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Anti-inflammatory, analgesic, and antipyretic activities of aqueous and methanol stem bark extracts of *Drypetes gossweileri* in persistent inflammatory pain induced by complete Freund's adjuvant in rats

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Abstract

Background: *Drypetes gossweileri* (Euphorbiaceae) is a medicinal plant used in Cameroon to treat toothache, gastritis, and pain. This study was conducted to evaluate the anti-inflammatory, analgesic, and anti-pyretic effects of the aqueous (AEDG) and methanol (MEDG) stem bark extracts in a persistent inflammatory pain model induced by Complete Freund's Adjuvant (CFA) in rats.

Methods: Inflammatory pain was induced by intra-plantar injection of 50 µL of CFA into the left hind paw of rats. AEDG and MEDG were given orally (100, 200, and 400 mg/kg) for 14 days after CFA administration. The anti-inflammatory, analgesic, and anti-pyretic effects were respectively measured using a digital caliper (paw edema), von Frey hair filaments (tactile allodynia), and a digital thermometer (fever). These parameters were recorded on 0, 1, 4, 7, 10, 13, and 14th days before and after CFA injection. On day 15, animals were sacrificed, brain and spinal cord collected for the measurement of oxidative stress parameters such as Nitric oxide (NO), superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT).

Results: Both AEDG and MEDG significantly ($p<0.05$, $p<0.01$, $p<0.001$) increased pain threshold, decreased paw edema and fever in animals along the treatment. NO and MDA were significantly ($p<0.001$) reduced in dose-dependent manner with MEDG in rats' spinal cord. Furthermore, SOD and CAT activity were significantly and dose-dependently increased in both brain and spinal cord with AEDG and MEDG.

Conclusion: Our results demonstrated that AEDG and MEDG exhibit anti-inflammatory, antihypernociceptive, and anti-pyretic activities, which may be associated with their modulatory influence on oxidative stress markers.

Keywords: antihypernociceptive; anti-inflammatory; antipyretic effects; antioxidative stress; *Drypetes gossweileri*; stem barks.

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Background

Inflammation, which is a naturally occurring defense mechanism, takes place during an injury or an infection to recognize and eliminate harmful and foreign stimuli, allowing healing [1]. As well, inflammation is characterized by redness, swelling, heat, loss of function, and pain [2]. According to the International Association of Study of Pain, pain is ''An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage'' [3]. Indeed, Physiological pain is an alarm signal allowing the body to prevent further injury [4] ; however, if pain becomes persistent within time and last for days or months in the absence of adequate treatment, it become a real debilitating disease having a negative socioeconomical impact on the patient quality of life [5, 6]. Moreover, inflammatory pain is always associated with chronic diseases such as rheumatoid arthritis, cancer, and inflammatory bowel disease. Chronic inflammatory pain worldwide affects 25 to 30% of the adult population [7 - 9], and this may be even more in populations living in developing countries where all cases are not statistically recorded due to their poor level of health care system [10]. Hence, during the inflammatory process, pain sensation is exacerbated by the attraction of a variety of immune cells species in the inflamed site, leading to the release of pain mediators such as serotonin, histamine, substance P, glutamate, proinflammatory cytokines, reactive oxygenated species [11-14]. Oxidative stress is a phenomenon which occurs due to the high production of free radicals, including reactive oxygen species (ROS) and manifested as an unbalance between prooxidant and antioxidant molecules, leading to the decrease of cellular defense mechanisms [15]. Therefore, overproduction of those free radicals (superoxide, oxygen anion, hydroxyl radical, peroxyl radicals, nitric oxide) can induce various deleterious effects such as lipid peroxidation, membrane lipid disruption, nucleic acid denaturation, and the general alteration of cell function [16, 17]. ROS are involved in the physiopathology of chronic diseases such as inflammatory bowel disease, chronic obstructive pulmonary disease, diabetes, cancer, and rheumatoid arthritis [18-20]. However, antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) prevent or inhibit cellular damage and protect cells against oxidative stress [15]. Thus, in persistent inflammation and eventually chronic pain, ROS production can activate many pathways. Consequently, the overexpression of various proinflammatory (growth factors, tumor necrosing factors alpha (TNF-α), interleukins 1β and 6 (IL-1β and IL-6)) mediators contributes to amplify and aggravate pain sensation [21, 22]. Nevertheless, many solutions have been found for the management of inflammatory pain, including the use of drugs such as opioids (morphine), non-steroidal (diclofenac, ibuprofen, indomethacin and celecoxib) and steroidal (dexamethasone) anti-inflammatory drugs [23, 24]. Unfortunately, the drugs used demonstrated a higher level of side effects such as chronic gastrointestinal ulcer, sedation, kidney failure, myocardial infarction, hepatotoxicity, addiction, tolerance, and dependence when consumed chronically [25]. Therefore, there is an urgent need for alternative anti-inflammatory and analgesic drugs with less side effects, available and affordable.

