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Evaluation of anti-inflammatory and analgesic effects on the extracts of different parts of *Excoecaria agallocha* L.

M. Babuselvam[1]*, S. Ravikumar[2], K.A Mohamed Farook[1], S. Abideen[1], M. Peer Mohamed[1] and M. Uthiraselvam[1]
[1] P.G. Department of Microbiology, Dr. Zakir Husain College, Ilayangudi, Sivagangai District, Tamil Nadu, India.
[2] School of Marine Sciences, Department of Marine Pharmacology, Alagappa University, Thondi campus, Thondi, Ramanathapuram District, Tamil Nadu, India.

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ABSTRACT

Scientific evaluated that, anti-inflammatory and analgesic activity of ethanol with water in the ratio of 3:1 extracts were obtained from different parts *viz.*, leaves, seeds and latex of *Excoecaria agallocha*. The latex was sequentially soxhlated with petroleum ether and methanol dried latex in anti-inflammatory processes and analgesic activity in two concentrations (250 mg/kg and 500 mg/kg). Preliminary phytochemical analysis showed that, presence of alkaloids, flavanoids, saponins were found maximum in the seed extract. Acute inflammatory studies showed that, latex, leaves and seed extracts of both concentration of chosen plant produced significant inhibition of carrageenin induced rat paw edema at 3rd hour ($p<0.005$) as compared to the control causing 63.15%, 62.15% and 69.69% respectively. In addition that, the seed extract at the concentration of 500 mg/kg showed maximum inhibition at 57.03% as compared to control in cotton pellet induced granuloma test. The analgesic activity of seed extract at the dose of 500 mg/kg. caused significant inhibitions in the acetic acid induced writhing. Moreover, the tail immersion model, seed extract at the concentration of 500 mg/kg. possess an maximum activity (80.29%) as compared to control.

INTRODUCTION

Inflammation is a process involving multiple factors acting in a complex network. The ingress of leukocytes into the site of inflammation is crucial for the pathogenesis of inflammatory conditions (Colditz, 1985; Kasama *et al.*, 1995). At the inflamed site, the recrited cells are activated to release many inflammatory mediators which elicit the initiation and maintenance of an inflammatory response, causing a change from the acute phase to the chronic phase of inflammation. Therefore, inhibition of the cellular reations is one of the targets that are generally used as an *in vitro* model for anti-inflammatory testing. Although, many anti-inflammatory and analgesics agents are present in the market, modern drug therapy is associated with some adverse effects like gastrointestinal irritation (Osadebe and Okoye, 2003, Jain *et al.*, 2002). Therefore, it is necessary to search for new drugs with less adverse effects. Medicinal plants have been used in the development of new drugs and continue to play an invaluable role in the progress of drug discovery. *Excoecaria agallocha* L. is a plant belongs to the family Euphorbiaceae family and it is widely found in subtropical regions. The selected plant species are used in the folk medicine to treat several disorders, mainly those that involve anti-inflammatory and analgesic process. The aim of this work was to evaluate the potential anti-inflammatory and analgesic activity from different part of *Excoecaria agallocha* extracts.

* *Corresponding Author*
Email: babu31993@gmail.com
Contact: +91 09791875657

METHODS

Preparation of extract from leaves and seed

Fresh elder leaves, latex and seed of Excoecaria agallocha were collected from Pichavaram mangrove forest of South East coast of India (Lat. 11°27'N; Long. 79°47'E). The shade dried parts of the whole plant (Leaf and seed) were coarsely powdered (500 g) and extracted with ethanol water mixture (3:1) for 48 hours in soxhlet apparatus. After evaporation of the solvent under reduced pressure, the extracts were obtained.

Preparation of the extract from latex

Latex was dried under room temperature, ground to small granules of dried latex (DL), and sequentially soxhlated with petroleum ether and methanol to get the methanolic extract of dried latex (MeDL) with a yield of 25%. The crude methanolic dried latex extract were triturated with gum acacia in normal saline (1:1) filtered and stored in sterilized plastic tubes in a refrigerator (4°C) for anti-inflammatory and analgesic activity.

Anti-inflammatory activity

Carrageenin-induced paw oedema in rats

Paw oedema was induced by the injection of 0.05 ml of a 1.0% carrageenin suspension in the subplantar region of the left hindpaw. An equivalent volume of saline solution was injected in the same region of the right hindpaw. The paw volume was determined before any treatment and measured at 1, 2, 3, 4, h after carrageenin injection with a plethysmometer (model 7150, UG) Basile, Italy). The extracts (250 and 500 mg/kg) were administered p.o 120 min before carrageenin injection. Aspirin (100 mg/kg) was administered as positive control for anti-inflammatory activity 60 min before challenging.

