



Antinociceptive effects of lead acetate in sciatic nerve chronic constriction injury model of peripheral neuropathy in male *Wistar* rats

Bamidele Victor Owoyele¹ · Ahmed Olalekan Bakare¹ · Maryam Tayo Ayinla¹ · Kehinde Ahmed Adeshina¹ · Damilola Onietan¹ · Saheed O. Azeez¹

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Abstract

The toxicological effects of lead and its compounds have overshadowed its possible health beneficial effects. Currently, the success rate for treating neuropathic pain has been very low. This study investigated the antinociceptive effects of orally administered low dose lead acetate in sciatic nerve ligated *Wistar* rats. Thirty *Wistar* rats randomly divided into five groups were used for this study. Chronic constriction injury (CCI) was used to induce neuropathic pain in *Wistar* rats. Allodynic and hyperalgesic signs were investigated using von Frey filaments and hotplate, respectively. Morris water maze test was used to assess the memory functions of the rats. The study revealed that oral administration of low-dose lead acetate significantly ($p < 0.05$) increased pain thresholds of ligated rats. CCI enhanced memory function in *Wistar* rats which was significantly decreased following lead acetate administration. The findings suggest that lead acetate possesses antinociceptive effects in peripherally induced neuropathic pain model in *Wistar* rats.

Keywords Allodynia · Hyperalgesia · Lead acetate · Memory · Neuropathic pain · Sciatic nerve

Introduction

Lesion or traumatic injury to the somatosensory nerves results in neuropathic pain (NP) conditions. About 7–10% of the general population (Colloca et al. 2017) is suffering from neuropathic pain with symptoms that include abnormal unpleasant sensations (dysesthesia), increased response to both the painful stimuli (hyperalgesia), and painless stimuli (allodynia) (Woolf and Mannion 1999).

Complex mechanisms that are involved in the development and maintenance of neuropathic pain complicate the therapeutic approaches in the treatment of NP patients. Opioids, non-

steroidal anti-inflammatory drugs (NSAIDs), anticonvulsant, antidepressant, and serotonin-norepinephrine reuptake inhibitors are the most common clinical lines of drugs used for the treatment of NP (Finnerup et al. 2010). Many of these drugs produces various deleterious side effects on patients. The side effects include cardiovascular dysfunctions, cognitive deficits, digestive tract abnormalities, addictions, as well as respiratory system dysfunctions (Ossipov et al. 2004; Tilo et al. 2006; Salvemini et al. 2011). The efficacy and effectiveness of drugs used for the treatment of chronic pain vary as 40–60% of patients have been reported to achieve partial relief (Dworkin et al. 2007; Finnerup et al. 2010). Hence, the continued search for a new therapeutic approach for the treatment of NP is crucial.

Lead (Pb) is ubiquitous and one of the earliest metals discovered by the human race. It is categorized as a highly toxic agent commonly transferred airborne (Fazli-Tabaei et al. 2003; Umar et al. 2016). The compounds of lead have been widely tagged as neurotoxic because numerous studies have reported lead-induced cognitive deficits using cellular models of learning and memory, vision and behavioral problems, and brain damage/mental retardation (Sanders et al. 2010; Umar et al. 2016). The toxic effect of lead has also been documented

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✉ Bamidele Victor Owoyele
owoyle@unilorin.edu.ng

¹ Neuroscience and Inflammation Unit, Department of Physiology, Faculty of Basic Medical Science, University of Ilorin, Ilorin, Kwara State, Nigeria

on the liver, kidney, and gastrointestinal system (Lamidi and Akefe 2017).

However, the therapeutic usage of lead acetate has been documented in the literature on various models of nociception. Nikfar and his colleagues (Nikfar et al. 1998) have reported that lead acetate mitigates the nociception induced by morphine and formalin injection. Further antinociceptive effects of lead acetate were noted on the hotplate (Bondy et al. 1985; Vickers and Paterson 1986) and tail-immersion (Burkey et al. 1994) models of nociceptive screening techniques. Currently, there is no report on the therapeutic value of lead compounds for the treatment of neuropathic pain. Therefore, this study was undertaken to investigate the effect of low dose lead acetate administration on the chronic constriction injury model of neuropathic pain in *Wistar* rats.

Materials and methods

Animals and treatment

Thirty randomly selected male *Wistar* rats weighing between 160 g $\pm$ 20 g were used for this study. The rats were divided into five (5) groups with each comprising of six animals. They were housed under standard conditions of daily light and dark cycles with free access to food and water. Normal saline, gabapentin, or lead acetate was orally administered to the rats once daily for 21 consecutive days. The lead acetate was purchased from Loba Chemie PVT LTD, Jehangir Villa, Colaba Mumbai, India.

