

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF *ELAEOCARPUS SPHAERICUS* LEAF EXTRACT

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ABSTRACT

In this study, we evaluated petroleum ether, chloroform, methanol and aqueous extract of *Elaeocarpus sphaericus* leaves for analgesic and anti-inflammatory potentials using carrageenan-induced paw oedema (inflammation) in rats and tail flick tests in mice. The methanol and aqueous extract of *Elaeocarpus sphaericus* leaves at all doses (50, 100, 200 mg/kg) showed significant ($p < 0.05$) percentage inhibition of oedema at 3rd hr of treatment when compared with control group but maximum percentage inhibition of oedema at dose 200mg/kg (ie. 46.21% and 41.66%) for both extracts. While the standard drug Diclofenac sodium (5mg/kg) showed significant inhibition of oedema at 3rd hr of treatment is 49.40%. For analgesic activity it is also concluded that the methanol and aqueous extract of *Elaeocarpus sphaericus* leaves at dose 100 mg/kg showed significant ($p < 0.05$) increase in tail flick response. Therefore, it can be inferred that the inhibitory effect of methanolic and aqueous extract at dose 200 mg/kg of *Elaeocarpus sphaericus* leaves on carrageenan induced inflammation may be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

Keywords: *Elaeocarpus sphaericus*, Analgesic and Anti-inflammatory

INTRODUCTION

Despite the progress made in medical research for the past decades, the treatment of many serious diseases is still problematic. Chronic inflammatory diseases and pain remains one of the world's major health problems¹. About one-third of the world's population suffers from persistent or recurrent pain which costing approximately \$100 billion each year in health care². Inflammation involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair and pain is a complex, multidimensional sensory experience that involves not only the transduction of noxious environmental stimuli but also cognitive and emotional processing by the brain³⁻⁵. So, Inflammation and pain has become the focus of global scientific research because of its implication in virtually all human and animal diseases.

Elaeocarpus sphaericus (syn. *Elaeocarpus ganitrus*) commonly known as *Rudraksha*, grown in Assam and Himalayan region of India for its attractive fruit stones and medicinal properties⁶. It is used in folk medicine in treatment of stress, anxiety, depression, palpitation, nerve pain, epilepsy, migraine, lack of concentration, asthma, hypertension, arthritis and liver diseases. According to the ayurvedic medicinal system, wearing of *Rudraksha* can have a positive effect on heart and nerves⁷⁻⁹. Methanolic extract of leaves shows the presence of alkaloids, steroids, flavanoids etc. The study has been designed to evaluate the analgesic & anti-inflammatory effect of *E. sphaericus* leaf extracts by using tail flick method in mice & carrageenan induced rat paw oedema in rats respectively.

MATERIALS AND METHODS

Plant collection

The leaves of *Elaeocarpus sphaericus* (*E. ganitrus*) were collected from Ch. Devi Lal herbal garden, khizrabad in the month of August, 2009 and authenticated from NISCAIR, New Delhi. The voucher specimen (2009-10/1341/143) of the plant was deposited at the college for further reference.

Preparation of extracts

Leaves of the whole plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (100g) of the powder was subjected to successive extraction with petroleum ether, chloroform, methanol and water in

Soxhlet apparatus. The extract was concentrated under reduced pressure using rotary evaporator until all the solvent has been removed. Percentage yield of all extracts of *E. sphaericus* was calculated in terms of w/w.

Animals used

Albino rats & Swiss mice were collected from ZINCO laboratory, Ambala. Albino rats and Swiss mice (either sex) weighing 150-200 and 25-30g respectively were procured from the animal house of M. M. College of Pharmacy, Mullana. The animals were kept in polypropylene cages (6 in each cage) at an ambient temperature of $25 \pm 2^\circ\text{C}$ and 55-65% relative humidity. A 12-12 hr light and dark schedule was maintained in the animal house. The mice had free access to water and were fed with commercially available feed. Food was withdrawn 1hr before the experiment. All experimental protocols were approved by the M. M. College of Pharmacy animal ethics committee.

