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ANALGESIC EFFECT OF A TOTAL AQUEOUS EXTRACT OF *TERMINALIA SUPERBA* ENGL. AND DIELS (COMBRETACEAE) STEM BARK IN MICE AND RATS

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ABSTRACT

Pain is an unpleasant sensation and an emotional experience in response to real or partial tissue damage. Steroidal and non-steroidal anti-inflammatory drugs, analgesics used in modern medicine to relieve pain lead to gastrointestinal disorders, kidney and skin toxicities. Therefore, the search for new effective analgesic substances is necessary. The use of medicinal plants is becoming an important path to explore in order to discover drugs that induce fewer side effects. This study, conducted on *Terminalia superba*, a plant used in traditional medicine for the treatment of pain and various ailments in Côte d'Ivoire, aims to evaluate the analgesic potential of a total aqueous extract of *Terminalia superba* stem bark (ETATs). *Mus musculus mice* (20-30 g) and albino rats *Rattus norvegicus*, *wistar* strain (100-120 g), aged 8-12 weeks, were used for these tests. ETATs was prepared by infusion of 100 g of *Terminalia superba* stem bark powder in 1 L of distilled water at 100 °C for 15 min. The analgesic activity of ETATs was evaluated using experimental models of induction of peripheral pain (acetic acid), central pain (hot plate) and formalin pain testing. ETATs (125, 250 and 500 mg/kg bw), ASPIRIN® (100 mg/kg bw) and 9 ‰ NaCl solution (10 mL/kg bw) were administered orally to animals and tramadol (30 mg/kg bw) was administered intraperitoneally. The results indicate that ETATs significantly inhibited the pain sensation induced by acetic acid injection by 54.28% and increased the latency time of leakage or licking of the mouse paw induced by the hot plate by 27.60 ± 1.50 seconds. ETATs showed a significant antinociceptive effect against formalin-induced pain at 2.5% in rats during neurogenic (37.36%) and inflammatory (74.43%) phases. The total aqueous extract of *Terminalia superba* has peripheral and central analgesic properties similar to those of ASPIRIN® and tramadol respectively in rats and mice.

KEYWORDS: Analgesic, mouse, pain, rat, *Terminalia superba*.

INTRODUCTION

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Its pharmacological management requires the use of analgesic drugs. These drugs, although effective, can cause serious side effects. The search for powerful analgesic agents with minimal side effects remains the goal of many scientific studies.[1] These side effects push people living in urban and rural areas to use medicinal plants for treatment because of the low cost, its easy access, the inadequacy of modern health infrastructure and the high cost of modern health techniques, habits and customs.[2] *Terminalia superba*, a plant used in traditional medicine for the treatment of various ailments[3,4,5,6,7] has been the subject of several scientific studies. This work focused on antidiabetic properties,[8; 9] analgesic,[10] antioxidant,[11,9] antihypertensives,[12,13] antibacterial and antifungal,[14] antiulcers and the acute oral toxicity.[15,16] However, despite these multiple therapeutic properties, no scientific study has yet been mentioned on analgesic activity of the total aqueous extract of *Terminalia superba* stem bark to confirm the use of this plant in the treatment of abdominal pain in Côte d'Ivoire. Therefore, this study aims to evaluate the analgesic potential of this extract in rats and mice.

MATERIALS AND METHODS

1. Materials

1.1. Plant

The plant material consists of *Terminalia superba* stem bark. The plant was identified thanks to samples preserved respectively under the numbers 2456 of June 4th, 1954 and 416 of April 3rd, 1974 at the national herbarium of Côte d'Ivoire and authenticated by the National Floristics Center (CNF) of Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire).

1.2. Animals

The animal material consists of albino rats (*Rattus norvegicus*), *Wistar* strain weighing between 100 g and 120 g and mice (*Mus musculus*) weighing 20-30 g. These animals were 8 to 12 weeks old and had free access to water and pellet food (IVOGRAIN® pellets). The photoperiod was 12 hours light and dark circle. These animals were treated according to good laboratory practices.[17]

1.3. Reagents and pharmacodynamic drugs

The reagents and pharmacodynamic substances used were acetylsalicylic acid (ASPIRIN®) (Cipharm, Côte d'Ivoire) and tramadol (TRABAR®) (Merckle, Blaubeuren-Weiler, Allemagne) as a reference anti-inflammatory and analgesic, formalin solution, acetic acid (Prolabo, France) and normal saline (NaCl, 9‰) as physiological reference solution.

