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Evaluation of Antalgic Activity and Trace Elements Analysis of *Trema guineensis* Extracts in Acetic Acid Induced in Rats

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Abstract

We evaluated centrally acting analgesic potential using tail immersion test and peripheral pharmacological actions using acetic acid induced writhing test in rats. The crude extracts of *Trema guineensis* leave plant were found to have significant analgesic activity at the intraperitoneal dose of 200 mg/kg body weight in two models. In the tail immersion test *Trema guineensis* increased significantly ($P < 0.001$) in comparison to the control group (NaCl). The ethanolic (7.57 ± 0.14 s) and aqueous (8.88 ± 0.12 s) extracts reached their maximum in reaction time successively at 30 and 45 min. The aim of this study is also to determine the trace elements content in suffering rats of pain induced by acetic acid, using Atomic Spectrophotometer Absorption. Our results showed that trace element concentrations in *Trema guineensis* ethanolic extract in rats were as follows: Zn (6.92 ± 0.39 mg/L), Fe (37.95 ± 0.04 µmol/L), Cu (8.16 ± 0.12 mg/L) and Mn (0.56 ± 0.12 mg/L). We found following values with aqueous extract compared to NaCl as control: Zn (5.03 ± 0.47 mg/L), Fe (31.08 ± 0.07 µmol/L), Cu (6.69 ± 0.53 mg/L) and Mn (0.51 ± 0.07 mg/L). The intake of trace elements by TGE would lessen the pain generated in acetic acid in rats; this action is much more remarkable with the ethanol extract.

Keywords

***Trema guineensis*, Oligosol, Trace Elements, Acetic Acid, Pain**

Subject Areas: **Biochemistry**

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1. Introduction

Nature has been a source of medicinal agents for thousands years and an impressive number of modern drugs have been isolated from natural sources; many of them based their use in folk medicine [1].

About 50% to 60% of pharmaceutical products are natural origin or synthesized from natural products [2] [3].

However, scientific studies have been conducted only to a limited extent with few medicinal plants [4]. That is also the case of *Trema guineensis* leaves which is empirically used for sore throat, cough and hypertension treatment [5].

The *in vivo* antiiflammatory of this plant has been demonstrated by KOUAKOU *et al*. (2015) [6]. Its phytochemical analysis indicated the presence of several secondary metabolites such as polyphenols, alkaloids, flavonoids, saponosids and tannins. The presence of these compounds could confer to the plant, several pharmacological activities including the analgesic activity. No scientific report regarding the *in vivo* analgesic activity of *Trema guineensis* Extract (TGE) has been published. That's why, we have now evaluated the analgesic properties of *Trema guineensis* ethanol and aqueous extracts in acetic acid induced in rats. The bioelements concentration (iron, zinc, copper and manganese) may be modified during the pain in rats after administration of various extracts of *Trema guineensis*.

We therefore evaluated the effect of extracts on trace elements in pain with rat model induced by acetic acid.

2. Material and Methods

2.1. Material

2.1.1. Plant Material

The fresh leaves of *Trema guineensis* were collected in Abobo (Abidjan) in 2015. The plant material was identified by Professor Aké Assi (Félix Houphouet University). They were dried under a shade during two weeks and pulverized using the crushing assistance (IKAMAG RCT®). The powder of leaves obtained, constituted our sample to be analyzed.

2.1.2. Drugs and Reagents

Acetic acid was obtained from Merck, Germany. Diclofenac sodium (Diclofam® MAX) was obtained from Square Pharmaceuticals Ltd., Cote d'Ivoire. Oligosol (Labcatal, industrial area of White Mount-France).

2.1.3. Aqueous Extract Preparation

The powder of *Trema guineensis* was used to prepare the various extracts.

One (1) hundred grams of the powder were extracted in 1 L of distilled water. The mixture obtained was then homogenized using a Mixor during 24 hours. The homogenate obtained is filtered successively twice on absorbent cotton then once on Wattman N˚1 filter paper. The filtrate was carried thereafter to evaporation in a drying oven with 50˚C during 48 hours. We obtained this way a powder which constituted the aqueous total extract used for the preparation of the various products concentrations [7].

