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CASE REPORT

Intestinal Obstruction Secondary to an Intra-Abdominal Foreign Body

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According to literature, the incidence of intestinal obstruction caused by internal abdominal hernia is very rare and has an occurrence rate of about 0.2-0.9%. Internal hernias are caused by defects that occur congenitally or as a result of surgery or trauma. It is still rarer for surgical instruments inadvertently left in the abdominal cavity after laparotomy to be the cause of internal herniation resulting in intestinal obstruction. A case of intestinal obstruction caused by an artery forceps left in the abdominal cavity after surgery is presented.
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Keywords*:* Internal hernia, foreign body, artery forceps, adhesions, intestinal obstruction

INTRODUCTION

From literature, it is known that intestinal obstruction caused by internal abdominal hernias is rare, with incidence rates of 0.2 – 0.9% at autopsy (Ghahremani, 1984) out of which about 0.5 – 5.8% are due to internal herniation (Pershad *et al.*, 1998). Internal abdominal hernias arise as a result of congenital defects due to anomalies of mesenteric fixation and intestinal rotation during foetal development or as a result of surgery or trauma (Yagmik *et al.*, 2009; Mathieu and Lucian, 2004). It is uncommon to have foreign bodies (e.g. surgical instruments) left behind in the abdominal cavity following laparotomy causing herniation and intestinal obstruction (Pershad *et al.*, 1998). Foreign materials, including surgical instruments and sponges left in the peritoneal cavity after laparotomy, are potentially dangerous medical errors (Lincourt, 2007). Retention of surgical instruments and materials in the abdominal cavity is uncommon because it is underreported and can carry serious medico-legal consequences (Karahasanoglu *et al.*, 2004; Berkowitz *et al.*, 2007; Ugochukwu and Edeh, 2011). Foreign bodies inadvertently retained in the abdominal cavity range from small gauzes and sponges (referred to as gossypiboma) to artery and tissue forceps, scissors, retractors, needles, spatulas and others (AORN, 2006; Wan *et al.*, 2009; Gibbs, 2011). Adhesions forming around these foreign bodies (gauzes and sponges) usually lead to intestinal obstruction (Lauwers and Hee, 2000). In the case of instruments, they are usually inert and can only cause intestinal obstruction if they compress a section of the bowel or the bowel is caught in the jaws of the instrument (Ugochukwu and Edeh, 2011). A case of intestinal obstruction as an outcome of an artery forceps being inadvertently left in the peritoneal cavity thus resulting in intestinal obstruction due to herniation of the small intestine through the handle/finger loop of the artery forceps is reported in this study.

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CASE REPORT

A 39-year-old woman presented with abdominal pain of 3 days and constipation of 2 days duration. The pain was aching in nature, constant, centrally located and so severe she had to stop all activity. There were no relieving or aggravating factors. This was followed by absolute constipation, anorexia, vomiting and fever.

She had surgery done in one of the hospitals in the Kumasi Metropolis three and a half months prior to presentation on account of a ruptured ectopic gestation. She was haemo-transfused during and after the surgery and was discharged a week later. Eight weeks after discharge she reported to her doctors with intermittent colicky pains, vomiting and constipation. She was seen on several occasions with the same complaints and was always given analgesics and sent back home. When the pains became constant, associated with vomiting and constipation she decided to report to Komfo Anokye Teaching Hospital (KATH) for further management.

Physical examination revealed a middle-aged woman with vital signs (including temperature, blood pressure and pulse rate) being normal. The abdomen appeared full with the presence of a Pfannenstiel incisional scar from the previous surgery and tender on palpation with guarding. The hernia orifices were intact and no bowel sounds were heard on auscultation. Digital rectal examination yielded normal findings. Her white blood cell count was 14.3 x 10^9/L and the renal function tests were normal. A chest radiograph showed no air under the diaphragm but a plain erect abdominal radiograph showed air-fluid levels with the outline of a metal instrument that looked like an artery forceps (Figure1). A diagnosis of intestinal obstruction secondary to an intra-abdominal foreign body (artery forceps) was made and the patient prepared for laparotomy.

