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## **Analgesic Effect of Aqueous and Hydroalcoholic Extracts of Three Congolese Medicinal Plants: *Hyptis suaveolens*, *Nauclea latifolia* and *Ocimum gratissimum***

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**Abstract:** Analgesic effect of aqueous and hydroalcoholic extracts of aeral parts of *Hyptis suaveolens*, *Nauclea latifolia* and *Ocimum gratissimum*, three plants used in congolese folk medicine in pain, were tested on acetic acid and hot plate tests. All extracts manifest analgesic effect on the two models used. The more active was the hydroalcoholic extract of *Ocimum gratissimum* which is not antagonized by naloxone and could potentiate analgesic effect of paracetamol.

**Key words:** *Hyptis suaveolens*, *Nauclea latifolia*, *Ocimum gratissimum*, analgesic, congolese

### **INTRODUCTION**

An ethnobotanical investigation realised in Brazzaville (capital of Republic of Congo) on medicinal plants used as analgesic between January and June 2003 revealed that *Hyptis suaveolens* (Lamiaceae), *Nauclea latifolia* (Rubiaceae), *Ocimum gratissimum* (Lamiaceae) are among the more used plants in pain (unpublished results). The others folk use of these three plants are asthma and fever, diabetes and hemorrhoid and diabetes and tooth algia, respectively for *Hyptis suaveolens*, *Nauclea latifolia* and *Ocimum gratissimum*<sup>[1,2]</sup>.

The aim of the present study was to confirm or infirm these data by studying analgesic effect of aqueous and hydroalcoholic extracts of these three plants on classical pharmacological methods of pain.

### **MATERIALS AND METHODS**

**Plant material:** *Hyptis suaveolens*, *Nauclea latifolia* and *Ocimum gratissimum* were collected from the area of Brazzaville (capital of Congo) in September 2003 and authenticated by the Center of Research on Vegetal Resources where a voucher specimen of each plant was preserved.

**Preparation of extracts:** The areal parts of *Hyptis suaveolens*, *Nauclea latifolia* and *Ocimum gratissimum*

were dried and powdered. Powdered plant material (60 g) was soaked in 250 mL of H<sub>2</sub>O-EtOH for 3 days and filtered. The filtrate was evaporated to dryness in vacuo to give a residue with a yield of 7.4, 5.7 and 4.0%, respectively for the three plants.

Aqueous extracts were also prepared after decoction of 60 g of powdered material plant in 300 mL of distilled water during 30 min.

### **Pharmacological studies**

**Animals:** Albino mice of both sexes, weighing 18-25 g were used. They were housed in standard environmental conditions, feed with standard mice and rat diet with water *ad libitum*. Animals were fasted overnight before use.

### **Preliminary acute toxicity and general observations:**

After oral administration of 200, 400, 800, 1200 and 1600 mg kg<sup>-1</sup> of aqueous and hydroalcoholic extracts animals were generally observed and mortality was determined for 24, 48 and 72 h.

**Acetic acid induced writhing movements:** Acetic acid induced writhing movements were induced in mice according to Koster *et al.*<sup>[3]</sup>. Mice received oral doses of extracts (400 and 800 mg kg<sup>-1</sup>) for aqueous and hydroalcoholic extracts of *Nauclea latifolia* and *Ocimum gratissimum* and for *Hyptis suaveolens* (50 and 200, 400 and 800 mg kg<sup>-1</sup>), respectively for hydroalcoholic and aqueous extracts, distilled water (0.5 mL 100 g<sup>-1</sup>) or

paracetamol (50 mg kg<sup>-1</sup>) prior to intraperitoneal acetic acid 0.6% (10 mL kg<sup>-1</sup>). The number of writhing movements of each mouse was determined for 10 min beginning 10 min after acetic acid injection.

