



# Anti-neuralgesic Effect of Ginsenoside Rg1 (GRg) in Chemotherapy-Induced Neuropathic Pain

Satbir Kaur<sup>1</sup>, Arunachalam Muthuraman<sup>2,3,4,\*</sup>

<sup>1</sup>Department of Pharmacology, Chaudhary Devi Lal College of Pharmacy, BD Sharma University of Health Science, Yamuna Nagar-135003, Haryana, India.

<sup>2</sup>Department of Pharmacology and Toxicology, Akal College of Pharmacy & Technical Education, Research scholar of IK Gujral Punjab Technical University (Kapurthala), Mastuana Sahib, Sangrur-147001, Punjab, India.

<sup>3</sup>Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru-570 015, Karnataka, India.

<sup>4</sup>Pharmacology Unit, Faculty of Pharmacy, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, Malaysia.

## Abstract

Ginsenoside Rg1 (GRg) is a natural bioactive flavonoid compound. It has potential action on the neuronal system and it prevents neurodegenerative disorders. The present study is focused to explore their role of GRg in paclitaxel-induced neuropathic pain. Neuropathic pain was induced by administration of paclitaxel dose 2 mg/kg, *i.p.* for 5 consecutive days in mice. The ginsenoside Rg1 (5 and 10 mg/kg; *i.v.*) and pregabalin (5 mg/kg; *i.v.*) were administered for 10 consecutive days. The neuralgic sensations were assessed by various pain assessment tests like acetone drop, pinprick, plantar, tail flick, and tail pinch test. All tests were performed on variable time intervals *i.e.*, 0, 4, 8, 12 and 16<sup>th</sup> day. The tissue biomarker changes *i.e.*, thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide anion, calcium, myeloperoxidase, and TNF- $\alpha$  level were estimated in sciatic nerve tissue. Treatment of GRg and pregabalin attenuated the paclitaxel-induced pain sensitivity in a dose-dependent manner along with tissue biomarkers changes. GRg has potential neuroprotective actions against paclitaxel-induced neuropathic pain due to its anti-oxidant; anti-inflammatory; and regulation of cytosolic calcium ion concentration.

**Keywords:** Allodynia, Ginsenoside, Hyperalgesia, Myeloperoxidase, Paclitaxel, Sciatic nerve.

## INTRODUCTION

Neuropathic pain is a progressive neurodegenerative disorder. It occurs due to the damage of central and peripheral neuronal tissue. It produces the unpleasant painful sensation and sometimes it induces the lack of sensation [1]. The treatment of neuropathic pain is complicated due to the involvement of multiple pathophysiological mechanism and complexity of drug prescription. The conventional medicines are treated the neuropathic pain by symptomatic manner. There are some common medicines are used to treat the neuropathic pain such as narcotic; anti-depressants; and anti-epileptic drugs. Even though, these approaches are not satisfactory for the management of neuropathic pain due to its potential side effects; lack of efficacy in certain neuropathic pain and cost-effectiveness. Therefore, alternative medicines are essential for the treatment of neuropathic pain. Numerous reports revealed that herbal medicine has a promising role in the prevention of neuropathic pain and their progress. In addition, it has neuroprotective action along with lacks of adverse effects for chronic usage; and low cost.

Paclitaxel is one of the natural anti-cancer agents and it is used for the treatment of breast cancer; lung cancer; and ovarian cancer. However, it causes serious adverse effects on host neurological system leads to cause the peripheral neuropathic pain. The mechanism of paclitaxel-induced neuropathic pain is the prevention of tubulin polymerization; calcium ion accumulation; raising the inflammatory cytokines including TNF- $\alpha$ , and activation of myeloperoxidase enzyme [2-4]. Experimentally, the administration of PT is documented to produce the neuropathic pain in rodents as well as in human [5-6]. The plant extracts are documented to prevents the progress of neuropathic pain-like *Acorus calamus*; *Artemisia dracunculoides*; *Butea monosperma*; *Citrullus colocynthis*; *Curcuma longa*; *Crocus sativus*; *Elaeagnus angustifolia*; *Ginkgo biloba*; *Mitragyna speciosa*; *Momordica charantia*; *Nigella sativa*; *Ocimum sanctum*; *Phyllanthus amarus*; and *Salvia officinalis* [7]. In addition, plant-derived constituents *i.e.*, celastrol [8], liquiritigenin [9], epigallocatechin gallate [10], tocotrienol [11], lycopene [12], thymoquinone [13], and resveratrol [14] are also produce the anti-nociceptive action.

Ginsenosides is one of the saponins type steroidal glycosides and it belongs from family of *Sapindaceae*. The treatment of ginsenosides Rg<sub>5</sub> and Rh<sub>3</sub> ameliorates the scopolamine-induced memory deficits [15]; multiple sclerosis and Parkinson's disease [16-17]; and ischemic stroke in human [18-19]. Thus, the role of ginsenosides in neuropathic pain remains to be explored. Therefore, the present study designed to evaluate the role of GRg in paclitaxel-induced neuropathic pain in mice.

## MATERIALS AND METHODS

### Animals

About 20-25 g and age of 10-month-old disease free male Swiss albino mice were used for the evaluation of GRg in paclitaxel-induced neuropathic pain in mice. Animals were kept at standard laboratory diet, temperature (65-75 °F; ~18-23 °C) and humidity (40-60 %) condition. The complete animal experimental protocol including acclimatized period, all the animals were kept in 12 hours light-dark cycles. The animals were allowed accessing the standard laboratory diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC; No.: ATRC/09/14). And, maintaining of animals was followed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India.

