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ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF THE ETHYL ACETATE FRACTION FROM *OZOROA PULCHERRIMA* SCHWEINF ROOTS METHANOLIC EXTRACT IN ANIMAL MODELS

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ABSTRACT
Ozoroa pulcherrima Schweinf, family Anacardiaceae, is traditionally used to treat various inflammatory diseases as conjunctivitis and intestinal helminthiasis. The aim of this study was to evaluate the analgesic and anti-inflammatory effects of the ethyl acetate fraction from *O. pulcherrima*roots methanolic extract (EAOp). The analgesic effect was evaluated using acetic acid or formalin-induced nociception in mice, and the tail immersion test in rats. The anti-inflammatory effect was tested in rats, by the carrageenan or histamine-induced paw oedema methods and by the cotton pellet-induced granuloma. EAOp dissolved in 1.5% DMSO was orally administered to animals at the doses of 25 and 50 mg/kg. Administration of EAOp to mice significantly ($P< 0.001$) reduces acetic acid-induced writhing with a maximum inhibition of 52.32% at 50 mg/kg, comparable to that of acetylsalicylic acid 100 mg/kg (49.58%).EAOp was effective on formalin-induced pain in both the two phases, with 44.05% inhibition at the dose of 50 mg/kg during the neurogenic phase. A significant increase of the threshold of sensitivity to heat during the tail immersion test was also recorded with a maximum of 51.89% inhibition of EAOp at 50 mg/kg at the 5th hour. Oral administration of EAOp at 25 mg/kg resulted in a significant reduction of carrageenan induced-paw oedema volume (61.54%) in a similar way to that of diclofenac10 mg/kg (53.85%) at the 3rd hour. EAOp was as effective (54.11% at 25 mg/kg and 58.90% at 50 mg/kg) as promethazine 1 mg/kg (60.96%) in inhibiting the histamine-induced inflammation. EAOp at the two doses also inhibits ($P< 0.001$) the granuloma formation induced by the cotton pellets. The findings from our present study demonstrate that EAOp possesses central and peripheral analgesic activity, as well as anti-inflammatory properties. EAOp could then serve a potential novel source of compounds effective in pain and inflammatory conditions.

Key words: *Ozoroa pulcherrima*, Analgesic activity, Antinociceptive activity, Anti-inflammatory activity, Medicinal plant.

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INTRODUCTION

Pain is a major public health problem and it limits the productivity and diminishes the quality of life. It's the primary reason for people to seek medical care (Dévora *et al*., 2015). The International Association for the Study of Pain (IASP) definition of pain, derived from Merskey (1968), is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or

described in terms of such damage. Inflammation is a defensive reaction of the body against infections and injuries. Oedema formation, leukocyte infiltration and granuloma formation represent typical features of inflammation (Sokeng *et al*., 2013a; Dimgom*et al*., 2017).Pain and inflammation are some of the most common manifestations of many diseases afflicting millions of people worldwide (Diaz *et al*., 2017). Inflammatory diseases are currently treated with steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) which are also used to relieve pain. Although they are effective, they are often associated with severe adverse side effects, such as gastrointestinal bleeding, peptic ulcers and renal morbidity (Ymele *et al*., 2011; Sokeng *et al*., 2013a, Diaz *et al*., 2017). There is, therefore, an urgent need todevelop drugs with less adverse effects. Plants are one of the most important sources of medicines. The use of natural remedies for the treatment of inflammatory and painful conditions have a long history, starting with Ayurvedic treatment, and extending to the European and other systems of traditional medicines. Plant drugs are known to play a vital role in the management of inflammatory diseases (Hemamalini *et al*., 2010).

Ozoroa pulcherrima Schweinf (syn.: *Heeria pulcherrrima* Schweinf), family Anacardiaceae, is a medicinal plant widely distributed from Guinea to Cameroon, in Central African Republic, in Ethiopia and in Sudan (Adjanohoun*et al*., 1996). In Benin, the stem and leaves are used to treat dystocia, hyperthermia, conjunctivitis, keratitis, cataract, blepharitis and trachoma, while in Cameroon, the root is used to treat dysmenorrhea and intestinal helminthiasis (Adjanohoun *et al*., 1989, 1996). Phytochemical studies of *O. pulcherrima* roots have resulted in the isolation and characterization of three alkylanacardicacids name dozo cardic A, ozorcardic A and ozorcardic B, while a ceramide named ozoromide has been isolated from its stem bark (Tsague*et al*., 2011a, 2011b, 2017). Essential oils, mainlyα-pinene, β-pinene, myrceneandlanosterolhave been extracted from the leaves and stems of *O. pulcherrima*, and have exhibited antiproliferative activity on breast cancer cells (Bogninou-Agbidinoukoun *et al*., 2016). Recently, our studies revealed that *O. pulcherrima* roots methanolic extract was effective against *S. mansoni*-induced liver inflammation in mice (Jatsa *et al*., 2018).The present study was carried out to evaluate the analgesic and the anti-inflammatory effects of the ethyl acetate fraction from *O. pulcherrima* roots

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Methanolic extract on experimentally induced pain and inflammation.

