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ANALGESIC, ANTI-INFLAMMATORY, ANTIPYRETIC EVALUATION OF ETHANOLIC EXTRACT OF LEAF OF *POLYALTHIA LONGIFOLIA*

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Abstract

Polyalthia longifolia leaves ethanolic extracts produced significant analgesic activity in both Hot plate and acetic acid induced writhing models in mice. In hot plate method percentage increase in reaction time was determined where as in acetic acid induced writhing model percentage decrease in writhings was determined. From the results obtained it can be concluded that *Polyalthia longifolia* leaves has analgesic, anti inflammatory and antipyretic activity.

Keywords: Analgesic, Anti-inflammatory, Antipyretic, Hot plate method, Acetic acid induced writhing model.

Introduction

In India numerous invaluable plants are used in ethnomedical practices as well as in Ayurveda and sidha. The pharmacological properties of many such plants are not sufficiently evaluated in the light of modern science.[1]

Non steroidal anti-inflammatory drugs (NSAID's) are widely used in the treatment of pain, pyrexia and inflammation. However these drugs have side effects especially on the gastro intestinal tract.[2] Through this evaluation of pharmacological activities in *Polyalthia longifolia* leaves for analgesic, anti-inflammatory and antipyretic activity in experimental animal models was taken up.

Polyalthia longifolia (*Annonaceae*) is used as an antipyretic agent in indigenous system of medicine. Recently a lactone with promising antibacterial activity has been isolated from the stem.[3]

The plant possesses significant analgesic, antiinflammatory and vasodepressant activities. Although this plant is used in traditional medicine to combat fever, its antipyretic effect has not been experimentally evaluated.[4]

In view of the reported active constituents present in the various parts of the plant *Polyalthia longifolia* like alkaloids, clerodane di-terpines, quercetin, bulbocapnin, β-sitosterol, stigmasterol campesterol, enihalimane diterpenes, and sesquiterpenoids.. An attempt is made to evaluate the analgesic, anti-inflammatory and antipyretic potential of the ethanolic extract of leaves of *Polyalthia longifolia.*

Materials, Methods

he acute toxicity study conducted for ethanolic extracts indicated that they are safe up to 2000

mg/kg body weight.

Polyalthia longifolia leaves ethanolic extracts produced significant analgesic activity in both Hot plate and acetic acid induced writhing models in mice. In hot plate method percentage increase in reaction time was determined where as in acetic acid induced writhing model percentage decrease in writhing was determined.

Materials and Methods

Collection and authentication of plant material

Leaves of *Polyalthia longifolia* belonging to the family opiliaceae were collected from parts of Wayanad District of Kerala. It was identified and confirmed by botanist.

Animal approval

The study was conducted after obtaining the approval from Institutional Animal Ethics Committee (IAEC), and the experimental procedure were in accordance to the guidelines of IAEC (No:688/02/c/CPCSEA).

Preparation of Extract [5,6, 7,8, 9]

The powdered leaves of *Polyalthia longifolia* were defatted with petroleum ether extracted with water successively by hot water reflux and continuous hot percolation method. The temperature and the duration of the extraction procedure were determined using investigational studies. An aliquot portion of the collected sample was transferred to a high grade round bottom flask and continuously refluxed for a calculated time period of three hours at a pre-determined extraction temperature of 60-70°C. Subsequently, alcohol precipitation was followed (using various concentration) and the precipitate obtained was redissolved in ethanol. The extracts were concentrated under reduced pressure and refrigerated.

Preliminary Phytochemical Studies

Preliminary phytochemical tests were performed for chemical constituents such as alkaloids, carbohydrates, glycosides, steroids, tannins, proteins and aminoacids, fixed oils and fats, flavonoids and saponins and it was found that flavonoids were present.

