Olufunmilayo O. Adeyemi*, Ismail O. Ishola, Elizabeth T. Adesanya and Destiny O. Alohan

# Antinociceptive and anti-inflammatory properties of *Tetracera alnifolia* Willd. (Dilleniaceae) hydroethanolic leaf extract

https://doi.org/10.1515/jbcpp-2016-0190
Received December 17, 2016; accepted August 4, 2018

**Abstract**

**Background:** *Tetracera alnifolia* Willd. (Dilleniaceae) is used in traditional African Medicine for the treatment of headache, abdominal pain, and rheumatism. Hence, this study sought to investigate the antinociceptive and anti-inflammatory effects of the hydroethanolic leaf extract of *T. alnifolia* (HeTA) in rodents.
**Methods:** Antinociceptive activity was evaluated using the acetic acid-induced writhing, formalin-/capsaicin-induced paw licking and hot plate tests in mice. The contribution of opioidergic, L-arginine-nitric oxide, and ATP-sensitive potassium channel pathways in HeTA-induced antinociception was also evaluated. The anti-inflammatory effect was assessed using the carrageenan-induced paw edema, xylene ear edema, cotton pellet granuloma, and complete Freund's adjuvant (CFA)-induced arthritis in rats.
**Results:** HeTA (100, 200, and 400 mg/kg, p.o.) produced significant ($p < 0.05$) decrease in mean number of acetic acid-induced writhing, time spent licking paw in formalin, and capsaicin tests as well as time course increase in nociceptive reaction latency in hot plate test. HeTA-induced antinociception was prevented by pretreatment of mice with naloxone (non-selective opioid receptor antagonist), L-arginine (nitric oxide precursor), or glibenclamide (ATP-sensitive potassium channel blocker). HeTA (100 mg/kg, p.o.) produced a significant anti-inflammatory effect against carrageenan-induced rat paw edema (1–5 h), xylene-induced ear edema, cotton pellet-induced granuloma formation, and CFA-induced arthritis in rats. The effects of HeTA in various models were similar to the effect of the standard reference drugs.
**Conclusions:** Findings from this study showed that HeTA possesses antinociceptive effect possibly mediated through peripheral opioid receptors with activation of L-arginine-nitric oxide and ATP-sensitive potassium channel pathway as well as anti-inflammatory activity.

**Keywords:** ATP-sensitive potassium channel; inflammation; L-arginine nitric oxide; nociception; opioid; receptor.

***Corresponding author: Olufunmilayo O. Adeyemi,** Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria, Phone: +2348034459618, E-mail: ooadeyemi@cmul.edu.ng
**Ismail O. Ishola, Elizabeth T. Adesanya and Destiny O. Alohan:** Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria

## Introduction

Inflammation is a major cause of morbidity throughout the world [1]. If untreated, it may lead to various associated diseases like arthritis and atherosclerosis [2]. The management of inflammatory disorders involves the use of non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs. Although NSAIDs are very effective, their use is associated with a broad spectrum of adverse reactions in the liver, kidney, cardiovascular system, skin, and gut [3]. Several Nigerian medicinal plants have been reported to exert notable antinociceptive and anti-inflammatory activities without producing considerable untoward effects [4, 5]. Despite the availability of a substantial number of potent synthetic drugs, medicinal plants are still playing a key role in the discovery of novel and effective drug molecules [6].

*Tetracera alnifolia* Willd. (Dilleniaceae) is a wild plant found in West African forest as well as Gulf of Guinea and Cameroon [7]. In Ivory Coast, the plant is believed to have high therapeutic value in the treatment of pain. Leafy twigs are grind and mixed into a paste with palm oil for application in headache, abdominal pain, and rheumatism, etc. [7]. An alcoholic macerate of leafy twigs in palm wine is used in the treatment of asthma and pyrexia in Ivory Coast. Leaves crushed with salt and pimento are taken as an aphrodisiac [8]. In Zaïre, the young leaves are eaten as a vegetable [7]. Based on the folkloric uses of *T. alnifolia* in the management of painful and inflammatory conditions, this study was carried out to evaluate the antinociceptive

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

and anti-inflammatory potentials of the leaf extract of *T. alnifolia* using validated experimental models *in vivo*.

## Materials and methods

### Plant material

The fresh leaves of *T. alnifolia* were collected from Abatadu Village, Ikire, Osun state. The leaves were identified by Mr O. O. Oyebanji, a forestry expert of the herbarium section, and authenticated by Prof. J. D. Olowokudejo, both in the Department of Botany, University of Lagos, Akoka, Lagos State, Nigeria. A voucher specimen number LUH 6521 was deposited in the herbarium for reference.

### Preparation of plant extract

Fresh leaves of *T. alnifolia* were air dried at room temperature for 4 days and pulverized into powder. The powdered plant material (201 g) was extracted by maceration in 70% v/v ethanol in water for 72 h and then filtered, and the procedure was repeated for exhaustive extraction. The filtrate was evaporated under reduced pressure at 40 °C in a Buchi rotary evaporator (R-100, BÜCHI Labortechnik AG, Flawil, Switzerland), and the residue was oven dried at 40 °C to give a dark brown hydroethanolic extract. The extract yield per gram of dried powdered leaves was 11.99% w/w.

### Experiment animals

Male albino rats (140–170 g) and male albino mice (17–23 g) used in this study were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos. The animals were maintained in propylene cages in a 12 h light-dark cycle, with free access to feed (Livestock feeds, Ikeja, Nigeria) and water. The experimental procedures used in this study were approved by the Research Grants and Experimentation Ethics Committee of the College of Medicine, University of Lagos, Lagos, Nigeria (CM/COM/8/Vol. XXXII) and in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [9].

### Drugs and chemicals

Bovine serum albumin, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, rutin, aluminum chloride, ascorbic acid, hydrochloric acid, dimethylsulfoxide, complete Freund's adjuvant (CFA), carrageenan, glacial acetic acid, formalin, potassium ferric cyanide, iron (II) sulfate, L-arginine, $N^G$-nitro-L-arginine, naloxone, and diclofenac sodium were obtained from Sigma Aldrich (St. Louis, MO, USA), xylene from Alfa Aesar GmbH & Co. (Karlsruhe, Germany) and celecoxib from Pfizer Pharmaceutical (Karlsruhe, Berlin, Germany).

### Preliminary phytochemical screening

The preliminary qualitative and quantitative phytochemical analyses were carried out using the methods of Harbone [10] and Kim et al. [11].

### *In vitro* antioxidant assays

The *in vitro* antioxidant assays were carried out using 1 mg/mL of leaf extract in 95% methanol followed by serial dilutions. The 1,1-diphenyl-2-picryl-hydrazyl radical scavenging assay was carried out using the method of Atsumi et al. [12], and the nitric oxide scavenging assay was evaluated using the protocol of Leone et al. [13], whereas the ferric ion reducing capacity was evaluated using the procedure of Aiyegoro et al. [14].

### Acute toxicity test

The acute toxic effect of *T. alnifolia* was determined using the fixed dose protocol of the Organization of Economic Co-operation and Development guidelines for testing of chemicals, $TG_{420}$ [15] for oral administration. Eleven female mice were given *T. alnifolia* (250 mg/kg, p.o., n = 1; 2000 mg/kg, p.o., n = 5; and 5000 mg/kg, p.o., n = 5), and the same procedure was repeated for intraperitoneal administration of the extract. Behavioral signs of toxicity and mortality were observed following *T. alnifolia* administration during the first 30 min, then for the second, fourth, sixth hour, and once daily for 14 days for delayed toxicity or mortality.

