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Further Studies on the Antinociceptive Action of the Hydroalcoholic Extracts from Plants of the Genus *Phyllanthus*

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Abstract

The analgesic effects of the hydroalcoholic extracts (HEs) of *Phyllanthus urinaria*, *P. tenellus*, *P. niruri* and *P. sellowianus* have been investigated in several models of nociception in mice.

The HE of four species of *Phyllanthus* (1–90 mg kg^{-1}, i.p.) caused a dose-related inhibition of acetic acid-induced abdominal constriction in mice with ID50 values of 5·4, 8·5, 18·2 and 53·0 mg kg^{-1} and maximal inhibition (%) of 80 ± 2, 67 ± 8, 63 ± 8 and 50 ± 4 for *P. urinaria*, *P. niruri*, *P. tenellus* and *P. sellowianus*, respectively. In the formalin test, the HE of all *Phyllanthus* species (0·3–60 mg kg^{-1}, i.p.) caused graded inhibition of both phases of formalin-induced pain, but they were, however, more potent in relation to the second phase of the pain. The ID50 values (mg kg^{-1}) for the first phase were 20·0, 23·0, > 60, and > 60 for the *P. urinaria*, *P. tenellus*, *P. niruri* and *P. sellowianus*, respectively, and percentages of maximal inhibition were 63 ± 2, 70 ± 2, 41 ± 3 and 46 ± 4, respectively. The ID50 values (mg kg^{-1}) for the second phase were 0·71, 4·87, 7·7, 33·0, with maximal inhibition (%) of 91 ± 6, 97 ± 3, 97 ± 3 and 92 ± 6, respectively. Given orally, the HEs of species of *Phyllanthus* caused a significant antinociceptive profile, but they were about one-tenth to one-twentieth as potent when given intraperitoneally. However, the HEs of *Phyllanthus* failed to affect formalin-induced paw oedema and did not interfere with the performance of animals in the rota-rod test. Naloxone (5 mg kg^{-1}) completely reversed the analgesic effect caused by morphine (5 mg kg^{-1}), but had no effect against the analgesic effect of the HE of *Phyllanthus*. Furthermore, the HEs of *Phyllanthus* in contrast to morphine had no analgesic effect in either tail-flick or hot-plate tests.

Taken together, these findings confirm and extend our previous results and indicate that all studied HE of species of plant belonging to the genus *Phyllanthus* exhibit potent and long-lasting antinociceptive activity in several models of pain, including the neurogenic algesic component of the formalin test. The mechanism underlying their analgesic profile is presently unknown.

It has been reported that several species of plants belonging to the genus *Phyllanthus*, distributed in tropical and subtropical countries, are used in folk medicine for treatment of several diseases, including disturbances of kidney and bladder calculi, intestinal infections, diabetes and hepatitis B virus (Morton 1981; Oliver-Bever 1983; Unander et al 1990, 1991, 1992). Recent biochemical, pharmacological and clinical studies have confirmed and also extended the medicinal uses of species of genus *Phyllanthus* in traditional medicine (Calixto et al 1984; Venkateswaran et al 1987; Thyagarajan et al 1988, 1990; Blumberg et al 1989; Ogata et al 1992; Shead et al 1992). Studies from our group have shown that the hydroalcoholic extract (HE) of *Phyllanthus corcovadensis* (Gorski et al 1993), as well as the methanolic extract of callus culture of some species of *Phyllanthus* (Santos et al 1994), exhibit potent and dose-related systemic antinociceptive effects when tested in several models of nociception in mice.

In the present study, we have attempted to examine further the antinociceptive profile of HEs of four species of plants belonging to the genus *Phyllanthus*, including *P. urinaria*, *P. tenellus*, *P. niruri* and *P. sellowianus* in several models of nociception in mice. We have also investigated some of the mechanisms involved in the analgesic effect of *Phyllanthus* species.

