Inflammopharmacol (2018) 26:197–205
https://doi.org/10.1007/s10787-017-0376-6

Inflammopharmacology

ORIGINAL ARTICLE

Anti-hyperalgesic activity of the aqueous and methanol extracts of the leaves of *Pittosporum mannii* Hook on CFA-induced persistent inflammatory pain

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Received: 25 May 2017 / Accepted: 12 July 2017 / Published online: 29 August 2017

Abstract

Background Previous study showed that aqueous (AEPM) and methanol (MEPM) extracts from the leaves of *Pittosporum mannii* have analgesic effects in acute pain models. The present study evaluates the acute and chronic anti-hypernociceptive and anti-inflammatory effects of AEPM and MEPM in a model of persistent inflammatory pain.

Methods The third day after induction of inflammatory pain by subplantar injection of 100 µL of CFA in Wistar rats, AEPM and MEPM were administered orally (75, 150 and 300 mg/kg/day) and their anti-hyperalgesic and anti-inflammatory effects were follow in acute (1–24 h) and chronic (for 14 days) treatments. At the end of the chronic treatment, oxidative stress and liver parameters were assessed. Effects of plant extracts were also evaluated on nociception induced by Phorbol 12-Myristate 13-Acetate (PMA) and 8-bromo 3′,5′-cAMP (8-Br-cAMP) in mice.

Results AEPM and MEPM significantly reversed the mechanical hyperalgesia caused by CFA in acute and chronic treatment. Moreover, AEPM and MEPM also significantly reduced the nociception caused by PMA (60%) and 8-Br-cAMP (87%). Nevertheless, AEPM and MEPM failed to inhibit the paw edema caused by CFA. Plant extracts significantly reduced the nitric oxide content in the spinal cord and the plasmatic concentration of alanine aminotransferase. MEPM also significantly increased the glutathione content in the spinal cord.

Conclusion AEPM and MEPM given orally are effective in inhibiting mechanical hyperalgesia in persistent inflammatory pain caused by CFA. Their mechanisms of action seem to involve an interaction with PKC, PKA and nitric oxide pathways. These extracts might be devoid of hepatotoxic effects.

Keywords *Pittosporum mannii* · Aqueous and methanol extracts · Anti-hyperalgesia · Nitric oxide · PKC · PKA

Abbreviations

8-Br-cAMP	8-Bromo 3′,5′-cyclic adenosine monophosphate
AEPM	Aqueous extract of *Pittosporum mannii*
CFA	Complete Freund's adjuvant
PMA	Phorbol myristate acetate
MEPM	Methanol extract of *Pittosporum mannii*
PKA	Proteine kinase A
PKC	Protein kinase C
TRPV	Transient receptor potential vanilloid

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Introduction

Inflammatory pain occurs after injury of tissues at a cellular level. This can happen with wounds, burns, extreme cold, fractures, arthritis, autoimmune conditions, excessive stretching, infections and vasoconstriction. Inflammatory pain is characterized by sensitization of nociceptors resulting in hyperalgesia and allodynia, which are, respectively, the exacerbated pain intensity in response to painful stimuli, and pain to stimuli that is not normally painful (Millan 1999; Navarro et al. 2013). Inflammatory stimuli such as complete Freund's adjuvant (CFA) induce a cascade of inflammatory mediators (cytokines, PGE_2, etc.…) resulting in inflammatory hyperalgesia (Cunha et al. 2005). These inflammatory mediators are responsible for sensitization of nociceptors and activation of second messenger pathways (PKA and PKC) which reduce the nociceptor threshold and increase neuronal membrane excitability, facilitating the primary nociceptor activation and impulse transmission, resulting in hyperalgesia (Cury et al. 2011). Therefore, blockade of second messenger pathway activation may reduce hyperalgesia. Another important component of inflammatory pain is the oxidative stress with the generation of molecules such as hydrogen peroxide, superoxide anion, and peroxinitrite, which are produced in response to stimuli and can promote hyperalgesia (Keeble et al. 2009). Therefore, antioxidant inducers and ROS scavengers are expected to have or enhance antihyperalgesic activity (Singh and Vinayak 2015).

