

## Analgesic and acute inflammation properties of the aqueous extract of dried leaves of *Paullinia Pinnata* (Sapindaceae) Linn

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### Abstract

Inflammation is frequently associated with pain. Plants continue to be major resources for therapeutic compounds against various diseases including inflammation and pain. *Paullinia Pinnata* is used to treat several diseases, including rheumatism and abdominal pain. This study was undertaken to assess the analgesic and anti-inflammatory effects of *Paullinia Pinnata*. The analgesic activity was evaluated by using behaviour pain model in mice. The anti-inflammatory activity was carried out by using carrageenan, dextran, histamine and serotonin induced inflammation in rat. The extract was administered orally at a dose of 200 and 400 mg/kg. The results showed that the extract significantly ( $P < 0.001$ ) reduced the number of writhing induced by the acid acetic. The aqueous extract reduced significantly ( $P < 0.001$ ) the paw licking time in formalin model. The effect of the extract (200mg/kg) was significantly ( $P < 0.001$ ) reduced in the presence of naloxone, during the inflammatory phase. In addition, the extract significantly ( $P < 0.05$ ) increase latency time at all point time and all doses on nociception induced by hot plate. Concerning inflammation induced by carrageenan and dextran, the extract significantly ( $P < 0.001$ ) inhibited oedema during the experimental time at the dose of 200 mg/kg. The results suggested that *Paullinia pinnata* aqueous extract possess analgesic activities which may interfere in both peripheral and central pathway. The anti-inflammatory activities may be mediated by either inhibiting or by blocking the release of vasoactive substances like histamine, serotonin, kinins and prostaglandins. These results justify the traditional use of the plant in the treatment of pain and inflammation.

**Keywords:** *Paullinia pinnata*, nociception, edema, anti-inflammatory.

### Introduction

Pain is an alarmous signal for the protection of organism against tissue damage [1]. Inflammation is a pathophysiological response of living tissue to injuries that leads to the local increase of plasmatic fluid and blood cells [2]. Pain and inflammation are associated with numerous diseases, which can be healed by analgesic and anti-inflammatory drugs [3]. However most of these drugs are known to provoke many undesired effects, especially in the gastrointestinal tract [4] besides their exorbitant prices. For these reasons, the World Health Organisation (WHO) is encouraging research on medicinal plants and traditional medicine in developing countries, hence relieving them from the obligation of buying expensive drugs from abroad [5]. It is in this manner that we resolved to assess the analgesic and anti-inflammatory activities of

*Paullinia pinnata* (Sapindaceae), called "dzuikelong" in Baham people of Cameroon [6].

*Paullinia pinnata* is a tropical, perennial creeping plant in the form of a shrub or woody vine with a long, flexible stem covered with a rigid bark. The plant is easily recognizable by its leaves, which have five leaves with saw-toothed edges, with prominent veins, a winged rachis and petiole. This vine develops in undergrowth forest recruitment, in moist and semi-deciduous forests, in gallery forests, along creeks and in savannas [7]. It grows naturally in South Africa, Madagascar, Brazil and Jamaica. It is also found in Zimbabwe and Zambia, from Senegal to Cameroon and Mali [8,9].

The whole plant, leaves, stem and root are use as remedies. In Malie, leaves are used against snake bite, gonorrhoea, wound, malaria and intestinal worms [10]. In Ivory Coast, the stem of the plant is used to treat rheumatism [11]. In Ghana the plant I used as an aphrodisiac and to treat dysentery. Machated root is used to treat pulmonary diseases and is applied on meadow, fractures and abscess [12].

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In Cameroon the leaves of this plant is used against stomach and abdominal pain [13, 14].

The aim of the present study was to investigate the effects of the aqueous extract of dried leaves of *Paullinia pinnata* on acid acetic, formalin and hot plate induced nociception on one hand, then on carrageenan, dextran, histamine and serotonin induced acute inflammation in experimental mice and rat on other hand.

## Materials and methods

The fresh leaves were collected in March 2014, in Simbog, a locality of Yaoundé, Cameroon. The plant material was identified by National herbarium of Cameroon. The voucher specimen was registered under number 10702/SRFCam.