Study grouping was as follows:

- Group A: (normal control) 10 ml/Kg of normal saline
- Group B: (sham control) 10 ml/Kg of normal saline
- Group C: (ligated control) 10 ml/Kg of normal saline
- Group D: (ligated reference group) 30 mg/kg of gabapentin drug
- Group E: (ligated lead) 150 mg/kg of lead acetate

Sciatic nerve ligation

Neuropathic pain was induced in the rats by chronic constriction of the sciatic nerve using the method of Bennet and Xie (1988). Briefly, after anesthetizing the rats with 80 mg/kg of ketamine intraperitoneally, the left thighs were incised, and blunt cuts were made through the biceps exposing the sciatic nerve. The nerve was loosely ligated with catgut (size 4,0) at four different points with about 1 mm distance from each successive point. This was carefully performed to prevent the disruption of the epineural blood supply. The muscular and the skin layers were sutured using suturing silk thread. Sham group was also operated to expose the sciatic nerve only

without ligating it. Following the completion of the operation, the animals were allowed to recover with water and food *ad libitum*.

Behavioral tests

Hotplate test was used to assess thermal hyperalgesia, and mechanical allodynia sensation was assessed by the use of von Frey filament test, while the functionality of the sciatic nerve was assessed by sciatic functional index. All behavioral tests were carried out before the induction of neuropathic pain, 3rd day after ligation, 7th day, 14th day, and 21st day after oral administration of drugs.

Assessing thermal hyperalgesia

Thermal hyperalgesia was assessed as described in the work of Abdulmajeed and Owoyele (2015). The development of thermal hypersensitivity associated with neuropathic pain was measured using mean paw withdrawal threshold (PWL) from the hotplate (Model number: DB-1A, DB, China) maintained at 55 $\pm$ 0.5 °C. The time taken for the rats to jump off the surface of the hotplate was taken as the paw withdrawal latency. A cut-off time of 30 seconds was imposed to avoid injury to the paw.

Assessment of mechanical allodynia

von Frey filament (Cat No: 37450-275, Ugo Basile, Italy) was used to appraise the degree of mechanical hypersensitivity in animals by evaluating the paw withdrawal latency to the applied mechanical force as reported by Zhu and his colleague (Zhu et al. 2012). A series of ten (10) von Frey filaments were used one after the other in an ascending order. The ten filaments have incremental bending forces equivalent to 2, 4, 6, 8, 10, 15, 26, 60, 100, and 180 g forces, respectively. Rats were confined in a transparent Perspex box with wire mesh floors, and they were allowed 15 minutes of adaptation to the environment before the behavioral test. The filament was individually applied to the plantar surface of each hind paw of the rats in ascending orders. Paw withdrawal latency was defined by the mechanical force (in gram) obtained from three consecutive trial withdrawal, licking, or flinching of the hind limb.

Sciatic function index (SFI) test

SFI test was carried out to evaluate the degree of autotomy as an indication of dysfunction in the sciatic nerve. A printed foot image of the ligated hind limb was used to gauge the SFI using the formula derived by Bain et al. (1989). Briefly, animals were made to walk on a wooden walking track (an 8.2 cm by 42 cm corridor) with a floor covered with a sheet of white paper. The rat hind paws were inserted in blue ink before the

rats were placed on the wooden walking track. The footprint was analyzed using

$$\text{SFI} = -38.3 \times \text{PLF} + 109.5 \times \text{TSF} + 13.3 \times \text{ITF} - 8.8.$$

PLF (printed length factor) is a factor estimated from the printed foot length which is the distance between the third toe and hind pad; TSF (toe spread factor) is the factor estimated from toe spread which is a distance between first and fifth toe; ITF (intermediate toe spread factor) is the factor estimated from intermediate toe spread which is a distance between the second and fourth toe.

An SFI of 0 is normal and of -100 indicates total impairment

Assessment of reference memory

Morris water maze test was used to investigate the spatial memory function. A pool containing milky colored water, 150 cm in diameter, and 60 cm high, with an escape platform of 10 cm in diameter and 28 cm high was used. The pool was filled with milky water up to 30-cm mark, slightly above the escape platform, and divided into four equal quadrants. A large visible visual geometric cue was pasted on the pool wall of each quadrant. Escape platform was stationed at a permanent position within the designated escape quadrant throughout the training session and removed on the probe trial day. The water temperature was maintained at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ throughout the experimental periods. Rats were trained for 5 consecutive days, 5 sessions per day of 90 seconds per session. Probe trial was performed 24 hours (24 hrs) after the last training session for the assessment of reference memory in rats. The total number of times each rat enter the designated escape quadrant, the number of time that rat crossed over the spot of the escape platform was initially situated; time taken to reach the designated escape quadrant, time taken to reach the spot of escape platform, total time spent at the designated escape quadrant as well as total time spent around the spot where escape platform was initially situated were noted and recorded.

Biochemical assays

After 21 days of the injury to the sciatic nerve, the rats were anesthetized with ketamine hydrochloride, and then transcardiac perfusion with phosphate-buffered saline (PBS) (pH 7.4) was carried out. The sciatic nerve from ligated hind limb (1–2 cm proximal and about 1 cm distal to the point of ligation) was excised and washed with cold PBS and homogenized.