A variety of biological effects have been ascribed to *Pittosporum mannii* stem bark (Adjanohoun et al. 1996; Muthaura et al. 2007; Momeni et al. 2010; Njiaza et al. 2015). Much attention has been given to their antiulcerogenic (Wandji 2009) and spasmolytic activities (Njiaza et al. 2015). Previous study showed that the aqueous (AEPM) and the methanol (MEPM) extracts from the leaves of *P. mannii* have antinociceptive properties in acute pain models, including capsaicin- and glutamate-induced pain (Wandji et al. 2016). It has been shown that chronic pain either neuropathic or inflammatory is highly associated with overexpression and hypersensitization of TRPV1 and glutamate receptors (Woolf and Salter 2000; Ngueleffack et al. 2015). Thus, from the fact that AEPM and MEPM possess potent inhibitory activity on capsaicin- and glutamate- induced pain, it can be hypothesized that these extracts could exhibit good analgesic effect on chronic pain such as persistent inflammatory pain. Therefore, the present work was carried out to evaluate the curative analgesic and anti-inflammatory properties of AEPM and MEPM, using a model of inflammatory pain induced by CFA. Further, we evaluated the in vivo antioxidant, the hepatotoxic effect of *P. mannii* extracts as well as their mechanism of action using PMA- and 8-Br-cAMP-induced pain models.

Materials and methods

Plant material and extraction

The leaves of *P. mannii* were harvested in Batchingou in November 2011 and were identified at the Cameroon National Herbarium, Yaoundé by comparison to existing voucher specimen number 22420HNC. These leaves were dried in shade, ground and extracted as a decoction to yield 9.23% of aqueous extract (AEPM). The powdered plant material was also extracted as maceration at room temperature, using methanol and concentrated under rotary evaporator to yield 8.66% of methanol crude extract (MEPM). Previous phytochemical studies have showed that these extracts contain pittovidoside and 1-o-rhamnopyranosyl-3-acid acetoxymberbic 29-methylesther (Wandji et al. 2016). AEPM and MEPM were dissolved in distilled water upon use.

Animals

Swiss mice (20–30 g) and Wistar rats (150–200 g) of either sex obtained from the animal house of the Department of Animal Biology of University of Dschang were used in the present study. Experimental protocols used herein were approved by the laboratory Committee and conformed to the guidelines for the study of pain in awake animals established by the International Association for the Study of Pain. The number of animals and the intensity of noxious stimuli used were the minimum necessary to demonstrate the consistent effects. Experimental protocols used herein were conformed to 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 laboratory Committee on the 16/01/2014 with the file number 025/13/304FSa.

Drugs and chemicals

Diclofenac sodium (Denk-Pharma, Germany) was obtained from local pharmacy. Acid acetic and orthophosphoric acid were purchased from Merck (Germany), while thiobarbituric acid, complete Freund's adjuvant (CFA), sulfamilamide, naphthyl ethylene-diaminedihydrochloride, dithiobisnitrobenzoate, adrenaline, trichloroacetic acid, phorbol myristate acetate chelerytrine, 8-bromo 3′,5′-cAMP and H-89 were purchase from sigma-aldrich (Germany).

Anti-hyperalgesic and inflammatory activities

Complete Freund's adjuvant (CFA)-induced hyperalgesia and inflammation

Before any treatment, the baseline values of paw diameter and mechanical pain threshold were measured. Then, rats were injected in the plantar surface of the left hindpaw (i.pl.) with 100 µL of CFA (Ferreira et al. 2001). Forty-eight hours after CFA intraplantar injection, second baseline values were recorded and animals were divided in eight groups of seven animals each. The negative control group was treated with distilled water. The positive control group received diclofenac (5 mg/kg) orally. Groups 3–5 received AEPM at respective doses of 75, 150 and 300 mg/kg, while the remaining groups (6–8) were treated with the same doses of MEPM. In the acute treatment attempted to verify the time-course effect of *Pittosporum mannii* extracts, the diameter of the injected paw volume was measured at 1, 3, 5, 7 and 24-h post-treatment while the mechanical hyperalgesia was evaluated at 2, 4, 6, 8 and 24 h. To investigate the effects of the long-term (14 days) repeated treatment, the same animals were used. They were administered the same treatment once a day and the mechanical pain threshold or the paw diameter were measured every 3 days. Pain threshold was assessed with the Ugo Basile analgesy meter (No. 372157) while the paw diameter of each rat was measured by the aid of a digital caliper (FineScience Tools No. 30087-00). Animal weight was also measured daily during treatment.

At the end of the experiment, rats were killed after being fasted overnight. Blood samples were drawn from the abdominal arteries to the 10 mL of heparinised tubes, then centrifuged at 3000 rpm/min for 15 min, the clear non-hemolyzed supernatant serum was quickly removed and used for the estimation of serum enzymes. After blood collection, the spinal cord of each animal was quickly removed, rapidly weighed and frozen until analyzed. The spinal cord was homogenized at 15% in a Tris buffer (10 mM, pH 7.4). The homogenates were centrifuged at 10.000 rpm for 15 min at 4 °C. The supernatants were separated and used for biochemical analysis.

