Ginsenoside Rg1 Prevents the Chronic Constriction Injury (CCI) of Sciatic Nerve Induced Neuropathic Pain in Mice

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Abstract

Introduction: Ginsenoside Rg1 (GRg) is a natural bioactive flavonoid compound. It has potential action on neuronal system and it prevents the neurodegenerative diorders. The present study is focused on evaluating the role GRg in mice model of chronic constriction injury of sciatic nerve (CCI) induced neuropathic pain.

Method: The test compound *i.e.,* ginsenoside Rg1 (5 and 10 mg/kg; *i.v.*) and reference compound *i.e.,* pregabalin (5 mg/kg; *i.v.*) were administered for 10 consecutive days. The pain sensations were assessed by various tests like acetone drop, pin prick, plantar, tail flick and tail pinch test. All behavioural tests were performed on different time intervals *i.e.,* 0, 4, 8, 12 and 16th day. The biochemical changes like thio-barbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide anion, calcium, myeloperoxidase and TNF-α level were estimated in sciatic nerve tissue.

Result: Treatment of GRg and pregabalin attenuated the CCI induced pain response in a dose dependent manner. Further, it also ameliorated the tissue biochemical changes. Statistically significant ($p < 0.05$) results were observed in test and reference drug treated groups.

Conclusion: GRg has potential neuroprotective actions against CCI induced neuropathic pain. It may attribute to multiple pharmacological action *i.e.,* free radical scavenging; down regulation of TNF- α proteins; and reduction of calcium accumulation leads to produce the neuroprotective actions.

Keywords: Chronic constriction injury, Ginsenoside, Myeloperoxidase, Pregabalin, Superoxide anion, Sciatic nerve.

INTRODUCTION

Neuropathic pain is an unpleasant emotional experience with painful sensation. It is mainly caused by damage of somatosensory system [1]. The pathogenesis of neuropathic pain is due to the alteration of various neuronal impulses; plasticity; and subsequently accelerates the neurodegenerative process [2]. The various ion channels are responsible for the alteration of neuronal signal; synaptic plasticity; and enhancement of neuronal excitation such target channels are transient receptor potential of vanilloid receptor (TRPV) channel; nicotinic receptor channel; puringeric receptor mediated ion channel; and voltage sensitive calcium channels [3-4]. Our previous study revealed that, calcium channel alteration and accumulation of cytosolic calcium channels are contributes in the progress of neuropathic pain via activation of m-calpain and calmodulin proteins [5-6]. The regulation of intracellular calcium concentration is prevents the neuropathic pain disorders. Whereas, the alteration immunological system function are involves in the neuropathic pain disorders via damage of neuron and enhancement of neurodegeneration [7-8].

Moreover, the neuronal oxidative stress also develops by the calcium dependent and independent activation of free radicals formation i.e., superoxide anion ($\cdot O_2^-$); and peroxynitrite ($\cdot ONOO^-$) [8-9]. This radicals are also responsible for the stimulation of immunological action leads to enhance the tumour necrosis factor alpha (TNF-α) synthesis and release [10-11]. Thus, the sequential and/or subsequent actions of calcium accumulation; free radicals synthesis; and release of TNF-α proteins are contributes in the progress of oxidative stress; neuroinflammation; and

neurodegeneration respectively [11-12]. Crucially, it causes the neuropathic pain via alteration of synaptic plasticity and enhances the long term potentiating of different levels of neuronal system [13-14]. The administrations of conventional medicines are documented to reduce the neuropathic pain symptoms in human as well as in rodents [3, 15-16]. However, the conventional medicine produce the unwanted side effects and some time it produce the life threatening effects [17-19]. The various plats are reported to produce the anti-neuralgesic action like *Acorus calamus*; *Artemisia dracunculus*; *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* [20]. In addition, some of the herbal medicines like (+)-Borneol [21], thymoquinone [22], celastrol [23], liquiritigenin [24], epigallocatechin gallate [25], tocotrienol [26], lycopene [27] and resveratrol [28] are also documented to produce the anti-neuralgesic effect. Therefore, herbal molecule may produce the better effects as compared to conventional medicine for neuropathic patients.

Ginsenosides is one of the saponins type steroidal glycosides and it is belongs from family of *Sapindaceae*. It is found in *Gynostemma pentaphyllum* (*Cucurbitaceae*); ginseng or red ginseng; and panax (*Ginseng*). Ginsenoside Rb_1 is the most abundant form in *Panax quinquefolius* (American ginseng) and it shown the potent neuroprotective effects. The treatment of ginsenosides Rg_5 and Rh_3 ameliorates the scopolamine-induced memory deficits [29]; multiple sclerosis and parkinson's disease [30-

31]; and ischemic stroke in human [32-33]. But, the role of ginsenosides in neuropathic pain remains to be explored. Therefore, the present study designed to evaluate the role of ginsenoside Rg1 (GRg) in chronic constriction injury (CCI) of sciatic nerve induced neuropathic pain in mice.