According to the World Health Organization (WHO), about 80% of the people living in developing countries use traditional medicines exclusively for their health problems [26]. Nowadays, medicinal plants play a key role in cost-effective medical treatment and represent a rich source of bioactive molecules, especially to treat pain and inflammation-associated disorders [27- 29]. In light of the above-mentioned, plants such as *Drypetes gossweileri* (DG) commonly used in traditional medicine for the treatment of arthritis, ulcers, and pain disorders [30] may constitute an excellent alternative. *D. gossweileri* is a tree of approximately 30 to 40 meters in height with a diameter of 120 cm. It is a plant native to countries like Nigeria, Congo, Gabon, Central African Republic and Cameroon. *D. gossweileri* was shown from previous studies to contain secondary metabolites such as alkaloids, phenols, flavonoids, saponins, anthocyanins, anthraquinones, gossweilerine, sterols, and essential oils after the crude extract of the plant was screened [31]. Our previous study already provided evidence of the analgesic potential of AEDG on acute pain models induced in mice [32] and the acute toxicity carried out by Ngouana et al. [30], in another study strongly guides us in the doses used in this study. Therefore, this plant seems to be a promising alternative track. However, the mechanisms underlying its antihyperalgesic effect are still unknown. Hence, the objective of this work was to investigate the anti-inflammatory, analgesic, and antipyretic effects of *D. gossweileri* stem bark on persistent inflammatory pain and to elucidate its possible mode of action.

Methods

Plant collection and identification

Stem barks of *D. gossweileri* were collected during the dry season in Mefou and Akono subdivisions, Centre Region, Cameroon, in January 2024. The plant was identified and authenticated at the Cameroon National Herbarium in Yaoundé by Mr. Ngassop Eric. A voucher specimen was deposited with code number N° 5678/HNC. The stem barks were dried in the shade and crushed in a mechanical blender into a fine powder and kept for the extraction.

Plant extraction

Aqueous extract (AEDG) was obtained by decocting 1000 g of powder for 1hour into 5 liters of distilled water. After filtration with a Whatman paper N° 1, the filtrate was evaporated in an air oven at 45°C to obtain 198 g (19.8% yield). For the methanol extract (MEDG) preparation, 600 g of powder was macerated in 4 litres of methanol for 72 hours. After filtration using a Whatman paper N°. 1, the filtrate was evaporated through a rotary evaporator (66 rpm) at 45 °C. Thus, 96 g of extract was obtained with an extraction yield of 16%. Both extracts were kept in the freezer at 4°C until use.

Phytochemical analysis

In this section the method previously described by Rakib et al. [33] was performed for the qualitative phytochemical analyses, this, in order to determine the presence of metabolites such as saponins, flavonoids, steroids, tannins, phenols, alkaloids and cardiac glycosides.

Animals

Male adult Wistar rats weighing 200 to 250 g were used. They were obtained from the animal house of the Faculty of Health Sciences of the University of Buea and randomly assigned to individual treatment groups. They had free access to food and water. Experimental protocols used in this study were reviewed by the Ethical Committee at the Faculty of Science, University of Buea, and an ethical clearance number *UB-IACUC 17/2025* was

provided. All behavioral experiments were conducted blindly in order to reduce or avoid biases.

Chemicals

Complete Freund's adjuvant (CFA) was obtained from Sigma Chemical Co. (Taufkirchen, Germany); diclofenac sodium (Denk-Pharma, Germany) was obtained from local pharmacy. The aqueous, methanol extracts and diclofenac solutions were prepared by dissolving in distilled water.

Persistent inflammatory pain induction with complete Freund's adjuvant

Animals were divided into 9 groups (n=6) and treatment administered orally, distilled water (group 1) for negative control group, diclofenac (5mg/kg, *p.o*) for positive control (group 2), normal control group (group 3) as naive, treated groups received AEDG (group 4, 5 and 6) and MEDG (group 7, 8 and 9) at doses of 100, 200 and 400 mg/kg respectively. The animals were acclimatized for two days, and on day three, the baseline values of the animals for von Frey hair test, paw volume, and anal temperature were recorded. Then, they were constrained, the left hind paw skin was sterilized with 75% ethyl alcohol, and injected with 50 µL of CFA, except the naive group. Hence, animals were daily treated for 14 consecutive days, and mechanical allodynia withdrawal threshold test, paw oedema measurement, and anal temperature threshold were recorded on the 1st, 4, 7, 10th, and 13th days from the beginning of treatments. On the 14th day, the open-field test was carried out to analyze the rat's locomotor activity as previously described [34].

Different tests assessed during the treatment after CFA injection

Von Frey test

A series of von Frey filaments (Ugo Basile, Italy) calibrated in terms of different bending forces (0.08–300 g) was applied to the mid-plantar surface of the paw. The filaments were applied five times in the same region, starting with a mid-range filament (4 g). A brisk withdrawal of the paw was considered as a positive response. If the animal does not respond, another fiber with increased stiffness was selected and vice versa. The 50% paw withdrawal threshold (PWT) was calculated using a modified Dixon's up–down method [35].

Paw oedema measurement

The change in paw thickness (mm) was measured using a digital calibrated Vernier Caliper (Model 2061, Mututoyo Digimatic Caliper, Japan) before and after the CFA injection. A change in paw thickness was considered a measure of inflammation and was calculated as the percent inhibition of inflammation [36].

% Oedema inhibition = 100 (1-Vt/Vc)

Where: Vc = Oedema volume in the control group and Vt = Oedema volume in the test group.

Antipyretic test

Antipyretic activity in rats was evaluated by inserting a digital thermometer into their rectum, and the temperature was recorded before and after treatment, as previously described [37].