Phorbol myristate acetate (PMA)-induced nociception

The procedure used was similar to that described previously (Meotti et al. 2005). Mice were divided in six groups of six animals each and treated as follow: group 1 received distilled water by oral route, group 2 received chelerytrine (100 pmol/paw) used as positive control, groups 3 and 4 received AEPM at respective doses of 150 and 300 mg/kg while groups 5 and 6 were treated with MEPM at the same doses. One hour after extracts or 15 min after chelerytrine administrations, each animal received a volume of 20 µL of PMA (50 pmol/paw; *i.pl.*) prepared in saline. After the injection, mice were individually placed in a transparent observation chamber and the amount of time the animal spent licking the injected paw was timed with a chronometer from the 15th to 45th min post-injection and considered as indicative of nociception.

8-bromo 3′,5′-cAMP -Induced Nociception (8-Br-cAMP)

The procedure used was similar to that described previously (Siebel et al. 2004; Otuki et al. 2005). Animals were divided and treated as in the PMA test excepted that positive control received H-89 (10 nmol/paw). Twenty microliters of 8-Br-cAMP (10 nmol/paw) was injected intraplantarly (*i.pl.*) under the surface of the right hindpaw. Animals were immediately placed individually in a transparent observation chamber and observed for 10 min after 8-Br-cAMP injection. The amount of time the animal spent licking the injected paw was timed with a chronometer and considered as indicative of nociception.

Biochemical analysis

Tissue and plasma protein contents were determined using freshly prepared Biuret reagent (Gornall et al. 1949).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using commercial kits (INMESCO, Wiedtalstr.11.53577 neustadt/wield-Germany) based on the procedure described by the supplier.

Nitrite assay was done using Griess reagent as reported by Nguelefack-Mbuyo et al. (2010). Glutathione peroxidase (GP) activity was determined according to the method of Sehirli et al. (2008) with slight modification. 1 mL de $Na_2HPO_4{\cdot}2H_2O$ (0.3 M) and 0.1 mL of dithiobisnitrobenzoate (0.4 mg/mL into 1% of trisodic acid) were added to 250 µL of sample. After mixture, the absorbance was measured at 412 nm and the enzyme activity was calculated.

Superoxide dismutase (SOD) activity was measured as described by Dimo et al. (2006) with some modifications. The reaction mixture was consisted of 140 microliters of tissue sample, 1660 µL carbonate buffer (pH = 9) and 200 µL of adrenaline (0.3 mM). The absorbance of SOD was measured at 480 nm and was expressed as units/mg protein. One unit was defined as the enzyme activity that inhibited auto oxidation of adrenaline by 50%.

Catalase concentration was determined as indicated by Dimo et al. (2006). The reaction mixture was consisted of 25 µL of tissue sample, 375 µL phosphate buffer (10 mM, pH 7.4), 100 µL de H_2O_2 (50 mM), 1 mL of potassium

dichromate (5%) prepared in acetic acid à 1%. The last solution was added in the medium 1 min later.

Lipid peroxidation was assayed by determining the level of malondialdehyde, by measuring thiobarbituric reactive species using the method of Fofié et al. (2014). The reaction mixture consisted of 100 µL of tissue sample, 500 µL of orthophosphoric acid (1%), 500 µL of thiobarbituric acid prepared in trichloroacetic acid (1%). After homogenization, the reaction mixture was kept in a water bath (100 °C) for 15 min, and then cooled in ice water. Tubes were then centrifuged at 3000 rpm for 15 min and the absorbance of the supernatant was read at 532 nm against a blank.

Statistical analysis

Results are expressed as Mean ± SEM (standard error of the mean). One-way ANOVA followed by the Tukey's post-test was used to compare the averages of the various groups in the antinociceptive tests in mice and in biochemical analysis. Two-way ANOVA followed by Bonferroni post-test was used to compare different groups in the anti-hyperalgesic and the inflammatory tests. These analyses were performed with the aid of Graph Pad Prism software version 5.0.

Results

Effects of acute and chronic administration of *P. mannii* extracts on CFA-induced hyperalgesia

CFA injection in the animal paw led to hyperalgesia that was stable for more than 2 weeks. Oral administration of AEPM and MEPM significantly reduced the hyperalgesia both in acute and in chronic treatment. At all the doses used, a single administration induced a significant effect that started from the second hour post administration and lasted more than 24 h (Fig. 1a, b). Repeated administration of AEPM and MEPM at all the doses used significantly reduced the persistent mechanical hyperalgesia induced by CFA (Fig. 1c, d).