MATERIAL AND METHODS

Extraction and fractionation of the plant material

Ozoroa pulcherrima roots were harvested in the month of July 2012 in the locality of Wakwa near Ngaoundéré in the Adamawa region of Cameroon. A botanical identification of a plant sample was performed at the "National Herbarium" of Yaoundé, Cameroon. A voucher specimen of the plant is conserved in the "National Herbarium" under the reference 13667/SRF/Cam. *O. pulcherrima* roots were thoroughly washed with water and cut into pieces. They were air dried under shade to a constant weight. Dried roots were ground to a coarse powder and then stored in an air-tight container at room temperature until the time of usage. The powder (1500g) was submitted to maceration in methanol during 48 h. After filtration, the resulting filtrate was concentrated under reduced pressure and dried to give *O. pulcherrima* roots methanolic extract. This extract was solvent-partitioned into *n*-hexane and ethyl acetate. The resulting fractions were separately concentrated under reduced pressure and dried. This process allowed obtaining 24.80 g of *O. pulcherrima* ethyl acetate fraction (EAOp). This fraction was previously dissolved in 1.5% DMSO before administration to animals.

Preliminary phytochemical analysis

A qualitative chemical test of the ethyl acetate fraction from *O. pulcherrima* roots methanolic extract was conducted to identify phytochemical constituents in the fraction. The screening was performed for the identification of alkaloids, phenols, condensed and hydrolysable tannins, flavonoids, saponins, terpenoids, anthraquinones, cardiac glycosides, reducing sugars, lipids, steroids and triterpenes (Trease and Evans, 1983). The determination of the total phenolic content, as well as the flavonoid content in EAOp, was also conducted. The Folin-Ciocalteu's method was used for the determination of the total phenolic content expressed as mg of gallic acid equivalents (GAE) per g of dried extract (Mansouri *et al*., 2005).A spectrophotometric method was used for the determination of total flavonoid content expressed as mg rutin equivalents per g of dried extract (Pal *et al*., 2012).

Animals

Adult BALB/c mice (20 – 30 g) and Wistar rats (100 – 120 g) of both sex were used in the present study. They were bred in the animal house of the Laboratory of Animal Physiology, University of Yaoundé I, under standard laboratory conditions. They were kept in polypropylene cages in groups of five with free access to a standard diet for rodent and water. On the day of the experiment, after 14 h of fasting with water *ad libitum*, animals were acclimatized to the laboratory for at least 2

hours before performing any test and were used only once throughout the study.For each test, animals were divided into 4 groups of 5 animals each. Experimental protocols used herein comply with the guidelines for the study of pain in awake animals established by the International Association for the Study of Pain (IASP) and were approved by the Animal Ethical Committee of the Laboratory of Animal Physiology of the Faculty of Sciences, University of Yaoundé I – Cameroon.

Analgesic activity

Acetic acid-induced writhing

The test was carried out as described previously (Dongmo *et al*., 2005; Dingom *et al*., 2017). Mice were treated *per os* as follows: the negative control group received 1.5% DMSO (10 µL/g body weight) and the positive control group received 100 mg/kg of acetylsalicylic acid. Mice of the last two groups received EAOp at 25 or 50 mg/kg. Thirty minutes after the treatment, each mouse was intraperitoneally injected with a solution of 1%acetic acid (10 mL/kg body weight). Mice were individually placed in a transparent observation cageand we counted the number of writhing episodes for 30 min. The writhing activity consists of a contraction of the abdominal muscles together with a stretching of the hind limbs (Dévora *et al*., 2015).The percentage of inhibition was calculated using the formula:

$[(W_C – W_T) / W_C] \times 100$

W_C: mean of writhing in the control group; W_T: mean of writhing in the treated group

Formalin-induced nociception

This test was performed according to the protocol described by Hunskaar and Hole(1987). Thirty minutes after oral administration of 1.5% DMSO (negative control), EAOp at 25 or 50 mg/kg or indomethacin at 10 mg/kg (positive control), 20 µl of 1% formalin (formaldehyde 40% solution) was subcutaneously injected into the right hind paw aponevrosisof each mouse. The time (in seconds) the mice spent licking or biting the formalin-injected paw was recorded and taken as an indicator of pain response. The percentage of inhibition was then calculated. Responses were measured between 0 and 5 min (first phase) and 15 – 30min (second phase) after formalin injection, representing the neurogenic and inflammatory pain responses respectively.