2. Methods

2.1. Preparation of the total aqueous extract of *Terminalia superba*

The total aqueous extract of *Terminalia superba* stem bark (ETATs) was prepared according to the method.[15] The stem bark of *Terminalia superba* was washed with distilled water, cut into small pieces and dried in an oven (Heto, type CD 52- I, France) at 45 °C for 5 days and powdered using an electric grinder (Culati, France). 100 g of *Terminalia superba* stem bark powder were infused for 15 min in 1 L boiled distilled water. The aqueous solution obtained was filtered on hydrophilic cotton and on Whatman n°3 filter paper. Half a liter of boiled distilled water at 50 °C was added to the residue for another 10 minutes infusion. This solution was also filtered. The filtrates were evaporated and dried in an oven at 45 °C, for 48 h to obtain 11.56 g of black brown powder to prepare the total aqueous extract of *Terminalia superba* stem bark (ETATs).

2.2. Anti-nociceptive effect

These tests aim to determine the decrease in pain induced by chemical agents (acetic acid, formalin) or thermal (hot plate) in the presence of ETATs.

2.2.1. Peripheral analgesic activity by the abdominal contortion test or Writhing test

The protocol described by some authors,[18] and modified by others[19] was adopted to assess analgesic activity. Its principle is the intraperitoneal injection of 0.6% acetic acid in mice inducing a pain syndrome characterized by contortions (stretching movements of the hind paws and stretching movements of the dorso-ventral muscle). For this study, 5 homogeneous batches of 5 mice, previously fasted for 16 hours before the experiment, were formed. The animals in lot 1 (Control NaCl) were gavaged with normal saline (NaCl, 9 ‰) at 10 mL/kg bw. Mice in batches 2, 3 and 4 received by gavage ETATs at doses of 125 ; 250 and 500 mg/kg bw. The mice in lot 5 (positive control) were orally administered with acetylsalicylic acid (ASPIRIN®) at 100 mg/kg bw. One hour after administration of these different substances, an intraperitoneal injection of 0.6% acetic acid was performed to each mouse at dose of 0.2 mL per 20 g bw. After injection of acetic acid, the pain syndrome was counted for 20 minutes. The percentage inhibition of cramps that expresses the analgesic activity was determined according to the formula.[20]

% Inhibition= [(Nb $_{control}$ - Nb $_{treated}$) / Nb $_{control}$] X 100.

Nb $_{control}$: number of contortions of mice of the control lot (NaCl, 9 ‰).

Nb $_{treated}$: number of contortions of mice in the treated batch.

2.2.2. Central analgesic activity by hot plate test or hot plate test

The hot plate test was used to assess central analgesic activity according to the method previously described by some researchers with minor changes.[21] The principle was to explore the central analgesic activity of substances using a thermal stimulus at the level of the hind paw. A pre-test was performed and selected the mice for the test. The mice were deposited in an aluminum bowl on a hot plate at a temperature of 54 °C. The latency time set for leaking (jumping) or licking the back paw was determined using a stopwatch. Mice with a latency not exceeding 15 seconds were selected for testing. They were divided into 5 batches of 5 mice each. They were then, respectively treated with the physiological solution of 9 ‰ NaCl at a rate of 0.1 mL/10 g bw orally for lot 1 (control lot NaCl), by tramadol (reference analgesic) at the dose of 30 mg/kg bw intraperitoneally for lot 5 (positive control lot). Mice in batches 2, 3 and 4 received ETATs at doses of 125 ; 250 and 500 mg/kg bw orally, respectively. The latency time (in seconds) of each mouse was determined on the hot plate at intervals of 30 min, 1 h, 2 h and 3 h after administration of the different substances but taking into account the maximum time of stoppage of the test (30 seconds) in order to prevent tissue damage. For each dose, the percentage of latency inhibition was calculated using the formula.[22; 23]

% Inhibition = [(T $_{treated}$ –T $_{control}$) / (T $_{stop}$ - T $_{control}$] X 100.

T $_{treated}$: latency time of treated animals

T $_{control}$: latency time of control animals

T $_{stop}$: test downtime.