MATERIALS AND METHODS
Animals
The disease free male Swiss albino mice (20-25 g and age of 10 month) were used for the evaluation of GRg in CCI of sciatic nerve induced neuropathic pain in mice. All the animals were kept at standard laboratory diet, temperature (37 °C) and humidity condition. A 12 h light-dark cycle was maintained throughout the experimental protocol. The animals had free access to standard laboratory diet and water *ad libitum*. This research work was approved by the Institutional Animal Ethics Committee (IAEC; No.: ATRC/09/I4) and animal care 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 obtained from Pioneer Enterprise Mumbai, Maharashtra, India. N-naphthylethylenediamine; sulfanilamide; and 1,1,3,3-tetramethoxy propane were purchased 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 rest of the all other chemical reagents were obtained from S.D. Fine Chemicals, Mumbai, India.

Induction of peripheral neuropathy by chronic constriction injury
Neuropathic pain was induced in mice by CCI of sciatic nerve as described by Ma and Eisenach [34] with slight modification of Kaur *et al.* [35]. Briefly, mice were anaesthetized with thiopental sodium (40 mg/kg, *i.p.*). The hair lower back of right thigh region of the mice was removed and the skin was sterilized with 0.5 % w/v povidine solution. The skin and muscle layers were of the thigh region was incised and sciatic nerve was exposed. The four loose ligatures (silk thread no.: 4) were placed around the proximal portion of the sciatic nerve 1 cm from from the trifurcation part (i.e., before knee joint). The distance of 1 mm was maintained between each ligature around the sciatic nerve. The loose ligatures were applied until the short flick response appearance in ipsilateral (same side) hind paw. After completion of CCI procedure muscle and skin layers were sutured with silk thread and topical antibiotic powder was applied at once.

Experimental Protocol
Seven groups were employed in the present study. Each groups comprising eight Swiss albino mice (n=8). *Group I (Normal control):* Mice were not subjected to any surgical procedure and kept for 10 consecutive day's normal research laboratory conditions. *Group II (Sham control):* Mice were subjected to expose the right sciatic nerve without any nerve ligation by standard surgical procedure. This group was providing the information for nerve injury associated neuropathic pain with comparison of CCI control group. *Group III (CCI control):* Mice were subjected to expose and ligation the right sciatic nerve under anesthetic condition. The procedure was described in an induction of neuropathic pain section. *Group IV (GRg per se):* Mice were subjected to the administration of GRg (10 mg/Kg, *i.v.*) for 10 consecutive days in healthy normal Swiss albino mice. *Group V and VI (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. *Group VII (PreG; 5 mg/kg):* Mice were subjected to the intravenous administration of PreG (5 mg/Kg) for 10 consecutive days. All seven groups were employed for the assessment of behavioral and biochemical evaluations. All neurobehavioural tests were performed on different time intervals *i.e.,* 0, 4, 8, 12 and 16th day. On 16th day, all the animals were sacrificed. 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 the method of Choi *et al.* [36]. It is clinically resembles to thermal allodynia symptoms. Briefly, the mice were placed on wire mesh grid. The acetone (100 µl) 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.

Pin prick test
The mechanical pain sensation was assessed by the method of Erichsen and Blackburn-Munro [37]. Clinically, it is resemble to the pin point mechanical hyperalgesic symptoms. Briefly, the blunted needle was touched to the mid plantar surface of right hind paw. The intensity was generated until the detectable reflex withdrawal response in 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 as 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 method of Hargreaves *et al.* [38]. 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 as painful response. The cut off time was maintained at 20 second.

Tail flick test
The radiant heat sensation was assessed in tail part of the mice by the method of D'Aemour and Smith [39] with slight modification of Hargreaves et al. [38]. Clinically, it is resembles to 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 heat lamp source was considered as 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 tail part of the mice by the method of Takagi et al. [40]. Clinically, it is resembles to 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 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, thio-barbituric acid reactive substances (TBARS) & reduced glutathione (GSH); total calcium; tumor necrosis factor-alpha (TNF-α) estimation respectively. Superoxide anion was also estimated in sciatic nerve tissue sample. Further, surrounding muscular tissue was homogenated with phosphate buffer (pH 7.4) and used for the further estimation of myeloperoxidase (MPO) activity.

