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EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF *PHYLLANTHUS LONGIFLORUS* IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Anti inflammatory potential of aerial parts of *Phyllanthus longiflorus* was investigated in the present study using acute and chronic inflammatory models. Carrageenan, dextran and formalin were used as an edematogenic agent to induce hind paw edema in rats and the mean increase in paw volume were measured using plethysmometer. Results showed that methanol extract of *Phyllanthus longiflorus* at the doses of 100 and 200 mg/kg body weight significantly reduced in hind paw edema induced by Carrageenan, dextran and formalin. Therefore, it is concluded that *Phyllanthus longiflorus* is useful in the management of inflammatory diseases.

KEYWORDS: ***Phyllanthus longiflorus*, carrageenan, formalin, anti inflammatory.**

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INTRODUCTION

Plants synthesize a wide variety of phytochemicals which performs their biological activities. These phytochemicals also have therapeutic effect in humans, hence, it can be refined to produce drugs. Most of the drugs used in modern medicine originated from plants. Plants based drugs are cost effective, less toxic and free from side effects, therefore, used world wide to treat a wide range of diseases, from headache to cancer. *Phyllanthus longiflorus* Heyne ex Hook f, Synonym- *Reidia longiflora* Gamble; *Reidia ovalifolia* wright, commonly known as Nallapulatti in Tamil, is a small bush with obliquely obovate – oblong leaves, pink flowers and small capsular fruits (0.4 cm long) and distributed in the Western Ghats of Tamilnadu[1]. Many members of the genus *Phyllanthus* were reported to possess anti tumor[2], anti inflammatory[3], hepatoprotective[4], diuretic[5], and anti bacterial[6] properties. The same authors have reported anti convulsant[7] and diuretic activity[8] of *P.longiflorus*. In the present study methanol extract of PHL (MPHL) was evaluated for its possible anti inflammatory activity. The activity was assessed on acute (carrageenan, dextran induced rat paw edema) and chronic (formalin induced rat paw edema) inflammatory models.

MATERIALS AND METHODS

Plant collection and authentication

Fresh aerial parts of *P. longiflorus* were collected from the Western Ghats of Tamilnadu, India during February 2008. It was authenticated by Dr.V.Chelladurai, Govt. Research officer, Botany C.C.R.A.S. Govt. of India, (Retired), Tirunelveli, Tamilnadu, India. A voucher specimen (PHL001) has been deposited in our laboratory for future reference.

Preparation of extract

The collected aerial parts were dried under shade for fifteen days, pulverized in to coarse powdered using mechanical grinder and about 1.5 kg of powder was extracted exhaustively with methanol by continuous hot extraction method using Soxhlet apparatus[9]. The solvent was removed under reduced pressure and dried using a rotary evaporator. The solid mass obtained was preserved in desiccator until further use. The extract was subjected to different phytochemical tests to identify the nature of chemical constituent present in the extract[10].

Animals

Male albino rats of Wister strain (120-150 g) and male Swiss albino mice (25-30 g) were procured from the central animal house of our institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 20°C; relative humidity 60-70%) in a 12 h light-dark cycle. The animals were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 12 h before and during the experimental hours. The protocol for the present study was approved by institutional animal ethics committee (Approval no. 509/02/C/CPCSEA).

Acute toxicity study

Acute toxicity study was carried out by Graded dose technique. The extract was administered in graded doses of 100 to 2000 mg/kg body weight by intraperitoneal route. The animals were observed continuously for the first 1 hr, intermittently for the next 4 hrs for any behavioral changes like sedation, loss of righting reflex, hyper activity, convulsion and periodically for first after 24 hrs for mortality and the study was continued for fourteen days[11].

Evaluation of anti inflammatory activity

Carrageenan induced paw edema model

Animals were divided in to four groups of six each. Group I received aqueous tween 80 (*p.o.*), Group II received diclofenac sodium (10 mg/kg, *i.p.*), Groups III and IV 100 and 200 mg/kg of methanol extract of PHL respectively. The anti-inflammatory activity was investigated on carrageenan induced edema by using the method described by winter's et al[12]. The

animals were injected with 0.05 ml of 1% suspension of carrageenan in the hind paw. The volume of the hind paw was measured before and 3 hr after carrageenan injection by mercury displacement method using plethysmometer.

Dextran induced paw edema model

The animals were treated as similar to carrageenan induced paw edema model, except that in place of carrageenan, dextran was utilized as edematogenic agent[13].

