A hydro-ethanolic extract of Synedrella nodiflora (L.) Gaertn ameliorates hyperalgesia and allodynia in vincristine-induced neuropathic pain in rats

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A hydro-ethanolic extract of *Synedrella nodiflora* (L.) Gaertn ameliorates hyperalgesia and allodynia in vincristine-induced neuropathic pain in rats

Abstract

Background: The hydro-ethanolic extract of *Synedrella nodiflora* (L.) Gaertn whole plant has demonstrated analgesic effects in acute pain models. The extract has also demonstrated anticonvulsant effects in murine models of experimental epilepsy. The present study illustrates an evaluation of the hydro-ethanolic extract of the plant for possible analgesic properties in hyperalgesia and allodynia associated with vincristine-induced neuropathy in rats.

Method: Neuropathic pain was induced in Sprague-Dawley rats by injecting 100 μg/kg of vincristine sulphate on alternative days for 6 days (days 0, 2, 4, 8, 10 and 12). Vincristine-induced cold allodynia, mechanical hyperalgesia and thermal hyperalgesia were measured pre-vincristine administration and on days 15, 17 and 19 post-vincristine administration. The rats were then treated with *S. nodiflora* extract (SNE) (100, 300 and 1000 mg/kg), pregabalin (10, 30 and 100 mg/kg) and distilled water as vehicle daily for 5 days and pain thresholds were measured on alternate days for 3 days.

Results: SNE and pregabalin produced analgesic properties observed as increased paw withdrawal latencies to mechanical, tactile, cold water stimuli and thermal hyperalgesic tests during the 5 days of treatment.

Conclusions: The findings suggest that hydro-ethanolic extract of *S. nodiflora* possesses anti-hyperalgesic and anti-allodynic effects in vincristine-induced neuropathic pain in rats.

Keywords: allodynia; cold; hyperalgesia; neuropathic; pain; pregabalin; *Synedrella nodiflora*; tactile; thermal; vincristine.

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Introduction

Neuropathic pain is caused by a lesion or dysfunction of the peripheral or central nervous system [1]. Major causes of neuropathic pain include diabetes mellitus [2, 3], shingles [4], multiple sclerosis [5], spinal cord injury [6], stroke [7–9], HIV infection [10], cancer [11–13], cancer chemotherapy [14, 15] and persistent postsurgical pain [16], as well as common conditions, such as lumbar or cervical radiculopathies, and traumatic or postsurgical nerve injuries [17]. About 30%–40% of cancer patients treated with chemotherapeutic agents such as vincristine [18], paclitaxel [19] and cisplatin [20] present peripheral neuropathy which often is responsible for the discontinuation of therapy [21].

Pharmacological management remains the most important therapeutic option for chronic neuropathic pain and the recommended treatment strategies are represented by tricyclic antidepressants (amitriptyline), anticonvulsants [gabapentin, pregabalin (PGB) and carbamazepine] and analgesics (tramadol and fentanyl patches) [22–26]. All these drugs have limited efficacy in pain and/or dysaesthesia relief and severe side effects interfere with effective symptom control [22]. Therefore, for an effective treatment of neuropathic pain, there is still a need to obtain therapeutic agents which possess a greater level of tolerability and safety.

Synedrella nodiflora (L.) Gaertn (family Asteraceae) is a common weed found along the banks of rivers, streams and along the roadsides [27]. In Ghana, the whole plant is boiled and the aqueous extract drunk for the treatment of epilepsy, while the leaves are used for threatened abortion, hiccup, laxative and fodder for livestock [27, 28]. The hydro-ethanolic extract of the whole plant has been found to possess anticonvulsant [29], sedative [30], in vitro antioxidant and free radical scavenging properties [31, 32], as well as anti-nociceptive properties [33]. Current toxicological assessment of the hydro-ethanolic extract from the plant suggests that it has a low toxicity profile [34]. In the present study, an animal model of vincristine-induced neuropathic pain was used to examine the potential analgesic property of hydro-ethanolic extract of *S. nodiflora* in neuropathic pain.

Materials and methods

Drugs and chemicals

Pregabalin (Lyrica®) was purchased from Pfizer Pharmaceuticals (New York, NY, USA) and vincristine sulphate (Vinlon®) from Celon Laboratories Limited (Hyderabad, India).

Plant collection and extraction

The samples of the plant were collected from the Botanical Gardens, University of Ghana, Accra in August 2012 and were identified and authenticated at Ghana Herbarium, Department of Botany, University of Ghana, Legon, Accra, where a voucher specimen (PA01/UGSOP/GH12) was kept. The hydro-ethanolic extract was prepared as previously described [29]. Briefly, the samples of the collected plant were air-dried for 7 days and powdered. The weighed quantities of the powder were cold macerated with 70% v/v of ethanol in water. The hydro-ethanolic extract was then evaporated to a syrupy mass under reduced pressure, air-dried, kept in a desiccator and the percent yield calculated. The resultant product was subsequently referred to as the extract or SNE.

Experimental animals and housing

Male Sprague-Dawley rats (Hsd:SD strain), weighing 150–200 g and 6–8 weeks old, were obtained from and maintained at the Department of Animal Experimentation, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, where all the experimental procedures were performed. All animal procedures and techniques used in these studies were approved by the Scientific and Technical Committee (STC) of the Noguchi Memorial Institute for Medical Research [reference number STC-6 (1) 2012-13] and also by the Noguchi Institutional Animal Care and Use Committee (NIACUC), College of Health Sciences, University of Ghana with protocol number NIACUC-2012-01-1E. It was also ensured that all the experiments carried out on animals conformed to the Organization for Economic Cooperation and Development (OECD) guidelines. The animals were housed in groups of five in stainless steel cages (34 cm×47 cm×18 cm) with soft wood shavings as bedding, fed normal commercial pellet diet (AGRIMAT, Kumasi), provided with water ad libitum and maintained under laboratory conditions (temperature 22±2 °C, relative humidity 60%–70%, and 1–2-h light-dark cycle). All experiments were performed during the day between the hours of 8:00 and 15:00.

