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Ethanolic Root Extract of *Jatropha curcas* L. Relieves Muscle Pain in Rats

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Abstract

The roots of Jatropha curcas are used traditionally to treat pain. The present study evaluated the analgesic property of ethanolic extract of Jatropha curcas (JAT) in acute and chronic muscle pain models. Acute muscle hyperalgesia was induced by injecting 3% carrageenan into the gastrocnemius muscles of rats. Twelve hours after the injection, baseline muscle hyperalgesia was measured and the rats were then treated orally with 30, 100 and 300 mg/kg/day JAT; intraperitoneally with 1, 3 and 10 mg/kg/day morphine and normal saline. A change in pain threshold was assessed with the grip strength analgesimeter. The effect of JAT on chronic muscle hyperalgesia was evaluated by injecting 3% carrageenan into the gastrocnemius muscles of the separate groups of rats. Two weeks later, baseline pain thresholds were measured and rats were treated with 30, 100 and 300 mg/kg/day JAT; 1, 3 and 10 mg/kg/day morphine and normal saline. Pain threshold was assessed again by measuring the latency to paw withdrawal in the ipsilateral and contralateral paws. JAT and morphine increased the grip strength in the acute muscle hyperalgesia test. Chronic muscle hyperalgesia was also significantly reduced by JAT and morphine. JAT relieves muscle pain in rats.

Keywords: *hyperalgesia, muscle, carrageenan, kaolin*

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INTRODUCTION

Jatropha curcas (Euphorbiaceae) commonly referred to as the physic nut is a pest and drought-resistant monoecious shrub that produces seeds containing about 34.4% biofuel [1]. The roots are used as antimicrobials (active against pneumonia, gonorrhoea and syphilis), antitumour agent and products for the treatment of skin diseases [2]. Decoction of the roots is employed as a mouthwash for bleeding gums, toothache, eczema, ringworm, scabies and dysentery [3, 4]. It is also reported that the methanol root extract exhibit anti-diarrhoeal activity in mice by inhibiting prostaglandin biosynthesis and has the ability to reduce osmotic pressure [5]. Mujumdar and Misar [6], reported the anti-inflammatory properties of the roots of *Jatropha curcas* in mice and rats. Again alcoholic extracts of the leaves, stem bark and roots have been shown to demonstrate antinociceptive properties in the tail flick model [7]. Since the mechanisms of pain and hyperalgesia induced by heat (tail flick) and by injecting inflammatory agents into tissues like muscle, and joints (musculoskeletal pain) represent different pathophysiological processes [8]. We evaluated the antinociceptive properties of the roots of *Jatropha curcas* in muscle pain models in rats. Musculoskeletal pain disorders such as arthritis and fibromyalgia are difficult to treat

clinically and therefore constitute a major global clinical problem [9]. It is of paramount interest to scientist to find alternate sources of drugs for musculoskeletal pain treatment to augment the already existing pharmacotherapies hence the screening of *Jatrophs curcas* for its analgesic effect on muscular pain.

MATERIALS AND METHODS

Sprague-Dawley rats (200–250 g) of both sexes were obtained from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana and housed in groups of five in stainless steel cages with soft wood shavings as bedding. The animals were fed with normal commercial pellet diet (AGRICARE, Kumasi), given water ad libitum and maintained under optimum laboratory conditions. All procedures and techniques used in the studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [10]. All protocols used were approved by the Departmental Ethics Committee of the Department of Biomedical and Forensic Sciences, University of Cape Coast.

Drugs and Chemicals

Carrageenan sulphate was obtained from Sigma–Aldrich Inc., St. Louis, MO, USA and morphine hydrochloride was obtained from Phyto-Riker, Accra, Ghana.

Preparation of Ethanolic Root Extract of *Jatropha curcas*

Roots of *Jatropha curcas* were harvested from Kete-Krachi in the Volta region of Ghana and identified by a botanist in the School of Biological Sciences. A voucher specimen has been deposited in the herbarium of the School of Biological Sciences. The roots were dried under a shade for 14 days and pulverized into fine powder.

