Sachin Goyal et al. / Journal of Pharmacy Research 2016,10(11),683-691

Research Article
ISSN: 0974-6943

Available online through
http://jprsolutions.info

Evaluation of the protective effects of *Sapindus trifoliatus* aqueous extract on vincristine induced neuropathic pain in rats

Sachin Goyal[*1], Gopal Menaria[2], Dileep Kumar[1], Himanshu Paliwal[1], Shivali Singla[1], Gurudas Khilnani[3], Mukul Mathur[4]

[1]Department of Pharmacy, Mewar University, Gangrar, Chittorgarh, Rajasthan,India.
[2]Pacific College of Pharmacy, Udaipur, Rajasthan,India.
[3]RNT Medical College, Udaipur, Rajasthan,India.
[4]SMS Medical College, Jaipur, Rajasthan,India.

Received on:30-09-2016; Revised on: 27-10-2016; Accepted on: 31-10-2016

ABSTRACT

Aim: Evidences have been generated over past years that saponins possess anti-convulsant, neuroprotective, anti-neuralgic, anti-nociceptive, anti-migraine and anti-inflammatory effects. It has been documented that saponin is one of the major constituents in aqueous extract of *Sapindus trifoliatus*. Present study investigated the protective effects of *Sapindus trifoliatus* aqueous extract (AE-ST) on vincristine induced neuropathic pain in rats. **Materials and methods:** Seven groups, each comprising of six SD rats, were employed in the present study. Painful neuropathy was induced in rats by administration of vincristine sulfate (75 µg/kg, i.p.) for 10 consecutive days. Spontaneous, mechanical, chemical and thermal nociceptive sensations were assessed at different time intervals i.e., 0, 3, 7, 9, 14 and 21. Biochemical alterations were determined on 21st day along with histopathological evaluations. Vincristine rats were co-administered aqueous extract of *Sapindus trifoliatus* (300, 100 and 30 mg/kg, p.o.) and pregabalin (10 mg/kg, p.o.) for 21 consecutive days. The data from the behavioral results were statistically analyzed by two-way ANOVA followed by Bonferonni's post test. Data from the biochemical results were statistically analyzed by one-way ANOVA followed by Tukey's multiple range test. Comparison results $p < 0.05$ were considered statistically significant. **Results:** Administration of AE-ST significantly ($p < 0.05$) attenuated vincristine induced development of painful behavioral, biochemical and histological changes in a dose dependent manner similar to that of standard drug pregabalin as compared to vincristine control. **Conclusion:** On the basis of data in hand, it may be concluded that AE-ST has potential ameliorating role in vincristine induced neuropathy which may be attributed to its multiple actions including anti-oxidative, anti-inflammatory and neuroprotective actions.

KEYWORDS: *Sapindus trifoliatus*, Neuropathic pain, Vincristine, Oxidative stress, Neuroprotective

1. INTRODUCTION

The International Association for the Study of Pain (IASP) defines neuropathic pain as ''pain initiated or caused by a primary lesion or dysfunction or transitory perturbation in the peripheral or central nervous system'' and is characterized by spontaneous pain, hyperalgesia, allodynia, which persist for a long time even after the resolution of an initial injury.[1-3] Various anti-cancer chemotherapeutic agents including vincristine, oxaliplatin, paclitaxel and cisplatin are well reported to produce peripheral neuropathic pain as their main side effect.[4-8] Dying-back neuropathy (distal axonopathy) has reported to be associated with toxic disturbance caused by chemotherapeutic agents like vincristine.[8]

Recently, some preclinical outcome have shown therapeutic efficacy of drugs from plant origin such as *Commiphora mukul*, *Cannabis sativa*, *Ocimum sanctum* and *Ginkgo biloba* in neuropathic pain.[9-12] This is also supported by few clinical studies which have evidenced the beneficial effect of herbal medicines in neuropathic pain syndrome.[13,14] Therefore, ample scope of the new herbal medicine to combat the management of neuropathic pain syndromes is expected.

Sapindus trifoliatus is very commonly used in Indian Ayurvedic healing system.[15] In folklore practice, some of the tribes of Orissa (India) use the decoction of the aerial parts of the plant for the treatment of diabetes mellitus. Fruits of *Sapindus trifoliatus* have been considered as a tonic, stomachic, alexipharmic and astringent

***Corresponding author.**
Dr. Sachin Goyal (M. Pharm, PhD, PDF)
Department of Pharmacy
NH – 79, Gangrar
Chittorgarh – 312901
Rajasthan (India)

and also useful in chronic dysentery, diorrhoea, cholera, hamicrania, tubercular glands, and paralysis and epileptic fits in children. The root is used as a collyrium in sore eyes and opthalmia. The seeds are employed to stimulate the uterus in childbirth and to increase mensuration.[16-20] *Sapindus trifoliatus* has various phytochemical constituents, of these saponins are regarded as the active component responsible for the observed pharmacological effects.[17,19,20]

Saponins play numerous functional and pathophysiological roles in the biological systems such as immune responses[21], anti-convulsant[22], neuroprotective and neuropathic pain[23] and phagocytic action[24]. Triterpenoid saponins have been reported to produce the anti-neuralgic effect[23]. *Sapindus trifoliatus* is also known to possess triterpenoid saponins[17,19,20]. Recent studies suggest that aqueous extract of *Sapindus trifoliatus* has an inhibitory activity on both peripheral and central pain mechanisms.[25] However, beneficial effect of *Sapindus trifoliatus* in the painful neuropathy remains to be explored. Therefore, the present study has been designed to investigate the ameliorative potential of aqueous extract of *Sapindus trifoliatus* in vincristine induced painful neuropathy in rats. Pregabalin (Lyrica™) a selective N-type voltage dependent calcium channel blocker, an anti-convulsant drug with significant analgesic and neuroprotective actions served as positive control in this study. [26,27]

2. METERIALS AND METHODS

2.1. Plant material

Pharmacognostically identified dried pericarps of fruits of *Sapindus trifoliatus* Linn., family Sapindaceae were collected from the local market and authenticated from Dr. M. S. Rathore, Professor and Head, Department of Botany, B. N. P. G. College, Udaipur, India.