### Drugs and Chemicals

Ginsenoside Rg1 (GRg: Purity  $\geq$  98 %) was obtained from Pioneer Enterprise Mumbai, Maharashtra, India. And, GRg dose was prepared with 50 mM of phosphate buffer. The paclitaxel injection was purchased from Bristol-Mayer Squipp, Mumbai. Sulfanilamide and 1,1,3,3-tetramethoxy propane were procured from Sisco Research Laboratories, Mumbai. Thiobarbituric acid and nitro blue tetrazolium (NBT) was procured from Sigma Aldrich Mumbai. Rat TNF-alpha ELISA kit was purchased from RayBio, Inc., USA. The remaining chemical reagents were obtained from S.D. Fine Chemicals, Mumbai, India with the analytical grade.

**Induction of peripheral neuropathy by paclitaxel administration**

Neuropathic pain was induced in mice by the administration of paclitaxel dose 2 mg/kg, *i.p.*; for 5 consecutive days in mice [5-6, 20]. Nociceptive pain threshold will be assessed at different time intervals *i.e.*, 0, 4, 8, 12 and 16<sup>th</sup> day.

**Experimental Protocol**

Six groups were employed in the present study. Each group comprising eight Swiss albino mice (n= 6). **Group I (Normal control):** Mice were not subjected to any drug administration. **Group II (Paclitaxel control):** Mice were subjected to administration of paclitaxel dose 2 mg/kg, *i.p.*; for 5 consecutive days. **Group III (GRg per se):** Mice were subjected to the administration of GRg (10 mg/Kg, *i.v.*) for 10 consecutive days in healthy Swiss albino mice. The drugs were administered via tail vein injection method. The location was selected from the mid part of the tail. And every day injection was applied right and left alternatively. **Group IV and V (GRg; 5 and 10 mg/kg):** Mice were subjected to the intravenous administration of GRg (5 and 10 mg/Kg) for 10 consecutive days respectively with PT treatment. Between, GRg and PT administration 10 minutes time intervals were maintained. **Group VI (PreG; 5 mg/kg):** Mice were subjected to the intravenous administration of PreG (5 mg/Kg) for 10 consecutive days. All six groups were employed for the assessment of behavioral and biochemical evaluations. All neurobehavioral tests were performed at different time intervals *i.e.*, 0, 4, 8, 12 and 16<sup>th</sup> day. On the 16<sup>th</sup> day, all the animals were sacrificed by cervical dislocation method. The sciatic nerve and surrounding tissue samples were collected for further biochemical evaluation.

**Behavioral evaluation****Acetone drop test**

The cold chemical sensitivity of the right hind paw was assessed by acetone drop tests described by Choi *et al.* [21]. It clinically resembles thermal allodynia symptoms. Briefly, the mice were placed on a wire mesh grid. The acetone (100  $\mu$ l) was sprayed on the plantar surface of the right hind paw of the mice after 5 minute accommodation period. The 1-minute duration was maintained for observation of acetone induced cold sensitive reaction. The pain sensitive reactions were scored *i.e.*, 1 for paw licking; 2 for shaking; 3 for right hind paw lifting duration less than 4 seconds; 4 for right hind paw lifting duration between 5 to 8 seconds; and 5 for right hind paw lifting duration above 8. The total score was noted as 15. Highest and lowest score depicts severe neuronal injury associated dysfunction of neuron and neuroprotection respectively.

**Pinprick test**

The mechanical pain sensation was assessed by pinprick test as a described method of Erichsen and Blackburn-Munro [22]. Clinically, it resembles the pinpoint mechanical hyperalgesic symptoms. Briefly, the blunted needle was touched to the mid-plantar surface of the right hind paw. The intensity was generated until the detectable reflex withdrawal response in the right hind paw of normal as well as neuropathic pain control animals. The needle was applied six times per minute. The quick withdrawal of the hind limb was considered a painful response. The cut off stimuli was applied only six times to avoid the unwanted tissue injury and development of wind-up phenomenon.

**Plantar test**

The radiant heat sensation was assessed in ipsilateral hind paw by the plantar test as described by Hargreaves *et al.* [23]. Clinically, it is mimicking the thermal hyperalgesic symptoms. Briefly, the right hind paw of mice was placed on the radiant heat lamp

source. The radiant heat sensitivity of the hind paws was noted as hind paw withdrawal latency. The brisk withdrawal of the hind limb was considered a painful response. The cut off time was maintained at 20 seconds.

**Tail flick test**

The radiant heat sensation was assessed in the tail part of the mice by tail flick test as a described method of D'Aemour and Smith [24] with a slight modification of Hargreaves *et al.* [23]. Clinically, it resembles central thermal sensation symptoms. Briefly, the 1 cm distance from the tail terminal region of mice was placed on the radiant heat lamp source. The radiant heat sensitivity of the tail was observed as the tail withdrawal latency. The quick withdrawal of the tail from the heat lamp source was considered as a painful response. The cut off stimuli was maintained for 15 seconds to avoid the potential tissue damage of the tail skin.

**Tail Pinch test**

The mechanical pain sensation was assessed in the tail part of the mice by tail pinch tests described by Takagi *et al.* [25]. Clinically, it resembles central mechanical pain sensation symptoms. Briefly, Hoffmann clamp was placed on the base of the tail. The screw of the Hoffmann clamp was adjusted to develop the mechanical pressure and elicit the painful sensation response within 5 s. The rising number of dislodgment attempt on the clamp was noted as a painful response. The cut-off time for the application of mechanical pressure was maintained for 10 s to prevent the potential tissue damage on the mice skin.