Evaluation of anti inflammatory activity was done by Formalin induced paw edema model, Croton oil ear edema model and carrageenan induced paw edema model respectively. In Formalin induced paw edema model mean change in paw volume and percentage protection were calculated. In croton oil ear edema model the difference between untreated ear and treated ear were determined which indicated degree of inflammatory edema. In carrageenan induced paw edema model the change in paw volume and percentage protection were calculated.

Results

Results of preliminary phytochemical screening:

Table 5:

SL. NO.	NAME OF THE TEST	OBSERVATION	CONCLUSION
		Ethanolic Extract	
I.	Tests for Steroids		
	Salkowski reaction	+	Steroids were present in ethanolic extract.
	Liebermann Burchard	+	
	Liberman's reaction	+	
II.	Tests for Saponins		Saponins were present in ethanolic extract.
	Foam test	+	
	Haemolytic test	+	

III.	Tests for Tannins and Phenolic Compounds Lead acetate test 5% Fe Cl_3 test Bromine water test Acetic acid solution test Potassium dichromate test	 + + + + +	Tannins were present in ethanolic extract.
V.	Tests for Flavonoids Shinoda test Lead acetate test Alkaline solution Ferric chloride test	 + + + +	Flavanoids were present in ethanolic extract.
VI.	Tests for Reducing Sugars Fehling's test Benedict's test	 - -	Reducing sugars were absent in ethanolic extract.

Thus we can conclude from above observations that ethanolic extract contain steroids, saponins, tannins, phenolic compounds and flavonoids. (Table No. 5.0)

5.1 Analgesic activity of polyalthia longifolia leaves

The ethanolic extracts of leaves of *Polyalthia longifolia* were evaluated for analgesic activity by hot plate and acetic acid induced writhing models, the results obtained are as follows,

5.1.1 Hot plate method:

The ethanolic extracts significantly and dose dependently protected the mice against thermally induced pain stimulus. All the extracts at various time intervals at which they were tested produced increase in reaction time. (Fig No.5.1).

The comparison of analgesic activity with the standard drug Tramadol at various time intervals is as follows. At 30 min, only PLEE 400 produced analgesic activity comparable ($P<0.05$) to that of standard. The percentage protection against thermally induced pain stimulus by PLEE 400 and the standard drug, tramadol was 84.34±5.22 and 68.84±6.74 respectively. At 45 min PLEE 400 produced analgesic activity comparable ($P<0.05$) to that of tramadol, the percentage protection was 75.92±7.21 and 81.42±5.30 respectively.

At 60 min PLEE 200 and 400 produced analgesic activity comparable ($P<0.05$) to that of tramadol. At 90, 120 and 180 min, all extracts at all doses produced analgesic activity better ($P<0.01$) than tramadol. (Table No. 5.1.1 and Fig. No. 5.1.1).

Table No. 5.1.1: Analgesic effect of *Polyalthia longifolia* ethanolic extract (PLEE) and tramadol in mice by hot plate method.

Treatment	Dose (mg/kg)	Percentage increase in reaction time					
		30 min	45 min	60 min	90 min	120 min	180 min
Standard (STD) (Tramadol)	5	67.74 ±5.84	80.32 ±4.10	77.74 ±6.40	70.67 ±7.00	65.32 ±7.47	30.58 ±8.07
PLEE 100	100	42.85 ±11.26	63.44 ±8.73	81.49 ±16.81	83.44 ±7.00**	80.30 ±6.67**	66.87 ±12.79**
PLEE 200	200	46.97 ±15.05	70.30 ±19.31	83.08 ±6.06*	80.87 ±6.67**	80.87 ±8.38**	66.67 ±14.91**
PLEE 400	400	84.34 ±5.22*	79.92 ±7.21	78.64 ±5.66*	85.35 ±5.21**	86.29 ±5.19**	47.12 ±18.48**

n=6, values represent mean ±SD

Where, PLEE 100, PLEE 200 and PLEE 400 indicates *Polyalthia longifolia* ethanolic extracts at doses 100, 200 and 400 mg/kg body weight respectively.

