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RESEARCH ARTICLE

Effects of an ethanol extract and the diterpene, xylopic acid, of *Xylopia aethiopica* fruits in murine models of musculoskeletal pain

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ABSTRACT

Context: Fruits of *Xylopia aethiopica* (Dunal) A. Rich. (Annonaceae) are used traditionally to manage arthritis, headache and other pain disorders.

Objective: The analgesic properties of the *X. aethiopica* ethanol fruit extract (XAE) and xylopic acid (XA) were evaluated in musculoskeletal pain models.

Materials and methods: Acute muscle pain was induced in gastrocnemius muscle of Sprague–Dawley rats with 3% carrageenan (i.m.). Rats received XAE (30–300 mg/kg), XA (10–100 mg/kg) or morphine (1–10 mg/kg) after 12 h. Effects of XAE and XA on muscle pain were assessed by measuring post-treatment grip strength of the rats. Chronic muscle pain was similarly induced, but drug treatment was on the eighth day and effects of XAE and XA assessed with Randall–Selitto test for hyperlagesia. Acute-skeletal pain was induced in knee joints of rats with 3% carrageenan-kaolin mixture and effects determined 12-h later. Similar induction protocol was used for chronic knee pain with treatment and measurement as done for chronic muscle pain.

Results: XAE and XA significantly and dose-dependently ameliorated both acute muscle (ED_{50} mg/kg: XAE = 22.9; XA = 6.2) and skeletal hyperalgesia (XAE = 39.9; XA = 17.7) induced by 3% carrageenan. Similarly, chronic skeletal hyperalgesia was reduced by XAE and XA treatment similar to morphine (ED_{50}: XAE = 13.0; XA = 4.6). This reduction was also seen in chronic muscle hyperalgesia (ED_{50}: XAE = 79.1; XA = 42.7). XAE and XA significantly reduced the spread of hyperalgesia to contralateral limbs in both models of chronic hyperalgesia.

Conclusion: These findings establish analgesic properties of the ethanol fruit extract of *X. aethiopica* and xylopic acid in musculoskeletal pain.

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Acute pain; carrageenan; chronic pain; kaolin; morphine; myalgia

Introduction

Musculoskeletal pain disorders such as arthritis, non-articular rheumatism, low-back disorders and fibromyalgia are a major clinical problem globally (Katz 2002; Bove et al. 2009). Despite its high prevalence and socioeconomic impact, musculoskeletal pain remains poorly understood and available treatment regimens are largely not-specific and insufficient (Breivik et al. 2006; Vavken & Dorotka 2011; Gerhardt et al. 2012). Currently, there is limited understanding of mechanisms that drive musculoskeletal pain and a limited repertoire of analgesics available to treat musculoskeletal pain (Bove et al. 2009).

Various plants are used traditionally for the treatment of musculoskeletal pain disorders; an example being *Xylopia aethiopica* (Dunal) A. Rich. (Annonaceae). The plant is a slim, tall, evergreen, aromatic tree that grows in Ghana, Democratic Republic of Congo, Ethiopia, Nigeria, Senegal and Uganda (Orwa et al. 2009). The fruit, commonly known as African pepper or locally as *Hwentia* (Twi), *Tso* (Ewe) and *Soo* (Ga), is used as a spice in the preparation of soup. It is also used traditionally for inflammatory conditions such as arthritis. In Ghanaian traditional medicine, the fruit extract is used for the treatment of cough, rheumatism, lumbago, headache, neuralgia and colic pain (Woode et al. 2013). The fruit of *Xylopia aethiopica* contains kauranes, a class of diterpenes, namely kaurenoic and xylopic acid (Ekong & Ogan, 1968). The extract and xylopic acid have shown antimicrobial and CNS depressant effects (Boakye-Yiadom et al. 1977; Biney et al. 2014).

Authors have previously reported the analgesic properties of the ethanol fruit extract of *Xylopia aethiopica* (XAE) and its constituent xylopic acid (XA) (Woode et al. 2012, 2013). Recently, the anti-allodynic and anti-hyperalgesic effects of XAE and XA in neuropathic pain have also been published (Ameyaw et al. 2014). In this study, the effect of XAE and XA on acute and chronic musculoskeletal pain has been evaluated in murine models.

Materials and methods

The dried fruits of *Xylopia aethiopica* were collected from the Botanical Gardens of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana (06°41′6.39″N; 01°33′45.35″W) between the months of August and December 2008. The fruits were authenticated by Dr. Kofi Annan of the Department of Herbal Medicine, Faculty of Pharmacy and

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Pharmaceutical Sciences, College of Health Sciences, KNUST. A voucher specimen (No. FP/09/77) has been kept at the herbarium of the Faculty.

Preparation of the ethanol extract of Xylopia aethiopica (XAE), isolation and purification of xylopic acid (15β-acetoxy-(-)-kaur-16-en-19-oic acid)

Preparation of the ethanol extract of *Xylopia aethiopica* and isolation and purification of xylopic acid (Figure 1) were as previously described (Adosraku & Oppong Kyekyeku 2011; Woode et al. 2013). Briefly, fresh unripe fruits of *Xylopia aethiopica* were shade dried, milled into a powder and extracted by cold maceration with 70% ethanol for 72 h. The resultant extract was concentrated into a semi-solid brownish mass and subsequently stored in a refrigerator until use.