Effects of acute and chronic administration of *P. mannii* on CFA-induced persistent inflammation

In both AEPM and MEPM treated groups, oral acute administration did not induced any significant variation of the paw diameter as compared to negative control group. In contrast, rats which received Diclofenac (5 mg/kg) showed a significant decrease in paw diameter when compared to control group. The significant effect started from the fifth hour post CFA injection and lasted more than 24 h (Fig. 2a, b). Similar results were obtained after repeated oral administration of plant extracts. Indeed, none of the two extracts after 14 days treatment, could significantly reduce the edema induced by the CFA injection (Fig. 2c, d). No significant difference in animal weight was observed between groups treated with extracts and control groups (data not shown).

Effects of *P. mannii* extracts on 8-bromo 3′,5′-cAMP -Induced Nociception

Pittosporum mannii extracts administered orally at the doses of 150 and 300 mg/kg induced a significant reduction of the nociception produced by 8-Br-cAMP. AEPM reduced the licking time by 80.60 and 58.78% at respective doses of 150 and 300 mg/kg. MEPM at the same respective doses, inhibited the nociception induced by 8-Br-cAMP by 77.27 and 90.90%. Dihydrochloride H-89 used as positive control exhibited an inhibition of 86.36% (Fig. 3a, b).

Effects of *P. mannii* extracts on phorbol myristate acetate (PMA)-induced nociception

Both AEPM and MEPM administered orally, induced a significant dose–dependent inhibition of the nociception resulted from the intraplantar injection of PMA. AEPM at the dose of 300 mg/kg produced a marked inhibition of 74.21% (Fig. 4a) whereas MEPM at the same dose showed an inhibition percentage of 58.52. The administration of chelerytrine used as positive control also significantly reduced the nociception induced by PMA by 85.81% (Fig. 4b).

Effects of *P. mannii* extracts on biochemical parameters

It is observed from Table 1 that repeated administration of AEPM and MEPM did not significantly affect the level of malondialdehyde, protein, catalase and the superoxide dismutase activity in the spinal cord, as compared to the negative control group.

Both extracts increased the tissue concentration of glutathione but the increase was only significant in the groups treated with MEPM at the doses of 150 and 300 mg/kg. The dose 150 mg/kg exhibited the best effect with an increase of 89.61%. Plant extracts treatment significantly reduced the level of nitric oxide in the spinal cord. Both extracts induced a dose-dependent effect with the maximal inhibitory percentages of 44.39 and 46.09 obtained, respectively, with AEPM and MEPM at the dose of 300 mg/kg (Table 1).

Oral administration of AEPM and MEPM caused a reduction in plasmatic concentration of alanine aminotransferase. This reduction was significant in groups treated