A quantity of 0.2 kg of the powdered material was macerated with 70% v/v ethanol for five days in cylindrical jars. The filtrate was concentrated with a rotary evaporator at a temperature of 50°C. The concentrate was dried over hot water bath to yield a solid mass of ethanol extract of roots of *Jatropha curcas* (percentage yield of 39.5% w/w) [14].

Effect of *Jatropha curcas* on Acute Muscle Pain

To determine the analgesic properties of ethanolic root extract of *Jatropha curcas* on acute muscle hyperalgesia, rats were grouped into seven with five rats per group. Acute muscle hyperalgesia was induced in these rats by injecting 100 µl of 3% carrageenan percutaneously into their left gastrocnemius muscles. Acute muscle hyperalgesia was confirmed in all the rats 12 h after the injection of the carrageenan. This was done using the grip force analyser as described elsewhere [11, 12]. Briefly, the magnitude of tensile force each rat exerted against a wire mesh grid with its hind paws when pulled gently in the caudal direction was measured. This was repeated three times for each rat at each time point and averaged to represent each rat's grip force for each time point. Following the acute muscle hyperalgesia confirmation, group 1, 2 and 3 rats were, respectively, treated orally with 30, 100 and 300 mg/kg daily with JAT. Group 4, 5 and 6 animals were also treated intraperitoneally with 1, 3 and 10 mg/kg morphine, respectively (positive control groups). Group 7 animals received normal saline (control group). Pain thresholds were measured again hourly for 3 h with the grip force analyser to determine any change in pain threshold resulting from the treatment of JAT and morphine.

Effect of *Jatropha curcas* on Chronic Muscle Pain

In a separate experiment to determine the analgesic effect of JAT on chronic muscle pain, animals were grouped into seven (group 8–14) with five animals in a group. The animals were injected with 100 µl of 3% carrageenan percutaneously into the left gastrocnemius muscles. The inflammation induced by the carrageenan was allowed to progress for 14 days to allow the development of chronic pain [13]. Chronic hyperalgesia was confirmed in all the rats by measuring the paw withdrawal latency for both paws of the animals in the Randall-Selitto test. Briefly, an analgesimeter (IITC Life Science Inc. Model 2888, Woodland Hills, CA, USA) was used to apply a linearly increasing pressure on the paw of each rat until it vocalized or forcefully withdrew its limbs [14]. The weight that produced the limb withdrawal or vocalization

was then recorded. A cut-off weight of 250 g was used to avoid tissue damage. After chronic muscle hyperalgesia has been confirmed, group 8, 9 and 10 rats were treated orally with 30, 100 and 300 mg/kg daily with JAT, respectively. Group 11, 12 and 13 animals were also treated intraperitoneally with 1, 3 and 10 mg/kg morphine, respectively. Group 14 animals received normal saline. Paw withdrawal latencies were again measured hourly for 3 h after the various drug treatments to determine the effects of the various treatments on pain threshold.

Statistical Analysis

All data are presented as mean ± S.E.M ($n = 5$). Raw data were 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 Tukey's post *hoc* test. Total nociceptive score for each treatment was calculated in arbitrary unit as the area under the curve (AUC). A p value of <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Effects of JAT on Acute Muscle Hyperalgesia

Acute muscle hyperalgesia was measured in the grip strength test 12 h after induction. JAT (30–300 mg/kg) significantly and dose-dependently (Figure 1b) inhibited the time-course of acute muscle hyperalgesia development ($F3,80 = 38.19$, $p < 0.0001$: Figure 1a) in the ipsilateral limb compared to normal saline treated group. Morphine (1–10 mg/kg) used as positive control also inhibited acute muscle hyperalgesia over the three hours of pain measurement ($F3,80 = 15.68$, $p < 0.0001$; Figure 1c). The middle and highest doses produced significant effect compared to the normal saline group (Figure 1d).

Figure 1: The analgesic effects of ethanolic extract of *Jatropha curcas* and morphine on acute muscle hyperalgesia.