2.2. Drugs and chemicals

Pregabalin was obtained as a gift sample (Torrent Research Centre, Gandhinagar). NBT (Nitro Blue Tetrazolium), EDTA (Ethylenediaminetetraacetic acid), H_2O_2 (Hydrogen peroxide), TCA (Trichloroacetic acid), DTNB [5, 5-dithiobis (2-nitrobenzoic acid)] and TBA (Thiobarbituric acid) were procured from Merck and S. D. Fine Chemicals Private Limited. All the chemicals used in the present study were of analytical grade.

2.3. Extraction

An amount of 100 g of the pericarp of fruit of *Sapindus trifoliatus* were soaked in 400 ml of distilled water for 16 h. The percolate was then decanted, centrifuged and filtered through Whatman (No. 1) filter paper to obtain clear extract (300 ml). This process of extraction was repeated again with same volume of distilled water. The percolates were pooled and lyophilized to give a brown colored powder (68% yield). Estimation of saponins present in the extract was calculated by HPLC method as described by Arulmozhi et al.[19]

2.4. Animal and maintenance

Male Sprague-Dawley rats of body weight between 200-230 g were used for neuropathic pain model. All experiments were approved by the Institutional Animal Ethics Committee (1622/PO/a/12/CPCSEA). Each rat was housed in plastic box cage individually with well controlled supplied air, humidity (< 70%) and temperature under a 12 h light/dark cycle with food and water *ad libitum*.

2.5. Induction of peripheral neuropathy by vincristine

Peripheral painful neuropathy was induced in rats by administration of vincristine as per the method of Authier et al.[28] Briefly, 75 µg/kg of vincristine was administered intraperitoneally for 10 consecutive days.

2.6. Experimental protocol

Seven groups, each comprising of six SD rats, were employed in the present study. Group I (Normal control): Rats were not subjected to any drug or vehicle administration and were kept for 21 days. Group II (Vincristine control): Rats were administered vincristine (VIN, 75 µg/ kg, i.p., daily, for 10 days). Group III (Vehicle in VIN): Carboxy methyl cellulose (CMC) (0.5% w/v, p.o.) was administered for 21 days in rats subjected to vincristine administration. Group IV, V and VI (AE-ST in VIN): Aqueous extract of Sapindus trifoliatus (300, 100 and 30 mg/kg, p.o., respectively) was administered for 21 days in rats subjected to vincristine administration. Group VII (Pregabalin in VIN): Pregabalin (10 mg/kg, p.o.) was administered for 21 days in rats subjected to vincristine administration. The dose selection was based on earlier reported oral toxicity studies of AE-ST. [18,29,30]

The behavioral tests, i.e., spontaneous pain, mechanical and cold allodynia, mechanical and thermal hyperalgesia were assessed on different days, i.e., day 0, 3, 7, 9, 14, and 21. On 21st day, all the animals were sacrificed subjected to biochemical and histopathological analysis for estimation of total protein, thiobarbituric acid reactive substances, reduced glutathione, catalase and superoxide dismutase along with axonal degeneration.

2.7. Behavioral examination

Nociceptive assays aimed at determining the severity of behavioral neuropathic parameters, namely spontaneous pain, allodynia and hyperalgesia were performed. The assays involved measurement of

the degree of spontaneous (ongoing) pain and tests of hind limb withdrawal to cold, thermal and mechanical stimuli (dynamic mechanical allodynia, cold allodynia, mechanical hyperalgesia and thermal hyperalgesia).

2.7.1. Spontaneous pain

Spontaneous pain was assessed for a total time period of 5 min as described previously by Choi et al. The operated rats were individually placed inside an observation cage and an initial acclimatization period of 10 min was given to each of the rat. The cumulative duration that the rat holds its ipsilateral paw off the floor was noted. The paw lifts associated with locomotion or body repositioning was not counted. For each measurement, three successive readings were taken without any elapse and the mean was calculated. [31]

2.7.2. Dynamic component of mechanical allodynia

Dynamic allodynic response was assessed according to the procedure described by Field et al. A positive dynamic allodynia response consisted of lifting the affected paw for a finite period of time in response to mild stroking on the planter surface using a cotton bud. This stimulus is non-noxious to a normally behaving rat. The latency to paw withdrawal was then noted. If no paw withdrawal was shown within 15 sec, the test was terminated and animal was assigned non-responsive. For each measurement, three successive readings were taken with 3 min elapsed between each test and mean was calculated. [32]

2.7.3. Cold allodynia

The rats demonstrating unilateral mononeuropathy were assessed for acute cold allodynia sensitivity using the acetone drop application technique, as described by Caudle et al. Few drops (100-200 µl) of freshly dispend acetone were squirted as a fine mist onto the mid-planter region of the affected paw. A cold allodynic response was assessed by noting the duration of the paw withdrawal response. For each measurement, the paw was sampled 3 times with 3 min elapsed between each test and the mean was calculated. [33]

2.7.4. Mechanical hyperalgesia

Mononeuropathic rats were assessed for mechanical hyperalgesia sensitivity according to the procedure described by Gonzalez et al. Hind paw withdrawal duration was measured after a mild pin-prick stimulus to the mid-planter surface of the ipsilateral hind paw. A withdrawal was defined as being abnormally prolonged if it lasted for at least 2 sec. The mean duration of withdrawal was taken from a set of three responses with 3 min elapsed between each test. [34]

2.7.5. Thermal hyperalgesia

Thermal nociceptive threshold, as an index of thermal hyperalgesia was assessed according to the procedure described by Eddy and Leimbach. The hot plate was pre-heated and maintained at a temperature of 52.5 ± 0.5°C. The operated rats were placed on the heated surface and the time interval between placements and shaking, licking or tucking of the affected hind paws was recorded as the latency response. If no paw withdrawal was shown within 22 sec, the test was terminated and animal was assigned non-responsive. For each measurement, three successive readings were taken with 3 min elapsed between each test and mean was calculated. [35]

2.8. Biochemical estimation

On the 21st day, immediately after the behavioral quantification, the animals were sacrificed by administering high dose of anesthesia. Portion of the sciatic nerve proximal to sciatic trifurcation was isolated immediately and rinse with ice cold normal saline. The uniformity among the different nerve samples was maintained by taking the constant weight of the respective samples. For histopathological studies, samples were stored in 10% formalin. Further, the rest of the samples were kept for biochemical estimation. A 10% (W/V) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 × g for 15 min and aliquots of supernatants were separated and further used for estimating total protein, thiobarbituric acid reactive substances, reduced glutathione, catalase and superoxide dismutase.

