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Anti-inflammatory, analgesic and anti-lipid peroxidative effects of *Rhaphidophora pertusa* (Roxb.) Schott. and *Epipremnum pinnatum* (Linn.) Engl. aerial parts

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The ethanol extracts of aerial parts of *Rhaphidophora pertusa* (Roxb.) Schott. and *Epipremnum pinnatum* (Linn.) Engl. (Family-Araceae) were evaluated for their anti-inflammatory activity in Wistar albino rats and analgesic effects in Swiss albino mice. Both the species produced significant inhibition of carrageenan-induced rat paw oedema when compared to the standard drug Indomethacin .They also showed a significant inhibition of acetic acid-induced writhing in mice at all the doses studied. The analgesic effect of *R. pertusa* was almost similar to that caused by the standard drug, aspirin. The ethanol extracts also showed significant anti-lipid peroxidant effects *in vitro* in rat liver homogenate. The acute toxicity study indicated that *R. pertusa* and *E. pinnatum* are fairly non-toxic. The results of the present study support the use of *R. pertusa* in traditional medicine of Kerala as an anti-inflammatory and analgesic agent and *E. pinnatum* may be included in the same category of medicinal plants.

Keywords : *Rhaphidophora pertusa*, *Epipremnum pinnatum*, Anti-inflammatory, Analgesic, Lipid peroxidation, Traditional medicine, *Anathipali.*

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Introduction

Herbal therapy although still an unwritten science is well established in some cultures and traditions and has become a way of life of almost 80% of the people in rural areas. Inflammatory and arthritic conditions are among those treated using traditional remedies, with considerable success.

Chronic inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. Although several modern drugs are used to treat these types of disorders, their prolonged use may cause severe adverse side effects on chronic administration[1], the most common being gastrointestinal bleeding and peptic ulcers[2]. Consequently there is a need to develop new anti-inflammatory agents with minimum side effects.

It is worthwhile to note that most of the present day analgesic drugs also exert a wide range of side effects[3]. Study of plant species that are traditionally used as pain killers should still be seen as a logical and fruitful research strategy, in the search of analgesic drugs[4].

Rhaphidophora pertusa (Roxb.) Schott. (Family-Araceae) is an attractive climber, cultivated throughout India as an ornamental plant. It is locally called as *Anathipali* or *Elithadi*[5]. The leaves, stem and seeds of the plant have medicinal use. In traditional medicine of Kerala its leaves are used to treat inflammatory conditions. The juice of the plant with black pepper is used extensively in traditional medicine as an antidote against the bite of Russell's Viper[6]. The juice of the plant along with juices of *Croton oblongifolius* Roxb. roots and fruits of *Momordica charantia* Linn. is applied to the bitten part. A perusal of literature revealed that although *R. pertusa* is widely used in traditional medicine as an anti-inflammatory and analgesic agent, these properties have not yet been scientifically evaluated (Fig. 1).

Epipremnum pinnatum (Linn.) Engl. (syn. *Rhaphidophora pinnatifida* Linn.) is another closely related Araceae member, from the Andamans

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collected by the scientists of the Institute during one of their plant exploration trips and established in the herbal garden of the Institute. Not much information is available about its medicinal use to treat inflammation and pain. It is likely that when bioactive compounds are found in one species, more species of the same genus may contain active compounds of a similar nature[7]. Prompted by this fact it was considered interesting to scientifically validate the anti-inflammatory and analgesic effects of *E. pinnatum* also. The present study was therefore, undertaken to evaluate and compare the anti-inflammatory and analgesic properties of these two plants (Fig.2).

Fig. 1—*Rhaphidophora pertusa*

Fig. 2—*Epipremnum pinnatum* branch with inflorescence

Materials and Methods

Plant materials

The aerial parts of both the plants were collected from the herbal garden of the Institute during January, 2006.They were authenticated by Dr Mathew Dan, plant taxonomist of the Institute and voucher specimens were preserved for future reference in the Herbarium of the Institute (TBGT 57007 dtd 21/1/2006 and TBGT 57008 dtd 24/1/2006).