Formalin induced paw edema model

The animals were divided into groups similar to carrageenan paw edema model. 1hr after administration of extract, formalin (0.1 ml of freshly prepared 2% formalin) was injected in to sub plantar area of ether anaesthetized rat[14]. The treatment was continued for 6 consecutive days and the edema was measured on 1 and 6 days. The percentage inhibition of paw edema was calculated for all the models using the following formula. % inhibition = 1-(Vt/Vc) × 100. Where Vt represent paw volume of PHL/Diclofenac, Vc represent paw volume of tween 80 treated control group.

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. The statistical analyses were performed by GraphPad InStat v.3.0.10.0 (GraphPad Software, Lajolla, CA, U.S.A). P<0.01 were considered significant.

Table 1

Anti inflammatory effect of methanol extracts of Phyllanthus longiflorus on Carrageenan, dextran and formalin induced paw edema model.

Treatment	Carrageenan		Dextran		Formalin	
	0 hr	3hr	0 hr	3hr	0 hr	6th day
Tween 80 10 ml/kg	0.141±0.13	0.257±0.63	0.142±0.16	0.310±0.42	0.148±0.11	0.385±0.65
Diclofenac 10mg/kg	0.147±0.36	0.102±0.72 (60.32)	0.147±0.52	0.131±1.76 (57.75)	0.140±0.17	0.223±0.73 (42.10)
MPHL 200mg/kg	0.146±0.09	0.132±0.39 (48.70)	0.145±1.81	0.169±0.11 (45.49)	0.143±1.16	0.230±0.36 (40.26)
MPHL 100mg/kg	0.142±0.06	0.146±0.45 (43.20)	0.141±0.96	0.177±0.62 (42.91)	0.140±1.95	0.245±0.52 (35.85)

n=6, mean ± SEM, P<0.01 Vs control, values in the parenthesis represents % inhibition of paw volume; zero hr- paw volume before the injection of edematogenic agent; 3 hr/6th day- paw volume after drug treatment.

RESULTS

Carrageenan induced paw edema

Methanol extract of *Phyllanthus longiflorus* at the doses 100 and 200 mg/kg, *p.o.* have significantly (P<0.01) reduced the carrageenan induced hind paw edema (table 1). The percentage inhibition of paw volume by PHL was 48.70% and 43.20% at the doses 200 and 100 mg/kg respectively, while for the reference drug, it was 60.32%.

Dextran induced edema

PHL also reduced dextran induced paw edema by 45.49% and 42.91% at the doses 200 and 100 mg/kg respectively, whilst for reference drug, it was 57.75% (table 1). , .

Formalin induced edema

The present study also revealed that PHL is effective against chronic inflammation. PHL exhibited 40.26% and 35.85% inhibition on formalin induced paw volume, where as diclofenac produced 42.10% inhibition (table 1).

DISCUSSION

Carrageenan and dextran are the acute inflammatory models while formalin induced paw edema is a chronic model[15]. Also The formalin induced paw edema closely resembles human arthritis[16]. Thus, the results obtained in the present study recommends that PHL is

affective in the treatment of arthritis and other acute as well as chronic inflammatory diseases. Carrageenan induced paw edema is thought to involve biphasic response. The first phase is related to the release of histamine, serotonin, kinins and 5-hydroxy tryptamine and in the second phase prostaglandin, cyclo-oxygenase, reactive oxygen species and some slow reacting substances are released. The release of these substances are peak at 3rd hr[17,18,19]. Dextran causes extravasations and edema formation[20], by releasing histamine and serotonin from mast cells., Formalin induced edema is also a biphasic response, the first phase is neurogenic, mediated through chemical nociceptive stimulation which involved the release of substance P and bradykinin. The second phase is a tissue mediated inflammatory phases which involved central and peripheral mechanism and mediated through the release of prostaglandin and histamine[21]. since, prostaglandins plays a key role in the late phase of inflammation and pain sensitivity, the existence of anti inflammatory activity by methanol extract of *Phyllanthus longiflorus* might involve the interference in the biosynthesis or/and release or/and activity of the above biochemicals, especially prostaglandins. As phytochemicals, flavanoids and phenolic compounds were reported to target prostaglandins[22], it can be concluded that the presence of flavanoids and phenolic compounds in the extract might be responsible for the anti inflammatory activity of aerial parts of *Phyllanthus longiflorus*. Further study is in progress to isolate the phytoconstituent responsible for the activity.

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