2.8.1 Estimation of total protein content

Protein concentration was estimated by biuret method using bovine serum albumin (BSA) as a standard.[36]The absorbance was determined spectrophotometrically at 540 nm.

2.8.2. Estimation of thiobarbituric acid reactive substances

The estimation of lipid peroxidation was done by measuring the thiobarbituric acid reactive substances as described by Wills. The amount of malondialdehyde (MDA), a measure of lipid peroxidation was measured spectrophotometrically by reaction with thiobarbituric acid at 532 nm. The values were calculated using molar extinction coefficient of chromophore (1.56 × 105 M.1 cm.1) and expressed as nmoles of MDA per milligram protein.[37]

2.8.3. Estimation of reduced glutathione levels

The reduced glutathione levels were measured according to the method of Ellman. Supernatant (1 ml) was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4°C for 1 h. The sample was centrifuged at 1,200 × g for 15 min at 4°C. To 1 ml of this

supernatant, 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) were added. The yellow color developed was immediately read spectrophotometrically at 412 nm. Results were calculated using molar extinction coefficient of chromophore (1.36 × 104 M.1 cm.1) and expressed as microgram GSH per milligram protein.[38]

2.8.4. Estimation of catalase

Catalase activity was assayed by the method of Luck, where the breakdown of hydrogen peroxide (H_2O_2) is measured at 240 nm. Briefly, assay mixture consisted of 3 ml of H_2O_2 phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and change in absorbance was recorded at 240 nm. The results were expressed as micromoles of H_2O_2 decomposed/ milligram of protein/ min (U/ mg protein).[39]

2.8.5. Estimation of superoxide dismutase

Superoxide dismutase activity was assayed by the method of Kono. Change in absorbance was measured spectrophotometrically at 560 nm.The results were expressed as SOD units/ milligram of protein. [40]

2.9. Histopathological evaluation

Samples of sciatic nerve were stored in the fixative solution (10% formalin) and cut into 4 mm thickness. Staining was carried out by using hematoxylin and eosin as described in the method of Sudoh et al. Nerve sections were analyzed qualitatively under light microscope (450 x) for axonal degeneration.[41]

2.10. Statistical analysis

All the results were expressed as mean ± SEM. The data from the behavioral results were statistically analyzed by two-way ANOVA followed by Bonferonni's post test. Data from the biochemical results were statistically analyzed by one-way ANOVA followed by Tukey's multiple range test. Comparison results $p < 0.05$ were considered statistically significant. The statistical software package Graph pad prism (Version 5.0) was used for the analysis.

3. RESULTS

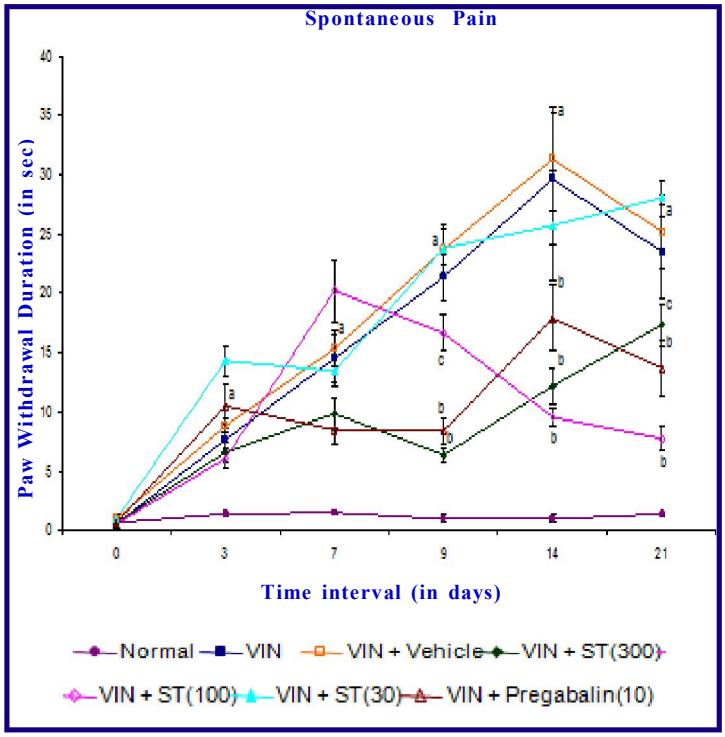
3.1. Behavioral examination

All animals included in this study exhibited characteristic neuropathic pain behavior in behavioral examination on day 3 of vincristine administration when compared with pre-vincristine values.

3.1.1. Effect on spontaneous pain: PWD

Vincristine administration resulted in a significant development of spontaneous pain as indicated by increase left hind paw withdrawal duration when compared to normal control (Figure 1). Administration of AE-ST (300 and 100 mg/kg, p.o.) attenuated vincristine induced increase in the spontaneous paw withdrawal duration in a dose dependent manner as compared to vincristine control but the response was observed after nine subsequent dosing. Treatment of pregabalin also produced similar effects. However, AE-ST (30 mg/kg, p.o.) and vehicle treated group did not show any changes in vincristine induced spontaneous pain behavior.

Figure 1: Effect of *S. trifoliatus* in reversing the spontaneous pain response in vincristine rats along with pregabalin.

