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Subchronic and chronic anti-inflammatory properties of the aqueous extract of dried leaves of *Paullinia Pinnata* (Sapindaceae) Linn

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ABSTRACT

Chronic painful inflammatory diseases affect great number of people worldwide and are a common cause of disability. In this study, *Paullinia Pinnata* was used to evaluate its anti-inflammatory effects in subchronic and chronic inflammation. Cotton pellet induced granuloma model was used to evaluate subchronic inflammation, while Complete Freund's adjuvant-induced oedema model was used for chronic inflammation. The extract was administered at the doses of 200 and 400 mg/kg. In subchronic inflammation, *Paullinia pinnata* reduced granuloma and exudate formation. The maximum effect was observed for the exudate with a percentage of inhibition of 48.02% ($p < 0.05$). In chronic inflammation, *Paullinia pinnata* reduced ($p< 0.001$) CFA-induced oedema. The maximum effect was obtained at the dose of 400 mg/kg with 40.86% as percentage of inhibition. This inhibition in both subchronic and chronic inflammation was associated with a modulation of serum biochemical parameters, as well as the oxidative stress for chronic study. Histological analyses showed a reduction in the synovial hyperplasia and mononuclear infiltration in *Paullinia pinnata* treated groups, which provides evidence for a chronic anti-inflammatory effect. The anti-inflammatory activities could be mediated either by the inhibition of the biosynthesis of proinflammatory mediators or the production of oxidative molecules to avoid granuloma formation.

Keywords: *Paullinia pinnata*, anti-inflammation, cotton pellet, Complete Freund's adjuvant.

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INTRODUCTION

Inflammation is a highly regulated protective response which helps in eliminating the initial cause of cell injury and initiates the process of repair [1]. When the organism's response is not efficient, the process migrates towards chronic inflammation. Chronic inflammatory disorders is an excessive inflammatory response characterized by the presence of activated and dysregulated macrophages which release multitude of mediators such as interleukins 1 and 6 (IL-1 and IL-6) and tumour necrosis factors α (TNFα), that play an important role in both maintenance and progression of the disease [2]. Although inflammation is beneficial in providing defence against infection, it may become unchecked in case of pathogenesis of chronic inflammatory disease [3]. Chronic inflammation leads to several systemic diseases such as rheumatoid arthritis, sarcoidosis, multiple sclerosis and many others. The systemic inflammatory response is also associated with the production of reactive oxygen species involving cells damage. Current treatment protocols used in the treatment of chronic inflammation involve steroidal and nonsteroidal anti-inflammatory drugs. Most of these treatments have severe side effects such as gastrointestinal bleeding, ulcers, and opportunistic infections due to immunosuppression [4]. Because of the high costs for treatment and care, the very long duration of illnesses correlated with mortality, chronic inflammation becomes a major concern. Taking into account this chronic aspect of the disease, the high side undesirable effects of current drugs, and the development of alternative therapy is required. Therefore, there is continuous research in exploration of natural drugs which have high therapeutic value, less associated side effects and easy access.

Paullinia pinnata (Sapindaceae), commonly called "dzuhkelong" in western region of Cameroon, 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. It grows naturally in South Africa, Madagascar, Brazil and Jamaica. It is also found in Zimbabwe and Zambia, from Senegal to Cameroon and Mali [5,6]. The whole plant, leaves, stem and root are used in folk medicine to treat rheumatism, stomach and abdominal pain, snake bite, gonorrhea, wound, malaria and intestinal worms [7,8]. The aim of the present study was then focus to evaluate the effects of the aqueous extract of dried leaves of *Paullinia pinnata* on cotton pellet induced granuloma and Complete Freund's adjuvant induced paw oedema in rat.

MATERIALS AND METHODS

The fresh leaves of *Paullinia pinnata* were collected at Eloumden II, a locality in Center Region of Cameroon, in June 2017. The identification of plant specimens was done at the Cameroon National Herbarium, where a voucher specimen was deposited under the reference number 66 934/HNC.

Animals

Adult Wistar rats of both sexes weighing 90 to 150 g and aged 7 to 8 weeks were used. They were bred in the animal house of the Faculty of Sciences, University of Yaoundé I, under standard laboratory conditions at 24 ± 2°C (12 hours light and 12 hours dark natural 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).

Preparation of extract

The extract was prepared according to the protocol developed by the Institute of Medical Research (IMPM). *Paullinia pinnata* leaves were dried at 38°C in the oven and powdered. The dried powerder (400g) was extracted with 1 L of distilled water for 48 hours, at room temperature, followed by filtration through a Whatman N°1 filter. The solvent (water) was removed by lyophilisation at -15°C to obtain 11,27g of residue. The extract yield average (weight/weight) was 2.82%.

Chemicals

Complete Freund's adjuvant (Sigma), indomethacin and dexamethasone were used. Total cholesterol, HDL-cholesterol were estimated according to standard colorimetric kits Immesco's protocol, triglycerides, ALAT, ASAT, PAL, GAMMA-GT, creatinine and bilirubin were measured using Fortress kinetic kit's protocol.