Experimental design

Induction of neuropathic pain with vincristine: The induction of vincristine-induced neuropathy in Sprague-Dawley rats was performed as described [35]. Briefly, an intraperitoneal injection of 100 μg/kg of vincristine sulphate was administered to the rats during two cycles of five consecutive working days (i.e., days 1–5 and days 8–12 with 2 days off). Baseline measurements of the reaction latency of maximum force applied until paw withdrawals or vocalisations indicative of pain were taken 30 min after the injection of the vincristine using the Randall-Selitto test.

Extract/drug treatment of vincristine-induced neuropathic pain: On days 15–19 after the induction of neuropathic pain with vincristine, the rats were treated daily with SNE (100–1000 mg/kg, p.o), PGB (10–100 mg/kg, p.o) or vehicle (distilled water). PGB, which is clinically used in the management of neuropathic pain and also as anticonvulsant, is an ideal reference drug since it shares similar pharmacological characteristics as the extract, which has also demonstrated anticonvulsant [29] and antinociceptive effects [33].

Behavioural assessment of neuropathic pain: Four set of pain assessment tests [tactile allodynia using the von Frey hairs (IITC Life Science Inc., Woodland Hills, CA, USA) of 4 g; cold allodynia using cold water at 4 °C; mechanical hyperalgesia using the Randall-Selitto (IITC Life Science Inc., Woodland Hills, CA, USA) test and thermal hyperalgesia using the hotplate test (Ugo Basile, Monvalle VA, Italy)] were used to evaluate the analgesic effects of SNE and PGB in the vincristine-induced neuropathic pain on days 15, 17 and 19. Baseline and post-drug treatments were assessed and percent maximum possible effects (%MPE) were calculated based on the formula:

$$\%\text{MPE} = \frac{\text{Post-drug latency} - \text{Pre-drug latency}}{\text{Cut-off latency} - \text{Pre-drug latency}} \times 100$$

Tactile allodynia: The effects of SNE (100–1000 mg/kg) and PGB (10–100 mg/kg) on static tactile allodynia was assessed using von Frey filaments with bending forces of 4 *g* as previously described [36]. The force at 4 *g* would not elicit paw withdrawals when applied to normal rats but will do so in chemotherapy-induced neuropathic rats, thus presenting tactile allodynia (pain induced by a normally innocuous stimulus) [37]. To facilitate the determination, all the animals were placed in a restrainer. The von Frey filament was applied to the mid-plantar area (avoiding the base of the tori) of each hind paw five times, with each application being held for 5 s. Withdrawal responses to the 4 *g* von Frey filament from both hind paws were counted and expressed as an overall percentage response, that is, if a rat withdrew six times out of a total 10 von Frey applications, this was recorded as 60% overall response to the 4 *g* von Frey filament. The overall response was presented as %MPE.

Cold allodynia: The analgesic effect of orally administered SNE (100–1000 mg/kg), PGB (10–100 mg/kg) and distilled water as the vehicle on cold allodynia was assessed by immersing the rat's tail into cold water (4 °C) as previously described [38]. The latency for a rat to withdrawal its tail was measured with a digital timer with a cut-off of 20 s. The %MPE was calculated from the measurement of pre- and post-drug tail withdrawal latencies as described above.

Mechanical hyperalgesia: The effect of SNE (100–1000 mg/kg), PGB (10–100 mg/kg) and the distilled water (vehicle) on mechanical hyperalgesia was measured with the Randall-Selitto paw pressure analgesimeter (IITC Life Science Model 2888 Woodland Hills, CA, USA) as previously described [39, 40]. Briefly, the hind paw of the rat 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 vocalisation. The force (*g*) obtained 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: Thermal hyperalgesic test was performed in the rats after the vincristine-induced neuropathy using the hotplate analgesiometer as previously described [41, 42]. Briefly, the animals were gently dropped onto the hot plate that was preheated to 55 °C and cut-off latency of 20 s was observed. Paw withdrawal latency was recorded using a timer that was started when the animal is released onto the preheated plate and stopped at the moment of withdrawal, shaking, or licking of either hind paw. This latency was recorded for each animal before drug/extract administrations (time 0) and at 30 min intervals after drug/extract administration for 3 h on days 15, 17 and 19.

Statistical analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. All data were presented as mean±SEM (n=5). The time-course curves were subjected to two-way (dose×time) analysis of variance (ANOVA) followed by a Bonferroni's post-hoc test. Total MPE was calculated in arbitrary units as the area under the curve (AUC) and these were also subjected to comparison by a one-way ANOVA followed by a Dunnett's multiple comparison test. A p-value ≤ 0.05 was considered statistically significant in all the analyses (one- or two-way ANOVA). All the graphs were plotted using Sigma-Plot for Windows Version 11.0 (Systat Software Inc., Germany)

Results

Induction of neuropathic pain by vincristine

An assessment of the paw withdrawal latencies to mechanical pain during the 10-day induction of neuropathic pain yielded a gradual decline of the latencies from day 1 to day 12. There was no significant difference between the various designated groups during the induction period ($p=0.8407$, $F_{6,35}=0.4490$, Figure 1B), although a two-way ANOVA followed by a Bonferroni's post-hoc test showed a significant difference ($p \leq 0.01$) in the SNE 300 mg/kg designated group in comparison to the vehicle-designated group. However, there was significant decline of the paw withdrawal latencies from day 1 to day 12 suggesting a reduced pain threshold and an induction of peripheral neuropathy by day 12 ($p<0.0001$; an unpaired two-tailed t-test, Figure 1B).