Estimation of TBARS
The thiobarbituric acid reactive substances (TBARS) was estimated by the method of Ohkawa et al. [41]. Briefly, 0.2 ml of supernatant of homogenate was mixed with 0.8 % w/v of thiobarbituric acid and test tubes were incubated for 1 h at 95 ^{0}C. The absorbance of pink colour chromogen was estimated spectrophotometrically 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 the method of Beutler et al. [42]. Briefly, 0.5 ml tissue homogenate was mixed

with freshly prepared DTNB (0.001 M) solution. The absorbance of yellow colour chromogen was estimated spectrophotometrically at 412 nm wavelength. The standard plot was prepared with 10-100 μg of GSH. The results of GSH concentrations were expressed as μg of GSH per mg of protein.

Estimation of total calcium
The total calcium levels were estimated in the sciatic nerve by the method of Severnghaus and Ferrebee, (1950) with slight modification of Muthuraman et al. [43]. Briefly, the sciatic nerve homogenate was mixed with 4 % trichloroacetic acid and centrifuged at $1500 \times g$ for 10 min. The supernatant was used for estimating the total calcium levels by atomic emission spectroscopy 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-α) level
The estimation of tumor necrosis factor-alpha (TNF-α) was done measured in the sciatic nerve homogenate as described by Muthuraman et al. [5]. Briefly, recombinant anti-Rat TNF-alpha was used for the reaction development. the procedure was followed as per the instruction of commercial rat TNF-alpha ELISA kit (RayBio, Inc., USA). The TNF-α standard plot was prepared by using 0 to 20,000 pg per ml of reference standard TNF-α samples. The absorbance of yellow colored formazan was estimated spectrophotometrically at 450 nm wavelength. The results were expressed as pictograms of TNF-α per mg of total protein.

Estimation of superoxide anion generation
The superoxide anion generation concentration was estimated by the method of Wang et al. [44] with slight modification of Muthuraman and Singh. [45]. Briefly, the sciatic nerve was treated with 5 ml phosphate buffered saline and 100 μM of NBT and incubated at 37ºC for 90 minutes. The NBT reduction changes are reflected in the changes of colour. The absorbance of purple-colored formazan was estimated spectrophotometrically at 540 nm wavelength. The quantity of NBT reduction = A x V / (T x Wt x ε x l). Where 'A' indicates the absorbance of blue formazan at 540 nm wavelength; 'V' indicates the volume of the solution; 'T' indicates the time period i.e., 90 minutes incubation with NBT; 'Wt' indicates the blotted wet weight of the sciatic nerve; 'ε' indicates the extinction coefficient of blue formazan i.e., 0.72 l per mmol per mm; and 'l' indicates the length of the light path. The results of NBT reduction were expressed as picomoles per minute per milligram wet weight of sciatic nerve.

Estimation of myeloperoxidase activity
The myeloperoxidase activity level in muscular tissue was estimated by the method of Patriarca et al. [46] with slight modification of Grisham et al. [47]. Briefly, the tissue homogenates were mixed reagent mixture i.e., DTNB to develop the yellow colored formazan. The absorbance of

yellow colored formazan was estimated spectrophotometrically at 460 nm wavelength. One unit of the myeloperoxidase activity is calculated by change of absorbance per min at pH 7.0 and 25 °C. One micromole of hydrogen peroxide used as substrate for MPO activity assessment. 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.* [48] method. Briefly, tissue samples were mixed with 5 ml of Lowry's reagent and 0.5 ml of Folin-Ciocalteu reagents. The test tubes were incubated at room temperature for 30 min. The absorbance of purple colour chromogen was estimated spectrophotometrically (UV-1800 UV-Vis spectrophotometer, SHIMADZU Corporation, Tokyo, Japan) 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 mean ± standard deviation (SD). Data obtained from behavioral tests were statistically analyzed using two-way analysis of variance (ANOVA) followed by Bonferonni's *post-hoc* analysis were applied by using Graph pad prism Version-5.0 software. The data of tissue biomarker *i.e.,* TBARS and GSH levels were analyzed using one way ANOVA followed by Tukey's multiple range tests were applied for *post-hoc* analysis by using Sigmastat Version-3.5 software. A probability value of $p < 0.05$ was considered to be statistically significant.

RESULTS
Role of GRg on CCI induced changes in acetone drop test
The performance of CCI of sciatic nerve resulted in a significant raise in thermal allodynic sensation as indication of increase in the scoring of chemical sensation when compared to the sham control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated CCI 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 (l0 mg/kg; *i.v.*) *per se* treated group did not show any significant ($p < 0.05$) changes in CCI induced thermal allodynia (Figure 1).

Role of GRg on CCI induced changes in pin prick test
The performance of CCI of sciatic nerve resulted in a significant raise in mechanical hyperalgesic sensation as indication of increase in the percentage withdrawal of right hind paw when compared to the sham control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated CCI 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 (l0 mg/kg; *i.v.*) *per se* treated group did

not show any significant ($p < 0.05$) changes in CCI induced mechanical hyperalgesia (Figure 2).