Estimation of glutamate and calcium level

Sciatic nerve homogenate was prepared with ice phosphate-buffered saline (PBS) (pH 7.4) and

centrifuged, and supernatant of homogenate was used to estimate the glutamate and calcium level using a colorimeter and UV/V spectrophotometer (model 752 N), respectively.

Histology study

Structural integrity of the hippocampus, kidney, and liver were investigated using hematoxylin and eosin (H&E) stain as described by Muthuraman and his colleague (Muthuraman et al. 2008). Each organ was fixed in 10% formalin solution, blocked and subsequently sectioned transversely into $5\text{ }\mu\text{m}$ before staining with H&E, and observed under light microscope.

Statistical analysis

All results were expressed as means $\pm$ SEM. Graphpad Prism version 5.0 was used to analyze all data. One-way analysis of variance (ANOVA) was used for the laboratory assay, while two-way analysis of variance was used to analyze the behavioural tests followed by Bonferroni post hoc multiple comparisons test with the significance level set at $p < 0.05$.

Results

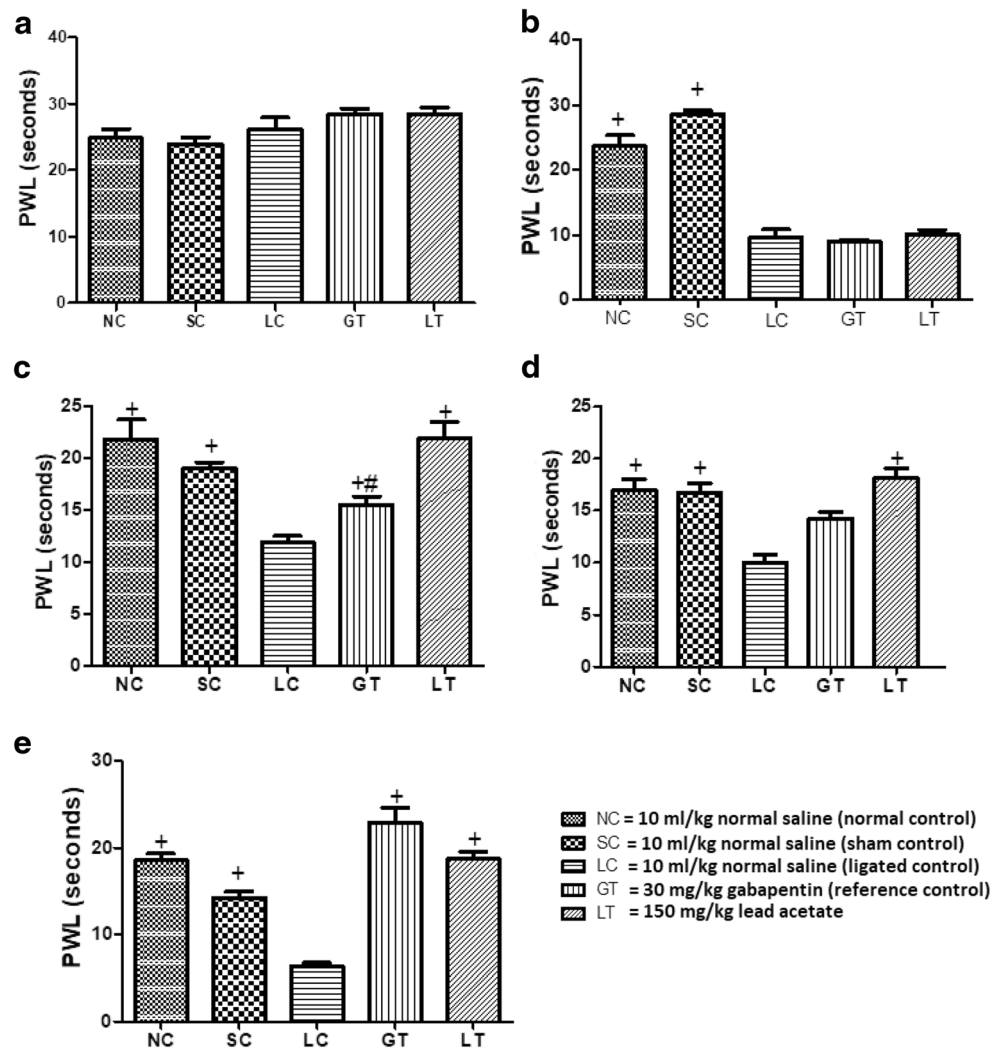
Thermal hyperalgesia test

Thermal hypersensitivity is a significant symptom of neuropathic pain. Sciatic nerve ligated rats showed significant ($p < 0.05$) increases in thermal hypersensitivity after sciatic nerve ligation compared with normal control (8.96 ± 0.32 vs 23.69 ± 1.70 seconds) and sham control (8.96 ± 0.32 vs 28.67 ± 0.56 seconds) groups. Administration of lead acetate attenuated the thermal hyperalgesia as there were significant ($p < 0.05$) differences between the group-administered lead acetate compared with the ligated control group. This was indicated by prolonged paw withdrawal latency from the hotplate (Fig. 1). The peak antinociceptive effect of lead acetate was observed on the seventh day of the post-ligation hotplate test (21.98 ± 1.53 seconds). Lead acetate completely abolished the thermal hyperalgesia experienced by the animal as there were no significant differences between the lead acetate-treated group and normal control group throughout the testing period.

