



## **ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITY OF PETROLEUM- ETHER EXTRACT OF *EUPATORIUM TRIPLINERVE VAHL***

**PARIMALA K , BINOY VARGHESE CHERIYAN AND VISWANATHAN S.**

**Department of Pharmacology, Meenakshi Medical College and Research Institute, Kanchipuram, TN.  
Meenakshi Academy of Higher Education and Research, Chennai-78**

### **ABSTRACT**

The petroleum-ether extract of *E.triplinerve Vahl* was subjected to preliminary phytochemical screening. Acute toxicity studies were carried out in Swiss albino mice. Analgesic activity of petroleum-ether extract was evaluated by well established models like acetic acid induced writhing, formalin test and tail immersion method in mice. The acute anti-inflammatory effect was studied by carrageenan induced hind paw edema method in rats. Phytochemical evaluation revealed the presence of sterols, alkaloids, tannins, phenols and glycosides. Acute toxicity studies showed that the extract was non-toxic upto a maximum dose of 2000 mg/kg body weight. Petroleum-ether extract exhibited significant inhibition of acetic acid induced writhing, reduced the paw-licking response time significantly in formalin test and increased the withdrawal latency time in tail immersion test. Carrageenan induced hind paw edema was significantly reduced in rats. The present study indicates that the petroleum-ether extract of *Eupatorium triplinerve Vahl* has potential antinociceptive and anti-inflammatory activity.

**Key words:** *Eupatorium triplinerve Vahl*, Antinociceptive, Anti-inflammatory

### **INTRODUCTION**

In indigenous system of medicine, the leaves of *Eupatorium triplinerve* are reported to be useful in pain and inflammatory disorder. However, its traditional claims have not been fully validated. Hence the present study was designed to evaluate the potential analgesic and anti-inflammatory activity of *Eupatorium triplinerve*.

*Eupatorium triplinerve Vahl* or *Eupatorium ayappana* familiarly known as *Ayappana* in Malayalam language belongs to the family Asteraceae and is an ornamental plant. The essential oil from the plant has been reported to possess a number of medicinal properties such as central nervous system (CNS) depressant, analgesic and sedative effects (Kokate et al. 1971). The

methanolic extract of *E.triplinerve* showed hepato protective effect and anti oxidant effect against carbon tetrachloride induced hepatotoxicity in rats (Bose et al. 2007). The ethanolic extract of the entire plant was active against *Bacillus subtilis* (Verpoorte et al. 1987). Marginal antimicrobial effect of petroleum-ether extract against various strains of bacteria and fungi were noted by Gupta et al (2002). An ethanolic extract and its fractions from *Eupatorium triplinerve* have been reported to exhibit analgesic effect in inflammatory model of pain. (Cheriyen et al. 2009).

The present study has been designed to investigate the petroleum-ether extract of

*Eupatorium triplinerve* for its antinociceptive and anti-inflammatory activity.

## MATERIALS AND METHODS

The leaves of *Eupatorium triplinerve* were collected from Kollam district, Kerala in the month of October 2006 and the sample was authenticated by herbarium department of Tropical Botanical Garden and Research Institute, (TBGRI) Trivandrum (Collection No:31691,31692,Account No:20391,20392). Voucher samples have been deposited at TBGRI Trivandrum and Department of Pharmacology, Meenakshi Medical College hospital & Research Institute, Kanchipuram.

### Animals

Swiss albino mice (25-30g) and male Wistar rats (175-200g) were procured from the institutional animal house. The animals had free access to standard pellet feed (Provomi) and water *ad libitum* under strict hygienic conditions and maintained in room temperature of  $25\pm 1^{\circ}\text{C}$ , relative humidity 45-55% and a 12:12h light/dark cycle. All the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the study protocol was approved by the institutional animal ethical committee.

### Preparation of extract

*Eupatorium triplinerve* Vahl leaves were shade dried and one kg of coarse powder was soaked in 4 litres of petroleum-ether for 3 days at room temperature. The extract was evaporated to dryness by using a rotary vacuum flash evaporator and the yield was 10% w/w.