Role of GRg on CCI induced changes in plantar test
The performance of CCI of sciatic nerve resulted in a significant raise in thermal hyperalgesic sensation as indication of decrease in right hind paw withdrawal threshold when compared to the sham control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated CCI induced decrease in 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 (l0 mg/kg; *i.v.*) *per se* treated group did not show any significant ($p < 0.05$) changes in CCI induced thermal hyperalgesia (Figure 3).

Role of GRg on CCI induced changes in tail flick test
The performance of CCI of sciatic nerve resulted in a significant raise in thermal hyperalgesic sensation as indication of decrease in tail withdrawal threshold when compared to the sham control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated CCI induced 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 (l0 mg/kg; *i.v.*) *per se* treated group did not show any significant ($p < 0.05$) changes in CCI induced thermal hyperalgesia (Figure 4).

Role of GRg on CCI induced changes in tail pinch test
The performance of CCI of sciatic nerve resulted in a significant raise in mechanical hyperalgesia as indication of increase in the number of dislodgement of Heffner's clamp from the tail when compared to the sham control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated CCI 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 (l0 mg/kg; *i.v.*) *per se* treated group did not show any significant ($p < 0.05$) changes in CCI induced mechanical hyperalgesia (Figure 5).

Role of GRg on CCI induced changes in tissue biomarker changes
The performance of CCI of sciatic nerve resulted in a significant increase in TBARS, total calcium, TNF-α, superoxide anion & MPO levels; and decrease in GSH content as indication of oxidative stress, inflammation and neuronal damage when compared to the sham control group. Administration of GRg (5 and 10 mg/kg, *i.v.*) attenuated CCI induced changes of 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 (l0 mg/kg; *i.v.*) *per se* treated group did not show any significant ($p < 0.05$) changes in CCI induced tissue biomarker changes (Table 1 and Table 2).

Table 1. Role of GRg on CCI induced biomarker changes in tissue supernatant.

Groups	TBARS (nM / mg of protein)	GSH (µg / mg of protein)	Total calcium (ppm / mg of protein)	TNF-α (pg / mg of protein)
Normal	2.98 ± 0.79	76.92 ± 2.05	3.03 ± 0.51	29.41 ± 0.29
Sham	3.06 ± 0.48	75.38 ± 2.23	2.96 ± 0.73	43.09 ± 0.19
CCI	7.94 ± 0.86 *	35.65 ± 1.89 *	23.94 ± 0.47 *	74.04 ± 0.46 *
GRg (10) *per se*	3.11 ± 0.74	74.97 ± 1.86	3.15 ± 0.69	31.79 ± 0.17
CCI + GRg (5)	4.96 ± 0.62 #	68.83 ± 2.14 #	12.47 ± 0.38 #	42.93 ± 0.22 #
CCI + GRg (10)	4.27 ± 0.57 #	73.75 ± 2.05 #	5.62 ± 0.71 #	36.76 ± 0.38 #
CCI + PreG (5)	3.51 ± 0.93 #	74.68 ± 1.94 #	3.97 ± 0.65 #	32.49 ± 0.61 #

Role of GRg on CCI induced biomarker changes in tissue supernatant. Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n=6 mice per group. * $p < 0.05$ Vs sham control group. # $p < 0.05$ Vs CCI control group. Abbreviation: CCI, chronic constriction injury; GRg, Ginsenoside Rg1; TBARS, thiobarbituric acid reactive substances; GSH, reduced glutathione.

Table 2. Role of GRg on CCI induced biomarker changes in tissue.

Groups	NBT reduction (pM / Min / mg of tissue)	MPO (unit / Min / mg of protein)
Normal	2.87 ± 1.04	11.63 ± 1.83
Sham	3.13 ± 1.23	12.04 ± 1.49
CCI	24.73 ± 0.97 *	137.72 ± 2.52 *
GRg (10) *per se*	2.92 ± 1.04	14.27 ± 1.37
CCI + GRg (5)	9.64 ± 1.09 #	53.91 ± 3.07 #
CCI + GRg (10)	4.59 ± 1.11 #	31.79 ± 2.85 #
CCI + PreG (5)	4.01 ± 0.93 #	17.58 ± 1.94 #