2.1.4. Ethanolic Extract Preparation

100 g of *Trema guineensis* powder were extracted in one liter (1 L) of ethanol-water mixture (70/30 v/v). Following unfolds as aqueous extraction.

Aqueous and ethanolic extracts obtained starting from these powders of leaves were used to make the studies of analgesic activity and trace elements analysis [8].

2.1.5. Experimental Animals

1) Animals

Wistar rats of either sex (weighing 145 - 250 g) were obtained from the animal house of FELIX Houphouet Boigny University, Abidjan. These animals were housed under standard environmental conditions. The rats were fed with FACI® (Fabrication d'Aliments de Côte d'Ivoire) pellets, groundnuts and dried fish. Their drink was tap water [9].

2.2. Methods

Analgesic Activity

1) Acetic acid-induced abdominal writhing test

The animals were divided into 5 groups of 5 rats each.

Group I: Saline water (0.9%); the healthy animals used as vehicle.

Group II: Saline water (0.9%); the negative control.

Group III: Piroxen (20 mg/kg).

Group IV: Aqueous extract of *Trema guineensis* (200 mg/kg, *i.p*).

Group V: Ethanolic extract of *Trema guineensis* (200 mg/kg, *i.p*).

30 minutes later, each rat was given 0.5 mL intraperitoneal injection of 0.6% acetic acid (10 mL/kg) except the group I which was the normal control. The writhing response per animal was recorded five minutes after acetic acid injection for duration of ten minutes. A writhe was indicated by abdominal contraction and stretching of the hind limbs [10] [11]. The analgesic activity was expressed as percentage inhibition of abdominal contraction between control group and extract treated groups. The percentage inhibition was calculated using formula [12]:

$$\left(N - Nt / N\right) \times 100$$

where

N: Average number of writhes in control group.

Nt: Average number of writhes in test group.

2) Tail immersion test

The procedure is based on the observation that diclofenac-like drug is selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55˚C.

Rats were randomly allotted to 5 groups of 5 animals each. The lower 5 cm portion of the tail was immersed in a water bath maintained at 55˚C ± 0.5˚C. Distilled water (0.9%) was given to the control while the reference group was given diclofenac. The remaining groups were administered 200 mg/kg i.p of *Trema guineensis* ethanolic and aqueous extracts. The time in seconds for tail withdrawal from the water was taken as the reaction time. Following administration of the samples, reaction time was measured at 0, 15, 30, 45 and 60 min [13] [14].

Trace elements analysis of *Trema guineensis* extracts in acetic acid induced in rats.

2.3. Analytical Procedures

2.3.1. Acetic-Acid Induced in Rats

After 16 hours fasting, the rats that lifted their tails from the hot water within around 22 s were selected for the study. The selected rats were then divided into five groups (n = 6). Group I received saline water (0.9%) intraperitoneally (i.p) and served as control (healthy animals). Group II served as negative control group and received saline water by i.p. Group III and IV animals received successively *Trema guineensis* ethanolic and aqueous extracts at dose of 200 mg/kg. With Group V, it was administered 0.5 mg/L of oligosol as standard drug of trace elements (Zn, Cu, Mn). Group VI was a standard drug (Hemafer) of iron. 1 h after-treatement, each rat was given 0.5 mL intraperitoneal injection of 0.6% acetic acid (10 mL/kg) except the group I which was the control. All animals were sacrificed and blood samples were collected into heparin-treated collection tubes.

2.3.2. Blood Analysis

Blood analysis included trace elements: total iron, zinc, copper and manganese.

The plasma was separated and de-proteinisation was done by placing 1.0 mL of plasma in the test tube and adding 3 mL of 2 M HCl [15]. Chloridric acid constituted the diluant of all the plasma (1:20).

The clear supernatant was aspirated into the flame atomic absorption spectrophotometer (AAS) with a SpectrAA 20 (Varian Techtron, Springvale, and AUS) after adjusting the wavelength at 324.8 nm, 248.3 nm, 279.5 nm and 307.6 nm for copper, iron, manganese and zinc estimation respectively. This model Flame Atomic Absorption Spectrophotometer (FAAS) equipped with hollow cathode lamps was used for trace elements determinations. The acetylene-air flame in the FAAS was used as described in the manufacturer's instructions for the spectrophotometer.