*Symbols represent statistical significance.** $P < 0.01$., *$P < 0.05$. as compared to tramadol.

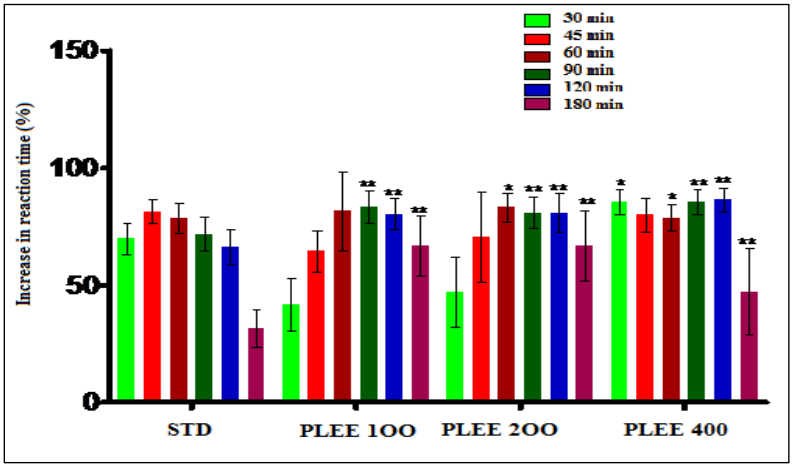

Fig 5.1.1 Effect of *Polyalthia longifolia* ethanolic extract in mice by hot plate method

5.1.2 Acetic acid induced writhing test:

Results of acetic acid induced writhing response in mice indicates that ethanolic extracts of *Polyalthia longifolia* produced analgesic activity in a dose dependent manner. PLEE 200, PLEE 400 and Aspirin produced significant ($P<0.01$) decrease in writhings induced by acetic acid when compared to control. PLEE 400 produced maximum ($P<0.01$) decrease in the number of writhes when compared with all other groups.

The percentage decrease in writhing by various extracts was compared to that of the standard drug aspirin. PLEE 400 produced maximum percentage decrease in writhing which was better ($P<0.01$) than that of standard .The percentage decrease in writhing ± SEM by PLEE 400 and aspirin were found to be 80.28±2.04 and 74.60±1.53 respectively**.** The ethanolic extract at lower dose did not produce significant decrease in writhing when compared to standard. (Table No. 5.1.2 and Fig. No.5.1.2,5.1.3)

Table No.5.1.2 :Effect of *Polyalthia longifolia* ethanolic extracts and Aspirin on acetic acid induced writhes in rats.

Treatment	Dose(mg/kg)	Number of writhes in 20 (min)	Percentage inhibition
Control	-	39.67± 3.18	-
PLEE 100	100	21.83 ±2.92^{**a}	44.25 ±7.46
PLEE 200	200	36.3± 1.36	7.23 ±3.48
PLEE 400	400	7.33 ±1.96$^{**a, **b}$	80.28 ±5.01$^{**c, **d}$
Aspirin	10	9.16 ±1.47^{**a}	74.60± 3.76

Values represent mean ±SD.

Where, PLEE 100, PLEE 200 and PLEE 400 indicate *Polyalthia longifolia* ethanolic extracts at doses 100, 200 and 400 mg/kg body weight respectively.

*Symbols represent statistical significance. ** $P < 0.01$., *$P < 0.05$. as compared to aspirin. . 'a' as compared with control, 'b' is comparision of PLEE 400 with other treatment groups, 'c' as compared with Aspirin and 'd' is comparision of PLEE 400 with other treatment groups.