Role of GRg on CCI induced biomarker changes in tissue. Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n=6 mice per group. * $p < 0.05$ Vs sham control group. # $p < 0.05$ Vs CCI control group. Abbreviation: CCI, chronic constriction injury; GRg, Ginsenoside Rg1; TBARS, thiobarbituric acid reactive substances; GSH, reduced glutathione.
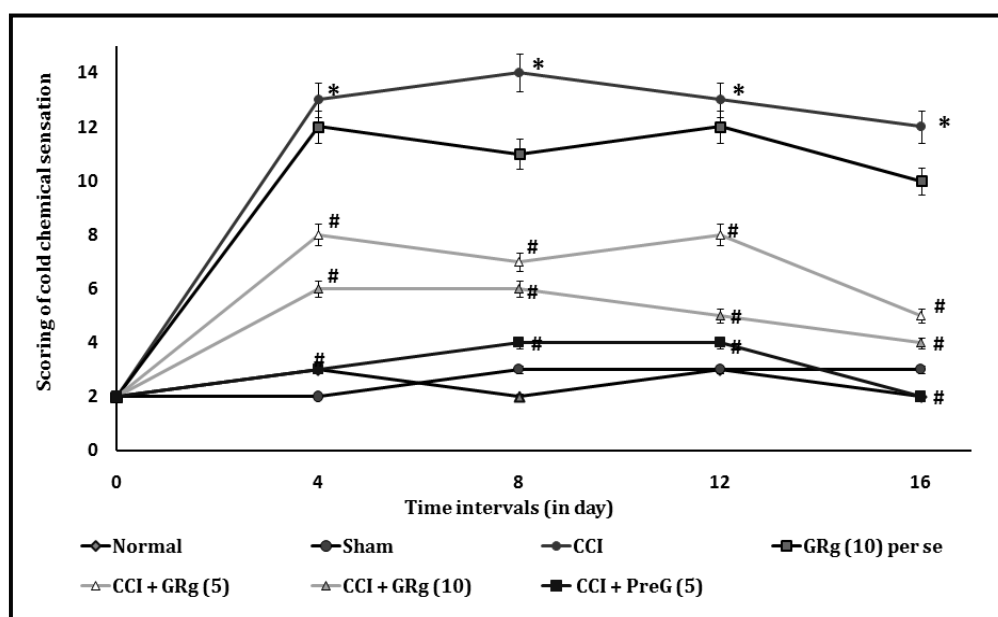
Figure 1. Role of GRg on CCI induced changes in acetone drop test (paw thermal allodynia). Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n=6 mice per group. * $p < 0.05$ Vs