Granulomatous tissue induction

The rats were divided into five groups (n=6). Pellets weighing approximately 40 mg each were made with 5 mm of dental cotton tampons. The pellets were sterilized and impregnated with 0.4 ml ampicillin water solution at the moment of implantation. Animals were anaesthetized and the pellets were subcutaneously introduced through an abdominal skin incision. Each group was treated daily, for six consecutive days, with different plants parts of Excoecaria agallocha (250 and 500 mg/kg) and aspirin 100 mg/kg by oral. On the seventh day, the animals were sacrificed, the pellets dissected out and granulomas dried at 60°C overnight to determine the dried weight. The difference between the initial and final weights was considered as the weight of the granulomatous tissues produced.

Analgesic activity

Acetic acid writhing method

This was performed according to Gaertner *et al.*, 1999. Rats were (n=6) injected intraperitoneally with 0.6% acetic acid at a dose of 10 ml/kg. The extract (250 and 500 mg/kg), aspirin (100 mg/kg) and distilled water (p.o.) were administered 30 min prior to treatment with acetic acid. The writhing induced by the acid, consisting of abdominal constrictions and hind limbs stretching, were counted from 30 min after a day latency period of 5 min. The percentage analgesic activity was calculated as follows:

$$\text{Percentage analgesic activity} = \frac{N - N1 \times 100}{N}$$

Where N is the average number of stretching of control per group. N1 is the average number of stretching of test per group.

Tail immersion

Tail immersion was conducted as described by Aydin *et al.*, 1999. Rats (n=6) were used. This involved immersing extreme 3 cm of the rat's tail in a water bath containing water at a temperature of 55±0.5°C. Within a few minutes, the rat reacted by withdrawing the tail. The reaction time was recorded with a stop watch. Each animal served as its own control and two readings were obtained for the control at 0 to 10 min interval. The average of the two values was the initial reaction time (Tb). The test groups were given extract (250 and 500 mg/kg, p.o) aspirin (100 mg/kg) and distilled water (p.o). The reaction time (Ta) for the test groups was taken at intervals 0.5, 1, 2, 4, and 6 h after a latency period of 30 min following the administration of the extract and drugs (Vogel and Vogel, 1997). The cut-off time, i.e. tome of no response was put at 120s. The reaction time was measured and calculated. The following calculation was:

$$\text{Percentage analgesic activity} = \frac{Ta - Tb \times 100\%}{Tb}$$

Phytochemical analysis

The extracts from different plant parts of Excoecaria agallocha were screened for the presence of phytochemical constituents by following the method of Safowora (1982) and Kepam (1986).

RESULTS

Our preliminary phytochemical tests showed that alkaloids, flavonoids, saponins, carboxylic acids, xanthoproteins, steroids, phenols, proteins and tannins were present in the different part of extracts. However, alkaloids and steroids are found maximum in latex, phenols and tannins are found maximum in leaves, alkaloids, flavanoids and saponins are found maximum in extracts from seed (Table 1). The anti-inflammatory activity of different parts of Excoecaria agallocha extracts were evaluated for testing the anti-inflammatory and analgesic drugs. For the acute inflammation, Control animals (n=6) treated with intraperitoneal administration of 0.9% saline showed a progressive time dependent swelling of the right hind paw after the sub planter administration of 0.05 ml of 1% carrageenin reaching its maximum at 2nd hour. Acute anti-inflammatory studies showed that latex, leaves and seeds extracts of two concentration of

Excoecaria agallocha produced a significant inhibition of carrageenin induced rat paw edema at 3rd hour ($p< 0.005$) as compared to the control groups (rats), causing 63.15 %, 62.15% and 69.69% respectively. Intraperitoneal administration of aspirin (100 mg/Kg) caused dose dependent inhibition of edema (Table 2). Crude extracts of latex, leaves and seed of Excoecaria agallocha (250, 500mg.kg), the extracts of latex, leaves and seed exhibited significant ($P<0.01$) reductions in paw edema volume of rats. The present results revealed that, in the chronic model (granuloumatous tissue induction) on the Excoecaria agallocha extracts of latex, leaf and seed at the dose level of 250 and 500 mg/Kg and standard drug aspirin (100 mg/Kg) showed decreased granuloma tissue and increased percentage of inhibition. The seed extract at the concentration of 500 mg/Kg showed maximum inhibition at 57.03% as compared to control (Table 3).

