Antinociceptive and anti-inflammatory effects of aqueous extracts of *Mallotus oppositifolium* leaves (Euphorbiaceae)

René KAMGANG [1*], Nkeh Benedicta CHUNGAG-ANYE [1], Eugène J NDEBIA [1], Roger ZINTCHEM [1]

[1]Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaounde I; P.O. Box 812 Yaounde – Cameroon.

ABSTRACT

Mallotus oppositifolium is used in Cameroonian traditional medicine for the management of pains and inflammation. The present work assesses the acute toxicity (LD_{50}), the pain-killing and anti-inflammatory activities of the aqueous extract of *M. oppositifolium* leaves. Pain was induced in mice by injection of 1 mL/100 g of 1% acetic acid and by the application of increasing pressure on albinos rat's paws using an analgesymeter. Inflammation was induced in rat's right hind paw by the injection of 0.05 mL of 1% carrageenan. The acute toxicity (LD_{50}) value of the extract in mice were > 5 g/kg *p.o.* Oral administration of aqueous extract of *M. oppositifolium* at doses of 350 mg/kg and 700 mg/kg significantly inhibited acetic acid-induced pain by 11% and 38% ($P<0.01$) respectively. Analgesymeter pressure-induced pain was reduced by 68% ($P<0.01$) and 46% ($P<0.05$) at these two dose levels. These results were comparable to those obtained with tramadol (weak central acting analgesic) suggesting that this plant could exercise their pain-killing effect by a central acting mechanism through the serotonin pathway. The extract (350 mg/kg) presented a minor anti-inflammatory property (41%, $p<0.05$) after 3 h following carrageenan injection. The results of the present study indicate that the aqueous extract of *Mallotus oppositifolium* has notable antinociceptive and slight anti-inflammatory properties.

Keywords: acute toxicity, analgesic, inflammation, *Mallotus oppositifolium aqueous extract.*

RESUME

Effets antinociceptive et anti-inflammatoire des extraits aqueux des feuilles de *Mallotus oppositifolium* (Euphorbiaceae).

Mallotus oppositifolium est une plante utilisée dans la médecine traditionnelle camerounaise pour le traitement des douleurs et des inflammations. La toxicité aiguë (DL_{50}), l'activité analgésique et anti-inflammatoire des extraits aqueux des feuilles de cette plante, administrés par voie orale, ont été évaluées. Les douleurs ont été induites chez des rats albinos par l'acide acétique à 1 % (1 mL/100 g, *i.p.*) et par application de pressions croissantes sur la patte à l'aide de l'analgésimètre. Les inflammations ont été produites sur la patte postérieure droite par injection locale de la carrageenan à 1 % (0,05 mL). La DL_{50} a été > 5 g/kg. Les extraits aqueux de *M. oppositifolium* à 350 et 700 mg/kg ont réduit les douleurs induites par l'acide acétique de 11 % et 38 % respectivement ($P < 0,01$), celles induites par l'analgésimètre de 68 % ($p<0,01$) et 46 % ($p < 0,05$) respectivement. Ces résultats comparables à ceux obtenus avec le tramadol (analgésique central mineur) amènent à penser que ces extraits auraient des effets anti-douleurs par un mécanisme central impliquant la voie de la sérotonine. L'extrait à 350 mg/kg a présenté 3 h après injection de la carrageenan une faible propriété anti-inflammatoire (41 %, $p < 0,05$). Les résultats de cette étude montrent que les extraits aqueux de *Mallotus oppositifolium* ont des propriétés antinociceptive importante et anti-inflammatoire mineure.

Mots clés : toxicité aiguë, analgésie, inflammation, extraits aqueux de *Mallotus oppositifolium.*

* Corresponding author address: General Endocrinology and Metabolism Systems (GEMS) Animal Physiology Laboratory, University of Yaounde I, P.O. Box 8127 Yaounde, Cameroon. E-Mail: rkamgang@uycdc.uninet.cm; gemskruy@yahoo.fr.