sham control group. # $p < 0.05$ Vs CCI control group. Abbreviation: CCI, chronic constriction injury; and GRg, Ginsenoside Rg1.
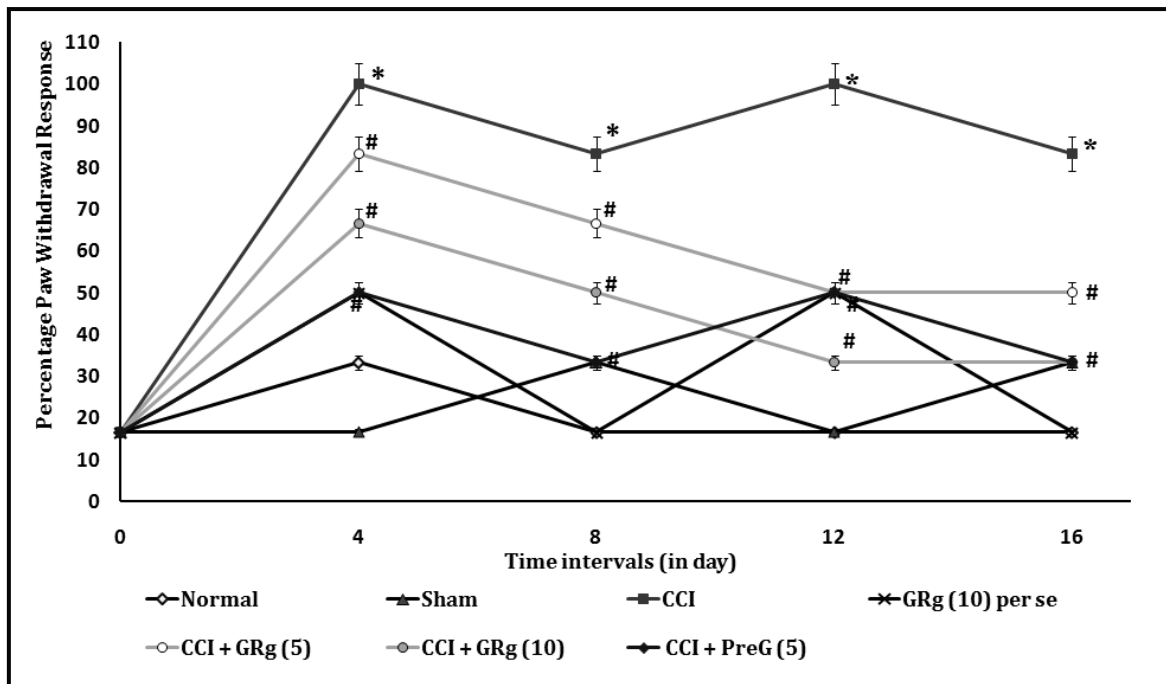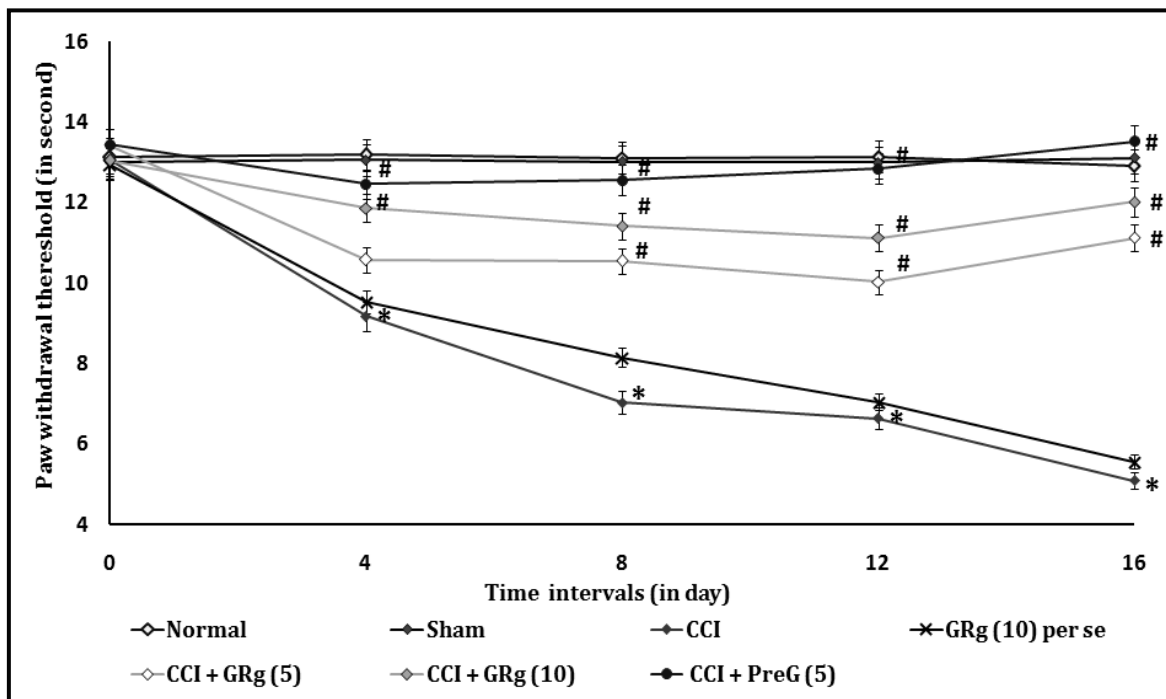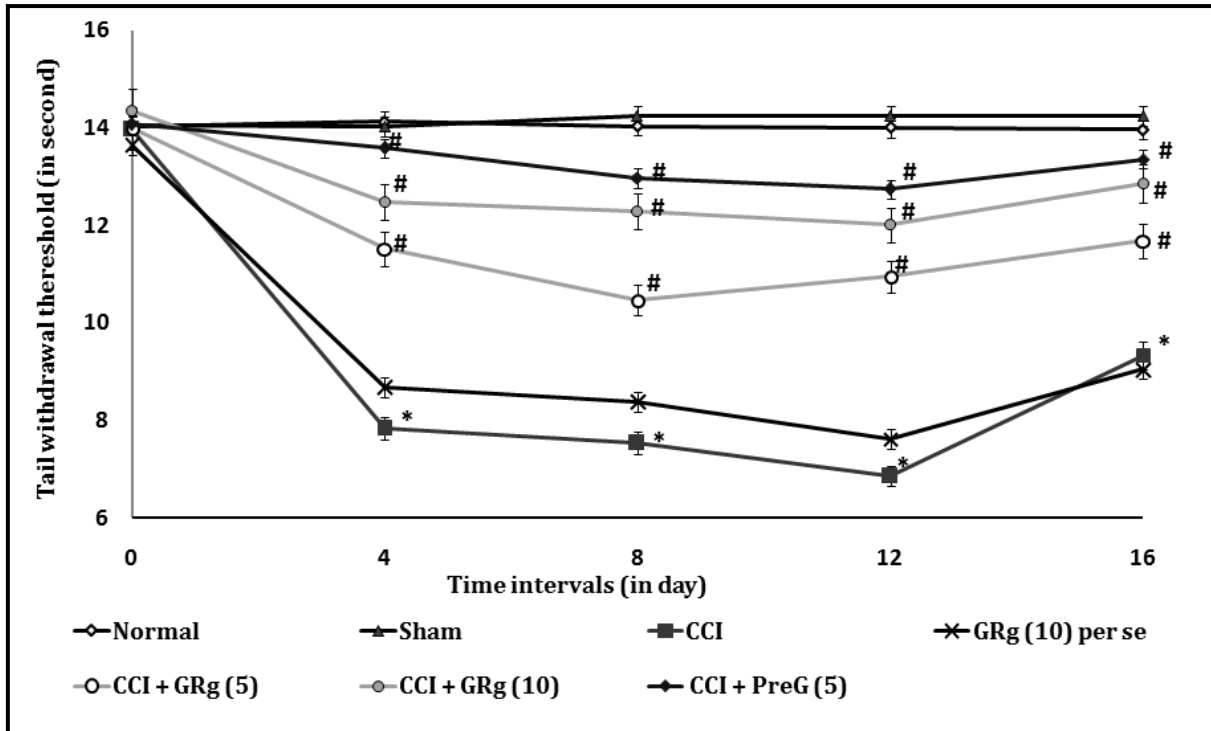
Figure 2. Role of GRg on CCI induced changes in pin prick test (paw mechanical hyperalgesia). Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n=6 mice per group. $^{*}p < 0.05$ Vs sham control group. $^{#}p < 0.05$ Vs CCI control group. Abbreviation: CCI, chronic constriction injury; and GRg, Ginsenoside Rg1.