**Hot plate test:** Mice were treated with oral doses of aqueous (400 and 800 mg kg<sup>-1</sup> for all extracts) and hydroalcoholic extracts, (400 and 800 mg kg<sup>-1</sup>) for *Nauclea latifolia* and *Ocimum gratissimum* and 50 and 200 mg kg<sup>-1</sup> for *Hyptis suaveolens*, distilled water (0.5 mL 100 g<sup>-1</sup>) and morphine (2 mg kg<sup>-1</sup>). The reaction time for each mouse was determined one hour after drugs administration with a cut-off period of 60s<sup>[4]</sup>.

**Study of potentiation of analgesic activity of paracetamol by hydroalcoholic extract of *Ocimum gratissimum*:** One hour after oral administration of hydroalcoholic extract of *Ocimum gratissimum* (200 mg kg<sup>-1</sup>), paracetamol (25 mg kg<sup>-1</sup>) and association paracetamol+hydroalcoholic extract of *Ocimum gratissimum* (12.5+200 or 25+200 mg kg<sup>-1</sup>) in mice, acetic acid (0.6%, 10 mL kg<sup>-1</sup>) was administered in mice and number of writhing movements was determined for 10 min beginning 10 min after acetic acid administration.

**Study of mechanism of analgesic effect of hydroalcoholic extract of *Ocimum gratissimum*:** The reaction time on the hot plate of mice treated with oral doses of hydroalcoholic extract of *Ocimum gratissimum* (400 mg kg<sup>-1</sup>), distilled water (0.5 mL 100 g<sup>-1</sup>) and morphine (2 mg kg<sup>-1</sup>) was determined in the presence of naloxone (1 mL kg<sup>-1</sup> ip) 1 h after administration of the drugs.

**Study of eventual tolerance with repeated administration of *Ocimum gratissimum*:** Mice were treated for 21 days with oral doses of hydroalcoholic extract of *Ocimum gratissimum* (400 mg kg<sup>-1</sup>), distilled water (0.5 mL 100 g<sup>-1</sup>) and morphine (2 mL kg<sup>-1</sup>). At the 7th, 14th and 21st day, the reaction time of each mouse on the hot plate was determined.

**Statistical analysis:** The data are expressed as mean±SE and the statistical significance between groups were analyzed by means of analysis of variance (ANOVA) followed by student t-test. P<0.05 were considered as indicative of significance.

## RESULTS AND DISCUSSION

Outside of ataxia observed with hydroalcoholic extract from the dose of 800 mg kg<sup>-1</sup>, all extracts used

Table 1: Effect of oral administration of aqueous (AE) and hydroalcoholic extracts (HAE.) of *Hyptis suaveolens*, *Nauclea latifolia* and *Ocimum gratissimum* on acetic acid induced writhing movements in mice

Treatments	Doses (mg kg <sup>-1</sup> )	Number of writhing <sup>a</sup> movements
Control	-	65.60±6.07
AE. <i>Hyptis suaveolens</i>	400	30.75±0.89***
	800	23.40±1.63***
HAE. <i>Hyptis suaveolens</i>	50	32.40±1.72***
	200	27.80±1.98***
AE. <i>Nauclea latifolia</i>	400	33.80±1.54***
	800	31.80±1.40***
HAE. <i>Nauclea latifolia</i>	400	34.80±1.28***
	800	27.80±1.04***
AE. <i>Ocimum gratissimum</i>	400	38.80±1.45***
	800	30.80±1.40***
HAE. <i>Ocimum gratissimum</i>	400	24.40±1.86***
	800	23.40±1.93***
Paracetamol	50	20.62±2.03***

Table 2: Effect of oral administration of aqueous (AE) and hydroalcoholic extracts (HAE) of *Hyptis suaveolens*, *Nauclea latifolia* and *Ocimum gratissimum* on reaction time on the hot plate test in mice

Treatments	Doses (mg kg <sup>-1</sup> )	Reaction time <sup>a</sup> (s)
Control	-	5.20±1.31
AE. <i>Hyptis suaveolens</i>	400	40.00±2.73***
	800	32.00±4.06***
HAE. <i>Hyptis suaveolens</i>	50	23.40±4.40***
	200	31.40±7.53***
AE. <i>Nauclea latifolia</i>	400	33.40±6.47***
	800	27.00±2.62***
HAE. <i>Nauclea latifolia</i>	400	35.20±2.95***
	800	27.00±2.62***
AE. <i>Ocimum gratissimum</i>	400	28.20±4.93***
	800	41.00±5.85***
HAE. <i>Ocimum gratissimum</i>	400	41.00±5.85***
	800	48.20±4.93***
Morphine	2	56.60±5.29***