Tactile allodynia

The daily administration of vincristine for 12 days (with a two off-day) produced a marked and prolonged dynamic

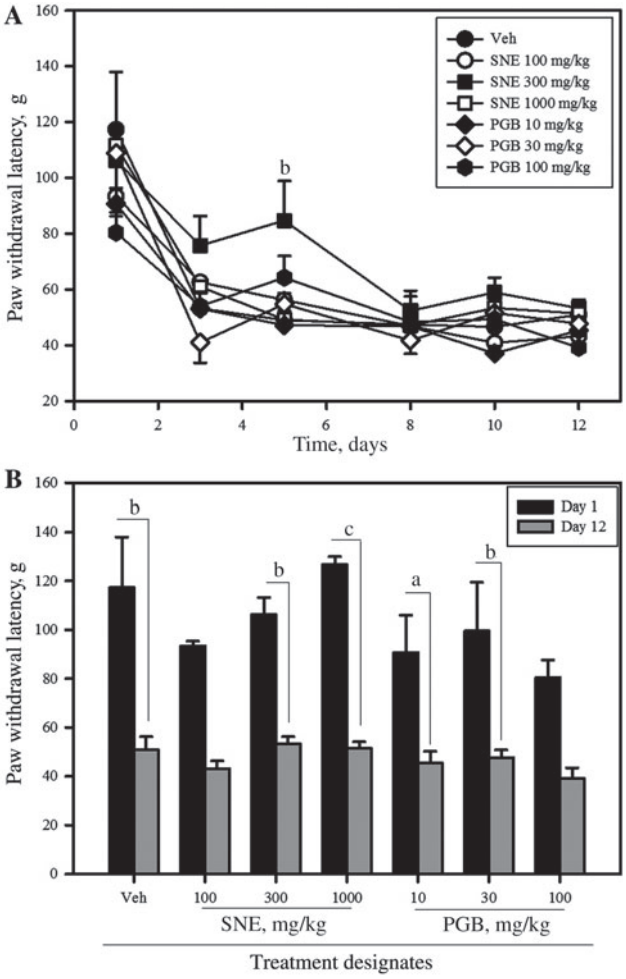

Figure 1 The effect of a daily intraperitoneal injection of vincristine sulphate (100 μg/kg) on the paw withdrawal thresholds in rats for 12 days.
(A) A time-course event from day 1, 3, 5, 8, 10 and 12. (B) A comparison of paw withdrawals on day 1 and day 12. Data are mean±SEM (n=5). [a]$p \leq 0.05$, [b]$p \leq 0.01$ and [c]$p \leq 0.001$ compared to vehicle group (two-way ANOVA followed by a Bonferroni's post-hoc test).

tactile allodynia in the rats. The vehicle control animals showed increased response to tactile allodynia compared to the treated animals. On day 15, none of the doses of SNE produced any significant anti-tactile allodynic effect in the neuropathic rats. On day 17 only SNE 300 mg/kg produced a significant ($p \leq 0.05–0.01$) effect and on day 19, both SNE 300 and 1000 mg/kg produced significant ($p \leq 0.01–0.001$) anti-tactile allodynic effect (Figure 2A). The overall anti-tactile allodynic effect of SNE was significant ($p=0.0041$, $F_{3,14}=7.020$) for only SNE 100 and 300 mg/kg and was not dose dependent (Figure 2B).

PGB produced similar effects as shown by SNE on day 15 (Figure 2C). However, on day 17 the effect produced by PGB was significant ($p \leq 0.05$) for 30 and 100 mg/kg dose (Figure 2C). On day 19, PGB dose-dependently produced significant ($p \leq 0.05–0.001$) anti-tactile allodynic effect (Figure 2C). The overall anti-allodynic effect of PGB as demonstrated by the von Frey's test was significant ($p=0.0086$, $F_{3,16}=5.513$) for only PGB 10 and 30 mg/kg and not dose-dependent (Figure 2D).

Cold allodynia

SNE increased the latency to tail withdrawal in the cold water and produced significant ($p \leq 0.01–0.001$) anti-allodynic effect at 100 and 300 mg/kg doses on days 15 and 17. However, on day 19, all the three doses of SNE (100, 300 and 1000 mg/kg) produced significant ($p \leq 0.01–0.001$) anti-cold allodynic effects (Figure 3A). The overall

