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Effects of Hydroalcoholic Leaf Extract of *Ficus Exasperata* Vahl (Moraceae) In Carrageenan/ Kaolin-Induced Acute and Chronic Musculoskeletal Pain in Rats

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ABSTRACT

Background: Leaf extracts of *Ficus exasperata* has been used traditionally in Ghana for the management of sprains, sore eyes, rheumatism and arthritis.

Objective: This study evaluates the scientific basis for the use of *Ficus exasperata* leaves in the management of acute and chronic musculoskeletal pain

Methods: The carrageenan-kaolin-induced hyperalgesia model was used to assess acute and chronic musculoskeletal pain using the Randall-Sellito and the grip strength tests using morphine as a reference drug.

Results: *F. exasperata* extract (30-300 mg kg^{-1}) significantly and dose-dependently decreased acute skeletal pain as well as chronic skeletal hyperalgesia in the ipsilateral and contralateral paws with maximum possible anti-hyperalgesia effects being achieved at the highest dose.
Furthermore, it completely reversed both acute and chronic muscle hyperalgesia in a significant and dose-dependent manner.

Conclusion: These findings thus support the usefulness of *Ficus exasperata* leaf extracts in the management of musculoskeletal pain.

Key words
Musculoskeletal pain, grip strength, hyperalgesia, morphine, carrageenan/kaolin

Effets de l'extrait hydroalcoolique de feuille de Ficus exasperata Vahl (Moraceae) En carraghénane / Kaolin induite aiguë et chronique douleur musculosquelettique chez les rats

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RÉSUMÉ

Contexte: Des extraits de feuilles de Ficus exasperata a été traditionnellement utilisé au Ghana pour la gestion des entorses, les yeux endoloris, les rhumatismes et l'arthrite.

Objectif: Cette étude évalue la base scientifique pour l'utilisation de Ficus exasperata laisse dans la gestion de la douleur musculosquelettique aiguë et chronique

Méthodes: Le induite kaolin-carraghénane modèle d'hyperalgésie a été utilisé pour évaluer la douleur musculo-squelettique aiguë et chronique à l'aide des tests de la force de préhension à l'aide de la morphine comme un médicament de référence Randall-Sellito et.

Résultats: F. extrait de exasperata (30-300 kg mg) de manière significative et dose-dépendante a diminué la douleur squelettique aiguë ainsi que l'hyperalgésie squelettique chronique dans les pattes ipsilatérales et controlatérales avec un maximum d'effets possibles anti-hyperalgésie atteints à la dose la plus élevée. En outre, il a complètement inversé la fois aiguë et chronique hyperalgésie musculaire de manière significative et dose-dépendante.

Conclusion: Ces résultats confirment donc l'utilité de Ficus extraits exasperata de feuilles dans la gestion de la douleur musculo-squelettique.

Mots clés: Douleurs musculo-squelettiques, la force de préhension, l'hyperalgésie, la morphine, carraghénane / kaolin

INTRODUCTION

Musculoskeletal pain creates a large socioeconomic burden by making work burdensome, increasing sick leave and can greatly affect quality of life [1-2] resulting in reduced productivity. It may manifest as lower back pain, myalgia, fibromyalgia, tendonitis and stress fractures.[3]

Ficus exasperata commonly referred to as the sandpaper tree is widely distributed in tropical Africa.[4] In Ghana, it is used for the treatment of rheumatism, arthritis and colic pains.[5, 6] In Nigeria, it has also been used as a mouthwash against thrush, inflammation of the gums and other mouth and throat ailments.[7]

The anti-nociceptive and anti-antipyretic effect of leaf extract has been reported by Woode *et al* in 2009.[8] Subsequently its anti arthritic effects in inflammatory models as well as anti-oxidant effects have also been reported by Abotsi *et al* in 2011.[9] There is also a report on its anti-bacterial and anti-ulcer effects.[10-11]

This present paper looks at the effect of a hydroalcoholic leaf extract of *Ficus Exasperata* in carrageenan-kaolin induced acute and chronic musculoskeletal pain in rats. This model is known to measure hyperalgesia directly from the site of injury which is very significant as far as painful clinical conditions that are aggravated by mechanical pressure at the site of injury is concerned.[12]

MATERIALS AND METHODS

Plant Collection and Extraction: Leaves of *F. exasperata* were collected from uncultivated fields on the campus of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana in September 2010. They were authenticated in the Department of Herbal Medicine of the university where a voucher specimen (No.FP/08/023) has been kept. The leaves were then shade-dried and later pulverized with a hammer mill. One kilogram of the resultant powdered material was cold macerated in four (4) litres of 70 % v/v of ethanol overnight. The hydro-alcoholic extract was then evaporated under reduced pressure to a syrupy mass using a rotary evaporator, dried in an oven to a semi-solid paste and kept in a desiccator. This is subsequently referred to as the extract or FEE.

Drugs and chemicals: Morphine hydrochloride was obtained from Phyto-Riker pharmaceuticals, Accra, Ghana, carrageenan sulphate and kaolin from Sigma-Aldrich Inc., St. Louis, MO, USA.