Mechanical allodynia test

Chronic constriction injury resulted in a high degree of mechanical allodynia in the ligated groups as they responded to the applied low mechanical von Frey filament force (Fig. 2).

Fig. 1 Antihyperalgesic effect of lead acetate on thermal stimulus. **(a)** Presurgical hotplate test. **(b)** 3rd day post-ligation baseline hotplate test. **(c)** 7th day oral administration of treatment post ligation hotplate test. **(d)** 14th day oral administration of treatment post-ligation hotplate test. **(e)** 21st day oral administration of treatment post-ligation hotplate test. Each value represents the mean $\pm$ SEM of each group; $+p < 0.05$ compared with the ligated control and $\#p < 0.05$ compared with normal control. *PWL* paw withdrawal latency, *NC* normal control (unligated), *SC* sham control, *LC* ligated control, *GT* gabapentin treatment, *LT* lead treatment

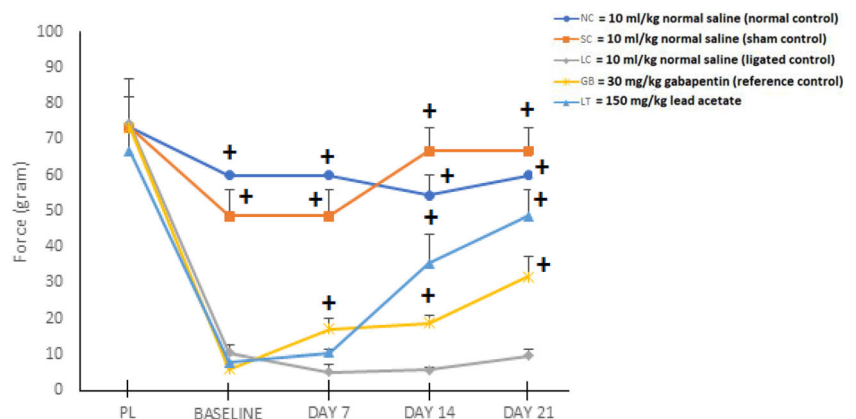


Lead acetate significantly ($p < 0.05$) decreased responses to low mechanical von Frey filament forces compared with the ligated control group progressively throughout the test period. The maximum effect of lead acetate was recorded on the 21st day of administration.

Sciatic functional index test

Sciatic functional index test showed the level of locomotive dysfunction in the various test groups. SFI was greatly pronounced in all the ligated groups following sciatic nerve

Fig. 2 Anti-allodynic effect of lead acetate. Each value represents the mean $\pm$ SEM of each group. $+p < 0.05$ compared with the ligated control. PL (pre-ligation): von Frey filament test before the chronic constriction of the sciatic nerve



ligation as indicated in Fig. 3. A very high negative value of SFI represents a greater degree of locomotive dysfunction. The normal and sham control groups have an optimal value of SFI, while the ligated test groups have marked increases in the value of SFI. Rats that were treated with lead acetate significantly ($p < 0.05$) improved the sciatic nerve functions compared with the ligated vehicle control group.

Morris Water Maze Test

Chronic constriction injury (CCI) of the sciatic nerve in *Wistar* rats results in increased reference memory retention. Ligated naïve group shows significant ($p < 0.05$) reduced time taken to reach the escape water quadrant and escape platform spot compared with all other groups as illustrated in Fig. 4a and b. There were also significant ($p < 0.05$) differences in the total time spent at both escape quadrant and around the spot of escape platform by ligated naïve group compared with all other groups. Although there were increases in the time taken by lead acetate-treated rats to reach the escape quadrant, it was insignificantly different ($p > 0.05$) compared with unligated naïve groups. The time spent in loitering around the escape quadrant as well as around the escape platform spot was insignificantly reduced in lead acetate group compared with the unligated naïve group. The ligated control group showed significant ($p < 0.05$) increases in the frequency of time swimming through the escape platform spot compared with other groups except the gabapentin-treated group. However, there were no significant differences ($p > 0.05$) between lead acetate-treated group and unligated naïve groups as indicated in Fig. 4e.

Histological study

Hippocampus

CCI induced increases but shrink myelinated hippocampal neurons compared with the unligated group. The degree of

vacuolation in the ligated rats hippocampal tissue was identical with that of an unligated control group. Treatment of ligated *Wistar* rats with gabapentin shows normal hippocampal myelinated neuronal size but reduced the population size. The hippocampus of the lead acetate-treated group shows the same degree of vacuolation compared with other groups, but there were significant increases in the number of swollen and distorted hippocampal myelinated neurons as indicated in Fig. 5.