### Animals

Young adults (3 months age) swiss albinos mice of both sexes weighing 20 to 30 g, and young adults (3 months age) wistar rats of both sexes weighing 90 to 150 g were used. They were bred in the animal house of the Faculty of Sciences, University of Yaoundé I, under standard laboratory conditions at 25 C (12 hours light and 12 hours dark cycles). All the animals were allowed to have free access to food (standard diet for rodent) and water. The animals were deprived of food 14 hours before the experiment, but allowed free access to water. The experiments were performed following the guidelines for the care of laboratory animals from the Cameroon National Ethical Committee (Ref. no Fw-IRB00001954). The animals were divided in groups of 6.

### Preparation of extract

The extract was prepared according to the protocol developed by the Institute of Medical Research (IMPM). The leaves of *Paullinia pinnata* were dried at 38 C in an oven and powdered. The dried powder (400g) was extracted with 1 L of distilled water for 48 hours, followed by filtration through a Whatman N 1 filter paper. The solvent (water) was removed by lyophilisation at -15 C to obtain 11.27g of residue. The average for extract yield (weight/weight) was 2.82%.

### Chemicals used

Acetic acid, formaldehyde (Roth), Complete Freund Adjuvant (Sigma), carrageenan (Sigma), dextran (Sigma), histamine (Sigma), serotonin (Sigma), paracetamol, tramadol, dexamethazone, naloxone and indomethacin molecules were used.

### Antinociceptive activity

### Acid acetic induced writhing test

The method used was described by Collier *et al.* [15]. Albinos mice were divided in 4 groups of 6 animals each. Mice of the first two groups received different doses of the plant extract (200 and 400mg/kg, *p.o.*). The two last groups of animals received standard drug paracetamol (20 mg/kg *p.o.*), and distilled water, respectively. Thirty minutes after treatment with tested products, pain was induced by intraperitoneal injection of 1 % acetic acid solution (10 mL/kg) and the number of abdominal writhing was counted for each mouse during 30 minutes after pain induction. The analgesic percentage was calculated using the ratio:  
(Control mean – Treated mean / Control mean) x 100 [16,17].

### Formalin test

This test was carried out according with the method described by Cha *et al.* [18]. Mice were divided in 7 groups of 5 animals. Mice of the first two groups received different doses of the extract (200 and 400 mg/kg, *p.o.*). The two next groups of animals received standard drugs tramadol (20mg/kg *p.o.*), and paracetamol (10mg/kg, *p.o.*) respectively. Mice of the sixth and seventh groups received naloxone (1mg/kg *i.p.*) and tramadol (20mg/kg *p.o.*) or naloxone (1mg/kg *i.p.*) and plant extract (200 mg/kg *p.o.*) respectively, with naloxone being injected 15 minutes before tramadol or the plant extract. The last group received vehicle. Thirty minutes after, the treatment of mice each animal was injected in the right hind paw aponeurosis with 20 µL of a formalin solution 1 %. The time taken to lick the injected paw was measured in two phases: the first five minutes and between the fifteenth and thirtieth minute after formalin injection. The analgesic percentage was calculated using the formula previously used in writhing test.

### Hot plate test

This test was performed following the method described by Vaz *et al.* [19]. Albinos mice were divided in 7 groups of 6 animals. Mice of the first two groups received different doses of the extract (200 or 400 mg/kg, *p.o.*). The two next groups of animals received standard drugs tramadol (20mg/kg *p.o.*), and paracetamol (20 mg/kg, *p.o.*) respectively. Mice of the fifth and the sixth groups received naloxone (1mg/kg *i.p.*) and tramadol (20mg/kg *p.o.*) or naloxone (1mg/kg *i.p.*) and plant extract (200 mg/kg *p.o.*) respectively, naloxone being injected 15 minutes before tramadol or the plant extract. The last group received vehicle. Thirty minutes after the treatment, each mice was placed into a glass cylinder placed on a hot plate thermostatically set at 55 ± 0.5 C. The time taken by the animal to lick the paw or to jump out of the plate was recorded before, then 30, 60, 120, 180, 240 and 300 minutes after drugs administration. The percentage of inhibition was expressed as follows:  $Tf - Ti / Ti \times 100$ , where Tf represents the mean time



after treatment of each group, and Ti, the mean time before treatment of each group.