Cotton pellets-induced granuloma

The subchronic inflammation of *Paullinia pinnata* was evaluated using cotton pellets induced granuloma according to the method described by Winter and Porter [9], with slight modifications. Wistar albinos rats of both sex were divided in 5 groups of 5 animals. The normal control group received distilled water; the treated groups received distilled water (1mL/100g), standard drug indomethacin (3 mg/kg), different doses of the plant extract (200 and 400 mg/kg). Cotton pellets **(**7 ± 1) mg were sterilized in an autoclave for 30 minutes at 120°C. Two cottons were implanted (*s.c*) into the ventral region, bilaterally below the axilla, one on each side of the rat, under light ketamine 1% anaesthesia associated with diazepam. Animals were kept under aseptic conditions for the entire duration of the study. The test substances were administered (*p.o*) for seven consecutive days after pellets implantation. On the eighth day, the animals were sacrificed by cervical dislocation. Blood was collected and the cotton pellets were carefully extracted and made free from extraneous tissues. The wet pellets were weighed, then the granuloma tissues was dried in an oven at 60 °C for 24 hours, and the dry weight was recorded. Blood samples were collected and centrifuged to get serum for the determination of ALAT, ASAT and PAL activity, the concentration of proteins, nitrites, creatinine and bilirubin. The difference between the wet pellet weight and dry pellet weight was considered to be the exudate amount. The granuloma weight was calculated by deducting the cotton pellet weight (7± 1) mg from the dry pellet weight and taken as a granuloma measure tissue formation. The percentage of inhibition was expressed as follows [10]:

$$\%\,\mathbf{INH} = \mathbf{100} - [\,(\mathbf{Xt}/\mathbf{Xc}) \times \mathbf{100}],$$

where:

%INH: Percentage of inhibition; **Xt:** Granuloma weight or exudate volume of test group;
Xc: Granuloma weight or exudate volume of group control.

Complete Freund's adjuvant induced paw oedema

The anti-inflammation effects of *Paullinia pinnata* in chronic inflammation was evaluated by Complete Freund's Adjuvant (CFA) induced paw oedema [11]. In this model, chronic inflammation was induced by injecting 0.1 mL of 1% W/V of CFA's solution under the back hind paw in all animals except the control and the pharmacological groups. Rats of both sexes were divided in 6 groups of 5 animals. Nine days after CFA's injection, the experimental groups were treated daily (*p.o*) with distilled water (1mL/100g), the plant extract at the doses of 200 and 400 mg/kg and dexamethasone (1mg/kg) during 12 days. The increase of the paw size was measured using a plethysmometer (UGO Basile n° 7140). The percentage of inflammation inhibition was expressed as follows [12]:

$$\mathbf{[(V_t - V_0)\ control - (V_t - V_0)\ treated] / (V_t - V_0)\ control]}$$

where $(V_t - V_0)$ control represents the mean difference in the volume of the paw increase of the rats of the control group and $(V_t - V_0)$ treated represents the mean difference in the volume of the paw increase of the rats of the treated group.

At the 22nd day, the rats were anaesthetized under light ether and blood samples were collected from the carotid section. Liver, spleen and kidney samples were collected for the evaluation of oxidative stress parameters (MDA, GHS, CAT, and SOD) proteins and nitrites. The serum prepared from the blood was used to determine the activity of ALAT; ASAT, PAL, the concentration of total proteins, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, creatinine and bilirubin. The joints were taken immediately and fixed in 10% formalin for histological analysis.

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.

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RESULTS

Granuloma and exudate formation studies

Plant extract at different doses and indomethacin was evaluated to know its potential in subchronic inflammation. The implantation of cotton pellets produced the formation of granuloma and exudate. The weight was 0.04±0.00mg and 0.15±0.02mg for the granuloma and the exudate, respectively. Table 1 showed that *Paullinia pinnata* at both doses (200 and 400 mg/kg) reduced the formation of granuloma and exudate. The maximum effect was observed at 400 mg/kg for the exudate with a percentage of inhibition of 48.02% ($p<0.05$). Meanwhile, the standard drug indomethacin significantly inhibited the granuloma and the exudate by 50% ($p <0.05$) and 55.92% ($p <0.01$) respectively.

Table 1: Effects of the aqueous extract of the leaves of *Paullinia pinnata* on the weight of the exudate and of the granuloma formation

Treatments	Doses (mg/kg)	Weight (mg)		Percentage of inhibition	
		Granuloma	Exudate	Granuloma	Exudate
Cotton + Water		0.04±0.00	0.15±0.02	-	-
Cotton+Indomethacin	3	0.02±0.01^{c}	0.07±0.01^{b}	**50.00**	**55.92**
Cotton + *P. pinnata*	200	0.02±0.00	0.11±0.03	**37. 50**	**26.32**
Cotton + *P. pinnata*	400	0.03±0.00	0.08±0.01^{c}	**25.00**	**48.02**

Each value represents the mean ± SEM, n = 5. $^{b}p<0.01$; $^{c}p<0.05$ significantly different with respect to the water group.

Paw oedema volume studies

Injection of CFA to rats induced a paw oedema of 2.3 ± 0.3 mL nine days later (Fig. 1). From day 9, administration of dexamethasone and *Paullinia pinnata* significantly ($p<0.01$) reduced the oedema throughout the experimental period. At the 21st day the percentage of inhibition was 44.85%, 33.22% and 40.86% respectively for dexamethasone, *Paullinia pinnata* at doses of 200 and 400 mg/kg.