2.2.3. Formalin test

The method of some researchers was used to assess the analgesic activity of the aqueous infusion of T*erminalia superba* stem bark.[24] Five batches of 5 rats were used. The rats were fasted 16 hours before the experiment. Then, each rat was placed in a transparent plastic observation cage one hour before the substances were given into the experimental room to allow it to get used to its environment. Rats in lot 1 (control lot NaCl) were gavaged with 9 ‰ NaCl at 10 mL/kg bw. Batches 2, 3 and 4 were orally administered with the total aqueous extract of *Terminalia superba* respectively at doses of 125 ; 250 and 500 mg/kg bw. Lot 5 (positive control lot) received by gavage the reference drug (ASPIRIN®) at 100 mg/kg bw. One hour after administration of the substances, an injection of 50 µL formalin (2.5%) was applied in the right rear paw. The nociceptive effect, resulting in the licking of the paw, was counted for 0-5 minutes for the first phase and between 15-30 minutes for the second phase. The percent inhibition was determined according to the following formula:[25]

% Inhibition= [(Nb $_{control}$ - Nb $_{treated}$) / Nb $_{control}$] X 100.

Nb $_{control}$: number of licks of the rat paw of the control lot (NaCl, 9 ‰).

Nb $_{treated}$: number of licks of the rat paw of the treated batch.

2.3. Statistical analysis

The results obtained were given as an average followed by the standard error on the mean (M ± SEM). The statistical analysis was performed using Graph Pad Prism 5.01 software (San Diego, Californie, USA). The One-Factor Analysis of Variance (ANOVA1) followed by the Tukey comparison test were used to identify differences between the treated and control groups. The values were significant for $p < 0.05$.

RESULTS

1. Effect of aqueous extract of *Terminalia superba* stem bark on acetic acid-induced abdominal contortions in mice

Table 1 shows the effects of total aqueous extract of *Terminalia superba* stem bark and ASPIRIN® on the mean number of contortions caused by intraperitoneal acetic acid injection in mice. This injection made it possible to count experimentally after 20 minutes 42 ± 2.51 contortions. The number of cramps induced by acetic acid 0.6% was significantly reduced respectively, in batches treated with the total aqueous extract of *Terminalia superba* stem bark from 42 ± 2.51 to 25.4 ± 1.69 at the dose of 125 mg/kg bw, then to 19.2 ± 2.91 at the dose of 250 mg/kg bw and finally to 24.6 ± 4.38 contortions at 500 mg/kg bw. This corresponds to inhibition percentages of 39.52%, 54.28% and 42.85% respectively compared to the control lot. The highest inhibition was observed in the batch treated with the total aqueous extract of *Terminalia superba* at 250 mg/kg bw. ASPIRIN® caused a 40% inhibition compared to the control group. Apart from the total aqueous extract of *Terminalia superba* stem bark at 250 mg/kg bw which has a higher analgesic effect than ASPIRIN®, the total aqueous extract of *Terminalia superba* stem bark at doses of 125 and 500 mg/kg bw has an analgesic effect similar to that of ASPIRIN®.

Table 1: Effect of total aqueous extract of *Terminalia superba* on abdominal contractions after acetic acid injection.

Treatment and doses (mg/kg bw)	Average number of contortions	% Inhibition
NaCl control	42 ± 2.51	-
ETATs 125	25.4 ± 1.69**	39.52
ETATs 250	19.2 ± 2.91***	54.28
ETATs 500	24.6 ± 4.38**	42.85
ASPIRIN® 100	25.2 ± 2.52**	40.00

** *p < 0.01 ;* *** *p < 0.001 ; n = 5 : Differences were significant when values of treated groups were compared to that of control group NaCl. ETATs : Total aqueous extract of Terminalia superba stem bark.*

2. Effect of total aqueous extract of *Terminalia superba* on latency in hot plate testing or hote plate test

Table 2 shows the effect of the total aqueous extract of *Terminalia superba* stem bark on the latency time of leakage or licking of the mouse paw induced by a thermal stimulus. The latency recorded in mice in the control lot NaCl is between 6.64 ± 1.04 and 8.02 ± 0.55 seconds during this study. The analgesic effect of the total aqueous extract of *Terminalia superba* stem bark at doses of 125 ; 250 and 500 mg/kg bw increased during this experiment. Thus, from 30 minutes to 3 hours, the analgesic percentage of the extract increased from 23.71 to 35.06% for 125 mg/kg bw, from 44.19 to 62.87% for 250 mg/kg bw and from 45.95 to 89.56% for 500 mg/kg bw. The analgesic power of the total aqueous extract of *Terminalia superba* stem bark is greater at the dose of 500 mg/kg bw. This effect is dose-dependent. Tramadol at 30 mg / kg of pc has a maximum effect of 100% thirty minutes after its administration and this effect gradually fades at the 3rd hour when there is 76.12% inhibition of the latency time.