At laparotomy, a loop of small bowel was found to have herniated through one handle/finger loop of the artery forceps at a distance of about 24cm from the ileo-caecal junction (Figure 2). This loop of bowel, measuring about 40 cm in length, was totally gangrenous (Figure 3). The artery forceps was otherwise lying freely in the peritoneal cavity without any adhesions. There were also no adhesions between the bowel loops. The gangrenous small bowel was resected en-bloc with the artery forceps and an end-to-end anastomosis done to restore bowel continuity. The post-operative period was uneventful and the patient was discharged to go home on the 5th post-operative day in a satisfactory condition. She was subsequently reviewed on two occasions after discharge and had no complaints on both occasions.

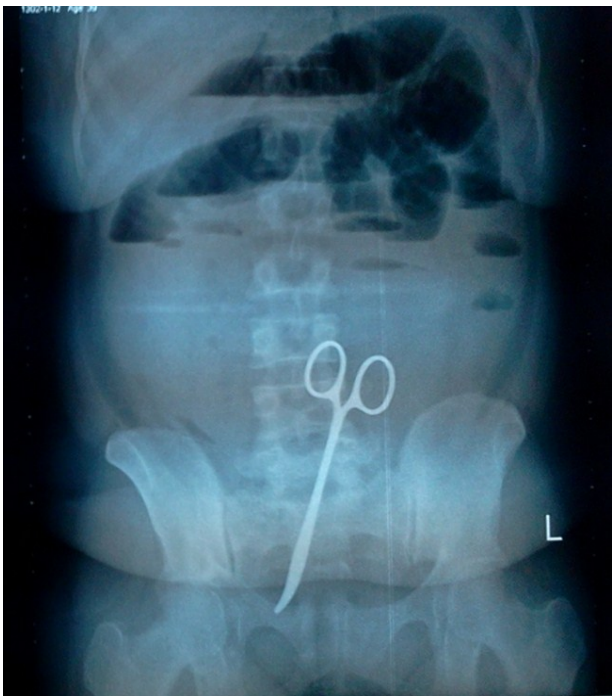

Figure 1: Plain erect abdominal radiograph showing an artery forceps in the peritoneal cavity. Also seen are air-fluid levels.

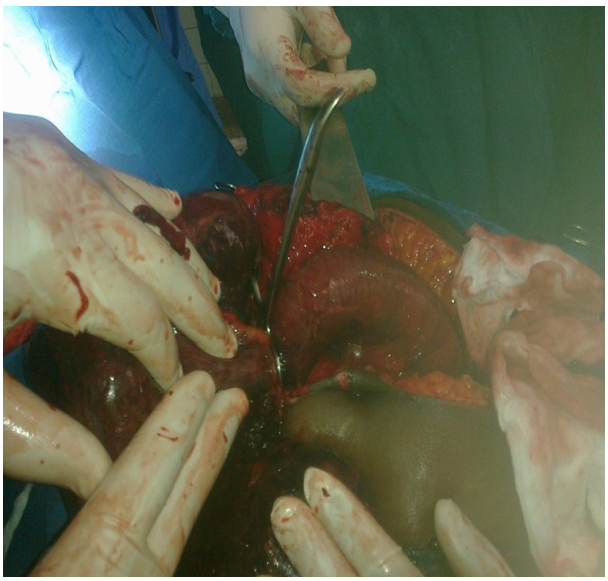

Figure 2: Artery forceps with the bowel herniating through one of the handle/finger loops