### Effect of treatment on spontaneous locomotor activity

The effect of HeTA (5000 mg/kg, p.o.; 500, 1000, and 2000 mg/kg, i.p.) on spontaneous locomotor activity was evaluated using the open field test, 1 h post-treatment. Briefly, the open field is an arena with walls to prevent escape. The field is marked with grid and square crossings. The lines divide the floor into sixteen 18 × 18 cm squares with a drawn central square (18 × 18 cm) in red. Each mouse was placed in the center and allowed to explore for 60 s and then observed for 3 min. The open field was cleaned between usages by each mouse using 10% v/v ethanol. The line crosses (frequency with which the mice crossed one of the grid lines with all four paws) were in 5 min after 1 min of habituation were recorded as an index of locomotor activity [16].

### Antinociceptive assays

**Acetic acid-induced mouse writhing test:** Thirty-six male albino mice (17–23 g) were divided into six groups of six animals each. Group 1: vehicle (10 mL/kg, p.o., normal saline), Groups 2–5: HeTA (50, 100, 200, or 400 mg/kg, p.o.), respectively, and Group 6: diclofenac (100 mg/kg, p.o., reference standard). One hour after drug administration, acetic acid (10 mL/kg, i.p., 0.6% v/v, in normal saline) was administered. The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) was

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

then counted for 30 min at 5 min intervals [4, 16]. A reduction in the number of writhes is an indication for antinociceptive property.

**Formalin-induced nociceptive test:** Mice that fasted overnight were divided into six groups (n = 6). Group 1: vehicle (10 mL/kg, p.o., normal saline), Groups 2–5: HeTA (50, 100, 200, or 400 mg/kg, p.o.), respectively, and Group 6: morphine sulfate (5 mg/kg, s.c.). Sixty minutes after oral drug treatment or 30 min after subcutaneous injection of morphine, formalin (100 μL of 1% solution) was injected subcutaneously into the right hind paw of each mouse. The time (in seconds) spent in licking and biting responses of the injected paw, indicative of pain, was recorded between 0–5 min (first phase) and 15–30 min (second phase) after formalin injection [4, 17]. The reaction time of the animals was compared with vehicle-treated control group and expressed as percent inhibition.

**Capsaicin-induced nociceptive test:** The method used was similar to that of formalin test. Mice that fasted overnight were divided into six groups (n = 6). Group 1: vehicle (10 mL/kg, p.o., normal saline), Groups 2–5: HeTA (50, 100, 200, or 400 mg/kg, p.o.), respectively, and Group 6: morphine sulfate (5 mg/kg, s.c.). Sixty minutes after oral administration or 30 min after subcutaneous injection of morphine, capsaicin (100 μL; 2 mg/mL) was injected subcutaneously into the right hind paw of each mouse. The time (in seconds) spent in licking and biting responses of the injected paw, indicative of pain, was recorded between 0–5 min (first phase) and 15–30 min (second phase) after capsaicin injection. The reaction time of the animals was compared with vehicle-treated control group and expressed as percent inhibition [18].

**Hot plate test:** The central antinociceptive effects of the hydroethanolic leaf extract of *T. alnifolia* (HeTA) was investigated using the hot plate test in accordance with the method described by Eddy and Leimbach [19]. Mice (18–23 g; n = 6) used in this experiment were initially screened by placing the animals in turn on a hot plate (Electrothermal Eng. Ltd.; catalogue number MH8514B; serial number 10021249) set at 55 ± 1 °C, and animals that failed to jump or flinch their paw within 5 s were discarded. Prior to treatment, the reaction time of each mouse was recorded at 0 and 15 min intervals. The average of the two readings was taken as the initial reaction time. Animals were divided into six groups of six mice each: Group 1: normal saline, vehicle (10 mL/kg, p.o.); Groups 2–5: HeTA (50, 100, 200, or 400 mg/kg, p.o.), respectively; and Group 6: morphine (5 mg/kg, s.c.). Nociceptive responses were recorded 30, 60, 90, 120, and 150 min post-treatment. A cut off time of 10 s was observed to prevent tissue damage [19].

**Elucidation of mechanism(s) of antinociceptive activity:** To elucidate the possible mechanism of action, HeTA (100 mg/kg, p.o.) [selected being the most active dosage in most paradigms] was administered 15 min after the administration of the various receptor antagonists.

**Involvement of opioid pathway:** The involvement of opioidergic pathway in the antinociceptive action of *T. alnifolia* was investigated through subcutaneous injection of naloxone (5 mg/kg, non-selective opioid receptor antagonist) [20, 21], 15 min before HeTA (100 mg/kg, p.o.), and after 1 h, acetic acid (10 mL/kg, i.p., 0.6% v/v in normal saline).

**Involvement of L-arginine-nitric oxide pathway:** The role of L-arginine-nitric oxide pathway in *T. alnifolia*-induced antinociception was evaluated through pretreatment of mice with L-arginine (750 mg/kg, i.p., nitric oxide precursor) and 15 min later, they received HeTA (100 mg/kg, p.o.) or vehicle (10 mL/kg, p.o.), and 1 h post treatment the acetic acid writhing test was carried out. In another study, mice were pretreated with $N^G$-nitro-L-arginine (10 mg/kg, i.p.; nitric oxide synthase inhibitor), and after 15 min, the animals received HeTA (100 mg/kg, p.o.) or vehicle (10 mL/kg, p.o.). One hour post treatment, the acetic acid writhing test was conducted [22].

**Involvement of ATP-sensitive potassium channels:** The involvement of ATP-sensitive potassium channels in HeTA-induced antinociception was investigated through pretreatment of mice with glibenclamide (10 mg/kg i.p.; an ATP-sensitive potassium channel inhibitor) [23]. Fifteen minutes later, HeTA (100 mg/kg, p.o.) was administered and 1 h later, acetic acid-induced nociception test was carried out.

## Anti-inflammatory activity

**Carrageenan-induced paw edema test:** The carrageenan-induced paw edema paradigm was carried out using the protocol of Winter et al. [24]. The initial right hind paw diameter of male rats (140–170 g) was determined using a vernier caliper. The animals were divided into five groups of five rats each and treated as follows: Group 1: normal saline, vehicle (10 mL/kg, p.o.), Groups 2–4: HeTA (100, 200, or 400 mg/kg, p.o.), respectively, and Group 5: diclofenac (100 mg/kg, p.o.). One hour post-treatment, 100 μL of carrageenan (1% w/v in normal saline) was injected into the right hind paw. The changes in paw size were recorded at 1, 2, 3, 4, 5, and 6 h after carrageenan injection (indicated the severity of edema). The percent inhibition of edema was determined for each animal in comparison to vehicle-treated controls [4].

**Xylene-induced ear edema:** Mice were allotted to five groups of five animals each: Group 1: normal saline, vehicle (10 mL/kg, p.o.), Groups 2–4: HeTA (100, 200, or 400 mg/kg, p.o.), respectively, and Group 5: diclofenac (100 mg/kg, p.o.). Thirty minutes post-treatment, edema was induced in each mouse by applying 30 μL of xylene to the inner surface of the right ear. Fifteen minutes later, the animals were killed under ether anesthesia, and then both ears were cut off, sized, and weighed. The mean of the difference between the right and left ears was determined for each group [4].

**Cotton pellet-induced granuloma:** Male rats (150–170 g) were anaesthetized by intraperitoneal injection of chloral hydrate (400 mg/kg). The groin region was carefully shaved and incised, and 20 mg of sterile cotton pellets was implanted on either sides of the groin region. The animals were thereafter divided into five groups of five rats each and treated for eight consecutive days with normal saline, vehicle (10 mL/kg, p.o.), HeTA (100, 200, or 400 mg/kg, p.o.), or celecoxib (30 mg/kg, p.o.). The rats were sacrificed under ether anesthesia on day 9, and the cotton pellets were removed, freed from extraneous tissue, weighed, and dried to a constant weight at 40 °C. The mean weights of wet and dried cotton pellet were recorded for each group [5, 25].