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Materials and Methods

Preparation of the crude extract

Botanical material was collected and classified by Dr Leila da Graça Amaral and Miss Mirian Ulyssea (Department of Botany, Universidade Federal of Santa Catarina). The dried leaves, stems and roots of *Phyllanthus urinaria*, *Phyllanthus niruri*, *Phyllanthus tenellus* and *Phyllanthus sellowianus* were minced and extracted with 50% ethanol-water in the proportion of 1 : 3, being stirred and macerated at room temperature (21 ± 3°C) for 15 days. The ethanol was evaporated and the extract was concentrated to the desired level and stored at −20°C. The extracts were dissolved in 0·9% NaCl solution at the desired concentration just before use.

Abdominal constriction response caused by intraperitoneal injection of dilute acetic acid

Male Swiss mice, 25–30 g, were kept in a temperature controlled environment (23 ± 2°C) with a 12-h light-dark cycle. Food and water were freely available. The abdominal constriction induced by intraperitoneal injection of acetic acid (0·6%), which consisted of a contraction of the abdominal muscle together with a stretching of hind limbs, was

carried out according to the procedures described previously (Santos et al 1994). Animals were pretreated with the HEs intraperitoneally (1–90 mg kg^{-1}) 30 min before, or orally (50–400 mg kg^{-1}) 60 min before the acetic acid injection. Control animals received a similar volume of 0·9% NaCl (10 mL kg^{-1}). All experiments were carried out at 20–22°C. After challenge, pairs of mice were placed in separate boxes and the number of abdominal constrictions was cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with HEs.

Formalin-induced pain

Male Swiss mice, 25–30 g, were used. The procedure was similar to that described previously (Hunskaar et al 1985, 1986; Murray et al 1988; Corrêa & Calixto 1993). Animals from the same strain were lightly anaesthetized with ether, except when used to analyse the first phase of formalin-induced pain, and 20 μL 2·5% formalin (0·92% formaldehyde) made up in phosphate-buffer was injected under the paw surface of the right hindpaw. Two mice (control and treated) were observed simultaneously from 0 to 30 min following formalin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of pain. The initial nociceptive scores normally peaked 5 min after formalin injection (first phase) and 15–30 min after formalin injection (second phase), representing the tonic and inflammatory pain responses, respectively (Hunskaar & Hole 1987). Animals were treated with HEs of *P. urinaria*, *P. niruri*, *P. tenellus* and *P. sellowianus* intraperitoneally (0·3–60 mg kg^{-1}) or orally (12–400 mg kg^{-1}), 30 and 60 min before formalin injection, respectively.

In an attempt to investigate the participation of the opioid system on the analgesic effect of these plants, separate groups of mice were treated with naloxone (5 mg kg^{-1}, i.p.) injected 10 min before administration of HE of *P. urinaria* (10 mg kg^{-1}, i.p.) and *P. niruri* (30 mg kg^{-1}, i.p.) or with morphine (5 mg kg^{-1}, s.c.) which was used as positive control. Control animals received only the vehicle used to dilute the HE (NaCl solution, 10 mL kg^{-1}). Following intraplantar injection of formalin, the animals were immediately placed into a glass cylinder of 20 cm in diameter, and the time spent licking the injected paw (second phase of formalin test) was determined. At the end of all experiments the animals were killed by cervical dislocation and the paws were cut at the knee and weighed.

Tail-flick test

Male Swiss mice, 25–30 g, were used. A radiant heat tail-flick analgesiometer was used to measure response latencies as described by D'Amour & Smith (1941), with minor modifications. Animals responded to a focused heat-stimulus by flicking or removing their inflicted tail, exposing a photocell in the apparatus immediately below the tail. The reaction time was recorded for control mice or for animals pretreated with morphine or with the HE from *Phyllanthus* species. An automatic 8-s cut-off was used to prevent tissue damage. The animals were selected 24 h previously on the basis of their reactivity in the model. A latency period of 20 s was defined as complete analysis. The animals received the HE of species of *Phyllanthus* administered orally (up to 500 mg kg^{-1}) or morphine (10 mg kg^{-1}, s.c.) 60 and 30 min before experiments. Control animals received the same volume of vehicle (10 mL kg^{-1}).