Experimental design

Tail flick test

Albino mice were divided into 14 groups, each group contains 6 animals. Test drugs were administered orally. The lower half of its tail dipped in beaker of cold water. The time in seconds for tail withdrawal from water was taken as reaction time. The reaction time was determined before and at 30 minute interval after administration of extract, vehicle and standard drug¹⁰.

Group 1: Vehicle control (2% gum acacia in normal saline)

Group 2: Diclofenac sodium (5mg/kg body weight)

Group 3-5: Leaf extracts (50,100, 200mg/kg body weight)

Paw oedema test

It is commonly employed method for screening anti-inflammatory activity in rats. In this experiment, acute inflammation was produced by subplanter injection of 0.1ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rat 1/2 hr after the oral administration of the test materials. The paw volume was measured by Plethysmometer at 1, 2, 3 hrs after the carrageenan injection. The extract was administered at 50,100, 200 mg/kg of the body weight. Diclofenac sodium suspended in 2% gum

acacia at dose of 5mg/kg was used as standard anti-inflammatory drug¹¹.

Group 1: Vehicle control (2% gum acacia in normal saline)

Group 2: Diclofenac sodium (5mg/kg body weight)

Group 3-5: Leaf extracts (50,100, 200mg/kg body weight)

Measurement of response

Paw volume measured before carrageenan injection (0 h). It was measured every hour till the 3 h after injection of carrageenan to each group. The difference between the initial and subsequent readings gave the actual oedema volume. The maximum inhibition was noted after 3 hr. so we consider reading of 3 hr only.

Percent inhibition of inflammation was calculated using the formula:

$$\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_t is the volume of paw oedema at corresponding time, V_c is hind paw volume (ml) with control group only.

Statistical analysis

The activities of the extracts were analyzed by one way ANOVA followed by Tukey's multiple comparison tests. The differences were considered significant at $p < 0.05$.

RESULTS

Preliminary phytochemical studies revealed the presence of alkaloids, steroids, flavanoids and glycosides also.

Analgesic activity

Tail flick method

The both (methanol and aqueous) leaf extracts of *Elaeocarpus sphaericus* at a dose 100 mg/kg showed significant ($p < 0.05$) increase in tail flick response when compared with vehicle from every 30 min. and continued till the 2nd hr of the study. (Table.1 & 2)

Table 1: Reaction time in seconds by tail flick response of methanol extract.

Groups	Dose	Before treatment (in seconds)	After treatment (in seconds)		
			30 min.	60 min.	120 min.
Control (vehicle)	2% gum acacia in normal saline	2.3±0.12	2.53±0.11	2.45±0.13	2.52±0.12
Diclofenac sodium	5 mg/kg.	3.05±0.08	4.56±0.22 ^a	5.46±0.13 ^a	4.98±0.17 ^a
Methanol extract	50mg/kg	2.26±0.09	2.95±0.09	2.80±0.11	2.85±0.10
Methanol extract	100mg/kg	3.0±0.06	4.5±0.05 ^{a,b}	4.85±0.07 ^{a,b}	4.90±0.05 ^{a,b}
Methanol extract	200mg/kg	2.48 ±0.08	4.03±0.06 ^{a,b}	4.23±0.07 ^{a,b}	4.13±0.06 ^{a,b}

Values are expressed as mean ± SEM. a= $p < 0.05$ vs control (vehicle); b= $p < 0.05$ vs Diclofenac sodium (Standard drug)

Table 2: Reaction time in seconds by tail flick response of aqueous extract

Groups	Dose	Before treatment (in seconds)	After treatment (in seconds)		
			30 min.	60 min.	120 min.
Control(vehicle)	2% gum acacia in normal saline	2.3±0.12	2.53±0.11	2.45±0.13	2.52±0.12
Diclofenac sodium	5 mg/kg.	3.05±0.08	4.56±0.22 ^a	5.46±0.13 ^a	4.98±0.17 ^a
Aqueous extract	50mg/kg	2.45±0.08	2.79±0.07	3.58±0.08 ^b	3.45±0.07 ^b
Aqueous extract	100mg/kg	2.70±0.05	3.01±0.10 ^b	3.90±0.07 ^{a,b}	3.78±0.07 ^{a,b}
Aqueous extract	200mg/kg	2.60±0.04	2.85±0.06 ^b	3.65±0.07 ^{a,b}	3.53±0.06 ^{a,b}

Values are expressed as mean ± SEM. a= $p < 0.05$ vs control (vehicle); b= $p < 0.05$ vs Diclofenac sodium (Standard drug)

Table 3: Change in paw volume on carrageenan induced rat paw oedema with methanol extract.

