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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF DIFFERENT EXTRACTS AND ISOLATED LIGNANS OF *PHYLLANTHUS AMARUS* SCHUM. & THONN. AERIAL PARTS.

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ABSTRACT

Phyllanthus amarus Schum. & Thonn. is an important plant of Indian Ayurvedic system of medicine and its various parts were used traditionally for the treatment of problems related to stomach, wounds, liver and spleen. The objective of this study was to evaluate the anti-inflammatory activity of hexane (PAHE), ethyl acetate (PAEA), methanolic (PAME) extracts and isolated lignans (phyllanthin (PAPH) & hypophyllanthin (PAHP)) of *P.amarus* aerial parts by using carrageenan induced hind-paw-oedema model. The tested extracts (125, 250 & 500mg/kg b.w) and lignans ($1.3x10^{-5}$ & $2.6x10^{-5}$ moles/kg b.w) produced dose dependent significant reduction ($p<0.05–0.001$) in carrageenan-induced rat paw oedema. The standard drug, Indomethacin at dose $1.3×10^{-5}$ moles/kg b.w reduced oedema by 62.01±0.22% and the total (AUC) paw oedema by 69.09±0.15% respectively. The results suggested that the tested extracts and lignans possess *in vivo* anti-inflammatory activity and the exhibited activity was comparable with the standard drug Indomethacin.

KEY WORDS: ***Phyllanthus amarus*, lignans, carrageenan and anti-inflammatory activity.**

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INTRODUCTION

Phyllanthus amarus Schum. & Thonn. belongs to the family Euphorbiaceae and is mentioned in the Indian Ayurvedic system of medicine for the treatment of various ailments like gastropathy, diarrhoea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds[1-5]. *P.amarus* is widely spread throughout the tropical and subtropical countries of the world including India. However, literature survey indicated no published reports on the anti-inflammatory activity of the successive extracts and isolated lignans of *P.amarus* aerial parts. Hence, a detailed study was carried out on the hexane (PAHE), ethyl acetate (PAEA), methanolic (PAME) extracts and isolated lignans (phyllanthin (PAPH) & hypophyllanthin (PAHP)) of *P.amarus* aerial parts for evaluating the anti-inflammatory activity by using carrageenan induced rat hind-paw-oedema model.

MATERIALS AND METHODS

(i)Drugs and chemicals

All the chemicals and reagents used were of analytical grade. Indomethacin, Sodium Carboxy Methyl cellulose (Na. CMC) and Carrageenan were purchased from Sigma chemicals, USA. HPLC grade solvents were obtained from Merck, India.

(ii)Collection, extraction and isolation of lignans

The plant material was collected in October 2010 from Paderu region, Visakhapatnam District, Andhra Pradesh and authenticated by Dr. M.Venkayya, Taxonomist. The Voucher specimen (BG/PMK/PA-10-10) was deposited in the herbarium, College of Pharmaceutical Sciences, Andhra University. The freshly collected aerial parts of the plant were shade dried and powdered. The powdered material (5 kg) was then subjected to successive soxhlet extraction with hexane, ethyl acetate and methanol. The solvent thus obtained was separately concentrated under vacuum at a temperature of 40 ºc by using a rotary evaporator (Buchi, Switzerland). The concentrated extracts were then collected and stored in dessicator for further studies. 25g of hexane extract was then fractionated over a column (100 cm length x 35mm diameter) of silica gel (60-120 mesh size). Gradient elution was done in the following sequence, hexane (100, v/v)→hexane-ethylacetate (95:5, v/v)→hexane-ethylacetate (90:10, v/v). The fractions (Fr150-189) collected for hexane-ethylacetate (90:10 v/v) showed the presence of lignans on TLC and these fractions were pooled together and concentrated. The lignan fraction was subjected to preparative separation using RP-HPLC (Waters Delta Prep-PDA, USA) for obtaining the pure (>95%) phyllanthin and hypophyllanthin.

(iii)Animals

Adult Wistar rats of either sex weighing 200-250gm were used in the present study and they were purchased from National Institute of Nutrition, Hyderabad, India. The animals were maintained under standard laboratory conditions at an ambient temperature of 23±2°C having 50±5% relative humidity with 12-h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

(iv)Acute toxicity studies

The acute toxicity studies were performed as per OECD-420 guidelines for doing limit test. Selected five groups of Wistar albino rats (n=6) of uniform weight are taken. They were given 2000 mg/kg b.w of the test extract (PAHE, PAEA & PAME)/compound (PAPH & PAHP) and are observed for 24 h for mortality. All the tested animals were observed closely for 24 h and then daily for 14 days, no mortality was observed. It was further supported by previous reports stating that the plant extracts belonging to Phyllanthus genus are nontoxic at dose of

5g/kg b.w[6]. Hence, I selected 500 mg/kg as maximum safety dose with descending dose levels with 2 fold interval i.e., 125 mg/kg and 250 mg/kg body weight of the test animal.