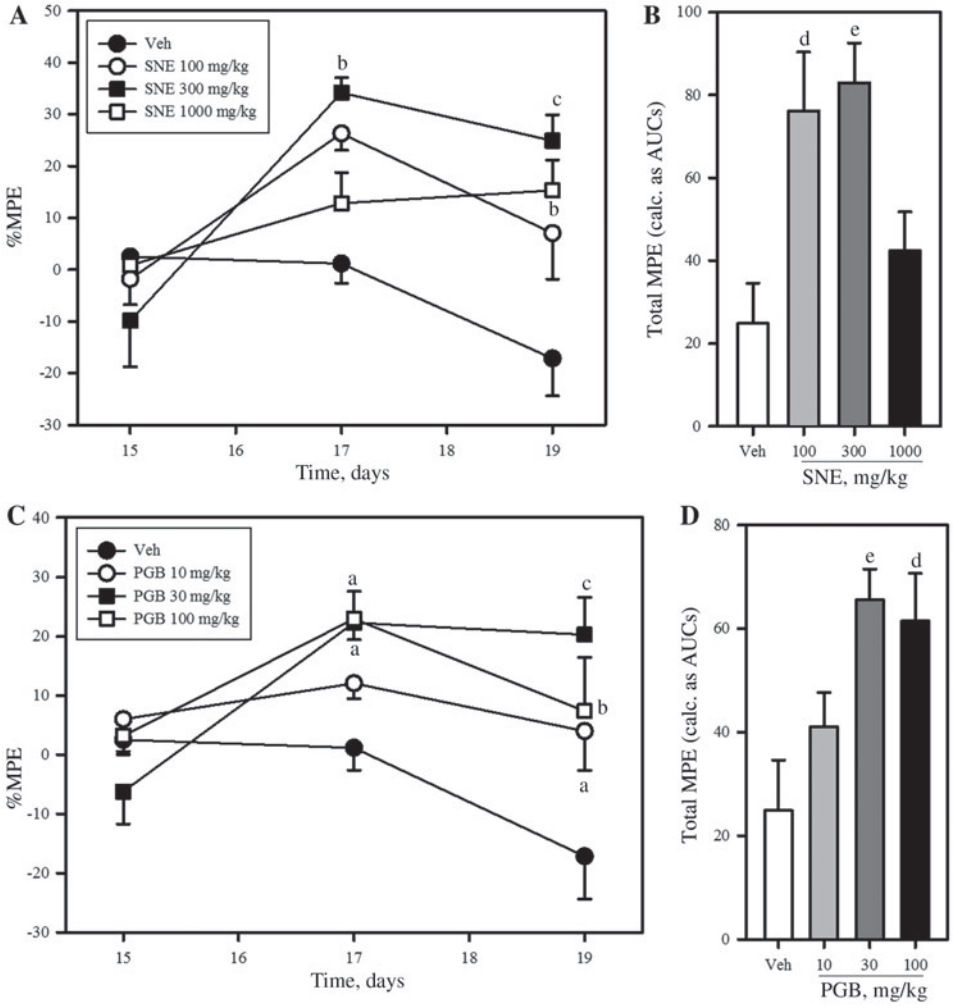

Figure 2 The effect of SNE (100–1000 mg/kg, p.o) and PGB (10–100 mg/kg, p.o) on tactile allodynia in vincristine-induced neuropathic rats. The left panels (A and C) represent a time-course effects of SNE (A) and PGB (C) on days 15, 17 and 19 after the induction of neuropathic pain. The right panels (B and C) also represent the total anti-tactile allodynic effects (total MPE calculated from the AUCs) of SNE (B) and PGB (D). Data are mean±SEM (n=5). [a]$p \leq 0.05$, [b]$p \leq 0.01$ and [c]$p \leq 0.001$ compared to vehicle group (two-way ANOVA followed by a Bonferroni's post-hoc test). [d]$p \leq 0.05$ and [e]$p \leq 0.01$ compared to vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test).

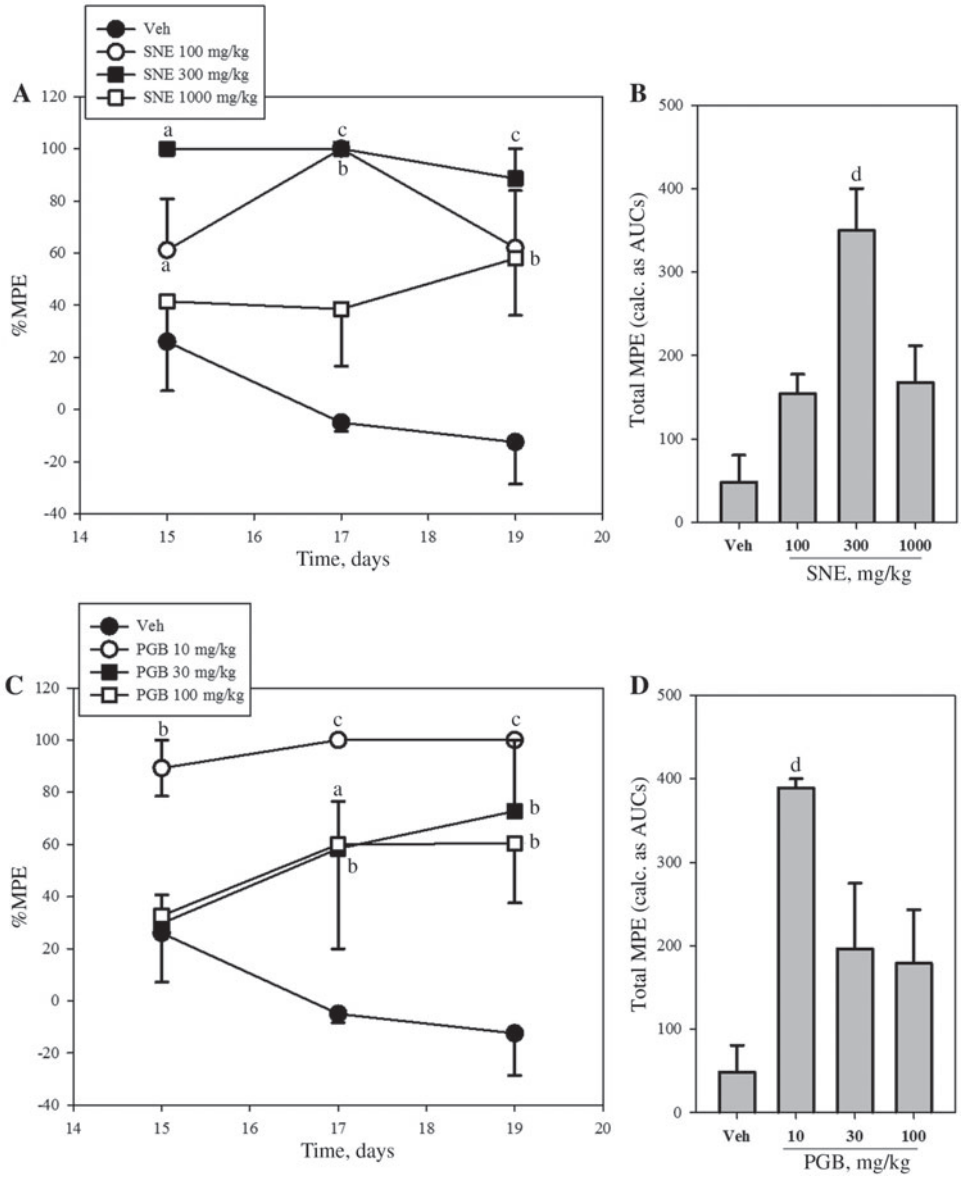

Figure 3 The effect of SNE (100–1000 mg/kg, p.o) and PGB (10–100 mg/kg, p.o) on cold allodynia in vincristine-induced neuropathic rats. The left panels (A and C) represent a time-course effects of SNE (A) and PGB (C) on days 15, 17 and 19 after the induction of neuropathic pain. The right panels (B and C) also represent the total anti-cold allodynic effects (total MPE calculated from the AUCs) of SNE (B) and PGB (D). Data are mean±SEM (n=5). [a]$p \leq 0.05$, [b]$p \leq 0.01$ and [c]$p \leq 0.001$ compared to vehicle group (two-way ANOVA followed by a Bonferroni's post-hoc test). [d]$p \leq 0.001$ compared to vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test).

anti-cold allodynic effect of SNE was only significant ($p=0.0014$, $F_{3,13}=9.491$) at 300 mg/kg dose level and not dose-dependent (Figure 3B).