Tail immersion-induced nociception

The test was performed as previously described by Vogel and Vogel(1997). The lower 5 cm portion of the tail of each rat was marked and they were treated *per os* with 1.5% DMSO (negative control), EAOp at 25 or 50 mg/kg or acetylsalicylic acid at 100 mg/kg (positive control). The tail of each rat was then immersed in a water bath maintained at 55 ± 1°C. The rats reacted within a few seconds by flicking or withdrawing their tail and this was measured with a stopwatch and recorded as there action time. Animals were tested before and at 0.5, 1, 2, 3,4 and 5 h after administration of substances. The cut-off time for tail immersion was 15 s to prevent tissue injuries. The percentage of inhibition was calculated using the formula below: [(Tf – Ti) / Ti] x 100

Tf:mean timeafter treatment in each group;Ti: mean time before treatment in each group

Anti-inflammatory activity

Carrageenan-induced paw oedema

The procedure used was similar to that described by Winter *et al*.(1962). Rats were treated *per os* with 1.5% DMSO (negative control), EAOp at 25 or 50 mg/kg or diclofenac at 100 mg/kg (positive control). Thirty minutes later, oedema was induced with the injection of 100µl of 1% carrageenan saline solution into the right hind paw. Paw volume was measured using a plethysmometer (UGO BASILE n° 7140) before carrageenan injection and after0.5, 1, 2, 3, 4 and 5h. The percentage of inhibition of the inflammation was expressed asfollows:

[[(Vt - V0) control - (Vt–V0) treated] / (Vt–V0) control] x 100

V0:mean paw volume for each group before carrageenan injection

Vt: mean paw volume for each group at a precise time after carrageenan injection

Histamine-induced paw oedema

This test was carried out following the method described by Mandal *et al*. (2000). Thirty minutes after oral administration of 1.5% DMSO (negative control), EAOp at 25 or 50 mg/kg or promethazine at 1 mg/kg (positive control) to rats, paw oedema was induced by a single injection of 100 µL of 1% histamine into the right hind paw. Paw volume was measured using a plethysmometer (UGO BASILE n° 7140) before histamine injection and after 1h. The paw oedema volume was then calculated and the percentage of inhibition of the inflammation determined.

Cotton pellet-induced granuloma

Cotton pellet-induced granuloma in rats was conducted according to the method described by Ismael *et al*.(1997). Granulomatous lesions were induced by surgically inserting sterile cotton pellets (7 ± 1 mg each) subcutaneously in both axilla regions of each rat following a single incision which was thereafter closed by interrupted sutures. After implantation of cotton pellets, 1.5% DMSO (negative control), EAOp at 25 or 50 mg/kg or indomethacin at 5 mg/kg (positive control) was administered orally to each rat, once daily for 7 consecutive days. On day 8, the cotton pellets were removed under anaesthesia, cleaned of extraneous tissue, weighed and dried at 40°C to a constant weight. The mean weights for different groups were determined. The

increase in dry weight of the pellets was taken as the measure of the granuloma formation. The percentage of inhibition of the inflammation was expressed as follows:
[[(W_G - W_C) control - (W_G - W_C) treated] / (W_G - W_C) control] x 100
W_G: granuloma weight; W_C: cotton weight

Statistical analysis

Data are expressed as mean ± SEM. Data were analysed using Graph Pad Prism version 7.00 for Windows, by one-way analysis of variance (ANOVA) and differences between groups were assessed using the Newman-Keuls multiple comparisons test multiple comparison post-test. Differences were considered significant from $P< 0.05$.

RESULTS

Phytochemical constituents of *Ozoroa pulcherrima* ethyl acetate fraction

Qualitative phytochemical analysis of *O. pulcherrima* ethyl acetate fraction (EAOp) revealed the presence of phenols, flavonoids, terpenoids, condensed and hydrolysable tannins, triterpenes, saponins, reducing sugars, cardiac glycosides, lipids and proteins. Alkaloids and anthraquinones were however absent in the fraction. In EAOp, the total phenolic content was 76.46 ± 0.01 mg gallic acid equivalent/g of dried extract (r^2 =0.9994) and total flavonoid content was 6.26 ± 0.31 mg rutin equivalent/g of dried extract (r^2= 0.9959).