INTRODUCTION

Mallotus oppositifolium (Euphorbiaceae) is a plant used in Southern and Eastern Cameroonian folk medicine for treating fever, tooth pain and decay. This plant is also used for its antidiarrhoeic properties (Kamgang et al., 2001). Leaves are recommended for migraines, wounds and haemorrhages, while leaves and the other aerial parts are used for dysentery (Mshana et al., 2000). For dental pain and decay, fresh leaves are ground to a paste and applied to the ailing tooth. Chemical analysis of methanol extract of the leaves revealed the presence of alkaloids, cardiac glycosides and phenolic compounds among which where flavonoids (Farombi et al., 2001). Since no investigation is available on the analgesic and antinflammatory properties of the aqueous extract of *Mallotus oppositifolium* leaves, the present study was undertaken to provide scientific validation of the claimed ethnopharmacological properties of the plant.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Mallotus oppositifolium* were harvested in Yaounde, Cameroon, and identified at the National Herbarium Yaounde, where a voucher specimen Nr HNC 16619 was deposited for future reference. The aqueous extract was prepared from fresh leaves thus: 100 g of ground plant material were macerated for 2 h in 500 mL hot distilled water. The aqueous filtrate was concentrated by evaporation at 40°C and yielded 20.75 g of dried plant materials.

Animals

Wistar rats (weighing 160-220 g) and Swiss mice (weighing 20-30 g) were bred in our animal house at room temperature. Food and water were provided *ad libitum*. Prior to treatment, the experimental animals were fasted overnight but were allowed free access to water.

Drugs

Aspirine (Aspegic®, Sanofi-Synthelabo, France), Indomethacine (Indocid®, Laboratoire Merck Sharp et Dohme, France), Morphine (Sigma-Aldrich-Quiinina S.A., Madrid-Spain), Tramadol (Laboratoire Pharmascience, France, Courbevoie) and plant extract were dissolved in distilled water, Carrageenan (Sigma Chemical Co, St Louis, USA) in physiological saline.

Acute toxicity (LD_{50})

The acute toxicity of the extract was evaluated in albino mice by the oral route. The method estimates the dose of the extract that will kill 50% of the animal population (LD_{50}) by the route given. Forty-eight albino mice were separated into six groups of eight mice each. Each group was fasted for 18 h after which they were treated orally with one of the increasing doses of the extract: 1, 4, 8, 12, 16 and 20 g/kg. The mice were then observed for 48 h for death. LD_{50} was calculated using the following formula:

$LD_{50} = X_s - d(\Sigma_p - ½)$ (Molle, 1986)

X_s: maximum lethal dose; d: interval between 2 successive doses; p: death ratio per group

Nociceptive activities

The analgesic activity was measured against chemical and mechanical stimulus.

Acetic acid-induced abdominal writhing test

Mice were divided into six groups each containing five animals. The first group served as a negative control and received distilled water. The second, the third and the fourth groups served as positive controls and received orally (*p.o.*) morphine (1.5 mg/kg), aspirin (100 mg/kg) and tramadol (25 mg/kg) respectively while the last two groups respectively received oral doses of 350 and 700 mg/kg aqueous extract of *M. oppositifolium*. One hour after administration of the test drugs, each group was injected intraperitoneally with 1% acetic acid (1 mL/100 g body weight). The number of writhing responses such as contortions and stretching (full extension of both hind paws) were recorded for 30 min. The results were evaluated by calculating the mean number of contortions per treated group compared with that of the control group. Protection rates were calculated as follows.

$$\text{Protection (\%)} = \frac{\overline{Nc} - \overline{Nt}}{\overline{Nc}} \times 100$$

$\overline{Nc}$: mean number of contortions of the control group;

$\overline{Nt}$: mean number of contortions per treated group.

Pressure test

In these experiments, the drugs cited above were tested at the same doses in rats using an Ugo Basile analgesymeter (N° 7200). Force was applied to the

left hind paw of experimental animals by an analgesymeter plunger which exerts 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, it got to a point where the animal struggled to free its paw. This was the level at which the animal felt pain. The weight causing pain before treatment and then 1, 2 and 3 h after treatment of animals with the various test drugs were determined. 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.

Carrageenan-induced paw oedema

Right hind paw swelling was induced on the rat by a subplantar injection of 0.05 mL of a solution of 1% sterile carrageenan in saline (Winter et al., 1962). The plant extract (350 and 700 mg/kg) and indomethacine (10 mg/kg) were administered orally 30 min before carrageenan injection. Control animals received the vehicle only. Inflammation was quantified by measuring the volume displaced by the injected paw, using a Plethysmometer (Ugo Basile N° 7140), at time before and 1, 2, 3, 4, 5 and 6 h after carrageenan injection. Oedema (V) and percentage inhibition (I %) of oedema were calculated as follows.

$$V = \overline{V_t} - \overline{V_0}$$

$$I\ (\%) = \frac{(\overline{V_t} - \overline{V_o})C - (\overline{V_t} - \overline{V_0})E}{(\overline{V_t} - \overline{V_0})C} \times 100$$

$\overline{V_0}$: right hind paw volume before subplantar injection of carrageenan

V_t :right hind paw volume at time t.

