ANALGESIC ACTIVITIES OF THE STEM BARK EXTRACT OF *TERMINALIA SUPERBA* ENGL. ET DIELS (COMBRETACEAE)

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Summary

Terminalia superba Engl. et Diels is a dense humid forest species, widespread from Guinea to Zaïre (Democratic Republic of Congo). The stem bark is used in folk medicine in the treatment of gastroenteritis, diabetes, female infertility and abdominal pain.The *n*-BuOH fraction of *Terminalia superba* was obtained from the dry stem bark of T. superba and its analgesic propriety investigated using acetic acid, formalin test and hot plate model. *n*-BuOH fraction of *T. superba* showed analgesic effect in a dose dependent (50-100 mg/kg; p.o) manner in the acetic acid test and in the second phase of formalin test which was comparable to the effects observed with acetylsalicylic acid (20 mg/kg). The results of this study lead credit to the traditional uses *T. superba*, especially as an analgesic. Phytochemical studies on this plant reveal the presence of terpenoids, flavonoids, tannin and these might be responsible for the analgesic activity of this plant.

Key words: *Terminalia superba*, analgesic, mice, terpenoids, flavonoids.

Introduction

Members of the Combretaceae are used for many medicinal purposes by traditional healers. These include treating abdominal disorders, abdominal pains, backache, bilharziasis, cough, colds, conjunctivitis, diarrhoea, dysmenorrhoea, earache, fattening babies, fever, headache, hookworm, infertility in women, leprosy, pneumonia, scorpion bite, snake bite, swelling caused by mumps, toothache, gastric ulcer, venereal diseases, heart diseases, dysentery and general weakness (1,2).

The Combretaceae consists of 18 genera, the largest of which are *Combretum* with about 370 species and *Terminalia* with about 200 species (3). Species of the *Terminalia* are most widely used for medicinal purposes, common and widely distributed throughout western and southern Africa (3). Among the *Terminalia*, we were interested in *Terminalia superba*. *Terminalia superba* Engl. et Diels is a dense humid forest species widespread from Guinea to Zaïre (Democratic Republic of Congo). *T. superba* is a large tree which 30-50 m high (4). Its stem bark is used in folk medicine in the treatment of gastroenteritis, diabetes, female infertility and abdominal pain (4). The genus *Terminalia* is known as a rich source of triterpenoids and their glycoside derivatives, flavonoids, tannins and other aromatic compounds (5-10).

In continuation of our phytochemical and pharmacological studies on bioactive constituents of African tropical plants (11,12), we have investigated the stem bark extract of *Terminalia superba* (Combretaceae).

The aim of our study therefore was to evaluate the analgesic activities of the extract obtained from this plant by in-vivo screening methods. This paper also deals with the preliminary phytochemical study in order to characterize the compounds which could be responsible of the activity.

Materials and Methods

Plant material

The stem bark of *Terminalia superba* (Combretaceae) was collected in May 2003 around Yaounde – Cameroon and identified by the Nole Tsabang (Institut de Recherches Médicales et d'Etudes des Plantes Médicinales, IMPM Yaoundé). A voucher specimen No. 19652/HNC collected by Leeuwenberg (No. 5963) has been deposited at Yaounde Herbarium, Cameroon.

Extraction

Dried powdered stem bark (100 g) of *T. superba was* refluxed with MeOH–H_2O (7: 3, 200 ml x 4) and evaporated to dryness yielding 20 g of MeOH–H_2O extract. This MeOH–H_2O extract was dissolved in water (200 ml) and partitioned successively with hexane, CH_2Cl_2 and water-saturated *n*-BuOH (each 3 x 200 ml) yielding after evaporation of the solvents the corresponding hexane (160 mg), CH_2Cl_2 (125 mg) and *n*-BuOH (2 g) fractions.

In order to identify the main chemical groups, the butanol fraction was chromatographed on TLC (silica gel plates 60 F_{254}) using AcOEt-HCOOH-CH_3COOH-H_2O (100:11:11:26) or $CHCl_3$–MeOH–H_2O (13: 7: 2; lower phase) as the mobile phase. TLC was observed under UV_{254nm} and UV_{366nm}, before and after spraying with natural products reagent NP/ PEG or Komarowsky reagent respectively. The pharmacological trials were carried out with dry material dissolved in 5% DMSO.