Liver

CCI induces mid-vacuolation and swollen hepatocyte in the liver tissue with a normal distribution of the hepatocytes compared with the unligated naïve group as indicated in Fig. 6. There was pronounced vacuolation of the liver in a group treated with gabapentin compared with ligated control and lead acetate-treated groups. However, the group administered with lead acetate showed increases in the number and congestion of liver hepatocytes (Fig. 6e).

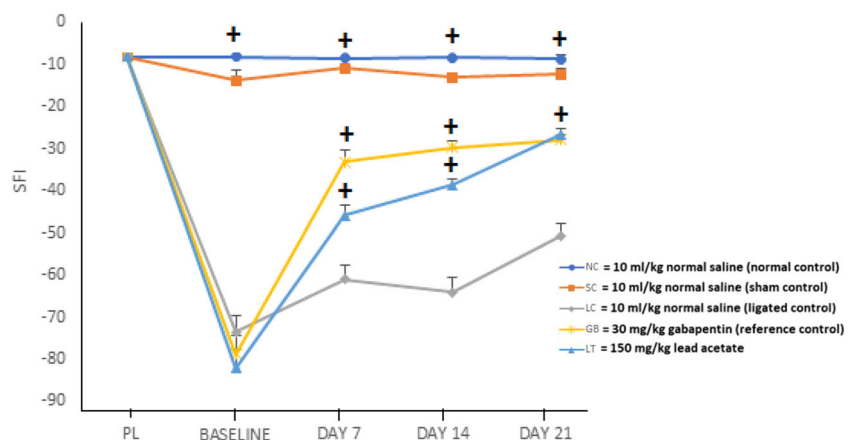
Kidney

There were no observable differences in the histological structure of both ligated and unligated groups. The renal corpuscle has a similar degree of vacuolation as indicated in Fig. 7. It can be deduced that CCI did not have a significant effect on the kidney. Contrarily, lead acetate-treated group showed mild increases in vacuolation in renal corpuscles.

Discussion

The burden of neuropathic pain on patients has continued to necessitate the quest for an effective therapeutic intervention to ameliorate the causative agent and restore the somatosensory neuron to normalcy. Antinociceptive effects of lead acetate on various nociceptive screening models for acute pain

Fig. 3 Lead acetate improved the sciatic nerve function. Sciatic function index (SFI): Each value represent the mean $\pm$ SEM of each group. + $p < 0.05$ compared with the ligated control. PL (pre-ligation): von Frey filament test before chronic constriction of the sciatic nerve