Hot-plate test

Male Swiss mice, 25–30 g, were used. The hot-plate was used to measure response latencies according to the method described by Eddy & Leimback (1953), with minor modifications. In these experiments the hot-plate (Ugo Basile, Model-DS 37) was maintained at 56 ± 1°C. Animals were placed into a glass cylinder of 24 cm diameter on the heated surface, and the time (s) between placement and shaking or licking of the paws or jumping was recorded as response latency. The reaction time was recorded for control mice or for animals pretreated with morphine (positive control) or pretreated with the HEs from *Phyllanthus* species. An automatic 5-s cut-off was used to prevent tissue damage. Animals were selected 24 h previously on the basis of their reactivity in the test. A latency period of 20 s was defined as complete analysis. Animals were treated with the HEs (up to 500 mg kg^{-1}, p.o.) or with morphine (10 mg kg^{-1}, s.c.) 60 and 30 min before experiments. Control animals received the same volume of vehicle (10 mL kg^{-1}).

Rota-rod test

Male Swiss mice, 25–30 g, were used. The apparatus consisted of a bar, with a diameter of 2·5 cm, subdivided into six compartments by disks 25 cm in diameter (Duham & Miya 1957). The bar rotated at a constant speed of 14 rev min^{-1}. The animals were selected 24 h previously by eliminating those mice which did not remain on the bar for two consecutive periods of 60 s. Animals were treated orally with NaCl (10 mL kg^{-1}) or with the HEs of several species of *Phyllanthus* (up to 500 mg kg^{-1}) 60 min before, and were retested. The time they remained on the rotating bar (maximum of 60 s) was recorded.

Drugs

The drugs used were: formalin, acetic acid, and morphine hydrochloride (Merck, Darmstadt) and naloxone hydrochloride (Dupont, Garden City, USA). All other reagents used were of a high grade of purity. All drugs and extracts were dissolved in 0·9% NaCl solution or in physiological buffered solution just before use.

Statistical analysis

The results are presented as mean ± s.e.m., and statistical significance between groups was analysed by means of analysis of variance followed by Dunnett's multiple comparison test. P values less than 0·05 were considered as indicative of significance. When appropriate, the ID50 values (the dose of extracts that reduced responses by 50% relative to control value) were estimated by graphical interpolation from individual experiments.

Results

Intraperitoneal injection of animals with the hydroalcoholic extracts of *P. urinaria*, *P. niruri*, *P. tenellus* and *P. sel-*

Table 1. Effect of the hydroalcoholic extracts of several species of *Phyllanthus*, given either intraperitoneally or orally, against acetic acid-induced abdominal constriction in mice.

Hydroalcoholic extracts	Intraperitoneal (mg kg^{-1})	Number of abdominal constrictions	Oral (mg kg^{-1})	Number of abdominal constrictions
P. urinaria	0	37·3 ± 1·7	0	36·0 ± 1·0
	3	31·6 ± 3·2	50	28·6 ± 3·6*
	10	7·6 ± 0·6**	100	23·1 ± 1·1**
	30	9·8 ± 2·9**	200	17·0 ± 3·5**
	—	—	400	18·5 ± 3·2**
P. niruri	0	37·2 ± 1·1	0	34·8 ± 0·8
	3	28·6 ± 1·3**	50	32·6 ± 2·1
	10	16·8 ± 2·4**	100	25·8 ± 1·8**
	30	13·8 ± 3·0**	200	25·7 ± 1·4**
	60	14·0 ± 2·5**	—	—
P. tenellus	0	37·5 ± 1·1	0	36·0 ± 1·0
	10	23·1 ± 2·0**	50	30·5 ± 2·0**
	30	13·0 ± 3·2**	100	25·0 ± 1·1**
	60	12·5 ± 3·2**	200	20·0 ± 2·4**
P. sellowianus	0	39·8 ± 1·9	0	38·6 ± 1·6
	10	27·0 ± 0·8**	50	37·3 ± 2·7
	30	23.8 ± 0·9**	100	35·0 ± 1·3
	60	20·3 ± 1·3**	200	27·5 ± 2·1**
	—	—	400	27·3 ± 1·8**

Each group represents the mean ± s.e.m. of six experiments. *$P < 0.05$, **$P < 0.01$ compared with control values.

