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Original Research Article

ANTI-INFLAMMATORY, ANTIPYRETIC AND ANALGESIC ACTIVITIES OF *EUCALYPTUS GLOBULUS* ESSENTIAL OIL IN ALBINO RATS

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Abstract: *Medicinal plants are effective natural anti-inflammatory, anti-pyretic, and anti-analgesic agents. Inflammation is a complicated biological defense reaction of vascular tissues to potentially damaging stimuli such as bacteria, damaged cells, or irritants. The current study aimed to establish the anti-inflammatory, antipyretic, and analgesic properties of E. globulus essential oil. In this research, 36 albino rats weighing 140-170g on average were employed. 36 rats were divided into three groups (control, standard and experimental group). Control and standard group consisted of 6 rats in each group whereas experimental group consisted of 24 rats. All rats in three groups were treated with carrageenan for induction of oedema for anti-inflammatory, brewer's yeast to induce pyrexia to check anti-pyretic activity and acetic acid to induce pain for analgesic activity. Control group rats were treated with normal saline for all 100u µl three activities. Standard group rats were treated with diclofenac for anti-inflammatory and analgesic activities and paracetamol for antipyretic activity. The essential oils were injected into abdominal muscles and intraperitoneal tissues. The maximum percentage of inhibition of leaves oil in anti-inflammatory action was 54.01%. The maximum percentage of suppression of fever by essential leaves oil was 75.44% in antipyretic efficacy. The highest percentage inhibition against pain in analgesic action was 25%. Significant anti-inflammatory, antipyretic, and analgesic effects were demonstrated at 50µl/L, 100µl/L, and 12.5µl/L doses, respectively, when compared to control group. From overall result, it was concluded that injection of E. globules leaves oil in abdominal and intraperitoneal muscle may possess significant anti-inflammatory, antipyretic and analgesic activities. Our study scientifically supports traditional use of E. globulus as a medicine.*

Keywords: Anti-inflammatory, antipyretic, analgesic, *Eucalyptus globulus*, pain, fever, leaf oil, diclofenac

Introduction

Eucalyptus globulus Labill is in the Myrtaceae family (Abbasi *et al*., 2020). The Tasmanian blue gum, or *E. globulus* Labill, is a native of Tasmania, the Bass Strait Islands, and southeast Australia. One of the most well-known and widely distributed fast-growing eucalypt species at the moment, it is thought to cover about 2.3 million acres worldwide (Silva *et al*., 2021). A tree called *E. globulus* can naturally reach heights of 60 to 80 metres. Brown or yellowish-brown coloured plants have mostly smooth bark that sheds in long strips, leaving a white or greyish surface that resists rot and can easily detach from the stem. The age of the tree has an impact on its leaf colour as well (Abbasi *et al*., 2020). Numerous monoterpenes and sesquiterpenes, as well as aromatic phenols, oxides, ethers, alcohols, esters, aldehydes, and ketones, such as 1,8-cineole (eucalyptol), citronellal, citronellol, citronellyl acetate, p-cymene, and eucamalol, are present in the complex composition of eucalyptus (Butnariu, 2021). Asthma, diabetes, and pulmonary tuberculosis are all treated with the essential oil extracted from *E. globulus* leaf leaves. Additionally, oils from its leaves, fruits, buds, and bark have been shown to have antibacterial, antioxidant, anti-inflammatory, antihelminthic, and anticancer properties (Mehta and Sharma, 2022). Eucalyptus oil can be used as an immunoregulatory agent against infectious diseases,

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according to the WHO, because it enhances innate cell-mediated immunity. A bioactive substance known as eucalyptus oil was extracted from Eucalyptus polybractea and is capable of acting as an antiviral agent against the influenza virus as well as inhibiting the avian influenza virus H11N9 in vapour and aerosol form. According to a report, eucalyptus oil exhibits in vitro antiviral activity, which enables it to inhibit enveloped viruses like the mumps and herpes (Asif *et al*., 2020; Alyas *et al*., 2020).

When used as an anti-inflammatory agent and to treat upper and lower respiratory illnesses in patients with severe asthma, eucalyptol has been shown to have anti-inflammatory properties (Alyas *et* al., 2020; Vecchio *et al*., 2016). Patients with severe, ongoing pain depend on a variety of complementary therapies while new medications with minimal or nonexistent side effects become available. Numerous studies have shown that EEO has analgesic properties (Gbenou *et al*., 2013; Lee *et al*., 2019; Mondal *et al*., 2021; Mworia *et al*., 2020). In general, *E. globulus* leaf extract showed in vivo antipyretic activities in rats, as shown by a decrease in rectal temperature in response to yeast-induced fever (Mworia *et al*., 2019).