PGB produced significant ($p \leq 0.01$) analgesic effect towards cold allodynia on day 15 only at a dose of 10 mg/kg (Figure 3C). However, all dose levels of PGB produced significant ($p \leq 0.05$–0.001) analgesic effect on days 17 and 19 (Figure 3C). The overall anti-cold allodynic of PGB was significant ($p=0.00012$, $F_{3,14}=9.372$) only at dose 10 mg/kg (Figure 3D).

Mechanical hyperalgesia

SNE (100, 300 and 1000 mg/kg) produced significant ($p \leq 0.0001$) analgesic effect in the Randall-Selitto mechanical hyperalgesic test on day 15. On day 15, only SNE (100 and 1000 mg/kg) produced significant ($p \leq 0.05$, $p \leq 0.001$, respectively) anti-hyperalgesic effect. However, on day 19, only the effect exhibited by SNE 1000 mg/kg was significant ($p \leq 0.001$) (Figure 4A). The overall anti-hyperalgesic effect against mechanical pain

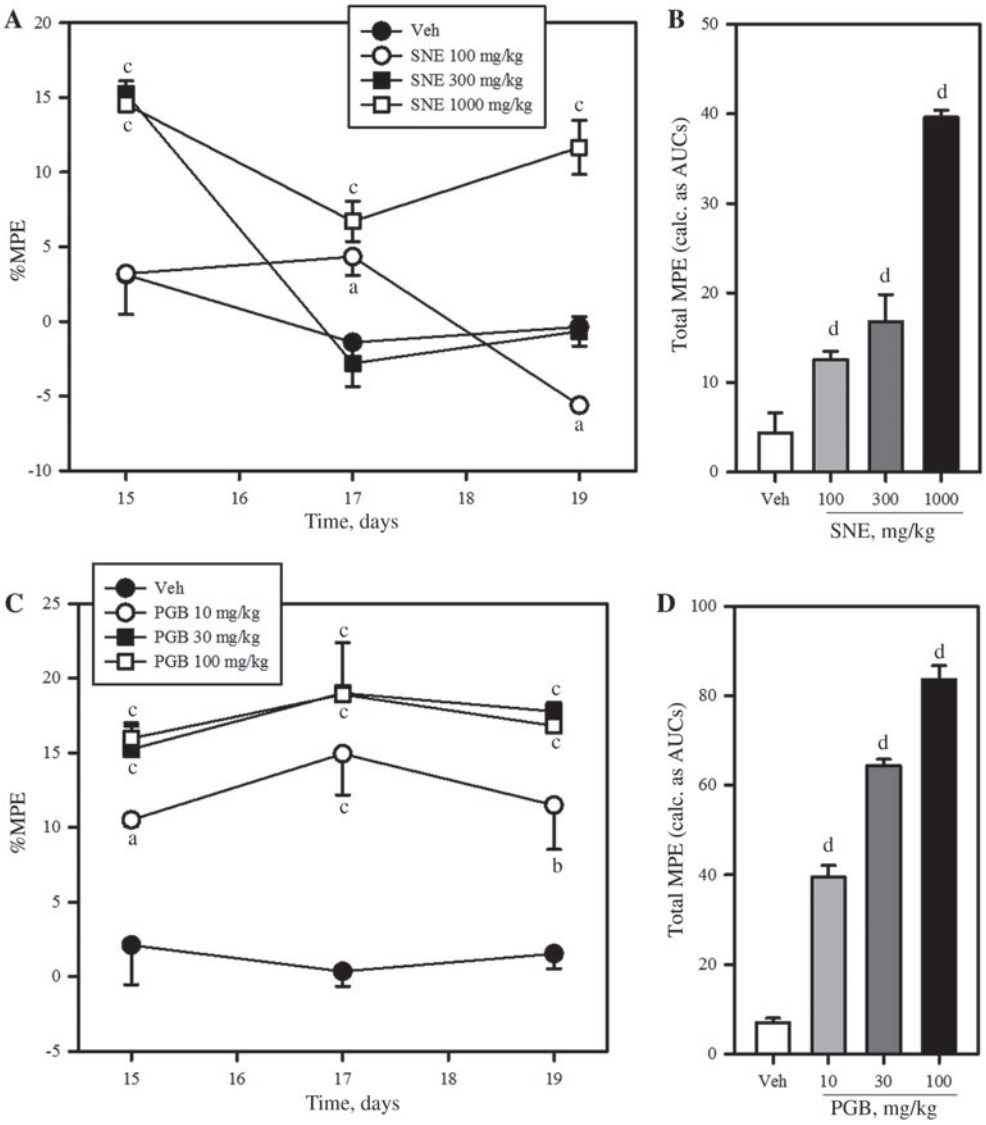

Figure 4 The effect of SNE (100–1000 mg/kg, p.o) and PGB (10–100 mg/kg, p.o) on mechanical hyperalgesia in vincristine-induced neuropathic rats.

The left panels (A and C) represent a time-course effects of SNE (A) and PGB (C) on days 15, 17 and 19 after the induction of neuropathic pain. The right panels (B and C) also represent the total anti-nociceptive effects (total MPE calculated from the AUCs) of SNE (B) and PGB (D). Data are mean±SEM (n=5). $^{a}p \leq 0.05$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.001$ compared to vehicle group (two-way ANOVA followed by a Bonferroni's post-hoc test). $^{d}p \leq 0.001$ compared to vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test).

was dose-dependently significant ($p<0.0001$, $F_{3,12}=1193$, Figure 4B).

PGB produced significant ($p \leq 0.05–0.001$) anti-hyperalgesic analgesic effect on days 15, 17 and 19 at all the dose levels tested (Figure 4C) and the overall analgesic effect was dose-dependently significant ($p<0.0001$, $F_{3,6}=221.5$, Figure 4D).