lowianus (1–90 mg kg^{-1}, i.p.) caused a dose-related inhibition of acetic acid-induced abdominal constriction response in mice (Table 1). The calculated ID50 values (and their 95% confidence limits) (mg kg^{-1}) were 5·4 (4·3–6·8), 8·5 (5·5–13·2), 18·2 (11·0–30·0) and 53·0 (45·0–61·0) and the maximal inhibitions (%) were 80 ± 2, 67 ± 8, 63 ± 8 and 50 ± 4 for the *P. urinaria*, *P. niruri*, *P. tenellus* and *P. sellowianus*, respectively. Given orally (50–400 mg kg^{-1}), the HEs also caused a significant inhibition against acetic acid-induced abdominal constrictions; however, they were less potent than when given intraperitoneally (Table 1). The antinociceptive profile of HEs administered intraperitoneally were long-lasting (2–3 h) (Table 2).

In the formalin test, the extracts of all species of *Phyllanthus* (0·3–90 mg kg^{-1}, i.p.) caused marked and dose-related inhibition against both phases of formalin-induced pain (Tables 3, 4, 5, 6). Moreover, their analgesic effects were more pronounced against the second phase of the pain model. The ID50 values (mg kg^{-1}) for the first phase were 20·0 (16·1–24·3), 23·0 (16·0–34·0), > 60, and > 60 for the *P. urinaria*, *P. tenellus*, *P. niruri* and *P. sellowianus* and the maximal inhibitions (%) were: 63 ± 2, 70 ± 2, 41 ± 3 and 46 ± 4, respectively (Tables 3, 4, 5, 6). The ID50 values (mg kg^{-1}) for the second phase were 0·71 (0·48–0·92), 4·87 (4·1–5·76), 7·7 (5·5–10·6) and 33·0 (26·0–42·0) and the maximal inhibitions were: 91 ± 6, 97 ± 3, 97 ± 3 and 92 ± 6, respectively, for the *P. urinaria*, *P. tenellus*, *P. niruri* and *P. sellowianus* (Tables 3, 4, 5, 6). Given orally (12–400 mg kg^{-1}), the HEs caused dose-related

Table 2. Time-course of antinociceptive effect of hydroalcoholic extracts of several species of *Phyllanthus*, given intraperitoneally, against acetic acid-induced abdominal constriction in mice.

Time (h)	Number of abdominal constrictions			
	Hydroalcoholic extract			
	P. urinaria (30 mg kg^{-1})	*P. tenellus* (30 mg kg^{-1})	*P. niruri* (30 mg kg^{-1})	*P. sellowianus* (30 mg kg^{-1})
0	34·2 ± 2·2	34·2 ± 2·2	34·2 ± 2·2	38·4 ± 1·4
0·5	7·7 ± 0·7**	12·6 ± 1·6**	13·6 ± 1·0**	20·0 ± 1·7**
1·0	14·3 ± 2·9**	18·6 ± 1·2**	15·3 ± 1·4**	20·3 ± 1·4**
2·0	21·6 ± 0·7**	26·0 ± 1·4*	15·3 ± 2·4**	23·6 ± 1·5**
3·0	29·3 ± 1·2	31·3 ± 2·9	23·0 ± 1·6**	32·0 ± 2·0
3·5	35·6 ± 0·7	34·3 ± 1·6	29·2 ± 1·2	31·3 ± 1·9
4·0	—	—	31·0 ± 0·8	37·0 ± 0·7

Each group represents mean ± s.e.m. of three to five experiments. *$P < 0.05$, **$P < 0.01$ compared with control values.

Table 3. Effect of hydroalcoholic extract of *P. urinaria* given either intraperitoneally or orally against the first phase (0–5 min) or against the second phase (15–30 min) and paw oedema in the mouse formalin test.