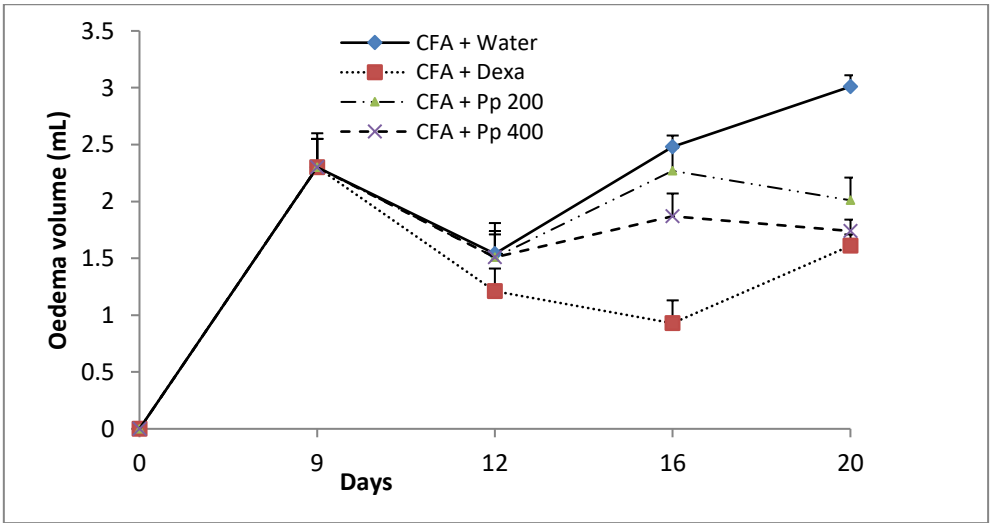

Figure 1: Effects of the aqueous extract of *Paullinia pinnata* on the paw volume after induction of inflammation by CFA. Each point represents the mean ± SEM. n=5 b; $^{b}p<0.01$ significantly different with respect to the group control. CFA: Complete Freund's adjuvant; Dexa: Dexamethasone; *Pp: Paullinia pinnata.*

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Histological studies

Histological observations in rats hind paw joints showed an apparent increase in the number of chondrocytes with mild synovial proliferation and infiltration of mononuclear cells contributing to cartilage erosion. Reduction in the volume of oedema in the experimental groups (treated with extract and dexamethasone) was accompanied by less affected cartilage and joint, less cellular infiltration and the reduction of the number of inflammatory cells at the level of the joint, when compared to negative control (Fig. 2).

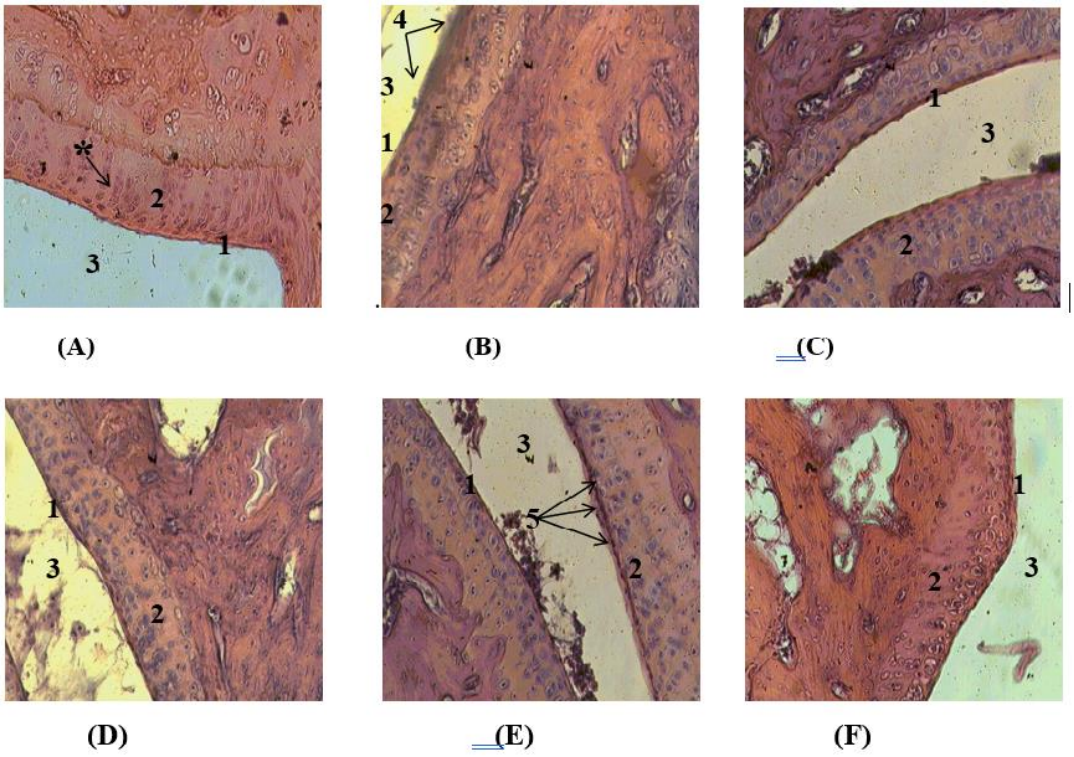

Figure 2: Effects of the aqueous extract of *Paullinia pinnata* on histological structure of rat paw joints after induction of inflammation by CFA.