To isolate xylopic acid, 2 kg of dried and powdered *Xylopia aethiopica* unripe fruits was exhaustively extracted by cold maceration in a percolator with petroleum ether for 72 h. The extract obtained was concentrated to half its volume with a rotary evaporator at 60 °C. The extract deposited crude crystals after 3 days; which was purified by recrystalization with a reflux condenser. The crystals obtained were characterized by high performance liquid chromatography tandem mass spectrometry (HPLC-MS) with a purity of 99.87%.

Drugs and chemicals

Morphine hydrochloride was obtained from Phyto-Riker, Accra, Ghana; Carrageenan sulfate and Kaolin from Sigma-Aldrich Inc., St. Louis, MO.

Animals

Sprague–Dawley rats (150–200 g, 12 weeks-old) of both sexes were obtained from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana. The animals were housed in groups of six in stainless steel cages ($34 \times 47 \times 18\ cm^3$) with soft wood shavings as bedding, fed with normal commercial pellet diet (Agricare Ltd, Kumasi, Ghana), given water *ad libitum* and maintained under laboratory conditions (temperature 24–25 °C, relative humidity 60–70%, and 12-h light-dark cycle) in the animal facility of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST). 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 (Animal Care and Use

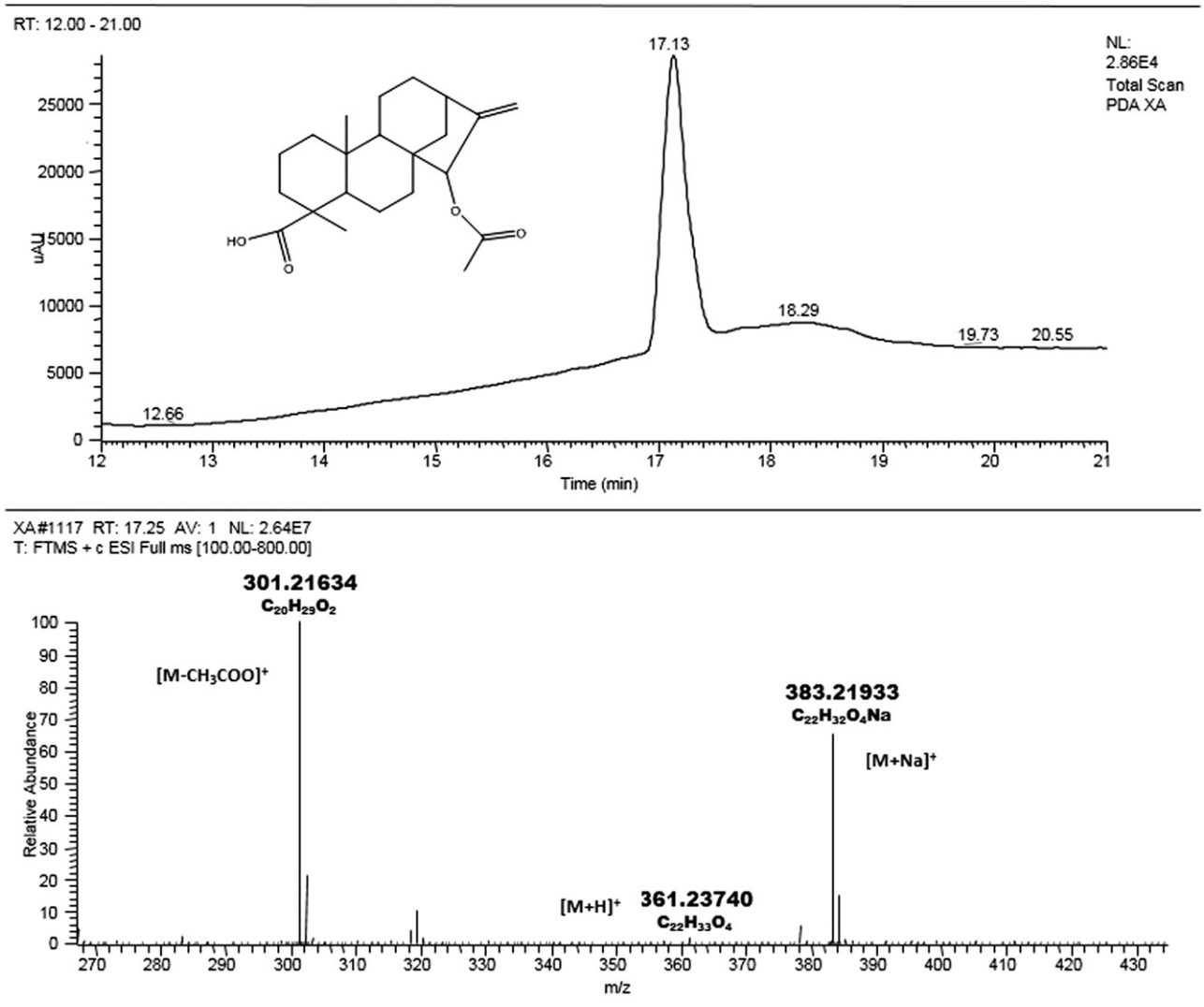

Figure 1. LC-MS profile and chemical structure of 15 β-acetoxy-(-)-kaur-16-en-19-oic acid (xylopic acid).

Committee, 1998). All protocols used were approved by the Departmental Ethics Committee (No.: FPPS/PCOL/0015/2010).