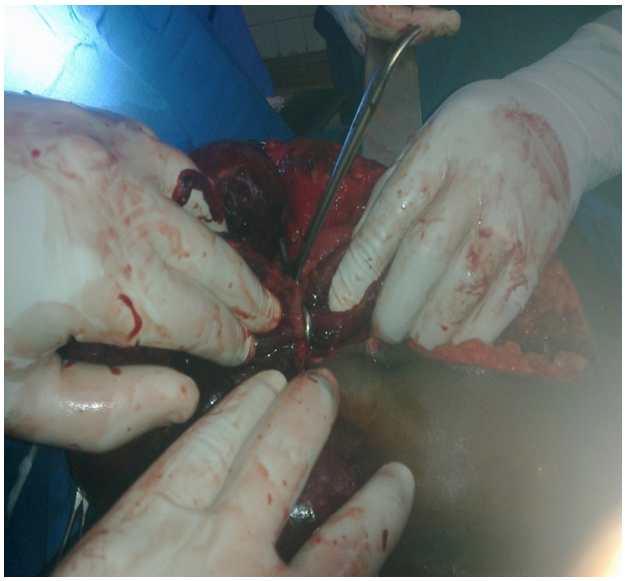

Figure 3: Herniation of loop of bowel through the handle loop, another view.

DISCUSSION

Even though there is paucity of data on retained surgical instruments and material in the sub-region, the problem is real and must be tackled holistically. The surgical team (surgeon, anaesthetist, scrub nurse, nurse runners and anyone involved in the operation) must be conversant with the risk factors associated with retention of surgical items during operating procedures.

Inadvertently retained surgical instruments or materials in the abdominal cavity are an uncommon but dangerous surgical error usually occurring during laparotomy (Gawande *et al.*, 2003). It is a serious and embarrassing occurrence in surgical practice and as such is under-reported and under-estimated (Asuquo *et al.*, 2006) probably due to medico-legal implications, the unwillingness of surgeons to publicize sucherrors and/or the complacency of colleagues in exposing the occurrence for fear of jeopardizing a professional life (Uguchukwu and Edeh, 2011). These retained materials and instruments in the peritoneal cavity can go undetected for years if they cause no problems and are usually found accidentally when the patient is being investigated for a different condition altogether (Nasir, 2009). The presence of foreign bodies in the abdominal cavity can lead to the formation of adhesions which are a common cause of small bowel obstruction in post-operative patients (Lauwers and Hee, 2000). Such materials or instruments can cause infection of the peritoneal cavity and perforations of hollow viscera and may result in severe morbidity or even mortality if measures are not taken to diagnose and remove the offending instrument or material (Lauwers and Hee, 2000; Kalovidouris *et al.*, 1999).

Foreign bodies unintentionally retained in the abdominal cavity include towels, artery forceps, pieces of broken instruments or irrigation sets and rubber tubes (Garg and Agarwal, 2010). The most common of foreign bodies left in the abdomen are small surgical sponges and towels, usually referred to as gossypibomas or textilomas (Rapaport and Haynes, 1990; Yildririm *et al.*, 2006). Several studies have been conducted to identify the risk factors for surgical material retention in patients after surgery and the symptoms caused by these materials. The three main risk factors for retention of a foreign body in the abdominal cavity after multivariate analysis of many factors include: emergency surgeries, unplanned changes in surgical procedure, and a higher body mass index (Gawande *et al.*, 2003). Patients with high BMI are likely to have large greater omentum hence the likelihood of a foreign body hiding underneath without being noticed. This patient had emergency surgery for a ruptured ectopic pregnancy and was obese (BMI of 35.2 kg m^2). She therefore had two out of the three identified risk factors for the retention of surgical instruments or material in the abdomen after laparotomy.

Other risk factors considered in literature which can lead to retention of surgical material after laparotomy include: lengthy surgical procedures, change in nursing staff during the procedure, poor communication among the operating team, operations performed late at night, more than one surgical team being involved in the operation, inexperienced and inadequate staff, staff fatigue, performance of a major procedure, unstable patient condition, the necessity to arrest massive intra-abdominal bleeding using multiple instruments and

packs of gauze, improper lighting in the theatre, hurried or non-meticulous sponge and instrument count, as well as absence of the surgeon at the time of wound closure (Murad and Basi, 2003; Wang *et al*., 2009; Dakubo *et al*., 2009). Knowledge of such risk factors is important to forestall unintentional leaving of surgical material and instruments after abdominal surgery. It is imperative that extreme care is taken during the performance of simple but vital tasks, such as counting of instruments and gauze to prevent them being left behind resulting in complications to the patient and cost to clinical practice in terms of law suits, morbidity and even mortality of patients.