Preparation of the plant extracts

The aerial parts were washed thoroughly with tap water, shade-dried and powdered. The powder (60g) of each of the two plant species was successively extracted separately with ethanol (600ml) overnight, at room temperature with constant stirring. The extracts were filtered and the filtrates were concentrated at 30°C under reduced pressure in a rotary evaporator. The yield (w/w) of the crude extracts was found to be 16.5% for *R. pertusa* and 18% for *E. pinnatum*. The crude extracts were suspended in 0.5% Tween-80 to required concentrations and used for the experiments. *R. pertusa* extract was referred to as RP and *E. pinnatum* extract as EP.

Experimental animals

Wistar albino male rats (175-220g) and Swiss albino mice (27-35g), were grouped and housed in polyacrylic cages (two animals per cage) and maintained under standard laboratory conditions (temperature 24-28°C, relative humidity 60-70% and 12h light dark cycles). They were fed commercial rat feed (Lipton India Ltd, Mumbai) and boiled water, *ad libitum*. All experiments involving animals were done according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee.

Carrageenan-induced paw oedema in rats

Anti-inflammatory activity of RP and EP was assessed by carrageenan induced paw oedema method[8]. Rats were divided into 6 groups (6 animals in each group). Animals of all the groups were injected with 0.1 ml of 1% carrageenan in 0.9% normal saline, under the plantar aponeurosis of the right hind paw. Group I animals (carrageenan control) received p.o., 0.5% of Tween-80, 30 min prior to carrageenan injection. Group II, the standard reference group was given p.o., an aqueous solution of Indomethacin (5mg/kg), 30 min prior to

carrageenan injection. Groups III and IV received p.o., 125 and 250mg/kg of RP, 30 min prior to carrageenan injection. Groups V and VI received EP (125 and 250 mg/kg p.o.), 30 min prior to carrageenan injection. The paw volume was measured plethysmographically just before and 3h after carrageenan injection. The percentage inhibition of oedema was calculated for each group with respect to its vehicle treated control group.

Acetic acid-induced writhing test in mice

Analgesia of RP and EP was assessed by the writhing test in mice[9]. Mice were divided into 6 groups (6 animals in each group). All the groups received i.p., 0.5% aqueous solution of acetic acid (10ml/kg). Group I, acetic acid control group received a single dose of 0.5% Tween-80 (0.5ml) p.o., 20 min prior to the administration of acetic acid. Group II, the standard control group received p.o., a single dose of aspirin (acetyl salicylic acid, 25mg/kg), 20 min prior to administration of acetic acid. Groups III and IV received p.o., a single dose of 125 and 250 mg/kg RP, 20 min prior to administration of acetic acid. Groups V and VI received 125 and 250 mg/kg of EP p.o., 30 min prior to the administration of acetic acid. The number of writhes (full extension of hind limb) per animal was recorded during the 20 min period, beginning 5 min after the injection of acetic acid.

***In vitro* anti-lipid peroxidation studies**

The antilipid peroxidant effect of RP and EP was studied *in vitro* following the modified methods of Yoshiyuki *et al*[10] and Masao *et al*[11]. The rat liver tissue (2g) was sliced and homogenated with 150mM KCL-Tris-HCl buffer (*p*H 7.2). The reaction mixture (in triplicate) was composed of 0.25ml liver homogenate, Tris-HCl buffer (*p*H7.2), o.1 mM ascorbic acid (AA), 4mM $FeCl_2$ and 0.05 ml of various concentrations of extracts (25, 50, 100, 200µg/ml). The mixture was incubated at 37°C for 1h in capped tubes. Then, 0.5 ml of 0.1N HCl, 0.2 ml of 9.8% sodium dodecyl sulphate (SDS), 0.9 ml of distilled water and 2ml of 0.6% thiobarbituric acid (TBA) were added to each tube and the tubes were vigorously shaken. Following this, all the tubes were placed in a boiling water bath at 100°C for 30 min. After cooling, the flocculent precipitate was removed by adding 5ml of n-butanol, mixed well and centrifuged at 1500 rpm for 20 min. The absorbance of the supernatant was measured at 532 nm.

Behavioral and toxic effects[12]

Three groups of 10 mice each were administered p.o., 125, 250 and 500mg/kg of the RP or EP extract. The animals were observed continuously for 1h for any gross behavioural changes, symptoms of toxicity and mortality, if any and intermittently for the next 6h and then again, 24h after dosing with RP or EP extract.

Statistical analysis[13]

Statistical comparison between control and treated groups was made using analysis of variance, followed by multiple comparisons.