The goal of the present study was to assess the medicinal value of plants for the treatment of inflammation, fever, and pain using an animal model (albino rats).

Material and methods

Sample collection

Leaves of *E. globulus* were collected from botanical garden, Quaid-e-Azam campus Punjab University, Lahore. The species were identified by botanists of IMBB department of The University of Lahore, Pakistan. Essential oil was extracted by hydro-distillation in a Clevenger apparatus. 100 g of E. globulus leaves were boiled for two hours in distilled water. The distillate was collected once the temperature had settled. The distillate was mixed with sodium chloride (5 g) to help separate the organic and aqueous phases. The mixture was then poured into a separate funnel, and the distillate was repeatedly cleaned with cyclohexane. After stirring, the organic phase was used to perform rotary evaporation to get the essential oil and get rid of the cyclohexane. After the yield estimate was calculated, the essential oil was kept at 4 °C (Belkhodja *et al*., 2022).

Experimental rats

Albino rats of either female and male sex (140-170 g) were used for all three activities. For experimental study, albino rats were purchased from (UVAS) University of Veterinary and Animal Sciences Lahore. Rats were kept in animal house, University of Lahore in polypropylene cages. Before experimental work, rats were kept in fasting condition. After that, they were given distilled water and balanced feed.

Drugs Used and Chemicals

- Normal saline 100 µl/L
- Diclofenac 50 µl/L
- *Eucalyptus globulus* leaf essential oil (oil) 100 µl/L
- Carrageenan (inflammation) 100 mg/kg
- Yeast (pyrexia) 100mg/kg
- Paracetamol 100mg/kg
- Acetic acid (writhing) 100mg/kg
- Distilled water 100 µl/L

Anti-inflammatory activity model

Firstly, all rats were treated with carrageenan in which 12.5 mg/kg, 25 mg/kg, 50 mg/kg and 100mg/kg was carrageenan injected into sub-plantar region of paw. Measurement of paw was taken before and after the induction of carrageenan. Group I was considered as control group. In control group rats were treated with normal saline 12.5 µl/L, 25 µl/L, 50 µl/L and 100 µl/L according to their body weights. Normal saline was injected into right hind paw. Volume of paw was measured in millimeter cube. Group II was considered as standard group. Rats of standard group were treated with diclofenac (12.5 µl/L, 25 µl/L, 50 µl/L and 100 µl/L). Group III was considered as experimental group. Rats of experimental group were treated with essential oil of *E. globulus* leaves 12.5 µl/L, 25 µl/L, 50 µl/L and 100µl/L 3hrs after the carrageenan injection. The volume of paw was measured after the interval of 1hr, 2hr and 3hr of treatment doses of essential oil. Anti-inflammatory activity was calculated by the given formula:

$$\text{Anti - inflammatory} = \frac{(Ct - Co) - (Ct - Co)\times 100\ C}{(Ct - Co)}$$

Where, Co= Reading of paw before carrageenan, Ct= Volume of the hind paw of after carrageenan (Ct-Co) = Volume of the hind paw of the treated group after carrageenan injection.

Anti-pyritic activity model

Firstly, all rats were treated with brewer's yeast, which were injected below the nape of neck 12.5, 25, 50 and 100µl/L according to their body weight. After the interval of 0, 1hr 2hrs, 3hrs and 4hrs temperature were measured with help of thermometer (rectum). Group I was considered as control group. In control group rats were treated with normal saline 12.5 µl/L, 25 µl/L, 50 µl/L and 100µl/L according to their body weights. Normal saline was injected into nape of the neck. Group II was considered as standard group. Rats of standard group were treated with paracetamol injection 12.5 mg/kg, 25 mg/kg, 50 mg/kg and 100 mg/kg in nape of the neck. After the interval of 0, 1hr, 2hrs, 3hrs and 4hrs temperature were measured with help of thermometer (rectum). Group III was considered as experimental group. The rats of experimental group were treated with leaves

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oil of *E. globulus* (12.5 µl/L, 25 µl/L, 50 µl/L and 100µl/L). After the interval of 0, 1hr, 2hrs, 3hrs and 4hrs temperature were measured with help of thermometer (rectum).

Percent production $= \frac{B - C_b \times 100}{B - A}$

Where, B = Temperature after pyrexia induction, Cb = Temperature after 1, 2 and 3 hours and A =Normal body temperature.