Analgesic activity

Effect of *Ozoroa pulcherrima* ethyl acetate fraction on acetic acid-induced writhing

Intra-peritoneal injection of acetic acid produced abdominal writhing in mice. Administration of *Ozoroa pulcherrima* ethyl acetate fraction to mice prevents the nociceptive action of acetic acid by significantly reducing abdominal writhing by 40.54% at 25 mg/kg or 52.32% at 50 mg/kg. The administration of acetylsalicylic acid at 100 mg/kg used as a positive control also significantly reduced the nociception by49.58%. EAOp administered at 50 mg/kg was as effective as the acetylsalicylic acid in reducing the pain induced by acetic acid (Table 1).

Effect of *Ozoroa pulcherrima* ethyl acetate fraction on formalin-induced nociception

The results of the effects of EAOp on formalin-induced paw pain in mice are presented on Table 2.EAOp at all doses significantly reduced the licking time of the formalin-injected paw in both the neurogenic and inflammatory phases. The maximum inhibition of 44.05% by EAOp at 50 mg/kg and 39.68% by EAOp at 25 mg/kg were recorded during the neurogenic and the inflammatory phases respectively. Indomethacin (10 mg/kg) failed to significantly reduce the licking time during the neurogenic phase but inhibited the pain during the inflammatory phase with a 54.62% inhibition. Moreover, indomethacin was more potent than EAOp at 50 mg/kg ($P< 0.05$) during this phase.

Effect of *Ozoroa pulcherrima* ethyl acetate fraction on tail immersion-induced nociception

The anti-nociceptive effect of EAOp on tail-immersion pain is presented in Table 3. At all doses, EAOP significantly increased the reaction time to the thermal stimulus during the five hours' experimental period. The maximum inhibitory effect was recorded at the dose of 50 mg/kg with 51.89% inhibition at the fifth hour ($P< 0.01$). Use as the reference drug, acetylsalicylic acid at 100 mg/kg did not exhibita significant anti-nociceptive activity. EAOp was then more efficient than this drug ($P< 0.05$, $P< 0.01$) in inhibiting the tail immersion-induced pain.

Anti-inflammatory activity

Effect of *Ozoroa pulcherrima* ethyl acetate fraction on carrageenan-induced paw oedema

The inhibitory effect of EAOp on carrageenan-induced paw oedema is shown in Table 4. The sub-plantar injection of carrageenan produced a localized oedema that reached to its maximum at the 3rdhourafterinjection.Oral administration of EAOp at 25 mg/kg resulted in significant reduction of paw oedema volume by 45.83, 61.54 and 54.54% at the, 2nd ($P< 0.05$), 3rd($P< 0.01$) and 4thhour ($P< 0.05$) respectively, after injection of carrageenan, as compared with that of the control group. The dose of 50 mg/kg of EAOp produced a decrease of the paw oedema volume, but this effect was not statistically significant. Used as the reference drug, diclofenac at 10 mg/kgshowed a similar inhibitory effect with a maximal inhibition percentage of 54.54% ($P< 0.05$) at the 4thhour after carrageenan injection. The anti-inflammatory effect of EAOp at 25 mg/kg was then similar to that of diclofenac.

Effect of *Ozoroa pulcherrima* ethyl acetate fraction on histamine-induced paw oedema

The sub-plantar injection of histamine produced, 1 hour after, a paw oedema. As shown in Figure 1, EAOp at all doses produced a significant reduction of the oedema volume as compared to that of the control. Inhibition percentages of 54.11% at 25 mg/kg ($P< 0.05$) and 58.90% at 50 mg/kg ($P< 0.01$)were recorded. Similarly, promethazine used as a standard anti-inflammatory drug, at 1 mg/kg, produced 60.96% inhibition of histamine-inducedoedema. EAOp was then as effective as promethazine in inhibiting the histamine-induced inflammation.

Effect of *Ozoroa pulcherrima* ethyl acetate fraction on cotton pellets-induced granuloma

The anti-inflammatory effect of EAOp on the

cotton pellet-induced granuloma is presented in Figure 2. EAOp significantly decreased the granuloma weight by 25.47% at 25 mg/kg ($P< 0.001$) and by 17.62% at 50 mg/kg ($P< 0.01$) as compared to that of the control. The reference drug indomethacin, at 5 mg/kg produced the highest inhibition of 39.85% and was then statistically more efficient than EAOp at 25 mg/kg ($P< 0.05$) and 50 mg/kg ($P< 0.01$).

Table 1. Effect of *Ozoroa pulcherrima* ethyl acetate fraction on acetic acid-induced writhing

Treatment	Dose (mg/kg)	Number of writhing	Percentage of inhibition (%)
Vehicle (1.5% DMSO)	/	73.00 ± 3.29	/
EAOp	25	43.40 ± 4.53***	40.54
EAOp	50	34.80 ± 2.56***	52.32
Acetylsalicylic acid	100	36.80 ± 3.83***	49.58

Data are expressed as mean ± SEM (n=5). ANOVA followed by Newman-Keuls multiple comparisons test was used for statistical analysis. ***$P < 0.001$: values are significantly different from the negativecontrol (Vehicle).