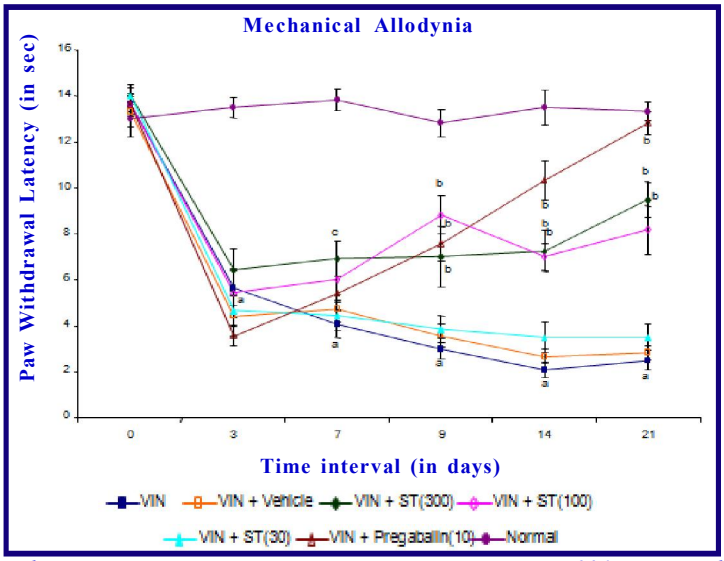


Values are mean ± SEM, n = 6 rats per group. a = P < 0.001 vs. normal control. b = P < 0.001 and c = P < 0.05 vs. vincristine control.

3.1.2. Effect on mechanical allodynia: PWL

Vincristine administration resulted in a significant development of mechanical allodynia as indicated by decrease in left hind paw withdrawal latency when compared to normal control (Figure 2). Administration of AE-ST (300 and 100 mg/kg, p.o.) attenuated vincristine induced decrease in the mechanical nociceptive latency i.e., mechanical allodynia from day 9 as compared to vincristine control (Figure 2). Similar effects were seen with pregabalin administration. However, AE-ST (30 mg/kg, p.o.) did not alter vincristine induced mechanical allodynia (Figure 2). Moreover, there was no statistical difference between the anti-allodynic effects of 300 and 100 mg/kg dose of AE-ST in vincristine administered rats. The vehicle administration did not modulate vincristine induced alteration in mechanical nociception.

Figure 2: Effect of *S. trifoliatus* in reversing the mechanical allodynia response in Vincristine rats along with pregabalin.

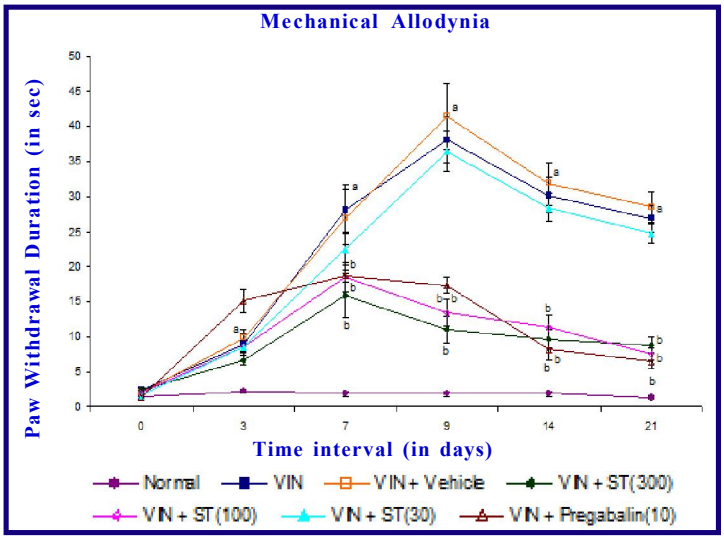


Values are mean ± SEM, n = 6 rats per group. a= P < 0.001 vs. normal control. b= P < 0.001 vs. vincristine control.

3.1.3. Effect on chemical allodynia: PWD

Vincristine administration resulted in the development of chemical allodynia as reflected by a significant increase in the left hind paw withdrawal duration in the acetone drop application compared to normal control (Figure 3). Administration of AE-ST (300 and 100 mg/kg p.o.) attenuated CCI induced increase in the withdrawal duration from day 7 in a significant manner as compared to vincristine control (Figure 3). Moreover, statistical difference was not observed between the anti-allodynic effects of 300 and 100 mg/kg dose of AE-ST in vincristine administered rats (Figure 3). Similar effects were seen with pregabalin administration. The AE-ST (30 mg/kg, p.o.) and vehicle administration did not modulate vincristine induced alteration in chemical nociception.

Figure 3: Effect of *S. trifoliatus* in reversing the chemical allodynia response in vincristine rats along with pregabalin.

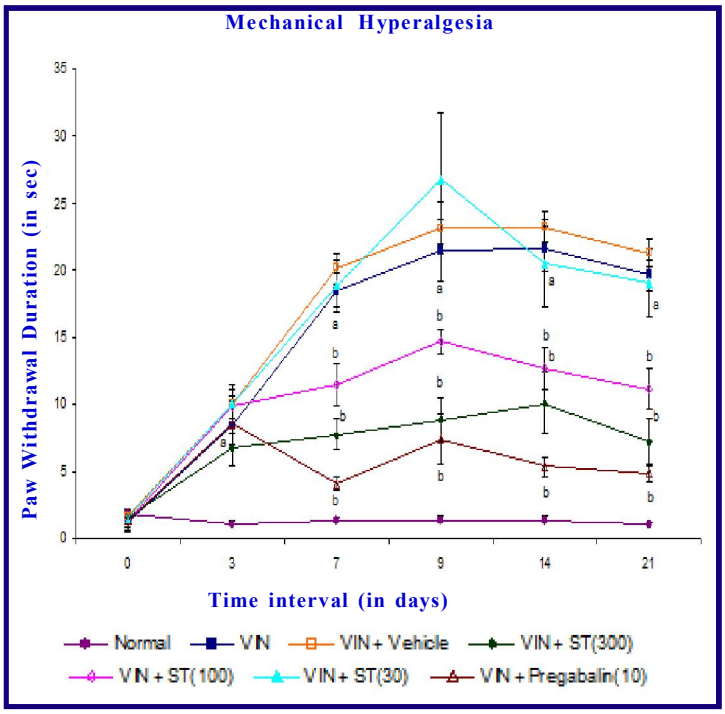


Values are mean ± SEM, n = 6 rats per group. a= P < 0.001 vs. normal control. b= P < 0.001 vs. vincristine control.

3.1.4. Effect on mechanical hyperalgesia: PWD

Vincristine administration resulted in the development of mechanical hyperalgesia as reflected by a significant increase in the left hind paw withdrawal duration in the pinprick test compared to normal control (Figure 4). Administration of AE-ST (300 and 100 mg/kg p.o.) attenuated vincristine induced increase in the withdrawal duration from day 7 in a dose dependent manner as compared to vincristine control (Figure 4). Similar effects were seen with pregabalin administration. However, administration of AE-ST (30 mg/kg, p.o.) and vehicle did not modulate vincristine induced alteration in mechanical hyperalgesic behavior.