(v)Carrageenan induced paw-inflammation model

Anti-inflammatory activity was evaluated in carrageenan induced rat paw oedema model and the paw thickness of each rat was measured using Zeitlin's apparatus[7,8]. Inflammation was induced in the right hind paw of each rat by sub-plantar injection of 1% carrageenan suspension (0.1ml). The left hind paw of the rat was injected 0.1ml of saline. Group-I received drug vehicle (1% sodium CMC). Group-II received standard drug Indomethacin at a dose of $1.3x10^{-5}$ moles/kg b.w. Group-III to Group-XI received orally hexane (PAHE), ethyl acetate (PAEA) and methanolic extracts (PAME) of *Phyllanthus amarus* aerial parts at doses of 125mg/kg, 250mg/kg and 500mg/kg b.w. Group-XII to Group-XIV received orally phyllanthin (PAPH) and hypophyllanthin (PAHP) at doses of $1.3x10^{-5}$ moles/kg and at $2.6x10^{-5}$ moles/kg b.w. in sodium carboxy methyl cellulose suspension 18 h and 2 h prior to the induction of oedema by carrageenan injection. After administration of these doses, each rat was injected with saline subcutaneously into the sub-plantar tissue of the left hind paw and with 1% carrageenan in saline subcutaneously into the sub-plantar tissue of right hind paw. The paw thickness of each rat was measured using Zeitlin's apparatus before carrageenan injection and every hour up to 6hrs after carrageenan injection. The percentage inhibition of paw oedema was calculated by using the following formula.

$$\% \text{ Increase in paw thickness} = \frac{Y_t - Y_0}{Y_0} \times 100$$

Y_t = Paw thickness at time t (1, 2, 3, 4, 5 and 6th) after injection

Y_o = Paw thickness at 0 hr (before injection).

(vi)Statistical analysis

Data of paw thickness was analyzed by using One-Way ANOVA followed by post hoc Dunnett's test using Graph pad Prism-5 software. The results are expressed as Mean ±S.E.M. $p<0.05$ was considered to be significant (*p< 0.05; **p < 0.01; ***p< 0.001).

RESULTS

The hexane, ethyl acetate, methanolic extracts and isolated lignans (phyllanthin and hypophyllanthin) of *Phyllanthus amarus* aerial parts at different tested doses showed significant (***p<0.001) dose dependent reduction of paw oedema when compared to drug vehicle treated control group in carrageenan induced paw inflammation model. Among all the tested groups phyllanthin showed prominent activity. The methanolic extract (PAME) showed better activity when compared to their ethylacetate (PAEA) and hexane (PAHE) extracts. The Percentage inhibition of the maximal paw oedema and Percentage inhibition of total (AUC) paw oedema during 6 hours were given in Table.1 and the Percentage inhibitions of the maximal paw oedema were graphically plotted as Fig.1, Fig.2, Fig.3 & Fig.4.

DISCUSSION

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury[9] and it involves a series of events that can be elicited by numerous stimuli, e.g.: infectious agents, ischemia, antigen- antibody interactions and chemical, thermal or mechanical injury. The response is accompanied by the clinical signs of erythema, oedema, hyperanalgesia and pain[10]. Acute hind paw oedema is induced in rats by injecting 0.1ml of 1%v/v carrageenan which reaches a peak oedema levels at 3-5 hours after Carrageenan injection. The development of oedema in the paw of the rat after the injection of carrageenan is due to the release of histamine, serotonin, prostaglandins[11,12]. The

order of anti-inflammatory activity of the three extracts (PAHE, PAEA & PAME) and two isolated compounds (PAPH & PAHP) of *P.amarus* aerial parts is in the following manner: Indomethacin>PAPH>PAHP>PAME>PAEA>PA HE. The above results clearly indicate that the different extracts and isolated lignans of *Phyllanthus amarus* aerial parts showed significant ($p<0.001$) dose dependent anti-inflammatory activity against carrageenan induced inflammation model in rats and these results in turn support the folkloric claims regarding the anti-inflammatory activity of *Phyllanthus amarus* and its medicinal values.

CONCLUSION

From the above study it was concluded that the hexane, ethylacetate, methanolic extracts and isolated lignans of *Phyllanthus amarus* aerial parts possessed anti-inflammatory activity in a dose dependent manner in carrageenan induced paw oedema model in rats. It was previously reported that the plant *P.amarus* consists of antioxidant phytochemicals like lignans, alkaloids, triterpenes, sterols, flavonoids, phenols and saponins[13-18] and these might contribute to the anti-inflammatory activity against carrageenan induced inflammation in rat hind paw model.