A: normal control rat showed the normal architecture of articular cartilage; **B**: control rat showed an apparent decrease in the number of chondrocytes with mononuclear cells infiltration contributing to the erosion of the cartilage, the presence of leukocytes infiltration and synovitis**; C**: indomethacin treated rat demonstrated the recovered histopathological profile towards normal architecture; **D**: rat treated with aqueous extract of *Paullinia pinnata* 200 mg/kg confirmed the normal architecture with less deformity of cartilage; **E** : rat treated with aqueous extract of *Paullinia pinnata* 400 mg/kg showed leukocytes infiltration (black arrows indicate inflammatory cells: leukocytes); **F**: pharmacological control rat showed normal architecture.

(1 = Joint capsule or synovial membrane covering the articular cartilage. 2 = Articular cartilage, * chondrocyte; 3 = Articular cavity, 4 = Black arrows indicate inflammatory cells (leukocytes); 5= Infiltration of mononuclear cells contributing to the erosion of cartilage.

Biochemical studies

Cotton pellets induced granuloma

Cotton pellets induced a non-significant increase of proteins (12.33%) and bilirubin (7.44%) levels in serum, accompanied by significant ($p < 0.001$) increase of ALAT, ASAT and PAL ($p < 0.05$) activity, creatinine ($p < 0.001$) and nitrites rates ($p < 0.05$), when compared to control group. The percentages of increase were respectively 104.32%, 77.33%, 47.53%, 156.36% and 60%. Treated rats with *Paullinia pinnata* extract at the doses of 200 and 400 mg/kg,as well as the reference drug (indomethacin) significantly restored the level of theses parameters towards normal values (Table 2).

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Table 2: Effects of the aqueous extract of the leaves of *Paullinia pinnata* on some serum parameters in cotton pellet induced granuloma

Treatments	Water	Cotton + Water	Cotton + Indomethacin	Cotton + *P. pinnata*	Cotton + *P. pinnata*
Doses (mg/kg)			3	200	400
Nitrites (mg/mL)	0.04±0. 01	0.06±0. 00$^{\beta}$ **(60.00%)**	0.02±0.00^{a} **(203.00%)**	0.04±0. 00^{b} **(60.00%)**	0.05±0.00 **(28.00%)**
Proteins (mg/mL)	107.40±25. 52	120.65±12. 81 **(12.33%)**	90.58±11. 30 **(33.19%)**	101.63±21. 58 **(18.71%)**	111.98±14. 90 **(7.74%)**
ALAT (U/l)	37.22±2. 02	76.05±3. 13$^{\alpha}$ **(104.32%)**	31.31±1.45^{a} **(142.89%)**	25.52±2. 01$^{a\beta}$ **(198.00%)**	30.94±1.84^{a} **(145.69%)**
ASAT (U/l)	74.95±4. 52	132.90±9.12$^{\alpha}$ **(77.31%)**	87.85±2.09^{a} **(51.28%)**	37.09±4.20$^{a\alpha}$ **(258.32%)**	109.90±1.56$^{c\alpha}$ **(20.93%)**
PAL (U/l)	163.70±1. 66	241.50±15.51$^{\theta}$ **(47.53%)**	93,83±7, 41^{a} **(147. 38%)**	161.80±9. 12^{c} **(49. 25%)**	42.50±26.36^{b} **(69.47%)**
Bilirubin (mg/dL)	6.64±0.89	6.18±0,.27 **(7.44%)**	6.75±0. 40 **(9.22%)**	6.28±1.67 **(1.62%)**	6. 62±2.16 **(7. 12%)**
Créatinine (mg/dL)	0.55±0. 12	1.41±0. 11$^{\alpha}$ **(156.36%)**	0.78±0.05^{b} **(80.77%)**	0.87±0.13^{c} **(62.06%)**	0.77±0.10^{b} **(83.12%)**

Each value represents the mean ± ESM, n = 5. ^{a}p<0.001 ; ^{b}p<0.01, ^{c}p<0.05 significant differences compared to the negative control. $^{\alpha}$p<0.001 ; $^{\beta}$p<0.01 ; $^{\theta}$p<0.05 significant differences compared to the normal control. The values in bold represent the percentages of inhibition.

Complete Freund's adjuvant induced paw oedema

The CFA model induced a significant (p< 0.001) increase in triglycerides (167.46%), total cholesterol (47.91 %), LDL-cholesterol (35.61%), and atherogenic index (13.58%). Meanwhile the HDL-cholesterol significantly (p< 0.001) decrease (36.20%) compared to normal control. The rates of these parameters were significantly depleted with the administration of *Paullinia pinnata* extract *a*nd dexamethasone. The percentages of inhibition were 35.77% for total cholesterol, 64.36% for LDL-cholesterol, 67.65% for triglycerides and 27.98% for atherogenic index, at the dose of 200 mg/kg. At the dose of 400 mg/kg, this percentage was 50.94% for total cholesterol, 59.50% for LDL-cholesterol, 67.65% for triglycerides and 10.84% for atherogenic index; and at least the dexamethasone showed for 21.47% for total cholesterol, 25.28% for LDL-cholesterol , 65.01% for triglycerides and 8.40% for atherogenic index. Whereas the concentration of HDL was significantly increased by 5.62% and 38.69% respectively the doses of 200 and 400 mg/kg; and 25.28% for indomethacin compared to the negative control. The dose of 400 mg/kg in healthy animals did not result in any significant effects on the lipid profile (Table 3). During the 21 days experimentation, FCA significantly (p<0.01) showed elevated transaminases activity (ALAT, ASAT, APL and gamma GT), and creatinine level in the negative control group, when compared to the normal control (Table 4).