Analgesic effects tested by Excoecaria agallocha extracts of latex, leaf and seed at different doses of 250 and 500 mg/kg on the writhing test in rats are shown in Table 3. Seed extract at the dose of 500 mg/kg and aspirin at the dose of 100 mg/kg exhibited significant inhibition compared with the control writhes at the rate of 84.6 and 66 % respectively in the acetic acid induced writhing. In addition, E. agallocha extracts at different doses of different parts of extracts are potentiated on the acetic acid induced analgesia. In the tail immersion test, an analgesic effect of E. agallocha extracts of latex, leaf and seed were observed at concentration of 250 and 500 mg/Kg. This results indicating that, the extract possesses an activity related to both inflammatory and non-inflammatory pain. It is proved by the present study that, seed extract at the dose of 500 mg/Kg possesses a maximum activity (80.29%) as compared to control. In addition, aspirin 100 mg/Kg also tested for comparison between different parts of E. agallocha and control (Table 5).

Table. 1: Phytochemical constituents of extracts of different plant parts from *Excoecaria agallocha*

Phytochemical constituents	Latex	Leaf	Seed
Alkaloids	++	-	++
Carboxylic acid	+	+	+
Coumarins	-	-	-
Flavanoids	-	-	++
Quinones	-	-	-
Phenols	-	++	+
Saponins	±	±	++
Xanthoproteins	+	+	+
Protein	+	+	+
Resins	-	-	-
Steroids	++	-	+
Tannins	-	++	++
Sugars	-	++	+

Table. 2: Anti-inflammatory effect of *Excoecaria agallocha* extracts in carrageeenan induced paw edema in rat.

Treatments	Dose (mg/kg)	1 Hour		2 Hour		3 Hour		4 Hour	
		Increase in paw volume (Mean ± SE)	% of inhibition	Increase in paw volume (Mean ± SE)	% of inhibition	Increase in paw volume (Mean ± SE)	% of inhibition	Increase in paw volume (Mean ± SE)	% of inhibition
Latex	250	0.24 ± 0.01	32.43	0.29 ± 0.01	35.53	0.18± 0.01	55.26	0.16± 0.01	57.89
	500	0.22 ± 0.01	43.24	0.17± 0.01	57.50	0.16± 0.01	57.89	0.14± 0.01	63.15
Leaves	250	0.23 ± 0.01	35.13	0.21± 0.01	50.00	0.18± 0.01	52.63	0.16± 0.01	57.89
	500	0.19 ± 0.01	48.6	0.16± 0.01	60.00	0.15± 0.01	60.52	0.15± 0.01	62.15
Seed	250	0.20 ± 0.01	39.39	0.23 ± 0.01	42.50	0.16 ± 0.01	55.16	0.13 ± 0.01	60.61
	500	0.18 ± 0.01	54.05	0.16 ± 0.01	60.52	0.14 ± 0.01	60.61	0.11 ± 0.01	69.69
Control	-	0.37 ± 0.01	-	0.41 ± 0.01	-	0.39 ± 0.01	-	0.38 ± 0.01	-
Aspirin	100	0.21± 0.01	33.6	0.18± 0.01	52.8	0.17± 0.01	59.8	0.15± 0.01	61.7

Values were found insignificant between treatments and found significant between duration of treatment

Table. 3: Effect of the *Excoecaria agallocha* extracts on cotton pellets induced granuloma pouch in rats.

Treatments	Dose (mg/kg)	Dry Weight of cotton pellet (mg)	Percentage of inhibition
Control	-	46.60 ± 1.16	-
Aspirin	100	22.4 ± 1.73	56.23
Latex	250	28.37 ± 0.87	40.02
	500	24.40 ± 1.15	50.41
Leaves	250	23.4 ± 0.85	52.32
	500	21.6 ± 0.81	56.32
Seed	250	27.6 ± 1.25	41.13
	500	20.6 ± 1.10	57.03

Values are found insignificant between treatments

Table 4. Analgesic activity (Acetic acid writhing test) of different parts of *Excoecaria agallocha* extracts in rats

Treatments	Acetic acid writhing in test animal		
	Dose (mg/Kg)	Number of writhes in 20 minutes	Percentage of inhibition
Control	3 ml.Kg^{-1}	46.78 ± 1.09	-
Aspirin	100	16.77 ± 1.07	66.00
Latex	250	20.49 ± 0.85	51.56
	500	13.63 ± 0.62	72.00
Leaves	250	20.50 ± 0.13	51.56
	500	11.05 ± 0.25	74.30
Seed	250	19.62 ± 0.87	57.00
	500	7.39 ± 0.88	84.6

Values are found insignificant between treatments

Table 5. Analgesic effect (Tail immersion test) of *Excoecaria agallocha* extracts in rats

