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Analgesic and anti-inflammatory activities of roots aqueous extract of *Pseudospondias microcarpa* (A. Rich.) Engl. (Anarcadiaceae) in mice and rats

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Abstract

Background: *Pseudospondias microcarpa (P. microcarpa)* is a tree belonging to the family Arnacardiaceae. This species is used in traditional medicine to treat pain, inflammation, malaria, and skin diseases. The present study was undertaken to evaluate the analgesic and anti-inflammatory properties of the aqueous extract of *P. microcarpa* roots.

Methods: Swiss mice and Wistar albino rats were used for the study. The aqueous extract (100, 300, and 600 mg/kg), standards, and water were administered by intragastric route. Pain was induced by intraperitoneal injection of acetic acid (1%, 10 mL/kg) or formalin paw injection (1%, 20 µL) solutions. Anti-inflammatory activity was carried out in experimental animals' models of acute inflammation using carrageenan (1%, 0.1mL) and arachidonic acid (0.1 mL, 0.2 M).

Results: The results showed that aqueous extract significantly inhibited ($P < 0.001$) the pain induced by acetic acid with 60.77% at the dose of 600 mg/kg. This dose of 600 mg/ kg also significantly reduced ($P < 0.01$) the neurogenic phase (50.81%) and the inflammatory phase (63.87%) of formalin-induced pain. In the inflammatory test, the aqueous extract of the roots of *P. microcarpa* significantly reduced carrageenan-induced paw edema at all hours with a maximum inhibition percentage of 79.40% (4h) at the dose of 600 mg/kg. Arachidonic acid-induced paw edema was significantly inhibited ($P < 0.001$) by the aqueous extract. The maximum inhibition was 82.35% at 600 mg/kg.

Conclusion: These results sufficiently demonstrate the analgesic and anti-inflammatory power of the aqueous extract of *P. microcarpa*. It should be noted that the analgesic property is both central and peripheral whereas the anti-inflammatory effect would be exerted by the cyclooxygenase and lipoxygenase pathways. This justifies the use of this plant in traditional medicine for the treatment of pain and inflammation.

Keywords: Arnacardiaceae; analgesic; anti-inflammatory; *Pseudospondias macrocarpa.*

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Background

Pain is defined as a physiological alarm signal that aims to protect the body against harmful stimuli that can cause tissue or organ damage and can be acute or chronic [1]. it is one of the first causes of medical consultation and characteristic of many pathologies and affects individuals of all ages [2]. Inflammation is a pathophysiological response to defense by the body against a threat or aggression. Pain and inflammation are common features of almost all other diseases [3]. The medical management of these pathologies involves a certain number of classes of drugs, namely analgesics or steroidal and non-steroidal anti-inflammatory drugs [4]. These different drugs cause side effects such as peptic ulcers, acute kidney failure, rashes, and allergies by prolonged use [4]. Also, some of these drugs are not available to a large number of the population due to their high cost. For these reasons, patients, especially those of the developing countries adopted phytotherapy for primary health care [5]. In Africa, phytotherapy is motivated by the existence of wide biodiversity. We also noticed that public interest in natural therapies has increased with the Covid-19 pandemic in Africa. Many plants belonging to the African flora, possess analgesic and anti-inflammatory properties; this is the case of *Pseudospondias macrocarpa* (A. Rich.) Engl. (Anarcadiaceae), a plant used in traditional African medicine and particularly in Cameroon as a sedative and for the treatment of pain, inflammation, constipation, and malaria [6]. However, no scientific study has been conducted on any pharmacological effect to justify the use of this plant in traditional treatment. The purpose of the present study was to investigate the possible analgesic and anti-inflammatory activities of the aqueous roots extract of *P. microcarpa*.

Methods

Plant material and extraction

The fresh roots of *Pseudospondias microcarpa*, were harvested at mount Eloundem, Yaoundé, Centre Region, Cameroon. The botanical identification was made at the National Herbarium of Cameroon in comparison with voucher specimen No. 23153/HNC. The roots aqueous extract of *Pseudospondias microcarpa* was prepared by boiling 1 kg of root powder in 10 liters of distilled water for 30 minutes. After filtration, the filtrate was lyophilized using a lyophilizer to obtain crude aqueous extract (yield of 3.04 g).

Experimental animals

Swiss mice (60 - 90 days; 20 - 25 g) and Wistar albino rats (75 – 90 days; 100 – 150 g) were used for assessment. They were bred in the animal house of the Laboratory of Biology and Physiology of Animals Organisms of the Faculty of Sciences, University of Douala, under standard conditions (ambient temperature, 12/12 hours light/ dark cycle). The animals received a standard diet and water *ad libitum.* The animals were fasted (with free access to water) overnight before dosing for anti-nociceptive and anti-inflammatory tests.