To date, the only two documented reports of surgical material being left in the abdominal cavity from literature in Ghana are from the Korle-Bu Teaching Hospital in Accra (Dakubo *et al*., 2009; Adu-Aryee *et al*., 2005). But for anecdotal accounts by surgeons to colleagues, there is no confirmation of any reports of retained surgical instruments or material in the abdominal cavity from Komfo Anokye Teaching Hospital, Kumasi, Ghana as such making this case report; the first to be reported from KATH. On the backdrop of evidence from available literature, surgeons need to have a high index of suspicion and consider retained surgical instruments or material, if after surgery; a patient has vague, non-specific abdominal signs and symptoms.

The symptomatology of retained foreign material, in the abdominal cavity gleaned from the world scientific literature reflects paucity of clinical signs and symptoms. The symptoms are usually non-specific and varied during the post-operative period until an emergency condition such as intestinal obstruction occurs as a result of adhesions caused by the retained surgical item. Wan et al., (2009) reported such signs and symptoms to include: vague abdominal pains or irritation, palpable mass, anorexia, weight loss, fatigue, fever, nausea, vomiting, rectal bleeding and so forth which are non-specific. This patient had abdominal pains and so was seen and treated with analgesics for several weeks before she decided to seek medical care at KATH when she developed vomiting and constipation in addition.

CONCLUSION

It is highly imperative that the surgical team sticks strictly to theatre etiquette of counting surgical material several times: once before starting the procedure, during the procedure, before the abdominal cavity is closed and at the end of the procedure as recommended by AORN, (2006). Furthermore, for cases identified as high risk, additional preventive measures should be considered as this will go a long way in minimizing retention of surgical items in the abdominal cavity, if not eliminating it altogether. However, for diagnosis of a retained surgical item to be made in a patient there should be a high index of suspicion from the part of the surgeon and not an over-reliance on any specific symptoms.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Adu-Aryee, N.A, Asumanu, E., Tetteh, A., Fordjour, E., Naaeder, S.B. (2005). Intestinal obstruction caused by retained surgical sponge: two case reports. GMJ 39: 37 – 38.

Aminian, A. (2008).Gossypiboma: a case report. Cases J 1: 220. Available from: http://www.casesjournal.com/content/1/1/220. Accessed on 10th April, 2013.

AORN (2006). Recommended practices for sponge, sharps and instrument counts. AORN 83:418, 421 – 424.

Asuquo, M.E., Ogbu, N., Udosen, J., Ekpo, R., Agbor, C., Ozinko, M., Emelike, K. (2006). Acute abdomen from gossypiboma: a case series and review of literature. Nigerian J Surg Res 8: 174 – 176.

Berkowitz, S., Marshall, H., Charles, A. (2007). Retained intra-abdominal surgical instruments: time to use nascent technology? Am Surg 73: 1083 - 1085.

Dakubo, J., Clegg-Lamptey, J.N., Hodasi, W.M., Obaka, H.E., Toboh, H., Asempa, W. (2009). An intra-abdominal gossypiboma.2009; 43: 43 – 45.

Garg, M., Aggarwal, A.D. (2010).A review of medicolegal consequences of gossypiboma. J Indian Acad Forensic Med 32: 358 - 361.

Ghahremani, G.G. (1984). Internal abdominal hernias.SurgClin North Am 64: 393 – 406.

Gibbs, V.C. (2011).Retained surgical items and minimally invasive surgery. World J Surg 35: 1532 – 1539.