The inflammation was accompanied by a significant decrease of serum, liver, spleen, kidney proteins and nitrites (Fig.3A and 3B; Fig. 4A and 4B). At the same time the oxidative stress parameters activity like SOD, CAT, the rate of GHS, and nitrites decrease in the tissues while MDA concentration increase (Fig. 4). *Paullinia pinnata* extract and dexamethasone treated groups significantly decreased the rate of hepatic and kidney function parameters, and MDA concentration. In the same way the enzymatic activity and the rate of oxidative molecules in liver, spleen and kidney increased. The pharmacological control group show no significant changes.

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Table 3: Effects of *Paullinia pinnata* of serum cholesterol content in CFA induced paw oedema

Treatment	Water	ACF + Water	ACF + Dexa	ACF + *P. pinnata*	ACF + *P. pinnata*	*P. pinnata*
Doses (mg/kg)			1	200	400	400
Total- Cholesterol (mg/dL)	119.90±4.44	160.20±0.60$^{\alpha}$ (35. 61%)	125. 80±4.94^{b} (21. 47%)	102.90±7.60^{a} (35. 77%)	78.58±9.33$^{\alpha a}$ (50. 94%)	118.70±3.17 **(1. 01%)**
HDL-Cholesterol (mg/dL)	22.33±0.48	14.24±0.50$^{\alpha}$ (36.20%)	17.84±0.24$^{\beta c}$ (25.28%)	15.04±0.92$^{\alpha}$ (5.62%)	19.75±1.25^{a} (38. 69%)	19.15±0. 48 **(16. 61%)**
LDL-Cholesterol (mg/dL)	96.28±4.83	142.40±0.70$^{\alpha}$ (47. 91%)	105. 80±0.40^{b} (34.59%)	86.64±7.66^{a} (64.36%)	57.67±9.81$^{\beta a}$ (59. 50%)	98. 18±2.77 **(1. 97%)**
Triglycerides (mg/dL)	6.70±0.38	17.92±1.40$^{\alpha}$ (167.46%)	10.86±0.50$^{\beta a}$ (65.01%)	5.80±0.18^{a} (67.65%)	5.79±0.19^{a} (67.69%)	6.92±0.33 **(3.28%)**
Atherogenic Index (mg/dL)	0.81±0.01	0.92±0.00$^{\theta}$ (13. 58%)	0.85±0.01 (8. 4%)	0.72±0.04 (27.98%)	0.83±0.04^{a} (10.84%)	0.83±0.02 **(2.47%)**

Each value represents the mean ± SEM, n = 5. $^{a}p<0.001$; $^{b}p<0.01$; $^{c}p<0.05$ significantly different with respect to the water group $^{\alpha}p<0.001$; $^{\beta}p<0.01$, $^{\theta}p<0.05$ significantly different with respect to the normal control group. Values in parenthesis represent the percentages of inhibition. Values in bold stand for deviation between the pharmacological group (*P. pinnata* 400 mg/kg) and the normal control group values. CFA: Complete Freund's. adjuvant; Dexa: Dexamethasone.

Table 4: Effects of aqueous extract of leaves of *Paullinia pinnata* on some markers of liver and kidney functions

Treatment	Water	CFA + Water	CFA + Dexa	CFA + *P. pinnata*	CFA + *P. pinnata*	*P. pinnata*
Doses (mg/kg)			1	200	400	400
ALAT (U/l)	30.56±2.59	56.36±3.76$^{\beta}$ (84. 42%)	28. 26±3.14^{a} (99.43%)	27. 88±4.68^{a} (102.15%)	24.79±2.43^{a} (127.35%)	41.29±6.67 **(35.11%)**
ASAT (U/l)	60.61±10.70	135.30±11.7$^{\alpha}$ (123. 30%)	81.13±2.45^{b} (66. 77%)	59.55±5.25^{a} (127.20%)	49.45±12.99^{a} (173.61%)	90.26±6.44 **(48.91%)**
PAL (U/l)	139.99±9.15	277.20±14.22$^{\beta}$ (40. 56%)	149.00±16.42 ^{b} (86.04%)	191.10±40.32 (45.05%)	154.90±1.50^{c} (78. 95%)	106.20±21.89 **(31.73%)**
GAMMA-GT (U/l)	2.18±10.70	5.81±11.72$^{\beta}$ (03. 68%)	2.11±0.60^{b} (175.36%)	1.76±0.25^{a} (231.11%)	2.74±0.30^{c} (112.04%)	2.94±0.67 **(34.86%)**
Creatinine (mg/dL)	0.60±0.09	1.22±0.19$^{\beta}$ (166. 51%)	0.61±0.04^{c} (100.33%)	0.69±0.04^{c} (77.10%)	0.56±0.08^{b} (118. 21%)	0.64±0.15 **(6.67%)**