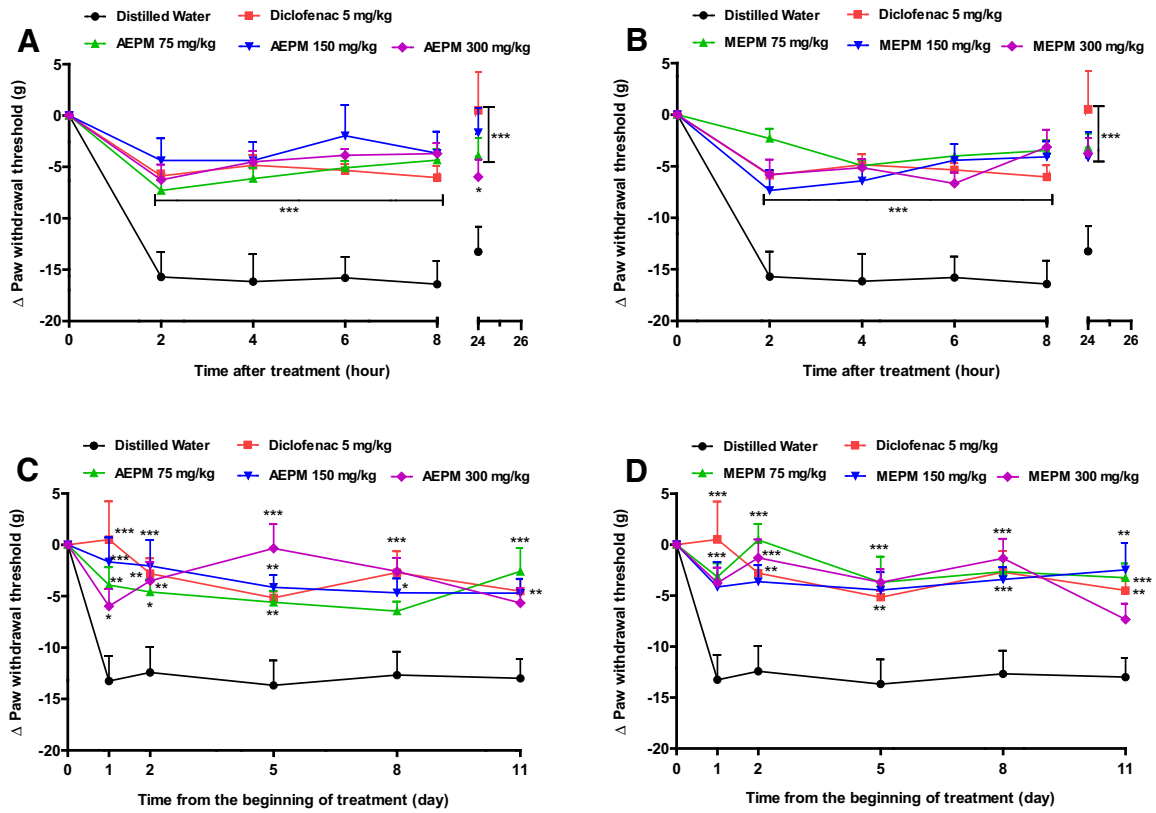

Fig. 1 Acute (**a**, **b**) and chronic (**c**, **d**) effects of the aqueous (**a**, **c**) and methanol (**b**, **d**) extracts of the leaves of *Pittosporum mannii* on CFA-induced hyperalgesia. Each point represents the mean ± standard error of the mean of seven individual values. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ significantly different compared to the control group (distilled water)

with AEPM at the dose of 150 mg/kg or with MEPM at the dose of 75 mg/kg. However, the same extracts did no significantly affect the plasmatic concentration of aspartate aminotransferase and proteins (Table 2).

Discussion

This study investigates the antihyper nociceptives properties of the AEPM and MEPM in model of persistent inflammatory pain and explores their possible mechanism of action. Treatment with AEPM and MEPM caused a marked inhibition of CFA-induced hind paw hyperalgesia. Anti-inflammatory effects was not observed during the acute and chronic treatments. These findings do not support the indigenous use of the leaves of the plant in the treatment of inflammation and further indicate the specificity of plant parts properties, as Momeni et al. (2010) reported instead the use of the stem bark of *P. mannii* for the treatment of inflammatory ailments. While working with *Pittosporum floribundum* a plant of the same genus as *Pittosporum mannii*, Yasodamma et al. (2015) showed that the leaf and seed methanol and aqueous extracts of the plant were not able to inhibit the acute edema induced by Carrageenan but extracts from the stem bark produced significant anti-inflammatory effects. These data corroborate results from the present work. Besides, the differential effect observe between leaves and stem bark extracts suggests a divergence in the chemical composition of the two plant parts although 1-*O*-rhamnopyranosyl-23-acetoxyimberbic acid 29-methyl ester was found both in the stem bark and the leaves (Nyongbela et al. 2013; Wandji et al. 2016).

Administration of *P. mannii* extracts significantly inhibited the hyperalgesia associated with the CFA-induced inflammation. Inflammatory stimuli such as CFA induce a cascade of inflammatory mediators such as cytokines and PGE_2, resulting in inflammatory hyperalgesia Cunha et al. (2005). These inflammatory mediators are responsible for the hypersensitization of nociceptors—a phenomenon that includes the appearance of inducible proteins—up-regulation and activation of various membrane and intracellular pathways, including cAMP, PKA and PKC, both at the periphery and at the central nervous system (Minami et al. 2006; Nguelefack et al. 2015). In fact, evidence now indicates that the hyperalgesia produced by CFA is mediated through the peripheral and central PKA and PKC stimulation (Malmberg et al. 1997;