Thermal hyperalgesia

On day 15, there was a significant difference ($p=0.0306$, $F_{3,16}=3.824$) between the SNE-treated rats and the vehicle control group. Also a two-way ANOVA followed by a Bonferroni's post-hoc test revealed significant effects at 1 h ($p \leq 0.05–0.01$; SNE 100 and 300 mg/kg) and 3 h post-SNE treatment (Figure 5A and 5B). On day 17, SNE significantly ($p<0.0001$, $F_{3,16}=18.96$) increased the paw withdrawal latencies during the 3-h test period (Figure 6A) and the overall anti-hyperalgesic effect of SNE on thermally induced pain was significantly high ($p \leq 0.0001$, $F_{3,16}=18.96$) and dose dependent (Figure 6B). SNE again produced significant anti-nociceptive effect by increasing the reaction latencies to thermal pain on day 19 (Figure 7A) and the overall anti-nociceptive effects of SNE on that day was

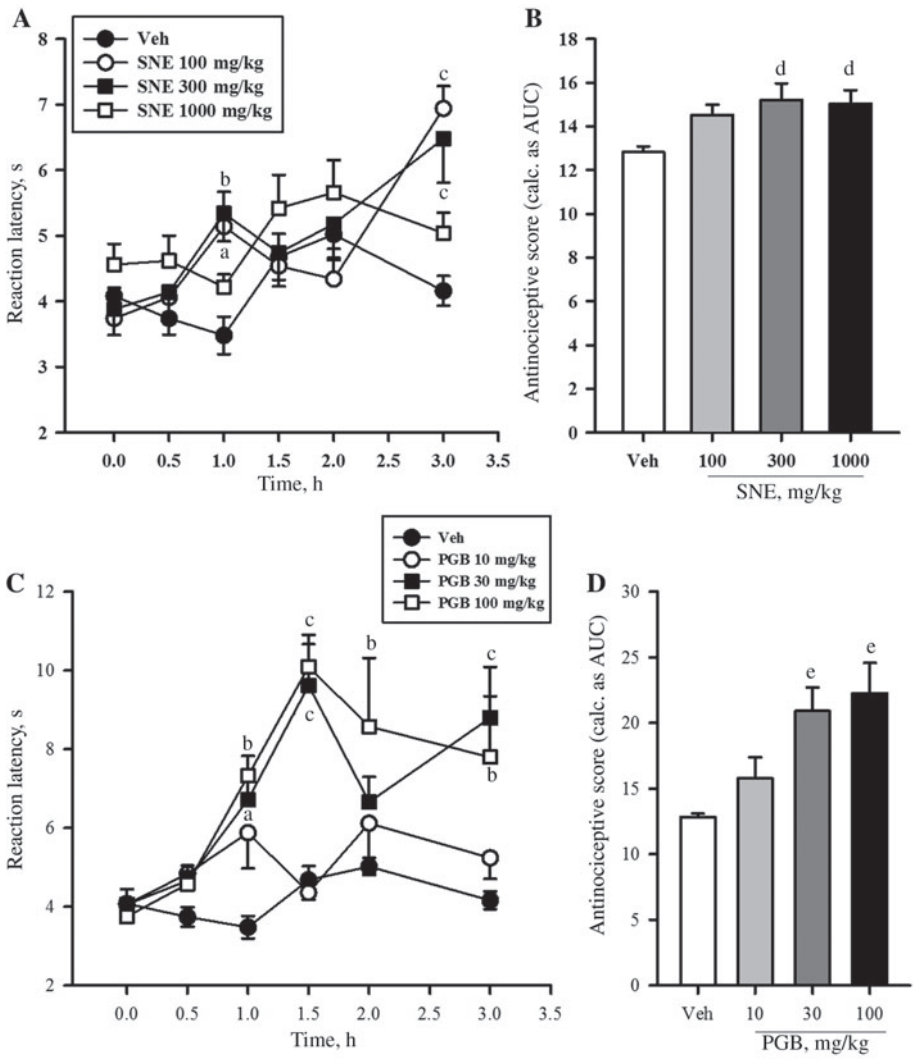

Figure 5 The effect of SNE (100–1000 mg/kg, p.o) and PGB (10–100 mg/kg, p.o) on thermal hyperalgesia in vincristine-induced neuropathic rats. The left panels (A and C) represent a time-course effects of SNE (A) and PGB (C) on day 15 after the induction of neuropathic pain. The right panels (B and C) also represent the total anti-nociceptive effects (total MPE calculated from the AUCs) of SNE (B) and PGB (D). Data are mean±SEM (n=5). $^{a}p \leq 0.05$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.001$ compared to vehicle group (two-way ANOVA followed by a Bonferroni's post-hoc test). $^{d}p \leq 0.05$ and $^{e}p \leq 0.01$ compared to vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test).

significant (p=0.0002, $F_{3,16}$=12.12) but not dose-dependent (Figure 7B).

PGB, on day 15, significantly (p=0.0027, $F_{3,15}$=7.519) and dose-dependently increased the paw withdrawal latencies and the total anti-nociceptive effect over the 3-h test period (Figure 5C). The anti-nociceptive effect was significant for only dose 30 and 100 mg/kg (Figure 5D). On day 17 and 19, PGB significantly (day 17: p<0.0001, $F_{3,15}$=29.03, Figure 6C and 6D; day 19: p<0.0001, $F_{3,15}$=19.99, Figure 7C and 7D) and dose-dependently produced anti-hyperalgesic effect during the 3-h test period post-drug treatment and the overall anti-hyperalgesic effects were significant ($p \leq 0.0001$) for all dose levels (Figures 6D and 7D).

Discussion

The present study aimed at investigating the possible analgesic effect of a hydro-ethanolic extract of *S. nodiflora* whole plant to alleviate hyperalgesia and allodynia in vincristine-induced neuropathic rats. The findings obtained clearly suggests that SNE possesses anti-hyperalgesic properties against mechanically and thermally induced hyperalgesia in the neuropathic rats. It also clearly demonstrates the anti-allodynic effect of SNE in ameliorating cold and tactile allodynia in the vincristine-induced neuropathic rats.