3. Effect of total aqueous extract of *Terminalia superba* on formalin-induced paw licking

Table 3 shows the anti-nociceptive effect of *Terminalia superba* and ASPIRIN® on the average number of licking of the paw induced in rats by formalin, 2.5%

injection. During the first phase (0-5 min), the sub-plantar injection of formalin into the right hind paw counted 18.2 ± 0.58 licks of the paw in the control lot NaCl. The number of licks of the right hind paw of rats treated with the total aqueous extract of *Terminalia superba* stem bark at doses of 125 ; 250 and 500 mg/kg bw was significantly inhibited by 35.16% ; 37.36% and 32.96% respectively compared to the control. ASPIRIN® also inhibited the number of paw licks of 41.76% compared to the control lot NaCl. During the second phase (15-30 min), the number of licks of the paw of the control lot NaCl was 106.4 ± 7.31. Treated with the aqueous total extract of *Terminalia superba* stem bark at doses of 125 ; 250 and 500 mg/kg bw and with aspirin® at 100 mg/kg bw, this number underwent a strong inhibition of 72.36% ; 68.04% ; 74.43 and 71.36% respectively. The total aqueous extract of *Terminalia superba* and ASPIRIN® presented substantially the same pain inhibitory power at this phase. The pain induced by formalin solution injection to rats was inhibited significantly by the aqueous total extract of *Terminalia superba* and ASPIRIN®.

Table 2: Effect of the total aqueous extract of *Terminalia superba* on the latency time of leakage or licking of the rear paw of the mouse induced by the hot plate.

Treatment and doses (mg/kg bw)	Average latency (s) / Analgesic percentage (%) parenthetically				
	0 h	30 min	1 h	2 h	3 h
NaCl control	6.64 ± 1.04	7.10 ± 1.09	7.52 ± 0.85	8.02 ± 0.55	7.01 ± 0.82
ETATs 125	7.70 ± 1.11	14.21 ± 1.33 (31.05)	12.85 ± 1.78 (23.71)	13.98 ± 1.79 (27.12)	15.53 ± 1.84 (37.06)
ETATs 250	8.46 ± 1.24	17.70 ± 4.21* (46.29)	18.81 ± 2.22*** (50.22)	21.84 ± 2.75** (62.87)	17.17 ± 3.31 (44.19)
ETATs 500	7.41 ± 1.44	19.19 ± 2.91* (52.79)	17.85 ± 1.43*** (45.95)	22.35 ± 2.80** (65.19)	27.60 ± 1.50*** (89.56)
Tramadol 30	8.67 ± 1.60	30 ± 0.00*** (100.00)	29.69 ± 0.31*** (98.62)	26.82 ± 1.97*** (85.53)	24.51 ± 3.37*** (76.12)

** p < 0.05 ; ** p < 0.01 ; *** p < 0.001 ; n = 5 : Differences were significant when values of treated groups were compared to that of control group, at the same corresponding time (same column). ETATs : Total aqueous extract of Terminalia superba stem bark.*

Table 3: Effect of the total aqueous extract of *Terminalia superba* on the licking of the paw induced by the formalin injection.

Treatment and doses (mg/kg bw)	First phase (0-5 min)		Second phase (15-30 min)	
	Average number of paw licks	% Inhibition	Average number of paw licks	% Inhibition
NaCl control	18.2 ± 0.58	-	106.4 ± 7.31	-
ETATs 125	11.8 ± 1.83	35.16	29.4 ± 4.25***	72.36
ETATs 250	11.4 ± 1.17	37.36	34 ± 8.46***	68.04
ETATs 500	12.2 ± 2.44	32.96	27.2 ± 2.85***	74.43
ASPIRIN® 100	10.6 ± 1.72*	41.76	29.8 ± 4.05***	71.99

** p < 0.05 ; *** p < 0.001 ; n = 5 : significant difference from the respective NaCl control for each phase. ETATs : Total aqueous extract of Terminalia superba stem bark.*

DISCUSSION

Abdominal contractions induced by acetic acid injection, hot plate test and formalin test were used to evaluate the analgesic effect of the total aqueous extract of *Terminalia superba* stem bark (ETATs).