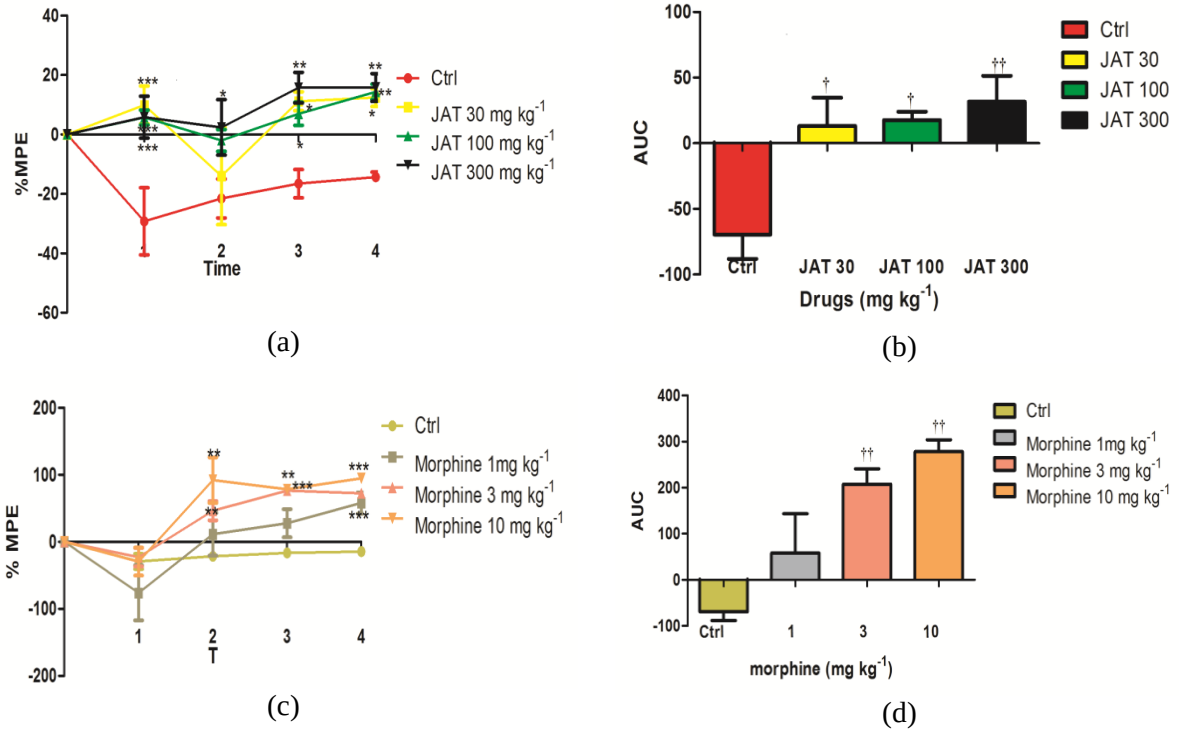


Fig. 1: *Effect of (a) JAT (30–300 mg kg-1 p.o.) and (c) Morphine (1–10 mg kg-1 i.p.) on the Time Course Curve and the AUC (b and d, Respectively) in Acute Muscle Pain. Data are Presented as Mean ± S.E.M. ***p < 0.001; ** p < 0.01; * p < 0.05, Compared to Vehicle-Treated Group (Ctrl) (Two-way ANOVA Followed by Bonferroni's Post hoc test). †† p < 0.01, † p < 0.001 Compared to Vehicle-Treated Group (One-way ANOVA Followed by Tukey's Post hoc test).*

Effect of JAT on Chronic Muscle Hyperalgesia

Chronic muscle hyperalgesia was measured from both the ipsilateral and contralateral paws fourteen days after pain induction. Chronic muscle hyperalgesia was significantly and dose-dependently attenuated by JAT (30–300 mg/kg) (ipsi: $F3,80 = 12.79$ $p < 0.0001$, Figure 2a and contra: $F3,80 = 14.66$, $p < 0.0001$, Figure 2c) in the ipsilateral and contralateral paws compared to normal saline treated groups. Significant analgesic effects were observed at the highest dose administered in both the ipsilateral (Figure 2b) and contralateral (Figure 2d) paws compared to normal saline treated groups.

Morphine blocked chronic muscle hyperalgesia in both paws of the animals (ipsi: $F3,80 = 78.81$, $p < 0.0001$, Figure 3a and contra: $F3,28 = 68.42$, $p < 0.0001$, Figure 3c): the maximum effect was achieved at the highest dose administered in both ipsilateral (Figure 3b) and contralateral paws (Figure 3d).