Results

Anti-inflammatory activity

The amount of oedema produced was quantitated after 3h of carrageenan injection. The group treated with Indomethacin (5mg/kg) showed maximum inhibition of oedema formation (83.33%). RP and EP at both doses (125 and 250mg/kg) studied significantly inhibited the carrageenan-induced paw oedema in rats i.e. 83.33 and 80.01% (RP), 76.66 and 66.66% (EP), respectively. The Indomethacin group and RP at 125mg/kg showed equal amount of inhibition of oedema formation. In the case of both RP and EP, increasing the dose further did not significantly increase the anti-inflammatory effects (Table 1).

Analgesic activity

Intraperitonial injection of acetic acid produced 32.0 ± 0.02 writhes in the control group, 20 min after injection. The per cent inhibition of writhing by groups pretreated with RP (125 and 250 mg/kg) and aspirin treated group (25mg/kg) was almost similar, i.e.92.80, 90.30 and 93.12%, respectively. EP (125, 250mg/kg) was less effective than RP in eliciting the analgesic response (67.50 and 62.50%),

Table 1—Effect of *R. pertusa* (RP) and *E. pinnatum* (EP) extract on carrageenan-induced paw oedema in rats

Treatment	Oral dose (mg/kg)	Difference in paw volume at 3h (ml)	Percentage inhibition of oedema
Carrageenan control	–	0.30 ± 0.03	–
Indomethacin	5	0.05 ±0.01	83.33**
RP	125	0.05 ±0.01	83.33**
RP	500	0.06 ±0.02	80.01**
EP	125	0.07 ±0.01	76.66**
EP	500	010 ±0.04	66.66**

Values are the mean ± S.D. n=6; ANOVA **$P \leq 0.01$ vs Carrageenan control

though it exhibited significant analgesic effects, compared to the acetic acid control group (Table 2).

***In vitro* lipid peroxidation**

There was significant increase of malondialdehyde (MDA) in $FeCl_2$-AA treated rat liver homogenate, compared to normal control without $FeCl_2$-AA. RP and EP showed very potent inhibition of $FeCl_2$-AA stimulated rat liver lipid peroxidation *in vitro* at 100µg/ml dose. All the other doses studied did not produce significant anti-lipid peroxidation effects *in vitro* (Table 3).

In the toxicity study, no mortality occurred within 24 h with the 3 doses of RP or EP tested. The LD_{50}, of both RP and EP was therefore, greater than 500mg/kg p.o., in mice (data not shown).

Discussion

Inflammation is a complex process and various mediators, eg. prostaglandins, leucotrienes, kinins, platelet activating factor, etc have been reported to be involved in the development of inflammatory diseases. Carrageenan assay is well suited for comparative bioassay of anti-inflammatory agents, since the relative potency estimates obtained from most drugs tend to reflect clinical experience[8]. The time course of oedema development in carrageenan induced paw oedema model in rats is generally represented by a biphasic curve[14]. The first phase occurs within an hour of injection and is partly due to the trauma of injection and also due to the serotonin component[15]. Prostaglandins play a major role in the development of the second phase of reaction which is measured around 3h time[16]. The presence of prostaglandin in the inflammatory exudates from the injected foot can be demonstrated[17]. The carrageenan induced paw oedema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit cyclooxygenase involved in prostaglandin synthesis[18]. Based on these reports, it is inferred that the inhibitory effect of RP and EP on carrageenan-induced inflammation in rats may be due to inhibition of the enzyme cyclooxygenase, leading to inhibition of prostaglandin synthesis.

Acetic acid-induced writhing response in mice is a simple and reliable model to evaluate peripheral type of analgesic action of herbal and other drugs rapidly. It was found that RP and EP significantly inhibited the acetic acid induced writhing response at 125 mg/kg showing 93 and 67% of inhibition of writhing. It has been reported that the abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins[19]. Therefore, it is possible that RP and EP exert the analgesic effect probably by inhibiting synthesis or action of prostaglandins.