**Biochemical estimation**

All the tissue samples were kept in the humidity chamber and maintained at 85 % relative humidity and 37° C. The 10 % w/v of sciatic nerve homogenate was prepared with 0.1 M Tris-HCl buffer (pH 7.4); deionised water; and phosphate buffer (pH 7.4) for total protein, thiobarbituric acid reactive substances (TBARS) & reduced glutathione (GSH); total calcium; tumor necrosis factor-alpha (TNF- $\alpha$ ) and myeloperoxidase (MPO) activity estimation respectively. Superoxide anion was also estimated in a sciatic nerve tissue sample.

**Estimation of TBARS**

The thiobarbituric acid reactive substances (TBARS) was estimated by Ohkawa *et al.* method [26]. The absorbance was estimated by spectrophotometrically (UV-1800 UV-Vis spectrophotometer, SHIMADZU Corporation, Tokyo, Japan) at 535 nm wavelength. A standard plot was prepared with 1-10 nM of 1, 1, 3, 3-tetramethoxy propane. The results of TBARS concentration were expressed as nM of MDA per mg of protein.

**Estimation of reduced glutathione (GSH) content**

The GSH content was estimated by Beutler *et al.* [27]. The absorbance was estimated by spectrophotometrically at 412 nm wavelength. The standard plot was prepared with 10-100  $\mu$ g of GSH. The results of GSH concentrations were expressed as  $\mu$ g of GSH per mg of protein.

**Estimation of total calcium**

The total calcium levels were estimated in the sciatic nerve by Severnghaus and Ferreebe [28] method with a slight modification of Muthuraman *et al.* [29]. The absorbance was estimated by spectrophotometrically at 556 nm wavelength. The standard plot was prepared with 100-1000 parts per million (ppm) of calcium. The results of total calcium were expressed as ppm per milligram of sciatic nerve tissue.

**Estimation of tumor necrosis factor-alpha (TNF- $\alpha$ ) level**

The estimation of tumor necrosis factor-alpha (TNF- $\alpha$ ) was done measured in the sciatic nerve homogenate as described by Muthuraman *et al.* [30]. The absorbance was estimated by spectrophotometrically at 450 nm wavelength. The TNF- $\alpha$  standard plot was prepared by using 0 to 20,000 pg per ml of reference standard TNF- $\alpha$  sample. The results were expressed as pictograms of TNF- $\alpha$  per mg of total protein.

#### Estimation of superoxide anion generation

The superoxide anion generation concentration was estimated by Wang *et al.* [31] method with a slight modification of Muthuraman and Singh [32]. The absorbance was estimated by spectrophotometrically at 540 nm wavelength. The results of NBT reduction were expressed as picomoles per minute per milligram wet weight of the sciatic nerve.

#### Estimation of myeloperoxidase activity

The myeloperoxidase activity level was estimated in the sciatic nerve sample by Patriarca *et al.* [33] method with a slight modification of the Grisham *et al.* [34]. The absorbance was estimated by spectrophotometrically at 460 nm wavelength. The results were expressed as myeloperoxidase activity units per milligram of protein at one minute.

#### Estimation of total protein content

The total protein content was estimated by Lowry's *et al.* method [35]. The absorbance was estimated by spectrophotometrically at 750 nm wavelength. The standard plot was prepared with 1-10 mg of bovine serum albumin. The results of total protein concentration were expressed as mg per ml of supernatant.

#### Statistical analysis

All the results were expressed as the mean  $\pm$  standard deviation (SD). Data obtained from behavioral tests were statistically analyzed using two-way analysis of variance (ANOVA) followed

by Bonferroni's *post-hoc* analysis were applied by using Graph pad prism Version-5.0 software. The data of tissue biomarkers were analyzed using one way ANOVA followed by Tukey's multiple range tests were applied for *post-hoc* analysis by using Sigmapstat Version-3.5 software. A probability value of  $p < 0.05$  was considered to be statistically significant.