### Anti-Inflammatory activity

#### Carrageenan-induced paw oedema

This test was carried out following to the method described by Lanher *et al.* [20]. In rats of both sexes, the hind paw oedema was induced by a single injection of 0.1 mL of 1% W/V of carrageenan in a normal saline solution under the back hind paw after thirty minutes pre-treated *p.o* with extract (200 and 400 mg/kg), indomethacin (10 mg/kg) as reference drug and distilled water (10 mL/kg). Paw volume was measured using a plethismometer (UGO Basile n 7140) before carrageenan injection, 30, and 1h interval for 5h. The percentage of inflammation inhibition was expressed as follows:

$[(Vt - Vo) \text{ control} - (Vt - Vo) \text{ treated}] / (Vt - Vo) \text{ control} \times 100$ , where Vo represents the initial mean paw volume, and Vt the mean paw volume at the “t” moment after induction of inflammation.

#### Dextran-induced paw oedema

This test was carried out following to the method described by Denny *et al.* [21]. Animals of both sexes were treated similar to the case of carrageenan induced paw oedema, except that in place of carrageenan and reference drug, dextran 0.1 mL of 1% W/V in a normal saline solution and cyproheptadin (20 mg/kg) were used. Paw volume was measured as described earlier before induction, 30 min, but rather at 1h and 2h later. The percentage of inflammation inhibition was expressed using the relation previously described.

#### Histamin-induced paw oedema

Animals of both sexes were treated similar to the case of carrageenan induced paw oedema, except that in place of carrageenan and reference drug, dextran 0.1 mL of 1% W/V in a

normal saline solution and promethazin 10 mg/kg) were used. Paw volume was measured as before histamine injection, 30 min and 1h later. The percentage of inflammation inhibition was expressed using the relation previously described.

#### Serotonin induced paw oedema

Animals of both sexes were treated similar to the case of carrageenan induced paw oedema, except that in place of carrageenan and reference drug, dextran 0.1 mL of 1% W/V in a normal saline solution and cortancyl (10 mg/kg) were used. Paw volume was measured as described earlier before induction and 30 min later. The percentage of inflammation inhibition was expressed using the relation previously described.

#### Statistical analysis

Values were expressed as mean ± SEM (Standard Error on Mean). One-away ANOVA, followed by the Turkey’s post-test, was used. Statistical analysis was performed using Graph pad Prism 5.01, and a P value less than 0.05, was considered as significant.

### Results

#### Antinociceptive activity

##### Acid acetic induced writhing test

Intraperitoneal injection of acetic acid induced abdominal constrictions in mice. Oral administration of the aqueous extract of *Paullinia pinnata* significantly reduced the number of mouse abdominal constrictions as compared to the control (Table 1). The highest inhibition percentage (39.67 %) was obtained with the plant extract administered at 200 mg/kg (p < 0.001). paracetamol (20 mg/kg), also significantly (p < 0.001) reduced constrictions by 52.83%.

**Table 1:** Effects of the aqueous extract of *Paullinia pinnata* leaves on pain induced by the acetic acid

Treatment	Doses(mg /kg)	Number of writhing	Analgesic percentages
Water	-	98.50 ± 2.84	
Paracetamol	20	45.66 ± 5.32 <sup>a</sup>	<b>52.83</b>
<i>P. pinnata</i>	200	58.83 ± 2.73 <sup>a</sup>	<b>39.67</b>
<i>P. pinnata</i>	400	84.16 ± 2.05 <sup>c</sup>	<b>14.33</b>

Each value represents the mean ± SEM n = 5. <sup>a</sup>P<0.001 ; <sup>c</sup>P<0.05 differences significantly different with respect to the water group.

#### Formalin test

In both neurogenic and inflammatory phases, the aqueous extract of *Paullinia pinnata* reduced the paw licking time significantly, with

the maximum inhibition 60,84 % (p<0.001) and 64.51% (p<0.001) during the neurogenic and the inflammatory phases respectively at the dose 200mg/kg with respect to group control. Tramadol also significantly displayed inhibition with 70.53 % (p<0.001) and 75.17



% ( $p < 0.001$ ) of inhibition during the neurogenic and the inflammatory phases respectively as compared to group control. In the presence of naloxone (1mg/kg), the plant extract (200mg/kg)

became less efficient to reduce the pain only during the inflammatory phase, meanwhile tramadol (20 mg/kg), failed to significantly reduce the licking time during both phases (Table 2).