Assessment of the effect of XAE and XA on acute skeletal pain

In all experiments, doses of XAE and XAE were selected based on their previously reported antinociceptive effects (Woode et al. 2012). Morphine served as reference antinociceptive agent due to its effects on both central and peripheral transmission of pain. The analgesic effect of XAE and XA were evaluated in acute skeletal pain models similar to that described (Skyba et al. 2005) with slight modification. On the test day, baseline compression thresholds of the ipsilateral knees were taken with an analgesimeter (IITC Life Science Inc. Model 2888, Woodland Hills, CA) by compressing the knee of the rat until the animal withdrew the limb forcefully or vocalized. The maximum compression force applied at withdrawal was recorded as the baseline compression threshold for the knee joint of the corresponding limb. Rats were then injected with 100 µL of a mixture containing 3% kaolin and 3% carrageenan intra-articularly into the left knee joint. Acute skeletal pain was established after 12 h in ipsilateral limbs by measuring the withdrawal of the limbs or vocalization after knee compression. Ten groups of rats received vehicle, XAE (30, 100 and 300 mg/kg *p.o.* 1 h), XA (10, 30 and 100 mg/kg *p.o.* 1 h) or morphine (1, 3 and 10 mg/kg i.p. 30 min) after primary hyperalgesia confirmation and knee compression thresholds were taken again hourly for 5 h. Total nociceptive score for each treatment was calculated in arbitrary unit as the area under the curve (AUC). To determine the percentage inhibition for each treatment, the following equation was used.

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

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:

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

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.

Evaluation of analgesic effect of XAE and XA on chronic skeletal pain

To evaluate the analgesic effect of XAE and XA on chronic skeletal pain (bilateral hyperalgesia), baseline compression thresholds of both paws were taken with an analgesimeter (IITC Life Science Inc. Model 2888, Woodland Hills, CA), which is based on the Randall–Selitto test (Randall & Selitto 1957). Ten groups of rats received intra-articular injection of 100 µL of a mixture containing 3% kaolin-carrageenan mixture. Animals were allowed eight days to develop chronic-knee inflammation (Radhakrishnan et al. 2003).

On the test day, chronic skeletal pain was established by measuring compression thresholds for both ipsilateral and contralateral paws. Ten groups of animals then received vehicle, XAE (30–300 mg/kg *p.o.*), XA (10–100 mg/kg *p.o.*) or morphine (1–10 mg/kg i.p.) after bilateral hyperalgesia confirmation and compression thresholds were taken again hourly for 5 h.

Analgesic effects of XAE and XA acute muscle pain

Assessment of the analgesic properties of XAE and XA on acute muscle pain was done by measuring hind limb grip strength of rats with a grip force analyzer; as described earlier (Kehl et al. 2003; Skyba et al. 2005; Tillu et al. 2008). The force analyzer 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. The peak force exerted by the rat before it released its grasp from the wire mesh grid was registered by a force transducer and recorded. Three consecutive hind limb grip force measurements (10 s apart) were obtained for each rat at each time point and averaged to represent each rat's grip force for each time point. After baseline grip strength measurements, the animals received 100 µL of 3% carrageenan percutaneously into their left gastrocnemius muscles. Ten groups of rats were separately treated with XAE (30–300 mg/kg *p.o.* 1 h), XA (10–100 mg/kg *p.o.*), morphine (1–10 mg/kg i.p. 30 min) or vehicle after primary hyperalgesia (acute pain) confirmation at the 12th h and grip strength measurements were taken again hourly for 5 h.

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 % Maximum Possible Effect (MPE):

$$\%\ MPE = (post\text{-}drug\ treatment - pre\text{-}drug\ treatment)/(baseline - pre\text{-}drug\ treatment).$$

Analgesic effect of XAE and XA on chronic muscle pain

The possible analgesic effect of XAE and XA in chronic muscle pain model was assessed by injecting 100 µL of 3% carrageenan percutaneously into the left gastrocnemius muscle of the rats. The animals were allowed eight days to develop chronic muscle pain (Radhakrishnan et al. 2003). On the test day, chronic muscle pain was established by measuring compression thresholds for both paws in the Randall–Selitto model as described before. The animals were grouped into ten and the separate groups of animals were treated with XAE (30–300 mg/kg *p.o.),* XA (10–100 mg/kg *p.o.*), morphine (1–10 mg/kg i.p.) or vehicle after chronic muscle pain confirmation and compression thresholds were taken again hourly for 5 h.

Statistical analysis

All data are presented as mean ± S.E.M ($n = 7$–8). 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.

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.

The fitted midpoints (ED_{50}s) of dose-response curves were compared statistically using F test. GraphPad Prism for Windows version 6.0 (GraphPad Software, San Diego, CA) was used for all

statistical analyzes and ED_{50} determinations. $p \leq 0.05$ was considered statistically significant.

Results

Effect of XAE and XA on acute skeletal pain

Acute knee (skeletal) hyperalgesia induced by mixture of 3% kaolin-carrageenan was measured with analgesimeter based on the Randall–Sellito model. XAE (30-300 mg/kg) significantly and dose-dependently reduced acute knee hyperalgesia ($F_{3,16} = 22.22$, $p < 0.0001$; Figure 2a). The highest MPE was of XAE was observed at the highest dose (Figure 2a, *inset*). Similarly, XA (10–100 mg/kg) significantly and dose-dependently reduced acute knee hyperalgesia ($F_{3,16} = 21.55$, $p < 0.0001$; Figure 2b) with the highest dose producing the highest MPE (Figure 2b, *inset*). Morphine (1–10 mg/kg) used as control significantly ($F_{3,16} = 16.18$, $p < 0.0001$; Figure 2c) and dose-dependently (Figure 2c, *inset*) reduced acute skeletal hyperalgesia.