% inhibition = (Tb – Ta /Tb) x 100

Where: Tb = Rectal temperature before treatment and Ta = rectal temperature after treatment.

Open field test

Each animal was placed in the corner of the open field (100×100×50 cm), and the number of squares crossed during a 5-minute testing session was recorded. The open field area was properly cleaned with 75% alcohol before the next animal was placed in the device area [38]. Afterwards, on day15th, animals were anesthetized with a combination of ketamine/xylazine (2 mL/kg and 1 mL/kg, respectively) and sacrificed. The spinal cord and brain were carefully and rapidly collected. The two hemispheres of the brain were separated, and the right brain was collected and weighed. Organs were crushed and homogenized at 15% in cold PBS and centrifuged at 10,000 rpm for 20 minutes at 4°C [39]. The supernatant was collected and used for biochemical analysis.

Biochemical analysis (antioxidant assay)

The oxidative stress markers, such as malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT), and nitric oxide (NO), were measured in the animal brain and spinal cord supernatant. Thus, lipid peroxidation was determined by the MDA level in the supernatant using the method previously described by Karimi et al. [40]. CAT and SOD assays were performed following the protocol previously documented [41, 42]. The total nitrate/nitrite in brain and spinal cord supernatant was estimated by calorimetric method using Griess reagent according to the procedure conducted by Arab et al. [43].

Statistical analysis

Data were expressed as mean ± SEM for six animals per group (n=6). One-way ANOVA followed by Tukey's post-test was used for one variable parameter. Two-way ANOVA-repeated measure followed by Bonferroni post-test, was used to analyze two variables' parameters. $p < 0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Prism 5.01.

Results

Qualitative phytochemicals analyses

The results obtained from the phytochemical analyses of AEDG and MEDG revealed the presence of saponins, steroids, tannins, alkaloids and phenols (Table 1)

Anti-pyretic activity of AEDG and MEDG in CFA induced persistent inflammatory pain

As shown in Figure 1, the antipyretic effect was significant ($p<0.001$) in both extracts. However, AEDG significantly ($p<0.05$; $p<0.01$) reduced fever at all the doses for the first day of treatment: at the dose of 200 mg/kg on day 4, and on day 13 at the doses of 200 and 400 mg/kg (Figure 1A), while MEDG significantly ($p<0.05$; $p<0.01$) reduced fever only on the first day at the doses of 200 and 400 mg/kg respectively as compared to the control group (Figure

1B). Likewise, diclofenac at the dose of 5 mg/kg also significantly ($p<0.001$) decreased fever in rats.

Effect of AEDG and MEDG on paw edema in CFA-induced persistent inflammatory pain

The oedema induced by sub-plantar administration of CFA was significantly ($p<0.001$) reduced by both extracts (Figure 2). A significant anti-inflammatory effect of the aqueous extract (AEDG) was observed starting from the 100 mg/kg dose, with sustained activity up to day 13 (Figure 2A). The most pronounced effect, however, was achieved with the methanolic extract (MEDG) at 200 mg/kg, showing a 33.87% inhibition of oedema compared to the vehicle-treated group.

Effect of AEDG and MEDG on mechanical allodynia with Von Frey hair in CFA-induced chronic inflammatory pain

Figure 3 below shows that both extracts significantly ($p<0.001$) increased the animal pain threshold from day 7 to day 13. However, the highest (82.43%) effect was obtained with MEDG at the dose of 400 mg/kg on day 10 as compared to the distilled water group (Figure 3B). Meanwhile, the effect of diclofenac 5 mg/kg was also significantly increased ($p<0.001$; $p<0.05$) from day one until day ten as compared to the vehicle group.

Effect of AEDG and MEDG on locomotor activity using the open field test on CFA-induced persistent inflammatory pain

It can be observed in Figure 4 that the mobility of animals treated with both AEDG and MEDG was not significantly affected during the experiment as compared to the distilled water group. However, the mobility in the diclofenac group was significantly ($p<0.05$) reduced following the open field test.

Biochemical analysis in brain and spinal cord supernatant

Effect of AEDG and MEDG on superoxide dismutase (SOD) activity in rat brain and spinal cord

As it can be observed in Figure 5, both extracts significantly ($p<0.01$; $p<0.001$) increased SOD activity in the brain and spinal cord at almost all doses. However, in the brain, MEDG significantly ($p<0.01$; $p<0.001$) and in a dose-dependent manner increased SOD activity with a higher effect at the dose of 400 mg/kg (Figure 5B). Regarding the spinal cord, SOD activity was significantly increased with the highest effect at the dose of 200 mg/kg for MEDG as compared with the vehicle group receiving distilled water (Figure 5D).

Effect of AEDG and MEDG on catalase activity in rat brain and spinal cord

The level of catalase activity in both brain and spinal tissue was significantly ($p<0.05$; $p<0.01$) increased by both extracts, as shown in Figure 6. Furthermore, in the brain, CAT activity was noteworthy ($p<0.01$) with MEDG treatment (Figure 6B) at the doses of 200 and 400 mg/kg compared to the distilled water group. Meanwhile, in the spinal cord, the catalase activity was dose-dependent ($p<0.05$; $p<0.01$) with the higher ($p<0.01$) activity noted at the dose of 400 mg/kg of MEDG (Figure 6D).