Each value represents the mean ± SEM, n = 5. $^{a}p<0.001$; $^{b}p<0.01$; $^{c}p<0.05$ significantly different with respect to the water group. $^{\alpha}p<0.001$; $^{\beta}p<0.01$significantly different with respect to the normal control group Values in parenthesis represent the percentages of inhibition. Values in bold stand for deviation between the pharmacological group (*P. pinnata* 400 mg/kg) and the normal control group values. CFA: Complete Freund's. adjuvant; Dexa: Dexamethasone

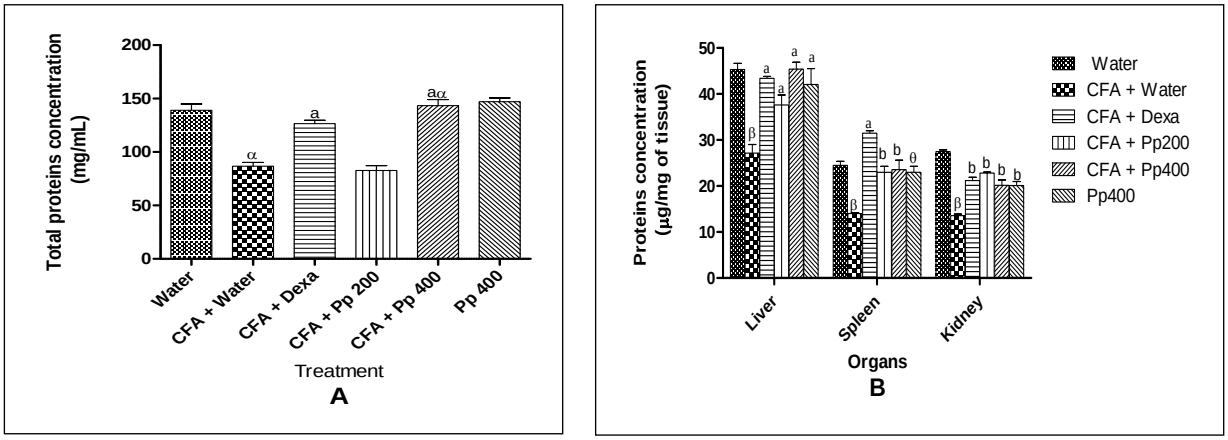

Figure 3: Effects of the aqueous extract of *Paullinia pinnata* on proteins concentration in blood (**A**) and organs (**B**). Each bar represents the mean ± SEM, n = 5. $^{a}p<0.001$; $^{b}p<0.01$; significantly different with respect to the negative group. $^{\alpha}p<0.001$; $^{\beta}p<0.01$; $^{\theta}p<0.05$ significantly different with respect to the normal control group. Pp: *Paullinia pinnata;* CFA: Complete Freund's adjuvant; Dexa: Dexamethasone

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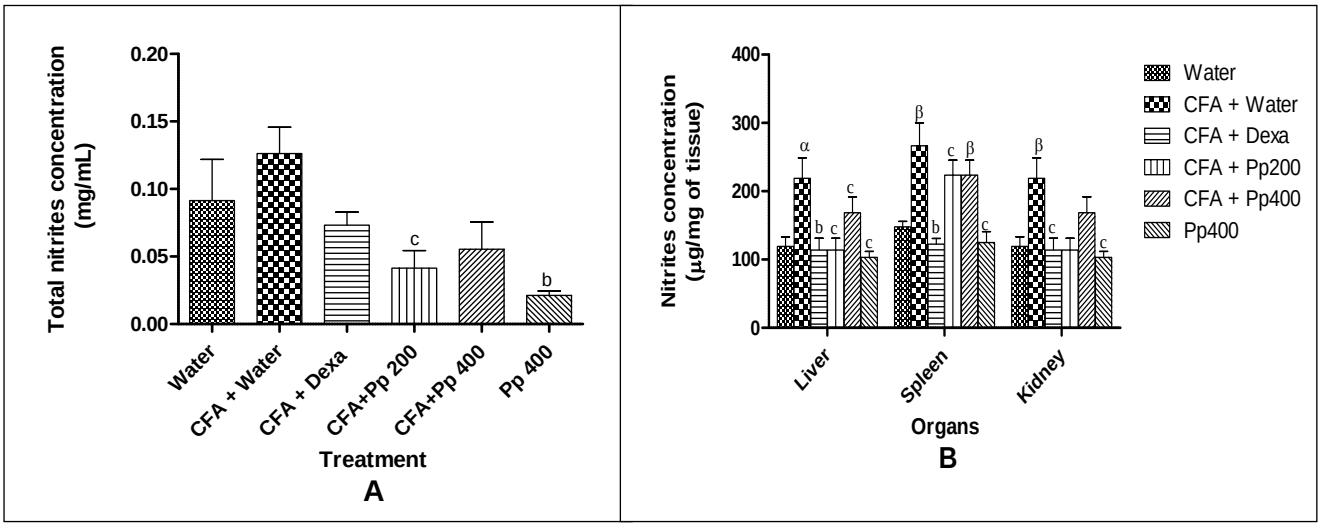