The optimum working was 0.02 - 5 µg/mL for Mn 0.1 - 24 µg/mL for Cu, 0.01 - 2 µg/mL for Zn, and 0.06 - 15 µg/mL for Fe. The concentrations were displayed electronically and the results were expressed in mg/L.

2.3.3. Statistical Analysis

The values expressed as Mean ± SEM from 6 or 5 animals. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnet, s t-test, $P < 0.05$ were considered as significant.

3. Results

3.1. Acetic Acid-Induced Abdominal Writhing Test

Ethanolic and aqueous extracts significantly decreased the writhes number when compared to the control (**Table 1**). Ethanolic extract in dose of 200 mg/kg body weight ($g \cdot kg^{-1}b \cdot wt$) reduced the writhes numbers by 41.44%.

The group of treated rats with piroxen in dose of 20 mg/kg body weight was 06.20 ± 1.30 writhes compared to 22.20 ± 3.63 writhes of the control group; thus piroxen reduced the writhes by 72.07%.

3.2. Immersion Method of Tail Rat

In this study, the analgesic effects of ethanolic and aqueous leaves extracts of *Trema guineensis* increased significantly ($P < 0.001$) in comparison to the control group. The maximum effect of aqueous extract was observed at dose of 200 mg/kg at 45 min, which showed a reaction time 6.61 ± 0.16 seconds as compared to NaCl group which showed a reaction time 5.87 ± 0.13 seconds (**Table 2**).

The ethanolic extract (7.57 ± 0.14 s) and the standard drug (8.88 ± 0.12 s) reach their maximum time successively to 30 and 45 min.

3.3. Bioelements Concentration

Trace elements analysis of *Trema guineensis* extracts in acetic acid induced in rats indicated in **Table 3**.

Table 1. Effect of *Trema guineensis* extracts on acetic acid induced writhing in Wistar rats.

Groups N = 5 animals	Doses ($g \cdot kg^{-1}b \cdot wt$)	Mean of writhes number ± S.E.M.	Writhes inhibition (%)
NaCl	-	22.20 ± 3.63	-
NaCl + acetic acid	-	49.41 ± 2.61***	-
Piroxen	20	06.20 ± 1.30***	72.07
Aqueous extract+ acetic acid	200	15.20 ± 0.84***	31.53
Ethanolic extract+ acetic acid	200	13.00 ± 2.24***	41.44

***Indicates statistical difference between the vehicle group (NaCl) and the other treated groups. $P < 0.001$ n = 5 in each group.

Table 2. Effect of ethanolic and aqueous extracts of *Trema guineensis* on tail immersion test in rats.

Groups N = 5 animals	Dose (mg/kg)	Basal reaction time (0 s)	Reaction time (min)			
			15	30	45	60
NaCl	-	3.21 ± 0.18	3.50 ± 0.18	4.48 ± 0.15	5.87 ± 0.13	3.60 ± 0.14
NaCl + acetic acid	-	1.33 ± 0.11**	1.49 ± 0.25***	1.89 ± 0.19***	2.3 ± 0.28***	2.45 ± 0.31*
Aqueous extract + acetic acid	200	2.94 ± 0.15**	4.52 ± 0.17***	5.30 ± 0.14***	6.61 ± 0.16***	5.02 ± 0.14***
Ethanolic extract + acetic acid	200	3.49 ± 0.15**	4.98 ± 0.11***	7.57 ± 0.14***	5.61 ± 0.14ns	4.55 ± 0.12***
Piroxen+ acetic acid	10	3.00 ± 0.12*	4.47 ± 0.16***	6.59 ± 0.16***	8.88 ± 0.12***	7.08 ± 0.12***

*, ** and ***Indicate difference with vehicle treated group at $P < 0.05$, 0.01 and 0.001 respectively. Values are given as mean ± SEM of 5 animals each.

Table 3. Trace elements contents in plasma from treated rats with *Trema guineensis* extracts, at dose of 200 mg/kg after acetic acid administration.

