ANTINOCICEPTIVE EFFECTS OF THE METHANOL EXTRACT OF *UAPACA GUINEENSIS* (EUPHORBIACEAE) STILT ROOT BARK

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Summary

The traditional therapeutic indications for the use of *Uapaca guineensis* (euphorbiaceae) have been investigated. The methanol extract of *Uapaca guineensis* was evaluated for analgesic effects in animal models. The extract showed significant protective effects against formalin- and acetic acid-induced pain in the mouse. It also produced a significant analgesic activity in pressure-induced pain test and the hot plate test in the rats. The results of the present study suggest that the antinociceptive effect of the methanol extract of *Uapaca guineensis* may be dependent on central analgesic mechanisms.

Keywords: *Uapaca guineensis*, pain, analgesia, writhing, antinociceptive activity

Introduction

Uapaca guineensis (Euphorbiaceae) is a plant used in Cameroonian folk medicine for treating fever, inflammation, pain, skin diseases and sexual dysfunction[1]. Locally known as '*Assam*' in the Beti language[2], this plant is confined with others of the same genus to the humid forests in the central region of Cameroon[3] Preliminary phytochemical studies revealed the presence of terpenoids, saponins, alkaloids and sterols. Based on the literature findings, no investigation is available on the analgesic properties of *Uapaca guineensis*. The objective of the present study was to investigate the analgesic properties of *Uapaca guineensis* using the writhing, formalin, hot plate and pressure tests in order to provide scientific validation of the claimed analgesic properties of this plant.

Materials

Plant material

The whole stilt roots of *Uapaca guineensis* were collected in Yaoundé - Cameroon, in September, 2006. Identification and authentification of the plant material was done at the National Herbarium, Yaoundé, where a voucher specimen is registered (No. 41501/HNC).

Preparation of the methanol extract of the stilt root bark of *Uapaca guineensis* (MEUG)

MUEG was prepared by soaking the air-dried powdered stilt root bark of *Uapaca guineensis* in methanol in the ratio 1:20 (w/v) for 72 hours. The solution was collected and filtered using Whatman paper No 1 filter paper. The filtrate was kept while the residue was discarded. The methanol from the filtered supernatant was evaporated and in the process the liquid extract obtained separated into two phases: a readily soluble phase and a relatively insoluble phase. The soluble phase was recovered and air dried to a brown powder. The dried extract was dissolved to prepare stock solutions of doses 62mg/kg and 124mg/kg in a 5% DMSO[4] solution for use in all experiments.

Animals

Swiss albino mice of either sex weighing 20–25 g and Wistar rats of 180-200 g were obtained from our animal house. They were housed under standard environmental conditions of temperature (27±1°C) and 12:12h light/dark cycle. All animals had free access to water and standard rat chow.

Drugs

Indomethacin (Indocid®, Laboratoire Merck Sharp et Dohme, France), Morphine (Sigma-Aldrich-Quiinina S.A. Madrid-Spain), Tramadol (Laboratoire Pharmascience, France, Couveboie), and plant extract were dissolved in distilled water, Carrageenan (Sigma Chemical Co, St Louis, USA) and Naloxone (Narcan®, laboratoire Dupont-nemours, France) in physiological saline.

Methods

Acetic acid-induced writhing test

This test was performed as described by Fontenele et al. (1996)[5]. Mice were randomly separated into groups of five and each group was pre-treated with one of the following: saline, indomethacin (10mg/kg), tramadol (25 mg/kg), morphine (10 mg/kg) or MEUG (62 mg/kg and 124 mg/kg) *po* respectively. Pain was induced by the intraperitoneal injection of 0,1 ml/10g acetic acid (1% v/v) one hour after test drugs which is within the efficient therapeutic duration range of morphine. Animals reacted with a characteristic stretching behaviour called writhing i.e. contractions of abdomen, turning of trunk and extension of hind limbs. The number of writhing movements was recorded for 30 minutes. The results were evaluated by calculating the mean number of contortions per treated group compared with that of the control group. Pain inhibition rates were calculated as follows:

$$Pain\ \ inhibition\ \ (\%) = \frac{\overline{Nc} - \overline{Nt}}{\overline{Nc}} \times 100$$