Figure 4: Effects of the aqueous extract of *Paullinia pinnata* on proteins concentration in blood (**A**) and organs (**B**). Each bar represents the mean ± SEM, n = 5. $^{b}p<0.01$; $^{c}p<0.05$ significantly different with respect to the negative group. $^{\alpha}p<0.001$; $^{\beta}p<0.01$ significantly different with respect to the normal control group. Pp: *Paullinia pinnata;* . CFA: Complete Freund's. adjuvant; Dexa: Dexamethasone

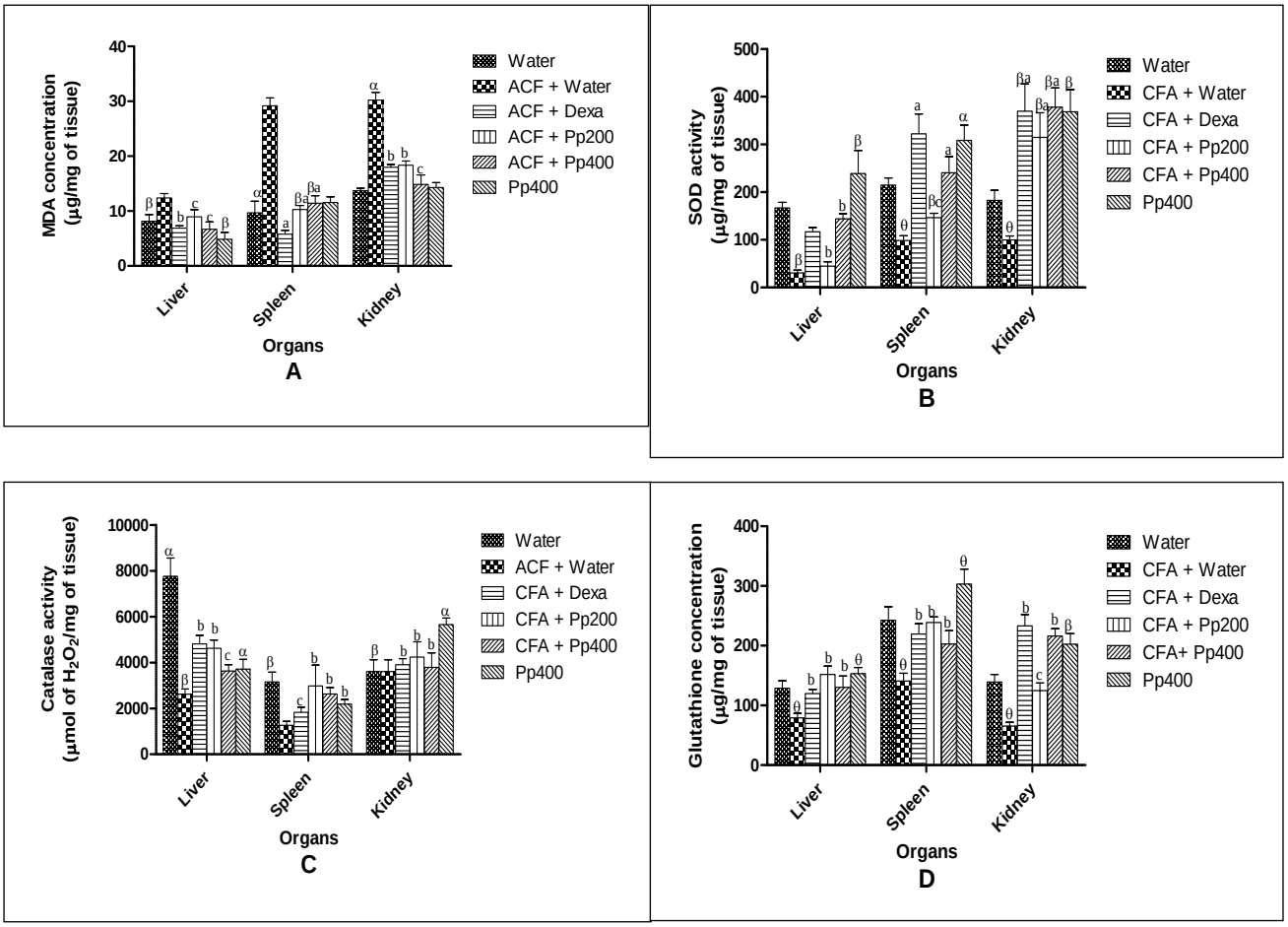

Figure 5: Effects of the aqueous extract of *Paullinia pinnata* on MDA (**A**); SOD **(B)**, catalase (**C**) activity and glutathione (**D**) rate in organs. Each bar represents the mean ± SEM, n = 5. $^{a}p<0.001$; $^{b}p<0.01$; $^{c}p<0.05$ significantly different with respect to the negative group. $^{\alpha}p<0.001$; $^{\beta}p<0.01$; $^{\theta}p<0.05$ significantly different with respect to the normal control group. Pp: *Paullinia pinnata;* . CFA: Complete Freund's. adjuvant; Dexa: Dexamethasone