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

**CFA-induced arthritis:** Unilateral arthritis was induced by intraplantar injection of CFA into the right hind paw. The CFA contained heat-killed *Mycobacterium tuberculosis* suspended in paraffin oil (1 mg/mL; F5881, Sigma Aldrich, St. Louis, MO, USA). Test animals received 100 µL CFA, and vehicle-treated control were injected with the same volume of saline. One hour post-CFA injection, animals were divided into five groups of six rats each and treated for 21 days as follows: Group 1: normal saline, vehicle (10 mL/kg, p.o.), Groups 2–4: HeTA (100, 200, or 400 mg/kg, p.o.), respectively, and Group 5: celecoxib (30 mg/kg, p.o.). CFA-induced unilateral paw arthritis at the site of injection. The inflammatory reaction to CFA was assessed by measuring paw diameter with the aid of a vernier caliper on days 4, 8, 12, 16, and 20. Edema was defined as the difference between the size of CFA-injected paw and the contralateral hind paw [26].

# Results

## Preliminary phytochemical analysis of HeTA

The results of the preliminary qualitative phytochemical analysis of HeTA revealed the presence of flavonoids, alkaloids, phenols, saponins, tannins, anthraquinones, steroids, and phlobatanins. Moreover, the equivalents of total phenolic content, total flavonoid content, and total antioxidant capacity of HeTA were 15.15 ± 0.54 mg GAE/100 g, 6.41 ± 0.13 mg RE/100 g, and 68.88 ± 0.17 mg GAE/100 g, respectively (GAE: gallic acid equivalent; RE: rutin equivalent).

## *In vitro* free radical scavenging assay

The hydrogen donating ability of HeTA was determined by DPPH free radical. The values for mean inhibitory concentrations ($IC_{50}$) obtained for DPPH assay were 41.00 and 51.50 µg/mL for HeTA and ascorbic acid, respectively. The results showed that the hydrogen donating ability of HeTA was lower than that of AA. In another series of experiments, the nitric oxide scavenging effect of HeTA was assayed. The ($IC_{50}$) for HeTA and ascorbic acid were 61.00 and 41.50 µg/mL. Ferric ion reducing power assay measures the electron donating capacity of an antioxidant. The absorbance measured at 700 nm of the resultant blue-green-colored solution is proportional to the amount of $Fe^{2+}$ in the system. An effective concentration ($EC_{50}$) is 88.00 and 65.50 µg/mL of HeTA and AA, respectively.

## Acute toxicity tests

Acute oral administration of HeTA up to 5000 mg/kg neither induced toxic behaviors nor mortality. However, intraperitoneal injection of the extract produced a median lethal dose ($LD_{50}$) of 467 mg/kg.

## Effect on spontaneous locomotor activity

In the open field test, oral administration of HETA (5000 mg/kg) produced no significant change in spontaneous motor activity. However, intraperitoneal injection of HeTA (500, 1000, or 2000 mg/kg) produced significant and dose dependent decrease in the spontaneous motor activity (Table 1) confirming its toxicity when administered parenterally.

## Acetic acid-induced mouse writhing test

As shown in Figure 1, intraperitoneal injection of acetic acid caused 75.40 ± 1.56 abdominal constrictions over a period of 30 min. However, pretreatment of mice with

**Table 1:** Effect of HeTA treatment on spontaneous motor activity in open field test.

| Treatments | No. of line crosses |
|---|---|
| Vehicle 10 mL/kg | 69.60 ± 9.522 |
| HeTA 5000 mg/kg, p.o. | 61.40 ± 18.73 |
| HeTA 500 mg/kg, i.p. | 31.20 ± 14.90[a] |
| HeTA 1000 mg/kg, i.p. | 8.60 ± 2.98[b] |
| HeTA 2000 mg/kg, i.p. | 3.50 ± 0.50[b] |

HeTA, hydroethanolic leaf extract of *T. alnifolia*. Values are expressed as mean ± SEM (n = 5); [a]$p < 0.05$; [b]$p < 0.001$ vs. vehicle control-treated, one-way ANOVA followed by Tukey's post hoc multiple comparison test.

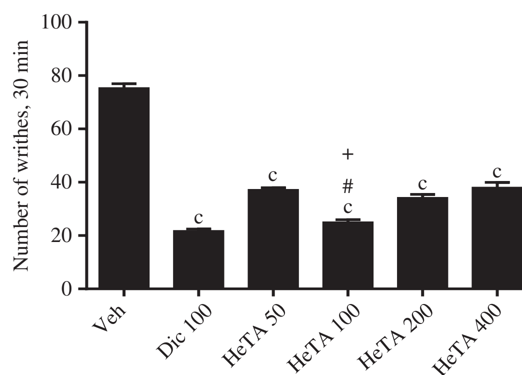



**Figure 1:** Effect of HeTA on acetic acid-induced mouse writhing. Values are expressed as mean ± SEM (n = 6). [c]$p < 0.001$ vs. vehicle control-treated; [#]$p < 0.001$ vs. HeTA 50 mg/kg; [+]$p < 0.001$ vs. HeTA 400 mg/kg using one-way ANOVA followed by Tukey's post hoc multiple comparison test.

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

HeTA (50, 100, 200, or 400 mg/kg) 1 h before intraperitoneal injection of acetic acid produced significant [$F(5, 36) = 230.70$, $p < 0.001$] inhibition of writhes by 50.60%, 73.70%, 54.60%, and 49.60%, respectively. Diclofenac produced 71.00% inhibition of writhes, which was similar to the effect of HeTA (100 mg/kg; 73.70%).

## Formalin-induced nociceptive test

Intraplantar injection of formalin into the right hind paw of mice produced a biphasic biting or licking behavior of the injected paw. Oral administration of HeTA (50, 100, 200, or 400 mg/kg) significantly reduced the duration of paw licking by 49.50%, 68.30%, 62.20%, and 44.30%, respectively, relative to vehicle-treated control in the early phase (0–5 min). The decrease in paw licking time (83.30%) produced by morphine was significantly higher than that of HeTA 100 mg/kg (Figure 2A). Moreover, one-way ANOVA revealed significant effect of treatment [$F(5, 30) = 533.00$, $p < 0.0001$]. Post hoc analysis showed an inverted U dose-related effect of HeTA, with peak effect seen at 100 mg/kg. As shown in Figure 2B, pretreatment of mice with HeTA produced significant inverted U-dose related inhibition of formalin-induced inflammatory pain (second phase) in mice. HeTA (50, 100, 200, and 400 mg/kg) treatment inhibited formalin-induced nociception by 52.70%, 72.40%, 61.00%, and 54.00%, respectively. Subcutaneous injection of morphine reduced the paw licking time by 91.50%. In addition, one-way ANOVA showed significant effect of HeTA or morphine treatment in the second phase [$F(5, 30) = 497.00$, $p < 0.0001$] (Figure 2B).