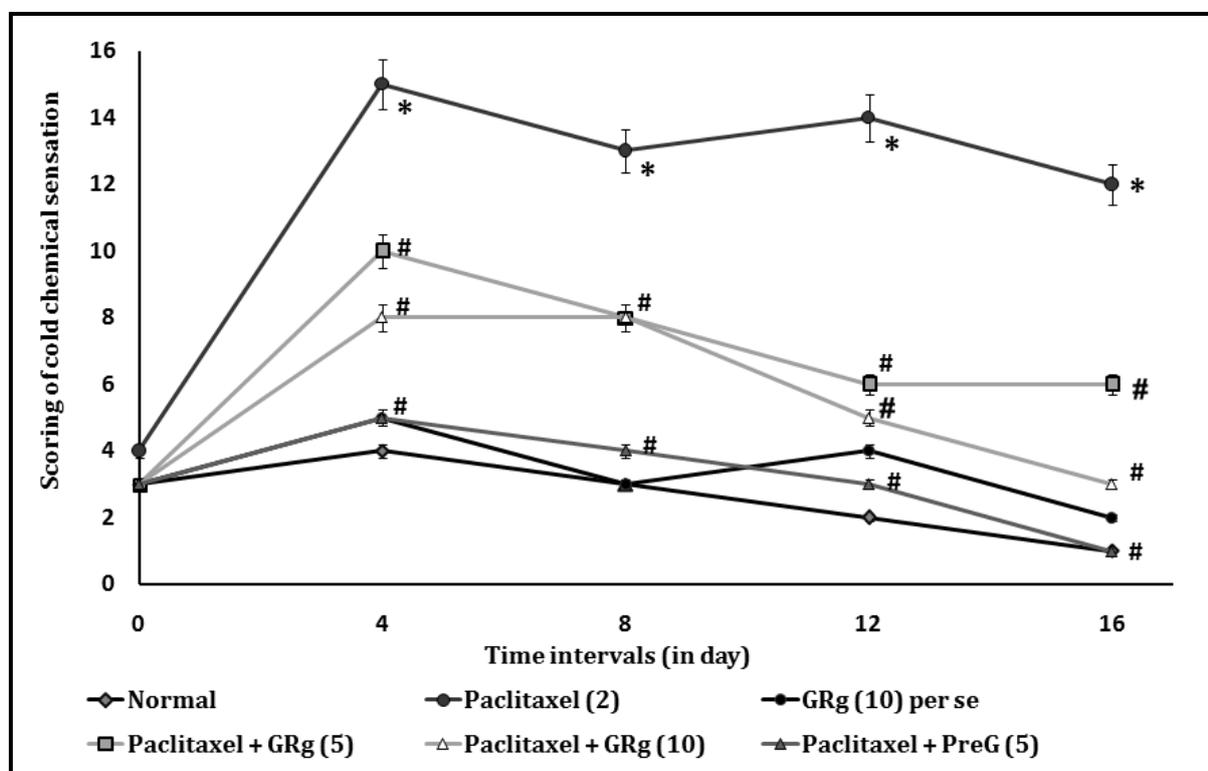
## RESULTS

#### Role of GRg on paclitaxel-induced changes in an acetone drop test

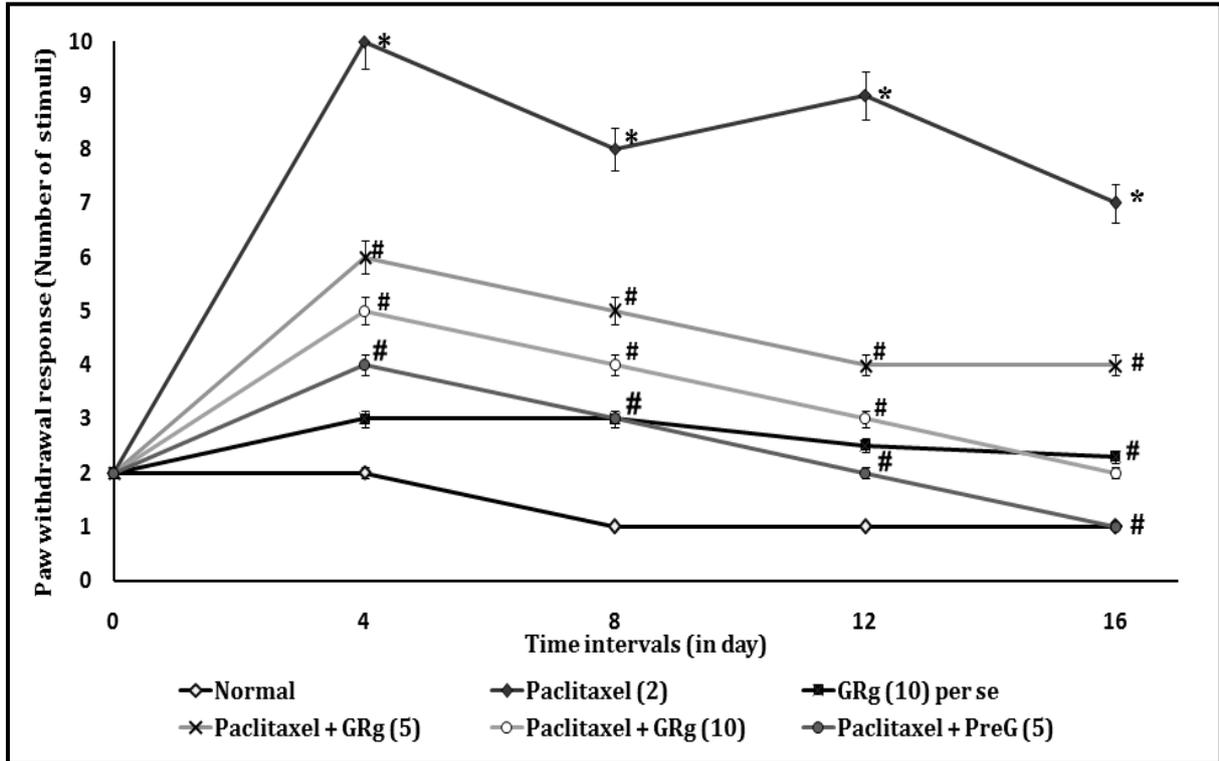
The administration of paclitaxel (2 mg/kg, *i.p.* for 5 consecutive days) resulted to significant ( $p < 0.05$ ) rise the thermal allodynic sensation as an indication of an increase in the scoring of chemical sensation when compared to the normal control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated the paclitaxel-induced increase in the scoring of chemical sensation in a dose-dependent manner. The effect of GRg is comparable and similar to the pregabalin treatment group. However, vehicle and GRg (10 mg/kg; *i.v.*) *per se* treated group did not show any significant ( $p < 0.05$ ) changes in paclitaxel-induced thermal allodynia (Figure 1).