Table 2. Effect of *Ozoroa pulcherrima* ethyl acetate fraction on formalin-induced nociception

Treatment	Dose (mg/kg)	Neurogenic phase(0 – 5 min)		Inflammatory phase(15 – 30 min)	
		Licking time (s)	Inhibition (%)	Licking time (s)	Inhibition (%)
Vehicle	/	90.72 ± 12.24	/	88.18 ± 6.23	/
EAOp	25	53.31 ± 6.61*	41.23	54.07 ± 3.96***	38.69
EAOp	50	50.75 ± 6.42*	44.05	62.55 ± 5.11**, £	29.07
Indomethacin	10	72.33 ± 11.21	20.27	40.02 ± 4.90***	54.62

Data are expressed as mean ± SEM (n=5). ANOVA followed by Newman-Keuls multiple comparisons test was used for statistical analysis. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$: values are significantly different from the negative control (Vehicle: 1.5% DMSO); £$P< 0.05$: values are significantly different from the positive control (indomethacin 10 mg/kg).

Table 3. Effect of *Ozoroa pulcherrima* ethyl acetate fraction on tail immersion-induced nociception

Treatment	Dose (mg/kg)	Reaction time (seconds)						
		0 h	0.5 h	1 h	2 h	3 h	4 h	5h
Vehicle (1.5% DMSO)	/	1.78 ± 0.15	1.59 ± 0.12	1.57 ± 0.22	1.58 ± 0.20	1.60 ± 0.12	1.60 ± 0.10	1.85 ± 0.12
EAOp	25	1.61 ± 0.18	2.06 ± 0.25 (28.36)	2.24 ± 0.15 (39.30)	2.38 ± 0.13*, £ (48.43)	2.29 ± 0.17* (42.66)	2.09 ± 0.28 (29.60)	2.34 ± 0.18*, £ (45.77)
EAOp	50	1.64 ± 0.23	2.24 ± 0.08* (37.00)	2.41 ± 0.20* (47.13)	2.43 ± 0.19**, £ (48.59)	2.17 ± 0.05 (32.72)	2.43 ± 0.16*, ££ (48.35)	2.49 ± 0.08**, ££ (51.89)
Acetylsalicylic acid	100	1.69 ± 0.10	1.64 ± 0.20	1.89 ± 0.28 (11.73)	1.76 ± 0.17 (4.26)	1.62 ± 0.23	1.40 ± 0.14	1.74 ± 0.09

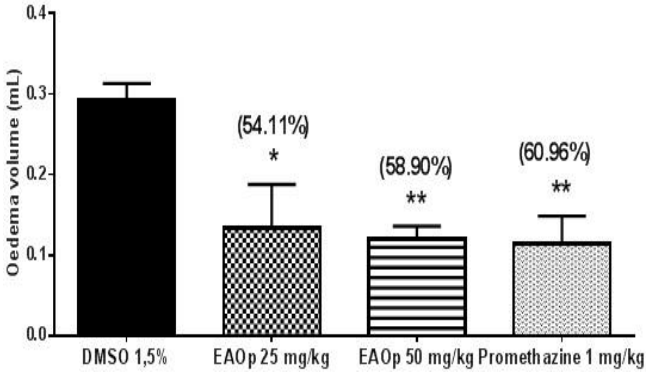
Data are expressed as mean ± SEM (n=5). Values in brackets represent the percentage of inhibition (%) of the pain. ANOVA followed by Newman-Keuls multiple comparisons test was used for statistical analysis. *$P < 0.05$, **$P < 0.01$: values are significantly different from the negative control (Vehicle: 1.5% DMSO); £$P< 0.05$, ££$P< 0.01$: values are significantly different from the positive control (Acetylsalicylic acid 100 mg/kg).

Table 4. Effect of *Ozoroa pulcherrima* ethyl acetate fraction on carrageenan-induced paw oedema

Treatment	Dose (mg/kg)	Paw oedema volume (mL)					
		0.5 h	1 h	2 h	3 h	4 h	5h
Vehicle (1.5% DMSO)	/	0.13 ± 0.01	0.17 ± 0.01	0.24 ± 0.03	0.26 ± 0.02	0.22 ± 0.02	0.17 ± 0.02
EAOp	25	0.10 ± 0.01 (22.22)	0.12 ± 0.01 (29.41)	0.13 ± 0.01* (45.83)	0.10 ± 0.02** (61.54)	0.10 ± 0.03* (54.54)	0.12 ± 0.03 (29.41)
EAOp	50	0.08 ± 0.02 (36.51)	0.14 ± 0.02 (17.65)	0.17 ± 0.02 (29.17)	0.18 ± 0.04 (30.77)	0.14 ± 0.04 (36.36)	0.12 ± 0.01 (29.41)
Diclofenac	10	0.12 ± 0.01 (3.17)	0.12 ± 0.01 (29.41)	0.12 ± 0.01** (50.00)	0.12 ± 0.03* (53.85)	0.10 ± 0.02* (54.54)	0.10 ± 0.02 (41.18)