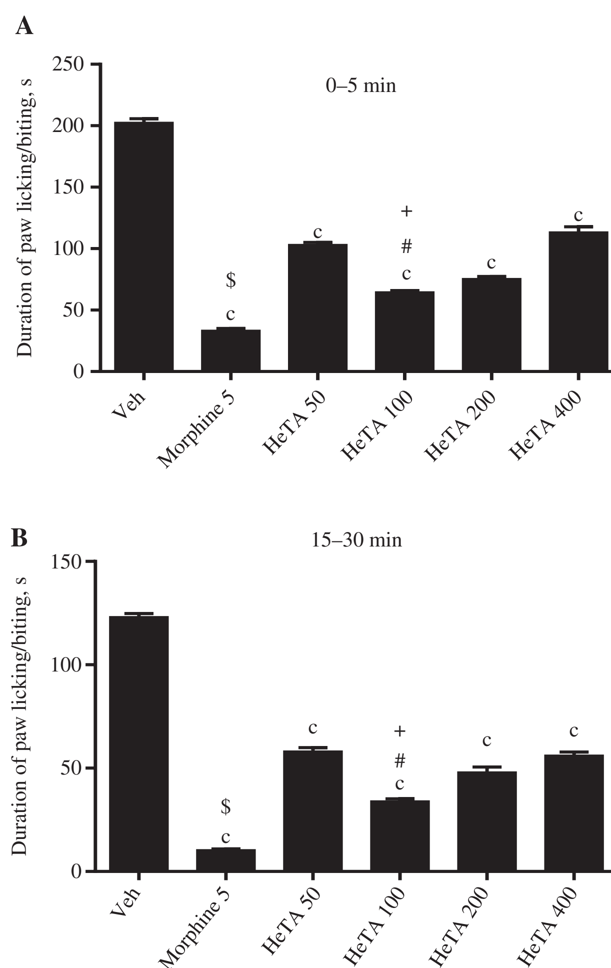



**Figure 2:** Effect of HeTA on formalin-induced paw licking in mice: (A) early phase and (B) late phase of nociception.
Values are expressed as mean ± SEM (n = 6). $^{c}p < 0.001$ vs. vehicle control-treated; $^{\#}p < 0.001$ vs. HeTA 50 mg/kg; $^{+}p < 0.001$ vs. HeTA 400 mg/kg; $^{\$}p < 0.001$ vs. HeTA 100 mg/kg using one-way ANOVA followed by Tukey's post hoc multiple comparison test.

## Capsaicin-induced nociceptive test

Intraplantar injection of capsaicin into the right hind paw of mice produced a biphasic biting or licking behavior of the injected paw (103.40 ± 1.69 s; early phase) and (95.60 ± 1.63 s; late phase). Oral administration of HeTA (50, 100, 200, and 400 mg/kg) significantly reduced the duration of paw licking by 44.88%, 64.60%, 58.60%, and 48.28%, respectively, relative to vehicle-treated control in the early phase (0–5 min), which was comparatively similar to the effect of diclofenac (71.70%) but lower than morphine (83.50% inhibition). One-way ANOVA revealed significant effect of treatment [$F(6, 54) = 538.9$, $p < 0.001$] (Table 2). In addition, HeTA (50, 100, 200, and 400 mg/kg) produced 55.20%, 67.30%, 62.30%, and 50.62% inhibition of capsaicin-induced nociception in the late phase (15–30 min), respectively. This effect is similar to that of diclofenac (74.00%) but lower than that of morphine (90.30%). One-way ANOVA showed significant effect of treatment in the second phase [$F(6, 54) = 538.9$, $p < 0.0001$] (Table 2).

## Hot plate test

The placement of vehicle-treated control mice on the hot plate elicited nociceptive reaction. Two-way ANOVA showed significant effect of treatment [$F(5, 144) = 33.36$, $p < 0.001$] (Table 3). Pretreatment of mice with HeTA (50, 100, 200, and 400 mg/kg) produced significant ($p < 0.05$, $p < 0.01$, $p < 0.001$) and time course increase in reaction time, which peaked at 100 mg/kg (14.40% maximum

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

**Table 2:** Effect of HeTA on capsaicin-induced pain in mice.

| Treatment, mg/kg | Early phase (0–5 min) | | Late phase (15–30 min) | |
|---|---|---|---|---|
| | Licking time, s | Inhibition, % | Licking time, s | Inhibition, % |
| Vehicle 10 mL/kg | 103.40 ± 1.69 | | 95.60 ± 1.63 | |
| HeTA 50 | 57.00 ± 2.000[a] | 44.88 | 42.80 ± 2.48[a] | 55.20 |
| HeTA 100 | 36.60 ± 3.07[a,b] | 64.60 | 31.20 ± 1.463[a,b] | 67.30 |
| HeTA 200 | 42.80 ± 2.354[a] | 58.60 | 36.00 ± 1.732[a] | 62.30 |
| HeTA 400 | 53.47 ± 2.76[c] | 48.28 | 47.55 ± 3.23[a] | 50.62 |
| Morphine 5 | 17.00 ± 0.95[a,c] | 83.50 | 9.20 ± 0.66[a,c] | 90.30 |
| Diclofenac 100 | 29.20 ± 0.58[a] | 71.70 | 24.80 ± 1.59[a] | 74.00 |

HeTA, hydroethanolic leaf extract of *T. alnifolia*. Values are mean ± SEM (n = 6); [a]$p < 0.001$ vs. vehicle control-treated; [b]$p < 0.001$ vs. HeTA 50 mg/kg; [c]$p < 0.01$ vs. HeTA 100 mg/kg treated, using one-way ANOVA followed by Tukey's post hoc multiple comparison test.

**Table 3:** Effect of HeTA on hot plate-induced nociception in mice.

| Treatment, mg/kg | 0 min | 30 min | 60 min | 90 min | 120 min | 150 min |
|---|---|---|---|---|---|---|
| Vehicle, mL/kg | 1.86 ± 0.26 | 1.30 ± 0.04 | 1.28 ± 0.18 | 1.12 ± 0.10 | 1.40 ± 0.24 | 1.37 ± 0.10 |
| Morphine 5 | 1.87 ± 0.12 | 6.10 ± 0.50[c] | 5.240.49[c] | 3.91 ± 0.44[c] | 3.14 ± 0.22[c] | 2.04 ± 0.27 |
| | | (52%) | (41.40%) | (25%) | (15.60%) | (2%) |
| HeTA 50 | 1.81 ± 0.32 | 2.20 ± 0.25[c] | 2.16 ± 0.24[c] | 2.04 ± 0.27[c] | 1.81 ± 0.25[c] | 1.41 ± 0.09 |
| | | (4.80%) | (4.30%) | (2.80%) | (0.00%) | (4.80%) |
| HeTA 100 | 1.81 ± 0.32 | 2.90 ± 0.18[c,d] | 3.04 ± 0.15[c,d] | 2.47 ± 0.23[b,d] | 1.56 ± 0.16[d] | 1.43 ± 0.17 |
| | | (13.30%) | (14.40%) | (8%) | (3.00%) | (4.60%) |
| HeTA 200 | 1.61 ± 0.15 | 2.50 ± 0.24[b] | 2.23 ± 0.15 | 1.96 ± 0.18 | 1.62 ± 0.18 | 1.37 ± 0.08 |
| | | (10.60%) | (7.30%) | (4.10%) | (0.11%) | (0.00%) |
| HeTA 400 | 1.90 ± 0.23 | 2.40 ± 0.09[a] | 2.50 ± 0.12[b] | 2.20 ± 0.67 | 1.70 ± 0.10 | 1.50 ± 0.23 |
| | | (5.20%) | (7.40%) | (3.70%) | (–2.40%) | (4.90%) |

HeTA, hydroethanolic leaf extract of *T. alnifolia*. Values are mean ± SEM (n = 6); [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$ vs. vehicle control-treated; [d]$p < 0.001$ vs. morphine using two-way ANOVA followed by Tukey's post hoc multiple comparison test.

possible effect) at 60 min. Post hoc analysis revealed that the antinociceptive effect of HeTA was significantly lower than that of morphine (5 mg/kg) as shown in Table 3.

## Elucidation of mechanism of HeTA-induced antinociception in mice

### Involvement of opioidergic system

The results depicted in Figure 3 show that the pretreatment of mice with naloxone (5 mg/kg, s.c.) prevented the antinociceptive effect elicited by HeTA (100 mg/kg) in writhing test. Two-way ANOVA revealed significant effect of HeTA treatment [$F(1, 16) = 172.2$, $p < 0.0001$], naloxone pretreatment [$F(1, 16) = 141.8$, $p < 0.0001$], and naloxone pretreatment × HeTA treatment interaction [$F(1, 16) = 215.1$, $p < 0.0001$]. Similarly, Tukey's post hoc test showed that the antinociceptive effect of HeTA in writhing test was significantly ($p < 0.001$) blocked by naloxone.