#### Role of GRg on paclitaxel-induced changes in pinprick test

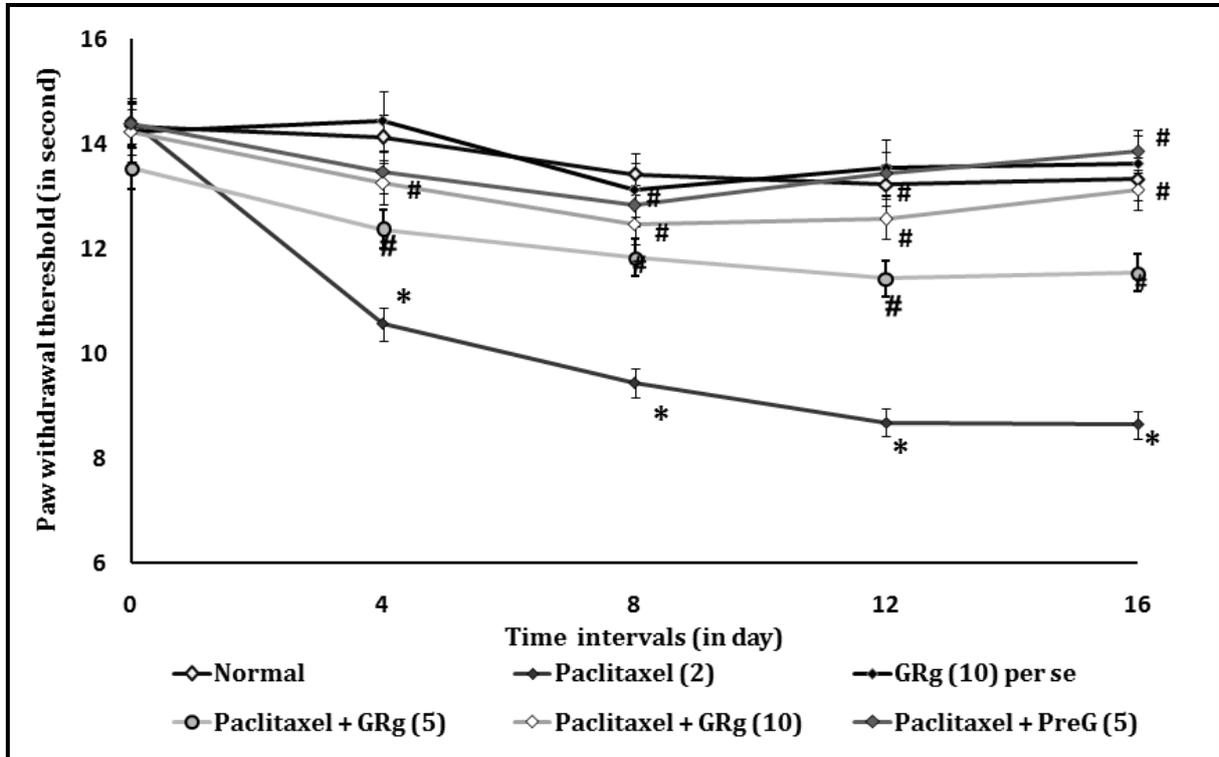
The administration of paclitaxel (2 mg/kg, *i.p.* for 5 consecutive days) resulted to significant ( $p < 0.05$ ) rise the mechanical hyperalgesic sensation as an indication of an increase in the percentage withdrawal of right hind paw when compared to the normal control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated the paclitaxel-induced increase in the paw withdrawal response in a dose-dependent manner. The effect of GRg is comparable and similar to the pregabalin treatment group. However, vehicle and GRg (10 mg/kg; *i.v.*) *per se* treated group did not show any significant ( $p < 0.05$ ) changes in paclitaxel-induced mechanical hyperalgesia (Figure 2).



**Figure 1:** Role of GRg on paclitaxel-induced changes in acetone drop test (paw thermal allodynia). Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean  $\pm$  SD, n = 6 mice per group. \* $p < 0.05$  Vs normal control group. # $p < 0.05$  Vs paclitaxel control group.



**Figure 2:** Role of GRg on paclitaxel-induced changes in pinprick test (paw mechanical hyperalgesia). Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean  $\pm$  SD, n = 6 mice per group. \* $p$  < 0.05 Vs normal control group. # $p$  < 0.05 Vs paclitaxel control group.



**Figure 3:** Role of GRg on paclitaxel-induced changes in plantar test (paw heat hyperalgesia). Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean  $\pm$  SD, n = 6 mice per group. \* $p$  < 0.05 Vs normal control group. # $p$  < 0.05 Vs paclitaxel control group.