Groups	Dose	Decrease in paw volume (ml)			Percentage inhibition
		Initial reading	3 hrs After drug administration	After drug administration	
Control (vehicle)	2% gum acacia in normal saline	1.74±0.10	1.68±0.10	-----	-----
Diclofenac sodium	5 mg/kg.	1.57±0.10	0.85±0.04 ^a	-----	49.40%
Methanol extract	50mg/kg	1.24±0.04	1.1±0.02	-----	34.52%
Methanol extract	100mg/kg	1.06±0.04	0.96±0.02 ^a	-----	42.85%
Methanol extract	200mg/kg	1.14±0.06	0.87 ±0.02 ^{a,b}	-----	46.21%

Values are expressed as mean ± SEM. a= $p < 0.05$ vs control (vehicle); b= $p < 0.05$ vs Diclofenac (Standard drug)

Table 4: Change in paw volume on carrageenan induced rat paw oedema with aqueous extract.

Groups	Dose	Decrease in paw volume (ml)			Percentage inhibition
		Initial reading	3 hrs After drug administration	After drug administration	
Control (vehicle)	2% gum acacia in normal saline	1.74±0.10	1.68±0.10	-----	-----
Diclofenac sodium	5 mg/kg.	1.57±0.10	0.85±0.04 ^a	-----	49.40%
Aqueous extract	50mg/kg	1.27±0.09	1.15±0.03 ^{a,b}	-----	31.54%

Aqueous extract	100mg/kg	1.24±0.07	1.10 ±0.03 ^{a,b}	34.52%
Aqueous extract	200mg/kg	1.06±0.04	0.98±0.03 ^{a,b}	41.66%

Values are expressed as mean ± SEM. a=p<0.05 vs control (vehicle); b= p<0.05 vs Diclofenac sodium (Standard drug)

Anti-inflammatory activity

Carrageenan-induced paw oedema

In the vehicle treated rats, sub plantar injection of 1% carrageenan suspension produced a local oedema reaching its maximum at 3 hr. Both leaf extracts (methanol and aqueous) of *Elaeocarpus sphaericus* significantly (p<0.05) inhibited the paw oedema at all doses tested when compared with vehicle treated group. Maximum percentage inhibition effect of both extracts at 200 mg/kg dose was 46.31% and 41.66% and diclofenac sodium (49.40%), the reference anti-inflammatory agent (Table 3 and 4).

DISCUSSION & CONCLUSION

Well authenticated leaves of *Elaeocarpus sphaericus* were subjected to successive solvent extraction in the increasing order of polarity with a view to segregate their constituents on the basis of polarity. Aqueous extract was observed to give higher yield of extractives.

Analgesic activity of various extracts was evaluated using a standard model- Tail flick model. The tail flick test is widely and reliably used model. Another advantage of this method is that there is minimal interanimal variation. Anti-inflammatory activity of various extracts was evaluated using Paw-oedema model. This method is simple, easy, short lasting and reproducible¹². Carrageenan induced rat paw oedema test is highly sensitive to non steroidal anti-inflammatory drugs and has long been accepted as a useful tool for investigating new drug therapies. Oedema, which develops in rat paw after carrageenan injection, is a biphasic event. The initial phase is attributed to the release of histamine and serotonin, oedema maintained between the 1st and 2nd phase is due to release of kinin like substances and the 2nd phase due to the release of prostaglandins like compound¹³. Prostaglandins (PGs) play a major role in the development of the second phase of inflammatory reaction which is measured at 3rd hr¹⁴. It can be inferred that the inhibitory effect of methanol & aqueous extract (200mg/kg) of *Elaeocarpus sphaericus* on carrageenan induced inflammation may be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

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