Groups N = 6 animals	Dose/concentration ($g \cdot kg^{-1}b \cdot wt$ or mg/mL)	Fe (µmol/L)	Zn (mg/L)	Cu (mg/L)	Mn (mg/L)
NaCl	-	25.51 ± 0.26ns	3.20 ± 0.26*	5.12 ± 0.96*	0.20 ± 0.07**
NaCl + acetic acid	-	24.90 ± 0.19	1.72 ± 0.24	2.78 ± 0.37	0.07 ± 0.12
Aq Ext + acetic acid	200	31.08 ± 0.07***	5.03 ± 0.47***	6.69 ± 0.53***	0.51 ± 0.07***
Eth Ext + acetic acid	200	37.95 ± 0.04***	6.92 ± 0.39***	8.16 ± 0.12***	0.56 ± 0.12***
Oligosol + acetic acid	0.4	-	7.37 ± 0.49***	8.91 ± 0.16***	0.66 ± 0.37***
Hemafer + acetic acid	50	47.67 ± 0.18***	-	-	-

Each value is the average of several rats ± SEM (standard error of the mean) N = 5 rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. One-way ANOVA followed by Dunnet multiple statistically significant comparisons. Test compared to control (NaCl).

4. Discussion

Any injury or tissue damage is associated with pain. Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptors site of pain while centrally acting analgesics not only raise the threshold for pain but also alter the physiological response to pain and suppress the animals anxiety and apprehension. Pain is an essential prelude to the repair process [16].

The acetic acid induced writhing method is an effective method to evaluate peripherally active analgesics. The abdominal constriction response induced by acetic acid is a sensitive method to test peripherally acting analgesics. Hyperalgesia, induced by the injection of acetic acid, is characterized by contraction of the abdominal muscle accompanied by body elongation and an extension of forelimbs [17]. Tail immersion response believed to be spinally mediated reflex.

Various peripherally acting analgesic drugs such as ibuprofen, aspirin, Piroxicam (Piroxen) and indomethacin have been reported to inhibit acetic acid induced writhing [18] [19].

In the two models used, through the data showed that both extracts significantly inhibited the pain created in rats compared to control NaCl but the ethanol extract had a superior analgesic effect than the aqueous extract.

The observed analgesic activities of *Trema guineensis* may be due mainly to flavonoids and alcaloids contained in this plant. Previous report has demonstrated that the leaves of *Trema guineensis* were rich in flavonoids and alkaloids. Much of its therapeutic activity is attributed to these flavonoids and alkaloids [20].

In trace elements study we used Abdominal Writhing and Tail immersion tests as an animal model to induce acute pain and evaluated our plant components (Cu, Mn, Zn and Fe) during pain induced by acetic acid. The variation of these trace elements concentration would be due to several shave.

N-methyl-D-aspartate (NMDA) receptor is one of the major receptors in pain processes and many studies have shown competitive or noncompetitive inhibitors of it can reduce pain sensation [21]-[23]. So may be in our study, zinc induced its analgesic effect by blocking NMDA receptors. On the other hand Zinc reduced the release of glutamate by increasing GABA as an inhibitory neurotransmitter and this can be another possible way that induced analgesic effect of Zn [24] [25].

Several pathologies including atherosclerosis and arthritic are accompanied by painful process. Copper is used for pain treatment associated with these pathologies. The acetic acid used to induce pain, causes gastric ulcer in rats according to Jainu *et al.* (2006). The manganese might possess gastro-protective activity [26] [27].

The trace elements values obtained compared with untreated animals group (NaCl + acetic-acid) revealed *Trema guineensis* extracts efficacy in ameliorating diseases accompanied by acute pain.

In the iron case there is no significant difference between the healthy group (25.51 ± 0.26 s) and the group having received acetic acid and untreated (24.90 ± 0.19 s); this implies the pain created by acetic acid did not influence iron concentration in rats.

However ethanolic and aqueous extracts administration to certain groups of rats (treated rats), supported a significant increase of iron concentration.

These values compared with witness group (NaCl + acetic acid) translate a good supplementation of *Trema guineensis* sheets extracts into iron.

5. Conclusion

We can confirm that ethanolic and aqueous extracts of *Trema guineensis* whole are endowed with central and peripheral analgesic properties. However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of extracts can be conducted for further pharmacological such as the research of the mechanisms involved in the central and peripheral analgesic effect. The intake of trace elements by TGE would lessen the pain generated in acetic acid in rats.

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