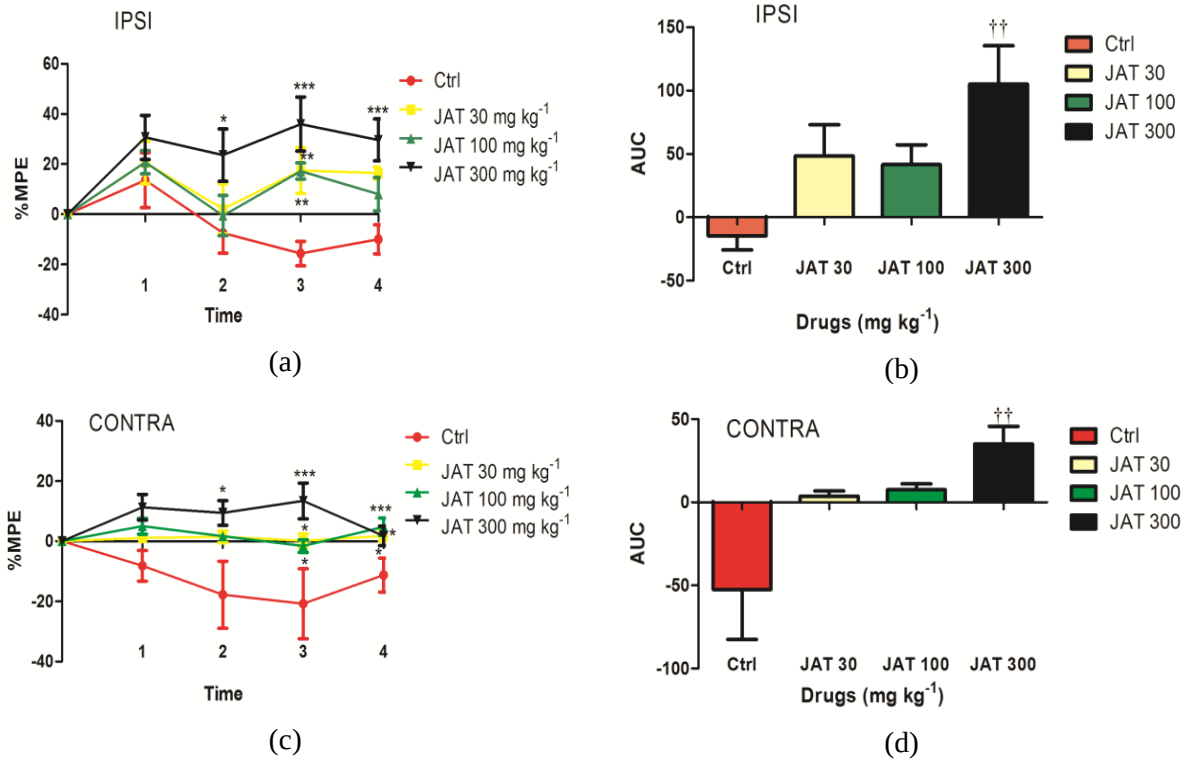
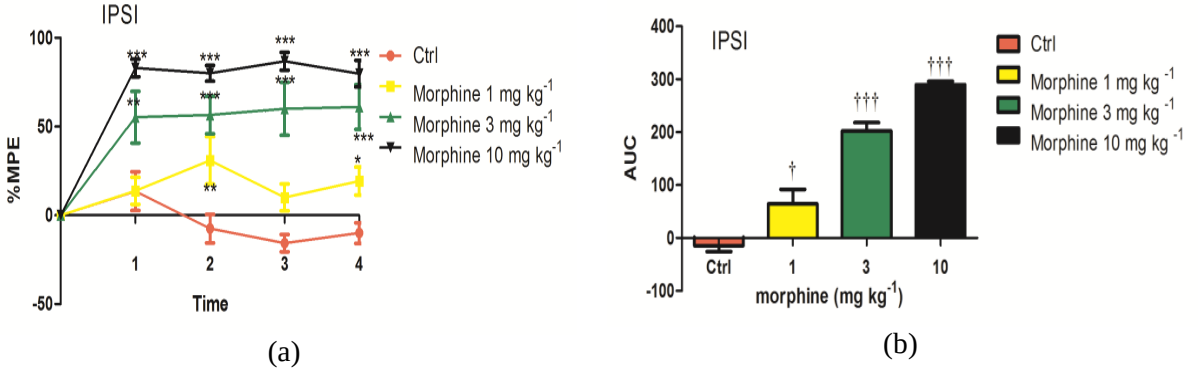
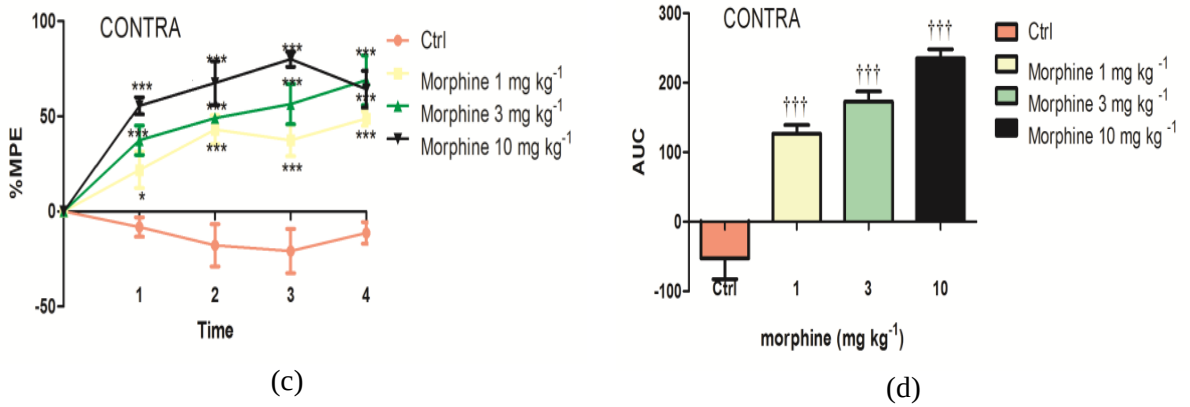