Figure 4: Effect of *S. trifoliatus* in reversing the mechanical hyperalgesia response in vincristine rats along with pregabalin.

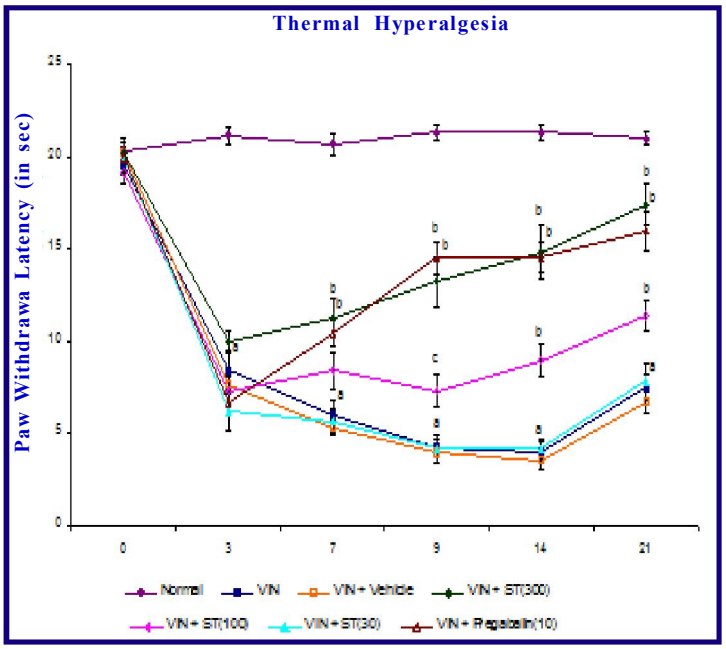


Values are mean ± SEM, n = 6 rats per group. a = P < 0.001 vs. normal control. b = P < 0.001 vs. vincristine control.

3.1.5. Effect on thermal hyperalgesia: PWL

Vincristine administration resulted in a significant development of thermal hyperalgesia reflected by a significant decrease in the left hind paw withdrawal latency in the hot plate test compared to normal control (Figure 5). Administration of AE-ST (300 and 100 mg/kg, p.o.) attenuated vincristine induced decrease in the thermal nociceptive latency i.e., thermal hyperalgesia from day 7 in dose dependent manner as compared to vincristine control (Figure 5). Similar effects were seen with pregabalin administration. However, AE-ST (30 mg/kg, p.o.) and vehicle did not alter vincristine induced thermal hyperalgesia (Figure 5).

Figure 5: Effect of *S. trifoliatus* in reversing the thermal hyperalgesia response in vincristine rats along with pregabalin.



Values are mean ± SEM, n = 6 rats per group. a = P < 0.001 vs. normal control. b = P < 0.001 and c = P < 0.05 vs. vincristine control.

3.2. Effect of AE-ST on oxidative stress markers

Vincristine administration resulted in a significant decrease in the levels of reduced glutathione, superoxide dismutase, catalase and increase in the levels of thiobarbituric acid reactive substances, as compared to normal control (Table 1). Administration of the AE-ST (300 and 100 mg/kg, p.o.) attenuated vincristine induced decrease in the levels of reduced glutathione, superoxide dismutase, catalase and increase in the level of thiobarbituric acid reactive substances in a significant manner as compared to vincristine control (Table 1). On the other hand, 30 mg/kg dose of AE-ST did not attenuate vincristine induced alteration in oxidative stress markers (Table 1). However, vehicle treated group did not show any significant changes in vincristine induced oxidative stress marker changes.

Table 1: Effect of AE-ST on oxidative stress markers in vincristine induced neuropathic pain.

Groups	Total protein (mg/ml)	LPO (nmol/mg protein)	GSH (µg/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Normal	1.13 ± 0.16	1.75 ± 0.08	32.47 ± 1.81	10.66 ± 0.12	7.75 ± 0.13
Vincristine	1.11 ± 0.05	5.88 ± 1.02[a]	11.52 ± 1.52[a]	4.83 ± 0.2 [a]	1.67 ± 0.18[a]
VIN + vehicle	1.12 ± 0.07	5.71 ± 0.82[a]	13.36 ± 1.21[a]	4.34 ± 0.19 [a]	1.59 ± 0.19[a]
VIN + ST 300	1.14 ± 0.09	1.77 ± 0.33[b]	29.36 ± 1.97[b]	10.49 ± 0.16[b]	5.54 ± 0.1[b]
VIN + ST 100	1.08 ± 0.05	2.04 ± 0.22[b]	24.33 ± 1.36[b]	8.77 ± 0.11[b]	5.14 ± 0.29[b]
VIN + ST 30	1.09 ± 0.08	4.34 ± 1.04	15.26 ± 1.32	6.03 ± 0.35	2.66 ± 0.15
VIN +Pregabalin10	1.07 ± 0.06	1.99 ± 0.34[b]	22.53 ± 1.69[b]	9.77 ± 0.14[b]	5.43 ± 0.37[b]

Values are mean ± SEM, n = 6 rats per group. LPO = Lipid peroxide, GSH = Reduced glutathione, SOD = Superoxide dismutase, CAT = Catalase, VIN = Vincristine. a P < 0.001 vs. normal control. b P < 0.001 vs. vincristine control.

3.3. Effect of AE-ST on histopathological changes

Vincristine administration resulted in abnormal histopathological changes assessed in the longitudinal section of sciatic nerve as indicated by the derangement of fibers and axonal swelling when compared to normal control (Figure 6a-b). Administration of AE-ST (300, 100 and 30 mg/kg, p.o.) attenuated vincristine induced abnormal histopathological changes (derangement of fibers and axonal swelling) in a dose dependent manner as compared to vincristine control (Figure 6c-e). Treatment of pregabalin also produced similar effects (Figure 6f).