Figure 3. Role of GRg on CCI induced changes in plantar test (paw 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 sham control group. $^{#}p < 0.05$ Vs CCI control group. Abbreviation: CCI, chronic constriction injury; and GRg, Ginsenoside Rg1.

Figure 4. Role of GRg on CCI 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 sham control group. $^{#}p < 0.05$ Vs CCI control group. Abbreviation: CCI, chronic constriction injury; and GRg, Ginsenoside Rg1.
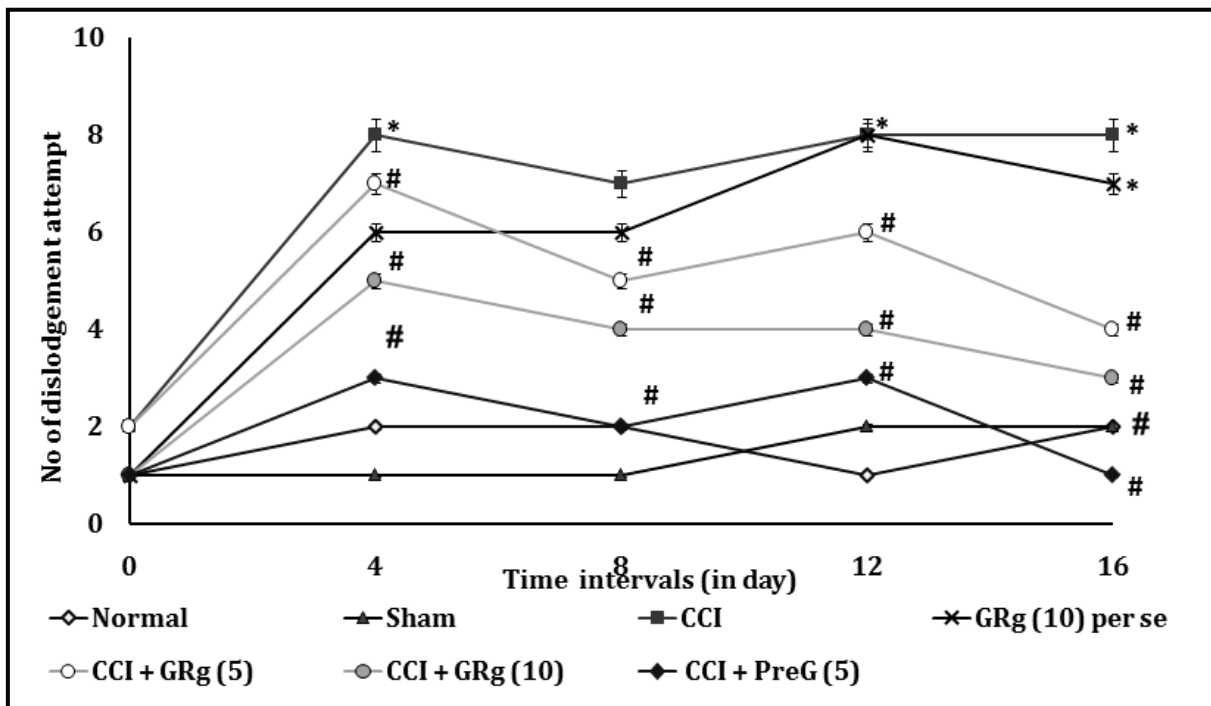

Figure 5. Role of GRg on CCI induced changes in tail pinch test (tail mechanical hyperalgesia). Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n=6 mice per group. $^{*}p < 0.05$ Vs sham control group. $^{#}p < 0.05$ Vs CCI control group. Abbreviation: CCI, chronic constriction injury; and GRg, Ginsenoside Rg1.