Gawande, A.A., Studdert, E.M., Orav, E.J., Brennan, T.A., Zinner, M.J. (2003).Risk factors for retained instruments and sponges after surgery. New Eng J Med 348: 229 – 235.

Kalovidouris, A., Kehagias, D., Moulopoulos, L., Gouliamos, A., Pentea, S., Vlahos, L. (1999). Abdominal retained surgical sponges: CT appearance. Eur Radiol 9: 1407 – 1410.

Karahasanoglu, T., Unal, E., Memisoglu, K., Sahinler, I., Atkovar, G. (2004).Laparoscopic removal of a retained surgical instrument. J Laparoendosc Adv Surg Tech 4: 241 – 243.

Lata, I., Kapoor, D., Sahu, S. (2011).Gossypiboma, a rare cause of acute abdomen: a case report and review of literature. Int J Crit Ill Inj Sci. 1: 157 – 160.

Lauwers, P.R., Hee, R.H.V. (2000).Intraperitoneal gossypibomas: the need to count sponges. World J Surg 24: 521 – 527.

Lincourt, A.E., Harrell, A., Cristiano, J., Sechrist, C., Kercher, K., Heniford, B.T. (2007). Retained foreign bodies after surgery. J Surg Res 138: 170 – 174.

Mathieu, D., Lucian, A. (2004).Internal abdominal herniations. AJR 183: 397 – 404.

Murad, M., Basi, V. (2003).Gossypiboma – a case report. Pak J Med Sci. 19: 138 – 140.

Nasir, G.A.A. (2009).Missed instrument and surgical sponge (gauze and pack).Internet J Surg 20.

Pershad, J., Simmons, G. T., Chung, D., Frye, T., Marques, M. B. (1998).Two acute pediatric abdominal catastrophes from strangulated left paraduodenal hernias. Pediatr EmergCare 14: 347 – 349.

Rappaport, W., Haynes, K. (1990).The retained surgical sponge following intra-abdominal surgery. A continuing problem. Arch Surg 125: 405 -407.

Stawicki, S.P., Seamon, M.J., Martin, N.D. (2008).Retained surgical foreign bodies: a synopsis. OPUS 12 Scientist 2: 1 – 6.

Ugochukwu, A.I., Edeh, A.J. (2011). Retained intra-abdominal artery forceps – an unusual cause of intestinal strangulation. North Am J Med Sci3: 339 – 343.

Ukwenya, A.Y., Dogo, P.M., Ahmed, A., Nmadu, P.T. (2006). The retained surgical sponge following laparotomy; forgotten at surgery, often forgotten at diagnosis. Our experience. Nigerian J Surg Res 8: 164 – 168.

Wang, C.F., Cipolla, J., Seamon, M.J., Lindsey, D.E., Stawicki, S.P. (2009). Gastro-intestinal complications related to retained surgical foreign bodies (RSFB): a concise review. OPUS 12 Scientist 3: 11 – 18.

Wan, W., Le, T., Riskin, L., Macario, A. (2009).Improving safety in the operating room: a systematic literature review of retained surgical sponges. Cur Opin Anaesthesiol 22: 207 – 214.

Whang, G., Mogel, G.T., Tsai, J, Palmer, S.L. (2009).Left behind: unintentionally retained surgically placed foreign bodies and how to reduce their incidence – pictorial review. AJR 193: S79 – S89.

Yagnik, V., Patel, P., Patel, A. (2009).Congenital internal hernia: a rare cause of small-bowel obstruction. The Internet J Surg. 20 (1).

Yildirim, S., Tarim, A., Nursal, T.Z., Yildirim, T., Caliskan, K., Torer, N., Karagulle, E., Noyan, T., Moray, G., Haberal, M. (2006). Retained surgical sponge (gossypiboma) after intraabdominal or retroperitoneal surgery: 14 cases treated at a single center. Langenbecks Arch Surg 391: 390 – 395.