Effect of XAE and XA on chronic skeletal pain

Chronic skeletal hyperalgesia was measured in the ipsilateral and contralateral paws on the eighth day post 3% kaolin-carrageenan mixture injection. A decrease in paw withdrawal reflex of the ipsilateral and contralateral (*insets of* Figure 3) paws to a mechanical source of stimulus was seen across all treatment groups on the test day indicating secondary skeletal hyperalgesia. This decrease in paw withdrawal reflexes remained significant for the control group throughout the experiment. Chronic skeletal hyperalgesia was significantly and dose-dependently attenuated by XAE (30–300 mg/kg) (ipsi: $F_{3,16} = 20.68$, $p < 0.0001$, contra: $F_{3,16} = 10.08$, $p = 0.0006$) in the ipsilateral and contralateral paws (Figure 3a).

XA administration produced significant and dose-dependent effects (ipsi: $F_{3,16} = 15.98$, $p < 0.0001$, contra: $F_{3,16} = 16.55$, $p < 0.0001$) in the ipsilateral and contralateral paws (Figure 3c). Maximum possible chronic skeletal anti-hyperalgesia was achieved at the highest dose (Figure 3d). Morphine (1–10 mg/kg) significantly and dose-dependently (Figure 3f) attenuated chronic skeletal hyperalgesia (ipsi: $F_{3,16} = 19.09$, $p < 0.0001$, contra: $F_{3,16} = 9.5$, $p = 0.0008$) in the ipsilateral and contralateral paws (Figure 3e).

The rank of potency in the acute and chronic musculoskeletal pain was in the order morphine > XA > XAE (Table 1; Figure 6).

Effects of XAE and XA acute muscle pain

Acute muscle hyperalgesia was measured in the grip strength assay after 12 h of induction. XAE (30–300 mg/kg) significantly and dose-dependently (Figure 4a) reduced acute muscle hyperalgesia ($F_{3,16} = 38.19$, $p < 0.0001$: Figure 4a). Also, XA (10–100 mg/kg) significantly ($F_{3,16} = 53.49$., $p < 0.0001$; Figure 4b) and dose-dependently (Figure 4b) reduced acute muscle hyperalgesia seen as an increase in paw withdrawal latency. In a similar fashion, morphine (1–10 mg/kg) used as control, significantly ($F_{3,16} = 37.88$, $p < 0.0001$; Figure 4c) and dose-dependently (Figure 4c, *inset*) reduced acute muscle hyperalgesia.

Effects of XAE and XA chronic muscle pain

Chronic muscle hyperalgesia was measured from both the ipsilateral and contralateral paws (insets) eight days after pain induction. The hyperalgesia induced in the contralateral paws was indicative of referred pain from muscle injury and central pain mechanism. Chronic muscle hyperalgesia was significantly and dose-dependently attenuated by XAE (30–300 mg/kg) (ipsi: $F_{3,16} = 10.83$, $p = 0.0004$, contra: $F_{3,16} = 29.55$, $p < 0.0001$, Figure 5a) in the ipsilateral and contralateral paws, XA (ipsi: $F_{3,16} = 23.57$, $p < 0.0001$, contra: $F_{3,16} = 22.72$, $p < 0.0001$, Figure 5c) and morphine (ipsi: $F_{3,16} = 6.14$, $p = 0.0056$, contra: $F_{3,16} = 15.93$, $p < 0.0001$, Figure 5e).

The extract (Figure 5b), xylopic acid (Figure 5d) and morphine (Figure 5f), were thus effective in attenuating hyperalgesia in the