HE (mg kg^{-1})	Licking (s)		Paw weight (mg)
	0–5 min	15–30 min	
Intraperitoneal			
0	67·8 ± 1·7	176·6 ± 7·0	65·5 ± 8·8
0·3	—	119·0 ± 7·0**	62· ± 3·2
1	—	70·6 ± 8·4**	59·0 ± 4·0
3	—	42·0 ± 4·8**	70·2 ± 4·1
10	45·1 ± 2·1**	15·6 ± 10·5**	58·4 ± 6·8
30	26·8 ± 1·3**	—	—
60	25·8 ± 1·2**	—	—
Oral			
0	60·8 ± 2·9	140·6 ± 7·0	85·3 ± 3·3
12	—	111·5 ± 8·0**	84·8 ± 7·6
25	—	48·1 ± 6·5**	82·3 ± 6·8
50	43·6 ± 4·4**	42·5 ± 0·9**	78·0 ± 5·9
100	39·0 ± 3·1**	47·2 ± 4·7**	81·4 ± 2·1
200	34·3 ± 3·6**	—	—
400	37·0 ± 3·1**	—	—

Each group represents the mean ± s.e.m. of six experiments. **$P < 0.01$ compared with control values.

Table 4. Effect of hydroalcoholic extract of *P. niruri* given either intraperitoneally or orally against the first phase (0–5 min) or against the second phase (15–30 min) and paw oedema in the mouse formalin test.

HE (mg kg^{-1})	Licking (s)		Paw weight (mg)
	0–5 min	15–30 min	
Intraperitoneal			
0	71·8 ± 2·8	176·6 ± 4·2	56·9 ± 4·9
1	—	170·0 ± 2·4**	52·6 ± 5·7
3	—	119·0 ± 3·1**	56·0 ± 4·5
10	60·0 ± 2·0**	78·8 ± 5·3**	58·0 ± 7·8
30	48·0 ± 1·5**	5·16 ± 3·2**	50·8 ± 7·0
60	42·5 ± 1·3**	—	—
90	44.0 ± 1·1**	—	—
Oral			
0	56·5 ± 2·1	147·1 ± 5·3	59·9 ± 4·5
25	—	131·6 ± 4·4	52·0 ± 5·7
50	49·0 ± 0·9*	78·0 ± 9·0**	51·7 ± 9·0
100	41·8 ± 2·3**	31·6 ± 9·5**	62·6 ± 7·7
200	36·3 ± 2·3**	34·5 ± 7·1**	56·6 ± 4·1
400	37·2 ± 2·2**	—	—

Each group represents the mean ± s.e.m. of six experiments. *$P < 0.05$, **$P < 0.01$ compared with control values.

inhibition against both phases of formalin-induced nociception, but they were about one-tenth to one-twentieth as potent than when given intraperitoneally; however, all the tested HEs of species from *Phyllanthus* failed to affect the oedematogenic response associated with the second phase of the formalin test in mice (Tables 3, 4, 5, 6).

Table 7 shows that HEs of the four species of *Phyllanthus* (up to 500 mg kg^{-1}, p.o.) were virtually ineffective against the tail-flick and hot-plate tests, in conditions where morphine (10 mg kg^{-1}, s.c.) caused a marked increase in the pain latency in both tests. The analgesic effects of morphine (5 mg kg^{-1}), but not those of the HEs of species of *Phyllanthus*, were fully reversed by previous treatment of animals

Table 5. Effect of hydroalcoholic extract of *P. tenellus* given either intraperitoneally or orally against the first phase (0–5 min) or against the second phase (15–30 min) and paw oedema in the mouse formalin test.

HE (mg kg^{-1})	Licking (s)		Paw weight (mg)
	0–5 min	15–30 min	
Intraperitoneal			
0	67·8 ± 1·6	172·8 ± 5·5	66·1 ± 8·3
1	—	160·6 ± 4·9	67·2 ± 4·4
3	—	124·5 ± 6·4**	71·2 ± 4·7
5	—	85.0 ± 6·7**	65·4 ± 6·6
10	47·5 ± 2·6**	19·0 ± 7·4**	50·0 ± 5·3
30	29·1 ± 2·4**	5·3 ± 4·3**	54·4 ± 2·5
60	20·8 ± 1·5**	—	—
90	22.0 ± 1·3**	—	—
Oral			
0	60·8 ± 2·8	140·0 ± 7·9	66·0 ± 6·8
25	—	104·3 ± 5·5	57·0 ± 4·4
50	42·8 ± 3·2**	56·8 ± 5·3**	58·4 ± 7·4
100	39·5 ± 3·1**	42·5 ± 6·5**	52·9 ± 9·3
200	36·8 ± 1·2**	48·3 ± 2·7**	51·9 ± 5·1
400	38·5 ± 1·0**	—	—

Each group represents the mean ± s.e.m. of six experiments. *$P < 0.05$, **$P < 0.01$ compared with control values.

