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The Analgesic and Anti-Inflammatory Activities of the Extracts of *Phyllanthus reticulatus*

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Abstract

The petroleum ether, ethyl acetate, and methanol extracts of *Phyllanthus reticulatus* Poir. (Euphorbiaceae) were chosen for pharmacological screening. In the acetic acid-induced writhing test, the ethyl acetate extract in doses of 150 and 300 mg/kg showed 51.23 and 65.12% inhibition of writhing, respectively. A significant elongation of tail-flick time was evident both in the ethyl acetate and the methanol extracts (42.38 and 60.49%) only at the 300 mg/kg dose level. In carrageenan-induced rat paw edema model, the methanol extract at the 300 mg/kg dose level showed 40.03% inhibition of edema at the end of 4 h. These results demonstrated that the extracts of *P. reticulatus* possess significant analgesic and anti-inflammatory properties.

Keywords: Acetic acid-induced writhing response, anti-inflammatory, carrageenan-induced rat paw edema assay, Euphorbiaceae, *Phyllanthus reticulatus*, radiant heat tail-flick method.

Introduction

Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can easily be induced (Sosa et al., 2002). The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses (Mattison et al., 1998). Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary.

Phyllanthus reticulatus Poir. (Synonym *Kriganelia reticulata* Bail, Bengali-Chitki, Assamese-Amluki) is a large, often scandent, shrub of the family Euphorbiaceae (Ghani, 2003). The plant grows throughout tropical areas of India, Bangladesh, China, and the Malay Islands (Kirtikar & Basu, 1980). The leaves and bark are used as astringent and diuretic. Juice of leaves is used for the treatment of diarrhea in children (Ghani, 2003). The bark showed significant antiviral (Renuka et al., 1998) and antiplasmodial activity (Omulokoli et al., 1997). The antibacterial potential of the aerial parts of this plant has recently been evaluated (Direkbusarakom et al., 1998). Previous phytochemical investigations showed the presence of octasanol, butenil, glochidonol, and many other crystalline compounds in the roots (Joshi et al., 1981). The stem and leaves contain 21-α-hydroxy-friedel-4(23)-en-3-one and other triterpenoids, including friedelin, β-sitosterol, friedelan-3β-ol, and betulinic acid (Ahmed et al., 1991; Hui et al., 1976).

Although *P. reticulatus* has traditionally been used in the treatment of many types of pain and inflammatory conditions in Bangladesh, no scientific report is available to date to validate these folkloric uses. As a part of our continuing studies on the medicinal plants of Bangladesh (Rouf et al., 2006; Uddin et al., 2004, 2005, 2006; Datta et al., 2004a,b; Rahman et al., 2004), we now report on the analgesic and anti-inflammatory activities of different extracts of *P. reticulatus*.

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Materials and Methods

Plant material

The aerial parts of *P. reticulatus* were collected at flowering stage from Dhaka during May 2003 and identified (Voucher specimen No. DUH-725:19) by Professor Abul Hasan, Department of Botany, University of Dhaka, Bangladesh.

Extraction

The sun-dried and ground plant materials (2.5 kg) were successively extracted by maceration over a 48 h period with petroleum ether (60–80°), ethyl acetate, and, finally, with methanol at room temperature. The extracts were filtered and concentrated with a rotary evaporator at 40–50°C and reduced pressure and subsequently defatted to obtain the dried petroleum ether (PHRPE), ethyl acetate (PHREA), and methanol (PHRME) extracts. The yields of the extracts were 0.44, 0.68, and 0.98%, respectively.

Preparation of the test materials

The extracts were separately triturated by the addition of a 100 mg of Tween 80. After proper mixing of the extract and Tween 80, distilled water was slowly added to give suspension.

Initial phytochemical screening

The crude extracts were investigated for the possible presence of three broad chemical classes of compounds, namely alkaloids, flavonoids, and glycosides by Dragendorffs, cyanidin, and Brontrager test, respectively (Kokate, 1977).

Experimental animals

Long-Evans rats (150–200 g) and Swiss albino mice (25–30 g) of both sexes were obtained from the Animal Research Branch of the International Centre for Diarrhoal Diseases and Research, Bangladesh (ICDDRB). The animals were kept in polyvinyl cages (BIK industries, India) under controlled room temperature (25 ± 2°C; relative humidity 65–75%) with the condition of natural (12 h) light and dark schedule and supplied with ICDDRB formulated food pellets and water *ad libitum*. To keep the hydration rate constant, the food and water were withdrawn 12 h before and during the experiments. All ethical manners for use of laboratory animals were considered carefully.

Drugs

The following chemicals and drugs were used: aminopyrine (Sigma-Aldrich), acetic acid (Merck, Germany), morphine (Jayson Pharmaceuticals Ltd., Bangladesh), carrageenan (Sigma-Aldrich) and phenylbutazone (Sigma-Aldrich).