### Preliminary Chemical test

The petroleum ether extract of *Eupatorium triplinerve* leaves was subjected to qualitative chemical investigation for the identification of phyto constituents (Khandelwal et al. 2000) like steroids, tannins, alkaloids, carbohydrates, flavonoids and glycosides using appropriate reagents.

### Acute toxicity studies

Acute oral toxicity studies were performed according to Organization for Economic Cooperation and Development (OECD 423) guidelines (Ecobichon, 1997). Albino mice of either sex weighing 25-30 g and of 90 days of age were used for this purpose. Tween-80 1% v/v was used as vehicle to suspend the petroleum-ether extract. The petroleum-ether extract was administered in a dose of 2g/kg orally to a group of three mice. The animals were continuously observed for changes in autonomic or behavioral responses for 6hrs. The animals were kept under observation for 14 days to detect any mortality.

### Antinociceptive activity

Acetic acid induced writhing, formalin induced nociception and hot water tail immersion methods were used to evaluate the potential antinociceptive activity of petroleum-ether extract of leaves of *Eupatorium triplinerve*. The petroleum-ether extract was prepared as a suspension in 1% tween 80 and administered orally to the experimental animals. A group of animals treated with vehicle (1% tween 80) served as control. Morphine (5mg and 10mg/kg sc) was used as a standard drug for comparison.

### Acetic acid induced abdominal constriction

Mice were orally treated with petroleum-ether extract of *Eupatorium triplinerve* in different doses (50,100 and 200 mg/kg). Sixty minutes later acetic acid (0.6% v/v in saline) was injected intra peritoneally in a dose 10 ml/kg. (Koster et al. 1959). The number of abdominal constrictions (writhing) in mice was counted for 15 min following acetic acid injection. Any significant reduction in the number of abdominal constriction by any treatment compared to vehicle treated animals was considered as antinociceptive response. The percentage inhibition of writhings compared to vehicle treatment was calculated using the formula  $(C-T/C) \times 100$  where C is the number of abdominal constrictions recorded in vehicle treated animals and T is the number of abdominal constrictions in the treatment group. The highest dose employed (200mg/kg) was chosen for further antinociceptive assessments.

**Formalin-induced paw-licking**

20 microlitres of 1% formalin in saline was administered s.c. in the plantar surface of the left hind paw of mice (Dubuisson and Dennis, 1997), 60 min after treatment with petroleum-ether extract of *Eupatorium triplinerve* (200mg/kg). The time spent in licking or biting the injected paw was recorded every 5 min for a period of 30 minutes. The early phase of nociceptive response normally peaks from 0-10 min and the late phase from 10-30 min after formalin injection. The paw licking time in mice after petroleum-ether extract/morphine (5mg/kg) treatment was compared with that of vehicle treated mice. The percent inhibition of paw licking time compared with vehicle treatment was calculated using the formula,

Percent inhibition =  $C-T/C \times 100$ , where C=Biting/paw licking response time (seconds) in vehicle treated group and T= Biting/paw licking response time (seconds) in petroleum-ether treated group.

**Tail immersion test**

Tail immersion test (Sewell and Spencer, 1976) was used to evaluate the antinociceptive activity of *Eupatorium triplinerve* in a dose of 200mg/kg p.o. The tail of the mouse was immersed in a water bath maintained at  $55 \pm 0.5^\circ \text{C}$ . Time for withdrawal of the tail was taken as the reaction time. A cut off period of 10 seconds was maintained. The reaction time was measured just before administration of test substances (0 min), then at intervals of 30 min up to a period of 90 min. The increase in latency period was compared with the vehicle treated group. Morphine in a dose of 10mg/kg s.c was used as a standard drug.

**Anti-inflammatory activity: Carrageenan-induced hind paw edema**

Male Wistar rats (150-175 g) were fasted overnight and the paw edema was developed by injecting carrageenan 0.1ml of 1%w/v suspension in sterile normal saline into the subplantar tissue of the right hind paw (Winter et al. 1962). One hour before carrageenan injection petroleum-ether extract of *Eupatorium triplinerve* suspended in tween 80 was administered orally in a dose of 200mg/kg. The control group of rats received 1ml of the vehicle.