Animals: Sprague-Dawley rats (190–200 g) of both sexes were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana and housed in the animal facility of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST). The animals were housed in groups of six in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions (temperature 24-25 C, relative humidity 60-70 %, and 12 hour light-dark cycle). All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication no. 85 - 23, 1985, revised 1996). All protocols used were approved by the Departmental Ethics Committee.

Effect of FEE on acute and chronic muscle pain

Hyperalgesia was induced by injecting 100 µl of 3 % w/v carrageenan percutaneously into the right gastrocnemius muscle of rats as described by Radhakrishnan.[13] Acute muscle pain developed 24 hours after injection and chronic muscle pain developed 8 days after injection. This was observed as a decrease in grip strength using a grip force analyser. The analyser measured the amount of tensile force each rat exerted against a wire mesh grid with its hind paws when pulled gently in the caudal direction.[12,14] Readings were taken before induction of hyperalgesia and at 0 hours after primary hyperalgesia confirmation and 5 conservative 1 hour intervals after drug treatment.

Chronic muscle pain was assessed by measuring paw withdrawal thresholds [15-18] using an analgesimeter (Model No. 15776, Ugo Basile, Comerio, Varese, Italy). Paw withdrawal was obtained by applying linearly, a perspex cone to the knee joint distal to the assaulted gastrocnemius muscle until the animal withdrew the paw or vocalised. The minimum pressure (grams) obtained before withdrawal was taken as the paw withdrawal threshold (PWT). Readings were taken at 0-5 hours after drug treatment on both ipsilateral and contralateral knee joints.

Reduction in grip force after percutaneous carrageenan relative to baseline grip force levels provides an index of the reduction in nociceptive threshold and was calculated as % MPE:

% MPE = (post-drug treatment — pre-drug treatment)/ (baseline – pre-drug treatment).

Effect of FEE in acute and chronic skeletal pain

Hyperalgesia was induced by injecting a 3 % w/v kaolin-carrageenan mixture into the right knee joint of the animals as described by Radhakrishnan.[13] Mechanical hyperalgesia was measured as paw withdrawal thresholds (PWT) using an analgesimeter (Model No. 15776, Ugo Basile, Comerio, Varese, Italy) as described previously.[16-17] By linear application of increasing pressure at the knee joint using a blunt perspex cone 24 h after assault, acute skeletal pain was measured as the minimum pressure required to elicit paw withdrawal or vocalisation. Chronic skeletal pain was measured as described above after 8 days but with readings taken from dorsal region of both ipsilateral and contralateral hind paws. Readings were taken at 0-5 hours after drug treatment.

In all experiments with the analgesimeter, 250 g was set as the cut-off point to prevent tissue damage to the limbs.

In all four experiments, animals were grouped as follows for each single experiment:

Group I Control Group (normal saline treatment)

Group II/III/IV Treated with FEE 30/100/300 mg kg^{-1}

Group V/VI/VI Treated with Morphine 1/3/10 mg kg^{-1}

Extract was prepared in 2 % w/v tragacanth mucilage. Drug solutions and suspensions were prepared such that not more than 1-ml of extract was given orally and not more than 0.5 ml of the standard drugs were injected intraperitoneally. All drugs were freshly prepared.

Statistical analysis

In all experiments, a sample size of seven to eight animals (n=7-8) were used. All data are presented as mean ± S.E.M. Raw data for the mechanical hyperalgesia in the Randall-Selitto tests was calculated as the percentage change in maximum possible effect (% MPE). The time-course curves were subjected to two-way (treatment × time) repeated measures analysis of variance (ANOVA) with Holm-Sidak's post hoc *test*

Total nociceptive score for each treatment was calculated in arbitrary unit as the area under the curve (AUC) from time course curves. To determine the percentage inhibition for each treatment, the following equation was used.

$$\%\ inhibition = \left(\frac{AUC_{control} - AUC_{treatment}}{AUC_{control}} \right) \times 100$$

Differences in AUCs were analyzed using one-way ANOVA with drug treatment as a between- subjects factor. Further comparisons between vehicle- and drug-treated groups were performed using the Holm-Sidak's test.

Doses for 50 % of the maximal effect (ED_{50}) for each drug were determined by using an iterative computer least squares method, with the following nonlinear regression (three parameter logistic) using the equation:

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogED_{50} - X)}\right)}$$

Where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape.

The fitted midpoints (ED_{50}s) of the curves were compared statistically using F test.[19-20] GraphPad Prism for Windows version 6.0 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and ED_{50} determinations. $P < 0.05$ was considered statistically significant.

RESULTS

Acute muscle hyperalgesia

A decrease in grip strength twenty-four (24) hours post carrageenan was observed in all groups of animals for acute muscle pain indicating the development of primary hyperalgesia. FEE (30-300 mg kg^{-1}) significantly and dose-dependently (Figure 1a, *inset*) reduced acute muscle hyperalgesia ($F_{3,16}$=*14.51, P*<0.0001: Figure 1a) seen as an increase in paw withdrawal latency. Also morphine (1-10 mg kg^{-1}) used as control significantly ($F_{3,16}$=*9.85, P*=0.0006; Fig. 1b) and dose-dependently (Fig. 1b, *inset*) reduced acute muscle hyperalgesia.