Analgesic activity model

Acetic acid induced writhing. Group I was considered as control group. In control group rats were treated with normal saline 12.5mg/kg, 25mg/kg, 50mg/kg and 100 mg/kg before 1 hrs treated with acetic acid, according to their body weights. Normal saline was injected in the intra peritoneal cavity. Group II was considered as standard group. Rats of standard group were treated with diclofenac (12.5mg/kg, 25mg/kg, 50mg/kg and 100 mg/kg) was given 1-hour beore the administration of acetic acid. Group III was considered as experimental group. Rats of experimental group were treated with leaves oil of *E. globulus* (12.5 µl/L, 25 µl/L, 50 µl/L and 100 µl/L) before 1hr treated with acetic acid. A writhing was recorded with the help of stopwatch.

Analgesic activity $= \frac{N_c - N_t \, X100}{N_c}$

Analgesic activity = Where, Nc= Control group writhe and Nt = Treated group writhe.

Statistical analysis

ANOVA was used for the statistical analysis, and repeated comparisons confirmed that there was a significant difference in the activities (P 0.05). However, a difference with a P value of less than 0.05 was deemed significant.

Results

Carrageenan induces paw edema of *E. globulus*

In the antipyretic activity, the *Eucalyptus globulus* leaf oil showed significant results (P>0.05) at a dose of 50µl/L results was shown highest inhibition value at 1hrs, 2hrs and 3hrs and study was most effective at dose 50µl/L to reduced inflammations.(table 1)

Antipyretic activity of essential oil of E. globulus

In the antipyretic activity, the *Eucalyptus globulus* leaf oil showed significant results (P>0.05) at a dose level of 100µl/L recorded the highest antipyretic effects, which was 85.43%, 78% 72% and 75% at 1hr, 2hrs, 3hrs and 4hrs respectively (table II).

Analgesic activity of essential oil of *E. globulus*

In the antipyretic activity, the *Eucalyptus globulus* leaf showed significant results (P>0.05) at a dose level of 12.2µl/L, 25µl/L, 50µl/L and 100µl/L were shown 14±0.26, 18.83±0.87, 18.50±0.76 and 18.67±0.56 writhing mean with 25.65%, 0, 1.75% and 0.85% writhing inhibition (table III).

Table 1. Anti-inflammatory of *E. globulus*

Groups	Treatment	Pre-inflammation	Post-inflammation	1hr	2hrs	3hrs
Group-I	**Control group**	3.33±0.17	4.05±0.12	3.74±0.11	3.60±0.09	3.53±0.09
Group-II	**Standard group**	2.94±0.14	5.65±.31	5.29±0.32	4.97±0.33	4.41±0.21
	Treated at (12.5 µl/L)	2.76±0.12	6.30±0.29	5.77±0.29	5.03±0.09	4.59±0.17
Group-III	**Treated at (25 µl/L)**	3.15±0.08	5.97±0.1	5.97±0.1	5.48±0.09	5.07±0.16
	Treated at (50 µl/L)	2.41±0.1	6.45±0.12	5.76±0.12	5.35±0.04	5.02±0.08
	Treated at (100 µl/L)	2.29±0.07	5.79±0.19	5.32±0.06	5.09±0.07	4.67±0.2
	p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Anti-inflammatory of *E. globulus* leaves oil Values are mean ±S.D; n=4 in each group *P*< 0.0001compare to control group

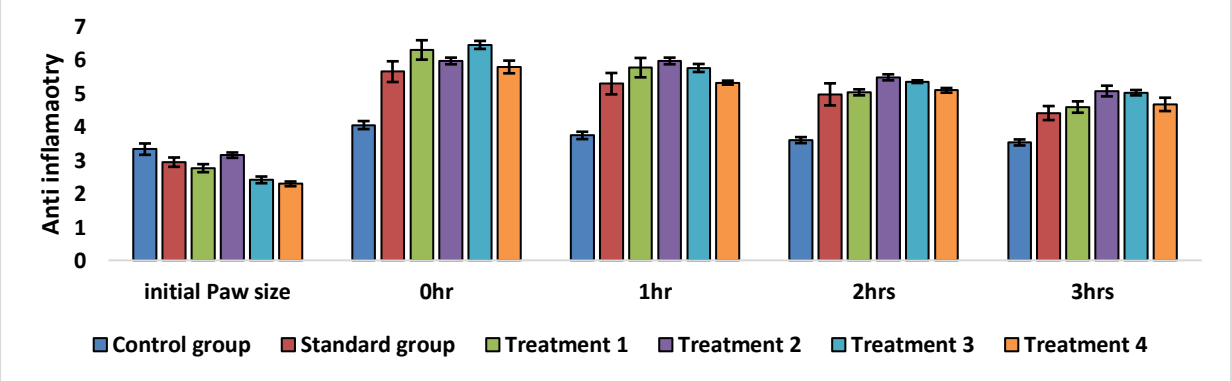

Figure I Anti inflammatory activity

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Table 2. Anti-Pyretic Activity of *E. globulus*