RP and EP significantly inhibited *in vitro* lipid peroxidation in rat liver homogenate. It has become evident that non-enzymatic or unspecified lipid peroxidation occurs during experimental inflammation in rats. Lipid peroxides may be

Table 2—Effect of *R. pertusa* (RP) and *E. pinnatum* (EP) extract of acetic acid-induced writhing response in mice

Treatment	Oral Dose (mg/kg)	Mean number of writhes in 30 min	Percent inhibitio of writhes
Acetic acid control	-	32.0 ± 0.20	-
Acetyl salicylic acid	25	2.2 ± 0.70	93.12**
RP	125	2.3 ± 0.31	92.80**
RP	250	3.1 ± 0.27	90.30**
EP	125	10.4 ± 0.51	67.50**
EP	250	12.0 ± 0.33	62.50**

Values are the mean ± S.D. n=6; ANOVA **$P \leq 0.01$ vs acetic acid control

Table 3—Inhibitory effect of *R. pertusa* (RP) and *E. pinnatum* (EP) extract on $FeCl_2$-ascorbic acid- induced lipid peroxidation in rat liver homogenate in vitro

Groups	Plant extract concentration (µg/ml)	MDA (n mol/mg protein)	MDA inhibition (%)
Normal control	-	1.35 ±0.01	-
$FeCl_2$-AA control	-	2.31 ± 0.02	-
$FeCl_2$-AA + RP	25	1.52 ± 0.09	34.19
$FeCl_2$-AA + RP	50	1.29 ± 0.07	44.15
$FeCl_2$-AA + RP	100	0.07 ± 0.02	96.97**
$FeCl_2$-AA + RP	200	1.69 ± 0.01	26.84
$FeCl_2$-AA + EP	25	2.16 ± 0.03	6.49
$FeCl_2$-AA + EP	50	2.07 ± 0.04	10.39
$FeCl_2$-AA + EP	100	0.17 ± 0.03	92.64**
$FeCl_2$-AA + EP	200	1.45 ± 0.01	37.20

Values are the mean ±S.D. n=3; ANOVA **$P \leq 0.01$ vs $FeCl_2$-AA control

pro-inflammatory and can damage tissues directly[20]. Protection against free radical-induced lipid peroxidation by plant extracts is of great significance for their traditional use against inflammatory disorders, many of which are associated with membrane damage and tissue recovery[21]. There is abundant evidence that peroxidative decomposition of structural lipids in cellular and subcellular membrane is catastrophic in a living system. Lipid peroxidation results in mitochondrial swelling and disintegration[22]. Disintegration of lysosomes has been correlated with the peroxidative decomposition of lysosomal lipids[23]. It can therefore, be concluded from the present study that the beneficial effects of RP and EP may be from their role in the stabilization of lysosomes. The combination of anti-inflammatory and analgesic effects of RP and EP indicate the likelihood of intervention of prostaglandin synthesis as prostaglandins have been established as a common mediator in all these responses. However, this possibility remains to be investigated in detail.

The acute toxicity study indicated that RP and EP are fairly non-toxic. This is not surprising, as *R. pertusa* is being used in traditional medicine of Kerala as an antidote and anti-inflammatory agent.

The active compounds responsible for the anti-inflammatory and analgesic activites of *R. pertusa* and *E. pinnatum* remain to be identified. A perusal of literature revealed the paucity of phytochemical studies on these two plant species. Preliminary phytochemical studies at our laboratory indicated the presence of tannins in them. Earlier reports showed the latex of *Calotropis procera* (Ait.) R. Br., containing tannins showed potent anti-inflammatory effects[24]. *Betula pendula* Roth and *Corylus avellana* Linn. having very high tannin content showed remarkable anti-inflammatory activity[25]. Volatile oils, resins, flavonoids and terpenoids isolated from plant extracts are known to produce anti-inflammatory and analgesic effects[26]. Condensed tannin and polysaccharides are known for the anti-inflammatory effects of *Rumex acetosa* Linn. and *Rumex patientia* Linn.[27]. It is likely that *R. pertusa* and *E. pinnatum* may contain some or most of the above mentioned phytocompounds which are responsible for their anti-inflammatory and analgesic effects. Detailed studies are warranted in this direction to decipher the exact nature of the phytochemical compounds responsible for their anti-inflammatory and analgesic effects.

Conclusion

The findings of this study have demonstrated that *R. pertusa* and *E. pinnatum* possess potent anti-inflammatory and analgesic activity. The results also furnish evidence that the beneficial effects of both the species may be from their role in the stabilization of lysosomes and free radical scavenging.

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