The extract [ED_{50}: 31.23±11.91] was found to be less potent than morphine [ED_{50} 0.29±0.11] after comparing the ED_{50}s obtained by non-linear regression ($F_{1,26}$=*6.52, P*=0.0168: Figure 5a)

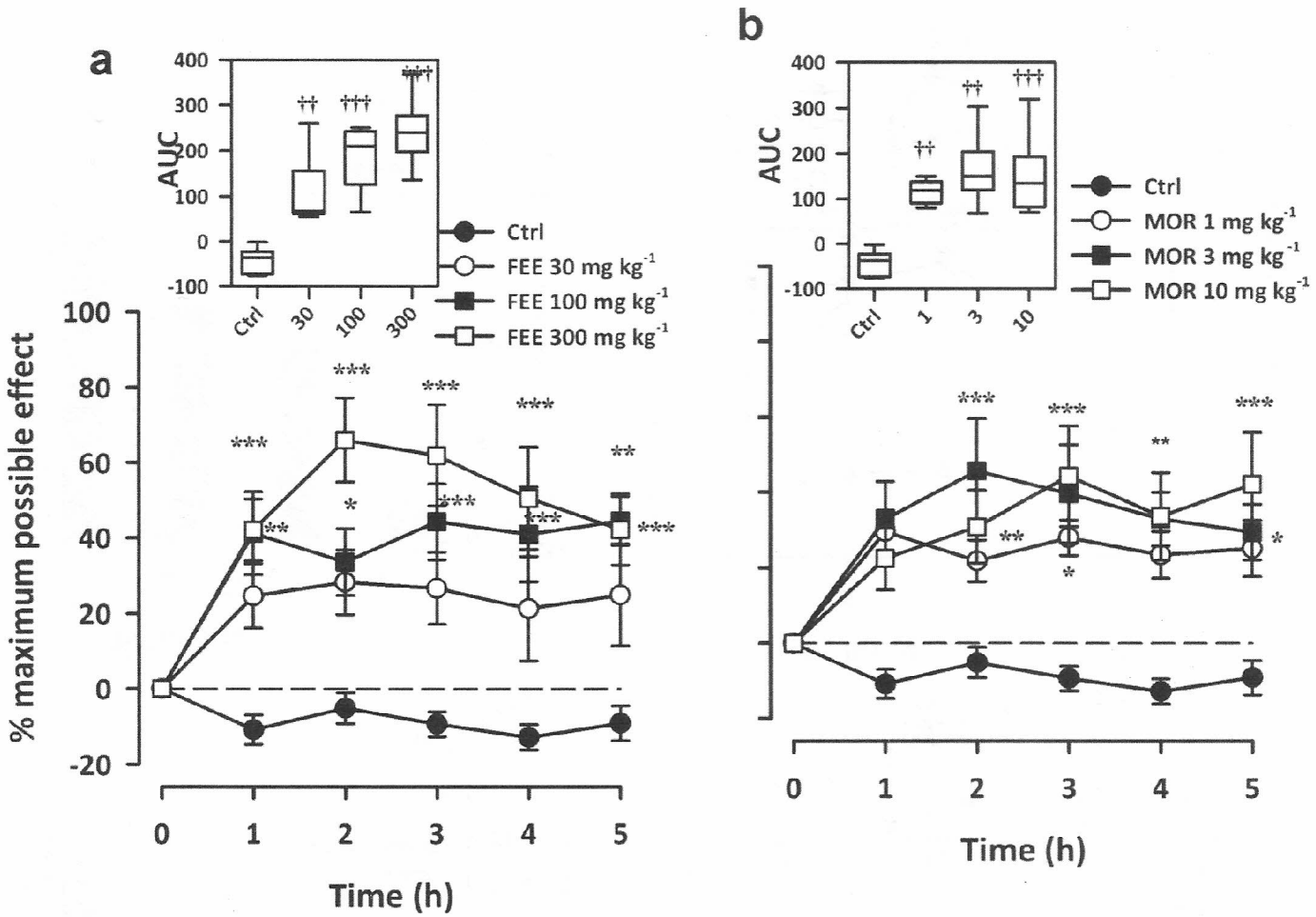

Figure 1 Effect of (a) FEE (30-300 mg kg^{-1} *p.o*) and morphine (b) (1-10 mg kg^{-1} *i.p.*) on the time course curve of grip strength test measured ipsilaterally in rats. Each point represents Mean ± S.E.M (n = 7). *P < 0.05, **P < 0.01, ***P < 0.001 compared to respective controls (two-way repeated measures ANOVA followed by Holm-Sidak's post hoc test); Insets are box and whisker plots of the AUCs derived from the respective time course curves. The lower and upper margins of the box represent the 25 and 75th percentiles, with the extended arms representing the 10 and 90th percentiles, respectively. The median is shown as the horizontal line within the box. *P< 0.05, **P < 0.01,***P < 0.001 (one-way ANOVA followed by Holm-Sidak's post hoc)

Chronic muscle hyperalgesia

An injection of 3% carrageenan-kaolin mixture was found to induce chronic hyperalgesia eight (8) days after the injection which was indicated by a significant drop in both the ipsilateral and contralateral paws withdrawal thresholds (PWT) (insets) on day 8 as compared to baseline readings (Day 0).

