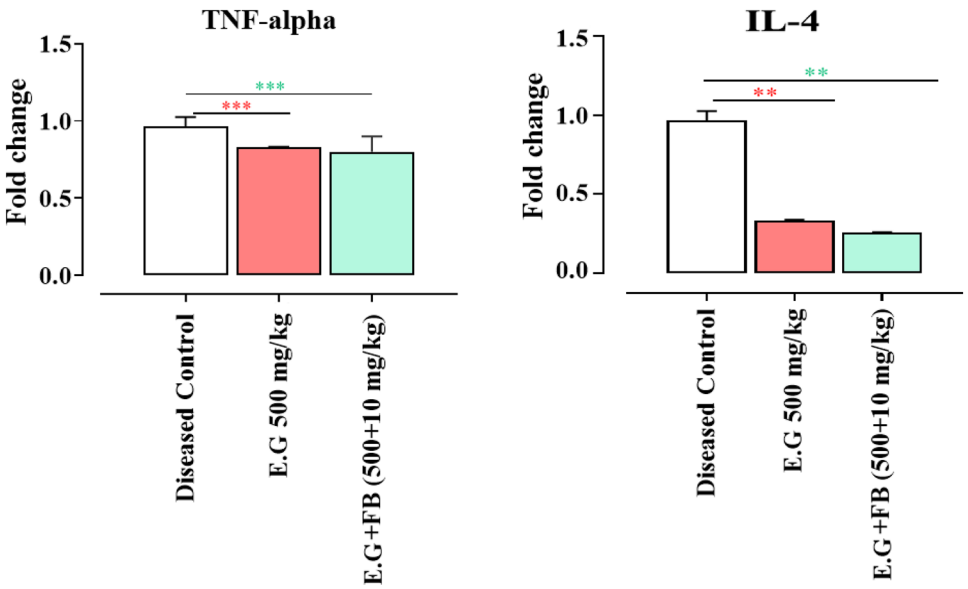

Fig. 9 Effect of *Eucalyptus globulus* oil and Flurbiprofen treatments alone and in combination on the expression of TNF-α and IL-4. Values shown are mean ± SEM of three independent experiments (n = 3). Where # = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ respectively. Red and green colours indicate comparison of 500 mg/kg and 500 + 10 mg/kg treatments with the diseased control group respectively

acetic acid-induced pain model (Sugimoto et al. 2016). *Eucalyptus globulus* oil has established peripheral effects which reduces the writhes (Silva et al. 2003). Limonene, the second most bioactive molecule detected in *E. globulus* oil has been proved to have anti-nociceptive effects (Adriana Estrella et al. 2021). In the current study, combination treatment showed significantly better analgesic effects which are suggested to be due to reduced levels as well as actions of PGE2 on peripheral nerves.

Fever is a complex physiologic response which may be caused by infection or aseptic stimuli. When prostaglandins (PGE2) accumulates in the hypothalamus pre-optic region, it causes elevation in body temperature (Mworia et al. 2019). In the current study, combination treatment (500 + 10 mg/kg) showed significantly better anti-pyretic activity than other treatment groups. This may be due to the flurbiprofen which inhibit prostaglandin E2 synthesis *via* COX inhibition in hypothalamus region (Mworia et al. 2019).

Cotton pellet-induced granuloma represents three stages of inflammation. The transudative phase in vascular permeability increases, capillary fluids develop and fill the pellets. The exudative phase which occurs between 3 and 72 h involves proteins migration around granuloma. The third phase (proliferative phase), which is characterised by an

increase in the dry weight of the pellet, as a result of recruitment of fibroblasts, neutrophils, and macrophages (Sokeng et al. 2020). 1,8-Cineole is known to inhibit granuloma formation (Martins et al. 2017). Moreover, essential oil of *Citrus aurantium L.* containing limonene has been shown to be effective regarding the transudate and granuloma formation amount (Khodabakhsh et al. 2015). NSAIDs also have anti-proliferative effects which decrease granuloma formation (de Morais Oliveira-Tintino et al. 2018). In the current study, combination treatment (500 + 10 mg/kg) inhibited granuloma formation. This could be due to multi-targeted actions of bioactive molecules of *Eucalyptus globulus* oil and flurbiprofen.

Features of Complete Freund's adjuvant (CFA)-induced arthritis pathologically match with rheumatoid arthritis (RA) (Andleeb et al. 2022). CFA injection can trigger release of number of inflammatory cytokines including, IL-1, IL-6, and COX-2 (Zhang et al. 2020). IL-1, IL-6, and TNF-alpha are among the few pro-inflammatory mediators which are elevated in RA (Andleeb et al. 2022; Asif et al. 2020a). The increased production of free radicals by activated neutrophils and macrophages is a key factor in the pathogenesis of RA (Mahdi et al. 2018). Moreover, IL-4 has been revealed to have both pro- and anti-inflammatory effects. On one side it decreases monocyte and macrophage functions and induces a Th2 type response. On the other side it causes recruitment of inflammatory cells by increasing the expression of cell adhesion molecules (VCAM-1) on the endothelial surface and increases eosinophils, macrophages and B cells chemo-attraction (Ratthé et al. 2009). In the current study, combination treatment (500 + 10 mg/kg) showed significantly better anti-arthritic effects as compared with other treatment groups. These effects may be due to cytokines inhibitory effects of eucalyptol (Silva et al. 2003) and flurbiprofen which blocks COX-2 expression at the site of inflammation. Overall, this causes possible decrease in number of inflammatory cells and cytokines release at the site of inflammation.

Conclusion

The current research demonstrates that eucalyptol rich *Eucalyptus globulus* essential oil in combination with flurbiprofen has potent anti-inflammatory, analgesic and antipyretic effects. Down-regulation of expression and actions of pro-inflammatory cytokines (e.g., TNF-α and IL-4) with subsequent inhibition of cellular events controlled by these mediators is proposed to be the major pathway. Further studies to formulate a stable dosage form of this combination and exploration of its effects in various chronic inflammatory disorders is undergoing in our research laboratory.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest Authors do not declare conflict of interest in the current work.

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