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DISCUSSION

The aqueous extract of *Paullinia pinnata* was evaluated for its anti-inflammatory activity in subchronic and chronic models. Cotton pellets induced granuloma was used as a subchronic model, well known as an indicative of proliferative phase of inflammation involving macrophages, neutrophils, fibroblast cells and collagen formation which are the basic sources of highly vascularized reddish mass, termed as granulation tissue [10]. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibres and suppressing mucopolysaccharides [13]. The extract of *Paullinia pinnata* as indomethacin a non-steroidal anti-inflammatory drug, showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions. These results corroborate those obtained by Suresha *et al*, [10], on varied anti-inflammatory activity of indomethacin in different experimental models. To better understand the mechanism of action of the aqueous extract of *Paullinia pinnata*, we evaluated his effects in curative treatment on the inflammation induced by the CFA. CFA-induced paw oedema is the most extensively experimental model of rheumatoid arthritis to investigate the clinical and pathogenic changes, which are similar to human arthritis, and also to study disease processes for the screening of new drugs [14]. Further, this method helps in determining the correlation between efficacy of therapeutic agents and rheumatoid arthritis model [15]. CFA administered under the paw leads to an arthritis characterized by an increase in paw volume, synovitis and swelling near the ankle joints confirming the expansion of arthritis which was measured as oedema [16]. This arthritis may be divided into three phases such as human rheumatoid arthritis. These phases begin with the onset of oedema without involvement of the joint, followed by synovitis and finally destruction of the joint. The process involves the increase of vascular permeability, leakage of element into the interstitial space and passage of macrophages, lymphocytes into inflamed tissue where number of inflammatory mediators like cytokines, histamine, 5 hydroxytryptamine, bradykinin, free radicals, various chemotactic factor, interferons and prostaglandin are released. These mediators, mainly the release of free radicals are liable for damage and destruction of joints, bones, cartilage and associated with pain, which can lead to several disabilities [17]. During inflammatory diseases and particularly those of the joints, other mechanisms involve leukocytes and phagocytes, preparing a complex with the mediators, releasing the lysosomal enzymes and causing the injury of cartilage and other tissue [18]. Indeed, the phagocytes accumulate in the joint produce the superoxide radicals and the hydrogen peroxide which leads to a failure of the endogenous antioxidants thus causing the damage of the synovial fluid, cartilage and other joint components [19].

In addition, CFA induces the systemic inflammation which also allows the production of NO in response to the bacterial products released. This is associated with the liberation by activated macrophages and lymphocytes, of oxygen free radicals and reactive nitrogen species together in the milieu cause a deletary syndrome when the balance between reactive oxygen species production and antioxidant defence is lost, lead to cells damage in liver, spleen and Kidney tissues and others, and responsible of the oxidative stress [20]. The defence response is accompanied by a set of endogenous antioxidant enzymes released, such as SOD, GSH, CAT and others, to prevent and neutralize the free radicals induced damage [20,21]. Lipid peroxidation (LPO) is a common feature of rheumatic arthritis. MDA (as an indicator of lipid peroxidation) is used to evaluate the LPO. MDA is a pro-oxidant factor which determines the oxidative stress present in the rats. More often, the high rate of serum ALAT, ASAT, APL and bilirubin during chronic inflammation is symptomatic to hepatocytes, nephrocytes and spleen cells damage. Therefore liver, spleen and kidney impairments are characteristics of the CFA model induced rheumatoid arthritis. Moreover, the model of arthritis in associated with alteration of the lipid profile such as triglycerides, total cholesterol, LDL-cholesterol, atherogenic index, the accumulation of HDL-cholesterol and the increase of the level nitrites and proteins. The anti-inflammatory components can act through several steps of pathophysiology by inhibiting the biosynthesis of proinflammatory mediators or the production of oxidative molecules to avoid cellular dysfunction and unwanted pathological conditions including RA, damage and destruction of bone, cartilage and associated with pain, which can lead to several disabilities. A good antirheumatic agent must be able to block one or more of these phases [16]. In the current study animals treated with dexamethasone or aqueous extract of the leaves of *Paullinia pinnata*, significantly reduced the oedema formed in comparison with the normal control animals. These are associated with the increase of the activity of SOD, GSH, and CAT. Whereas MDA decreased in liver, spleen and kidney. In the same way, the activity of transaminases decreased. Moreover, the lipid profile was restablished and the range of nitrites and proteins, hepatic and kidney parameters were restored. The reduction of oedema in treated rats could be due to inhibition of neutrophil infiltration, inflammatory exudate formation and cartilage erosion at

the articular level. This is confirmed by histological observations of the joints which clearly showed that the cartilage layer is protected when treated with *Paullinia pinnata*. Similar results were obtained by Subhashis *et al*. [22], when studying curative and protective properties of crude gel of *Aloe vera* from sub-Himalayan West Bengal in chronic and acute inflammatory rats models. Previous phytochemical investigations studies have demonstrated that *Paullinia pinnata* contains catechic tannins, phenols and flavonoids that are natural antioxidants [23,24]. According to Calixto *et al.* [25], natural and synthetic antioxidants have anti-inflammatory activity. Assuming that the aqueous extract of *Paullinia. pinnata* contains these secondary metabolites. Its effects on chronic inflammation could be also explained by the inhibitory action of these metabolites against cell degradations and the production of free radicals caused by ACF.

CONCLUSION

It has been demonstrated that *Paullinia pinnata* aqueous extract leaves is non-toxic and possess anti-inflammatory effects as well as in subchronic and chronic inflammation models. Its anti-inflammatory properties could act through inhibition of the biosynthesis of proinflammatory mediators and the production of oxidative molecules, providing indeed some evidence for it folk use and further exploitation for manufacture of new drugs.

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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