Intraperitoneal injection of vincristine to the rats over a total period of 10 days resulted in the induction

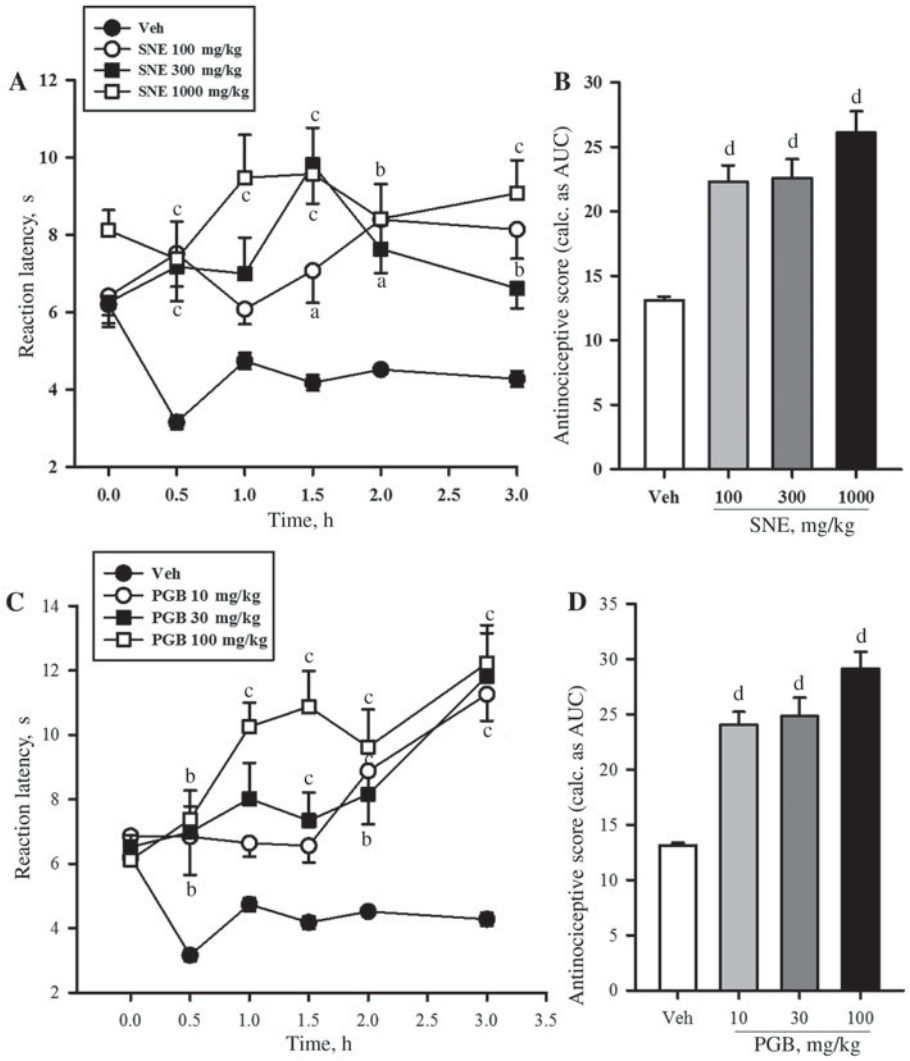

Figure 6 The effect of SNE (100–1000 mg/kg, p.o) and PGB (10–100 mg/kg, p.o) on thermal hyperalgesia in vincristine-induced neuropathic rats.

The left panels (A and C) represent a time-course effects of SNE (A) and PGB (C) on day 17 after the induction of neuropathic pain. The right panels (B and C) also represent the total anti-nociceptive effects (total MPE calculated from the AUCs) of SNE (B) and PGB (D). Data are mean±SEM (n=5). [a]$p \leq 0.05$, [b]$p \leq 0.01$ and [c]$p \leq 0.001$ compared to vehicle group (two-way ANOVA followed by a Bonferroni's post-hoc test). [d]$p \leq 0.001$ compared to vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test).

of neuropathic pain. This was demonstrated by the significant fall in the reaction latencies to thermal, cold and mechanical stimuli from the first to the last injection of vincristine. Various mechanisms have been implicated in the vincristine-induced neuropathy. Partial degeneration of the sensory nerves in the form of loss of intraepidermal warm and cool specific Aδ- and C-fibres in vincristine-induced neuropathy in rats has been associated with heat and cold allodynia [37, 43, 44]. The loss of these intraepidermal nerves has also been recognised in other neuropathic pain syndromes such as in diabetes mellitus, post-herpetic neuralgia and complex regional pain syndrome (CRPS) type-I [45]. Since SNE attenuated the neuropathy induced by the administration of vincristine, it is quite probable that it may do so by inhibiting pain stimuli propagation in the degenerated unmyelinated and myelinated C-, Aδ-, and Aβ-fibres.

Increases in 5-HT_{2A} receptors on the dorsal horn and dorsal root ganglia (DRG) neurons and sensitisation of both peripheral nociceptive fibres and spinal dorsal horn neurons have been implicated in vincristine-induced neuropathic pain [46, 47]. Thus, future work aimed at determining the mechanism of anti-nociceptive activity of the extract in vincristine-induced neuropathy can pursue the involvement of the serotonergic pathway. Free radical generation, secondary to an increase in cytosolic