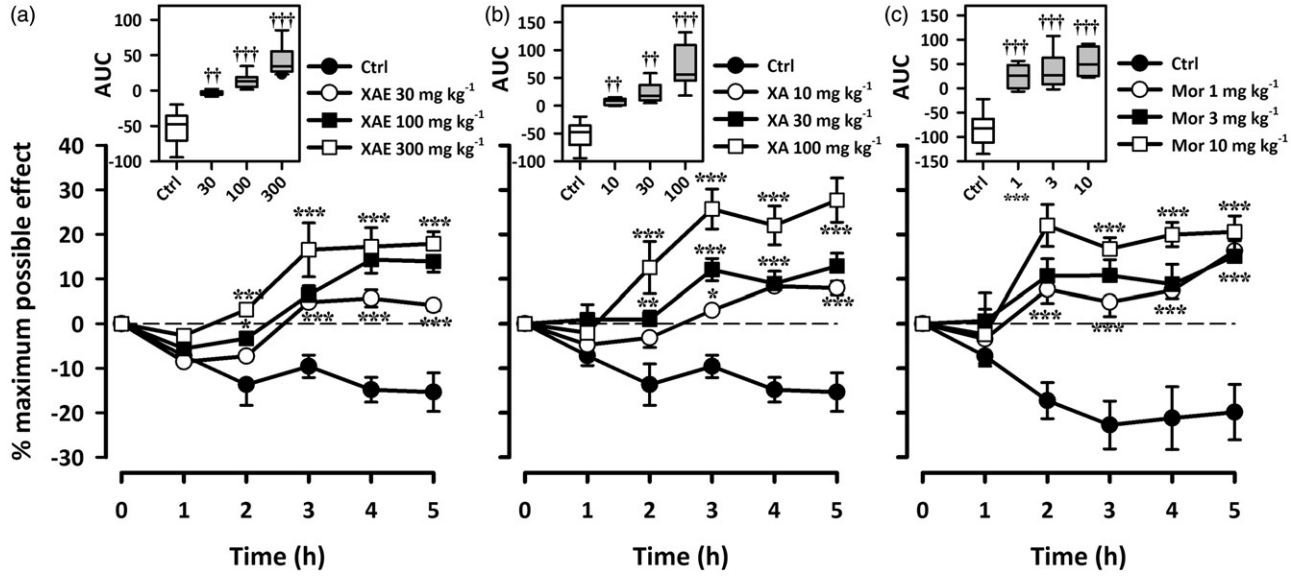

Figure 2. Effect of XAE (30–300 mg/kg *p.o*), XA (10–100 mg/kg *p.o*) and morphine (1–10 mg/kg i.p.) on the time course curve of paw withdrawal latency in the Randall–Sellito test (a, b and c) in acute knee pain. The box-and-whisker plots (*insets*) depict AUCs derived from the respective time course curves. The plots show the 25th and 75th percentiles, the median (horizontal line within the box), and the 10th and 90th percentiles (whiskers). Symbol represents outliers. Data are presented as mean ± S.E.M. ($n = 8$); ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$ compared to vehicle-treated group (Two-way ANOVA followed by Holm-Sidak's *post hoc* test). $^{\dagger\dagger\dagger}p < 0.001$ $^{\dagger\dagger}p < 0.01$ compared to vehicle-treated group (One-way ANOVA followed by Holm-Sidak's *post hoc* test).

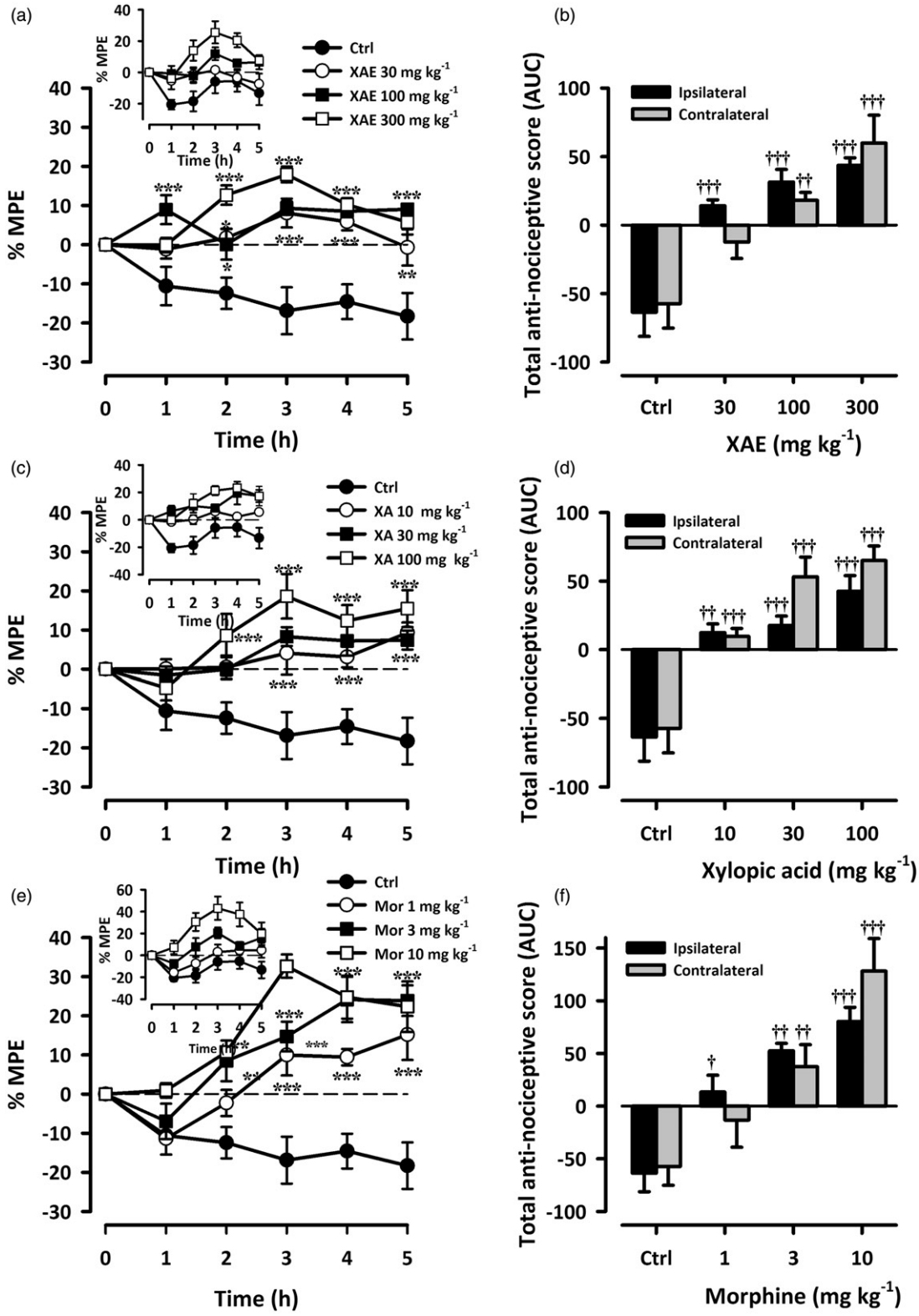

Figure 3. Effect of XAE (30–300 mg/kg *p.o.*), XA (10–100 mg/kg *p.o*) and morphine (1–10 mg/kg i.p.) on the time course curve of ipsilateral and contralateral paw (insets) withdrawal latency (a, c and e) and the AUC (b, d and f) in chronic knee pain. Data are presented as mean ± S.E.M. ($n = 8$); ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$ 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).

ipsilateral (ipsi) paw than the hyperalgesia in the contralateral (contra) paws.