Effect of AEDG and MEDG on malondialdehyde level in rat brain and spinal cord

As it can be seen in Figure 7, both aqueous and methanol extracts significantly ($p<0.05$; $p<0.01$; $p<0.001$) decreased the level of MDA in the rat's brain and spinal cord. However, the MDA level reduction in the brain was remarkable and dose-dependent in animals treated with MEDG, with the higher effect at the dose of 400 mg/kg compared to the vehicle group (Figure 7B). Accordingly, in the spinal cord, the most significant ($p<0.001$) effect was exhibited by the aqueous extract at the dose of 200 mg/kg as observed in Figure 7A.

Effect of AEDG and MEDG on nitrate level in rat brain and spinal cord

As shown in Figure 8, the level of nitrate was significantly ($p<0.05$; $p<0.01$) reduced by both extracts in both animal's brain and spinal cord. However, the level of NO in the brain was notably ($p<0.05$; $p<0.01$) and dose-dependently reduced in the animals treated with MEDG, with the highest effect induced at the dose of 200 mg/kg as compared to the vehicle group receiving distilled water (Figure 8B). At the level of the spinal cord, a significant ($p<0.05$) decrease of NO was observed with methanol extract at the dose of 200 mg/kg (Figure 8D). Surprisingly, diclofenac failed to induce a significant reduction effect in both brain and spinal cord as viewed in Figure 8.

Discussion

Our previous work on this plant showed that, when administered orally, AEDG induced a significant analgesic effect in acute pain models such as acetic acid and formalin tests [32], translating its peripheral and central acting drug. However, no study has been reported on DG extracts' activity on persistent pain conditions. So, the aim of the present study was to evaluate the antipyretic, anti-inflammatory, and antihypernociceptive properties of AEDG and MEDG on a persistent inflammatory model induced by CFA. It appears from the findings that both extracts possessed antipyretic, anti-inflammatory, and antihypernociceptive effects, which may be at least partially due to their ability to modulate oxidative stress parameters.

The administration of CFA under the animal hind paw is known to be associated with inflammation characterized by edema, redness, fever, and persistent pain [44]. During the inflammatory process, the release of free radicals such as hydrogen peroxide (H_2O_2) and NO) and activation of proinflammatory mediators such as TNF-α, IL1β and IL6 lead to the increased production of prostaglandins which is one of the main actions that elevate the fever by stimulating the hypothalamus [45]. However, it is well documented that non-steroidal anti-inflammatory (NSAIDS) drugs such as aspirin, diclofenac, and ibuprofen prevent the production of prostaglandins by targeting cyclooxygenases (COX) [46-48]. Our results on the antipyretic effect of AEDG and MEDG revealed that both extracts significantly reduced fever in animals, similar to diclofenac used as a positive control. This suggests that the decrease of fever observed may be due to their ability to inhibit pyrogenic agents through potentially the blockage of COX activity. Complete Freund's adjuvant injection under the rat's paw induces a prolonged edema that becomes maximal from the first day and persists for many days and can be related to an increased vascular permeability, infiltration of immune cells, followed by granuloma formation and the release of various proinflammatory mediators

such as cytokines, histamine, glutamate and prostaglandins [49, 50]. It appears in this study that both extracts during all the experimental period, significantly inhibited animal paw edema, demonstrating the interaction of the extracts with the edema mechanism described above. Persistent inflammatory pain is characterized by hyperalgesia and allodynia. In other words, pain perceived in this case is caused by central sensitization of the spinal cord and brain [51-53]. Indeed, Voltage-gated sodium channels are known to induce currents that influence the action potential of the pain threshold in the central nervous system. Previous studies reported that products such as ROS can increase mechanical allodynia through their ability to suppress potassium channels activity, induce higher sensitization of sodium and calcium channels, and reduce the activity of antioxidant enzymes such as SOD and CAT leading to the decrease in pain threshold and exacerbation of pain sensation [54-56]. Our results showed that giving orally, both extracts significantly inhibited mechanical allodynia by increasing the pain withdrawal threshold of animals. This may be explained by the capacity of the extracts to interfere with the process of ROS production or through the increase of antioxidant enzymes activity.

In order to determine the mechanism of action by which the extracts may induce an anti-inflammatory effect, the anti-oxidative stress parameters such as SOD, CAT, MDA, and NO were measured. Oxidative stress is when there is an imbalance of free radicals (including ROS) and antioxidants. This oxidative stress leads to the release of pro-inflammatory mediators and enhances a persistent inflammatory state. Likewise, this inflammatory state promotes the production of more free radicals such as superoxide radical (O_2^-), H_2O_2 radical causing oxidative stress and sustaining this state [57]. However, the body has put in place powerful mechanisms, including endogenous enzymes such as SOD and CAT, to regulate oxidative stress. The crucial role of SOD cannot be underrated, as it scavenges superoxide radical, thereby preventing the formation of other ROS like hydrogen peroxide, which could contribute to further tissue damage and pain [58, 59]. As well, it has been demonstrated that overexpression of SOD leads to reduction of allodynia and hyperalgesia by modulating the production of pro-inflammatory mediators such as TNF-α, IL-1β, IL-6, and cyclooxygenase in persistent inflammation [60, 61]. Our findings depicted that the aqueous and methanol extracts of the plant significantly increased the activity of SOD and may then explain the anti-inflammatory and analgesic effects observed. As SOD, CAT is also an antioxidant which catalyzes the decomposition of hydrogen peroxide into water and oxygen leading to the reduction of ROS levels in the body, thus, regulating oxidative stress and inflammation [62, 63]. In the present study, both extracts significantly increased catalase activity. This action may also partly justify their anti-inflammatory effect.