Analgesic activity of Pseudospondias microcarpa aqueous extract

Acetic acid test

The acetic acid-induced writhing pain was investigated in mice using the method described by Koster *et al.* [7]. Intraperitoneal injection of acetic acid solution (1%, 10 mL/kg) in mice causes pain that is manifested by abdominal writhing and stretching of the body. These manifestations can be decreased or stopped by the administration of an analgesic substance. Mice were treated orally with distilled water 10 mL/kg (control); paracetamol at a dose of 100 mg/k[1] body weight (standard drug) or aqueous extract of *Pseudospondias microcarpa* at doses of 100, 300 and 600 mg/kg body weight. Thirty minutes after administration of the substances, pain was induced by intra-peritoneal injection of acetic acid solution in each mouse. Abdominal writhings or stretching of the body which begin 5 minutes following injection, were counted for 30 minutes. The percentage of pain inhibition was calculated by the following formula [8]:

$$PI\ (\%) = [(N- N')/N] \times 100$$

PI = percentage of inhibition; N= the contractions mean of the control group; N'= contractions mean of the treated group.

Formalin test

The method previously described by Tjolsen et al. [9] was used. The mice were fasted 12 hours before the experiment. The mice were treated with vehicle (distilled water 10 mL/kg); tramadol (20 mg/kg body weight) or aqueous extract of *Pseudospondias microcarpa* (100, 300 and 600 mg/kg body weight). Thirty minutes after oral administration of drugs, each mouse received an injection of formalin (20 µL of 1%) into the sub-plantar of the right hind paw of the animals. The duration spent licking the paw, considered as indicative of pain, was recorded in two periods: the first period (neurogenic phase) extends for the first 5 minutes after the formalin injection and the second (inflammatory phase) from 20 to 30 minutes. The percentage of pain inhibition was calculated by the following formula:

$$PI\ (\%) = [\ (T-T')/T]x100$$

PI = percentage of inhibition; T = average licking time in the control group; T'= average licking time in the experimental group considered.

Anti-inflammatory activity of the aqueous extract of Pseudospondias microcarpa

Carrageenan induced paw edema test

The method described by Winter *et al*. [10] was used. The rats were subjected to fasting 12 hours before the experiment. They were treated with distilled water 10 mL/kg (negative control), or indomethacin at a dose of 10mg/kg of body weight (positive control) or aqueous extract of *Pseudospondias microcarpa* at doses of 100, 300, and 600 mg/kgof body weight. Acute inflammation was induced in rats by a single injection into the plantar aponeuvrosis of the right hind paw, of 0.1 mL of freshly prepared 1% carrageenan in normal

saline, 30 minutes before dosing. The initial volume (V_0) of the paw was measured before induction of edema and the volumes of the inflamed paw were recorded at 1/2, 1, 2, 3, 4, 5, and 6 h after injection of the carrageenan using the Ugo Basile 7510 plethysmometer. Anti-inflammatory activity was calculated as a percentage reduction of paw volume of treated rats by the following formula [11]:

$$PI(\%) = \frac{(Vt-Vo)control - (Vt-Vo)tested}{(Vt-Vo)control} \times 100$$

PI = percentage of inhibition; Vt = mean volume of edema in each group at a time t; Vo = mean volume of edema in each group before treatment.

Arachidonic acid-induced paw edema test

The animals were distributed as described on the carrageenan test above. Edema was induced by injection into the right plantar aponeuvrosis of the hind paw of rat, an arachidonic acid (0.1 mL) dissolved in carbonate buffer (0.2 M, pH: 8.43-8.56),1-hour post-administration of the substances [12]. Volumes of edema were measured before and half hour after administration of arachidonic acid. Anti-inflammatory activity was calculated as a percentage reduction in edema in rats treated with aqueous extract compared to control rats according to the formula [13].

Statistical analysis

Data were expressed as mean ± SEM. The calculation of the averages and percentages of inhibition was done using the Microsoft Excel 2007 workbook. For statistical evaluation, one-way and two ways analysis of variance (ANOVA) followed by Dennett's post hoc test was used in the analgesic test to compare the means. Bonferroni post test was used in inflammatory tests to compare the groups' edema volume means. The difference was considered significant at $P < 0.05$.