**Table 2:** Effects of the aqueous extract of *Paullinia pinnata* leaves on pain induced by formalin

Treatment	Doses (mg /kg)	Neurogenic phase (0- 5mn)		Inflammatory (15 – 30 mn)	
		Time(s)	Percentages	Time(s)	Percentages
Water	-	105.25 ± 3.28		114.14 ± 2.48	
Paracetamol	20	25.00 ± 5.95 <sup>a</sup>	<b>76.24</b>	35.20 ± 2.40 <sup>a</sup>	<b>69.23</b>
Trabar	20	31.00 ± 4.42 <sup>a</sup>	<b>70.53</b>	28.40 ± 9.76 <sup>a</sup>	<b>75.17</b>
<i>P. pinnata</i>	200	41.20 ± 10.82 <sup>a</sup>	<b>60.84</b>	40.60 ± 12.32 <sup>a</sup>	<b>64.51</b>
<i>P. pinnata</i>	400	41.80 ± 12.67 <sup>a</sup>	<b>60.27</b>	55.20 ± 12.37 <sup>b</sup>	<b>51.75</b>
NLX+Tramadol	1+20	53.80 ± 13.80 <sup>b</sup>	<b>48.46</b>	76.00 ± 4.91 <sup>3</sup>	<b>33.57</b>
NLX+Pp200	1+200	51.40 ± 6.46 <sup>a</sup>	<b>51.14</b>	103.00 ± 13.23 <sup>1</sup>	<b>9.97</b>

Each value represents the mean ± SEM, n = 5. <sup>a</sup>P<0.001 ; <sup>b</sup>P<0.01 differences significantly different with respect to the water group. ; <sup>1</sup>P<0.001; <sup>3</sup>P<0. 05 differences significantly different with respect to Trabar .Values in parenthesis represent the percentages of inhibition. Pp: *Paullinia pinnata* ; NLX : Naloxone.

### Hot plate test

The plant aqueous extract on hot plate test at the doses of 100, 200 or 400 mg/ kg significantly increased latency time reaction, during all the five hours of experimentation. The maximum inhibitory effect was obtained at the dose of 200mg/kg with 74.63 % ( $p < 0.05$ ) of inhibition at the fifth hour, compared to 67.15 % ( $p < 0.05$ ) for Tramadol (20 mg /kg) during the same hour. In the presence of Naloxone (1mg/kg), tramadol failed to increase significantly the latency time (Table 3) while the analgesic efficiency of the plant (200 mg/kg) has not significantly changed.

### Anti-inflammatory tests

#### Effects of *Paullinia pinnata* on carrageenan induced paw oedema

When compared with the control, the aqueous extract of *Paullinia pinnata* at the dose of 200 mg/kg and the reference drug significantly reduced the paw oedema during all the five hours after carrageenan injection. The maximal percentage of inhibition induced by the extract was 42.05% ( $p < 0.05$ ) at dose 200 mg/kg at the first hour. The effect of diclofenac ( reference drug) at the same hour was 27.97% ( $p < 0.05$ ) (Table 4).

**Table 3:** Effects of the aqueous extract of *Paullinia pinnata* leaves on pain induced by the hot plate

Treatment	Doses (mg /kg)	Reaction time (s)					
		0.5h	1h	2h	3h	4h	5h
Water	-	0.91 ± 0.19	1.96 ± 0.62	2.60 ± 0.21	1.48 ± 0.32	01.66 ± 0.12	01.98 ± 0.95
Tramadol	20	3.10 ± 0.16 (44.33%)	4.77 ± 0.33 <sup>c</sup> (68.82%)	5.00 ± 0.52 <sup>c</sup> (72.70%)	4.81 ± 0.30 <sup>c</sup> (69.40%)	4.67 ± 0.32 <sup>c</sup> (67.08%)	4.65 ± 0.67 <sup>c</sup> (67.15%)
<i>P. pinnata</i>	200	2.35 ± 0.56 (38.75%)	2.12 ± 0.42 (33.75%)	1.43 ± 0.32 (22.92%)	2.94 ± 0.82 (47.95%)	3.20 ± 0.80 (47.31%)	4.66 ± 1.39 (74.63%)
<i>P. pinnata</i>	400	2.29 ± 0.15 (36.31%)	3.25 ± 0.17 (56.84%)	4.35 ± 0.59 (74.49%)	5.09 ± 1.20 <sup>c</sup> (88.70%)	3.43 ± 0.96 (59.09%)	4.10 ± 1.37 (70.96%)
NLX+Tramadol	1+20	0.32 ± 0.10 (3.88%)	0.32 ± 0.04 <sup>1</sup> (4.74%)	1.04 ± 0.62 (13.43%)	1.58 ± 0.64 <sup>c</sup> (-19.80%)	0.50 ± 0.03 (6.53%)	1.03 ± 0.25 <sup>c</sup> (-16.05%)
NLX+Pp	1+200	1.40 ± 0.14 (53.84%)	2.29 ± 0.15 (27.23%)	1.84 ± 0.39 <sup>b</sup> (29.87%)	1.92 ± 0.55 (40.16%)	2.66 ± 0.30 (20.89%)	2.02 ± 0.33 (22.23%)