Chronic muscle hyperalgesia was significantly and dose-dependently attenuated by FEE (30-300 mg kg^{-1}) (ipsi: $F_{3,16}$=28.71, P<0.0001, contra: $F_{3,16}$=34.87, P<0.0001, Figure 2a) in the ipsilateral and contralateral paws and morphine (ipsi: $F_{3,16}$=6.14, P= 0.0056, contra: $F_{3,16}$=15.93, P<0.0001, Figure 2b). Furthermore, FEE was able to produce complete reversal of the chronic hyperalgesia with the maximum dose producing a mean effect of 318.50±35.06 ipsilaterally and 294.20±22.04 contralaterally.

There was no significant difference ($F_{1,26}$=0.06, P=0.8151) between the potency of the extract ipsilaterally [ED_{50}: 26.54±10.12]and contralaterally [ED_{50}: 22.57±8.61]. Morphine also did not show any significant difference ($F_{1,26}$=1.66, P=0.2095) in potency when compared ipsilaterally [ED_{50}: 1.09±0.41] and contralaterally [ED_{50}: 0.59±0.21]. Morphine was however significantly more potent than FEE when the ED_{50}s were compared ipsilaterally ($F_{1,26}$=0.06, P=0.8151) and contralaterally ($F_{1,26}$=0.06, P=0.8151) (Figure 5c).