The order of potency was in the order morphine > xylopic acid > extract (Table 1; Figure 6).

Discussion

In this study, it has been shown that XA and XAE are effective in attenuating acute and chronic musculoskeletal pain in murine models. The mechanisms of pain and hyperalgesia induced by injecting inflammatory agents into different tissues like muscle, joint and skin are different and represent different pathophysiological processes (Sluka 2002; Radhakrishnan et al. 2003). That is why despite the reported analgesic effects of XAE and XA (Woode et al. 2012, 2013; Ameyaw et al. 2014), the current study evaluated their specific potential in reducing musculoskeletal pain. The acute and chronic musculoskeletal pain models used in this study are more closely related to fibromyalgia and arthritic pain experienced in humans (Skyba et al. 2005).

The acute phase of the musculoskeletal pain model is due to cell infiltration (notably neutrophil) and the action of glutamate, aspartate, prostaglandin E_2 (PGE_2) and citrulline. This is seen as mild hemorrhage and edema at 4 h after injection of carrageenan and carrageenan-kaolin mixture (Yang et al. 1996). It was observed in this study that treatment with XAE, XA and morphine blocked acute muscle pain in rats. This suggests that XAE and XA may have some action against inflammatory pain mediators like glutamate, aspartate, prostaglandin E_2 (PGE_2) and citrulline: this may not be far-fetched since XAE and XA have been shown to act against inflammatory pain mediators such as glutamate, bradykinin and prostaglandin E_2 (Woode et al. 2012, 2013).

This study also revealed that XAE, XA and morphine were effective in reducing chronic musculoskeletal pain in the animal models. This type of pain is due to macrophage infiltration with a few scattered mast cells, production and release of glutamate, substance P, calcitonin gene-related peptide and prostanoids on dorsal horn neurons and supraspinal structures. These mediators produce central sensitization resulting in wind-up activity. XAE and XA possibly inhibited the pain mediators as part of their analgesic action. No evidence of inflammatory cell infiltrates in the contralateral knee joint or muscle tissues in chronic musculoskeletal pain models have been reported. The pain in the contralateral paws/limbs is therefore due to spinal and supraspinal neural changes with little or no primary afferent drive or input from the site of injury (ipsilateral limbs) once chronic pain has been developed (Coderre & Melzack 1985; Sluka et al. 2001). XAE and XA may also have activated the descending inhibitory pain control system in the rostral ventral medulla (a group of nuclei with bilateral spinal projections and wide receptive fields covering the contralateral limbs) since the descending inhibitory control of pain is impaired in people with chronic musculoskeletal pain (Radhakrishnan et al. 2003; Arendt-Nielsen et al. 2008). The observation that XAE and XA also exert their analgesic effects partly by enhancing central mechanisms for mitigating pain also give credence to this suggestion (Woode et al. 2013).

Table 1. Summary of the effect of *Xylopia* extract, xylopic acid and morphine in acute and chronic musculoskeletal pain in rats.

	Xylopia extract		Xylopic acid		Morphine	
	ED_{50} mg/kg	E_{max}	ED_{50} mg/kg	E_{max}	ED_{50} mg/kg	E_{max}
Acute muscle pain	22.9 ± 1.6	65.4	6.2 ± 1.0	63.1	0.9 ± 0.1	77.7
Chronic muscle pain						
Ipsilateral paw	79.1 ± 0.4	25.9	42.7 ± 1.8	31.4	7.4 ± 1.4	33.9
Contralateral paw	28.9 ± 1.6	16.7	8.1 ± 1.1	15.6	1.9 ± 0.5	33.8
Acute knee pain	39.9 ± 1.8	20.8	17.7 ± 1.5	28.2	0.3 ± 0.13	26.2
Chronic knee pain						
Ipsilateral paw	13.0 ± 1.3	21.5	4.6 ± 0.9	20.4	1.1 ± 0.21	31.0
Contralateral paw	79.8 ± 2.2	28.7	9.5 ± 1.2	27.1	6.4 ± 1.2	59.8

Values are expressed as mean ± S.E.M. ($n = 7$–8). ED_{50}s ± S.E.M. were obtained by least-square nonlinear regression as described under *statistical analysis*.