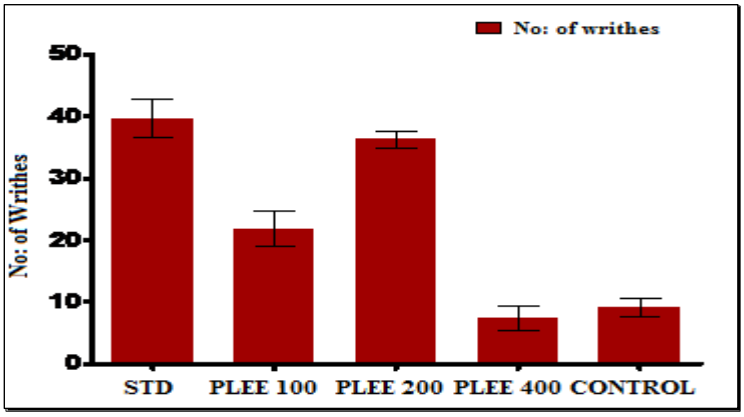

Figure No.5.1.2 Analgesic activity of *Polyalthia longifolia* on acetic acid induced writhes. Where n=6, values are mean ±SEM,

PLEE 100, PLEE 200, 400, indicates *Polyalthia longifolia* ethanolic extracts at doses 100, 200 and 400 mg/kg body weight respectively.

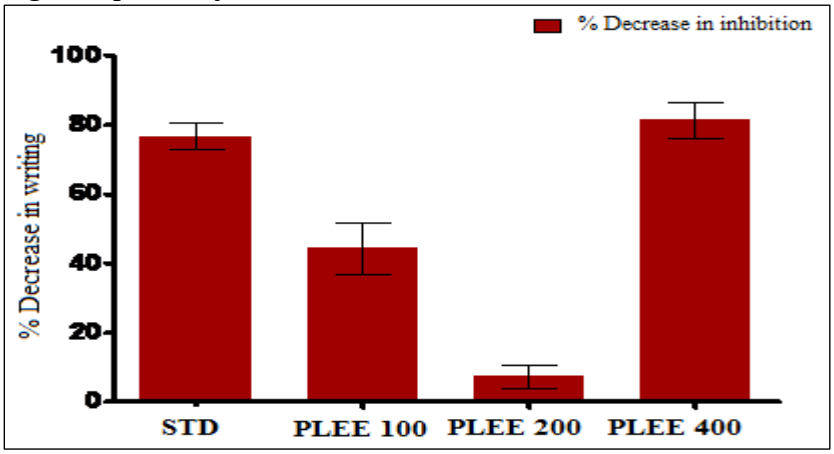

Figure No. 5.1.3 Analgesic activity of *Polyalthia longifolia* **on acetic acid induced writhes.**

Where, n=6, values are mean ±SEM, PLEE 100, PLEE 200, 400 indicates *Polyalthia longifolia* aqueous and alcoholic extracts at doses 200 and 400 mg/kg body weight respectively.

5.2 ANTI-INFLAMMATORY ACTIVITY OF POLYALTHIA LONGIFOLIA ETHANOLIC EXTRACT (PLEE)

5.2.1 FORMALIN INDUCED PAW EDEMA MODEL

Table 5.2.1: Acute anti-inflammatory activity of the PLEE (*Polyalthia longifolia ethanolic extract)* and Ibuprofen (reference drug) on formalin induced paw edema in Wistar rats.

Group	N	30 min	60 min	120 min	180 min	240 min	300 min
Control	**6**	1.25 ±0.014	1.30 ±0.01	1.34±0.03	1.32 ±0.056	1.26 ±0.084	1.24 ±0.070
PLEE 100	**6**	1.09 ±0.013	1.15 ±0.049	1.20 ±0.021	1.14 ±0.049	1.12* ±0.070	1.06* ±0.021
PLEE 200	**6**	1.08 ±0.056	1.17 ±0.014	1.25 ±0.042	1.23±0.035	1.10* ±0.014	1.00* ±0.028
PLEE 400	**6**	1.10±0.056	1.20±0.014	1.27±0.042	1.24±0.035	1.11* ±0.014	1.00* ±0.028
Ibuprofen (100mg/kg)	**6**	1.25 ±0.007	1.31 ±0.028	1.33 ±0.028	1.20 ±0.007	1.02 ±0.014	0.93* ±0.035