Treatments	Hot plate method		
	Dose (mg/Kg)	Mean Reaction Time (S)	Percentage of inhibition
Control	3 ml/Kg	2.646 ± 0.47	--
Aspirin	100	16.57 ± 0.54	62.72
Latex	250	4.689 ± 0.52	48.0
	500	6.701 ± 0.39	65.8
Leaves	250	3.927 ± 0.24	40.1
	500	5.92 ± 0.24	61.25
Seed	250	18.16 ± 0.53	72.72
	500	18.57 ± 0.90	80.29

Values are found significant between treatments (P>0.05)

DISCUSSION

It is well established that prostaglandins, by virture of their activity as modulators of inflammatory responses, have a major role in the inflammatory mechanisms. The carrageenin-induced hind paw edema in rat is known to be sensitive to cyclooxygenase inhibitors, but not to lipoxygenase inhibitors, and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the cycloxygenase involved in prostaglandins synthesis. It has been demonstrated that, the suppression of carrageenin-induced hind paw edema correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents (Di Rosa, 1972). The present studies indicate that, the extracts of different parts of E. agallocha possess anti-inflammatory activity against the carrageenin induced paw edema in rats. The seed extract of E. agallocha significantly inhibited paw edema as an inflammatory agent. This is coincides with the inhibition of the production of prostaglandins. This inhibition of synthesis of prostaglandins might be due to the presence of alkaloids, flavanoids, saponins and tannins.

Previous studies reported that, plants are rich in alkaloids triterpenoids, flavanoids and saponins which showed present anti-inflammatory activity (Haihua Shu *et al.,* 2006; Theophile *et al.,* 2006; Borgi *et al.,* 2007) Preliminary phytochemical analysis performed in this study shows the present of triterpenoids, saponins, polyphenols, steroids, tannins and flavanoids in the seed extract of E. agallocha. The anti-inflammatory activity action of triterpenoids has been reported by many researchers (Vazquez *et al.,* 1996; Suh *et al.,* 1998'; Huss *et al.,* 2002). Saponnins has also been reported to have anti-inflammatory activities by inhibition of the enzymes iNos, Cox-2 and lipoxygenase (Bermejo Benito *et al.,* 1998; Li and Chu, 1999; Kim *et al.,* 2002). Therefore, it seems that analgesic and anti-inflmmatory profile of E. agallocha seed might be related to the triterpenoids and saponins present in seed. In conclusion, the seed extract of E. agallocha, which contains triterpenoids, Saponins and simple phenols, possesses inflammatory effects.

The analgesic activities of E. agallocha extracts of different parts were also studied by the present study. It reveals that, E. agallocha extract of seed significantly decreased the number of writhes in 20 minutes and also increased the percentage of inhibition in acetic acid writhing test in test animals. Therefore, it is likely that E. agallocha extracts might suppress the formation of prostaglandins or antagonizes the action of these substances and thus exerts its analgesic activity in acetic acid induced writhing test. The tail immersion method is considered to be selective for opioid like compounds in several animal species (Janssen *et al.,* 1963). It could suggest that, the extract may possess both anti-inflammatory and analgesic activities probably mediated through common mechanisms. Experimental evidence obtained in the present study indicates that, E. agallocha seed extract (500 mg/kg) significantly delayed the reaction times of the rat used in the 'hot plate' analgesic test method. Moreover, these observations tend to suggest that E. agallocha seed extract possesses centrally and peripherally mediated analgesic properties. The peripheral analgesic effect of the plant extract may be mediated via inhibition of cyclooxygenase and lipoxygenase, while the central analgesic action of the plant extracts may be mediated through inhibition of central pain receptors. Although the results of the present study are inconclusive, they tend to suggest that E. agallocha extracts are probable produces its anti-inflammatory effect by inhibiting the

release, synthesis and/or production of inflammatory mediators, including polypeptide kinins, prostaglandins and so forth, like aspirin. The present investigation suspected that, presence of alkaloids and flavanoids in seed extracts from E. agallocha potent analgesic effect with less toxicity.

CONCLUSION

In conclusion, the results of present study showed that the extract of different parts of Excoecaria agallocha possesses anti-inflammatory activity an acute inflammatory processes. Its anti-inflammatory effect could be produced by the alkaloids, flavanoids, saponins and tannins were present in the chosen extracts. The present study showed that Excoecaria agallocha extracts also possesses analgesic effect, which can be very useful in painful haemorrhoid. The present findings proved the tradition use of E. agallocha is an anti-inflammatory and analgesic drug in Indian folk medicine.

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