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RESEARCH ARTICLE

ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *POLYCARPAEA CORYMBOSA* LAM. (CARYOPHYLLACEAE)

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Abstract

Polycarpaea corymbosa is one of the medicinal plants having great potency in treating certain common diseases traditionally like jaundice, ulcer, demulcent and astringent. In view of identifying its active components responsible for its medicinal value, attempt is made for the quantitative analysis of phytochemicals by GC – MS. The crude methanolic aerial and root extract (200 mg/kg b.wt.) of *P.corymbosa* was investigated for anti-inflammatory experimental models.Oral administration of aerial and root extract in mice did not produce any toxicity at 2000mg/kg b.wt. dose level. In carrageenan and histamine induced inflammation, administration of *P.corymbosa* (aerial and root) as well as indomethacin (25 mg/kg b.wt.) reduced rat paw oedema significantly at 3, 4 and 5h. The present study has confirmed the anti-inflammatory activity of *P.corymbosa* due to presence of bioactive phytoconstitutes and provides pharmacological evidence in favor of traditional claim of *P.corymbosa* as anti inflammatory agent.

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Introduction

Medicinal plants have been known for millennia and are considered as a rich source of pharmaceutical agents for the prevention and treatment of diseases and ailments. According to WHO, more than 80% of the population within developing countries uses herbal and other traditional medicines to treat their common ailments [WHO, 2013]. Plants in the world may maintain an important position in the production of raw materials either directly for crude drugs or as the bioactive compounds in the formulation (Tiwari, 2008). Medicinal plants have great value to phytochemists because of their medicinal properties (Oladosu *et al.*, 2011). Throughout the world many plant species are used for the treatment of inflammation and other diseases. Despite the availability of anti-inflammatory agents search for newer therapeutic agent from the natural plants is still progressing due to presence of diverse chemical substances that have an better alternative and safer effect on inflammation without or lesser side effects. Therefore, screening and development of drugs for their anti-inflammatory activity is the need of hour and there are many efforts for finding anti-inflammatory drugs from indigenous medicinal plants (Srinivasan *et al.*, 2001).

Polycarpaea corymbosa Lam. (Caryophyllaceae) commonly known as Nilaisedachi (Tamil). Mostly found on open, often moist, sandy soils, less often in grassy places on mountain slopes above 1200 m sea level. Flavonoids and phenolic compounds, widely distributed in the plants have been reported to exert multiple biological effects, including antioxidant (Sindhu, 2013), anti-inflammatory, anticarcinogenic, etc. Traditionally *P.corymbosa* possesses different therapeutic uses such as jaundice, ulcer, demulcent and astringent (Chetty, 2008). Hence, in the present study, methanol extract of aerial and root parts of *P.corymbosa* Lam. were investigated for anti-inflammatory activity using *in vivo* animal models.

2 MATERIALS AND METHODS

2.1 Plant materials collection and identification

Polycarpaea corymbosa Lam. (Caryophyllaceae) were collected from Chennimalai, Erode dist., Tamil Nadu, India. The specimen was authenticated at Botanical Survey of India (No. BSI SCR 5/23/2011-12 Tech.1391), Coimbatore and is documented in our laboratory. The shade dried, powdered aerial and root parts were stored in air-tight container.

2.2 Extraction of plant material

The powdered aerial and root of the plant (50g) was exhaustively extracted with methanol using soxhlet apparatus for 72 h. The extract was concentrated under reduced pressure using rotary evaporator and stored at 2˚C - 8 ˚C until the completion of pharmacological studies. The yield of extract was noted (21.62 and 32.6 % w/w for aerial and root respectively).

2.3 Experimental animals

Female Wistar albino rats (180-200 g) were used to assess anti-inflammatory of aerial and root methanolic extract of *P.corymbosa*. All animals were kept and maintained under standard laboratory conditions (temperature, 22 ± 2 ˚C; humidity, 45 ± 5 ˚C and 12 h light: 12 h dark cycle). The animals were fed with standard laboratory diet and allowed to drink water *ad libitum*. The experimental protocols were approved by institutional Animal Ethical Committee (Reference no. 659/02/a/CPCSEA).