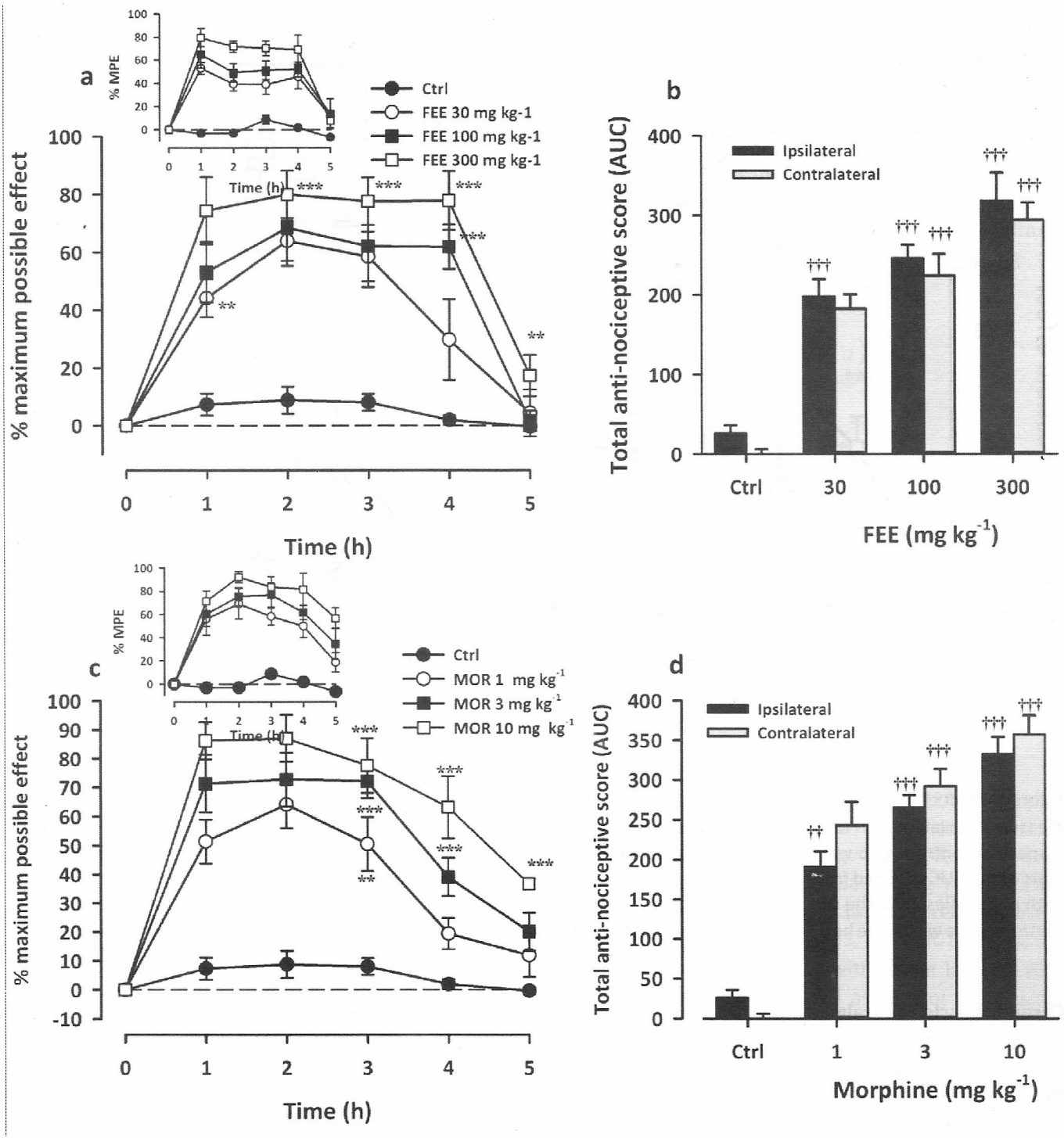

Figure 2 Effect of FEE (30-300 mg kg^{-1} *p.o.*) and morphine (1-10 mg kg^{-1} *i.p.*) on the time course curve of ipsilateral and contralateral paws (insert) withdrawal latency using the Randall-Sellito test (a and c) and the AUC (b and d) in chronic muscle pain. Data is presented as mean ± S.E.M. (n = 7); ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ compared to vehicle-treated group (Two-way ANOVA followed by Holm-Sidak's *post hoc* test). $^{\dagger\dagger\dagger}P < 0.001$ $^{\dagger}P < 0.01$ compared to vehicle-treated group (One-way ANOVA followed by Holm-Sidak's *post hoc* test).

Acute knee (skeletal) hyperalgesia

FEE (30-300 mg kg^{-1}) significantly and dose-dependently increased paw withdrawal threshold (PWT) at the knee joint ($F_{3,16}$=26.77, P<0.0001) (Figure 3a) with the highest dose giving a mean MPE of 241.70±37.23 (Figure 3a, *inset*). Morphine (1-10 mg kg^{-1}) also significantly ($F_{3,16}$=23.76, P<0.0001; Figure 3b) and dose-dependently (Figure 3b, *inset*) reduced acute knee hyperalgesia

A comparison of the ED_{50}s of FEE [ED_{50} 14.29±5.44] and morphine [ED_{50} 0.59±0.22] obtained by non-linear regression (Figure 5b) showed no significant difference ($F_{1,23}$=2.98, P=0.0980) in their potencies.