Another group of rats was administered with the standard drug diclofenac 10mg/kg i.p 30 minutes prior to carrageenan injection.

The diameter of paw was measured by using a digital Vernier calipers before administration of carrageenan and at hourly intervals upto 6 hr after the administration of carrageenan. The edema thickness (in mm) at various time intervals was calculated by subtracting the zero hour reading and represented as mean increase in paw diameter (mm).

Percentage reduction of edema was calculated using the formula  $(C-T/C \times 100)$ , where C is the mean increase in paw diameter in control group and T is the mean increase in paw diameter of the treatment group.

**Statistics**

The data were expressed as mean  $\pm$  standard error of mean (S.E.M). The results were analysed by one way Analysis of Variance followed by Post hoc Bonferroni Test. A value of  $p < 0.05$  was considered significant.

**RESULTS****Phytochemical Screening**

The percentage yield of petroleum-ether extract of leaves of *Eupatorium triplinerve* was found to be 10.6%w/w. The chemical tests indicate the presence of sterols, carbohydrates, tannins, phenols, glycosides and alkaloids in the petroleum-ether extract.

**Acute toxicity studies**

There was no significant alteration in autonomic or behavioral responses in the mice treated with petroleum-ether extract of the leaves of *Eupatorium triplinerve*. No mortality was recorded in these animals up to 14 days. Thus the petroleum-ether extract was found to be non-toxic up to dose of 2g/kg body weight.

**Antinociceptive activity****Acetic acid induced abdominal constrictions**

The mean number of abdominal constrictions in vehicle treated control animals was  $36.50 \pm 0.42$  (Table 1:). A significant reduction in the number of

abdominal constrictions was recorded for morphine treated mice with the mean value being  $4.83 \pm 0.54$  and the percentage inhibition of nociception was 86.76%. A dose dependent reduction in the number of abdominal constrictions was noticed after the administration of petroleum-ether extract of *E.triplinerve*. The reduction was significant with 100mg/kg ( $30.45 \pm 0.56$ ) and 200mg/kg ( $14.83 \pm 1.49$ ) of the extract. In the above doses, the percentage inhibition of nociception were 16.57% and 59.36% respectively.

### Formalin -induced nociception

In vehicle treated control animals the paw licking response time was  $46.33 \pm 2.61$  sec in early phase (0-10 min) and  $85.8 \pm 3.58$  sec in the late phase (10-30 min). In morphine treated animals the paw licking response time was significantly reduced both in the early ( $10 \pm 0.73$  sec) and late phase ( $3.33 \pm 0.49$  sec). A significant reduction in the paw licking response time was evident in the early phase ( $37.16 \pm 1.16$  sec) and late phase ( $27.33 \pm 5.31$  sec) after treatment with petroleum-ether extract of *Eupatorium triplinerve*. The extract nearly produced 19.79 % and 68.98 % inhibition of nociceptive response in the early and late phases respectively (Table 2:).

### Tail Immersion Test

The mean reaction time in the vehicle treated mice during the observation periods of 30, 60 and 90 min were  $1.78 \pm 0.07$ ,  $1.73 \pm 0.04$  and  $1.76 \pm 0.02$  sec. respectively (Table 3:).

Morphine treatment significantly increased the reaction time in all the observation periods. The reaction time with 200 mg/kg petroleum-ether extract of *Eupatorium triplinerve* was also significantly increased when compared with the vehicle treated mice. It showed maximum latency period ( $2.76 \pm 0.10$  sec.) at 60 minutes.

### Carrageenan induced hind paw edema

Intraplantar carrageenan administration increased the diameter of the paw significantly over the period of observation in vehicle treated control animals.(Table 4).The increase was significantly less at all observation periods with diclofenac treatment. A maximum of 48% inhibition was observed at five hours. In a similar fashion treatment with petroleum-ether extract of *E.triplinerve* also attenuated the increase in paw diameter due to carrageenan administration. A maximum of 26% inhibition was observed at 5<sup>th</sup> hour.