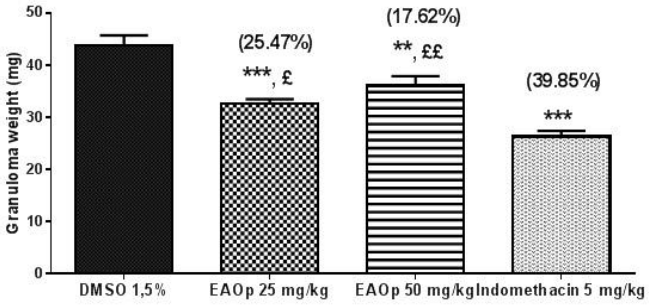
Data are expressed as mean ± SEM (n=5). Values in brackets represent the percentage of inhibition (%) of the inflammation. ANOVA followed by Newman-Keuls multiple comparisons test was used for statistical analysis. *$P < 0.05$, **$P < 0.01$: values are significantly different from the negative control (Vehicle: 1.5% DMSO)

Fig 1. Effect of *Ozoroa pulcherrima* ethyl acetate fraction on histamine-induced paw oedema.



Data are expressed as mean ± SEM (n=5). Values in brackets represent the percentage of inhibition (%) of the inflammation. ANOVA followed by Newman-Keuls multiple comparisons test was used for statistical analysis. **P* < 0.05, ***P* < 0.01: values are significantly different from the negative control (Vehicle: 1.5% DMSO)

Fig 2. Effect of *Ozoroa pulcherrima* ethyl acetate fraction on cotton pellets-induced granuloma.



Data are expressed as mean ± SEM (n=5). Values in brackets represent the percentage of inhibition (%) of the inflammation. ANOVA followed by Newman-Keuls multiple comparisons test was used for statistical analysis. ***P* < 0.01, ****P* < 0.001: values are significantly different from the negative control (Vehicle: 1.5% DMSO). $^{£}P$ < 0.05, $^{££}P$ < 0.01: values are significantly different from the positive control (Indomethacin 5 mg/kg).

DISCUSSION

To evaluate the analgesic activity of *O. pulcherrima* ethyl acetate fraction (EAOp), we designed different tests to measure different types of pain: chemical stimuli by acetic acid-induced writhing and formalin test as well as a thermal stimulus by the tail immersion test. The writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like phenylquinone or acetic acid in mice (Gawade, 2012). We first evaluate the anti-nociceptive effect of EAOp in acetic acid-induced abdominal writhing in mice, which is a visceral pain model. This model is reliable, simple, sensitive and particularly suitable for evaluating even weaker analgesics (Le Bars *et al*., 2001). After acetic acid injection, mice show episodes of abdominal constriction followed by extension of the hind limbs. Writhing is an overt response to the intense pain induced by irritant principles via nociceptors. In fact, acetic acid causes algesia by releasing of noxious endogenous substances including histamine, serotonin, bradykinin, substance P and prostaglandins which then excite the pain nerve endings leading to the abdominal writhing (Dévora *et al*., 2015; Buhkari *et al*, 2016; Bribi *et al*., 2017). It has been demonstrated that the intraperitoneal injection of acetic acid is associated with an increase of the concentration of prostaglandins PGE2 and PGF2α in the peritoneal fluid (Dévora*et al*., 2015; Buhkari *et al*., 2016, Tatiya*et al*., 2017). Furthermore, acetic acid injection induces a release of TNF-α, interleukin-1β and interleukin-8 by resident peritoneal macrophages and mast cells (Cavalcante-Silva *et al*., 2014; Boakye-Gyasi *et al*.,2017). The analgesic activity of a test compound is thus inferring from the decrease in the frequency of writhing. In this study, EAOp administered orally at the doses of 25 and 50 mg/kg significantly decreases the number of writhing induced by acetic acid in mice. EAOp inhibited the number of writhing as the standard drug acetylsalicylic acid at 100 mg/kg. Acetylsalicylic acid inhibits cyclooxygenase in peripheral tissues, thus, interfering with the mechanism of transduction in primary afferent nociceptors via inhibiting the synthesis of prostaglandins (Hirose *et al*., 1984). EAOp could exert its analgesic action by interfering with the synthesis and the release of endogenous substances, the blockage of their effect or the desensitization of the sensory C-fibres that excite pain nerve endings. Several medicinal plants used as analgesic such as *Smilax canariensis* (Dévora et al., 2015), *Buddle jacrispa* (Bukhari et al., 2016), *Bridelia retusa* (Tatiya et al., 2017), *Fumaria capreolata* (Bribi et al., 2017), *Paulinia pinnata* (Dingom et al., 2017) and *Ziziphusabys sinica* (Boakye-Gyasi et al., 2017)have been shown to decrease abdominal constriction induced by acetic acid. The acetic acid-inducedwriting test is effective but it is non-selective since it equally detects some non-analgesics such as antihistamines, muscle relaxants and monoamine oxidase inhibitors (Le Bars*et al*., 2001). Therefore, in order to obtain more specific evidence of the nociceptive activity of EAOp, formalin test was used. Formalin test is particularly very useful for the evaluation of new analgesics since it encompasses neurogenic, inflammatory and central mechanisms of nociception (Boakye-Gyasi et al., 2017).A distinct biphasic pain reaction usuallyresults when formalin is injected into mice hind paws.The early phase that corresponds to neurogenic pain is mediatedby the central nervous system via direct activation of peripheral nociceptors while the inflammatory pain occurs in the late phase and is accompanied by the release of inflammatory mediators such as histamine, serotonin, bradykinin and prostaglandins at the peripheralsite (Hunskaar and Hole, 1987; Tjølsen *et al*., 1992). In the