Fig. 2: *Effect of JAT (30–300 mg kg-1 p.o.) on the Time Course Curve of (a) Ipsilateral (Ipsi) and (c) Contralateral (Contra) Paw Withdrawal Latency in the Chronic Muscle Hyperalgesia and the AUC of (b) Ipsilateral and (d) Contralateral Paws. Data are Presented as Mean ± S.E.M. (n = 5); ***p < 0.001; ** p < 0.01; *p < 0.05 Compared to Vehicle-Treated Group (Ctrl) (Two-way ANOVA Followed by Bonferroni's Post hoc test). †† p < 0.001, Compared to Vehicle-Treated Group (One-way ANOVA Followed by Tukey's Post hoc test).*





(c) (d)

Fig. 3: *Effect of Morphine (1–10 mg kg-1) on the Time Course Curve of (a) Ipsilateral and (c) Contralateral Paws Withdrawal Latency and the Respective AUC (b and d) in Chronic Muscle Pain. Data are Presented as Mean ± S.E.M.; ***p < 0.001; ** p < 0.01; *p < 0.05 Compared to Vehicle-Treated Group (Ctrl) (Two-Way ANOVA Followed by Bonferroni's Post hoc test). †††p < 0.001, †p < 0.05, Compared to Vehicle-Treated Group (One-Way ANOVA Followed by Tukey's Post hoc test).*

DISCUSSION

Ethanolic root extract of *Jatropha curcas* exhibited antinociceptive properties in acute and chronic muscle pain models in rats. The muscle pain models in rats employed investigated fibromyalgia type of pain experienced in man [12]. Ethanolic root extract of *Jatropha curcas* may therefore be used to manage such types of pain. Morphine, the standard drug produced similar effects. The acute muscle hyperalgesia was caused by inflammation and myonecrosis largely mediated by neutrophils [13]. JAT may have inhibited acute muscle hyperalgesia by blocking the neuronal activation and sensitization of the produced inflammatory pain mediators such neutrophils.