C: control group; *E*: essays group

Statistical analysis

The results are expressed as mean ± SEM. Data was analyzed statistically by ANOVA (analysis of variance) followed by Dunnett's multiple comparison tests. P values less than 0.05 were considered as indicative of significance.

RESULTS

Acute toxicity study (LD_{50})

In the acute toxicity test, no death was observed up to 8 g/kg. The LD_{50} estimated was 14 mg/kg.

Antinociceptive effects

Acetic acid-induced abdominal writhing test

The number of writhings and stretchings induced by intraperitoneal injection of 1% acetic acid was not significantly attenuated by the oral administration of *M. oppositifolium* extract (350 mg/kg). Whereas a significant protective effect (63%,

Table 1: Effect of leaves aqueous extracts of *Mallotus oppositifolium* on acetic acid-induced writhing.

Group	Dose (mg/kg p.o.)	Writhing	% Protection
Control	-	148 ± 14	-
Aspirin	100	77 ± 8 **	48
Tramadol	25	113 10*	24
Morphine	1.5	58 ± 8**	61
M. Oppositifolium	350	131 ± 17	12
M. Oppositifolium	700	91 ± 6**	63

Significant difference vs control: *P<0.05; **P<0.01; n = 5

Table 2: Effect of aqueous extracts of leavea of *Mallotus oppositifolium* on weight (g.f) causing pain induced by analgesymeter pressure.

Group	Dose (mg/kg p.o.)	Before administration	After administration		
			1 h	2 h	3 h
Control	-	86 ± 5	80 ± 9 (-7)	92 ± 5 (7)	91 ± 7 (6)
Aspirin	100	89 ± 4	105 ± 11 (18)	102 ± 6 (15)	105 ± 5 (18)
Tramadol	25	78 ± 5	92 ± 5 (18)	130 ± 4** (67)	118 ± 6** (51)
Morphine	1.5	81 ± 5	107 ± 7* (32)	136 ± 2** (68)	108 ± 3* (33)
Mallotus oppositifolium	350	68 ± 8	71 ± 2 (5)	93 ± 12* (37)	106 ± 5** (56)
Mallotus oppositifolium	700	67 ± 8	78 ± 7 (16)	79 ± 10 (18)	98 ± 6* (46)

Significant difference vs. control: *P<0.05; **P<0.01;n = 5; ( ): % Protection

p<0.01) was observed with 700 mg/kg of extract (Table 1). The reference drugs also produced significant protective effects against acetic acid-induced pain. Morphine and Aspirin had 61% and 48% (P<0.01) respectively while tramadol produced only 24% inhibition (p<0.05).

Pressure test

Orally administrated aqueous extracts of *M. oppositifolium* leaves aqueous extracts significantly increased the ability of the treated rat to withstand greater pressure, when compared with the control animals (Table 2). Three hours following their administration, extracts (350 and 700 mg/kg) reduced pressure-induced pain (p<0.05 and p<0.01, respectively). A significant analgesic effect (p<0.05) was observed two hours after 350 mg/kg extract administration; by the third hour its effect was comparable to that of tramadol (25 mg/kg). Aspirin (100 mg/kg) did not show significant antinociceptive effect on this type of pain.

Carrageenan- induced paw oedema

Paw swelling took place progressively 30 min after injection of 1% carrageenan. The maximum inflammatory effect was observed after at hours (0.61 ± 0.07 mL *vs* 0.18 ± 0.03 mL at t_o). Paw oedema was powerfully reduced (p<0.01) by indomethacine after two hours (Table 3). Four hours after test components administration, *M. oppositifolium* extract showed a minor inhibition of oedema formation only at the dose of 350 mg/kg (29%, p<0.05).

4. DISCUSSION

The purpose of the present paper was to establish the scientific rationale for the traditional use of the aqueous extracts of *Mallotus oppositifolium* leaves in treating pains. Peripheral and central antinociceptive effects were investigated using acetic acid-induced writhing and pressure tests, respectively. Carrageenan-induced paw oedema was selected to represent a model of acute inflammation. Acute toxicity (LD_{50}) of the extract was evaluated.

Since the LD_{50} value is largely higher than 5 g/kg, the extract is considered safe for practical purposes in the laboratory (Lockes, 1983) and for all medicinal uses according to WHO criteria (Diezi, 1992). The assayed doses represented 1/40 and 1/20 of the oral LD_{50}.