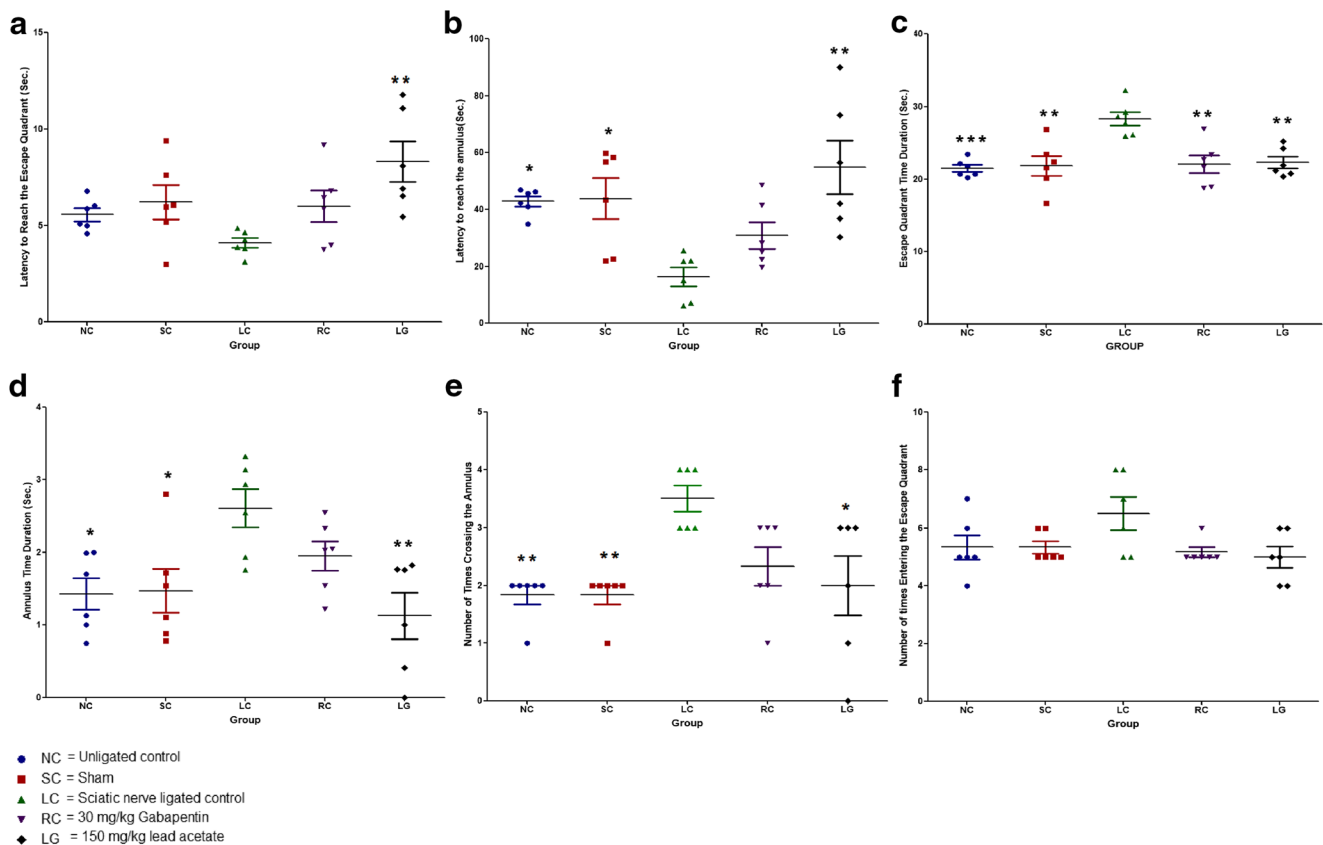


Fig. 4 Lead acetate reversed increases reference memory function in water Morris test in the sciatic nerve chronic constriction injury model of neuropathic pain. **(a)** Time taken by the rats to enter the escape water quadrant. **(b)** Time taken to reach the escape platform spot. **(c)** Time spent by the rats at the escape water quadrant. **(d)** Time spent by the rats around the spot where escape platform was initially situated in the water Morris; **(e)** Number of the rats crossover the exact spot of escape platform in the

escape water quadrant of the Morris water. **(f)** The number of time rats visit the escape quadrant of the Morris water. Each value represents the mean $\pm$ standard error of mean (SEM.) of each group. * $p < 0.05$ compared with the ligated control (LC); # $p < 0.05$ compared with lead acetate administered group (LG). NC unligated control, SC sham, LC sciatic nerve chronic constriction injury group, GT gabapentin treatment group, LT Lead treatment group

have been reported in the literature (Vickers and Paterson 1986; Burkey et al. 1994; Nikfar et al. 1998). The current study investigated the potential benefits of lead acetate in the chronic constriction injury model of pain which is a type of neuropathic pain in Wistar rats. Hyperalgesia and allodynia have been the most prevailing reported symptoms that are often associated with neuropathic pain patients, and they can easily be elicited in animal studies (Abdulmajeed and Owoyele 2015; Jiang et al. 2018). Findings from this study revealed the antinociceptive effects of lead acetate in sciatic nerve ligated model of neuropathic pain. This was shown by the attenuation of hyperalgesia and allodynia as well as improved sciatic function index.

Hotplate test remains one of the reliable tools that is used for the screening of the antinociceptive effect of most biochemical substances (Ishola et al. 2014). In this study, lead acetate increased the threshold to thermal stimulus. This indicates that low doses of lead acetate could act centrally at both the spinal and supraspinal levels. The effectiveness of lead acetate is quite surprising as it completely reverses the thermal

threshold of the ligated rats back to normal. There is no indication that the observed effect was one of the neurotoxic effects of lead acetate because there is no indication of loss of sensory sensation throughout the study.

Amelioration of mechanical allodynia and improved sciatic nerve function as observed in the study is an indication of antinociceptive effects by lead acetate. The use of von Frey filaments in assessing tactile allodynia is widely reported in the literature (Zhang et al. 2017). This study shows that lead acetate possesses a stronger anti-allodynic effect than gabapentin, one of the commonly used anticonvulsant drugs that are also employed for the management of neuropathic pain. A sciatic functional index is a measure of locomotive dysfunction as a result of a chronically constricted sciatic nerve. The index can serve as a measure of functional recovery of sciatic nerve from the nerve injury (Monte-Raso et al. 2008). Lead acetate improved the functional recovery of the sciatic nerve. These antinociceptive properties of low-dose lead acetate suggests that it might be a good adjuvant for the treatment of neuropathic pain. The findings also indicate that