Results

Analgesic activity

Effect of aqueous extract of Pseudospondias microcarpa on acetic acid-induced pain

Analgesic effect of the aqueous extract of *P. microcarpa* roots against acetic acid-induced pain was assessed (Figure 1). Pretreatment with aqueous extract at doses of 100 to 600 mg/kg body weight showed a significant ($P<0.001$) reduction of the number of abdominal contortions induced by intraperitoneal injection of acetic acid in mice. This reduction was 60.77% at 600 mg/kg compared to control. Paracetamol (100 mg/kg) used as standard drug showed an inhibition percentage of 45.81%.

Effect of Aqueous Extract of Pseudospondias microcarpa on formalin-induced pain

Injection of 20 µL of formalin under the surface of the right paw generated two distinct phases (neurogenic and inflammatory) pain in mice. The aqueous plant extract (100 – 600 mg/kg) significantly ($P<0.01$) reduced the time spend to lick paw in each phase of the formalin test. The maximum inhibition percentage was 50.81% (600 mg/kg) at the first phase and 63.87 % (600 mg/kg) at the second phase (Figure 2). The reference drug, tramadol (20 mg/kg) also significantly ($P<0.001$) reduced pain in both phases with 70.03% and 80.92% inhibition respectively.

Anti-inflammatory activity

Effect of Aqueous Extract of P. microcarpa on Carrageenan-induced paw edema

The aqueous extract of *P. microcarpa* at different doses tested reduced paw volume edema induced by carrageenan compared to control. This reduction was very significant ($p < 0.001$) for all doses tested with a maximum of 59.20% (6 h), 72.40% (6 h) and 79.40% (4 h) respectively at doses 100, 300 and 600 mg/kg of body weight (Table 1). Indomethacin (10 mg/kg), the reference drug, significantly inhibited rat paw edema and the maximum inhibition percentage was 65.90% (5 h).

Effect of aqueous extract of P. microcarpa on arachidonic acid-induced paw edema

The aqueous extract of *P. microcarpa* at different doses tested inhibited arachidonic acid-induced paw edema compared to control. This inhibition was significant ($p < 0.001$) with inhibition percentages of 61.18% (100 mg/kg); 71.18% (300 mg/kg) and 82.35% (600 mg/kg), thirty minutes later. Indomethacin (10 mg/kg) used as the reference substance also caused a very significant ($p < 0.001$) decrease in arachidonic acid-induced edema with an inhibition percentage of 59.41% thirty minutes later (Table 2).

Discussion

Intraperitoneal injection of acetic acid in mice causes pain characterized by abdominal contortions and stretching of the body. This pain is due either by stimulation of chemo-sensitive nociceptors or by irritation of the visceral surface that results in the release of algogenic substances such as histamine, bradykinin, prostaglandins, and serotonin [14]. The aqueous extract of *P. microcarpa* significantly inhibited acetic acid-induced abdominal contortions which decreased from 75 abdominal contortions in the control group to 29 abdominal contortions at the doses of 600 mg/kg (60.77%) group. The analgesic effect of the aqueous extract of *P. microcarpa* on acetic acid-induced pain could be due to either an inhibitory action on acid-sensitive nociceptive receptors or to an inhibition of the production of algogens. Paracetamol, a reference peripheral analgesic drug, also inhibited acetic acid-induced pain (45.81%). Paracetamol reduces prostaglandin synthesis by inhibition at the peroxidase site of the enzyme prostaglandin H_2 synthase [15]. Since all analgesics have the property of inhibiting abdominal contractions induced by acetic acid, this test is useful for performing the first sorting of substances with an analgesic action [16] but does not allow to precisely indicate the site of action (central or peripheral) of the test substance.

The formalin test, one used to evaluate the central analgesic was performed. Intraplantar injection of formalin in mice causes biphasic pain: the first phase known as the neurogenic phase begins immediately after injection of formalin and lasts 5 minutes. The second phase took place between 20 and 30 minutes after formalin injection and is known as the inflammatory phase [17].

The neurogenic phase is due to the direct stimulation of C fibers and the release of substance P [18,19]. The inflammatory phase is mediated by the release of chemical mediators such as prostaglandins, serotonin, histamine, and kinins [20]. The aqueous extract of *P. microcarpa* (100, 300, and 600 mg/kg) significantly inhibited both phases of formalin-induced pain. Tramadol significantly ($p < 0.001$) inhibited formalin-induced pain during both phases. Tramadol, an agonist, activates μ receptors inhibiting the reuptake of serotonin and norepinephrine [21]. These findings suggest that aqueous extract of *P. microcarpa* would act as a central analgesic by inhibiting the release of chemical mediators. Phytochemistry study of *P microcarpa*, demonstrated the presence in this plant of flavonoids and triterpenes that can inhibit phospholipase A_2 and block the metabolism of arachidonic acid and cyclooxygenase enzymes [22, 23].