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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

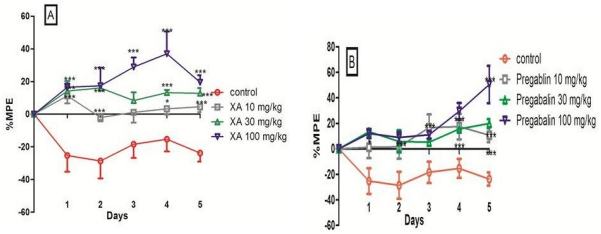

Figure 1: The time-course curve of the effects of daily dosing of xylopic acid (10-100 mg/kg), pregabalin (10-100 mg/kg) and cremophor solution (control) on established paclitaxel-induced mechanical hyperalgesia. Graph A shows the effect of daily systemic administration of 10-100 mg/kg xylopic acid or 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).

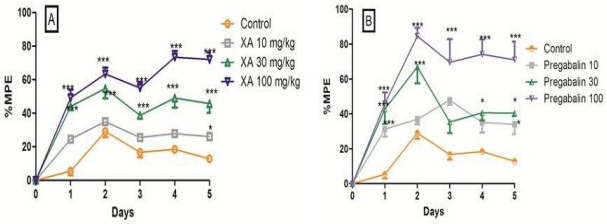

Figure 2: The time-course curve of the effects of daily dosing of xylopic acid (10-100 mg kg^{-1}), pregabalin (10-100 mg kg^{-1}) and cremophor solution (control) on established paclitaxel-induced thermal hyperalgesia. Graph A shows the effect of daily systemic administration of 10-100 mg kg^{-1} xylopic 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

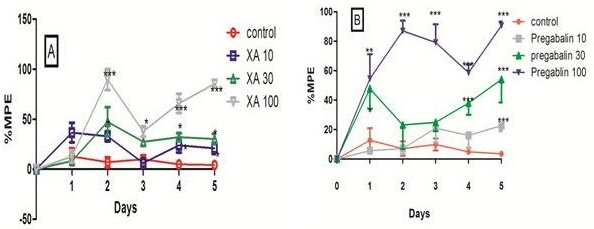

Figure 3: The time-course curve of the effects of daily dosing of xylopic acid (10-100 mg kg^{-1}), pregabalin (10-100 mg kg^{-1}) and cremophor solution (control) on established paclitaxel-induced cold allodynia. Graph A shows the effect of daily systemic administration of 10-100 mg kg^{-1} xylopic 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.

REFERENCES

Adaramoye OA, Sakar J, Singh N, Meena S, Changkija B, Yadav PP, Kanojiya S, Sinha S (2011). Antiproliferative action of *Xylopia aethiopica* fruit extract on human cervical cancer cells. *Phytother Res*. **25**(10), 1558-1563.

Akerley W, Glantz M, Choy H, Rege V, Sambandam S, Joseph P, Yee L, Rodrigues B, Wingate P,Leone L (1998). Phase I trial of weekly paclitaxel in advanced lung cancer. *J Clin Oncol*.**16**(1):153–158.

Ameyaw EO, Agyei PEO, Boampong JN, Koranteng KA, Kyei S (2013). Ethanolic root extract of *Jatropha curcas* L. ameliorates paclitaxel-induced neuropathic pain in rats. *Research & Reviews: A Journal of Pharmacology*. 3(2): ISSN: 2230-9861.

Ami N, Okamoto K, Oshima H (2012). Analgesic effect of magnetic stimulation on paclitaxel -induced peripheral neuropathic pain in mice. *Brain Res*. **1461**:24-9.

Bray D, Hollenbeck PJ(1988). Growth cone motility and guidance.*Annu. Rev. Cell Biol*. **4**:43-61.

Burgos E, Gómez-Nicola D, Pascual D, Martín MI, Nieto-Sampedro M, Goicoechea C

(2012). Cannabinoid agonist WIN 55,212-2 prevents the development of paclitaxel-induced peripheral neuropathy in rats. Possible involvement of spinal glial cells. *Eur J Pharmacol.***682**(1): 62-72.