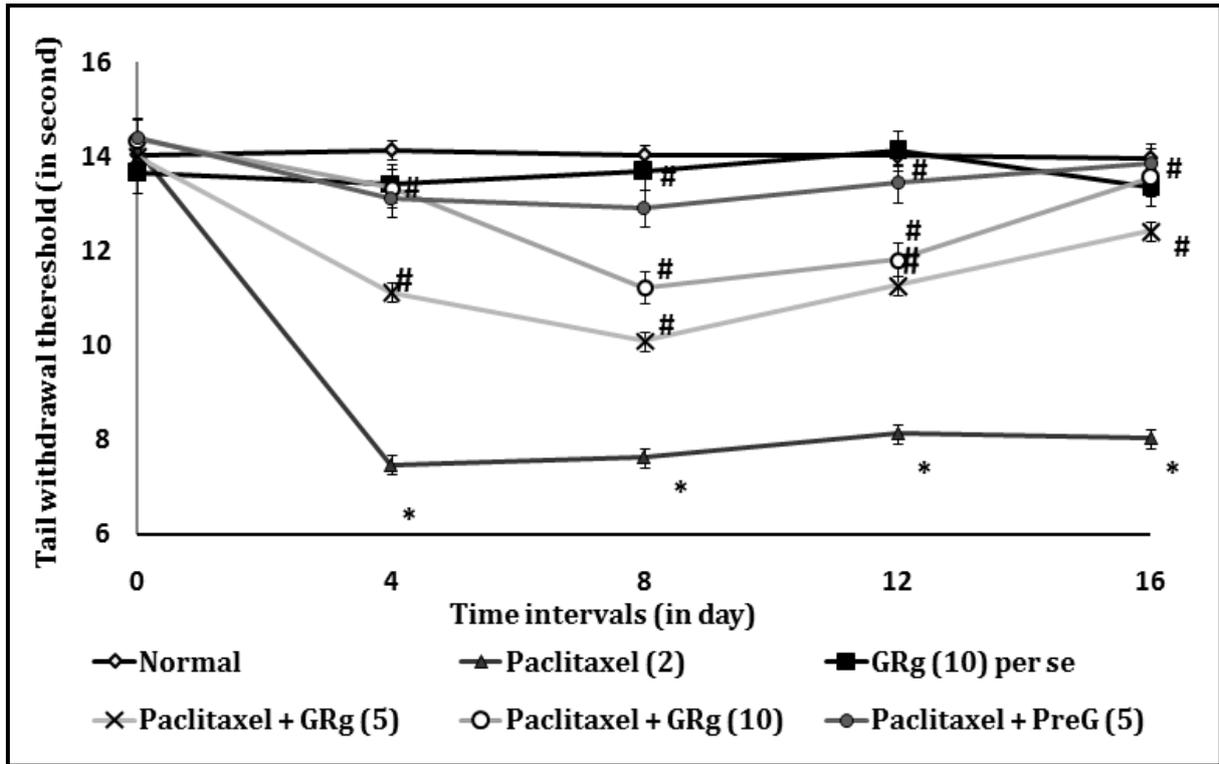


Figure 4: Role of GRg on paclitaxel-induced changes in tail flick test (tail heat hyperalgesia). Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n = 6 mice per group. \**p* < 0.05 Vs normal control group. #*p* < 0.05 Vs paclitaxel control group.

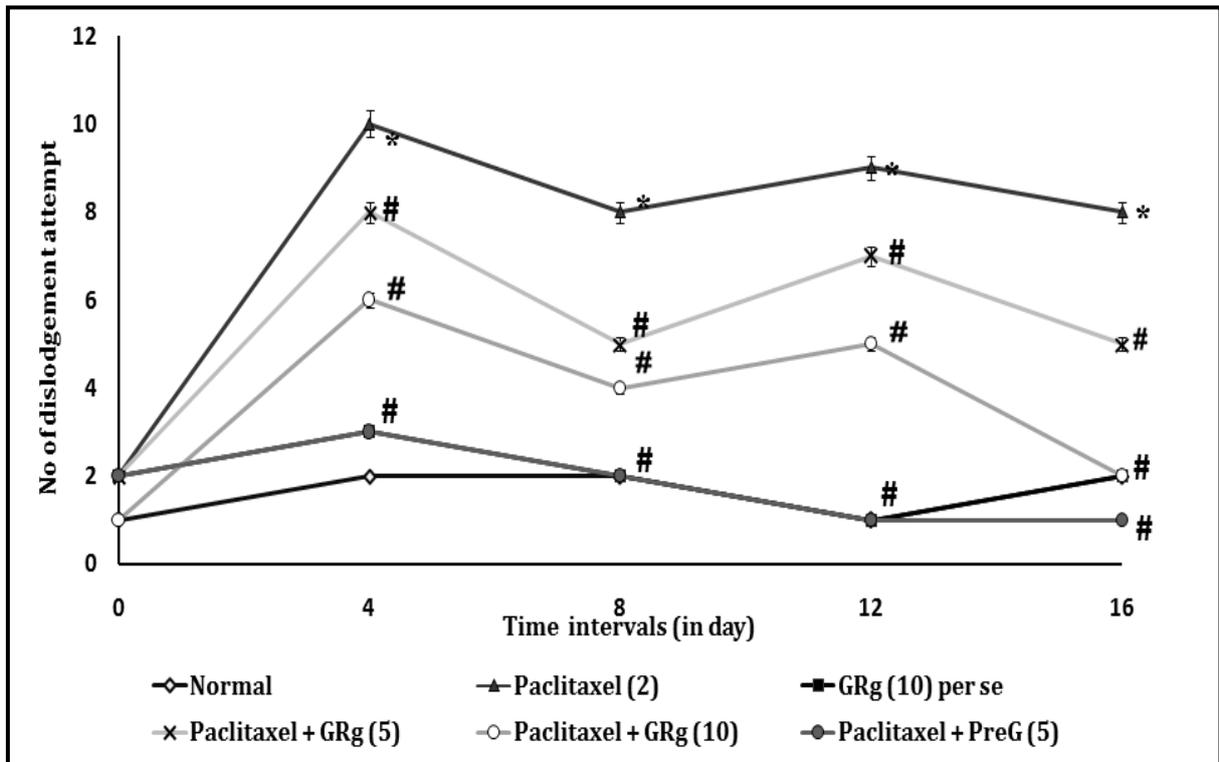


Figure 5: Role of GRg on paclitaxel-induced changes in tail pinch test (tail mechanical hyperalgesia). Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n = 6 mice per group. \**p* < 0.05 Vs normal control group. #*p* < 0.05 Vs paclitaxel control group.