The effectiveness of the aqueous extract on visceral pain induced by acetic acid and on the late phase of formalin-induced pain all conducted by mediators of inflammation, suggests that this extract has anti-inflammatory activity. To explore this activity, carrageenan, and arachidonic acid-induced paw edema were performed. Carrageenan-induced rat paw edema is an appropriate experimental model for evaluating the anti-inflammatory properties of natural products [24]. The injection of carrageenan causes a triphasic edematous response. The first phase, which runs from 30 minutes to 1 hour after an injection of the phlogogen, is mainly due to a release of histamine and serotonin [25]. The second phase, which goes on between 2 to 3 h after carrageenan injection, is characterized by the release of inflammatory mediators such as kinins including bradykinin. The last phase extends from 4 h to 6 h is characterized by the release of prostaglandins and leukotrienes [26]. In our study, the aqueous extract significantly inhibited inflammation during the first phase, the maximum inhibition percentage was 65% (1 h) at the dose of 600 mg/kg body weight. The second phase of edema was significantly inhibited by the aqueous extract of *P. microcarpa* with a maximum inhibition percentage of 69.50% (3 h) at a dose of 600 mg/kg. Indomethacin, a nonsteroidal anti-inflammatory drug (NSAID), also inhibited this phase with a maximum inhibition of 60.10%. It is known that NSAID significantly inhibits the synthesis of mediators of the second phase by blocking the kallicrein responsible for the bioconversion of hepatic kininogens into kinins [27]. These results suggest that the aqueous extract would have, like NSAIDs, an inhibitory effect on kinin synthesis; this effect could also be related to the presence of steroids in this extract [28]. The third phase of carrageenan-induced edema was significantly inhibited by all doses of the aqueous extract of *P. microcarpa*. The maximum inhibition was 59.20% (6 h), 72.40 % (6 h) and 79.40% (4 h) respectively at doses 100, 300 and 600 mg/kg body weight. Indomethacin used as the reference substance also significantly inhibited this phase of inflammation with a maximum inhibition percentage of 65.90% (5 h). Indomethacin is a nonsteroidal anti-inflammatory drug and a known inhibitor of cyclooxygenase II, histamine synthesis, serotonin release, and bradykinin effects [29]. The aqueous extract could act as indomethacin by inhibiting cyclooxygenase II and/or the above mediators. The results of the present study show that the extract produced an anti-inflammatory effect during all hours following the injection of carrageenan.

Arachidonic acid-induced paw edema is the best *in vivo* model used to distinguish between cyclooxygenase and lipoxygenase inhibitors [30]. This edema is reduced by inhibitors of arachidonic acid metabolism and corticosteroids. However, it's insensitive to selective cyclooxygenase inhibitors [12, 28]. In the present work, edema caused by arachidonic acid was significantly inhibited by the aqueous extract of *P. microcarpa* with a maximum inhibition percentage of 82.35% (600 mg/kg) after 30 mn and one hour. Indomethacin, a cyclooxygenase inhibitor, caused inhibition of arachidonic acid-induced paw edema with a percentage of only 59.41%. These results suggest that this extract has an inhibitory effect on lipoxygenases. It's known that saponins, phenolic compounds, and other secondary metabolites present in the aqueous extract of *P. microcarpa* block the degradation of arachidonic acid by the cyclooxygenase and/or lipoxygenase pathways, preventing the production of prostaglandins, leukotrienes, and thromboxanes responsible for inflammation [29,30].

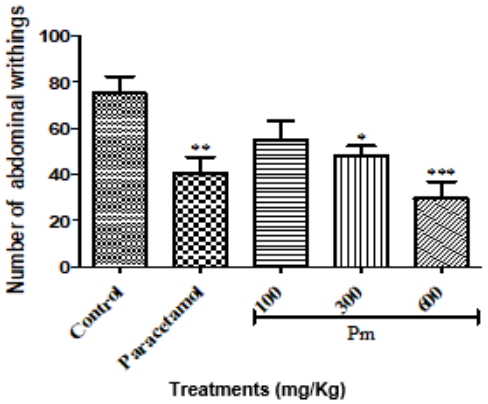

Figure 1. Anti-nociceptive effect of roots aqueous extract of *Pseudospondias microcarpa* on acetic acid-induced writhing.
Each bar represents a mean ± S.E.M. of five rats for each group. ***P< 0.001; **p<0.01; *P< 0.05; statistically significant from control group; Pm = *Pseudospondias microcarpa*.