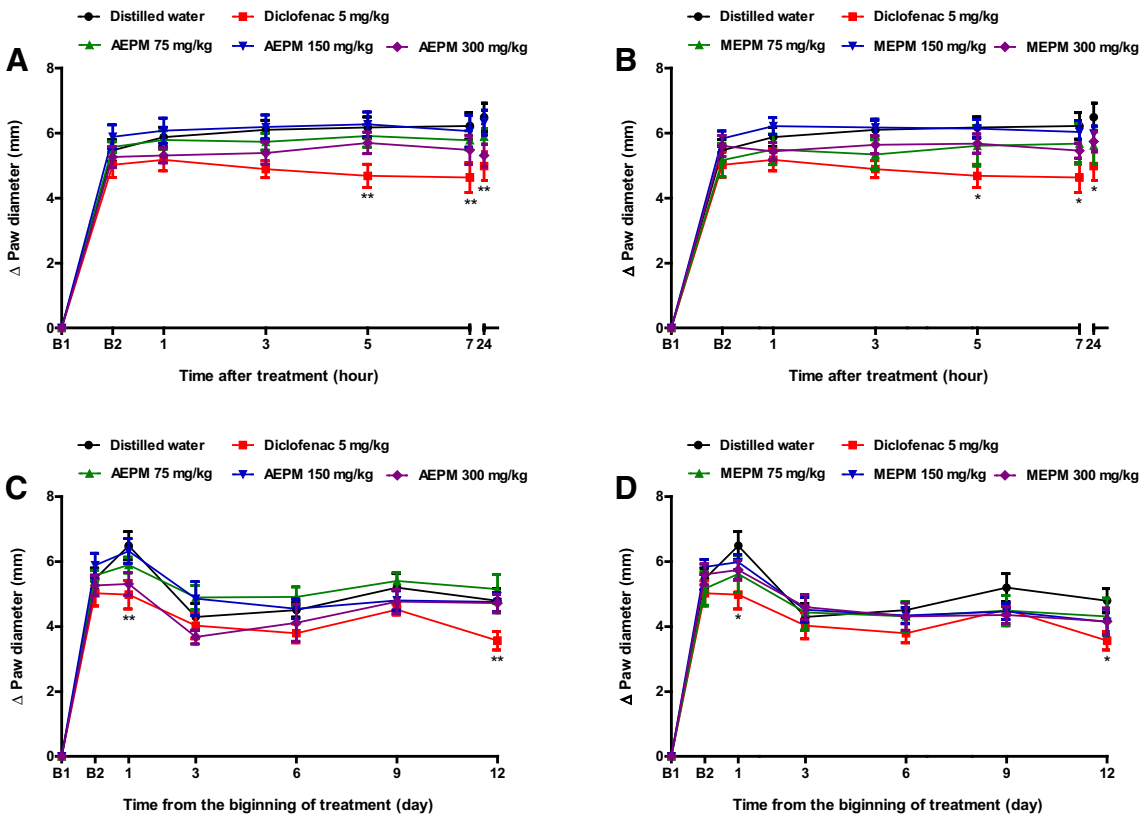

Fig. 2 Acute (**a**, **b**) and chronic (**c**, **d**) effects of the aqueous (**a**, **c**) and methanol (**b**, **d**) extracts of the leaves of *Pittosporum mannii* on CFA-induced inflammation. Each point represents the mean ± standard error of the mean of seven individual values. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ significantly different compared to the control group (distilled water)

Aley and Levine 1999; Sluka and Audette 2006; Kopach et al. 2013) and there is even a cross-talk between the two pathways (Huang et al. 2015). In addition, it has been demonstrated that inhibitors of PKC prevent the phosphorylation of receptors like TRPV1, reducing the sensitization of this capsaicin sensitive receptor (Ferreira et al. 2004; Calixto et al. 2005; Meotti et al. 2005; Nguelefack et al. 2015). Therefore, it could be taught that AEMP and MEPM induce their anti-hyperalgesic effect by inhibiting the pathways involving PKC and PKA. To verify this hypothesis, AEPM and MEPM were tested on nociception induced by intraplantar injection of 8-Br-cAMP a direct activators of PKA or PMA a direct activators of PKC. Interestingly, oral treatment with *P. mannii* extracts significantly inhibited the nociception produced into mouse paw. These findings suggest that AEPM and MEPM may interact with kinase pathways, blocking the activity of both PKC and, in a special manner, PKA. But this seems unlikely since it is well known that PKC activation plays a pivotal role in the inflammatory process (Savkovic et al. 2003; Kim et al. 2013; Ren et al. 2014). Thus, if the *P. mannii* extracts inhibited PKC and PKA, they should be able to induce anti-inflammatory activity. It is therefore, possible that AEPM and MEPM act downstream the protein kinases. As such they may be acting directly on nociceptors or analgesic receptors as previously shown (Wandji et al. 2016). Besides it was observed that both in chronic analgesic testing and in acute test using 8-Br-cAMP, AEPM induced a non-dose-dependent effect. This findings corroborate previous results obtained by Wandji et al. (2016) showing an *U* shaped dose response relationship of this plant extracts. The *U* shaped dose–response relationships has been documented in numerous biological, toxicological, and pharmacological investigations (Calabrese and Baldwin 2001) and has been linked to either maximal stimulation or activation of different type of receptor at higher doses.