Burkhart CA, Berman JW, Swindell CS, Horwitz SB. (1994). Relationship between the structure of taxol and other taxaneson induction of tumor necrosis factor-alpha gene expression and cytotoxicity. *Cancer Res.***54**(22): 5779-82.

Cavalcanti BC, Bezerra DP, Magalhaes HIF, Moraes MO, Lima MA, Silveira ER, Câmara CA, Rao VS, Pessoa C, Costa-Lotufo LV (2009). Kauren-19-oic acid induces DNA damage followed by apoptosis in human leukemia cells.*J Appl Toxicol.* **29**(7):560–568.

Cavaletti G, Cavalletti E, Oggioni N, Sottani C, Minoia C, D'Incalci M, Zucchetti M, Marmiroli P, Tredici G (2000).Distribution of paclitaxel within the nervous system of the rat after repeated intravenous administration. *Neurotoxicology.* **21**(13):389-393.

Choi SS, Koh UW, Nam JS, Shin JW, Leem JG, Suh JH (2013). Effect of ethyl pyruvate on paclitaxel-induced neuropathic pain in rats. *Korean J Pain.* **26**(2):135-141.

Costa R, Motta EM, Dutra RC, Manjavachi MN, Bento AF, Malinsky FR, Pesquero JB, Calixto JB (2011). Anti-nociceptive effect of kinin B1 and B2 receptor antagonists on peripheral neuropathy induced by paclitaxel in mice. *Br J Pharmacol.* **164**(2b): 681–693.

Deng L, Guindon J, Vemuri KV, Thakur GA, White FA, Makriyannis ,Hohmann AG (2012). The maintenance of cisplatin- and paclitaxel-induced mechanical and cold allodyniais suppressed by cannabinoid CB2 receptor activation and independent of CXCR4 signaling in models of chemotherapy-induced peripheral neuropathy. *Mol Pain.* **8**:71.

Dougherty PM, Cata JP, Cordella JV (2004). Taxo-linduced sensory disturbance is characterized by preferential impairment of myelinated fiber function in cancer patients. *Pain.* **109**(1-2): 132–142.

Fidanboylu M, Griffiths LA, Flatters SJL (2011). Global Inhibition of Reactive Oxygen Species (ROS) Inhibits Paclitaxel-Induced Painful Peripheral Neuropathy. *PLoS ONE.***6**(9): e25212.

Flatters SJ, Bennett GJ (2006). Studies of peripheral sensory nerves in paclitaxelinduced painful peripheral neuropathy: evidence for mitochondrial dysfunction. *Pain.* **122**(3):245–257.

Flatters SJL, Bennett GJ (2004). Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. *Pain.* 109:150–161.

Forsyth PA, Balmaceda C, Peterson K, Seidman AD, Brasher P, DeAngelis LM (1997). Prospective study of paclitaxel-induced peripheral neuropathy with quantitative sensory testing. *J Neurooncol.* **35**(1): 47–53.

Jaggi AS, Singh N (2012). Mechanisms in cancer-chemotherapeutic drugsinduced peripheral neuropathy. *Toxicology*, 2012,**291**(1-3):1–9.

Kim SK, Park JH, Bae SJ, Bae SJ, Kim JH, Hwang BG, Min BI, Park DS, Na HS (2005). Effects of electroacupuncture on cold allodynia in a rat model of neuropathic pain: mediation by spinal adrenergic and serotonergic receptors. *Exp. Neurol.* **195**(2): 430-436.

Kobayashi N, Mundel P (1998). A role of microtubules during the formation of cell processes in neuronal and non-neuronal cells. *Cell Tissue Res.* **291**(2):163-174.

Kumar N, Laferriere A, Yu JS Poon T, Coderre TJ (2010). Metabotropic glutamate receptors (mGluRs) regulate noxious stimulus-induced glutamate release in the spinal cord dorsal horn of rats with neuropathic and inflammatory pain. *J Neurochem.***114**(1): 281-290.