2.4 Acute toxicity

Acute oral toxicity study was performed as per OECD-423 guidelines (OECD, 2001). Swiss albino mice (n =6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the aerial and root extracts were administered orally at the dose level of 5mg/kg body weight (1000, 2000, 3000, 4000 and 5000mg/kg b.wt) by oral gavage and observed for 14 days. Mortality was not observed at 2000 mg/kg body weight which was considered as LD_{50} cut off dose (Ecobichon, 1997).

2.5 Carrageenan induced rat paw oedema

Acute inflammation was produced by sub-plantar injection of 0.1 mL of 1% w/v suspension of carrageenan in normal saline, in the right hind paw of the rats, 1 h after oral administration of the doses of aerial and root methanolic extract, positive (Indomethacin) and negative control (carrageenan) substances to the overnight fasted rats of respective groups. The rats were divided into four groups of six animals in each group. Group I served as control and group II as negative control which received carrageenan (1%); group III served as positive control which received Indomethacin (25 mg/kg b.wt); group IV and V served as extract treatment group which received methanolic aerial and root extract at 200 mg/kg b.wt. The paw volume was measured plethysmographically by Vernier calliper (Inco- Niviqure, Version 60.1, India) at 0, 1, 3, 4 and 5 h after the carrageenan injection. The indomethacin and plant extract were freshly suspended in Carboxy Methyl Cellulose (CMC) just before oral administration (Patra *et al.*, 2009).

2.6 Histamine induced paw edema

Inflammation of hind paw was induced by injecting 0.1 ml of histamine (1 mg/ml) in normal saline into the subplantar region of right hind paw (Singh *et al.*, 2003). Group I served as control and group II as negative control which received histamine (1%); group III served as positive control which received Indomethacin (25 mg/kg b.wt); group IV and V served as extract treated group which received methanolic aerial and root extract at 200 mg/kg b.wt. All the plant extract treatments were given 1 hr before histamine injection. The paw volume was measured with digital plethysmometer at 1, 2, 3, 4 and 5 hr after histamine injection (Amresha *et al.*, 2007).

2.7 Statistical analysis

All the data were expressed as Mean ± SEM and evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test and the value $P < 0.05$ were considered as statistically significant.

3 RESULTS AND DISCUSSION

In rat, oral administration of methanolic extract of *P.corymbosa* at the dose level of 2000 mg/kg b.wt does not exhibited any signs of toxicity and mortality up to 14 days. This indicates that the aerial and root methanolic extract of *P.corymbosa* was nontoxic in mice upto an oral dose of 2000 mg/kg of b.wt. Therefore, further the biological evaluation was carried out using 200 mg/kg b.wt dose level.

Anti-inflammatory effects of aerial and root methanolic extracts of *Polycarpaea corymbosa* on carrageenan-induced rat paw edema

Group	Treatment	Dose (mg/kg b.w., Oral)	Hind paw edema at time (h) after carrageenan (% of inhibition)					
			0h	1h	2h	3h	4h	5h
I	Control	-	4.13±0.14	4.08±0.09	3.88±0.09	4.15±0.1	4.15±0.1	3.95±0.07
II	Negative control (Carrageenan)	1%	3.18±0.07***	3.78±0.06***	3.53±0.07	3.48±0.07	3.41±0.11	3.37±0.23
III	Positive control (Indomethacin)	25	3.76±0.06	3.8±0.08	3.5±0.09	3.45±0.11	3.31±0.06	3.13±0.1
IV	Aerial extract	200	4±0.06	4.26±0.09	4.03±0.08	3.93±0.08	3.79±0.07	3.65±0.09
V	Root extract	200	3.45±0.12***	3.6±0.1*	3.4±0.13*	3.58±0.11***	3.46±0.11***	3.33±0.09*

Values are given as Mean ± SEM for groups of six animals each.
*P<0.05, **P<0.01 and *** P<0.001 as compared to control vs oedema, control rats and drug treated rats.