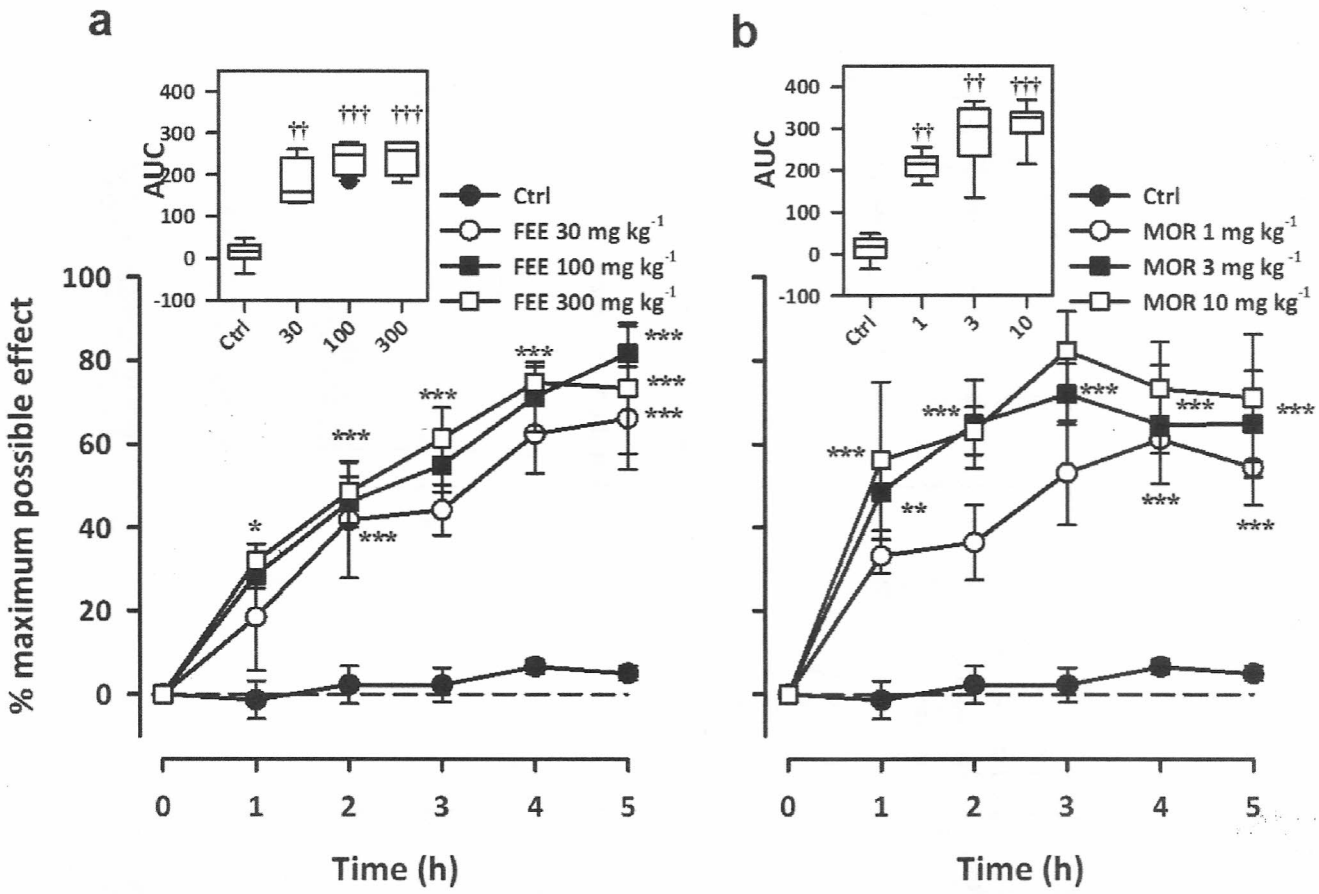

Figure 3 Effect of FEE (30-300 mg kg^{-1} *p.o*) and morphine (1-10 mg kg^{-1} *i.p.*) on the time course curve of paw withdrawal latency in the Randall-Sellito test (a and b) in acute skeletal pain. Each point represents Mean ± S.E.M (n = 7). *P < 0.05, **P < 0.01, ***P < 0.001 compared to respective controls (two-way repeated measures ANOVA followed by Holm-Sidak's test. post hoc); Insets are box and whisker plots of the AUCs derived from the respective time course curves. The lower and upper margins of the box represent the 25 and 75th percentiles, with the extended arms representing the 10 and 90th percentiles, respectively. The median is shown as the horizontal line within the box. *P< 0.05,**P< 0.01,***P<0.001 (one-way ANOVA followed by Holm-Sidak's post hoc)

Chronic knee (skeletal) hyperalgesia

There was a decrease in paw withdrawal threshold of both ipsilateral and contralateral (insets) paws to a mechanical stimulus in on the test day. This was present in all the animals indicating secondary skeletal hyperalgesia.

FEE (30-300 mg kg^{-1}) significantly and dose-dependently (ipsi: $F_{3,16}$=24.07, P< 0.0001, contra: $F_{3,16}$=94.90, P<0.0001) (Figure 4a) decreased chronic skeletal hyperalgesia in the ipsilateral and contralateral paws (Figure 4a). Maximum possible chronic skeletal anti-hyperalgesia was achieved at the highest dose (Figure 4b). Morphine (1-10 mg kg^{-1}) significantly and dose-dependently (Figure 4c) attenuated chronic skeletal hyperalgesia (ipsi: $F_{3,16}$=60.86, P< 0.0001, contra:

$F_{3,16}$=56.31, P< 0.0001) in the ipsilateral and contralateral paws (Figure 4d).

There was no significant difference ($F_{1,26}$=0.48, P=0.4975) between the potencies of the extract ipsilaterally [ED_{50}: 29.56±11.27] and contralaterally [ED_{50}: 43.64±16.63] (Figure 5d). Morphine also did not show any significant difference ($F_{1,26}$=0.52, P=0.4796) in potency when compared ipsilaterally [ED_{50}: 1.11±0.42] and contralaterally [ED_{50}: 0.79±0.30]. Morphine was however significantly more potent than FEE when the ED50s were compared ipsilaterally ($F_{1,26}$=11.25, P=0.0025) and contralaterally ($F_{1,26}$=36.96, P<0.0001).