Table 6. Effect of hydroalcoholic extract of *P. sellowianus* given either intraperitoneally or orally against the first phase (0 to 5 min) or against the second phase (15 to 30 min) and paw oedema in the mouse formalin test.

HE (mg kg^{-1})	Licking (s)		Paw weight (mg)
	0–5 min	15–30 min	
Intraperitoneal			
0	71·8 ± 2·8	172·0 ± 4·6	71·6 ± 3·6
10	69·5 ± 3·9	174·1 ± 4·6	65·5 ± 6·7
30	59·8 ± 1·5**	98·0 ± 10·1**	61·0 ± 2·0
60	40·6 ± 2·2**	13·1 ± 9·0**	57·6 ± 6·9
90	45.0 ± 2·1**	—	—
Oral			
0	60·8 ± 2·8	140·0 ± 7·9	54·5 ± 6·1
25	—	126·0 ± 9·0**	55·8 ± 6·2
50	53·1 ± 3·7	61·5 ± 7·1**	46·5 ± 6·1
100	42·5 ± 3.0**	12·0 ± 3·5**	52·5 ± 6·0
200	38·1 ± 2·4**	26·0 ± 2·9**	49·7 ± 6·6
400	37·0 ± 1·7**	—	—

Each group represents the mean ± s.e.m. of six experiments. **$P < 0.01$ compared with control values.

with naloxone (5 mg kg^{-1}, i.p.) when analysed against the second phase of formalin-induced pain (Fig. 1). Also, the HEs of species of *Phyllanthus* (up to 500 mg kg^{-1}, p.o.) did not cause any significant effect in the motor co-ordination of animals when tested in the rota-rod model (Table 8).

Discussion

Previous studies of our group have demonstrated that HE obtained from the leaves, stems and roots of *Phyllanthus corcovadensis* (Euphorbiaceae), given either intraperitoneally or orally, exhibit potent and dose-related antinociceptive activity when analysed in several models of nociception in mice (Gorski et al 1993). We have also demonstrated similar potent analgesic effect for the methanolic extracts of callus culture in-vitro obtained from *P. tenellus*, *P. corcovadensis* and *P. niruri* (Santos et al 1994). In the present study we have confirmed and also extended these initial observations by demonstrating that the HEs of the leaves, stems and roots of four additional species of *Phyllanthus*, *P. urinaria*, *P. tenellus*, *P. niruri* and *P. sellowianus*, all had potent and dose-related analgesic effects either when given intraperitoneally or orally to mice. In addition, their analgesic effects were long-lasting and

Table 7. Effect of treatment with morphine (s.c.) and hydroalcoholic extract (p.o.) of several species of *Phyllanthus* in the hot-plate and tail-flick tests in mice.

Treatment	Dose (mg kg^{-1})	Latency (s)	
		Hot-plate	Tail-flick
Vehicle (NaCl)	0	4·5 ± 0·5	5·1 ± 0·3
P. tenellus	500	4·3 ± 0·3	5·5 ± 0·6
P. niruri	500	4·1 ± 0·6	5·3 ± 0·5
P. sellowianus	500	4·1 ± 0·6	5·0 ± 0·3
P. urinaria	500	4·0 ± 0·6	5·3 ± 0·5
Morphine	10	19·4 ± 0·6**	23·9 ± 1·5**

Each group represents the mean ± s.e.m. of six experiments. **$P < 0.01$ compared with control values.