$\overline{Nc}$**:** mean number of writhing of the control group

$\overline{Nt}$**:** mean number of writhing per treated group

In another set of trials, naloxone (1 mg/kg) a specific antagonist of morphinometic receptors was administered *sc* 15 min test drugs in order to determine if the observed pharmacological effect was of central origin.

Formalin test

This test causes local tissue injury to the paw in which it is injected and has been used as a model for the study of tonic pain and localized inflammatory pain[6,7]. This test was carried out as described by[8] with a few modifications. Animals were injected subcutneously with 20µl of formalin into the dorsal hind paw. The drugs cited above were administered *po* 30 min before formalin injection. Each group was composed of five mice though each mouse was placed individually in a plexiglass cage for observation after treatment. The time the mice spent licking or biting the injected paw or leg was recorded on the basis of the response pattern described by[9]. Two distinct periods of intensive licking activity were identified[10] and scored separately. The first period (early phase) was recorded 1-5 min after the injection of formalin and the second period (late phase) was recorded 15-30 min[11] after the injection. In a second series of experiments, naloxone, an opioid antagonist, was injected just before formalin injection[12] and the effects of the same drugs as previously tested. The percentage inhibition of licking was calculated using the formula:

$$Pain\ inhibition\ \ (\%) = \frac{Tc - \overline{Tt}}{\overline{Tc}} \times 100$$

$\overline{Tc}$: Mean time of licking of the control group

$\overline{Tt}$: Mean time of licking per treated group

Hot plate test

This test was carried out as describe by Wagh et al. 2006[13]. In these experiments, the drugs cited above were tested in mice using Eddy's hot plate (Ugo Basile 7250). The temperature was maintained at 55 ± 0.2 ºC – a temperature which is hot enough to cause discomfort but cause no tissue damage. Animals licked their forelegs and/or jumped as an indication of pain. Experimental conditions and test drugs were the same as those used in the writhing test. Each animal served as its own control: the reaction time was determined before treatment and twice with an interval of 10 min after treatment. The various responses (jumping and licking) were recorded at 1h, 2h and 4h after treatment of animals with test drugs. In another set of experiments, animals were pre-treatment with naloxone. Pain inhibition rates were calculated as follows:

$$Pain\ inhibition\ (\%) = \frac{\overline{Tt} - \overline{To}}{\overline{To}} \times 100$$

$\overline{To}$: The reaction time of animals before administration of drugs

$\overline{Tt}$: The reaction time of animals after the administration of drugs

Pressure-induced pain

In these experiments, the drugs cited above were tested at the same doses in rats using an Ugo Basile Analgesy Meter (N° 7200). Force was applied to the left hind paw of experimental animals by an Analgesy Meter plunger which exerted a constantly increasing force on the rat paw. The rat was suspended vertically while its left hind paw was placed between the plinth and the plunger. As the applied force increased, a point was reached where the animal struggled to free its paw. This indicated the level at which the animal felt pain (Ito et al, 2001). The weight causing pain before treatment and then 1h, 2h and 4h after treatment of animals with the various test drugs was determined. Pain protection rates were calculated as follows.

$$\text{Protection.}(\%) = \frac{\overline{Ft} - \overline{Fo}}{\overline{Fo}} \times 100$$

$\overline{Fo}$: Force where the animal struggles to free its paw before administration of drugs.

$\overline{Ft}$: Force where the animal struggles to free its paw after administration of drugs.

Statistical analysis

Data obtained for each set of anti-nociceptive models was expressed as mean ± S.D and analyzed by one-way ANOVA followed by Dunnett's multiple comparison tests. Level of significance was set at $P<0.05$. All statistical manipulations were carried out using Graph Padinstat® Prism 3.0 (USA) statistical software.