Each value represents the mean ± SEM, n = 5. <sup>b</sup>P<0.01 ; <sup>c</sup>P<0.05 differences significantly different with respect to T<sub>0</sub>. ; <sup>1</sup>P<0.001 differences significantly different with respect to Trabar .Values in parenthesis represent the percentages of inhibition. Pp: *Paullinia pinnata*; NLX: Naloxone.

**Table 4:** Effects of the aqueous extract of *Paullinia pinnata* leaves on inflammation induced by carrageenan

Treatment	Doses (mg/kg)	Oedema volume ( $\Delta V$ mL)					
		0.5 h	1 h	2 h	3 h	4 h	5 h
Water	-	0.85±0.06	0.99±0.07	1.23±0.08	1.58±0.06	1.86±0.03	1.90±0.05
Diclofenac	1	0.66±0.07 (21.88%)	0.71±0.07 <sup>c</sup> (27.97%)	0.77±0.03 <sup>b</sup> (37.60%)	0.96±0.06 <sup>b</sup> (39.29%)	0.77±0.05 <sup>b</sup> (58.67%)	0.95±0.06 <sup>b</sup> (49.89%)
<i>P. pinnata</i>	200	0.80±0.08 (5.17%)	0.57±0.06 <sup>b</sup> (42.05%)	0.87±0.09 <sup>b</sup> (29.01%)	1.35±0.02 <sup>c</sup> (14.73%)	1.30±0.05 <sup>b</sup> (30.40%)	1.54±0.06 <sup>b</sup> (18.54%)
<i>P. pinnata</i>	400	0.59±0.06 (30.25%)	0.42±0.06 <sup>c</sup> (57.74%)	1.19±0.02 (3.40%)	1.40±0.08 (11.39%)	1.65±0.06 (10.81%)	1.57±0.03 (16.94%)

Each value represents the mean ± SEM, n = 5. <sup>b</sup>P<0.01 ; <sup>c</sup>P<0.01 differences significantly different with respect to the water group. Values in parenthesis represent the percentages of inhibition.

### Effects of *Paullinia pinnata* on dextran induced paw oedema

The aqueous extract of *Paullinia pinnata* at the dose of 400 mg/kg as well as the reference drug significantly reduced the paw oedema thirty minutes and one hour after dextran injection. The percentage

of inhibition was 14.65% (p < 0.05) and 28.44% (p < 0.01) at the 30<sup>th</sup> minute of dose 200 and 400 mg/kg respectively. The maximal effect was observed one hour after inflammation induction with percentage of inhibition of 19.03% (p<0.01), and 38.97% (p<0.01) at the doses of 200 and 400 mg/kg respectively. Cyproheptadine exhibited an inhibition of 48.27% and 67.97% at thirty minutes and one hour respectively (Table 4).

**Table 4:** Effects of the aqueous extract of *Paullinia pinnata* leaves on inflammation induced by dextran

t	t	Oedema volume ( $\Delta V$ mL)			Percentages of inhibition		
		0.5h	1 h	2 h	0,5 h	1 h	2h
Water	-	0.69± 0.05	0.66 ±0.02	0.66±0.03	--	--	--
Cyproheptadine	1	0.36±0.09 <sup>b</sup>	0.21±0.09 <sup>b</sup>	0.43±0.01 <sup>b</sup>	48.27	67.97	34.54
<i>P. pinnata</i>	200	0.59±0.04 <sup>b</sup>	0.53±0.06 <sup>a</sup>	0.76±0.05	14.65	19.03	--
<i>P. pinnata</i>	400	0.49±0.03 <sup>a</sup>	0.40±0.03 <sup>a</sup>	0.63±0.09	28.44	38.97	4.24

Each value represents the mean ± SEM, n = 5. <sup>a</sup>P<0.01 ; <sup>b</sup>P<0.01 differences significantly different with respect to the water group.