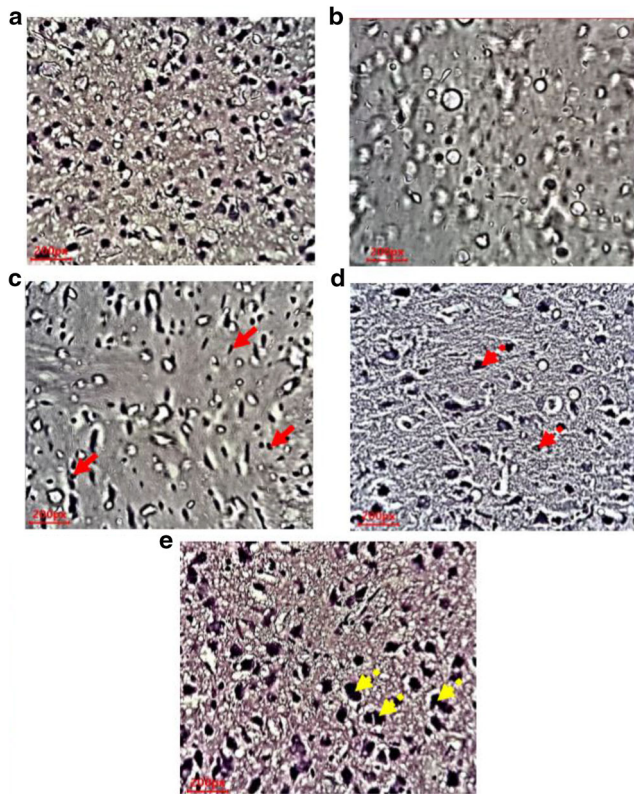


Fig. 5 Representative light micrographs of coronal sections of the rat hippocampus are shown. **(a and b)** Unligated and sham control hippocampus. **(c)** Ligated control hippocampus. **(d)** CCI gabapentin-treated hippocampus, and **(e)** CCI lead acetate-treated hippocampus in rats. Increased, shrunken myelinated neuronal cells (red arrows), reduced but normal myelinated neuronal hippocampal cells (red broken arrows), and increased and swollen myelinated hippocampal neurons (yellow broken arrows). Abnormal appearance of neurons. (Hematoxylin and Eosin stain, $\times 400$, Scale bar = $200\ \mu\text{m}$)

the recovery of locomotive dysfunction in rats is relatively the same for both lead acetate- and gabapentin-treated group.

The cognitive dimension of pain includes both motivation and memory perspectives. Body of evidence has shown that the development of neuropathic pain involve the basic neuronal mechanism of memory formation (Sandkühler and Lee 2013). CCI resulted in increased spatial reference memory in the *Wistar* rats. The untreated ligated rats demonstrated enhanced reference memory which may explain increased and long-lasting guiding behavior observed in those rats. This may also be attributed to increased perception of pain stimuli. This is in line with the view suggesting that maladaptive pain induces effective learning (Rainville 2002; Miguez et al. 2010; Sacktor 2010). The study showed that lead acetate reversed the enhanced memory retention possibly by inhibiting long-term potentiation (LTP). Inhibition of mediators that results in LTP during retention and consolidation processes have been suggested to fully prevent the development of neuropathic pain (Dableh et al.

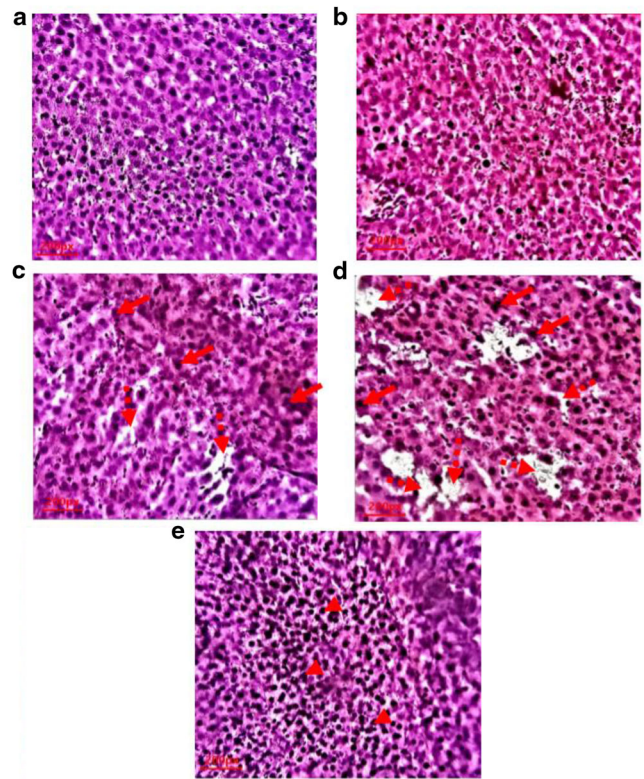


Fig. 6 Effect of lead acetate on hepatic cells in CCI-induced model of neuropathic pain. **(a)** Unligated control group showing normal hepatocytes in hepatic tissue. **(b)** Sham control group has similar hepatic tissue morphology as in unligated control. **(c)** Ligated (CCI) control hepatic tissue presenting little vacuolation (red broken arrow) and swollen of hepatocyte cells (red arrow). **(d)** Gabapentin-treated group shows severe vacuolation (red broken arrow) and swollen of hepatocyte cells (red arrow). **(e)** Lead acetate showed reduced vacuolation in liver tissue and increases the hepatocyte cells (red arrowhead) (hematoxylin and eosin stain, $\times 400$)

2011). Inhibition of such memory mediators has also been linked with mitigation of mechanical allodynic symptom (Li et al. 2010; Miguez et al. 2010). On this account, this study suggests that lead acetate reversed the enhanced memory function due to CCI. On the other hand, inhibition of the enhanced reference memory may be as a result of the neurotoxic effect of the lead acetate on the hippocampal region as increased swelling and reduced myelinated neurons were reported in the histological study. However, this may be beneficial in the neuropathic pain condition.