RESULTS

Analgesic activity on acetic acid-induced writhing

The results presented in figure 1 show the effects of the MEUG on abdominal constrictions in mice. The extract exhibited significant ($p<0.05$) antinociceptive activity which was not greatly increased at higher dose (62 and 164 mg/kg). Morphine (10 mg/kg), tramadol (25 mg/kg) and indomethacin (10 mg/kg) significantly decreased the number of contortions and stretching of mice induced by acetic acid.

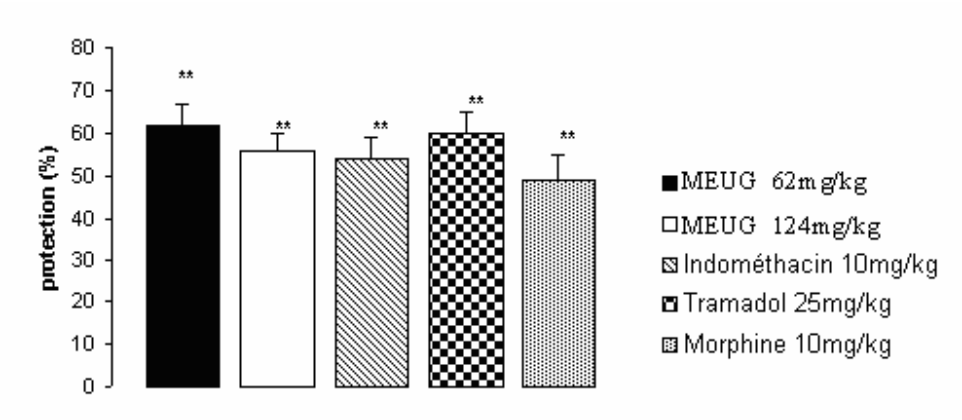

Figure 1: Antinociceptive effect of oral treatment with MEUG on acetic acid induced writhing in mice. Results are shown as the mean ± SEM. n=5. (** P<0,01) compared to control group

However, the analgesic effect of MEUG (62 and 124 mg/kg), Morphine (10 mg/kg) and tramadol (25 mg/kg) were completely antagonized by naloxone though the effects of indomethacin were not modified (fig. 2).

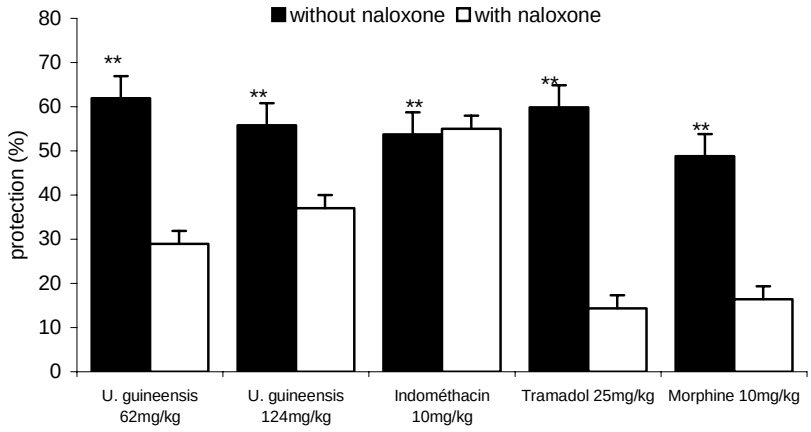

Figure 2: Antinociceptive effect of oral treatment with MEUG in mice pre-treated with naloxone of naloxone on acetic acid-induced writhing in mice. Results are shown as the mean ± SEM. n=5. (** P<0,01) resuls are compared with control group

Analgesic activities on the formalin-induced pain

Formalin-induced pain response was in two phases, the early and late phases. All tested compounds showed a significant inhibition of formalin-induced pain – both the early and late phases (Table 1).