present study, the oral administration of EAOp significantly reduced the paw licking time in both the neurogenic and the inflammatory phase, while indomethacin inhibits the late phase. Our results indicate that EAOp displayeda central and peripheral action while the action of indomethacin, an NSAID is peripheral. This implies that EAOp may have a direct effect on nociceptors associated with the early phase of the pain and a modulatory effect on the synthesis and/or the release of inflammatory mediators. To corroborate that EAOp has a central analgesic action, the tail immersiontest was conducted. This test uses a short thermal stimulus and the behavioural responses measured are considered to be spinally or supra-spinally integrated responses, so it is suitable for evaluation of centrally butnot of peripherally acting analgesic drugs. Substances exhibiting a good anti-nociceptive effect in this method may be considered as potent central analgesics (Ymele *et al*., 2011; Muhammad, 2014; Dévora *et al*., 2015; Boakye-Gyasi *et al*., 2017). The central analgesics activate the release of endogenous peptides such as of prostaglandins, leukotrienes, bradykininsvia the periaqueductal grey matter (PAG), which is then carried to the spinal cord to inhibit the pain impulse transmission within the dorsal horn (Boakye-Gyasi *et al*., 2017; Diaz *et al*., 2017). Pain threshold in the tail immersion test was significantly increased by EAOp, suggesting that it may be acting spinally or supra spinally to interfere with the nociception process. Acetylsalicylic acid was not effective in the tail immersion-induced nociception test, since this model of pain is sensitive and specific for strong analgesics like opioids, while peripherally acting analgesics like acetylsalicylic acid are inactive (Diaz *et al*., 2017).

