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ORIGINAL ARTICLE

Effect of Xylopic Acid on Paclitaxel-induced Neuropathic pain in rats

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Xylopic acid, a diterpenoid isolated from the fruits of *Xylopia aethiopica* has demonstrated analgesic properties in acute pain models. It was therefore evaluated for its analgesic properties in paclitaxel-induced neuropathic pain, a type of pain difficult to treat clinically. Neuropathic pain was induced in rats by injecting 2 mg kg^{-1} of paclitaxel on alternative days for four days (days 0, 2, 4 and 6). Paclitaxel-induced cold allodynia, mechanical hyperalgesia and thermal hyperalgesia were measured during pre-paclitaxel administration and on day 16 post-paclitaxel administration. The rats were treated with xylopic acid (10, 30 and 100 mg kg^{-1}; groups 1-3), pregabalin (10, 30 and 100 mg kg^{-1}; groups 4-6) and vehicle (group 7) daily for 5 days. Pain thresholds were also measured daily for 5 days in the three models. Xylopic acid and pregabalin produced analgesic properties seen as increased paw withdrawal latencies to mechanical and cold water stimuli during the five days treatment. In addition, the two agents significantly ($P<0.05$) exhibited analgesic properties in the thermal hyperalgesia test. These data suggest that xylopic acid is an effective agent against paclitaxel-induced neuropathic pain.
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Keywords*:* Xylopic acid, paclitaxel, neuropathic, pregabalin, hyperalgesia, cold allodynia

INTRODUCTION

It is estimated that, more than half of patients with cancer are treated with chemotherapeutic agents such as taxanes (paclitaxel), platinum-based compounds and vinca alkaloids and about 40% of such patients are prone to neuropathic pain (Deng *et al.,* 2012). The incidence and severity of paclitaxel-induced neuropathic pain symptoms correlates with increasing cumulative doses of paclitaxel (Akerley *et al.,* 1998; Postma *et al.,* 1995). Paclitaxel, an anti-cancer drug was originally derived from the bark of the Western yew tree, *Taxus brevifolia*. It is used to treat several tumours including ovarian, breast and lung cancers. The antineoplastic activity of paclitaxel is thought to involve disruption of microtubule assembly; an important cellular component responsible for development and maintenance of neurons, mediation of axonal transport in the neurons and provision of structural support for neurons (Bray *et al.,* 1988; Kobayashi and Mundel, 1998). The most common clinical neurotoxicity associated with the use of paclitaxel is sensory peripheral neuropathy which is often dose-related and may begin as early as 24-72 hours after administration of high single dose of paclitaxel (Rowinsky *et al.,* 1993). Patients describe various sensory symptoms including mechanical allodynia, spontaneous pain, cold allodynia, numbness and tingling (Rowinsky *et al.,* 1993; Forsyth *et al.,* 1997; Dougherty *et al.,* 2004).

In an attempt to solve this enigma, several plants and isolated compounds from them have been tested against paclitaxel-induced neuropathic pain. Eth-

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anolic fruit extract of *Xylopia aethiopica* has been shown to possess anti-tumour properties (Adaramoye *et al.,* 2011). Xylopic acid, a major diterpene isolated from the fruits of *X. aethiopica* is devoid of anticancer properties but has shown antinociceptive properties in several animal models of pain (Cavalcanti *et al.,* 2009; Woode *et al.,* 2012). It is against this backdrop that the analgesic property of xylopic acid was evaluated in paclitaxel-induced neuropathic pain in rats.

MATERIALS AND METHODS

Experimental animals and housing

Sprague-Dawley rats (200–250 g) of both sexes were housed in stainless steel cages (n=5) for a week in the laboratory to acclimatize with the environment. The animals were fed with normal commercial pellet diet (AGRICCARE, Kumasi) and water *ad libitum* and kept under standard laboratory conditions. All experiments were performed during the day between the hours of 8:00 –15:00.

The 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 (NIH Publication No. 85 -23, 1985, revised 1996). All protocols used were approved by the Departmental Ethics Committee.

Drugs and Chemicals

Pregabalin (Lyrica®) was purchased from Pfizer Pharmaceuticals (Arzneimittelwerk Godecke, Freiburg, Germany), cremophor from Sigma-Aldrich Inc. (St. Louis, MO, USA) and paclitaxel (Intaxel®) from Fresinius Kabi Oncology (Badi, India).