Manthey CL, Brandes ME, Perera PY, Vogel SN (1992). Taxol increases steady-state levels of lipopolysaccharide-inducible genes and protein-tyrosine phosphorylation in murine macrophages. *J Immunol.* **149**(7): 2459-65.

Melli G, Taiana M, Camozzi F,Triolo D, Podini P, Quattrini A, Taroni F, Lauria G

(2008).Alpha-lipoic acid prevents mitochondrial damage and neurotoxicity in experimental chemotherapy neuropathy.*Exp Neurol.* **214**(2):276–284.

Miller JR (2004). GraphPad Version 4.0. Step-by-Step Examples, *GraphPad Software Inc., San Diego, CA.*

Motulsky HJ, Christopoulos A (2003). Fitting model to biological data using linear and nonlinear regression. A practical guide to curve fitting. *San Diego, CA, GraphPad Software Inc.*

Petrenko AB, Yamakura T, Baba H, Shimoji K (2003). The role of N-methyl-Daspartate (NMDA) receptors in pain: a review. *Anesth Analg.* **97**(4):1108–1116;

Postma TJ, Vermorken JB, Liefting AJ, Pinedo HM, Heimans JJ (1995).Paclitaxel-induced neuropathy. *Ann Onco.*, **6**(5):489–494.

Rowinsky EK, Chaudhry V, Cornblath DR, Donehower RC (1993). Neurotoxicity of Taxol.*J. Natl. Cancer Inst. Monogr.* **15**:107-115.

Rowinsky EK, Eisenhauer EA, Chaudhry V,Arbuck SG, Donehower RC (1993).Clinical toxicities encountered with paclitaxel (Taxol). *Semin Oncol.* 20(4 Suppl 3):1–15.

Schim JD (2009). Neuropathic Pain: Diagnosis and New Treatment Options. *22nd U.S. Psychiatric and Mental Health Congress.*

Somova LL, Shode FO, Moodley K, Govender Y. (2001) Cardiovascular and diuretic activity of kaurene derivatives of *Xylopia aethiopica* and *Alepidea amatymbica. J. Ethnopharmacol.***77** (2-3): 165-174.

Thirumal M, Srimanthula S, Kishore G, Vadivelan R, Anand Kumar AVS (2013). Analgesic and antipyretic effects of aqueous extract from *Clerodendrum inerme* (L.) Gaertn. leaves in animal models.*Der Pharmacia Lettre.* **5**(2): 315-323.

Woode E, Ameyaw EO, Ainooson GK, Wonder K.M. Abotsi, Eric Boakye-Gyasi and James Oppong Kyekyeku (2013). Analgesic Effects of an Ethanol Extract of the Fruits of *Xylopia aethiopica* and Xylopic Acid in Murine Models: Possible Mechanism(s). *Pharmacologia*, **4**:285-300

Woode E, Ameyaw EOA, Boakye-Gyasi E, Abotsi WK (2012). Analgesic effects of an ethanol extract of the fruits of *Xylopia aethiopica* (Dunal) A. Rich, (Annonaceae) and the major constituent, xylopic acid in murine models. *J Pharm Bioallied Sci.* **4**(4):291–301.

Xiao WH, Zheng H, Zheng FY, Nuydens R, Meert TF, Bennett GJ (2011). Mitochondrial abnormality in sensory, but not motor, axons in paclitaxel-evoked painful peripheral neuropathy in the rat. *Neuroscience.* **199**: 461–469.

Zhang H, Yoon SY, Zhang H, Dougherty PM (2012). Evidence that spinal astrocytes but not microglia contribute to the pathogenesis of Paclitaxel-induced painful neuropathy. *J Pain.***13**(3): 293-303.

Zheng H, Xiao WH, Bennett GJ (2011). Functional deficits in peripheral nerve mitochondria in rats with paclitaxel- and oxaliplatin-evoked painful peripheral neuropathy.*Exp Neurol.* **232**(2):154–161.

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