The plant extract showed analgesic effects which were comparable to results obtained with the reference compounds. Pre-treatment with naloxone, significantly blocked the early phase response (Fig 3) but not the late phase response (fig. 4) in all treated groups. Like Morphine the plant extract showed significantly reduced analgesic activity when animals were pre-treated with naloxone in both the early late phases. Naloxone had little effect on the analgesic effects of indomethacin in the late phase.

Table 1: Effect of oral treatment with MEUG on the formalin induced nociception in mice

group	Doses (mg/kg)	Licking time (s)		Inhibition (%)	
		Early phase (0-5min)	Late phase (20-30 min)	Early phase	Late phase
Control		95,6±2	36,1±4	-	-
MEUG					
	62	51,8±5**	25,2±3*	45,8	30,1
MEUG	124	54,4±5**	31,4±3	43,0	13,0
Indomethacin	10	46,2±2**	25,3±2*	51,7	29,9
Tramadol	25	48,5±2**	27,9±3*	49,3	22,7
Morphine	10	55,8±2**	23,2±2*	41,6	35,7

Results are shown as the mean ± SEM (n=5). Different from control (*P<0,05; ** P<0,01)

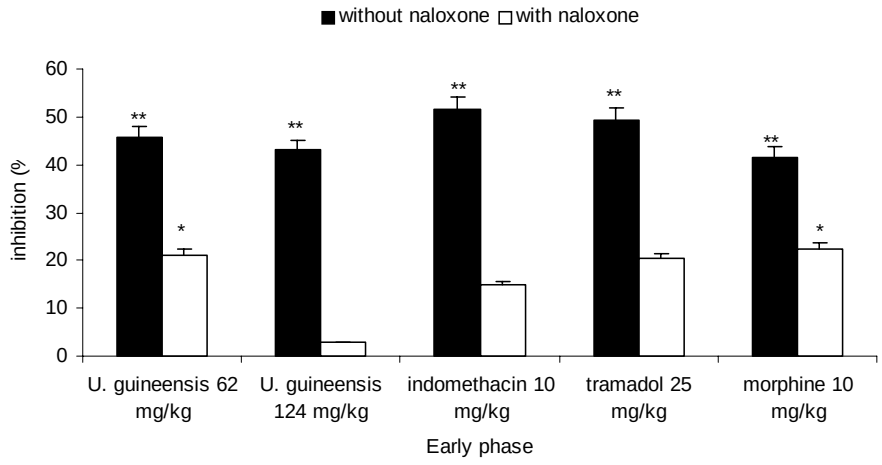

Fig 3: Early phase of formalin-induced nociception in mice with and without naloxone pre-treatment. Results are shown as the mean ± SEM. n=5. (** P<0,01; *P<0,05) results are compared with the controls group.