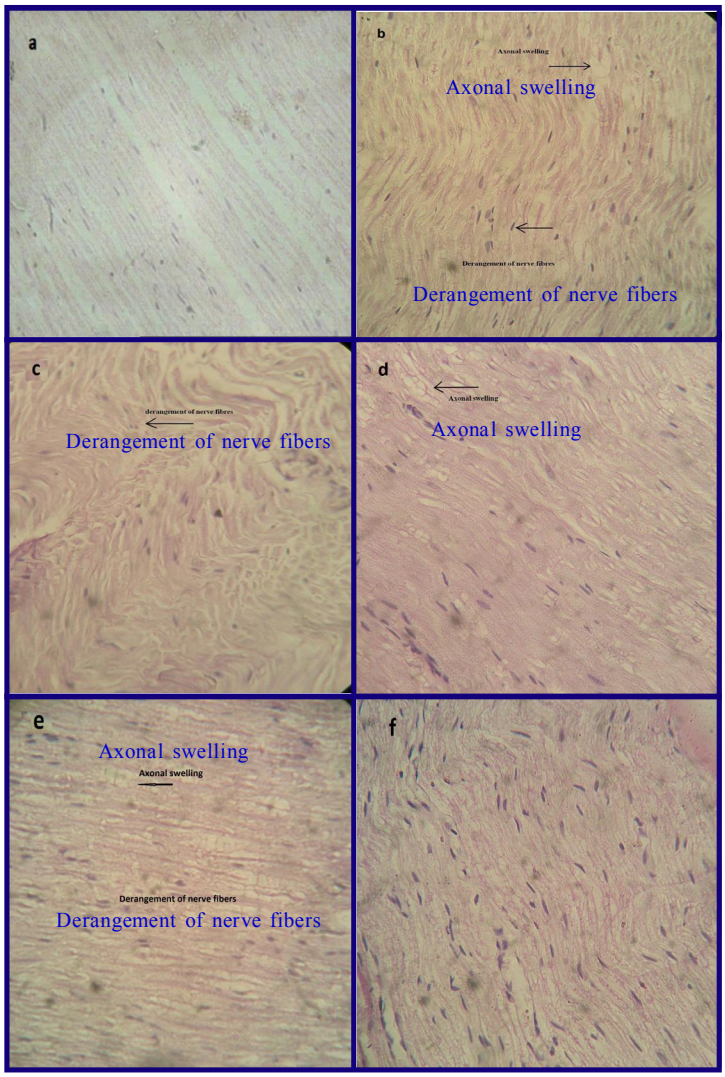


Figure 6a-f: Effect of AE-ST on histopathological changes. a to f show the longitudinal section of sciatic nerve of normal, vincristine, AE-ST (300, 100 and 30 mg/kg) and pregabalin (10 mg/kg) treated groups, respectively. Figure a, normal control. Figure b, vincristine control, shows vincristine induced axonal swelling and fiber arrangement. Figure c, d and e, treatment of AE-ST in vincristine rats (300, 100 and 30 mg/kg, p.o., respectively), show decrease in the vincristine induced histopathological changes (i.e., axonal swelling, derangement of nerve fibers) in dose dependent manner. Figure f, treatment of pregabalin in vincristine rats (10 mg/kg, p.o.) shows similar changes. Microscopic examinations were performed under 450 x light microcopy.

4. DISCUSSION

Earlier studies, did not reported any acute as well as sub-acute oral toxicity of aqueous extract of *Sapindus trifoliatus* at doses as high as 2000 mg/kg.[18,29,30] In the present study, aqueous extract of dried pericarps of fruits of *Sapindus trifoliatus* attenuated vincristine induced behavioral (i.e., spontaneous pain, mechanical and chemical allodynia and mechanical and thermal hyperalgesia), biochemical (i.e., thiobarbituric acid reactive substances, reduced glutathione, superoxide dismutase and catalase) as well as histopathological (axonal degeneration) changes in dose dependent manner. After vincristine treatment the behavioral alterations started from the day three and maximal nociceptive threshold was observed on ninth day. These observations are also reports from the other laboratories.[6,12,14,42-45]

Vincristine has been widely used as a chemotherapeutic agent for the management of various cancer disorders including Hodgkin's disease.[46] However, its clinical setting has been limited due to unavoidable painful neuropathy. It possesses the property of high binding affinity towards neuronal cytoskeleton protein i.e., b-tubulin, subsequently it has been documented to cause disruption of microtubule polymerizations eventually leading to neurotoxicity as well as cancer preventive actions.[47] Molecular mechanism of vincristine has also been reported to modulate the cellular Ca^{2+} levels, reactive oxygen species and free radical generation, release of inflammatory mediators, leading to neuronal cytotoxicity.[48] Vincristine induced activation of caspases and calpains has been shown the axonal degeneration by alteration of stability of axonal cytoskeleton proteins.[4,48] However it is difficult to say at this point, what is the sequence of deleterious events in vincristine induced neuropathic pain? Crucially, axonal degeneration has been reported due to these events, suggesting that cellular oxidant and inflammatory mediators play a key role in the pathogenesis of painful neuropathy.[12,43,49] Therefore, vincristine induced biochemical and histopathological alternations noted in this study.

In the present investigation, vincristine induced behavioral, biochemical and histopathological changes have been attenuated by administration of aqueous extract of *Sapindus trifoliatus* in dose dependent manner. Saponin is one of the major constituents in aqueous extract of *Sapindus trifoliatus* and it has been documented that saponins possess anti-convulsant, neuroprotective and neuropathic pain, anti-neuralgic, anti-nociceptive, anti-migraine, and anti-inflammatory effects.[17,19,20,22,23,25,50,51]

On the basis of data in hand and with support from literature, therefore, it may be proposed that aqueous extract of *Sapindus trifoliatus* dried pericarps produced ameliorative effect in vincristine induced painful peripheral neuropathy which may be attributed to its multiple effects viz; anti-oxidative, anti-inflammatory and neuroprotective actions manifested in the terms of alteration of vincristine induced behavioral (spontaneous pain, allodynia and hyperalgesia), biochemical (thiobarbituric acid reactive substances, reduced glutathione, superoxide dismutase and catalase) as well as histopathological changes (axonal degeneration) in dose dependent manner.

Pregabalin is a well known agent being currently used clinically to manage neuropathic pain of various etiologies.[52-54] Although pregabalin mediated beneficial effects are proposed to be potentially mediated via inhibition of voltage gated calcium channels. In addition pregabalin has also been shown to possess anti-oxidative as well as anti-inflammatory actions.[55,56] The present data also supports earlier reports.