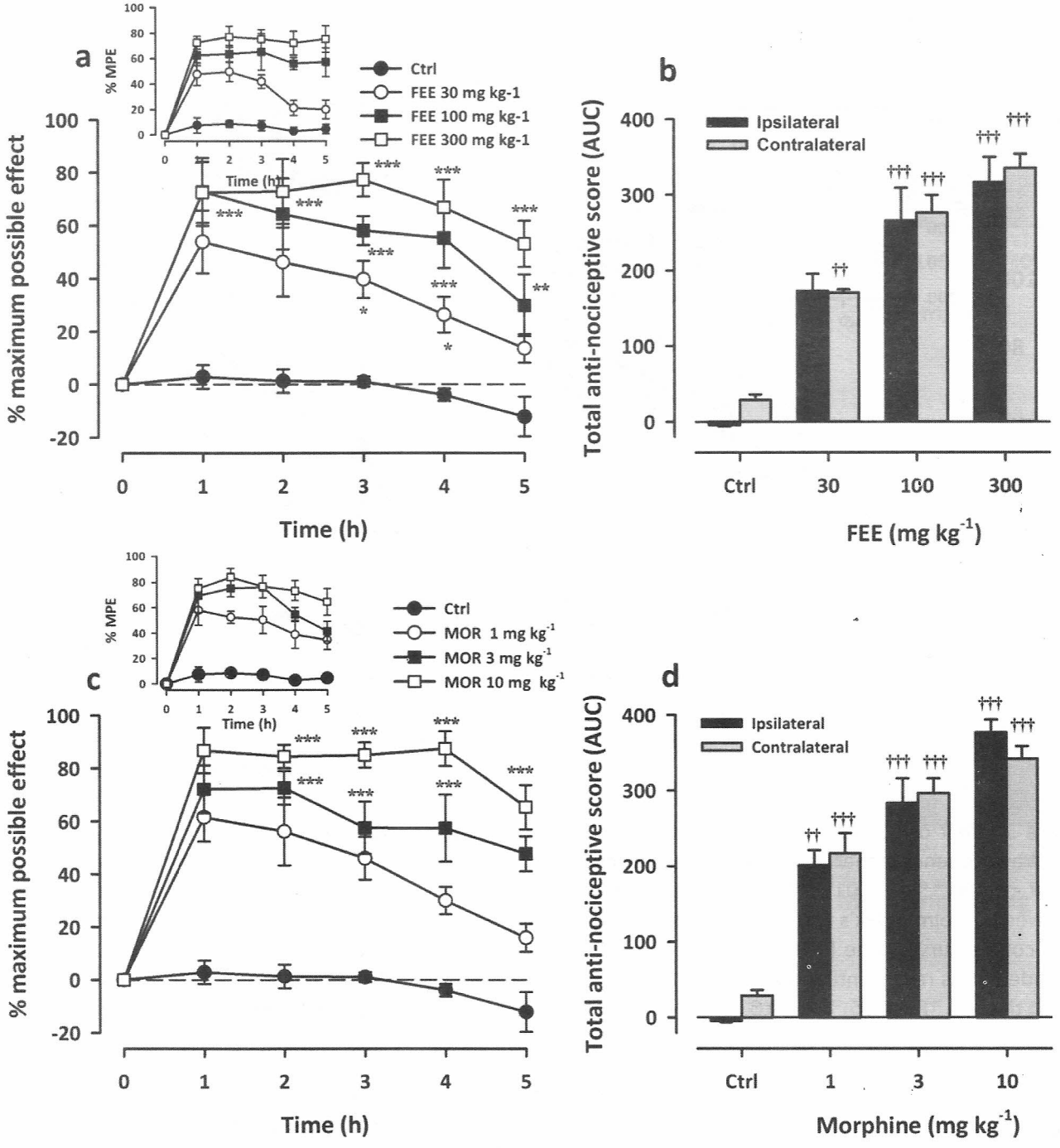

Figure 4 Effect of FEE (30-300 mg kg^{-1} *p.o.*) and morphine (1-10 mg kg^{-1} *i.p.*) on the time course curve of ipsilateral and contralateral paw (insert) withdrawal latency (a and c) and the AUC (b and d) in chronic skeletal pain. Data is presented as mean ± S.E.M. (n = 7); ***P < 0.001; ** P < 0.01; *P < 0.05 compared to vehicle-treated group (Two-way ANOVA followed by Holm-Sidak's *post hoc* test). $^{\dagger\dagger\dagger}P$<0.001 $^{\dagger}P$<0.05 compared to vehicle-treated group (One-way ANOVA followed by Holm-Sidak's *post hoc* test).