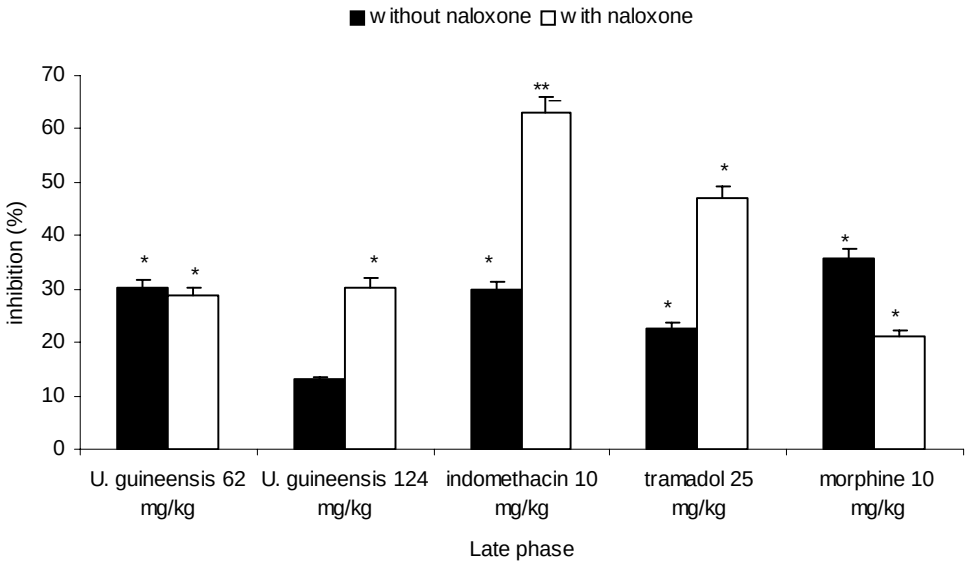

Fig 4: Late phase of formalin-induced nociception in mice with and without naloxone pre-treatment. Results are shown as the mean ± SEM. n=5. (** P<0,01; *P<0,05) results are compared with the control group

Analgesic activity by hot plate method

The result presented in table 2 show that MEUG extract at doses of 62 and 124 mg/kg significantly increased the reaction time of mice though to a smaller extent compared to results obtained with morphine (10 mg/kg) and tramadol (25 mg/kg). Indomethacin (10 mg/kg) failed to increase reaction time to pain in tested animals. However, with naloxone pre-treatment, the analgesic effect of MEUG (62 and 124) mg/kg), Morphine (10 mg/kg) and tramadol (25 mg/kg) against thermal pain were significantly antagonised (table 3).

Table 2 : Antinociceptive effect of oral treatment with MEUG by the hot plate test in mice

Group	Doses (mg/kg)	Reaction time ± SEM (Inhibition %)			
		0h	1h	2h	4h
Control		7,2±1,0	8,7±0,7 (21,5)	7,3±1,0 (2,2)	7,8±0,4 (9,5)
MEUG	62	9,8±1,6	15,9±1,3* (62,4)	13,5±2,1* (38,2)	11,5±1,5 (18,0)
MEUG	124	7,5±1,0	9,7±2,5* (28,7)	9,3±2,2* (23,3)	9,3±1,9* (23,3)
Indomethacin	10	7,5±1,2	8,3±1,6 (11,0)	8,4±0,8 (12,6)	7,9±1,0 (6,15)
Tramadol	25	6,2±0,9	10,4±1* (68,3)	10,8±0,3** (75,1)	11,6±1** (87,4)
Morphine	10	7,4±0,8	13,1±1,7** (77,0)	13,6±1,6** (84,8)	13,8±2,1** (87,3)

Results are shown as the mean ± SEM for five animals each group.

Different from control (*P<0,05; ** P<0,01).

Table 3: Analgesic effects by the hot plate method in mice pre-treated with naloxone

Group	Doses (mg/kg)	Inhibition (%)					
		1h		2h		4h	
		Without naloxone	With naloxone	Without naloxone	With naloxone	Without naloxone	With naloxone
Control		21,5	7,3	2,2	4,41	9,5	10,2
MEUG	62	62,4	40,3	38,2	28,3	18,0	43,5
MEUG	124	28,6	13,8	23,3	11,4	23,3	14,6
Indomethacin	10	11,0	13,4	12,6	12,0	6,1	4,1
Tramadol	25	68,3	17,3	75,1	34,6	87,4	25,5
Morphine	10	77,0	22,6	84,6	38,3	87,3	13,1

n : 5 per group

Analgesic activity on pressure-induced pain

MEUG at doses of 62 and 124 mg/kg significantly increased the ability of animals to bear pressure-induced pain. Morphine (10 mg/kg) and tramadol (25 mg/kg) were more effective at reducing sensitivity to pain while indomethacin (10 mg/kg) showed little protective effects in this model of pain (table 4).