Extraction and purification of xylopic acid ((15-β -Acetoxy-(-)-kaur-16-en-19-oic Acid)

Xylopic acid was extracted according to the process described by Woode *et al.,* (2012). Briefly, 360 mg of the fruit of *Xylopia aethiopica* was macerated with 5 L of petroleum ether (40–60 °C) and allowed to stand for three days. The petroleum ether was drained and concentrated with rotary evaporator at a temperature of 50°C. To facilitate crystallization of xylopic acid, ethyl acetate was added to the concentrate. Crystals (xylopic acid) formed after three days were washed with petroleum ether at 40–60°C. Crude xylopic acid was purified in 96% ethanol. The yield of the xylopic acid was 1.41%. The purity of the isolated xylopic acid was 95% with high performance liquid chromatography.

Paclitaxel Administration

Rats were allowed to acclimatize to the behavioural testing environment and baseline measurements of mechanical, thermal and cold stimuli were performed. Neuropathic pain was induced in the rats by intraperitoneal (i.p.) injection of paclitaxel (2 mg kg^{-1}) dissolved in saline on four alternate days (days 0, 2, 4 and 6) as described by Ameyaw *et al.,* (2013); Flatters and Bennett, (2004). On day 16 post-paclitaxel treatments, xylopic acid (10, 30 and 100 mg kg^{-1} dissolved in cremophor; groups 1-3), pregabalin (10, 30 and 100 mg kg^{-1}; groups 4-6) and cremophore solution (group 7) were administered to the rats after confirmation of neuropathic pain in the various tests. The effect of xylopic acid, pregabalin and cremophor treatments on paclitaxel-induced neuropathic pain were evaluated in the Randall-Sellito paw pressure-, thermal tail immersion- and cold- allodynia tests.

Behavioural assessment of neuropathic pain

Mechanical hypersensitivity

The effect of xylopic acid (10-100 mg kg^{-1}), pregabalin (10-100 mg kg^{-1}) and cremophor solution on mechanical hyperalgesia was measured with the Randall-Selitto paw pressure analgesimeter (IITC Life Science Model 2888 Woodland Hills, CA, USA) as previously described by Woode *et al.,* (2012). The rat's hind paw was placed into a pressure applicator, and a steadily increasing pressure stimulus (maximum cut-off of 250 g) was applied to the dorsal surface of the paw until withdrawal or vocalization. This was recorded as the nociceptive threshold value. For each animal, two recordings were made for each hind paw, and the data were reported as the mean of both hind paw values.

Thermal Hyperalgesia

The tail immersion test was used to determine the effect of xylopic acid (10-100 mg kg^{-1}), pregabalin (10-100 mg kg^{-1}) and cremophore solution on ther-

mal hyperalgesia (Thirumal *et al.,* 2013). The distal portion of the tail (3 - 4 cm) of the rat was immersed in hot water maintained at 52°C temperature until the tail was withdrawn. The duration of immersion was recorded and a cut-off time of 10 s was used.

Cold allodynia

The analgesic effect of xylopic acid (10-100 mg kg^{-1}), pregabalin (10-100 mg kg^{-1}) and cremophor solution on cold allodynia was assessed by immersing the rat's hind paw into cold water (4.5°C). The latency for a rat to withdraw its paw was measured with a digital timer as described by Kim *et al.,* (2005). Only one hind paw was assessed during each immersion at a time with a cut-off time of 20 s. For each animal, two recordings were made for each hind paw, and the withdrawal responses were reported as the mean of both hind paw values.

Statistical analysis

Data were analyzed with GraphPad Prism Version 5 (GraphPad Software, San Diego, CA, USA). The results are presented as mean ± S.E.M. The time-course curves were subjected to two-way (treatment × time) repeated measures of analysis of variance (ANOVA) with Bonferronis's *post hoc* 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) 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}) of the curves were compared statistically using F-test (Miller, 2003; Motulsky and Christopoulos, 2003). ED_{50} determinations were also done with GraphPad Prism Version 5. For all comparisons, a $P < 0.05$ was considered statistically significant.

RESULTS

Injection of a cumulative dose of 8 mg kg^{-1} of paclitaxel into rats produced neuropathic pain that lasted weeks after the injection. Neuropathic pain was confirmed in the mechanical hyperalgesia, cold allodynia and thermal hyperalgesia models on the 16th day post paclitaxel injection. Xylopic acid (10-100 mg kg^{-1}) produced significant ($P<0.0001$) analgesic properties in the Randall-Sellito test (Figure 1A). Treatment of rats with 100 mg kg^{-1} xylopic acid reversed the mechanical hyperalgesia significantly from day two to five. The 10 and 30 mg kg^{-1} xylopic acid treatments significantly reversed the mechanical hyperalgesia except for day two. Pregabalin (10-100 mg kg^{-1}) similarly produced analgesic properties in this model (Figure 1B). The potency of xylopic acid in this model was 2.54 times the potency of pregabalin (Table 1).