5. CONSCLUSION

From experimental data collected it is concluded that *Sapindus trifoliatus* aqueous extract has potential ameliorating role in vincristine induced neuropathic pain syndrome which may be attributed to its multiple effects viz; anti-oxidative, anti-inflammatory and neuroprotective actions. This study presents the first preliminary report of *Sapindus trifoliatus* on neuropathic pain. Nevertheless, further studies are needed to explore full potential, exact mechanism of action and phytoconstituents of this extract in painful neuropathy.

REFERENCES

1. Burakgazi AZ, Messersmith W, Vaidya D, Hauer P, Koke A, Polydefkis M. Longitudinal assessment of oxaliplatin-induced neuropathy. Neurology 2011; 77(10): 980-6.
2. O'Connor AB, Dworkin RH. Treatment of neuropathic pain: an overview of recent guidelines. Am J Med 2009; 122: S22-S32.
3. Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms and management. Lancet 1999; 353: 1959-64.
4. Muthuraman A, Singh N. Attenuating effect of *Acorus calamus* extract in chronic constriction injury induced neuropathic pain in rats: an evidence of anti-oxidative, anti-inflammatory, neuroprotective and calcium inhibitory effects. BMC Complement Altern Med 2011; 11: 24-37.
5. Gomber S, Dewan P, Chhonker D. Vincristine induced neurotoxicity in cancer patients. Indian J Pediatr 2010; 77: 97-100.

6. Kiguchi N, Maeda T, Kobayashi Y, Saika F, Kishioka S. Involvement of inflammatory mediators in neuropathic pain caused by vincristine. Int Rev Neurobiol 2009; 85: 179-90.
7. Sioka C, Kyritsis AP. Central and peripheral nervous system toxicity of common chemotherapeutic agents. Cancer Chemother Pharmacol 2009; 63: 761-67.
8. Argyriou AA, Koltzenburg M, Polychronopoulos P, Papapetropoulos S, Kalofonos HP. Peripheral nerve damage associated with administration of taxanes in patients with cancer. Crit Rev Oncol Hematol 2008; 66: 218-28.
9. Goyal S, Khilnani G, Singhvi I, Singla S, Khilnani AK. Guggulipid of *Commiphora mukul*, with antiallodynic and antihyperalgesic activities in both sciatic nerve and spinal nerve ligation models of neuropathic pain. Pharm Biol 2013; 51(12): 1487-98.
10. Kim YS, Park HJ, Kim TK, Moon DE, Lee HJ. The effects of *Ginkgo biloba* extract EGb 761 on mechanical and cold allodynia in a rat model of neuropathic pain. Anesth Analg 2009; 108(6): 1958-63.
11. Comelli F, Giagnoni G, Bettoni I, Colleoni M, Costa B. Antihyperalgesic effect of a *Cannabis sativa extract* in a rat model of neuropathic pain: mechanisms involved. Phytother Res 2008; 22(8): 1017-24.
12. Muthuraman A, Diwan V, Jaggi AS, Singh N, Singh D. Ameliorative effects of *Ocimum sanctum* in sciatic nerve transaction-induced neuropathy in rats. J Ethnopharmacol 2008a; 120(1): 56-62.
13. Chen W, Luo YF, Liu JP. Topical herbal medicine for treatment of diabetic peripheral neuropathy: a systematic review of randomized controlled trials. Forsch Komplementmed 2011; 18: 134-45.
14. Ellis RJ, Toperoff W, Vaida F, Gonzales J, Gouaux B, Bentley H, Atkinson JH. Smoked medicinal cannabis for neuropathic pain in HIV: a randomized, crossover clinical trial. Neuropsychopharmacol 2009; 34(3): 672-80.
15. Rao GHJ, Lakshmi P. Sapindus trifoliatus: A Review. Int J Pharm Technol 2012; 4(3): 2201-14.
16. Meena VN, Rajakohila M, Syndia LAM. Multifacetious uses of soapnut tree - A mini review. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012; 3(1): 420-24.
17. Sharma A, Sati SC, Sati OP. Chemical constituents and bioactivities of genus Sapindus. Int J Res Ayurveda Pharm 2011; 2: 403-9.
18. Kishore DV, Pinto J, Mini KV. Anti ulcer activity of leaf extract of *Sapindus trifoliatus* Linn. International Journal of Advances in Pharmaceutical Sciences 2010; 1(1): 104-7.
19. Arulmozhi DK, Veeranjaneyulu A, Bodhankar SL, Arora SK. Pharmacological Investigations of *Sapindus trifoliatus* in various in vitro and in vivo models of inflammation. Indian J Pharmacol 2005a; 37(2): 96-102.
20. Albiero ALM, Bacchi EM, Mourao KSM. Caracterizacao anatomica das folhas, frutos esementes de *Sapindus saponaria* L. (Sapindaceae). Acta Scientiarum 2001; 23: 549-60.
21. Zhai L, Li Y, Wang W. Enhancement of humoral immune responses to inactivated New castle disease and avian influenza vaccines by oral administration of ginseng stem and leaf saponins in chickens. Poultry Sci 2011; 90: 1955-59.
22. Jalsrai A, Grecksch G, Becker A. Evaluation of the effects of *Astragalus mongholicus* Bunge saponin extract on central nervous system functions. J Ethnopharmacol 2010; 131: 544-49.
23. Kaur G, Jaggi AS, Singh N. Exploring the potential effect of *Ocimum sanctum* in vincristine induced neuropathic pain in rats. J Brachial Plex Peripher Nerve Inj 2010; 5: 3-11.
24. Kang KA, Kang JH, Yang MP. Ginseng total saponin enhances the phagocytic capacity of canine peripheral blood phagocytes in vitro. Am J Chin Med 2008; 36: 329-41.
25. Arulmozhi DK, Veeranjaneyulu A, Bodhankar SL, Arora SK. Investigations into the anti-nociceptive activity of *Sapindus trifoliatus* in various pain models. J Pharm Pharmacol 2004; 56(5): 655-61.
26. Xiao W, Boroujerdi A, Bennett GJ, Luo ZD. Chemotherapy evoked painful peripheral neuropathy: analgesic effects of gabapentin and effects on expression of the alpha-2-delta type-1 calcium channel subunit. Neuroscience 2007; 144(2): 714-20.
27. Gilron I, Flatters SJ. Gabapentin and pregabalin for the treatment of neuropathic pain: A review of laboratory and clinical evidence. Pain Res Manag 2006; 11: 16A-29A.
28. Authier N, Coudore F, Eschalier A, Fialip J. Pain related behaviour during vincristine-induced neuropathy in rats. NeuroReport 1999; 10: 965-68.
29. Jayasree T, Naveen A, Chandrasekhar N. Evaluation of muscle relaxant activity of aqueous extract of Sapindus trifoliatus (pericarp) in swiss albino mice. J Chem Pharm Res 2012; 4(4): 1960-64.
30. Jayasree T, Ghouse B, Chandrasekhar N. Evaluation into antiepileptic activity of aqueous extract of Sapindus trifoliatus in swiss albino rats. Asian J Phar Biol Res 2011; 1(4): 459-63.
31. Choi Y, Yoon YW, Na HS. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain 1994; 59: 369-76.