**Table1: Effect of Eupatorium triplinerve extract on acetic acid induced abdominal constrictions in mice**

Treatment	Number of abdominal constriction
Vehicle (Tween 80 1% v/v), p.o	$36.50 \pm 0.42$
Morphine 1 mg/kg s.c	$4.83 \pm 0.54^*$ (86.76%)
<i>E.triplinerve</i> Petroleum ether extract 50mg/kg p.o	$35.62 \pm 0.21$ (2.41%)
<i>E.triplinerve</i> Petroleum ether extract 100mg/kg p.o	$30.45 \pm 0.56^*$ (16.57%)
<i>E.triplinerve</i> Petroleum ether extract 200mg/kg p.o	$14.83 \pm 1.49^*$ (59.36%)

Each value represents the mean  $\pm$  SEM of six observations

\* $p < 0.05$  compared to vehicle treatment (one way Anova followed by Bonferonni test)

The value in parenthesis indicates the percentage inhibition of nociception.

**Table 2: Effect of Eupatorium triplinerve extract on formalin induced nociception in mice**

Treatment	Dose (mg/kg)	Early phase 0-10min	Late phase 10-30min
Vehicle (Tween -80 1% v/v )	0.1ml/10g.p.o	$46.33 \pm 2.61$	$85.8 \pm 3.58$
Morphine	10mg.sc	$10 \pm 0.73^*$ (82%)	$3.33 \pm 0.49^*$ (96.11%)
<i>E.triplinerve</i> Pet-Ether Extract	200mg.p.o	$37.16 \pm 1.16^*$ (19.79%)	$27.33 \pm 5.31^*$ (68.98%)

Each value is presented as mean  $\pm$  SEM of six observations

\* $p < 0.05$  compared to vehicle treatment (one way Anova followed by Bonferonni test)

The value in parenthesis indicates the percentage inhibition of nociception.

**Table 3:** Effect of *Eupatorium triplinerve* extract on Thermal nociception in mice

Treatment mg/kg	Reaction time in seconds			
	0 hours	30 min	60 min	90 min
Vehicle(Tween 80, 1%v/v, p.o)	1.71±0.03	1.78±0.07	1.73±0.04	1.76±0.02
Morphine 10mg,sc	1.80±0.05	2.30±0.03*	4.13±0.08*	8.16±0.08*
<i>E.triplinerve</i>	1.92±0.07	2.03±0.07*	2.76±0.10*	2.42±0.05*
Pet-Ether Extract 200mg,p.o				

Each value is presented as mean ± SEM of six observations.

\* $p < 0.05$  compared to vehicle treatment (one way Anova followed by Bonferonni test)

**Table 4:** Effect of *Eupatorium triplinerve* extract on Carrageenan induced paw edema

Treatment	Increase in paw diameter (mm)				
	1 hour	2 hour	3 hour	4 hour	5 hour
Vehicle (Tween 80, 1% v/v, p.o)	1.43±0.08	2.20±0.27	2.27±0.28	2.31±0.35	2.31±0.39
Diclofenac 10mg/kg ,s.c	1.29±0.3 (9.79 %)	1.49±0.48* (32.27%)	1.38±0.49* (39.2%)	1.31±0.49* (43.29%)	1.20±0.35* (48.05%)
<i>E.triplinerve</i>	1.30±0.12 (9.09%)	1.90±0.15* (13.63%)	1.91±0.19* (15.85%)	1.81±0.88* (21.64%)	1.71±0.79* (25.97%)
Pet-Ether extract 200 mg/kg,p.o					

Each value is presented as mean ± SEM of six observations.

\* $p < 0.05$  compared to vehicle treatment (one way Anova followed by Bonferonni test)

The value in parenthesis indicates the percentage reduction of paw edema.