A sustained thermal hyperalgesia was observed in the control rats but not animals treated with xylopic acid and pregabalin. Xylopic acid at doses of 100 and 30 mg kg^{-1} reduced significantly the thermal hyperalgesia during the five days daily treatments (Figure 2A). On the contrary, the lowest dose of xylopic acid did not produce any significant thermal

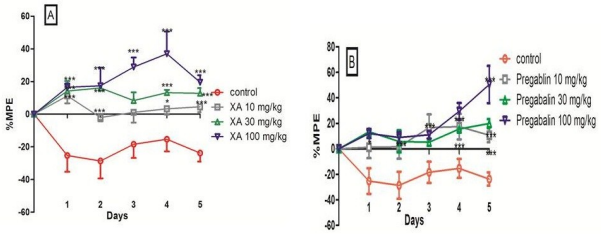

Figure 1: The time-course curve of the effects of daily dosing of xylopic acid (10-100 mg/kg), pregabalin (10-100 mg/kg) and cremophor solution (control) on established paclitaxel-induced mechanical hyperalgesia. Graph A shows the effect of daily systemic administration of 10-100 mg/kg xylopic acid or vehicle and B shows daily systemic administration of 10-100 mg/kg pregabalin or vehicle for five days. Each point represents Mean ± S.E.M (n = 5); *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, compared to respective controls (Two-way repeated measures ANOVA followed by Bonferroni's post hoc).

hyperalgesia at any time point. Pregabalin, similar to xylopic acid produced significant ($P<0.0001$) reversal of thermal hyperalgesia (Figure 2B). Xylopic acid was 4.3 times more potent than pregabalin (Table 1) in the thermal hyperalgesia test.

The latency to paw withdrawal to cold stimulus was significantly prolonged after treating the animals with xylopic acid (10-100 mg kg^{-1}; Figure 3A) and pregabalin (10-100 mg kg^{-1}; Figure 3B) compared to vehicle treated animals. The analgesic effect of pregabalin was significant at all the time points and dose-dependent. In this model, xylopic acid was 2.4 times potent than pregabalin (Table 1).

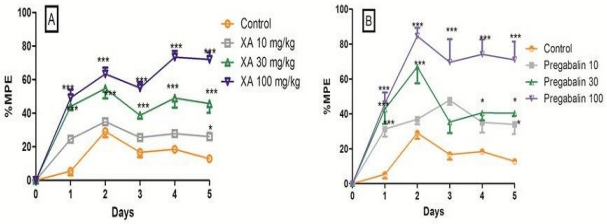

Figure 2: The time-course curve of the effects of daily dosing of xylopic acid (10-100 mg kg^{-1}), pregabalin (10-100 mg kg^{-1}) and cremophor solution (control) on established paclitaxel-induced thermal hyperalgesia. Graph A shows the effect of daily systemic administration of 10-100 mg kg^{-1} xylopic acid or vehicle and B shows daily systemic administration of 10-100 mg kg^{-1} pregabalin or vehicle for five days. Each point represents Mean ± S.E.M (n = 5); *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, compared to respective controls (Two-way repeated measures ANOVA followed by Bonferroni's post hoc).

Table 1: Respective ED_{50} (mg kg^{-1} ± S.E.M.) for xylopic acid and pregabalin in cold allodynia, mechanical and thermal hyperalgesia tests

Test	Pregabalin	Xylopic acid
Mechanical hyperalgesia (Randall-Sellito test)	7.18±0.51	18.21±0.38
Thermal hyperalgesia (Tail immersion test)	4.32±0.96	16.09±0.95
Cold allodynia	8.1±0.99	19.33±0.85

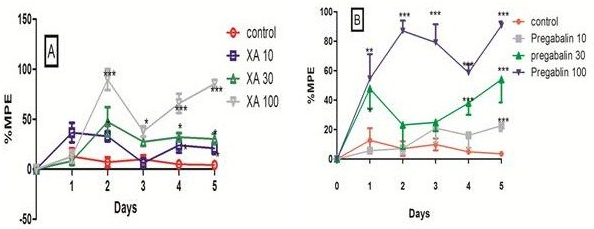

Figure 3: The time-course curve of the effects of daily dosing of xylopic acid (10-100 mg kg^{-1}), pregabalin (10-100 mg kg^{-1}) and cremophor solution (control) on established paclitaxel-induced cold allodynia. Graph A shows the effect of daily systemic administration of 10-100 mg kg^{-1} xylopic acid or vehicle and B shows daily systemic administration of 10-100 mg kg^{-1} pregabalin or vehicle for five days. Each point represents Mean ± S.E.M (n = 5); *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, compared to respective controls (Two-way repeated measures ANOVA followed by Bonferroni's post hoc).