Animals

Swiss Albino mice (20–30 g) of either sex were used for this study. The animals were fed with rat pellet food and water supplied ad libitum. All animals were acclimatized to the laboratory environment for at least 1 week before the experimental session. The "principle of laboratory animal care" was followed in this study (13).

Acetic acid-induced pain

This test was performed as described by Collier et al.(14)and Fontenele et al.(15). Acetic acid (0.7% v/v) was administered i.p. in a volume of 0.1 ml/10 g. Vehicle (saline), acetylsalicylic acid (20 mg/kg) and *T. superba* extract (50, 100 and 200 mg/kg) were administered p.o. 30 min before acetic acid injection. Each group was composed of 7 mice. The number of writhing and stretching produced in each group for the succeeding 15 min was counted and compared to the response in the control group as described by Koster et al. (16). Immediately after the injection of the algic compound, each animal was isolated in an individual box (24 x 11 x 10 cm) to be observed during 15 min. The number of writhing and stretching was recorded and permitted to express the percentage of protection using the following ratio: (Control mean — treated mean) x 100/control mean.

Formalin induced nociceptive response

This test was carried out as described by Hunskaar and Hole (17). Animals were injected subcutaneously with 20 µl of formalin into the dorsal hind paw. *T. superba* extract (50, 100 and 200 mg/kg), vehicle (saline 10 ml/kg) and acetylsalicylic acid (20 mg/kg) were administered p.o. 30 min before formalin injection. Each group was composed of 7 mice. The time the mice spent licking or biting the injected paw or leg was recorded. On the basis of the response pattern described by Tjolsen et al.(18), two distinct periods of intensive licking activity were identified and scored separately. The first period (early phase) was recorded 0-5 min after the injection of formalin and the second period (late phase) was recorded 20-30 min after the injection. The percentage inhibition of licking was calculated by the formula: (C — T)/(C) x 100; where C represents the vehicle treated control group value for each phase and T represents the treated group value for each phase.

Hot plates model

The mice were first treated with different doses of *T. superba* extract (50, 100 and 200 mg/kg) after 1 h of extract administration, they were placed on a hot plate maintained at 55 ± 1.0 °C. The time taken by the animals to lick the fore or hind paw or jump out of the plate was taken as the reaction time. Morphine (5 mg/kg, s.c.) was used as reference drug. The mice which reacted within 15s and which did not show large variation tested on four separated occasions were selected for studies.

Statistical analysis

The results were expressed as mean ± s.e.m. The statistical analysis involving two groups was performed by means of non parametric test of Mann-Whitney, whereas ANOVA, followed by Dunnett comparison test, was used in order to compare more than two groups. All data were processed with SPSS software (Sigma stat 2.03). $P < 0.05$ significant.

Results

Chemical analysis

A preliminary chemical analysis was carried out on the n-BuOH fraction of the MeOH-H_2O extract of the stem bark of *T. superba*. After spraying the TLC plate by NP/PEG reagent many orange, yellow and fluorescence blue zones were observed in the Rf range 0.30 – 0.80 indicating the presence of a number of flavonoids.

Another TLC plate after spraying by Komaroswky reagent and heating to about 100°C revealed many violet zones were observed, indicating the presence of terpenoids and a large band brown characteristic of tannins.

Analgesic effects

As can be seen from Table 1, butanolic fraction of *T. superba* significantly reduced the number of writhings and stretchings induced by a 0.7% acetic acid solution in dose dependent manner. This protective effect reached 39.1, 55.1 and 62.2% at doses of 50, 100 and 200 mg/kg, respectively. Acetylsalicylic acid exerted a significant protective effect, inducing a protection of 44.1 % at a dose of 20 mg/kg.

Table1:Analgesic effect of the butanolic fraction from *T. superba* on the acetic acid-induced nociception.

Group	Dose (mg/kg)	No. writings (Mean ± s.e.m)	% Inhibition
Control		31.3 ± 0	
Acetylsalicylic acid	20	17.2 ± 4.0*	44.9
T. superba	50	19.0 ± 3.0*	39.1
T. superba	100	14.0 ± 3.0*	55.1
T. superba	200	11.8 ± 4.0 *	62.2

Values are mean ± S.E.M; n = 7; * P< 0.05; as compared to the control.