## DISCUSSION

The present study was undertaken to scientifically validate the traditional claims of *Eupatorium triplinerve* Vahl with particular reference to its antinociceptive and anti-inflammatory effects. Different types of nociception, like visceral nociception (acetic acid induced abdominal constriction), thermal nociception (tail immersion test), neurogenic and inflammatory nociception (formalin induced paw licking) were included in the study. The anti-inflammatory effect was studied by carrageenan induced hind paw edema method.

In acute toxicity testing no mortality was observed in mice even in a dose of 2g/kg of petroleum-ether extract of *E.triplinerve* which indicates the safe nature of the extract.

Acetic acid induced abdominal constriction (Koster et al. 1959) is regarded as a very sensitive method which induces minimal noxious stimulus.

The advantage of this method is that even compounds with weaker analgesic property can be detected from the results of this test (Bentley et al. 1981). The associated nociceptive response is believed to involve the release of endogenous mediators such as bradykinin and prostanooids which stimulate the nociceptive endings. These neuronal fibres are sensitive to both narcotic analgesics and NSAID. The mean number of abdominal constrictions after acetic acid injection in mice was significantly reduced by petroleum-ether extract of *E. triplinerve* revealing its antinociceptive effect.

Formalin induced paw licking is a persistent-pain model used to evaluate neurogenic and inflammatory nociception in 2 phases (i.e.) early (0-10 min) and late phase (10-30 min) respectively (Hunskar and Hole, 1987). The early phase of formalin response is attributed to direct stimulation

of nociceptors. The late phase is due to an inflammatory reaction caused by tissue injury leading to the release of histamine, serotonin, prostaglandin and excitatory amino acids (Dubuisson and Dennis, 1997). Centrally acting analgesic drugs like narcotic analgesics inhibit both the phases while peripherally acting drugs such as steroids and NSAID suppress mainly the late phase. Petroleum-ether extract of *E.triplinerve* significantly attenuated both the phases of formalin induced paw licking response time. However the inhibition was more (68%) in the late phase when compared to the early phase. This reveals that the *Eupatorium triplinerve* may be more effective in alleviating pain due to inflammation.

Thermal nociception is found to represent pain of high intensity and is amenable only to potent analgesic like opioids. In the present study morphine was effective in increasing the reaction time of mice in hot water tail immersion test. Thermal nociception in mice was also significantly reduced by petroleum-ether extract of *E. triplinerve* although to a lesser degree.

Carrageenan induced paw-edema model has gained greater importance and support over the years, because edema induced by carrageenan is reported to have been inhibited by majority of the steroidal and the non-steroidal anti-inflammatory drugs. Carrageenan induced paw-edema has a

biphasic effect. The first phase (0-3hr) is due to release of histamine and serotonin, plateau phase is maintained by a kinin like substance (3hours) and late phase (4.5-5hr) of inflammation is attributed to prostaglandin release (Vane and Botting, 1998). In the initial phase, the mast cells are activated and degranulated releasing histamine and serotonin. These mediators increase the vascular permeability of blood vessel that facilitates the infiltration of neutrophils, accumulation of plasma fluids and proteins into the interstitial spaces. This is followed by the release of kinin after certain time. These events lead to the development of oedema which is reduced by anti-inflammatory agents.

Administration of *E.triplinerve* extract has consistently reduced the paw edema in rats after carrageenan administration (Table 4). This observation indicates the potent anti-inflammatory effect of petroleum-ether extract of *E.triplinerve*. Thus the present study has revealed the potential analgesic and anti-inflammatory effects of petroleum-ether extract of *E.triplinerve*. Moreover, the present results are in agreement with an earlier study of Cheriyan et al. (2009), who reported a similar finding with the alcoholic extract of *E.triplinerve* and both the findings unequivocally confirm the presence of active principles mediating anti-inflammatory and antinociceptive effects in this plant.

## CONCLUSION

The traditional claims of the usefulness of *E.triplinerve* in the treatment of inflammation and pain has been scientifically validated by the results

of the present study. Further investigations may help us to identify the active principle(s) responsible for the above effects of *E.triplinerve*.

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