DISCUSSION

Neuropathic pain induced with a cumulative dose of 8 mg kg^{-1} paclitaxel administered in four injections resulted in significant cold allodynia, mechanical and thermal hyperalgesia. Xylopic acid and pregabalin, the standard drug, inhibited the hyperalgesia associated with thermal and mechanical stimulation as well as the cold allodynia associated with cold water stimulus. Pharmakokinetically, paclitaxel formulated as Cremophor-ethanol (Taxol) preparation distributes in the central and peripheral nervous system in rats following its administration (Cavaletti *et al.,* 2000). Paclitaxel accumulates in the dorsal root ganglia and the brain at very low concentrations. Accumulation has been reported in the sciatic nerve and spinal cord at intermediate concentrations (Cavaletti *et al.,* 2000).

The neuropathy in this study after low dose paclitaxel administration was due to atypical (swollen and vacuolated) mitochondria in peripheral sensory axons—both C-fibrer and myelinated axons and a loss of intra-epidermal nerve fibres (Fidanboylu *et al.,* 2011). Allodynia caused by paclitaxel neurotoxicity is as a result of apoptosis in the dorsal root ganglion neurons (Seong *et al.,*

2013). Paclitaxel induces morphological changes (swollen and vacuolated mitochondria) and dysfunction (reduced respiration and energy production) of mitochondria in axons, which then alters intracellular calcium levels and initiates apoptotic pathways (Flatters *et al.,* 2006; Melli *et al.,* 2008; Xiao *et al.,* 2011; Zheng *et al.,* 2011). The exact mechanism of xylopic acid in this model cannot be pointed out but it is likely that as a calcium channel antagonist (Somova *et al.,* 2001), it inhibited calcium channels to stabilize the nerve membrane. Pregabalin is effective both experimentally and clinically in the management of neuropathic pain. Its action is as a result of antagonist effect on $\alpha^{2\text{-}\delta 1}$ Ca^{2+} channel subunit of N-type voltage dependent calcium channels. Inhibition of calcium channels prevent neuronal excitability and other cellular enzymatic cascade reactions that lead to pain sensation (Schim, 2009; Kumar *et al.,* 2010).

The effect of xylopic acid on pro-inflammatory pain mediators and cytokines cannot be ruled out. Several reports indicate that paclitaxel evokes pro-inflammatory pain mediators and cytokines, including bradykinin and TNF-α as well as the activation of microglial and astroglial cells (Costa *et al.,* 2011; Burkhart *et al.,* 1994; Manthey *et al.,* 1992; Zhang *et al.,* 2012; Burgos *et al.,* 2012). It has been reported that xylopic acid inhibits the nociceptive effects of bradykinin and glutamate (Woode *et al.,* 2013). The blocking of the effects of these pain mediators may contribute to the observed analgesic properties in the mechanical and thermal hyperalgesia as well as cold allodynia tests. Glutamatergic neurotransmission and N-methyl-D-aspartate (NMDA) receptors are involved in paclitaxel-induced neuropathic pain (Jaggi *et al.,* 2012). Peripheral nerve damage results in glutamate/NMDA receptor-mediated sensitization and spontaneous activity of primary afferents, and causes hyper-excitability of dorsal horn neurons and down-regulation of glial glutamate transporters (i.e. GLAST and GLT-1) in the spinal dorsal horn (Petrenko *et al.,* 2003; Zhang *et al.,* 2012).

In addition, xylopic acid suppresses pain via the opioidergic nociceptive pathway (Woode *et al.,* 2013) and this may partly contribute to the analgesic properties of xylopic acid in this model. Agents such as morphine that blocks the opioidergic nociceptive pathway have been shown to inhibit paclitaxel-induced neuropathic pain (Ami *et al.,* 2012).

CONCLUSIONS

The data presented indicate that xylopic acid exerts analgesic properties in paclitaxel-induced neuropathic pain in rats and may be useful in managing neuropathic pain associated with chemotherapy in man.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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