Acute toxicity study

Acute toxicity of the extracts was studied by the method described by Ganapathy et al. (2002). The test compounds were administered to different groups of experimental animals with increasing dose starting from 100 mg/kg body weight. No adverse effect or mortality was detected in Swiss albino mice up to 2 g/kg body weight from any of the orally administered extracts of PHR during the 24 h observation period.

Acetic acid-induced writhing test

The peripheral analgesic activity of different extracts of PHR was determined by the acetic acid-induced writhing inhibition method (Whittle, 1964). The prescreened Swiss albino mice employed for this experiment were divided into groups as shown in Table 1. The extracts were administered orally at 150 and 300 mg/kg kg body weight. The inhibition of writhing in mice by the plant extracts was compared with the inhibition of writhing by a standard analgesic, aminopyrine, given orally at a dose of 50 mg/kg. Acetic acid (0.7%) at a dose of 0.1 ml/10 g was administered intraperitoneally (i.p.) to create pain sensation. The number of writhess was calculated for 10 min immediately after the acetic acid injection. The percentage of pain protection was calculated.

The percent protection of writhing by both test and standard drug was calculated according to the following equation.

$$\text{Percent protection} = 100 - \left(\frac{X_t}{X_c}\right) \times 100$$

where, X_t = Average number of writhes in treated group; X_c = Average number of writhes in control group.

Radiant heat tail-flick method

The central analgesic activity was determined by measuring drug-induced changes in the sensitivity of the prescreened (reaction time: 2–4 sec) mice to heat stress applied to their tails (D'Amour & Smith, 1941). The animals were divided into groups as shown in Table 1. A Medicraft Analgesiometer Mask-N was employed for this experiment. Intensity of the current passing through the naked nicrome wire was 5 ampere. The distance between the heat source and the tail skin was 1.5 cm, and cut-off reaction time was fixed at 10 sec to avoid tissue damage. The extracts were administered orally at 150 and 300 mg/kg body weight. Morphine at a dose of 2 mg/kg body weight subcutaneously was used as the standard analgesic for comparing the tail-flick latencies of crude extracts. Tail-flick latency was measured 1 h after the drug administration.

Table 1. Analgesic activity of different extracts of *Phyllanthus reticulatus* on acetic acid-induced writhing response and radiant heat tail-flick model in mice.

	Acetic acid induced writhing response in mice			Radiant heat tail flick	
Group	Dose[a] (mg/kg body weight)	Writhings[b]	% Inhibition	Reaction time (sec)[b]	% of elongation
Control	–	27.0 ± 1.31	–	4.05 ± 0.20	–
PHRPE	150	25.1 ± 1.26	7.09	4.85 ± 0.23	19.75
	300	24.5 ± 1.06	9.25	4.97 ± 0.29	22.63
PHREA	150	$13.2 \pm 0.89^{**}$	51.23	5.58 ± 0.86	37.86
	300	$9.42 \pm 0.55^{**}$	65.12	5.77 ± 0.65	42.38
PHRME	150	$21.2 \pm 2.33^{*}$	21.60	5.57 ± 0.29	37.44
	300	$20.8 \pm 2.16^{*}$	22.83	$6.5 \pm 0.28^{**}$	60.49
Aminopyrine	50	$4.0 \pm 0.52^{**}$	85.18	–	–
Morphine	2[c]	–	–	$7.97 \pm 0.63^{**}$	96.7
One-way ANOVA	F	36.0		5.82	
	Df	7, 40		7, 40	
	P	< 0.001		< 0.05	

[a]1 h after drug treatment, mice were injected i.p. with 0.7% (v/v) acetic acid (0.1 ml/10 g); immediately after the injection.
[b]The number of writhes was counted for 10 min; values are mean ± SEM ($n = 6$); one-way ANOVA; $^{**}P < 0.01$, $^{*}P < 0.05$ compared to control.
[c]Morphine, 2 mg/kg body weight was administered subcutaneously.

Anti-inflammatory study

In this experiment, carrageenan-induced rat hind paw edema was used as the animal model of acute inflammation (Winter et al., 1962). The animals were divided into groups as shown in Table 2. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats, 1 h after oral administration of the test materials. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 1, 2, 3, 4, and 24 h after the carrageenan injection. The extracts were administered orally at 150 and 300 mg/kg body weight. Phenylbutazone suspended in 2% gum acacia at a dose of 80 mg/kg orally was used as the standard anti-inflammatory drug. The percent of inhibition of edema volume was obtained by the following equation:

$$\% \text{ Inhibition} = \frac{[(\text{Predrug reading} - \text{Postdrug reading}) \times 100]}{\text{Predrug reading}}$$

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A *P* value <0.05 was considered significant.