### Effects of *Paullinia pinnata* on histamine induced paw oedema

The anti-oedematous activity was 68.05% in rats treated with the reference drug. The aqueous extract of the leaves of *Paullinia pinnata* at the doses of 200 and 400 mg / kg, did not induce any significant anti-inflammatory activity on histamine-induced oedema (Table 5).

**Table 5:** Effects of the aqueous extract of *Paullinia pinnata* leaves on inflammation induced by histamine

		Oedema volume ( $\Delta V$ mL)	Percentage of inhibition
		1 h	1 h
Water	-	1.69± 0.09	--
Promethazine	1	0.54±0.03 <sup>b</sup>	68.05
<i>P. pinnata</i>	200	1.57±0.03	7.07
<i>P. pinnata</i>	400	1.58±0.06	6.60

Each value represents the mean ± SEM, n = 5. <sup>b</sup>P<0.01 differences significantly different with respect to the water group. (-) = negative value (no inhibition)

### Effects of *Paullinia pinnata* on serotonin induced paw oedema

The aqueous extract of *Paullinia pinnata* at the dose of 400 mg/kg significantly reduced oedema during the 30 minutes later, whereas the reference drug did not show any significant inhibition. The



percentage of inhibition was 42.09% ( $p < 0.05$ ), and 43.22% ( $p < 0.05$ ) at doses 200 and 400 mg / kg respectively (Table 6).

**Table 6:** Effects of the aqueous extract of *Paullinia pinnata* leaves on inflammation induced by serotonin.

		Average volume of the paw (mL)		Oedema volume ( $\Delta V$ mL)	Percentage of inhibition
		0 h	0.5 h	0.5 h	0.5 h
Water	-	2.37±0.03	3.08±0.08	0.70± 0.08	--
Cortancyl	1	2.43±0.04	2.96±0.03	0.53±0.03	24.85
<i>P. pinnata</i>	200	2.14±0.03	2.55±0.05	0.41±0.05 <sup>b</sup>	42.09
<i>P. pinnata</i>	400	2.16±0.03	2.56±0.02	0.40±0.02 <sup>b</sup>	43.22

Each value represents the mean ± SEM, n = 5; <sup>b</sup>P<0.01 differences significantly different with respect to the water group.

## Discussion

The aim of this study was to evaluate antinociceptive and anti-inflammatory activities of *Paullinia pinnata* in mice and rat.

The assessment of analgesic activity demonstrates that oral administration of *Paullinia pinnata* at doses of 100, 200 and 400 mg/kg produce significant inhibition in acid acetic model induced writhing test, formalin induced licking pain and the hot plate test.

In the model of acid acetic induced abdominal writhing which is commonly known as a typical visceral, tissues and muscles pain model [22], the pain is mainly due to the direct stimulation of the peripheral channels fibres like and C fibres which transmit the nociception message. The algic process leads to release arachidonic acid and by cyclooxygenase pathway the nociception mediators biosynthesis such as Prostaglandin, bradykinin, cytokine (TNF, IL and IFN) [23,24]. Thus, the analgesic effects of the aqueous extract of *Paullinia pinnata* in acid acetic abdominal constrictions could be attributed, partly, to the inhibition of biosyntheses of endogenous mediators, and also could be due to its anti-inflammatory effect by inhibition of algic cytokines as in the visceral pain model. The antinociceptive effect could result to direct inhibitory activity on nerve endings which led of the blockage of the transmission by the algic message.

The formalin test is believed to be a more valid analgesic model which is better correlate with clinical pain [25]. This model was used to know whether the antinociceptive effect of *Paullinia pinnata* is exerted at the peripheral or central level of the nervous system. In fact, in the formalin test, the analgesic effect is biphasic: the earlier phase was due to the direct stimulation of the sensory nerve fibres which release the P substance, a neurotransmitter which facilitate the transmission of the nociceptive message, while the pain in the late phase was due to the release of inflammatory mediators like histamine, serotonin, prostaglandins, bradykinins, and leukotrienes [22,25,26]. In both neurogenic and inflammatory phases, the aqueous extract of *Paullinia pinnata* reduced significantly the paw licking time. This result suggested that analgesic effect could be due on both peripheral and central pathway of the nervous system involving algic and inflammatory mediators. In the presence of naloxone which is known to bind specifically to opioid receptors, the effect of *Paullinia pinnata*