Anti-inflammatory activity of aerial and root extract of *P.corymbosa* against carrageenan and histamine induced paw oedema in rats was tabulated in Table 1, 2. The extract exerted anti-inflammatory activity (P<0.05) with both root and aerial parts of plant extracts when compared to that of the standard drug Indomethacin (25 mg/kg b.wt). The oedema volume increased progressively and the maximum paw volume was attained at 2h after injection. Highest activity was produced at 5th h. Methanol aerial extract (200 mg/kg b.wt) produced highest activity. Among these different extracts, aerial extract showed maximum inhibition in inflammation (3.65±0.09) as compared to control group (3.37±0.23), which was comparable with that of standard, Indomethacin (3.13±0.1) (Table 1). In case of histamine induced paw oedema, the methanol aerial extract produces maximum inhibition at 5th h.

Inflammation is the common phenomenon and it is a reaction of living tissues towards injury (Saleem *et al.*, 2011). Carrageenan induced inflammation is a useful model for the estimation of anti- inflammatory effect of the plant extracts (Ratheesh *et al.*, 2007). The development of oedema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin, prostaglandin (Georgewill *et al.*, 2010; Georgewill and Georgewill, 2010; Saleem *et al.*, 2011). Histamine is an important chemical mediator that may cause inflammation, vasodilatation, increased vascular permeability, decreased peripheral resistance, airway smooth muscle contraction, and itching sensory nerve stimulation (Estelle, 2003). Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo* (Opie, 1962; Umapathy *et al.* 2010). Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. *P.corymbosa* methanolic aerial and root extract showed significant (P<0.05) anti-inflammatory activity at dose level of 200 mg/kg b.wt. The significant anti-inflammatory effect of P.*corymbosa* extract at the dose level of 200 mg/kg b.wt was comparable with Indomethacin. The result is quite similar to the one observed for Indomethacin at 25mg/kg b.wt the standard drug. GC-MS analysis of *P. corymbosa* aerial part revealed the presence of 5-Hydroxymethylfurfural. This compound might have positive role in anti-inflammatory effect (Sindhu *et al.*, 2013). In the present study the anti-inflammatory activity of *P.corymbosa* can be attributed to the presence of above chemical constituents.

Anti-inflammatory effects of aerial and root methanolic extracts of *Polycarpaea corymbosa* on Histamine-induced rat paw edema

Group	Treatment	Dose (mg/kg b.w., Oral)	Hind paw edema at time (h) after Histamine (% of inhibition)					
			0h	1h	2h	3h	4h	5h
I	Control	-	4.13±0.14	4.08±0.09	3.88±0.09	4.15±0.1	4.15±0.1	3.95±0.07
II	Negative control (Histamine)	1%	3.3±0.07***	3.68±0.06***	3.51±0.07**	3.48±0.07	3.41±0.08***	3.25±0.11**
III	Positive control (Indomethacin)	25	3.8±0.08	3.95±0.04**	3.76±0.09	3.75±0.14**	3.6±0.13	3.55±0.1
IV	Aerial extract	200	3.95±0.06	4.13±0.07	3.96±0.04	3.95±0.08	3.63±0.12	3.48±3.11
V	Root extract	200	3.61±0.14**	3.73±0.11*	3.61±0.11	3.5±0.08	3.46±0.08*	3.4±0.1***

Values are given as Mean ± SEM for groups of six animals each.
*P<0.05 , **P<0.01 and *** P<0.001 as compared to control vs oedema, control rats and drug treated rats.

4 CONCLUSION

The result of present study authentifies the folklore information on the anti-inflammatory property of the aerial and root part extracts of *P.corymbosa*. It seems that methanolic extract of *P.corymbosa* significantly inhibits the acute phase of inflammation *via* blockage of the mediators that cause oedema. However, it did not show any effect on the proliferative phase of inflammation, in carrageenan and histamine induced inflammation granuloma formation. Considering these results, methanolic extract of *P.corymbosa* may represent a potential new source of drugs for the treatment of anti-inflammatory pain. Further research is therefore obviously required to purify and identify the structure of the active principle(s), as well as to determine its anti-inflammatory properties.

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