During persistent inflammatory pain, it has been described that an increase in lipid peroxidation is involved in the oxidation of polyunsaturated fatty acids found in cell membranes by free radicals, giving rise to the production of MDA [64]. This situation provokes the damage of the cellular membrane, inducing a change in membrane permeability and eventually leading to cell death. MDA acts as a potent proinflammatory molecule as it directly stimulates the production of proinflammatory cytokines as well as activation of nuclear factor kappa B (NF-κB), a transcription factor involved in proinflammatory gene expression, resulting in the amplification and persistence of inflammation [65, 66]. In the present work, MDA level in both the brain and spinal cord was significantly reduced, demonstrating the effectiveness of extracts in the inhibition of the inflammation. Additionally, NO is a key player in oxidative stress and persistent inflammation. In conditions of oxidative stress, inducible nitric oxide synthase (iNOS) leads to the formation of peroxynitrite from excess NO. Higher nitrate levels are associated with persistent inflammatory pain by stimulating the release of proinflammatory cytokines by glial cells at the level of the central nervous system, oxidizes proteins and lipids [67, 68]. These proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, are also known to induce the production of NO; therefore, this continuous cycle sustains persistent pain and inflammation [69]. However, the presence of compounds such as alkaloids, tannins, phenols, and steroids and especially anthraquinones may be responsible for the anti-inflammatory effect as they have been shown to reduce nitrate levels, MDA levels as well as increase SOD and CAT in previous studies [70, 71]. Additionally, the fact that diclofenac which is a peripheral acting drug used in this work failed to reduce oxidative stress markers such as NO clearly demonstrate that some secondary metabolites such as alkaloids (known as central nervous system acting drugs) present in the extracts may interfered with oxidative stress markers production or activity at the central nervous system level [72].

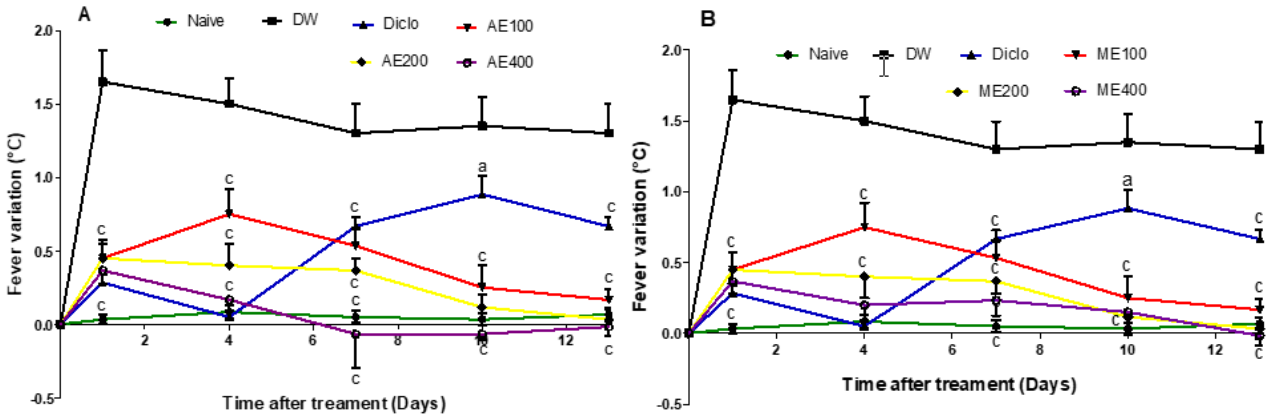

Figure 1. Antipyretic effect of oral administration of the AEDG and MEDG on CFA-induced persistent inflammatory pain.

Panel A presents the effect of aqueous extract, while Panel B shows the effect of the methanolic extract in rats. The values are expressed as mean ± SEM (n=6), [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ compared to the distilled water group. DW= distilled water; DICLO = diclofenac.