The results show that the aqueous extract of leaves of *M. oppositifolium* (700 mg/kg) significantly inhibited writhing syndrome induced by acetic acid in mice, indicating peripheral analgesic effect of the plant (Atta et al., 1997). In peripheral tissues, prostaglandins and kinines are suggested to play an important role in the pain process (Hajare et al., 2000) and writhing induced by chemical substances injected intraperitoneally was said to be due to sensitization of chemosensitive nociceptors by prostaglandins (Maria Elena et al., 1997). This test also confirms the peripheral action of Aspirin (Rang et al., 1995).

Pre-treatment of rats with *Mallotus oppositifolium* extract inhibited pain caused mechanically by a constantly increasing pressure on rat paw by the plunger and plinth of the analgesymeter. The nociceptors could be sensitized by sensory nerves; therefore it is more likely that opioid-like analgesic drugs may be more effective in inhibiting mechanically induced pain (Nkeh et al., 2002). The involvement of endogenous substances such as prostaglandins may be minimized in this model (Dongmo et al., 2001). These results suggest that *Mallotus oppositifolium* would exercise pain killing by acting like opioid drugs such as Tramadol (weak central acting analgesic) and morphine. The effects *M. oppositifolium* aqueous extracts of leaves, three hours after administration were quite comparable to 25 mg/kg Tramadol which acts through serotonergic pathway by inhibiting neuronal reuptake of serotonin (Oliva et al., 2002). Aspirin did not show analgesic effect on this model of pain. This corroborates the previous study that aspirin and aspirin like drugs are ineffective both against pain due to sensory nerve stimulation (Flower et al., 1985).

In the carrageenan experimental inflammation the initial phase of paw oedema is mediated by histamine and serotonin, while the later phase is suspected to be due to arachidonate metabolites producing oedema dependent on mobilisation of neutrophiles (Hwang et al., 1996; Lo et al., 1987). Although the cyclooxygenase and lipoxygenase pathways are both involved in the inflammation process, inhibitors of cyclooxygenase are more effective in inhibiting carrageenan-induced inflammation than lipoxygenase inhibitors (Flower et al., 1985). In our study, oedematous response was significantly suppressed in rat pre-treated with *M. oppositifolium* only 3h 30 min after the carrageenan injection. These results suggest that *M. oppositifolium* could slow down the later phase of the inflammatory process and probably by slight inhibition of cyclooxygenase activity which is involved in the formation of prostaglandins.

The present study showed that aqueous *Mallotus oppositifolium* leaf extract has remarkable peripheral and central analgesic activities which seem to confirm the use of leaves for pains in folk medicine, while its antiflammatory effects are weak. Since some flavonoids have antiinflammatory and

Table 3: Anti-inflammatory activity of aqueous extracts of *Mallotus oppositifolium* leaves in carrageenan-induced hind paw oedema: expressed in paw volume variation (ΔV: mL)

Group	Dose (mg/kg, po)	0,5h (Carrageenan)	1h	2h	3h	4h	5h	6h
Control	-	0,18± 0.03	0.35± 0.06	0.52± 0.08	0.61± 0.07	0.55± 0.05	0.51± 0.06	0.47± 0.07
Indomethacine	10	0.20± 0.04 (-11)	0.20± 0.01 (43)	0.20± 0.02** (62)	0.11± 0.02** (82)	0.08± 0.04** (85)	0.21± 0.03** (60)	0.28± 0.02** (43)
M. oppositifolium	350	0.11± 0.02 (39)	0.21± 0.06 (40)	0.43± 0.08 (17)	0.45± 0.09 (18)	0.37± 0.04* (29)	0.40± 0.05 (23)	0.37± 0.04 (26)
M. oppositifolium	700	0.16± 0.02 (6)	0.23± 0.03 (31)	0.42± 0.09 (19)	0.49± 0.09 (20)	0.40± 0.06 (25)	0.42± 0.04 (19)	0.42± 0.06 (12)

Significative difference vs. control: *P<0.05; **P<0.01; n = 5; ( ) = % Protection

antinociceptive activities (Reanmongkol et al., 1994; Reanmongkol et al., 1995) other studies are going on in order to identify alkaloid and flavonoid components and to establish the possible mechanism of action.

REFERENCES

Atta AH, Alkofahi A (1997) Anti-nocive and anti-inflammatory effects of some Jordanian medicinal plant extracts. Journal of Ethnopharmacology 60, 117-124.