In the formalin test (Table 2) inhibition occured as well in the first phase as in the second phase. The inhibition at the early phase reached 59.4 and 51.0 % at a dose of 50 mg/kg and 200 mg/kg respetively. The dose-dependent protective effect observed in the second phase reached 30.61, 41.30 and 63.75 % at doses of 50, 100 and 200 mg/kg. Acetylsalicylic acid was efficient on the late phase (37.7% inhibition at 20 mg/kg).

Table 2: Analgesic effect of the butanolic fraction from *T. superba* on the formalin induced nociception

Group	Dose (mg/kg)	Licking times (s)		% inhibition	
		Early phase (1-6 min)	Late phase (20-30 min)	Early phase	Late phase
Control		77.1 ± 9.6	56.5 ± 5.0		
Acetylsalicylic acid	20	67.0 ± 4.6	35.3 ± 1.8 *	13.1	37.50
T. superba	50	31.3 ± 7.9	39.2 ± 8.0	59.4	30.52
T. superba	100	39.7 ± 5.5*	17.2 ± 2.5 *	48.5	41.30
T. superba	200	37.8 ± 1.0*	20.3 ± 5.8 *	51.0	63.95

Values are mean ± S.E.M; n = 7; * P< 0.05; as compared to the control.

One hour after treatment of the mice with the BuOH fraction of *T. superba* and morphine (i.p, 5 mg/kg), the latency to jumping or licking the hindpaws on a hot plate was determined. Table 3 shows that the treatment with *T. superba* has no effect while the latency times was significantly altered by treatment with morphine (5mg/kg).

Table3: Effect of the butanolic fraction from *T. superba* on the hot-plate model.

Group	Dose (mg/kg)	Latency times (s)
Control		5.16 ± 0.8
Morphin	5	18.0 ± 0.5*
T. superba	50	5.1 ± 0.9
T. superba	100	5.3 ± 0.9
T. superba	200	5.6 ± 0.6

Values are mean ± S.E.M; n = 7; * P< 0.05; as compared to the control.

Discussion

The objective of the present work was to study the antinociceptive activity of *n*-BuOH fraction of *T. superba* using the writhing and formalin test in mice. Acetic acid produces a painful reaction and acute inflammation in the peritoneal area. The stimulation of peritoneal nociceptors is indirect and occurs through the release of endogenous substances, which stimulate nerve endings (19). A great increase occurs in prostaglandins E_2 and F_{2a} levels in peritoneal fluid after acetic acid injection, and the analgesic effect of substances similar to aspirin could be due to the blockade of prostaglandin synthesis. *n*-BuOH fraction of *T. superba* was able to significantly and dose-dependently inhibit the abdominal contractions induced by acetic acid, as well as the second phase of the response to formalin. The formalin test is considered a model for chronic pain (20). In this test, animals present two distinct nociceptive behaviour phases, which probably involve different stimuli. The first phase initiates immediately after formalin injection and lasts about 5 min, resulting from chemical stimulation of nociceptors. The second phase initiates about 20 min after formalin injection, lasts 20 to 40 min and seems to depend on a peripheral mechanism as well as a central one. While substance P and bradykinin are involved in the first phase, histamine, 5-HT, prostaglandins and bradykinin are involved in the second phase. The effect of *n*-BuOH fraction of *T. superba* was significant in the first and the second phase, indicating an action related to the inflammatory process.

n-BuOH fraction of *T. superba* showed no effect in the hotplate test, which involves higher brain functions and consists of responses to nociceptive stimuli organized at a supraspinal level (21). Morphine which is a centrally acting drug inhibited hot plate induced pain while analgesic drugs such as aspirin and paracetamol do not have any effect in this test (18).

Considering the relationship between the nature of the active principles and the pharmacological effects, another objective was the identified the type the compounds contained in the plant extract. In our phytochemical experiments, we have shown that *T. superba* fraction contain flavonoids, terpenoids and tannins. Because it has been reported that flavonoids and terpenoids have analgesic effect and because a number of previous studies have suggested that flavonoids may interact directly with the prostaglandin system (22,23), terpenoids and flavonoids can be responsible for the analgesic effects of *T. superba*.

In conclusion, n-BuOH fraction of *T. superba* stem bark could be beneficial in the management of pain. Thus, the results of this study confirm the traditional uses of *T. superba*. The terpenoids and flavonoids might be responsible for the analgesic activity.

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