No. of Groups	Treatment	Initial temperature	0hr	1hr	2hrs	3hrs	4hrs
Group-I	Control group	98.08±0.3	100.82±0.47	100.62±0.47	100.40±0.45	100.15±0.42	99.98±0.42
Group-II	Standard group	97.77±0.16	100.43±0.44	99.03±0.3	98.57±0.22	97.93±0.17	97.78±0.17
	Treated at (12.5 µl/L)	99.23±0.32	100.05±0.5	98.85±0.25	98.38±0.14	97.88±0.17	97.12±0.31
	Treated at (25 µl/L)	99.32±0.25	100.90±0.26	99.57±0.27	99.10±0.19	98.87±0.12	98.17±0.18
Group-III	Treated at (50 µl/L)	98.68±0.49	100.22±0.36	99.32±0.14	99.02±0.22	98.23±0.59	97.05±0.62
	Treated at (100 µl/L)	99.05±0.21	99.78±0.28	98.28±0.29	96.83±0.95	95.90±0.79	95.28±0.76
	p-value	0.004	0.3265	0.0002	0.0004	<0.0001	<0.0001

Antipyretic activity of *E. globulus* of leaves oil
Values are mean ±S.D; n=4 in each group $P<0.005$ compared to control group.

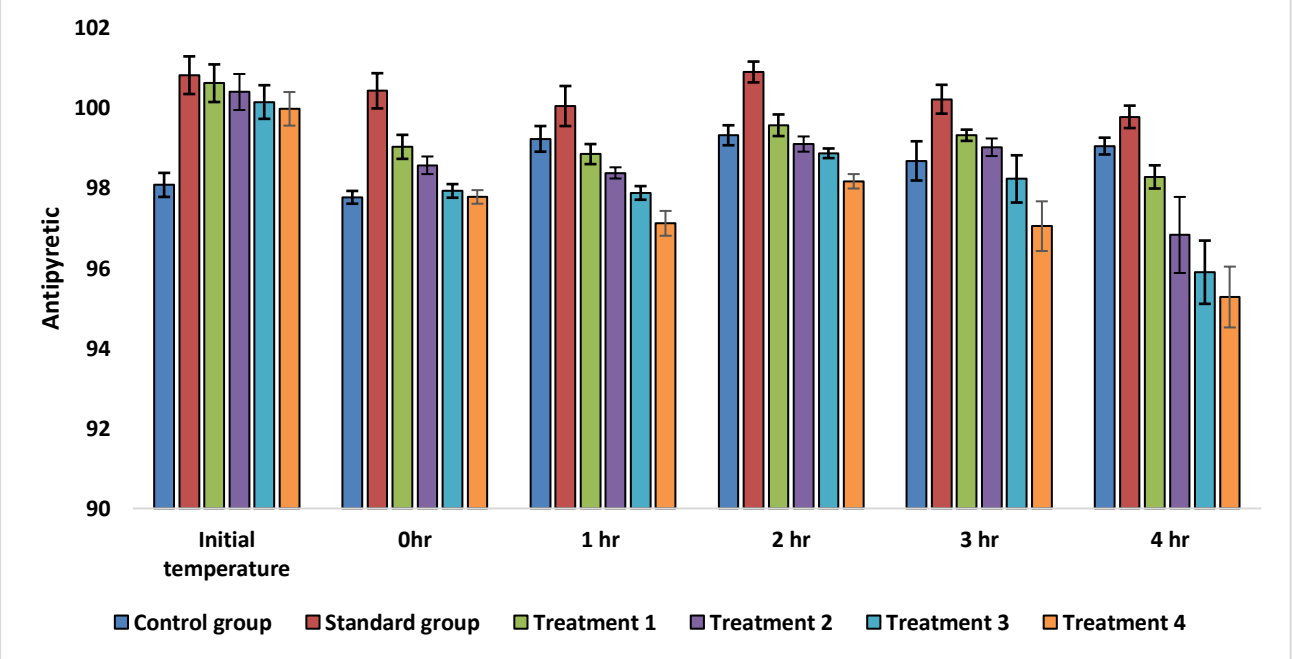

Figure II Antipyretic activity

Table III. Analgesic activity of *E. globulus*

Treatments	Analgesic Time	Writhing (%)
Control group	18.83±0.4	0
Standard group	12.17±0.91	35.36
Treated at (12.5 µl/L)	14.00±0.26	25.65
Treated at (25 µl/L)	18.83±0.87	0
Treated at (50 µl/L)	18.50±0.76	1.75
Treated at (100 µl/L)	18.67±0.56	0.85
p-value	< 0.0001	

Analgesic effect of *E. globulus* leaves oil
Values are mean ±S.D; n=4 in each group $P< 0.0001$compare to control group.