The chronic muscle hyperalgesia was due to epimysial and perimysial chronic inflammation in the ipsilateral limbs characterized by the presence of macrophages and few scattered mast cells. In addition to these immune cells, inflammatory pain mediators namely glutamate, aspartate, prostaglandin-E_2 (PGE2) and citrulline are also involved in the chronic muscle hyperalgesia [14, 15]. Prostaglandin- E_2 plays an important role in the development and maintenance of chronic muscle pain. The produced PGE2 in the spinal cord together with increased sensitivity of peripheral nociceptors as a result of the injected carrageenan causes central sensitization spinally and/or supraspinally by activating the neurons of the dorsal horn [16].

This manifests behaviourally in the rats as secondary hyperalgesia in the areas adjacent to the injury and in distal locations [17].The referred/secondary hyperalgesia observed in the contralateral limbs is therefore due to sensitization of neurons of spinal and supraspinal locations. JAT and morphine blocked chronic muscle hyperalgesia in rats. This signifies the possible antagonistic effect of JAT on inflammatory pain mediators like glutamate, aspartate, prostaglandin-E_2 (PGE2) and citrulline and activation of central processes that act to down regulate pain.

Opioid agonists such as morphine relieve musculoskeletal hyperalgesia by agonistic effect on opioid receptors located spinally and supraspinally in the rostral ventrolateral medulla and periaqueductal grey [18, 19].

Similar to the chronic muscle hyperalgesia, the chronic skeletal hyperalgesia involved supraspinal activation of descending facilitatiory pain pathway [20]. This may suggest a central anti-hyperalgesic mechanism of JAT on the descending modulatory pain pathway. In conclusion, ethanolic root extract of *Jatropha curcas* relieves acute and chronic muscle pain in rats.

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REFERENCES

1. Achten W, Verchot L, Franken Y, *et al*. *Biomass Bioenergy* 2008; 32: 1063–84p.
2. Henning R. *IK Notes* 2002; 47: 1–4p http://www.worldbank.org/afr/ik/iknt47.pdf.
3. Duke J, Ayensu E. MI: Reference Publications, Inc. Algonac; 1985.
4. Openshaw K. *Biomass Bioenergy* 2000; 19: 1–15p.
5. Mujumdar A, Misar A, Salaskar M, Upadhye A. *J. Nat. Remed*. 2001; 1: 89–93p.
6. Mujumdar A, Misar A. *J. Ethnopharm.* 2004; 90: 11–15p.
7. Bhavesh SN, Krishnakumar NP. *Malaysian J. Pharm. Sci*. 2010; 8: 23–8p.
8. Schaible H, Schmidt R, Willis W. *Exp. Brain Res.* 1987; 66: 489–99p.
9. Afable R, Ettinger JW. *Drugs Aging* 1993; 6: 49–59p.
10. Institute of Laboratory Animal Research C.O.L.S., National Research Council. *Guide for the Care and Use of Laboratory Animals*. 7th edition, Washington, D.C.: The National Academies Press.; 1996. ISBN: 1996; 9780309588690.
11. Kehl LJ, Hamamoto DT, Wacnik PW, *et al*. *Pain* 2003; 103: 175–86p.
12. Skyba DA, Radhakrishnan R, Sluka KA. *J. Pain* 2005; 6: 41–7p.
13. Radhakrishnan R, Moore SA, Sluka KA. *Pain* 2003; 104: 567–77p.
14. Woode E, Ameyaw EO, Boakye-Gyasi E, *et al*. *J. Pharm. Bioallied Sci.* 2012; 4: 291–301p.
15. Yang LC, Marsala M, Yaksh TL. *Pain* 1996; 67: 345–54p.
16. Hoheisel U, Mense S, Simons DG, *et al*. *Neurosci. Lett.* 1993; 153: 9–12p.
17. Sluka K. *J. Neurosci.* 2002; 22: 5687–93p.
18. Fields H, Basbaum A. In: Melzack R, Wall PD, editors. *Textbook of Pain*. London: Churchill Livingstone; 1999. 308–38p.
19. DeSantana J, da Silva L, Sluka K. *Pain* 2009; 148: 84–93p.