The method of contractions induced by intraperitoneal injection of acetic acid in mice was used to study the peripheral analgesic effect of ETATs. ETATs inhibited abdominal contractions significantly with an inhibition percentage of 54.28% at a dose of 250 mg/kg bw. This analgesic effect could be related to the inhibition of the release of chemical mediators. Indeed, the pain caused by the injection of acetic acid is due to the release of serotonin, histamine, bradykinin, substance P and prostaglandins ($PGE_{2\alpha}$, $PGF_{2\alpha}$). These chemical mediators stimulate peripheral nociceptive neurons and induce increased vascular permeability.[26; 27] However, in this study, ETATs significantly decreased the number of abdominal contortions, suggesting that ETATs may decrease the release of these chemical mediators. Significant inhibition of the number of contortions shows that the total aqueous extract of *Terminalia superba* stem bark has a peripheral analgesic effect. These results are similar to those obtained by some researchers with the ethanolic extract of *Annona vepretorum* (25-100 mg/kg bw) which caused a 92% inhibition of acetic acid-induced contortions in mice.[28] Methanol extract from *Bryophyllum pinnatum* leaves (100-900 mg/kg bw) significantly inhibited acetic acid-induced contortions in mice by 83.79%.[29]

The central analgesic effect of this extract at doses of 125 ; 250 and 500 mg/kg bw increased during this experiment. Thus, from 30 minutes to 3 hours after oral administration of ETATs, the analgesic percentage of ETATs varied from 23.71 to 89.56%. ETATs inhibited hot plate-induced pain as did tramadol, a morphine derivative with central analgesic action reference in this study with a 100% inhibition percentage at the 1st hour. The effect of this extract could involve neuronal structures, because according to some authors, the hot plate method involves a spinal reflex, but also involves neuronal structures and makes it possible to identify central analgesics.[30] Thermal stimuli are selectively inhibited by central analgesics and not by peripheral analgesics.[31,32] ETATs therefore has a central analgesic activity due to the significant inhibition of pain caused by the hot plate. These results are similar to those of various authors. Indeed, according to some researchers, the hydroethanol extract of *Euterpe oleracea* administered orally at the respective doses of 30, 100 or 300 mg / kg bw has a central analgesic effect dose dependent on 39.1 ± 10 ; 51.9 ± 9.5 and 94.7 ± 4.4%.[33] Finally, researchers showed that arachic acid ethyl ester isolated from Cameroonian propolis significantly inhibits the sensation of pain caused by the hot plate in rats with an analgesic percentage of 81% compared to the control rat that received refined palm oil.[34] On the other hand, these results are contrary to those of the methanol extract *Bryophllum pinnatum* leaves which had no analgesic activity against thermal stimuli.[29]

The formalin test was used to evaluate the anti-nociceptive effect of ETATs. The pain induced by formalin solution injection to rats was inhibited by ETATs with percentage inhibitions ranging from 32.96 to 37.36% for the 1st phase and from 68.04 to 74.43% for the 2nd phase. This result suggests that ETATs could suppress the early and late phases of the acute inflammatory response. Indeed, the injection of formalin solution into rats causes a biphasic response. The first phase is triggered immediately after the injection of formalin solution and is characterized by the stimulation of C fibers and the release of substance P and bradykinin. The second phase is due to local inflammatory pain caused by the production of serotonin, histamine and prostaglandins.[35,36] Central analgesics such as opioids inhibit both phases while peripheral analgesics (NSAIDs) with the exception of aspirin and paracetamol antagonize the inflammatory phase.[37; 38] This study showed that ETATs inhibited both phases caused by the formalin solution injection with a significant analgesic effect in the second phase. This result suggests that the extract acts like NSAIDs and could also act like opioids. Thus, the extract could reduce the production of the different chemical mediators involved in both phases of the inflammatory response. These results are similar to those obtained with the hydroalcoholic extract from the seed decoction of *Euterpe oleracea* fruits, at doses ranging from 30 to 300 mg /kg of pc, which inhibited respectively the neurogenic and inflammatory phases of pain caused by the formalin solution of 36.4 ± 5.3 seconds and 90.1 ± 15.2 seconds.[33] The same is true for the methanol extract of *Persicaria hydropiper* leaves (20-75 mg/kg bw) which inhibited the nociceptive pain induced by formalin solution in mice of 94.02%.[39] Finally, the combined extract of hydroalcoholic extracts of *Malva sylvestris*, *Carum carvi* and *Medicago sativa* in the proportions (300 / 300 / 150 mg/kg bw) significantly inhibited the nociception in the acute phase of the formalin test in rats.[40]

All of these results relating to the peripheral and central analgesic effects as well as the anti-nociceptive effect corroborate those of some researchers which showed that the butanolic fraction from the methanol extract of *Terminalia superba* at doses between 50 and 200 mg/kg bw has analgesic effects.[10]

CONCLUSION

This study showed that the total aqueous extract of stem bark of *Terminalia superba* has remarkable peripheral and central analgesic properties similar to those of ASPIRIN® and tramadol. These results may justify the traditional use of this plant by traditherapists in the treatment of abdominal pain, pain related to peptic ulcers and headaches.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to disclose.

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