Diezi J (1992) Principe de base et repercussion clinique. In: Schoderet M. Pharmacologie: des principes fondamentaux aux applications thérapeutiques. Eds Frison-Roche, Paris, Slatkine, Genève 2ème éd. pp33-35.

Dongmo AB, Kamanyi A, Anchang MS, Chungag-Anye N B, Njamen D, Nguelefack TB, Nole T, Wagner H (2001) Anti-inflammatory and analgesic properties of the stem bark extract of *Erythrophleum suaveolens* (Caesalpiniaceae), Guillemin α Perrottet. Journal of Ethnopharmacology 7(2-3), 137-141.

Farombi EO, Ogundipe OO, Moody JO (2001) Antioxidant and anti-inflammatory activities of *Mallotus oppositifolium* in model systems. Afr J Med Med Sci 30(3), 213-215.

Flower JR, Moncada S, Vane JR (1985) Analgesic, antipyretic and anti-inflammatory agents: Drugs employed in the treatment of gout. In: Goodman and Gilman, editors. The pharmalogical basic of therapeutics, 6th ed pp682-728.

Hajare SW, Suresh C, Tandan SK, Sarma J, Lal J, Telang AG (2000) Analgesic and antipyretic activities of *Dalbergia sissoo* leaves. Indian Journal of Pharmacology 32, 357-360.

Hwang S, Lam M, Li C, Shen T. (1996) Release of platelet activiting factor and ist involvement in the first phase of carrageenan rat foot edema. European Journal pharmacology 120, 33-41.

Kamgang R, Zintchem R, Dimo T, Yewah M Panjo. (2001) Les effets des extraits totaux aqueux de *Mallotus oppositifolium* et *Euphorbia hirta* (Euphorbiacée) sur l'activité contractile intestinale du rat. African Journal of science and technology; (AJST) 2(2), 8-11.

Lo TN, Sauf SS (1987) Carrageenan stimulated release of arachidonic acid and of lactate dehydrogénase from rat pleural cells. Biochemical Pharmacology 36, 2405-2513.

Lockes D. (1983) A new approach to acute toxicity testing. Archives of Toxicology 54, 275-287.

Maria Elena NG, Da JASE, Caden S, Antonio JL (1997) Analgesic and Anti-inflammatory Activities of the Aqueous Extract of *Plantango Major* L. International Journal of Pharmacognosy 35(2), 99-104.

Molle J (1986) Limites de tolérence et toxicités de quelques amino-acides (formes L et D). Amino-peptides-protéine, Cahier 24 ; Ed. A.E.C. pp207-232.

Mshana NR, Abbiw DK, Addae-Mensah I, Adjanohoun JE, Ahyimra, Ekpere JA, Enow-Orock EG, Gbilezo, Noamesi GK, Odie MA, Odunlami H, Oteng-Yeboah AA, Sarpong K, Safowara A, Taekie AN (2000) Traditional Medicine and pharmacopoeia contribution to the revision of ethnobotanical and floristic studies in Ghana. Organization of African Unity/Scientific, Technical and Research Commission p243.

Nkeh B, Njamen D, Wandji J, Fomum Z, Dongmo A, Nguelefack TB, Wansi B, Kamanyi A (2002) Anti inflammatory and analgesic effect of Drypermolundein A, a sesquiterpene lactone from *Drypetes molunduana.* Pharmaceutical Biology 40, 1-5.

Oliva P, Aurilio C, Massimo F, Grella A, Maione S, Grella E, Scafuro M, Rossi F, Berrino L (2002) The antiniciceptive effect of tramadol in the formalin test is mediated by the serotonergic component. European Journal of Pharmacology 445, 179-185.

Rang HP, Dale MM, Ritter JM (1995) Pharmacology. Churchill, Livinstone, London.

Reanmongkol W, Matsumoto K, Watanabe H, Subhadhirasakul S, Sakai S (1994) Antinociceptive and antipyretic effects of alkaloids extracted from the stem bark of *Hunteria zeylanica.* Biol Pharm Bull 17, 1345-1350.

Reanmongkol W, Matsumoto K, Watanabe H, Subhadhirasakul S, Takayama H, Sakai S (1995) Effects of alkaloids extracted from the stem bark of *Hunteria zeylanica* on acute inflammation in experimental animals. Biol Pharm Bull 18, 33-36.

Winter CA, Risley EA, Nuss GW (1962) Carrageenan-induced edema in hind paw of the rat as an essay for antiinflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544-552.

Received: 20/02/2005
Accepted: 07/07/2005