Several lines of evidence indicate that nitric oxide (NO) has a pivotal role in acute and chronic pain state at both peripheral and central levels. Indeed, NO is implicated in the synaptic plasticity, the central sensitization, the weakening of the descending inhibition by interfering with GABAergic and glycinergic inhibitory tone (Little et al. 2012). So, targeting NO production may be a good alternative for pain therapy. Interestingly, *P. mannii* extracts dose-dependently reduced the NO content in the spinal cord. This result indicates that the antihyperagesia activity

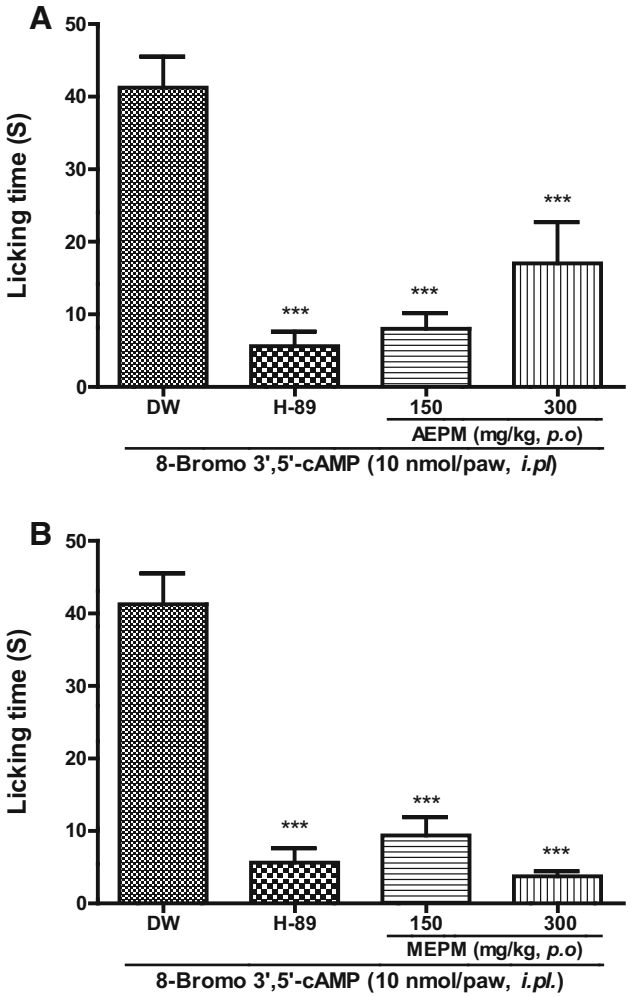

Fig. 3 Effects of the oral administration of the aqueous (AEPM) and methanol (MEPM) extracts of the leaves of *Pittosporum mannii* on the nociception induced by 8-Br-cAMP. Each point represents the mean ± standard error of the mean of six individual values. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ significantly different compared to the control group. *DW* distilled water

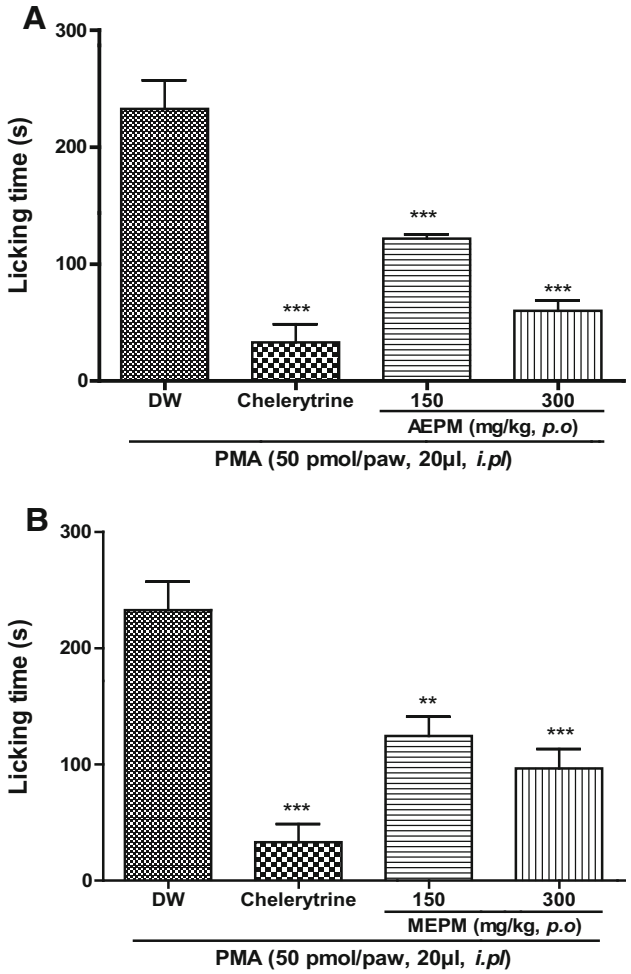

Fig. 4 Effects of the oral administration of the aqueous (AEPM) and methanol (MEPM) extracts of the leaves of *Pittosporum mannii* on the nociception induced by PMA. Each point represents the mean ± standard error of the mean of six individual values. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ significantly different compared to the control group. *DW* distilled water

of AEPM and MEPM may be due to the inhibition of NO production.