Table 4: Antinociceptive effect of the MEUG in pressure causing pain

Group	Doses (mg/kg)	Weight causing pain (g/f ± SEM) (Inhibition (%))				
		Before administration	After administration			
			1h	2h	3h	4h
Control		63±6	41±7 (-35)	39±5 (-38)	47±7 (-25)	45±5 (-28)
MEUG	62	57±9	90±12** (57)	82±18 (44)	66±16 (16)	80±17 (40)
MEUG	124	56±8	71±10 (27)	73±16 (30)	72±8 (29)	71±9 (27)
Indomethacine	10	87±10	74±8 (-15)	91±7 (5)	98±9 (13)	86±17 (1)
Tramadol	25	65±9	80±15 (23)	83±14 (28)	79±5 (21)	68±4 (5)
Morphine	10	61±9	108±14** (77)	96±14* (57)	77±5 (26)	95±4* (56)

Results are shown as the mean ± SEM, n=5. *P<0,05; ** P<0,01

Discussion

In the present study, MEUG was found to show remarkable antinococeptive properties using different pain models.

We have shown from our results that that MEUG significantly inhibited the writhing syndrome induced by acetic acid in mice, indicating the peripheral action of the plant extract. The abdominal writhing involves the production and release of arachidonic acid metabolites via cycloxygenase (COX) and prostaglandin (PGE_2) biosynthesis[14]. It has been demonstrated that COX-1 elicited acute writhing responses to noxious chemical stimuli[15]. Thus, the results led to the hypothesis that MEUG might play a role in the inhibition of prostaglandin synthesis. The analgesic potential of MEUG as shown by the acetic acid test was significant but not specific. The test did not indicate if the potential resulted from central and /or peripheral actions. To clarify this we carried out the formalin test.

The formalin test provides a model of the behavioural response to a moderate tonic, inflammatory pain and the nociceptive response closely resembles clinical pain[16]. In this test, animals presented two distinct nociceptive behavioural phases, which probably involve different stimuli. The first phase which begins immediately after formalin injection and lasts 3 to 5 min, is caused by the direct stimulation of nociceptors[17]. The second phase noted 15 to 20 min after formalin injection is a period of reduced nociceptive activity, while the late phase represents a period of inflammatory pain. The late phase of moderate pain starts about 20 min after formalin injection and may last up to 40 min after formalin injection[18] appears to activate NMDA receptors in the dorsal horn of the spinal cord[19]. Indeed, Malmberg and Yaksh, 1995[20] showed that formalin nociception was accompanied by a biphasic release of prostaglandins. In this study, MEUG significantly inhibited the early phase more than the late phase of formalin nociceptive activity which suggested that this extract may have an opioid-like analgesic activity. To verify this suggestion the hot plate and the pressure test were performed, these tests are suitable for identifying centrally and not peripherally acting analgesics.

The pre-treatment of rats with MEUG inhibited pain caused mechanically by a constantly increasing pressure on rat paw by the plunger and plinth of the analgesy Meter - an indication of the central acting properties of the plant This system provides a model for the study of non inflammatory pain. The involvement of endogenous substances such as prostaglandins may be minimized in this model[21]. The central protecting effect of MEUG were the similar as morphine and tramadol test results. It is therefore more likely that opioid-like analgesic drugs be more effective in inhibiting mechanically induced pain. MEUG is effective against pain due to sensory nerve stimulation and indeed, significantly blocked pain sensation at both doses used. Indomethacin did not show analgesic effect on this model of pain, this corroborates the previous study that Aspirin and NSAIDs are ineffective against pain due to sensory nerve stimulation [22]. The observed analgesic effect against thermal pain supports the role of central component in the analgesic activity of MUEG. To confirm this effect, the entire tests cited above were evaluated in the presence of naloxone, a specific antagonist of morphine receptors.

In the formalin test, writhing test and hot plate test, the analgesic affect of MEUG, morphine and tramadol were significantly antagonised by naloxone, suggesting a central acting property of MUEG. The present study showed that MEUG has remarkable central analgesic activities which seem to confirm the use of the plant for pains in traditional medicine.

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