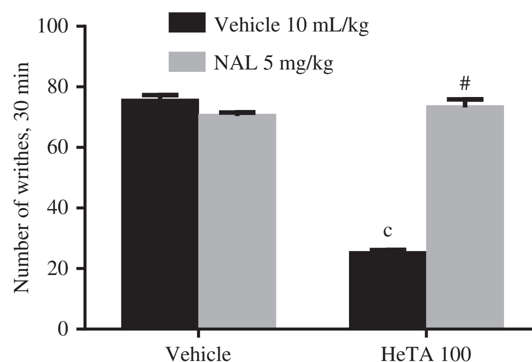



**Figure 3:** Effect of naloxone on HeTA-induced antinociception in acetic acid-induced writhing test.
Values are expressed as mean ± SEM (n = 6). [c]$p < 0.001$ vs. vehicle control-treated, [#]$p < 0.001$ vs. HeTA 100 mg/kg, using two-way ANOVA followed by Tukey's post hoc multiple comparison test.

### ATP-sensitive $K^+$ channel pathway involvement

Pretreatment of mice with glibenclamide (10 mg/kg; ATP-sensitive $K^+$ channels blocker, i.p.) prevented the

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

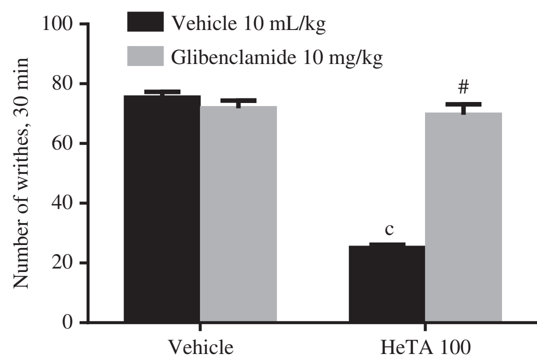
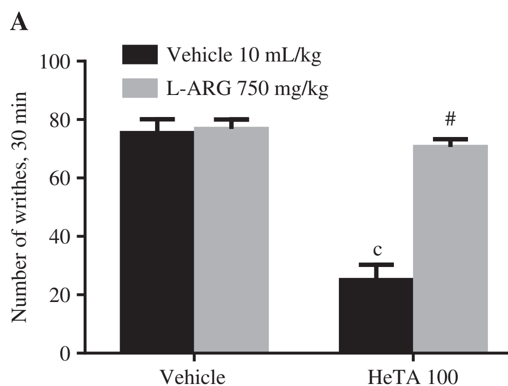



**Figure 4:** Effect of glibenclamide on HeTA-induced antinociception in the acetic acid-induced writhing test.
Values are expressed as mean ± SEM (n = 6). [c]p < 0.001 vs. vehicle control-treated, [#]p < 0.001 vs. glibenclamide using two-way ANOVA followed by Tukey's post hoc multiple comparison test.

antinociceptive effect elicited by HeTA in the mouse writhing assay. Two-way ANOVA revealed significant effects of HeTA treatment [$F(1, 16) = 116.4$, $p < 0.0001$], glibenclamide pretreatment [$F(1, 16) = 70.75$, $p < 0.0002$], and glibenclamide pretreatment × HeTA treatment interaction [$F(1, 16) = 97.78$, $p < 0.0001$] (Figure 4). Post hoc analysis showed that the reduction in number of writhes induced by HeTA was blocked by glibenclamide.

### Involvement of L-arginine-nitric oxide pathway

The pretreatment of mice with L-arginine (750 mg/kg; nitric oxide synthase precursor) prevented the antinociceptive effect elicited by HeTA (100 mg/kg) in writhing test. Two-way ANOVA revealed significant effects of HeTA treatment [$F(1, 16) = 140.1$, $p < 0.0001$], L-arginine pretreatment [$F(1, 16) = 96.63$, $p < 0.0001$], and L-arginine pretreatment × HeTA treatment interaction [$F(1, 16) = 85.46$, $p < 0.0001$] (Figure 5A). In addition, Tukey's post hoc test showed that the antinociceptive effect of HeTA in writhing test was significantly ($p < 0.001$) blocked by L-arginine (Figure 5A). The pretreatment of mice with $N^G$-nitro-L-arginine (10 mg/kg; nitric oxide synthase inhibitor, i.p.) did not affect HeTA-induced antinociceptive effect in the mouse writhing assay. Two-way ANOVA revealed a main effect of HeTA treatment [$F(1, 16) = 363.3$, $p < 0.0001$], but not $N^G$-nitro-L-arginine pretreatment [$F(1, 16) = 0.001$, $p = 0.9816$] nor $N^G$-nitro-L-arginine pretreatment × HeTA treatment interaction [$F(1, 16) = 0.180$, $p = 0.7996$]. Post hoc analysis indicated that the effect of HeTA was not blocked by $N^G$-nitro-L-arginine (Figure 5B).



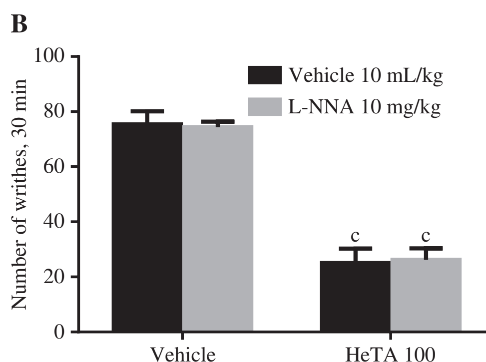




**Figure 5:** Effect of (A) L-Arginine or (B) L-nitro-arginine on HeTA-induced antinociception in the acetic acid writhing test.
Values are expressed as mean ± SEM (n = 6). [c]p < 0.001 vs. vehicle control-treated, [#]p < 0.001 vs. HeTA 100 mg/kg using two-way ANOVA followed by Tukey's post hoc multiple comparison test.

### Carrageenan-induced rat paw edema

The intraplantar injection of carrageenan in rats induced edema. The increase in paw size was evident 1 h after injection of carrageenan (0.32 ± 0.04 cm) and reached its peak at the second hour (0.68 ± 0.08 cm), which was sustained for 4 h (0.64 ± 0.03 cm). However, carrageenan-induced paw edema was reduced by pretreatment of rats with HeTA (200 or 400 mg/kg) with peak effects, 59.38% and 65.63%, recorded 4 h post-carrageenan injections. As expected, diclofenac inhibited carrageenan-induced paw edema by 60.94%. Moreover, two-way ANOVA revealed significant effects of treatments [$F(5, 120) = 3.036$, $p = 0.0129$)] (Figure 6). Post hoc analysis showed that HeTA (200 and 400 mg/kg) produced significant time course inhibition of carrageenan-induced edema in rats.

### Xylene-induced ear edema

As shown in Figure 7, xylene application to the ear increased the weight of the mouse ear suggestive of edema formation.

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

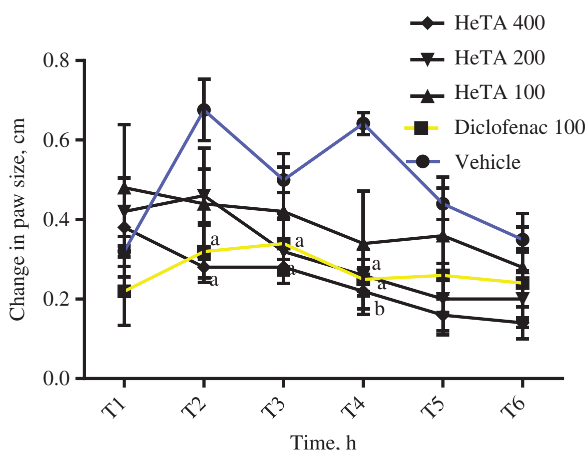



**Figure 6:** Effect of HeTA against carrageenan-induced rat paw edema. Values are expressed as mean ± SEM (n = 5). $^a$p < 0.05; p < 0.01 vs. vehicle-treated control. Statistical level of significance analysis by two-way ANOVA followed by Tukey's post hoc multiple comparison test.