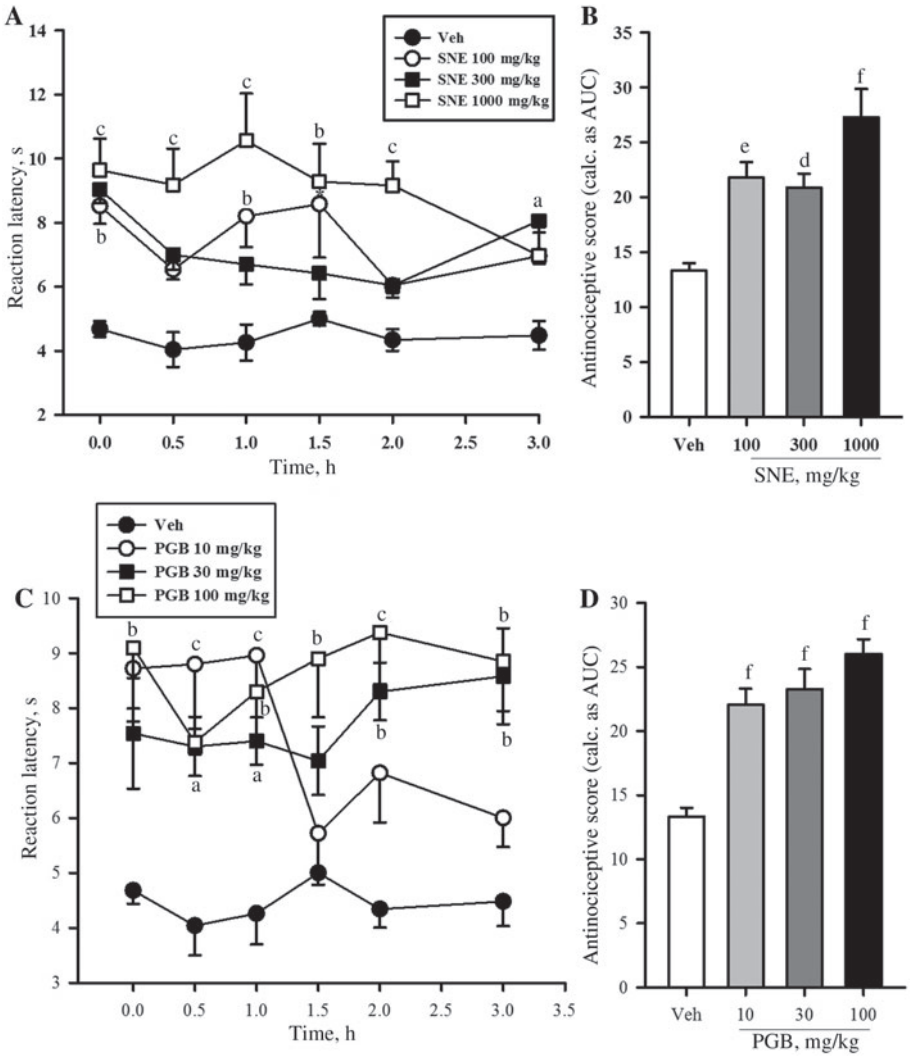

Figure 7 The effect of SNE (100–1000 mg/kg, p.o) and PGB (10–100 mg/kg, p.o) on thermal hyperalgesia in vincristine-induced neuropathic rats. The left panels (A and C) represent a time-course effects of SNE (A) and PGB (C) on day 17 after the induction of neuropathic pain. The right panels (B and C) also represent the total anti-nociceptive effects (total MPE calculated from the AUCs) of SNE (B) and PGB (D). Data are mean±SEM (n=5). [a]$p \leq 0.05$, [b]$p \leq 0.01$ and [c]$p \leq 0.001$ compared to vehicle group (two-way ANOVA followed by a Bonferroni's post-hoc test). [d]$p \leq 0.05$, [e]$p \leq 0.01$ and [f]$p \leq 0.001$ compared to vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test).

calcium, produces neuronal cytotoxicity in vincristine-induced neuropathy and the role of oxidative stress in the development of neuropathic pain in other cancer chemotherapy-induced neuropathic pain models has been well documented [42, 48–50]. There is sufficient evidence that the hydro-ethanolic extract and other solvent extracts from *S. nodiflora* possess significant anti-lipid peroxidation, free radical scavenging and antioxidant properties [31, 32, 51] and thus, the role of the antioxidant effect of SNE in this murine model of neuropathic pain requires further investigation [42].

Neuroinflammation, secondary to increased release of TNF-α and IL-1, IL-6 and NO from glial cells, macrophages and LC cells and subsequent hypersensitisation of primary sensory afferents, have also been implicated as mechanism of vincristine-induced neuropathy [43, 52–54]. A demonstrated anti-inflammatory property of SNE may be responsible for its beneficial effects towards the vincristine-induced neuropathic pain [33, 55].

Other mechanisms implicated in the vincristine-induced neuropathy involves increased sodium ion current in the DRG predisposing to paraesthesia and fasciculations [56, 57] and an increase in cytosolic calcium from extracellular (by channels) and intracellular stores from mitochondria [43, 53, 58].

The exact mechanism of the anti-hyperalgesic and anti-allodynic effects of SNE in vincristine-induced neuropathic pain in rats was not investigated in this study.

Thus, further research demonstrating clearly defined mechanism(s) by which SNE produced these effects in the rats is warranted. The extract has also been found to contain glycosides, saponins, tannins and alkaloids [29] and one or more of these phytoconstituents may be responsible for these effects. Therefore, an attempt should be made to isolate and characterise the active phytoconstituent(s) responsible for these anti-allodynic and anti-hyperalgesic activities.

The current findings are consistent with others that indicate that PGB is clinically effective in managing neuropathic pain [59–61]. It binds to α_2-δ_1 subunit of the voltage-gated calcium-gated channels, decreasing the release of glutamate, norepinephrine and substance P and preventing the binding of the neurotransmitter to their receptor, thus reducing the neuronal hyperexcitability [62, 63]. These effects and other cellular enzymatic cascade reactions induced by PGB may result in decreased sensitivity to pain. It is therefore possible that SNE, among other mechanisms, may have blocked pain in this model by inhibiting calcium channels similar to PGB.

In conclusion, the hydro-ethanolic extract of *S. nodiflora* possesses anti-hyperalgesic and anti-allodynic effects in vincristine-induced neuropathic pain in rats.

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Conflict of interest statement

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. The funding organisation(s) played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report or in the decision to submit the report for publication.
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