DISCUSSION

In the present study results revealed that, CCI of the sciatic nerve has produced the neuropathic pain by accelerating the thermal & mechanical hyperalgesia and allodynia in paw and tail region. It indicates that, CCI of sciatic nerve causing the neuronal excitation and accelerating the neuronal impulse. In addition, it also rising TBARS, total calcium, TNF-α, NBT reduction and MPO activity levels; whereas, the reduced glutathione levels were decreased. These changes are indicates the CCI mediated the pathogenesis are due to the activation free radical generation, lipid peroxidation, alteration of cellular calcium homeostasis, and rasing the inflammatory mediators associated neuroinflammation. The administration of natural medicines i.e., ginsenoside Rb1 (5 and 10 mg/Kg, *i.v.*) attentuated the CCI induced pain behavior and biochemical changes. It indicates that, ginsenoside Rb1 possess the potentent pain preventive action via neuroprotection.

The chronic constriction injury model is widely used model for the tesing of mononeuritic neuropathic pain [34, 49]. Clinically, it mimics the complex regional pain syndrome (CRPS) with involvement of multiple pathophysiological mechanisms in human as well as in animals [50] [51]. The peripheral nerve injury is also accelerates the neuronal signal in different region like peripheral nerve, spinal cord, and brain. In addition, various neurochemicals such as neurotransmitters i.e., serotonin, histamine and nor-adrenaline; ions i.e., sodium (Na^{2+}), potassium (K^+) and calcium (Ca^{2+}); pro-inflammatory mediatros i.e., TNF-α and interleukin (IL); and free redicals like superoxide anion, peroxy nitrite and hypochlorus radicals are contributes in the pathogenesis of neuropathic pain. Furthermore, it alters the sympathetic; para-sympathetic and non adrenergic-noncholinergic (NANC) neuronal systems [52-54]. Then, it alters the function of peripheral vascular system including nerve blood barriers and neurovascular units. Subsequently it enhances the ischemic environment for the peripheral nerve and it also enhance the accumulation of free radicals and neuroinflammations leads to aggrevates the neurodegeneration [7]. The rasing of free redicals known to cause the peroxidataion of cellular membrane lipids and alteration of ion channels/exchangers [43] Furthermore, the chronic activation of ion channels opening enhances the free cytosolic calcium ion (Ca^{2+}) accumulation [7, 55]. Then, it is inducing the mitochondrial and nuclear DNA dysfunction via activation m-calpain proteins and apoptotic proteins and cytokines including TNF-α protein [56-57]. Among all, the most vulnerable molecules for the neurodegeneration and neuronal death associated neuropathic pain is m-calpain and TNF-α protein [57-58].

The various natural plants are documented to produce the anti-neuralgesic effects via scvanging of free radicals; reduction of lipid peroxidation; decreasing of inflammatory mediator synthesis; and alteration oxidative stress related enzyme action like MPO, xanthine oxidase, glutathione reductase and glutathione peroxidase enzymes [55, 59-60]. Our previous research report also evidenced that, plant extract of various plant i.e *Acorus Calamus* [5,

59]; Butea monosperma [12]; *Swietenia mahagoni* [55]; *Ocimum sanctum* [50]; and *Vernonia cinerea* [61] have promising role in the management of neuropathic pain. In addition, phytoconstiturnts like Cannabinoids [62]; puerarin [63]; bulleyaconitine A [64]; (+)-borneol; thymoquinone; celastrol; liquiritigenin; epigallocatechin gallate; tocotrienol; lycopene and resveratrol [20] are also documented to produce the anti-neuralgesic effect viz free radical scavenging; reduction of TNF-α synthesis and regulation of cellular enzymatic defense system. In the present study, ginsenoside Rb1 ameliorates CCI induced neuropathic pain symptoms via reduction TBARS, calcium; TNF-α, superoxide anion and MPO levels; along with rasing the reduced glutathione level. Experimentally, ginsenoside reduced the free radicals, oxidative stress, neuroinflammation, cytokine production and neuronal death [65-67]. The present data and literature report revelas that, ginsenoside contributes in the potential neuroprotective action against the peripheral nerve injury. Hence, it is concluded that, ginsenoside Rb1 has the potential ameliorating effect in CCI of sciatic nerve injury induced neuropathic pain via anti-oxidant; anti-inflammatory; anti-cytokines and maintanence of cytosolic calcium ion concentration. Therefore, ginsenoside Rb1 is one of the newer molecules for the management of neuropathic pain disorders.

Author's contributions
Satbir Kaur: She has contributed in 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.

Conflict of interest
We declare that we have no conflict of interest.

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