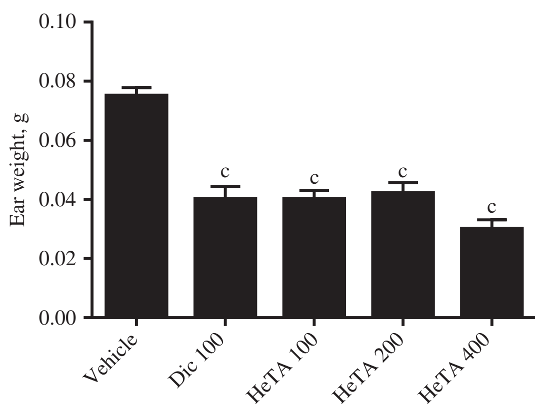



**Figure 7:** Effect of HeTA against xylene-induced ear edema in mice. Values are expressed as the mean ± SEM (n = 6); $^c$p < 0.001 vs. vehicle-treated control. Level of significance analysis by one-way ANOVA followed by Tukey's post hoc multiple comparison test.

However, the pretreatment of mice with HeTA (100, 200, or 400 mg/kg) produced significant [$F$(4, 19) = 20.14, p < 0.001] inhibition of ear edema by 46.67, 44.00% and 60.00%, respectively, when compared with vehicle-treated group. Prednisolone (a known steroidal anti-inflammatory drug) also significantly reduced the ear edema (46.67% inhibition).

### Cotton pellet-induced granuloma pouch

The granuloma tissue formation is a feature of chronic inflammation. As shown in Figure 8, subcutaneous cotton pellet implantation produced granuloma. HeTA (100 mg/kg) or celecoxib (COX-2 inhibitor; standard anti-inflammatory agent) exhibited significant (p < 0.05)

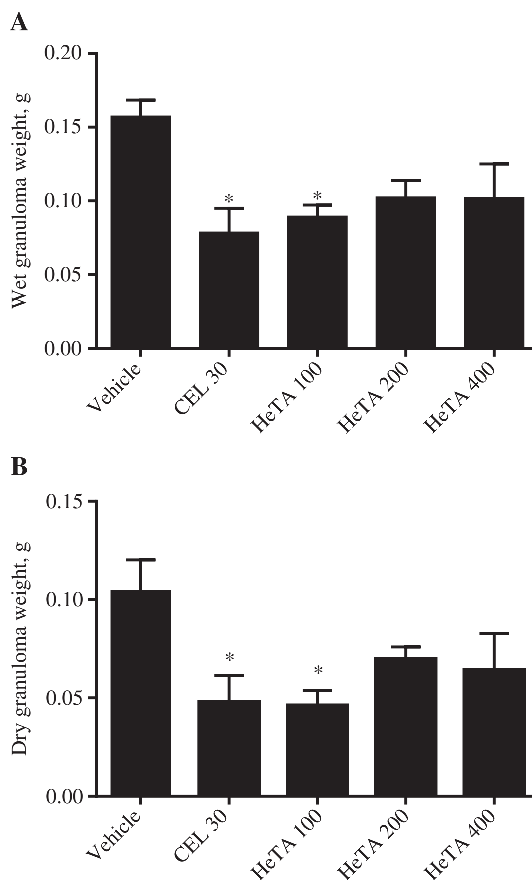



**Figure 8:** Effect of HeTA on cotton pellet-induced granuloma formation in rats: (A) wet granuloma weight and (B) dried granuloma weight. Values are expressed as the mean ± SEM (n = 6); $^*$p < 0.05 vs. vehicle-treated control. Level of significance analysis by one-way ANOVA followed by Tukey's post hoc multiple comparison test.

inhibition of both wet and dry granuloma formation (Figure 8A, B). HeTA (100 mg/kg) reduced wet and dry granuloma by 43.75% and 55.07%, respectively, while celecoxib reduced the formation of wet and dry granuloma by 50.00% and 56.02%.

### CFA-induced arthritis in rats

The intraplantar administration of CFA produced significant (p < 0.05) increase in the paw size in arthritic control rats when compared with normal control rats. Two-way ANOVA revealed significant effects of treatments [$F$(4, 118) = 13.08, p < 0.01]. In the first week after adjuvant injection, the increase in paw size (3.28 ± 0.18 mm; day 4) was significantly (p < 0.01) reduced by oral administration of HeTA (100 and 200 mg/kg). The anti-arthritic effect of HeTA peaked on day 20 with 55.59% [2.24-folds] inhibition of edema at 400 mg/kg (Figure 9), which was similar to the effect of celecoxib with 51.73% [1.87-folds].

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

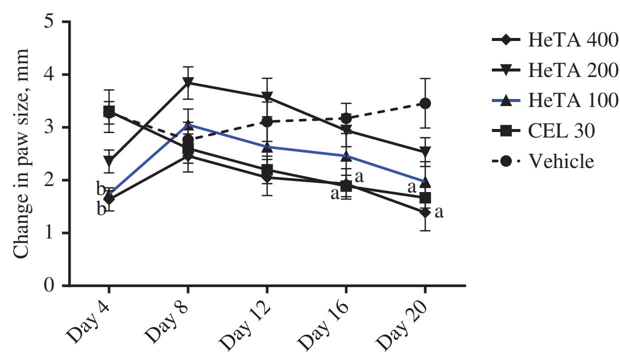



**Figure 9:** The effect of daily oral administration of HeTA against CFA-induced arthritis in rats.
Values are expressed as mean ± SEM (n = 6), [a]$p < 0.05$, [b]$p < 0.01$ vs. vehicle-treated arthritic rats. Level of significance analysis by two-way ANOVA followed by Tukey's post hoc multiple comparison test.

## Discussion

The results obtained from this study showed that the HETA possessed antinociceptive and anti-inflammatory activities in well-characterized models of pain and inflammation in rodents. The extract produced antinociceptive effect through reduction of mean number of writhes in acetic acid-induced writhing, paw licking in formalin, and capsaicin models as well as time course increase in pain threshold or reaction time in hot plate test. This effect was prevented by naloxone (opioid receptor antagonist), glibenclamide (ATP-sensitive potassium channel blocker) and L-arginine (precursor of nitric oxide). Similarly, *T. alnifolia* produced anti-inflammatory effect by decreasing the swelling in carrageenan- and xylene-induced edema; inhibition of granulocyte infiltration in cotton pellet-induced granuloma as well as reduction of CFA-induced chronic inflammation in rats.

Acetic acid-induced writhing test is a widely used model to investigate peripheral antinociceptive effect of a drug. Intraperitoneal injection of acetic acid increases pain mediators, such as prostaglandins, histamine, serotonin, substance P, and cytokines [27, 28]. Thus, increasing vascular permeability leads to abdominal constriction or writhing through stimulation of nociceptive receptors [29]. In this study, intraperitoneal injection of acetic acid induced mouse writhing reflex suggestive of pain, which was ameliorated by the pretreatment of mice with *T. alnifolia* leaf extract indicating antinociceptive activity. Due to the non-specific effect of acetic acid model, i.e. muscle relaxants and sedatives also reduce the number writhes, hence, formalin-induced nociceptive test was carried out.