Table 3: Potentiation of paracetamol effect on acetic acid induced writhing movements by hydroalcoholic extract (HAE) of *Ocimum gratissimum*

Treatments	Doses (mg kg <sup>-1</sup> )	Number of writhing movements <sup>a</sup>
Control	-	65.60±6.07
Paracetamol	125	28.25±2.21***
	25	23.40±3.23***
HAE <i>Ocimum gratissimum</i>	200	29.80±2.28***
Paracetamol+	125	22.60±1.16***
H.A.E. <i>Ocimum gratissimum</i>	200	
Paracetamol+	25	18.00±1.64***
H.A.E. <i>Ocimum gratissimum</i>	200	

Table 4: Effect of oral administration of hydroalcoholic extract (HAE) of *Ocimum gratissimum* on reaction time on hot plate in the presence of naloxone

Treatments	Doses (mg kg <sup>-1</sup> )	Number of writhing movements <sup>a</sup>
Control	-	5.20±1.31
HAE. <i>Ocimum gratissimum</i>	400	28.20±2.00***
morphine	2	56.60±5.29***
HAE. <i>Ocimum gratissimum</i> +	400	24.00±2.34***
Naloxone	1	
Morphine+	2	18.00±1.64 ***
Naloxone	1	

<sup>a</sup>Values are mean±SEM with n=5

\*\*\*Student t-test, p<0.001, different from control

Table 5: Effect of repeated oral administration of hydroalcoholic extract (HAE) of *Ocimum gratissimum* on reaction time on hot plate in mice

Treatments	Doses (mg kg <sup>-1</sup> )	Reaction time <sup>a</sup> (s)			
		Initial	Day 7th	Day 14th	Day 21st
Control		5.20±1.31	5.25±1.35	5.15±1.25	5.20±1.31
HAE. <i>Ocimum gratissimum</i>	400	30.80±6.28	35.60±6.74	34.25±5.67	32.25±4.33
Morphine	2	56.60±5.25	41.75±5.85	30.25±2.38*	26.75±6.07*

<sup>a</sup>Values are mean±SEM with n=5 \*Student t-test, p < 0.05, different from initial morphine value

were well tolerated. All extracts at the doses used reduced significantly (as paracetamol) the number of writhing movements induced by acetic acid in mice compared to control (Table 1-5). In the same way, all extracts demonstrate in the hot plate test, antinociceptive activity as morphine, a well known opioid receptors agonist, suggesting that the extracts possesses also a central analgesic effect<sup>[5]</sup>.

Among these preparations, hydroalcoholic extract of *Ocimum gratissimum* manifest the more important activity. This extract potentiate profoundly the analgesic activity of paracetamol in acetic acid test. Furthermore, contrary to analgesic effect of morphine which is reduced by naloxone (an antagonist of opioid receptors) and by repeated administration, antinociceptive activity of hydroalcoholic extract of *Ocimum gratissimum* is not influenced by naloxone and did not manifest tolerance phenomenon which is a characteristic feature of all the opioid drugs and represents major limitations in their clinical use<sup>[6]</sup>.

Phytochemical screening of hydroalcoholic extract of *Ocimum gratissimum* revealed the presence of flavonoids, saponins, alkaloids, steroids and polyphenols. Several of these compounds would exert analgesic effect<sup>[7]</sup>.

In conclusion, this study demonstrated that aqueous and hydroalcoholic extracts of *Hyptis suaveolens*, *Nauclea latifolia* and *Ocimum gratissimum* possess a significant analgesic effect, confirming their folk use in pain. Hydroalcoholic extract of *Ocimum gratissimum* manifest the more important effect which could potentiate the low dose of paracetamol. This effect is not mediated by opioid receptors.

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