Results and Discussion

Initial qualitative phytochemical screening of the extracts of *P. reticulatus* (PHR) revealed the presence of flavonoids and glycosides. In the acetic acid-induced writhing test, the ethyl acetate extract of PHR (150 and 300 mg/kg) showed a significant ($P < 0.001$) reduction in the number of writhes with 51.23 and 65.12% of inhibition, respectively (Table 1). Maximum inhibition was observed at the dose of 300 mg/kg. In the radiant heat tail-flick model, the tail-flick elongations observed with the ethyl acetate and methanol fractions of PHR at 300 mg/kg dose were 42.38 and 60.49%, respectively (Table 1). The result was found to be statistically significant ($P < 0.01$) only for the methanol extract of PHR in comparison to the control. In the carrageenan-induced rat paw edema test (Table 2) for acute inflammation, the ethyl acetate and methanol extracts of PHR in doses of 150 mg and 300 mg/kg showed 25.53, 31.13 and 28.23, 40.03% inhibition of edema, respectively, at the end of 4 h. The results were statistically significant ($P < 0.0001$).

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. The response is thought to involve local peritoneal cells and is mediated by the prostaglandin pathways (Ronaldo et al., 2000). The ethyl acetate extract showed significant antinociceptive activity, indicating the presence of analgesic principles that might be intervening with the prostaglandin pathways.

In the tail-flick method, both the ethyl acetate and methanol fractions increased the stress tolerance capacity of the animals and hence indicate the possible involvement of a higher center (Whittle, 1964). The analgesic activity of PHR was found to be more significant in the acetic acid induced model ($P < 0.001$) than in the tail-flick model ($P < 0.05$). The carrageenan-induced paw edema in rats is believed to be biphasic (Vinegar et al., 1960). The first phase is due to the release of histamine or serotonin, and the second phase is caused by the release of

Table 2. Anti-inflammatory activity of different extracts of *Phyllanthus reticulatus* on carrageenan-induced rat paw edema.

Group	Dose (mg/kg)[a]	Carrageenan induced rat paw edema[b] Mean ± SEM (% inhibition of paw volume)				
		1 h	2 h	3 h	4 h	24 h
Control	–	72.8 ± 1.46	95.2 ± 2.37	113.0 ± 2.16	121.0 ± 2.71	68.2 ± 2.08
PHRPE	150	65.0 ± 0.7* (10.71)	85.2 ± 2.13* (10.5)	99.0 ± 1.64** (12.69)	105.0 ± 2.25** (13.83)	65.2 ± 1.53 (4.39)
	300	63.6 ± 1.5** (12.63)	82.6 ± 2.2** (13.23)	97.0 ± 1.79** (14.46)	102.0 ± 2.62** (15.65)	63.0 ± 1.64 (7.62)
PHREA	150	59.8 ± 1.83** (17.85)	77.2 ± 2.5** (18.9)	90.4 ± 1.66** (20.28)	90.4 ± 1.66** (25.53)	64.0 ± 1.82 (6.15)
	300	55.6 ± 2.01** (23.62)	70.0 ± 2.10** (24.36)	83.6 ± 1.60** (26.27)	83.6 ± 1.60** (31.13)	63.8 ± 1.74 (6.45)
PHRME	150	56.4 ± 2.44** (22.52)	70.6 ± 2.01** (25.84)	84.0 ± 1.95** (25.92)	86.4 ± 2.25** (28.23)	65.4 ± 2.29 (4.10)
	300	50.2 ± 2.8** (31.04)	62.8 ± 2.73** (34.03)	70.4 ± 2.01** (37.91)	72.8 ± 1.96** (40.03)	63.4 ± 3.66 (7.03)
PBZ	80	46.6 ± 1.57** (35.98)	54.2 ± 1.88** (43.06)	65.6 ± 2.14** (42.15)	78.8 ± 1.83** (35.09)	55.8 ± 2.15** (18.18)
One-way ANOVA	F	19.9	33.2	68.6	55.1	2.58
	df	7, 32	7, 32	7, 32	7, 32	7, 32
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.05

[a] 1 h after treatment of test compounds, p. o., carrageenan was administered in rat hind paw.
[b] Values are Mean ± SEM ($n = 6$); paw volume is expressed in change of height (in mm) of Hg bath (in parentheses, % inhibition of edema). One-way ANOVA; $^{**}P < 0.01$, $^{*}P < 0.05$ compared to control. PBZ = Phenylbutazone.

bradykinin, protease, prostaglandin, and lysosome (Crunkhorn & Meacock, 1971). Therefore, it can be assumed that the inhibitory effect of different extracts of PHR on carrageenan-induced inflammation could be due to the inhibition of the enzyme cyclooxygenase, leading to the inhibition of prostaglandin synthesis.

The present study on different extracts of *P. reticulatus* has demonstrated that this plant has significant analgesic and anti-inflammatory properties, and it justifies the traditional use of this plant in the treatment of various types of pains and inflammation.

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