The formalin test for nociception involves moderate, continuous pain generated by injured tissue. In this way, it differs from most traditional tests of nociception that rely upon brief stimuli of threshold intensity. The response to formalin shows an early and a late phase. The early phase seems to be caused predominantly by C-fiber activation due to the peripheral stimulus, while the late phase appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord [30]. In this study, intraplantar injection of formalin induced a biphasic lifting, licking, biting, and flinching/shaking behavior of the injected paw, which is in agreement with previous studies [4, 31]. Pretreatment of mice with *T. alnifolia* inhibited both phases thus confirming a peripheral mechanism of action while also indicating the role of a central mechanism of antinociceptive effect. It is of interest to note that the peak effect was observed at 100 mg/kg in both acetic and formalin tests such that the effect was more significant than that of 400 mg/kg. To ascertain the mechanism of *T. alnifolia*-induced antinociception, capsaicin-induced nociception was carried out. Capsaicin selectively stimulates nociceptive neurons by increasing the influx of ions, such as calcium, in dorsal root ganglion neurons, and activates the transient receptor potential vanilloid type 1 (TRPV1) [32–34] and has been widely used to study pain-related events. Oral administration of *T. alnifolia* showed a significant suppression of capsaicin-induced paw licking. The suppression of TRPV1 activation provides a therapeutic option to reduce inflammation and pain in different animal disease models through mechanisms involving dampening of TRPV1 activation and signaling events [35]. To confirm the involvement of central mechanism in the antinociceptive effect of *T. alnifolia*, the hot plate test was used based on the fact that centrally acting analgesic drugs elevate pain threshold of rodents to heat [4]. The hot plate is a more complex pain model, producing two behavioral components (paw licking and jumping) considered to be supraspinally integrated responses [36]. In this study, *T. alnifolia* 100 mg/kg produced the peak increment in animal pain threshold but not as effective as morphine, thus confirming a central mechanism of action.

The involvement of opioid receptors in the antinociceptive effect of the extract (100 mg/kg) was evaluated through pretreatment of mice with naloxone (non-selective opioid receptor antagonist) in acetic acid nociceptive model. Subcutaneous injection of naloxone reversed the antinociceptive effect of *T. alnifolia* and confirmed the involvement of opioidergic pathway in antinociceptive effect of extract. Opioid receptors produced antinociceptive effect through

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

activation of L-arginine-nitric oxide-cGMP pathway [37, 38]. In this study, the pretreatment of mice with L-arginine prevented *T. alnifolia* antinociceptive effect. Conversely, the pretreatment with $N^G$-nitro-L-arginine (neuronal nitric oxide inhibitor) failed to reverse *T. alnifolia*-induced antinociception. Therefore, *T. alnifolia* probably produced its antinociceptive effect through the L-arginine/NO/cGMP signaling pathway similar to the effect of diclofenac [21]. The possible involvement of ATP-sensitive $K^+$ channels in peripheral antinociception induced by *T. alnifolia* was also elucidated. Glibenclamide (specific $K^+_{ATP}$ blocker) prevented the antinociceptive effect of the extract. Summarily, these data suggest that the antinociceptive effect of *T. alnifolia* involved the participation of opioid receptors and L-arginine-nitric oxide/$K_{ATP}$ pathways.

The anti-inflammatory effect of *T. alnifolia* was evaluated in this study using the carrageenan, xylene, cotton pellet, and CFA models of inflammation. Carrageenan-induced paw edema is a useful model to assess the contribution of inflammatory mediators and has been described as a biphasic event [39]. The initial phase of edema (0–1 h) had been attributed to the release of histamine, serotonin, and bradykinin [40]. The second accelerating phase of swelling (1–6 h) has been correlated with the elevated production of prostaglandins as well as induction of inducible cyclooxygenase (COX-2) in the hind paw [41, 42]. *T. alnifolia* failed to reduce edema formation in the early phase, which was similar to the effect of NSAIDs. However, the extract significantly attenuated the second accelerating phase (2–5 h) of edema, which suggests possible inhibition of prostaglandin production in the hind paw. The acute anti-inflammatory effect of the extract was investigated using the xylene-induced ear edema test. Instillation of xylene into the ear increases vascular permeability possibly through induction of prostaglandins, which release neuropeptides that activate its receptor, causing neurogenic inflammation [43]. One of the neuropeptides, called substance P, is a potent vasodilator that acts by releasing nitric oxide from endothelial cells, which causes vasodilation and plasma exudation [44]. In this study, *T. alnifolia* significantly reduced the ear edema.

The effect of the extract on chronic inflammatory conditions was evaluated using the classic cotton pellet-induced granuloma and CFA-induced arthritis models in rats. The cotton pellet is used to evaluate the transudative phase, defined as the increase in wet weight of the pellet that occurred during the first 3 h and proliferative phase, measured as the increase in dry weight of the granuloma that occurs between 3 and 6 days after implantation [45]. Subcutaneous implantation of 20 mg of cotton pellet in the groin region induced granuloma formation. *T. alnifolia* (100 mg/kg) significantly reduced both the wet and dry weights of the pellet indicating anti-transudative and proliferative effects. This showed the potential of the extract to ameliorate chronic inflammation. The protective effect of the extract against chronic inflammation was further confirmed using the CFA-induced arthritis model. CFA is widely used to model chronic inflammation and is of considerable relevance to the study of pathophysiological and pharmacological control of inflammatory processes, as well as anti-inflammatory effect of drugs [46, 47]. CFA elicits joint swelling, synovial membrane inflammation, and cartilage destruction [46]. The present study showed that CFA induced paw swelling, which peaked on day 8 and was significantly reduced by *T. alnifolia*, similar to the effect of celecoxib.

Several studies have reported antinociceptive and anti-inflammatory effects of flavonoids and phenolic acids [48, 49]. Our preliminary phytochemical studies showed that *T. alnifolia* leaf extract is rich in flavonoid and polyphenolic compounds. It has been shown that phenolic compounds exert their antioxidant effects by decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radicals, such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non-radical species, and breaking chains to prevent continued hydrogen abstraction from the substance [50].

Moreover, the extract showed potent antioxidant activity evidenced in its ability to scavenge DPPH, nitric oxide, and ferric ion radicals. Hence, further studies are required to isolate the chemical constituents responsible for the observed effects. It is of interest to note that the extract possesses wide margin of safety following acute toxicity study and does not affect spontaneous motor activity up to 5000 mg/kg oral administration.

## Conclusions

The study showed that the HETA possessed antinociceptive property through opioid/L-arginine-nitric oxide/$K_{ATP}$ pathways as well as anti-inflammatory activity. This justifies the use of the plant extract in traditional African medicine for the treatment of painful and inflammatory disorders.

**Acknowledgments:** We acknowledged the technical assistance rendered by Mr. C. Micah of the Department of Pharmacology, Therapeutics and Toxicology, College of Medicine of the University of Lagos, Lagos, Nigeria

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
**Research funding:** None declared.
**Employment or leadership:** None declared.
**Honorarium:** None declared.
**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