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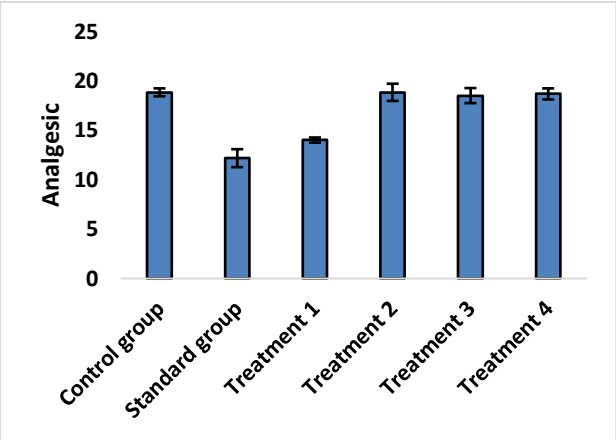

Figure III Analgesic activity

Discussion

In our study, *E. globulus* leaf oil was used to evaluate anti-inflammatory, antipyretic and analgesic activities on albino rats. Due to the drug's lag in entering rats' bodies, time lag arises in analgesic action (Parra *et al.,* 2019). Carrageenan's anti-inflammatory activity causes edoema in rat paws whereas brewer's yeast's antipyretic activity causes pyrexia in rats, and acetic acid's analgesic activity is used to assess the writhing test's analgesic effect (Alyas *et* al., 2022; Subedi *et al.,* 2016). In inflammation process main symptoms are fever and pain (Deka *et al.,* 2018). In our results, highest paw oedema inhibition of leaves oil of *E. globulus* was at dose of 50µl/L. Anti-inflammatory activity was dose dependent. The essential oil declined the paw edema induced by carrageenan injection when compared with the control group. The presence of chemical substances which are inflammatory process mediators results in increase in the vascular permeability at the site of inflammation, thus promoting accumulation of fluid in tissues and this resulted in oedema (Germolec *et al.,* 2018).

Brewer's yeast causes pyrexia in the body by boosting prostaglandin production when it is injected into the albino rats' nape of the neck. Brewer's yeast is used to create pathogenic fever, which is characterised by the generation of prostaglandins as its etiological cause. Synthesis of prostaglandins can be used to produce an antipyretic effect. Paracetamol and prostaglandins both work to suppress the cyclo-oxygenase enzyme's activity. To lower the fever, intraperitoneal injection of *E. globulus* leaf oil is used (Muhammad *et al.,* 2021). According to results the group that received leaf oil of *E. globulus* at a dose level of 100µl/L recorded the highest antipyretic effects, which was 85.43%, 78% 72% and 75% at 1hr, 2hrs, 3hrs and 4hrs respectively.

Acetic acid induction induces abdominal and visceral pain in rats, which generates prostaglandin pain from peritoneal fluid (Bairagi *et al.,* 2017). Acetic acid is used as a painkiller to block endogenous substances that cause pain and release from nerve endings (Subedi *et al.,* 2016). In contrast to the control group, our study found that intra-peritoneal treatment of the leaf oils of *E. globulus* dramatically reduced the number of acetic acid-induced writhes in rats. The maximum writhing inhibition and the highest analgesic effect were caused by the leaves oil of *E. globulus* (12.5 l/L).

Conclusion

E. globulus leaves oil may possess significant anti-inflammatory, antipyretic and analgesic activities due to its phytoconstitutents. Our study scientifically supports traditional use of *E. globulus* as a medicine. In future prospective, secondary metabolites present in plant will help us to understand identification and isolation of compound that can be clinically used. Natural compounds of plant are responsible for above activities. Due to the anti-inflammatory, antipyretic, and pain-relieving properties of its bioactive compounds, it was suggested that this essential oil be used in scientific research and industry as a natural alternative and drug.

Conflict of interest

The authors declared absence of conflict of interest.

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