**Role of GRg on paclitaxel-induced changes in plantar test**

The administration of paclitaxel (2 mg/kg, *i.p.* for 5 consecutive days) resulted to significant ( $p < 0.05$ ) rise the thermal hyperalgesic sensation as an indication of a decrease in right hind paw withdrawal threshold when compared to the normal control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated paclitaxel-induced decrease hind paw withdrawal threshold in a dose-dependent manner. The effect of GRg is comparable and similar to the pregabalin treatment group. However, vehicle and GRg (10 mg/kg; *i.v.*) *per se* treated group did not show any significant ( $p < 0.05$ ) changes in paclitaxel-induced thermal hyperalgesia (Figure 3).

**Role of GRg on paclitaxel-induced changes in tail flick test**

The administration of paclitaxel (2 mg/kg, *i.p.* for 5 consecutive days) resulted to significant ( $p < 0.05$ ) rise the thermal hyperalgesic sensation as an indication of a decrease in tail withdrawal threshold when compared to the normal control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated paclitaxel-induced a decrease in tail withdrawal threshold in a dose-dependent manner. The effect of GRg is comparable and similar to the pregabalin treatment group. However, vehicle and GRg (10 mg/kg; *i.v.*) *per se* treated group did not show any significant ( $p < 0.05$ ) changes in paclitaxel-induced thermal hyperalgesia (Figure 4).

**Role of GRg on paclitaxel-induced changes in tail pinch test**

The administration of paclitaxel (2 mg/kg, *i.p.* for 5 consecutive days) resulted to significant ( $p < 0.05$ ) rise the mechanical hyperalgesia as an indication of an increase in the number of dislodgement of Heffner's clamp from the tail when compared to the normal control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated the paclitaxel-induced increase in mechanical nociceptive pain threshold in a dose-dependent manner. The effect of GRg is comparable and similar to the pregabalin treatment group. However, vehicle and GRg (10 mg/kg; *i.v.*) *per se* treated group did not show any significant ( $p < 0.05$ ) changes in paclitaxel-induced mechanical hyperalgesia (Figure 5).

**Role of GRg on paclitaxel-induced changes in tissue biomarker changes**

The administration of paclitaxel (2 mg/kg, *i.p.* for 5 consecutive days) resulted to significant ( $p < 0.05$ ) rise the TBARS, total calcium, TNF- $\alpha$ , superoxide anion & MPO levels; and decrease in GSH content as indication of oxidative stress, inflammation and neuronal damage when compared to the normal control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated paclitaxel-induced changes of the above tissue biomarkers in a dose-dependent manner. The effect of GRg is comparable and similar to the pregabalin treatment group. However, vehicle and GRg (10 mg/kg; *i.v.*) *per se* treated group did not show any significant ( $p < 0.05$ ) changes in paclitaxel-induced tissue biomarker changes (Table 1 and Table 2).

**Table 1: Role of GRg on paclitaxel-induced biomarker changes in tissue supernatant.**

Groups	TBARS (nM / mg of protein)	GSH ( $\mu$ g / mg of protein)	Total calcium (ppm/mg of protein)	TNF- $\alpha$ (pg / mg of protein)
Normal	3.98 $\pm$ 0.49	78.22 $\pm$ 2.15	3.43 $\pm$ 0.62	30.43 $\pm$ 0.19
Paclitaxel(2)	8.54 $\pm$ 0.56 <sup>*</sup>	37.45 $\pm$ 1.69 <sup>*</sup>	26.14 $\pm$ 0.67 <sup>*</sup>	78.12 $\pm$ 0.36 <sup>*</sup>
GRg (10) <i>per se</i>	3.36 $\pm$ 0.54	76.37 $\pm$ 1.56	3.45 $\pm$ 0.29	34.49 $\pm$ 0.27
Paclitaxel+ GRg (5)	4.98 $\pm$ 0.52 <sup>#</sup>	70.93 $\pm$ 2.04 <sup>#</sup>	16.27 $\pm$ 0.18 <sup>#</sup>	44.92 $\pm$ 0.42 <sup>#</sup>
Paclitaxel+ GRg (10)	4.02 $\pm$ 0.17 <sup>#</sup>	73.25 $\pm$ 1.08 <sup>#</sup>	4.22 $\pm$ 0.52 <sup>#</sup>	34.24 $\pm$ 0.28 <sup>#</sup>
Paclitaxel+ PreG (5)	3.71 $\pm$ 0.73 <sup>#</sup>	75.58 $\pm$ 1.34 <sup>#</sup>	3.57 $\pm$ 0.15 <sup>#</sup>	31.98 $\pm$ 0.52 <sup>#</sup>

Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean  $\pm$  SD, n = 6 mice per group. <sup>\*</sup> $p < 0.05$  Vs normal group. <sup>#</sup> $p < 0.05$  Vs paclitaxel control group. Abbreviation: GRg, ginsenoside Rg1; TBARS, thiobarbituric acid reactive substances; GSH, reduced glutathione.