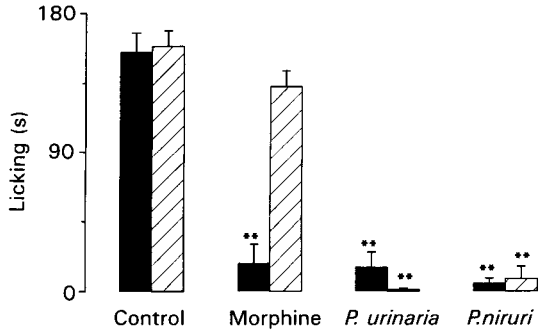

FIG. 1. Effect of naloxone on the antinociceptive profile caused by morphine (5 mg kg^{-1}, s.c.) or hydroalcoholic extract of *P. urinaria* (10 mg kg^{-1}, i.p.) and *P. niruri* (30 mg kg^{-1}, i.p.), analysed in the second phase of formalin-induced pain in mice. Each group represents the mean of 6 to 8 experiments and the vertical bars indicate the s.e.m. $^{**}P < 0.01$ compared with corresponding control value. ■ Vehicle; ▨ naloxone (5 mg kg^{-1}, i.p.).

occurred rapidly after their systemic administration. All studied HEs of species of *Phyllanthus*, including *P. corcovadensis* (Gorski et al 1993), are capable of attenuating, in a dose-related fashion, both the neurogenic and the inflammatory phases of the formalin-induced pain. Moreover, these extracts, even in higher doses, failed to interfere with the paw oedema formation associated with the second phase of the formalin test, and none of the HEs of *Phyllanthus* species was able to abolish completely the neurogenic pain response of the formalin test. This suggests that part of this nociceptive response in the formalin test involves mediators that are insensitive to active principles of *Phyllanthus*. It has been shown that the inflammatory pain associated with the second phase of the formalin test is accompanied by release of several inflammatory mediators (Hunskaar et al 1985; Abbott & Franklin 1986; Murray et al 1988; Chapman & Dickenson 1992; Corrêa & Calixto 1993). In addition, the second but not the first phase (neurogenic pain) of the formalin test can be attenuated in a concentration-dependent fashion by drugs known to inhibit cyclo-oxygenase metabolites derived from the arachidonic acid pathway. In contrast, morphine potently inhibits both phases of formalin-induced pain (Hunskaar et al 1985; Murray et al 1988; Shibata et al 1989; Corrêa & Calixto 1993; Gorski et al 1993).

As reported previously for the HE of *P. corcovadensis* (Gorski et al 1993) and for the methanolic extracts of callus culture of several species of *Phyllanthus* (Santos et al 1994), the mechanisms underlying the antinociceptive action of the HEs of species of *Phyllanthus* seems to be unrelated to the release or activation of opioid receptors. These observations arise from the view that the antinociceptive action of HEs of *Phyllanthus* species, in contrast to that reported for morphine, was not reversed by naloxone, an opioid antagonist. Secondly, the HEs of studied species of *Phyllanthus* were completely devoid of analgesic activity when analysed against the radiant heat in the tail-flick test as well as in the hot-plate test, in conditions where morphine caused marked analgesic effect. In all models of nociception, the HE of *P. urinaria* was the most potent, followed by *P. corcovadensis* > *P. tenellus* > *P. niruri* > *P. sellowianus*. The antinociceptive potency of the HE of *P. urinaria* against the second phase of the formalin test was about 3- and 4-fold that of morphine and indomethacin, respectively (Gorski et al 1993), and about 29- and 50-fold that of paracetamol and aspirin, respectively (results not shown). These observations might lead one to conclude that the active antinociceptive principles present in the plant of genus *Phyllanthus* are more concentrated in *P. urinaria*. Alternatively, the *P. urinaria* may contain other active principles not present in other species. Additional chemical and pharmacological studies are necessary to confirm these views.

Table 8. Effect of hydroalcoholic extract of species of *Phyllanthus* on the rota-rod test in mice.

Treatment	Dose (mg kg^{-1}, p.o.)	Performance on the rota-rod (s)
Vehicle (NaCl)	10	59·0 ± 2·2
P. tenellus	500	59·5 ± 1·1
P. niruri	500	59·5 ± 0·9
P. sellowianus	500	58·3 ± 2·2
P. urinaria	500	59·5 ± 1·1

Each group represents the mean ± s.e.m. of six experiments.

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