Data are the mean ± SEM values for six rats in each group.
*p < 0.05, **p < 0.01 as compared to the control.
At 400mg/kg dose (1.00±0.028), the activity of the extract showed almost similar activity compare to standard drugs

5.2.2 CROTON OIL EAR EDEMA MODEL

Table 5.2.2: Effect of PLEE on Croton oil ear edema in rats

Group	Dose (mg/kg)	N	Weight of Untreated ear (Right ear) (mg)	Weight of treated ear (Left ear) (mg)	Difference
Control	1ml/kg	6	37.53 ±1.08	25.02 ±1.17	13.17 ±1.24
PLEE 100	100mg/kg	6	37.49 ±0.37	28.14 ±0.28	9.35±0.09
PLEE 200	200mg/kg	6	37.02 ±0.51	29.04 ±1.20	7.98* ±0.85
PLEE 400	400mg/kg	6	36.82 ±0.44	30.66 ±0.63	6.16**±0.69
Ibuprofen	100mg/kg	6	37.43 ±0.64	32.47 ±0.57	4.95* ±0.11

Data are the mean ± SEM values for six rats in each group.
*p < 0.05, **p < 0.01 as compared to the control.

5.2.3 TURPENTINE OIL-INDUCED GRANULOMA POUCH BIOASSAY[13]

The effect of standard Ibuprofen at dose of 100 mg/kg and test drug PLEE at three different concentrations 100, 200 and 400 mg/kg b.w. on turpentine oil induced granuloma pouch bioassay and is tabulated in Table 5.2.3.
A dose dependent reduction in volume of exudate in ml was observed by PLEE extracts and the potency of anti-inflammatory activity was evaluated using percentage inhibition of inflammation brought about by PLEE. It was found to be:

PLEE 400 > PLEE 200 > PLEE 100. So it can be concluded PLEE exhibits antiinflammatory activity in a dose dependent manner.

Table 5.2.3: Effect of PLEE on turpentine oil induced granuloma in rats.

Groups	Treatment	Dose (mg/kg)	Volume of exudate (ml)	Percentage of inhibition
I	Control	-	3.49 ± 0.07	-
II	Ibuprofen Standard	100	0.96 ± 0.08[b]	60
III	PLEE 100	200	2.90 ± 0.09[b]	13
IV	PLEE 200	400	2.48 ± 0.11	29
V	PLEE 400	600	2.06 ± 0.18	40

Values are expressed as mean+SEM; number of animals used are 6 in each group; a P <0.001.

5.2.4 Antipyretic activity

Brewer ‘s yeast induced Pyrexia in Rats

Sl.No	Group	Dose (mg/kg)	No. of animals	3hours	6hours	9 hours
1	Yeast Control	1ml	6	2.25±0.08	2.50±0.07	2.73±0.08
2	Standard (Paracetamol)	100	6	0.98±0.18***	1.47±0.28**	1.77±0.18***
3	PLEE 100	100	6	1.31± 0.08	2.06±0.17	2.15±0.08
4	PLEE 200	200	6	1.37±0.08	1.72±0.13	1.75±0.12
5	PLEE 400	400	6	1.13±0.12	1.47±0.10	1.45±0.12

Actual change in rectal temperature (^{0}C)

Values are expressed as mean+SEM; number of animals used are 6 in each group; **P <0.01, ***P<0.001 (students t test) compared with yeast control.

PLEE - ***Polyalthia longifolia ethanolic extract***

In the present study, in the yeast control group the rise in temperature was consistent and significant in comparison to the initial values. *Polyalthia longifolia ethanolic extract* (PLEE 100,200,400) produced very good antipyretic effect in a dose-dependant manner and the observed effect was almost similar to that in the paracetamol-treated group.

Conclusion

From the results obtained it can be concluded that *Polyalthia longifolia* leaves has analgesic, anti inflammatory and antipyretic activity.

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