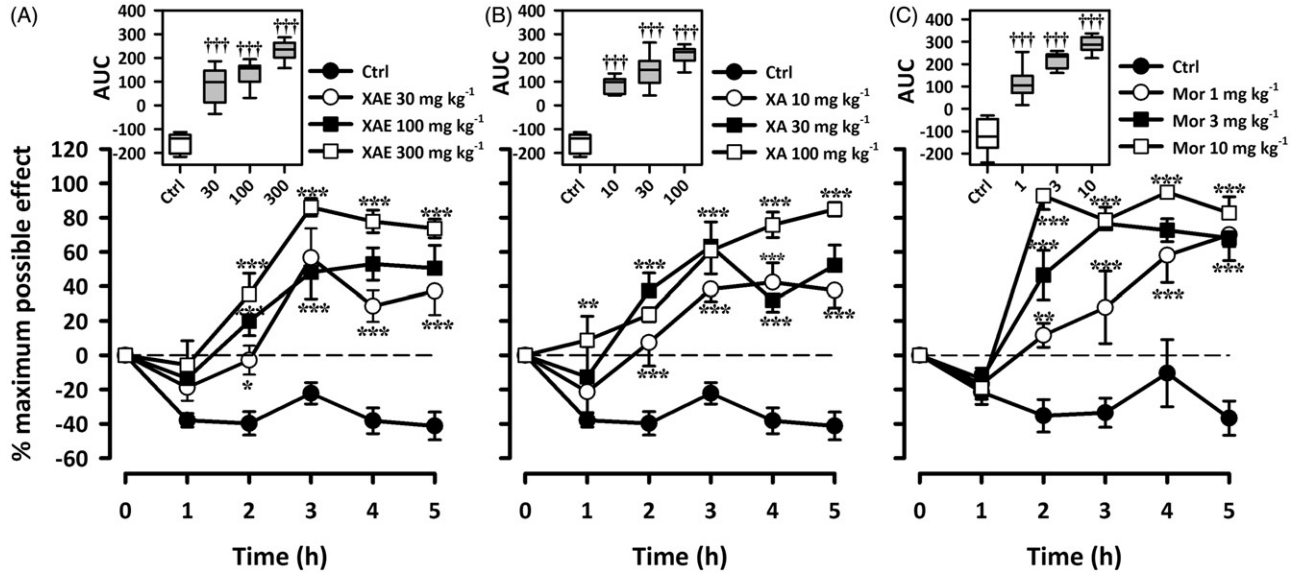

Figure 4. Effect of XAE (30–300 mg/kg *p.o.*), XA (10–100 mg/kg *p.o.*) and morphine (1–10 mg/kg i.p.) on the time course curve of grip strength test (a, c and e) and the AUC (b, d and f) in acute muscle pain. Data are presented as mean ± S.E.M. ($n = 8$). The box-and-whisker plots (insets) depict AUCs derived from the respective time course curves. The plots show the 25th and 75th percentiles, the median (horizontal line within the box), and the 10th and 90th percentiles (whiskers). ***$p < 0.001$; **$p < 0.01$; compared to vehicle-treated group (Two-way ANOVA followed by Holm-Sidak's *post hoc* test). $^{\dagger\dagger\dagger}p < 0.001$ compared to vehicle-treated group (One-way ANOVA followed by Holm-Sidak's *post hoc* test).