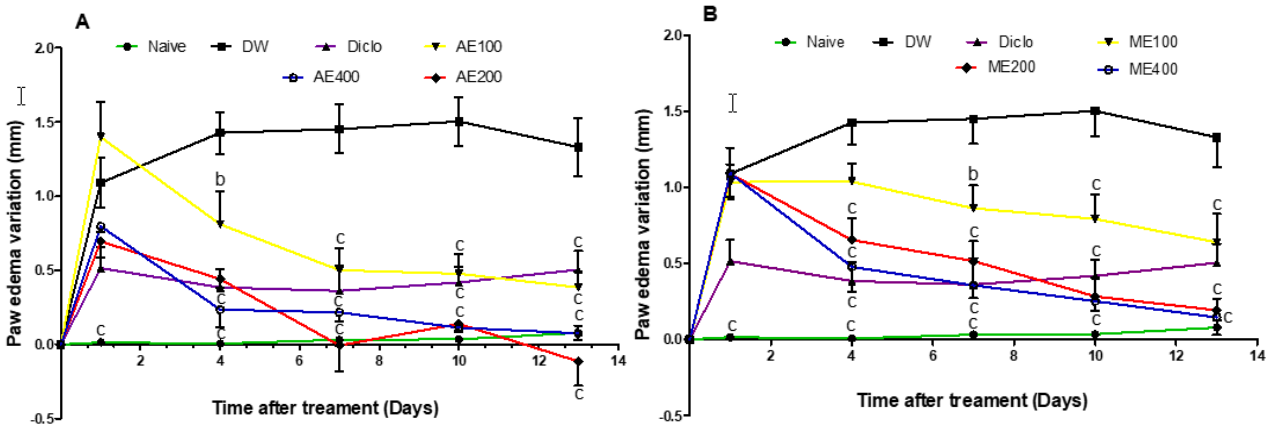

Figure 2. Effect of AEDG and MEDG on paw oedema in CFA-induced persistent inflammatory pain.

Panel A presents the effect of aqueous extract while panel B shows the effect of the methanolic extract in rats. The values are expressed as mean ± SEM (n=6), [a] p<0.05, [b] p<0.01, [c] p<0.001 compared to the distilled water group. DW = distilled water; DICLO = diclofenac.

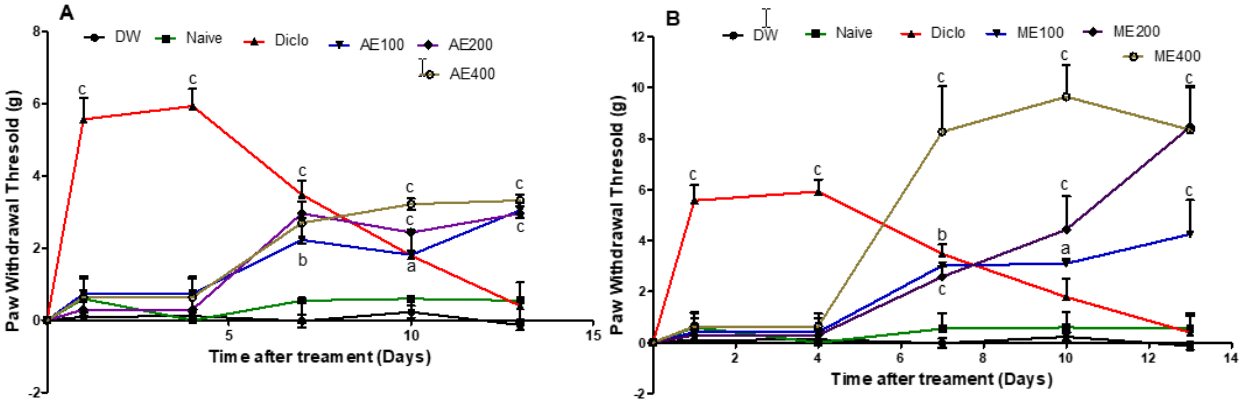

Figure 3. Effect of AEDG and MEDG on mechanical hyperalgesia in CFA-induced persistent inflammatory pain.

Panel A presents the effect of aqueous extract while panel B shows the effect of the methanolic extract in rats. The values are expressed as mean ± SEM (n=6), [a] p<0.05, [b] p<0.01, [c] p<0.001 compared to the distilled water group. DW = distilled water; DICLO = diclofenac.

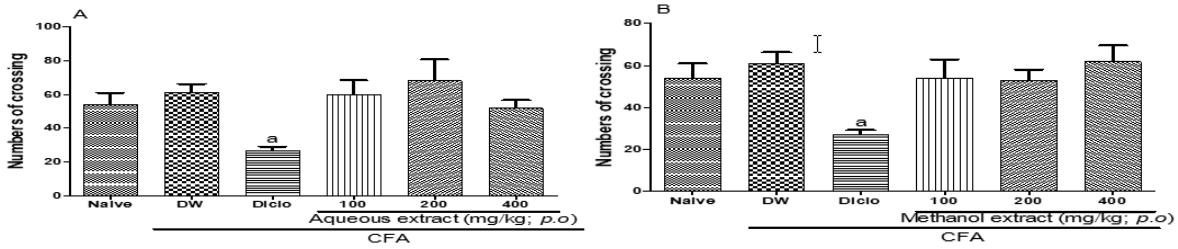

Figure 4. Effect of AEDG and MEDG on locomotor activity using the open field test on CFA-induced persistent inflammatory pain.

Panel A presents the effect of aqueous extract while panel B shows the effect the methanolic extract in rats. The values are expressed as mean ± SEM (n=6), [a] p<0.05, [b] p<0.01, [c] p<0.001 compared to the distilled water group. DW = distilled water; DICLO = diclofenac.