The results obtained in the present study suggest that EAOp may act via neurogenic pain as well as inflammatory pain. We, therefore, studied its effects in standard experimental models of inflammation. The carrageenan-induced paw inflammation is a suitable experimental animal model of acute inflammation and is sensitive to most clinically effective anti-inflammatory drugs. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from a biphasic release of various inflammatory mediators. In the early phase (1 - 2 h after carrageenan injection), the oedema production is mediated by histamine and serotonin while in the late phase (3 - 5 h after carrageenan injection) the vascular permeability is maintained by bradykinin and prostaglandins. These mediators contribute in the inflammatory response and induce pain. (Vinegar *et al*., 1969; Burchand De Haas, 1990; Akkol *et al*., 2007; Hemamalini *et al*., 2010; Sokeng *et al*., 2013a). The anti-inflammatory activity of EAOp against carrageenan-induced paw oedema was confirmed with reduced paw volume in the early (2h after carrageenan injection) and second phases (3 and 4h after carrageenan injection) in the same manner as diclofenac. The literature reports the anti-inflammatory activity of many medicinal plants against carrageenan-induced paw oedema (Akkol *et al*., 2007; Awaad *et al*., 2011; Ymele *et al*., 2011; Sokeng *et al*., 2013a, 2013b;Dévora*et al*., 2015; Diaz *et al*., 2017; Dingom*et al*., 2017;Tatiya*et al*., 2017). In the present study, EAOp was as effective as diclofenac, a potent NSAID which exerts its action via inhibition of prostaglandin synthesis by inhibiting cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Gan, 2010). Based on our result, the possible mechanism of the anti-inflammatory activity of EAOp might be its ability to inhibit the synthesis and/or the release of various inflammatory mediators such as histamine, serotonin, bradykinin and prostaglandins. The anti-inflammatory activity of EAOp in the early phase in the carrageenan-induced rat paw oedema was also confirmed in the histamine-induced paw oedema model. Histamine is the inflammatory mediator of the early phase of inflammation which promotes vasodilation and increases vascular permeability (Ymele *et al*., 2011; Dingom *et al*., 2017). In the present study, EAOp significantly inhibited the paw oedema formation produced by a subcutaneous injection of histamine. The anti-inflammatory effect of EAOp at 50 mg/kg was almost near to that of the standard drug promethazine. This result indicates that EAOp may interfere with the secretion and the action of histamine in inducing inflammation. In fact, promethazine is an antihistamine that inhibits the release of proinflammatory mediators from mast cells and basophils, the chemotaxis and activation of inflammatory cells, especially eosinophils, and the expression of adhesion molecules on epithelial cells (Assanasen and Naclerio, 2002). In order to assess the effect of EAOp on the activation of inflammatory cells during the process of inflammation, we conducted the cotton pellet-induced granuloma test which is a suitable method for studying the efficacy of drugs against the proliferative phase of inflammation (Parvataneni *et al*., 2005; Sokeng *et al*., 2013a). In this model, EAOp at doses of 25 and 50 mg/kg, effectively inhibited the development of the granulomatous tissues as compared to the control group. However, the standard drug indomethacin at 5 mg/kg was more effective than the fraction. EAOp may act by inhibiting neutrophils and macrophages migration or may inhibit the activity of fibroblasts and the synthesis of collagen, which are natural proliferative events of granuloma formation. Similar anti-inflammatory effects of medicinal plants have been reported in the literature (Sokeng *et al*., 2013a, 2013b;Tatiya *et al*., 2017).

The fact that the ethyl acetate fraction of *O. pulcherrima* roots methanolic extract (EAOp) produced good analgesic and anti-inflammatory activities leads us to conclude that these activities were possibly due to the presence of chemical compounds such as phenols, flavonoids, terpenoids, tannins, triterpenes, saponins, reducing sugars, cardiac glycosides, lipids and proteins

identified in this fraction. Phenolic compounds (phenols, flavonoids, tannins) are important bioactive components of medicinal plant extracts and are primarily natural antioxidants which act as reducing agents, metal chelators and singlet oxygen quenchers (Ahmed *et al*., 2014). With its good total phenolic content of 76.46 ± 0.01 mg GAE/g of dried extract, EAOp would be a good potential antioxidant. In fact, in a previous study, we have demonstrated the *in vivo* antioxidant capacity of *O. pulcherrima* roots methanolic extract (Jatsa *et al*., 2018). Pharmacological studies of others species of *Ozoroa* have revealed that the acidified 70% acetone crude extracts and the ethyl acetate fractions from *O. mucronata* and *O. paniculosa* exhibit good radical scavenging activities toward DPPH, ABTS and OH radicals. Moreover, these crude extracts inhibit the linoleic acid peroxidation and the 15-lipoxygenase enzyme(Ahmed *et al*., 2014). It has indeed been reported that anacardic acids from *O.mucronata* and *O. paniculosa* possess anti-inflammatory properties by inhibition of prostaglandin synthetase and lipoxygenase (LOX) (Kubo *et al*., 1987; Ha and Kubo, 2005). Since prostaglandins and LOX are deeply implicated in the analgesic and inflammatory processes, the analgesic and anti-inflammatory activities of EAOp could therefore be linked to the bioactive compounds alkylanarcadic acids isolated from *O. pulcherrima* (Tsague *et al*., 2011a, 2011b). The presence of saponins in EAOp could also explain its anti-nociceptive effect, since the capacity of saponins to inhibit abdominal constriction induced by *p*-benzoquinone in mice has been demonstrated (Akkol *et al*., 2007). EAOp contains other important secondary metabolites such as lipids, which may also participate in modulating the anti-inflammatory response (Mori and Beilin, 2004). So, the phytochemical constituents of EAOp might be responsible for its analgesic and anti-inflammatory activities and they may act individually or synergistically.

CONCLUSION

Results of the present study have clearly demonstrated that the ethyl acetate fraction of *O. pulcherrima* roots methanolic extract exhibits central and peripheral analgesic activity. It also possesses anti-inflammatory activity against both exudative and proliferative phases of inflammation. The beneficial effects of this plant may be correlated to the antioxidant activity of its phytochemical constituents. This study opens additional perspectives for the search of novel and safe drugs against inflammatory diseases.

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Nil

CONFLICT OF INTEREST

No interest

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