**Table 2: Role of GRg on paclitaxel-induced biomarker changes in tissue.**

Groups	NBT reduction (pM / Min / mg of tissue)	MPO (unit / Min / mg of protein)
Normal	3.47 $\pm$ 1.14	12.74 $\pm$ 1.73
Paclitaxel(2)	26.53 $\pm$ 0.27 <sup>*</sup>	112.24 $\pm$ 2.12 <sup>*</sup>
GRg (10) <i>per se</i>	2.70 $\pm$ 0.09	16.37 $\pm$ 1.47
Paclitaxel + GRg (5)	8.54 $\pm$ 1.19 <sup>#</sup>	54.96 $\pm$ 3.47 <sup>#</sup>
Paclitaxel + GRg (10)	4.79 $\pm$ 1.04 <sup>#</sup>	33.19 $\pm$ 2.35 <sup>#</sup>
Paclitaxel + PreG (5)	4.21 $\pm$ 0.43 <sup>#</sup>	18.16 $\pm$ 1.54 <sup>#</sup>

Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean  $\pm$  SD, n = 6 mice per group. <sup>\*</sup> $p < 0.05$  Vs normal group. <sup>#</sup> $p < 0.05$  Vs paclitaxel control group. Abbreviation: GRg, Ginsenoside Rg1; TBARS, thiobarbituric acid reactive substances; GSH, reduced glutathione.

## DISCUSSION

In the present study results revealed that, the administration of paclitaxel (2 mg/kg, *i.p.* for 5 consecutive days) significantly ( $p < 0.05$ ) produced the neuropathic pain by accelerating the thermal & mechanical hyperalgesia and allodynia in paw and tail region. It indicates that paclitaxel on sciatic nerve causing the neuronal excitation and accelerating the neuronal impulse. In addition, it also rising TBARS, total calcium, TNF- $\alpha$ , NBT reduction, and MPO activity levels; whereas, the reduced glutathione levels were decreased. These changes indicate the paclitaxel mediated the pathogenesis are due to the activation free radical generation, lipid peroxidation, alteration of cellular calcium homeostasis, and raising the inflammatory mediator's associated neuroinflammation. The administration of natural medicines *i.e.*, ginsenoside Rb1 (5 and 10 mg/Kg, *i.v.*) attenuated the paclitaxel-induced pain behavior and biochemical changes. It indicates that ginsenoside Rb1 possess the potential pain preventive action via neuroprotection.

The administration of paclitaxel (2 mg/kg, *i.p.* for 5 consecutive days) induced neuropathic pain model is a widely used model for the testing of polyneuritic neuropathic pain [36]. Clinically, the administration of paclitaxel mimics the complex regional pain syndrome (CRPS) in human as well as in animals [37-38]. The paclitaxel is one of the potent cancer chemotherapeutic agents cause neuronal damage and neurodegeneration [39-40]. The paclitaxel-induced peripheral neurodegeneration occurred by accumulation calcium ion concentration in the cytosol of the nerve tissue [41-42]; synthesis and release of inflammatory cytokines [43]; and alteration of neuronal myelin and tubulin proteins [44-45]. Even, alteration of the free radicals associated neurovascular system also contributes to the pathogenesis of paclitaxel induced neuropathic pain disorders [46]. The chronic alteration of neuronal microvascular environment makes the damage and degeneration of the peripheral nervous system [47-48]. Our previous research reports and other laboratory reports are documented that, plant extract and constituents are prevented from neuropathic pain disorders. Some plants *i.e.*, *Acorus calamus* [30, 49]; *Butea monosperma* [50]; *Swietenia mahagoni* [51]; *Ocimum sanctum* [52]; and *Vernonia cinerea* [53]; and phytoconstituents like cannabinoids [54]; puerarin [55]; bulleyaconitine A [56]; thymoquinone; epigallocatechin gallate; lycopen and resveratrol [7] are evidenced to produce the anti-neuralgic effect *viz* free radical scavenging; reduction of TNF- $\alpha$  synthesis and regulation of cellular enzymatic defense system. Experimentally, ginsenoside Rb1 also prevents the free radical formation, neuro-inflammation, cytokine production and neuronal apoptosis [57-58]. Neuropathic pain is mainly due to the neuronal excitation via activation of prolong neuronal calcium channel. GRg has a role in the release of  $\gamma$ -aminobutyric acid (GABA) and inhibition of GABA receptor synaptic neurotransmission [59]. In addition, GRg has neuroprotection against the cerebral ischemic injury via regulation of nuclear factor *i.e.*, peroxisome proliferated activated the receptor-gamma regulatory mechanism, reduction of reduced glutathione [60-61]. Similar results are obtained in the present study.

The present study results expressed that, GRg attenuates the paclitaxel-induced neuropathic pain by reduction TBARS, calcium; TNF- $\alpha$ , superoxide anion, and MPO levels; along with raising of reduced glutathione level. Based on the data in hand and literature evidence; it is concluded that ginsenoside Rb1 has potential ameliorating effect in paclitaxel-induced neuropathic pain *viz* anti-oxidant; anti-inflammatory; anti-cytokines and maintenance of cytosolic calcium ion concentration in the peripheral neuron. Therefore, ginsenoside Rb1 can be considered as one of the newer herbal candidates for the management of cancer chemotherapy-induced neuropathic pain disorders.

## Conflict of interest

None.

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## Author's contributions

*Satbir Kaur*: She has contributed to data collection and writing of the article.

*Arunachalam Muthuraman*: He has contributed to the design, analysis, and interpretation of data; and critical evaluation of manuscript preparation.

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