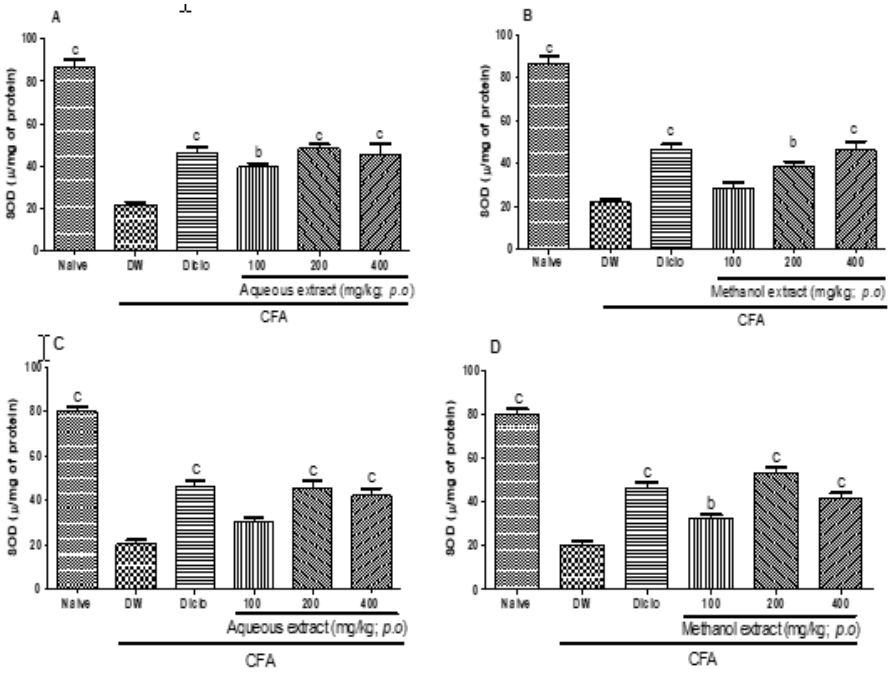

Figure 5. Effect of AEDG and MEDG on SOD activity in the brain (A, B) and spinal cord (C, D) of rats.

Panel (A, C) presents the effect of aqueous extract, while panel (B, D) shows the effect of the methanolic extract in rats. The values are expressed as mean ± SEM (n=6); [b] p<0.01, [c] p<0.001 as compared to the distilled water group. DW = distilled water; DICLO = diclofenac.

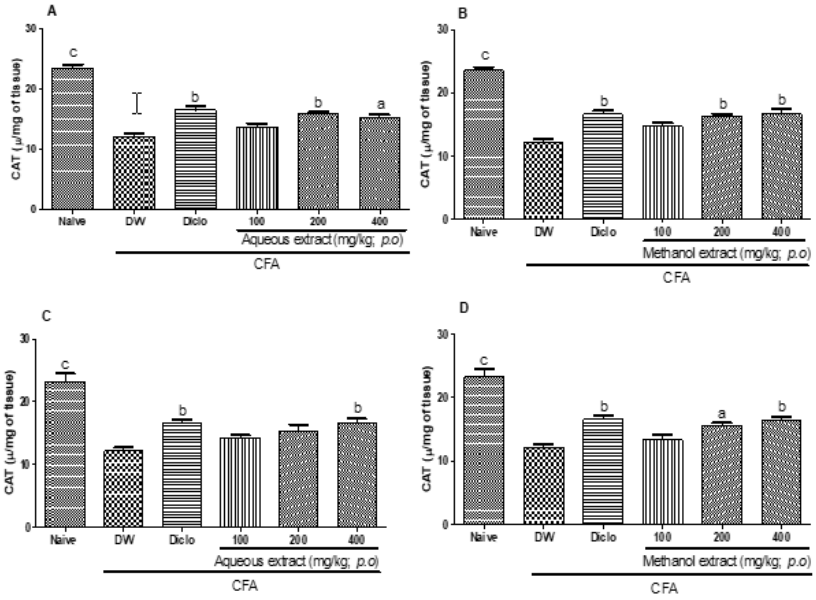

Figure 6. Effect of AEDG and MEDG on CAT activity in the brain (A, B) and spinal cord (C, D) of rats.

Panel (A, C) presents the effect of aqueous extract, while panel (B, D) shows the effect of the methanolic extract in rats. The values are expressed as mean ± SEM (n=6); a p<0.05, [b] p<0.01, [c] p<0.001as compared to the distilled water group. DW = distilled water; DICLO = diclofenac.