Free radicals are implicated in a number of pathological processes such as inflammation, aging, and pain. It has been demonstrated that combined antioxidant therapy is safe and effective therapy for pain relief (Cai et al. 2013). We evaluated whether *P. mannii* extracts could have additional antioxidant effects. AEPM and MEPM at all doses had no effect on the level of malondialdehyde, catalase and superoxide dismutase activity in the spinal cord. Although MEPM could significantly increase the glutathione concentration, it can be concluded that antioxidant activity do not account much for the analgesic effects of *P. mannii* leaves extracts.

The plasma ALT activity has been regarded as a reliable and sensitive marker of liver disease. ALT may also be a good indicator of overall health. The result obtained indicated no alteration of plasmatic transaminases, suggesting that 14 days treatment with AEPM and MEPM did not induce any liver toxicity. This further demonstrate the safety of extracts from the leaves of *P. mannii*.

Conclusion

The present results indicates that *P. mannii* aqueous and methanol extracts possess anti-hyperalgesic effects in models of persistence inflammatory pain in rat but lack anti-inflammatory activities. The precise mechanisms through which *P. mannii* exerts its action still to be elucidate, but they seem working downstream PKC and PKA and to involve the inhibition of nitric oxide synthesis in the

Table 1 Effects of *Pittosporum mannii* aqueous (AEPM) and methanol (MEPM) extracts on the SOD activity and glutathione, catalase, malondialdehyde, nitric oxide (NO) and proteins contents in spinal cord

Treatment	Dose (mg/kg)	SOD (mUI/mg protein)	Glutathione (μmol/g of tissue)	Catalase (mmol/g of tissue)	Malondialdehyde μmol/g of tissue)	NO (mmol/g of tissue)	Protein (mg/g of tissue)
Control	/	31.74 ± 10.5	9.338 ± 0.44	299.3 ± 29.80	788.2 ± 172.2	4.10 ± 1.36	141.2 ± 29.61
Diclofenac	5	27.87 ± 11.31	10.67 ± 0.587	301.8 ± 25.84	628.9 ± 221.7	2.71 ± 0.77	123.2 ± 16.72
AEPM	75	10.02 ± 5.26	10.85 ± 0.733	284.9 ± 11.14	722.5 ± 172,3	4.68 ± 1.34	142.1 ± 28.90
	150	25.23 ± 7.42	10.87 ± 0.652	274.7 ± 5.473	688.2 ± 220.1	2.30 ± 0.35**	143.1 ± 26.90
	300	21.04 ± 6.19	11.06 ± 0.697	292.6 ± 13.25	558.5 ± 191.1	2.28 ± 0.33**	144.2 ± 29.40
MEPM	75	21.89 ± 3.61	10.96 ± 0.845	300.1 ± 9.538	426.8 ± 142	2.30 ± 0.75	126.6 ± 32.17
	150	23.82 ± 6.00	17.71 ± 2.149***	298.0 ± 10.37	566.7 ± 209.7	2.39 ± 0.53*	176.1 ± 35.49
	300	42.98 ± 11.04	13.78 ± 2.00*	310.3 ± 14.05	719.7 ± 214.5	2.21 ± 0.58*	188.3 ± 40.59

Values are mean ± standard error of the mean of seven individual values

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significantly different compared to the control group

Table 2 Effect of *Pittosporum mannii* aqueous (AEPM) and methanol (MEPM) extracts on the plasmatic level of proteins, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Treatment	Dose (mg/kg)	Proteins (mg/mL)	ALT (UI/L)	AST (UI/L)
Control	/	53.27 ± 4.83	32.22 ± 4.67	60.43 ± 3.149
Diclofenac	5	49.46 ± 3.77	21.52 ± 2.12	52.35 ± 7.091
AEPM	75	61.98 ± 4.71	24.49 ± 2.93	50.02 ± 2.632
	150	65.60 ± 3.66	14.91 ± 3.14**	53.80 ± 2.785
	300	58.75 ± 1.86	23.02 ± 3.12	60.11 ± 5.820
MEPM	75	66.29 ± 3.41	17.03 ± 3.912*	60.88 ± 6.046
	150	62.40 ± 2.90	22.61 ± 2.320	51.85 ± 4.782
	300	55.62 ± 5.56	22.04 ± 3.72	55.42 ± 9.267

Values are mean ± standard error of the mean of seven individual values

* $p < 0.05$, ** $p < 0.01$ significantly different compared to the control group

spinal cord. Extracts from the leaves of *P. mannii* may be proper for pain treatment but not inflammation.

Compliance with ethical standards

Ethics approval and consent to participate Experimental protocols used herein were approved by the laboratory Committee and conformed to the guidelines for the study of pain in awake animals established by the International Association for the Study of Pain.

Conflict of interest All the authors declare no conflict of interest.

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