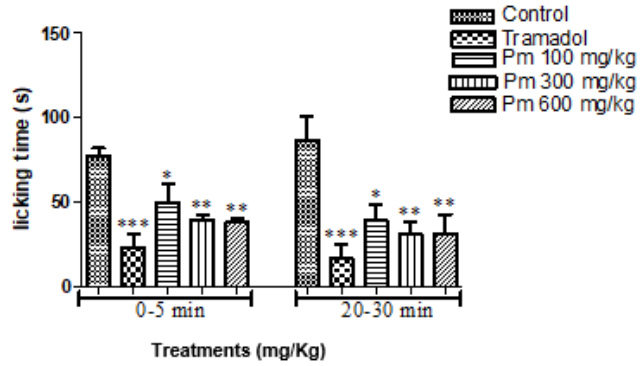

Figure 2. Anti-nociceptive effect of the roots aqueous extract of Pseudospondias *microcarpa* on formalin-induced test.
Each bar represents a mean ± S.E.M. of five rats for each group.; n = 5; *p<0.05; **p<0.01; ***p<0.001; statistically significant from control group. Pm = *Pseudospondias microcarpa*

Table 1. Effect of roots aqueous extract of *Pseudospondias microcarpa* on paw oedema induced by carrageenan

Treatment	Doses (mg/kg)	Mean volume of edema (ΔV) in mL						
	-	½ h	1 h	2 h	3 h	4 h	5 h	6 h
Distilled water	-	0.230 ± 0.02	0.314 ± 0.03	0.390 ± 0.06	0.466 ± 0.04	0.420 ± 0.04	0.370 ± 0.03	0.304 ± 0.04
Indometacin	10	0.140 ± 0.02 (39.10)	0.182 ± 0.03 (42.00)	0.186 ± 0.03*** (52.30)	0.186± 0.03*** (60.10)	0.180 ± 0.05*** (56.90)	0.126± 0.03*** (65.90)	0.120 ± 0.03** (60.50)
P. microcarpa	100	0.152 ± 0.03 (33.90)	0.172 ± 0.04* (45.20)	0.244 ± 0.03* (37.40)	0.258± 0.05*** (44.60)	0.200 ± 0.05*** (53.10)	0.162± 0.03*** (56.20)	0.124 ± 0.04** (59.20)
P. microcarpa	300	0.094 ± 0.01 (59.10)	0.138± 0.04** (56.10)	0.256 ± 0.02 (34.40)	0.294 ± 0.04** (36.90)	0,160 ± 0.04*** (61.70)	0.188 ± 0.04** (49.20)	0.084 ± 0.04 *** (72.40)
P. microcarpa	600	0.076 ± 0.02* (67.00)	0.110±0.03*** (65.00)	0.140 ± 0.05*** (64.10)	0.142 ± 0.04*** (69.50)	0.090 ± 0.04*** (79.40)	0.122 ± 0.02*** (67.00)	0.080 ± 0.02*** (73.70)

The values are expressed as mean ± S.E.M of five rats for each; each value in parenthesis indicates the percentage inhibition rate. Statistically significant from control group. *P< 0.05; **p<0.01; ***p< 0.001

Table 2. Effect of roots aqueous extract of *Pseudospondias microcarpa* on paw edema induced by arachidonic acid

Treatment	**Doses (mg/kg)**	**Mean volume of edema (ΔV) in mL**
		½ H
Distilled water	-	0.283 ± 0.02
Indometacin	10	0.115 ± 0.02 *** (59.41)
P. microcarpa	100	0.110 ± 0.01*** (61.18)
P. microcarpa	300	0.082 ± 0.02 *** (71.18)
P. microcarpa	600	0.050 ± 0.02 *** (82.35)

The values are expressed as mean ± S.E.M. of five rats for each group. Statistically significant from control group. ***p< 0.001.

Conclusion

In the context of this study, it emerges that, the aqueous extract of *P. microcarpa* has peripheral and central analgesic activity on models of pain induced by acetic acid and formalin. The aqueous extract of *P. microcarpa* has equally anti-inflammatory activity on acute inflammation patterns induced by carrageenan or arachidonic acid and justify its use in traditional medicine.

Abbreviations

SEM: standard error mean; HNC: National Herbarium of Cameroon

Authors' Contribution

HPMN carried out all the experiments; ELN managed students in the technical experiment, wrote and read the manuscript; CZB, helped to realize the experiment; ALFM helped to realize the experiment; ABD revised, corrected, and read the final manuscript; AGBA helped to choose the plant

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Conflict of interest

The authors declare no conflict of interest

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