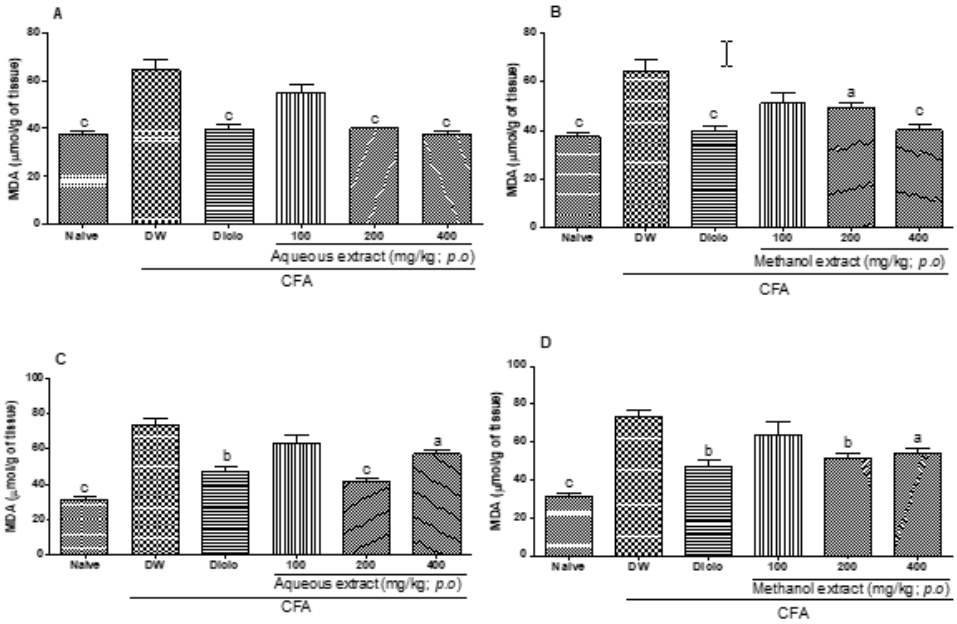

Figure 7. Effect of AEDG and MEDG on MDA activity in the brain (A, B) and spinal cord (C, D) of rats.
Panel (A, C) presents the effect of aqueous extract, while panel (B, D) shows the effect of the methanolic extract in rats. The values are expressed as mean ± SEM (n=6); [a] p<0.05, [b] p<0.01, [c] p<0.001as compared to the distilled water group. DW = distilled water; DICLO = diclofenac.

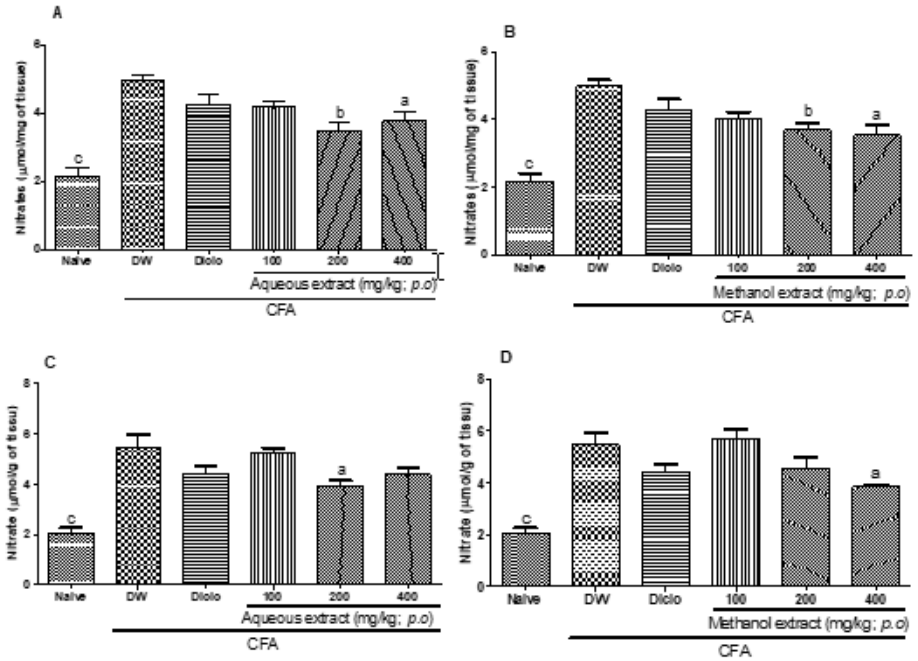

Figure 8. Effect of the aqueous and methanol extract on NO activity in the brain (A, B) and spinal cord (C, D) of rats.
Panel (A, C) presents the effect of aqueous extract, while panel (B, D) shows the effect of the methanolic extract in rats. The values are expressed as mean ± SEM (n=6); [a] p<0.05, [b] p<0.01, [c] p<0.001as compared to the distilled water group. DW = distilled water; DICLO = diclofenac.

Table 1. Phytochemical analysis of aqueous and methanol extracts of *Drypetes gossweileri* stem bark.

***Drypetes gossweileri* extracts**	**Saponins**	**Flavonoids**	**Steroids**	**Tannins**	**Alkaloids**	**Cardiac Glycosides**	**Phenols**
Aqueous	**+++**	**-**	**-**	**+++**	**++**	**-**	**-**
Methanol	**++**	**-**	**+++**	**++**	**++**	**-**	**+**

***+++** indicates the presence of **60-75%, ++ (50-55%), + (30-45%)** and **– (5-10%)** of the metabolites.*

Conclusion

This study demonstrates that AEDG and MEDG possess antipyretic, anti-inflammatory, and antihypernociceptive effects, which may be associated with their modulatory influence on oxidative stress markers. This may therefore explain the use of the plant in Cameroonian folk medicine.

Abbreviations

AEDG : Aqueous extract of *Drypetes gossweileri*
CAT : Catalase
CFA : Complete Freund Adjuvant
COX : Cyclooxygenase
H_2O_2 : Hydrogen peroxide
iNOS : inducible nitric oxide synthase
MDA : Malondialdehyde
MEDG : Methanolic
NO : Nitric oxide
NSAIDs: Non-steroidal anti-inflammatory drugs
ROS : Reactive Oxygenated Species
SOD : Superoxide Dismutase

Authors' Contribution

UEN, FDBT, and EAA designed, collected data, wrote the manuscript; FDBT, and EAA supervised the work and analyzed the data; BAW, MM, ENN, AJS, AFK, and TBN reviewed the paper draft.

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Conflict of interest

The authors declare no conflict of interest

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