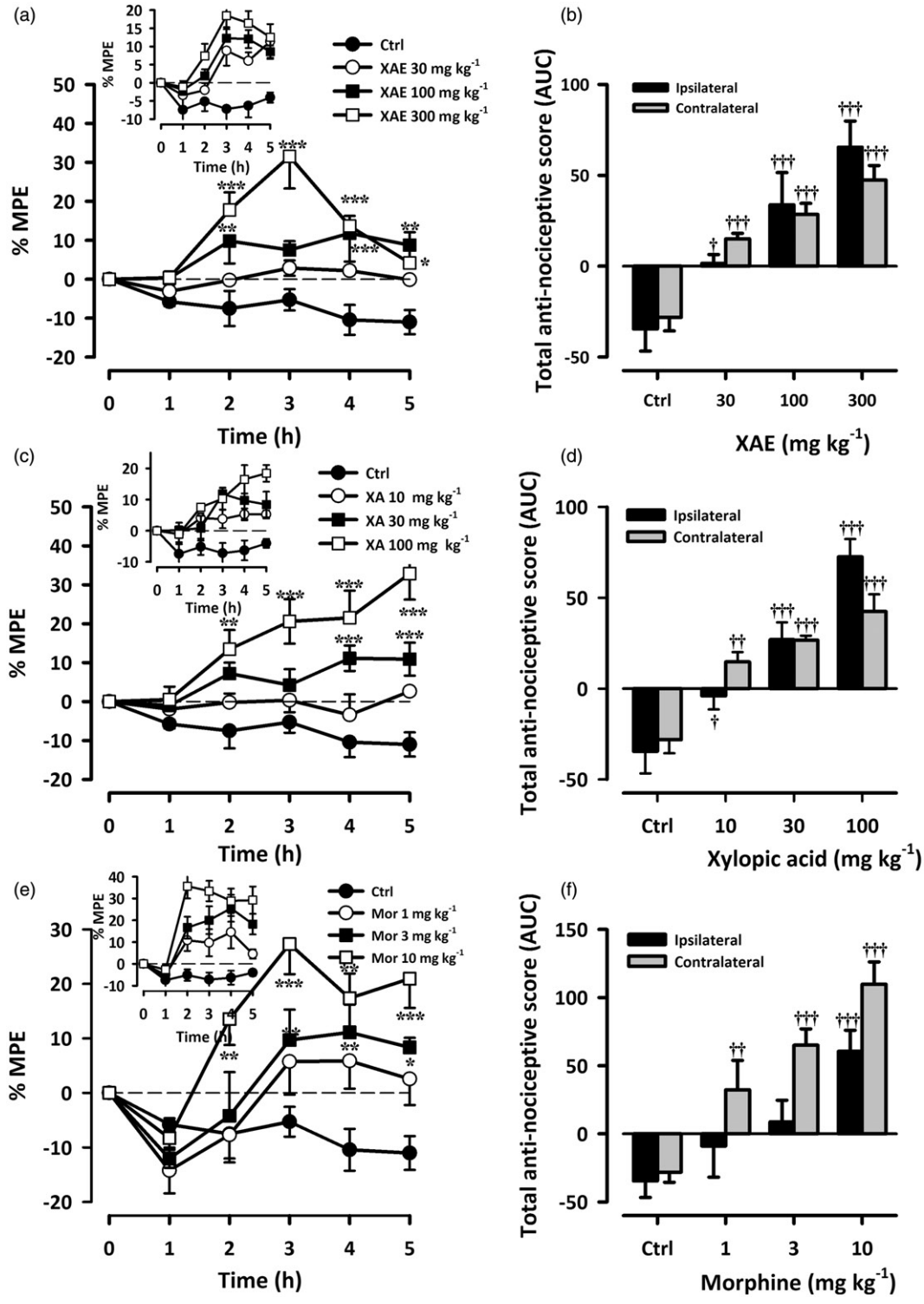

Figure 5. Effect of XAE (30–300 mg/kg *p.o.*), XA (10–100 mg/kg *p.o*) and morphine (1–10 mg/kg i.p.) on the time course curve of ipsilateral and contralateral paws (*insets*) withdrawal latency using the Randall–Sellito test (a, c and e) and the AUC (b, d and f) in chronic muscle pain. Data are presented as mean ± S.E.M. ($n = 8$); ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$ 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).

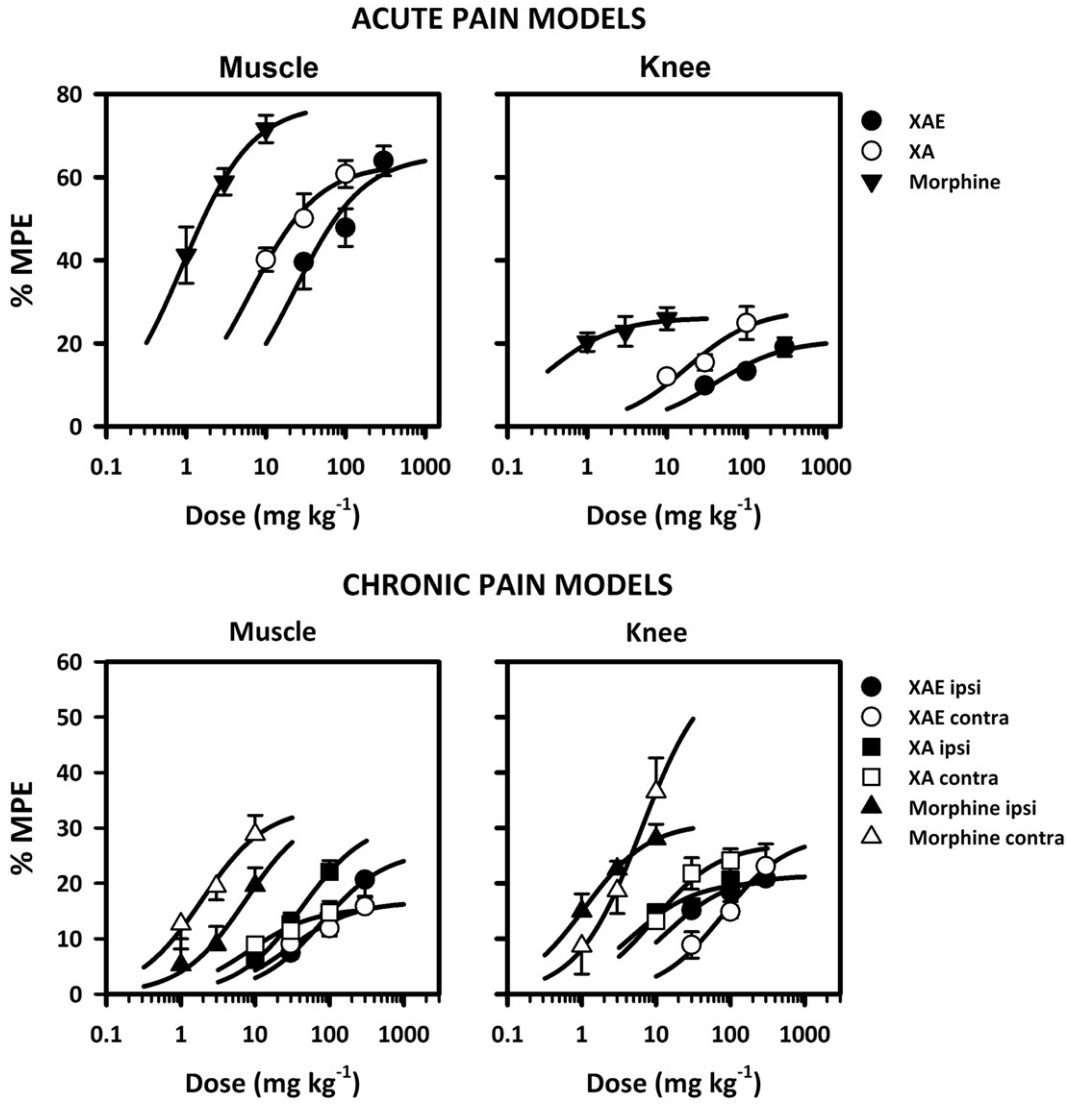

Figure 6. Dose-response curves of the effects of the extract, xylopic acid and morphine in the models used. Percentage MPEs were derived from the AUCs and the curves obtained by non-linear regression as described under *Materials and methods*.

Conclusion

This study has provided further scientific evidence on the antinociceptive properties of XAE and XA. More importantly, XAE and XA were effective in both acute and chronic musculoskeletal pain.

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Disclosure statement

The authors report no declarations of interest.

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