## References

1. Dewanjee S, Maiti A, Sahu R, Dua TK, Mandal SC. Study of anti-inflammatory and antinociceptive activity of hydroalcoholic extract of Schima wallichii bark. Pharm Biol 2009;47:402–7.
2. Ray SD, Ray S, Zia-Ul-Haq M, De Feo V, Dewanjee S. Pharmacological basis of the use of the root bark of Zizyphus nummularia Aubrev. (Rhamnaceae) as anti-inflammatory agent. BMC Compl Altern Med 2015;15:416.
3. Aronson JK. Meyler's side effects of analgesics and anti-inflammatory drugs. Amsterdam: Elsevier, 2009.
4. Ishola IO, Akindele AJ, Adeyemi OO. Analgesic and anti-inflammatory activities of Cnestis ferruginea Vahl ex DC (Connaraceae) methanolic root extract. J Ethnopharmacol 2011;135:55–62.
5. Ishola IO, Ikumawoyi VO, Afolayan GO, Olorife OJ. Antinociceptive and anti-inflammatory properties of hydroethanolic seed extract of Monodora myristica (Annonaceae) in rodents. West Afr J Pharm 2016;27:22–32.
6. Shah SM, Shah SM. Phytochemicals, antioxidant, antinociceptive and anti-inflammatory potential of the aqueous extract of Teucrium stocksianum bioss. BMC Compl Altern Med 2015;15:351.
7. Burkill HM. The useful plants of West Tropical Africa, vol. 1. Kew: Royal Botanic Gardens, 1985;15.
8. Bouquet A, Debray M. Plantes medicinales de la Cote d. Ivoire, Trav. Doc. ORSTOM. 1974;32.
9. NIH. Guide for the use of laboratory animals DHHS, PHS. NIH Publication No. 85-23 (1985 Revised).
10. Harborne JB. Phytochemical methods a guide to modern techniques of plant analysis. London, New York: Chapman and Hall Ltd, 1973;49–188.
11. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem 2003;81:321–6.
12. Atsumi T, Iwakura I, Kashiwagi Y, Fujisawa S, Ueha T. Free radical scavenging activity in the nonenzymatic fraction of human saliva: a simple DPPH assay showing the effect of physical exercise. Antioxid Redox Signal 1999;1:537–46.
13. Leone AM, Francis PL, Rhodes P, Moncada S. A rapid and simple method for the measurement of nitrite and nitrate in plasma by high performance capillary electrophoresis. Biochem Biophysics Res Comm 1994;200:951–7.
14. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Compl Alternat Med 2010;10:1–8.
15. OECD. Guidance document on acute oral toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No. 24, 2000.
16. Ishola IO, Akinyede AA, Sholarin AM. Antidepressant and anxiolytic properties of the methanolic extract of Momordica charantia Linn (Cucurbitaceae) and its mechanism of action. Drug Res (Stuttg) 2014;64:368–76.
17. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. Pain 1989;38:347–52.
18. Sakurada T, Katsumata K, Yogo H, Tan-No K, Sakurada S, Kisara K. Antinociception induced by CP 96,345, a non-peptide NK-1 receptor antagonist, in the mouse formalin and capsaicin tests. Neurosci Lett 1993;151:142–5.
19. Eddy NB, Leimbach D. Systemic analgesics. II. Dithienylbutenyl and dithienylbutylamines. J Pharmacol Exp Ther 1953;107:385–93.
20. Rodrigues AR, Duarte ID. The peripheral antinociceptive effect induced by morphine is associated with ATP-sensitive $K^+$ channels. Br J Pharmacol 2000;129:110–4.
21. Ishola IO, Awodele O, Olusayero AM, Ochieng CO. Mechanisms of analgesic and anti-inflammatory properties of Annona muricata Linn. (Annonaceae) fruit extract in rodents. J Med Food 2014;17:1375–82.
22. Galdino GS, Xavier CH, Almeida R, Silva G, Fontes MA, Menezes G, et al. The nitric oxide/$_c$GMP/$K_{ATP}$ pathway mediates systemic and central antinociception induced by resistance exercise in rats. Int J Neurosci 2015;125:765–73.
23. Venkataramanan PE, Parvathavarthini S, Viswanathan S. Role of ATP sensitive potassium channel on 7-hydroxy flavone induced antinociception and possible association with changes in glycaemic status. Indian J Exp Biol 2000;38:1172–4.
24. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962;111:544–7.
25. Ashford A, Penn GB. Effect of adrenalectomy on cotton pellet granuloma formation in the rat. J Pharm Pharmacol 1965;17:10–4.
26. Ishola IO, Akinyede A, Lawal SM, Popoola TD. Antinociceptive and anti-inflammatory effects of Olax subscorpioidea Oliv. (Olacaceae) leaf extract in rodents: possible mechanisms of antinociceptive action. West Afr J Pharm 2015;26:99–112.
27. Deraedt R, Jouquey S, Delevallée F, Flahaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol 1980;61:17–24.
28. Ikeda Y, Ueno A, Naraba H, Oh-ishi S. Involvement of vanilloid receptor $VR_1$ and prostanoids in the acid-induced writhing responses of mice. Life Sci 2001;69:2911–9.
29. Martínez V, Thakur S, Mogil JS, Taché Y, Mayer EA. Differential effects of chemical and mechanical colonic irritation on behavioral pain response to intraperitoneal acetic acid in mice. Pain 1999;81:179–86.
30. Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5–17.
31. Abbott FV, Franklin KB, Westbrook RF. The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain 1995;60:91–102.
32. Wood JN, Winter J, James IF, Rang HP, Yeats J, Bevan S. Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. J Neurosci 1988;8:3208–20.

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM

33. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000;288:306–13.
34. Fattori V, Hohmann MS, Rossaneis AC, Pinho-Ribeiro FA, Verri WA. Capsaicin: current understanding of its mechanisms and therapy of pain and other pre-clinical and clinical uses. Molecules 2016;21:844–76.
35. Yang Y, Yang H, Wang Z, Varadaraj K, Kumari SS, Mergler S, et al. Cannabinoid receptor 1 suppresses transient receptor potential vanilloid 1-induced inflammatory responses to corneal injury. Cell Signal 2013;25:501–11.
36. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev 2001;53:597–652.
37. Duarte ID, Lorenzetti BB, Ferreira SH. Peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway. Eur J Pharmacol 1990;186:289–93.
38. Ferreira SH, Duarte ID, Lorenzetti BB. The molecular mechanism of action of peripheral morphine analgesia: stimulation of the cGMP system via nitric oxide release. Eur J Pharmacol 1991;201:121–2.
39. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan edema in rats. J Pharmacol Exp Ther 1969;166: 96–103.
40. Di Rosa M, Giroud JP, Willoughby DA. Studies of mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol 1971;104:15–29.
41. Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, et al. Pharmacological and biochemical demonstration of the role of cyclooxygenase-2 in inflammation and pain. Proc Natl Acad Sci USA 1994;91:12013–7.
42. Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, et al. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br J Pharmacol 1996;118:829–38.
43. Banki E, Hajna Z, Kemeny A, Botz B, Nagy P, Bolcskei K, et al. The selective $PAC_1$ receptor agonist maxadilan inhibits neurogenic vasodilation and edema formation in the mouse skin. Neuropharmacology 2014;85:538–47.
44. Torres-Rêgo M, Furtado AA, Bitencourt MA, Lima MC, Andrade RC, Azevedo EP, et al. Anti-inflammatory activity of aqueous extract and bioactive compounds identified from the fruits of Hancornia speciosa Gomes (Apocynaceae). BMC Complement Altern Med 2016;16:275.
45. Swingle KF, Shideman FE. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. J Pharmacol Exp Ther 1972;183:226–34.
46. Calvino B, Crepon-Bernard MA, Le Bars D. Parallel clinical and behavioral studies of adjuvant-induced arthritis in the rat: possible relationship with “chronic pain”. Behav Brain Res 1987;24:11–29.
47. Liu YL, Lin HM, Zou R, Wu JC, Han R, Raymond LN, et al. Suppression of complete Freund’s adjuvant-induced adjuvant arthritis by cobratoxin. Acta Pharmacol Sin 2009;30:219–27.
48. Kim YW, Zhao RJ, Park SJ, Lee JR, Cho IJ, Yang CH, et al. Anti-inflammatory effects of liquiritigenin as a consequence of the inhibition of NF-kappaB-dependent iNOS and proinflammatory cytokines production. Br J Pharmacol 2008;154:165–73.
49. Fang J. Classification of fruits based on anthocyanin types and relevance to their health effects. Nutrition 2015;31:1301–6.
50. Elisha IL, Dzoyem JP, McGaw LJ, Botha FS, Eloff JN. The anti-arthritic, anti-inflammatory, antioxidant activity and relationships with total phenolics and total flavonoids of nine South African plants used traditionally to treat arthritis. BMC Complement Alt Med 2016;16:307.

Brought to you by | Göteborg University - University of Gothenburg
Authenticated
Download Date | 10/18/18 9:31 AM