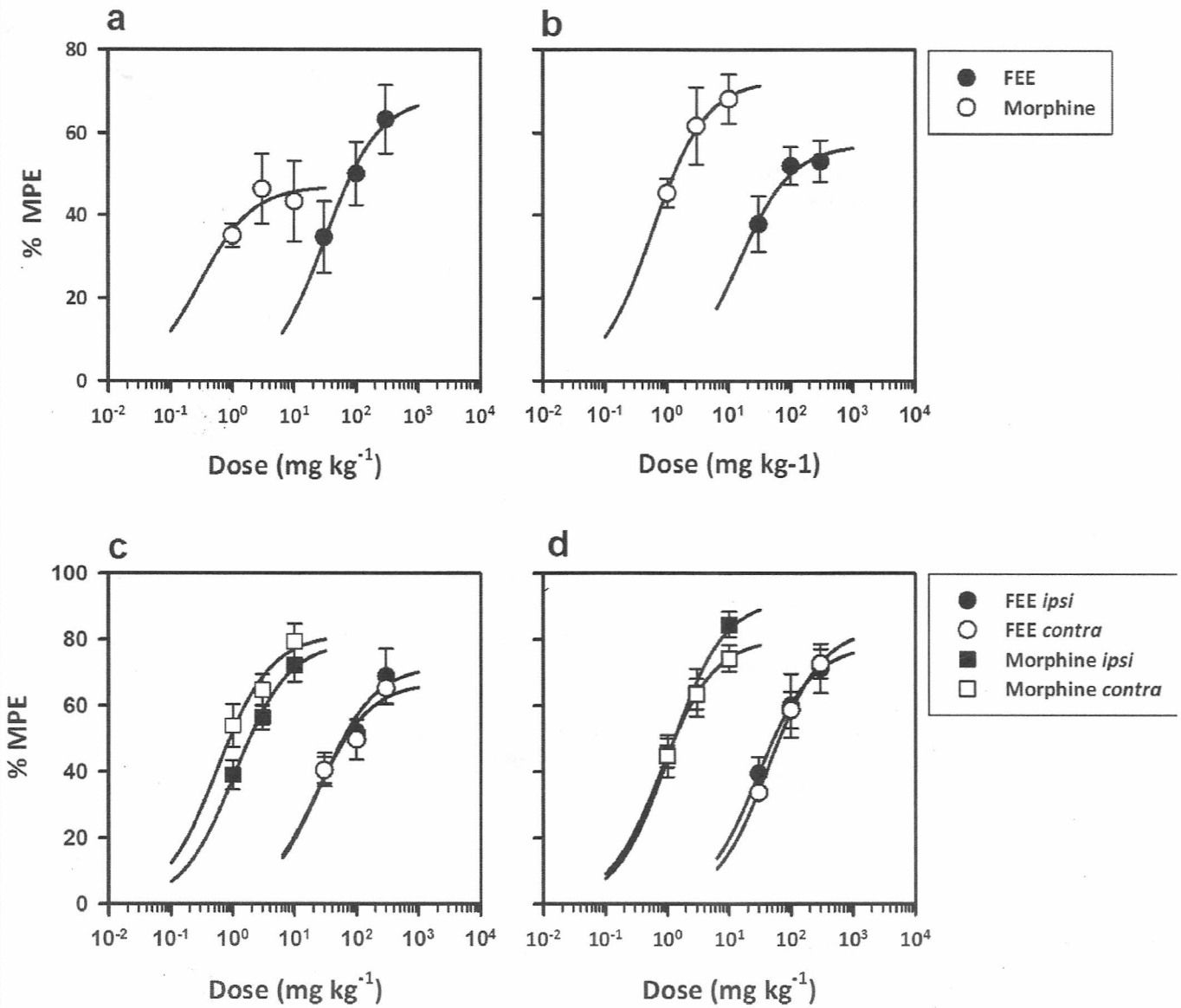

Figure 5 Dose-response curves of the effects of FEE and morphine in acute muscle pain (a), acute skeletal pain (b), chronic muscle pain (c) and chronic skeletal pain (d). Percentage MPEs were derived from the AUCs and the curves obtained by non-linear regression as described under Materials and Methods.

DISCUSSION

Oral administration of hydroalcoholic leaf extract of *Ficus exasperata* blocked both acute and chronic musculoskeletal pain induced by carrageenan-kaolin mixture. This carrageenan-kaolin model employed in this study measures hyperalgesia directly from the site of injury.[12]

When carrageenan is injected, it causes accumulation of neutrophils and release of inflammatory mediators like glutamate, prostaglandins, histamine and serotonin. Concomitant injection with kaolin causes immediate increase in glutamate and nitric oxide metabolites in the knee joint that persists for hours. These mediators sensitize primary efferent neurons hence resulting in primary hyperalgesia.[21]

In the study, both FEE and morphine were able to reverse the induced hyperalgesia both in acute and chronic musculoskeletal pain experiments confirming the model as useful for identifying novel substances for managing musculoskeletal pain.

The ability of FEE to block primary hyperalgesia may be due to the modulation of inflammatory mediators that are released when the muscle and joint were assaulted with carrageenan-kaolin mixture. This could be due to the ability of the extract (FEE) to suppress synthesis and or release of inflammatory pain mediators (kinin,

histamine, nitric oxide, serotonin etc.), interact with the inflammatory cells (e.g. mast cells and neurons) or inhibit peripheral cyclooxygenase activity to prevent release of prostaglandins. This is not surprising as the anti-inflammatory effects of *F. exasperata extract* have already been reported.[8]

Injection of carrageenan-kaolin mixture into deep tissues may activate neurons in the dorsal horn of the spinal cord leading to central sensitization either spinally or supraspinally. This together with the peripheral sensitization manifests as secondary hyperalgesia which persists to chronic hyperalgesia at sites adjacent or sometimes distal to the site of assault. The unilateral assault of both muscle and knee joints also lead to the development of chronic hyperalgesia and contralateral spread of hyperalgesia.[22-24]

Spinal changes accounting for the central sensitization following assault of a muscle or joint with carrageenan and kaolin is mediated by release of glutamate, aspartate, substance P, nitric oxide and prostaglandin E_2 in the dorsal horn as well as the induction of COX-2 mRNA in the lumbar spinal cord.[22,25]

The ability of the extract to reduce hyperalgesia at sites distal to the site of assault as well as in the contralateral limb suggests it might have central effects either spinally or supraspinally by modulating some or all of these mediators responsible for the central sensitization. This central analgesic effect of the extract could also be mediated by activating the opioid receptors in the descending inhibitory pathway in the brain or the nociceptive afferent neurons in the spinal cord since descending inhibitory control of pain is normally impaired in chronic musculoskeletal pain situations.[26,27]

CONCLUSION

This study has provided further scientific evidence to show that the hydroalcoholic leaf extract of *Ficus exasperata* has significant anti nociceptive activity in acute and chronic musculoskeletal pain and may be useful in the clinical management of musculoskeletal pain.

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