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Table 1
Effect of different extracts and isolated lignans of P.amarus aerial parts on carrageenan induced rat paw oedema.

Treatment/Dose	Groups	Percentage inhibition of the maximal paw oedema during 6 hours	Percentage inhibition of total AUC paw oedema during 6 hours
1% Na. CMC-1ml	Group I	0.0	0.0
Indomethacin-$1.3x10^{-5}$ moles/kg	Group II	62.01±0.22***	69.09±0.15***
PAHE-125mg/kg	Group III	22.92±0.47***	23.38±0.59***
PAHE-250mg/kg	Group IV	31.59±0.27***	35.35±0.36***
PAHE-500mg/kg	Group V	38.29±0.22***	38.62±0.62***
PAEA-125mg/kg	Group VI	19.22±0.53***	20.87±0.37***
PAEA-250mg/kg	Group VII	35.37±0.49***	38.67±0.41***
PAEA-500mg/kg	Group VIII	39.63±0.48***	42.33±0.64***
PAME-125mg/kg	Group IX	28.17±0.22***	29.90±0.19***
PAME-250mg/kg	Group X	38.25±0.45***	40.52±0.14***
PAME-500mg/kg	Group XI	42.71±0.25***	44.72±0.32***
PAPH-$1.3x10^{-5}$ moles/kg	Group XII	47.78±0.72***	51.12±0.94***
PAPH-$2.6x10^{-5}$ moles/kg	Group XIII	50.11±0.64***	54.25±0.47***
PAHP-$1.3x10^{-5}$ moles/kg	Group XIV	41.98±0.49***	47.62±0.56***
PAHP-$2.6x10^{-5}$ moles/kg	Group XV	46.62±0.86***	51.29±0.45***

Significance: ***P<0.001. All groups were compared with control group (Group I).
Values are expressed as Mean ± SEM. n=6 animals in each group.

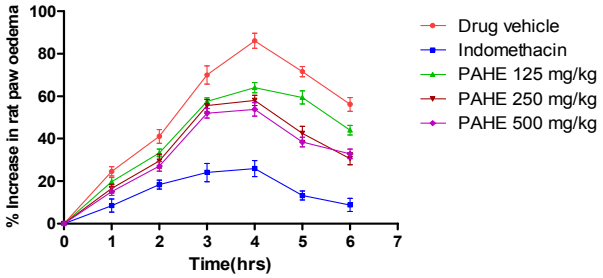

Figure 1
Effect of P.amarus hexane extract (PAHE) on the maximal oedema in carrageenan induced rats.

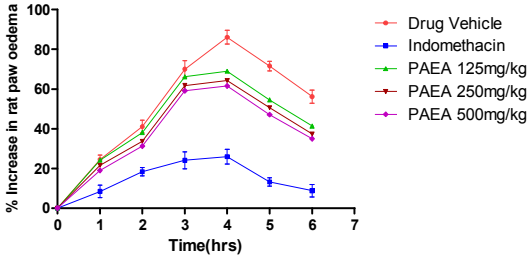

Figure 2
Effect of P.amarus ethylacetate extract (PAEA) on the maximal oedema in carrageenan induced rats.

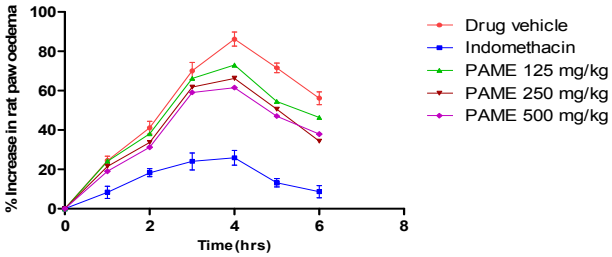

Figure 3
Effect of P.amarus methanolic extract (PAME) on the maximal oedema in carrageenan induced rats.

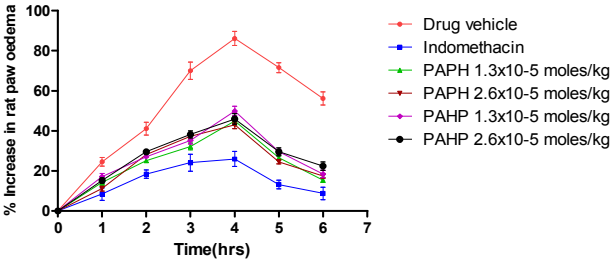

Figure 4
Effect of phyllanthin (PAPH) and hypophyllanthin (PAHP) on the maximal oedema in carrageenan induced rats.

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