32. Field MJ, McCleary S, Hughes J, Singh L. Gabapentin and pregabalin, but not morphine and amitriptyline, block both static and dynamic components of mechanical allodynia induced by streptozocin in the rat. Pain 1999; 80(1-2): 391-98.

33. Caudle RM, Mannes AJ, Benoliel R. Intrathecally administered cholera toxin blocks allodynia and hyperalgesia in persistent pain models. J Pain 2001; 2: 118-27.

34. Gonzalez MI, Field MJ, Hughes J, Singh L. Evaluation of selective NK(1) receptor antagonist CI-1021 in animal models of inflammatory and neuropathic pain. J Pharmacol Exp Ther 2000; 294(2): 444-50.

35. Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl and dithienylbutylamines. J Pharmacol Exp Ther 1953; 107: 385-93.

36. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem 1949; 177: 751-66.

37. Wills ED. Mechanism of lipid peroxide formation in animal. Biochem J 1966; 99: 667-76.

38. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70-77.

39. Luck H. Catalase. Methods of Enzymatic Analysis. In: Bergmeyer HU, Editor. New York: Academic Press; 1971. p. 885-93.

40. Kono Y. Generation of superoxide radical autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys 1978; 186(1): 189-95.

41. Sudoh Y, Desai SP, Haderer AE, Sudoh S, Gerner P, Anthony DC, Wang GK. Neurologic and histopathologic evaluation after high-volume intrathecal amitriptyline. Reg Anesth Pain Med 2004; 29(5): 434-40.

42. Muthuraman A, Ramesh M, Sood S. Development of animal model for vasculatic neuropathy: Induction by ischemic-reperfusion in the rat femoral artery. J Neurosci Methods 2010; 186: 215-21.

43. Muthuraman A, Jaggi AS, Singh N, Singh D. Ameliorative effects of amiloride and pralidoxime in chronic constriction in jury and vincristine induced painful neuropathy in rats. Eur J Pharmacol 2008b; 587(1-3): 104-11.

44. Kiguchi N, Maeda T, Kobayashi Y, Kishioka S. Up-regulation of tumor necrosis factor-alpha in spinal cord contributes to vincristine-induced mechanical allodynia in mice. Neurosci Lett 2008; 445: 140-43.

45. Joseph EK, Levine JD. Mitochondrial electron transport in models of neuropathic and inflammatory pain. Pain 2006; 121: 105-14.

46. Ozdemir N, Dogan M, Sendur MA, Yazici O, Abali H, Yazilitas D, Akinci MB, Aksoy S, Zengin N. Efficacy and safety of first line vincristine with doxorubicin, bleomycin and dacarbazine (ABOD) for hodgkin's lymphoma: a single institute experience. Asian Pac J Cancer Prev 2014; 15(20): 8715-18.

47. Swain SM, Arezzo JC. Neuropathy associated with microtubule inhibitors: diagnosis, incidence, and management. Clin Adv Hematol Oncol 2008; 6: 455-67.

48. Jaggi AS, Singh N. Mechanisms in cancer chemotherapeutic drugs induced peripheral neuropathy. Toxicology 2012; 291: 1-9.

49. Siau C, Bennett GJ. Dysregulation of neuronal calcium homeostasis in chemotherapy-evoked painful peripheral neuropathy. Anesth Analg 2006; 102: 1485-90.

50. Arulmozi DK, Veeranjaneyulu A, Bodhankar SL, Arora SK. Effect of *Sapindus trifoliatus* on hyperalgesic in vivo migraine models. Braz J Med Biol Res 2005b; 38(3): 469-75.

51. Arulmozi DK, Veeranjaneyulu A, Bodhankar SL, Arora SK. Investigations of *Sapindus trifoliatus* in dopaminergic and serotonergic systems, purative anti-migraine mechanisms. Indian J Pharmacol 2005c; 37(2): 120-25.

52. Navarro A, Saldana MT, Perez C, Torrades S, Rejas J. A cost consequences analysis of the effect of pregabalin in the treatment of peripheral neuropathic pain in routine medical practice in primary care settings. BMC Neurol 2011; 11: 7-18.

53. Plested M, Budhia S, Gabriel Z. Pregabalin, the lidocaine plaster and duloxetine in patients with refractory neuropathic pain: a systematic review. BMC Neurol 2010; 10: 116-29.

54. Perez C, Navarro A, Saldana MT, Masramon X, Rejas J. Pregabalin and gabapentin in matched patients with peripheral neuropathic pain in routine medical practice in a primary care setting: Findings from a cost consequences analysis in a nested case-control study. Clin Ther 2010; 32: 1357-1570.

55. Ha KY, Carragee E, Cheng I, Kwon SE, Kim YH. Pregabalin as a neuroprotector after spinal cord injury in rats: biochemical analysis and effect on glial cells. J Korean Med Sci 2011; 26: 404-11.

56. Ha KY, Kim YH, Rhyu KW, Kwon SE. Pregabalin as a neuroprotector after spinal cord injury in rats. Eur Spine J 2008; 17: 864-72.

Source of support: Nil, India, **Conflict of interest:** None Declared