aqueous extract (200 mg/kg) became less efficient to reduce the pain only during the inflammatory phase, meanwhile tramadol, a reference morphinomimetic drug, failed to significantly reduce the licking time during both phases. Similar result was obtained by Tatsinkou *et al.* [24]. on antinociceptive properties of the aqueous and methanol extracts of stem bark of *Petersianthus macrocarpus*. In our case, the result showed that the central analgesic effects of *Paullinia pinnata* extract could be dependant from opioids receptors, whereas the peripheral analgesic effect is independent, and involves a different mechanism of action. The nociception is also due on activation of some ionic channels receptors in sensory neurons that response to heat and acid, and lead to release P substance. In order to assess whether the *Paullinia pinnata* extract could act by this way, the pain was induced by the hot plate known as a central analgesic test. In this model, nociception results not only by opioids pathway, but also by P substance. The present results indicated that the extract increased latency time at all point time and all doses. Previous study showed similar results [24]. According to the present results, *Paullinia pinnata* aqueous extract effect could act by opioids pathway, and the blockage of the transduction and transmission of signal comes from heat receptors. This result corroborates the one obtained in the second phase of formalin test during which inflammatory mediators released, together trigger inflammatory process and pain.

With respect to anti-inflammatory effect of *Paullinia pinnata* aqueous extract, the carrageenan, dextran, histamine and serotonin model were used. Carrageenan-induced rat paw oedema is a suitable experimental animal model for investigating the anti-inflammatory effect of natural products [27]; and this involve three phases. The first phase (1 hr after Carrageenan induced) involves the release of serotonin and histamine from mast cells. The second phase (2hr) is provided by kinins and the third phase (3hr) is mediated by prostaglandins, the cyclooxygenase product and lipoxygenase products [28]. *Paullinia pinnata* significantly inhibited carrageenan-induced oedema during the 3 phases at the dose of 200 mg/kg. However, the dose 400 mg/kg significantly inhibited only the first phase. This inhibition could be due to the presence in the extract of bioactive compounds able to antagonize the effects of the mediators released during the first and second phases. Previous studies have demonstrated that sulfated polysaccharidic



fractions obtained from *Gracilaria* species showed an anti-inflammatory effect in carrageenan-induced paw oedema [29]. The results corroborate those obtained by the present work. The anti-inflammatory activity of *Paullinia pinnata* seems to exert more activity on the release of histamine and/or serotonin, the first mediators acting during the inflammatory reaction [30]. To verify these findings, the effect of the plant extract was performed on dextran, serotonin and histamine. It is well established that carrageenan and dextran induce rat paw oedema by different mechanisms. The dextran model is characterized by increase of vascular permeability, the activation of kinins, and the release of histamine and serotonin from mast cells, leading to osmotic oedema with low levels of proteins and neutrophils [31,32,33]. It is also known that during the inflammatory process, serotonin is primarily responsible for vasodilation and pro-inflammatory oedema by activation of HT-2 receptors, while histamine is more involved in pruritogenic processes and over causes vasodilatation mediated by the release of NO which acts on the increase of vascular dilatation and permeability, the synthesis and release of PGs, cytokines and reactive oxygen species ROS (which causes oxidative stress) and the contraction of endothelial cells [34]. In the present work, significant reduction in the volume of oedema was observed in comparison with rats treated with distilled water, suggesting that the *Paullinia pinnata* effects could involve blocking the histamine and/or serotonin receptors or inhibition of their release from mast cells. Since the results showed the decrease of the oedema, it could also be the depletion of NO resulting to the blockage of

histamin. Therefore, the histamine and serotonin model suggested that the effects on serotonin are more likely than effects on histamine, as the direct effect of histamine was not significantly prevented by *Paullinia pinnata* treatment at both doses (200 and 400 mg/kg). Thus, *Paullinia pinnata* appears to possess anti-permeability and anti-vasodilation.

## Conclusion

*Paullinia pinnata* aqueous extract leaves have been demonstrated to possess analgesic and anti-inflammatory activities providing some evidence for its folk use and further exploitation. Its antinociceptive properties may interfere in both peripheral and central pathway associated by the inhibition or the blockage of the release of proinflammatory mediators. However further investigations are required to identify the active components of the extract as well as the efficacy in sub-acute and chronic inflammatory process.

## Authors' contribution

DATP, KAB and BEAJ were involved in acquisition of different data. DATP, DDPD, DT and PK: were involved in design, interpretation and analysis of the data and the writing of the manuscript.

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