The toxicity effect of lead acetate was studied from the histological section of the hippocampus, liver, and kidney. Neurotoxicity (Mahmoud and Sayed 2016), hepatotoxicity (Abdou and Hassan 2014; Sayed Aly Mohamed Metwally 2015), and nephrotoxicity (Oyagbemi et al. 2015) of lead acetate at various doses have been widely reported in many experimental studies. The current study indicated that administered lead acetate dose may pose only a mild degree of neurotoxicity in the hippocampus and hepatotoxicity but not

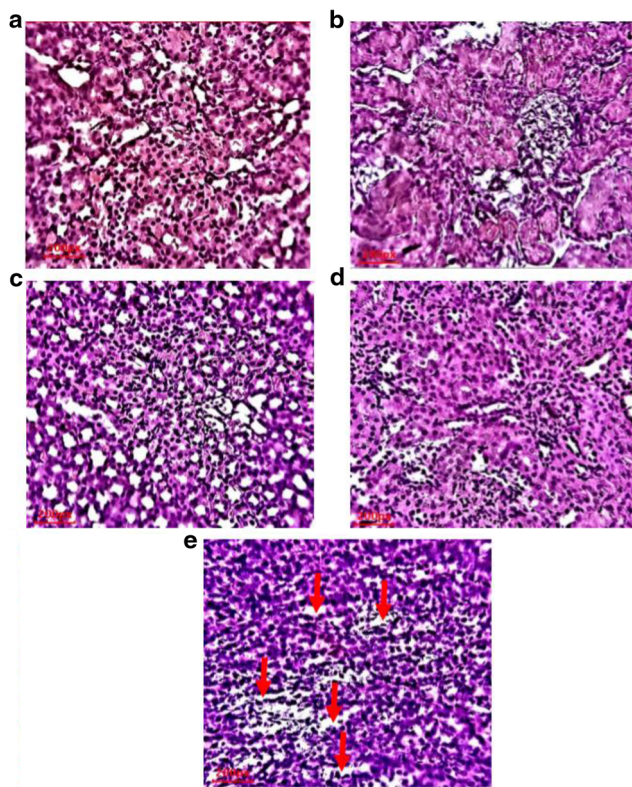


Fig. 7 Micrograph showing transverse section of Wistar rats kidney. (a and b) Unligated and sham control. (c) Ligated control. (d) CCI gabapentin-treated, and (e) CCI lead acetate-treated group. Increased vacuolation in the renal corpuscle (red arrows). (Hematoxylin and Eosin stain, $\times 400$, Scale bar = 200 μm)

nephrotoxicity. The mild neurotoxicity effect of lead on the hippocampus may be due to its counter effects on glial cells. Neuropathic pain has been reported to be characterized by increased activation and mobilization of glial cells (Yan and Weng 2013; Tiwari et al. 2014; Weng et al. 2014). However, loss of glial cells or reductions in their activities have been implicated as a mechanism that underpins maturation-dependant neurotoxicity of lead acetate (Zurich et al. 2002). Hence, the observed mild neurotoxic effect of lead acetate may be due to its inhibitory effect on neuropathic pain which ultimately affects the cells in the hippocampus. Likewise, the increase in the congestion of hepatocytes may account for a rigorous compensatory mechanism for the toxic effect of lead on the liver

In conclusion, this study revealed that lead acetate could be beneficial as an analgesic agent. It produces anti-nociceptive effects in rats induced with neuropathic pain. Severe neurotoxic and hepatotoxic effects were not observed following the administered doses of lead acetate. Since several investigative attempts are in progress to develop an effective therapeutic intervention for neuropathic pain, the findings of this study may serve as a pointer to the beneficial role of lead acetate in neuropathic pain conditions.

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Authors' contributions BVO conceived and designed the research. AOB, KA, DA, and SA conducted experiments and analyzed data. BVO and AOB contributed new reagents and analytical tools. BVO, AOB, and MA supervised the research and wrote the manuscript. All authors read and approved the manuscript and all data were generated in-house and no paper mill was used.

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Compliance with ethical standards

The study was conducted in accordance with the ARRIVE guidelines (Kilkenny et al. 2013). The protocol was also assessed and approved by the University of Ilorin Ethical Review Committee (UERC/ASN/2019/1744) before the commencement of the research.

Conflict of interest The authors declare that they have no conflict of interest.

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