Potential Impact of Ginsenoside Rg1 on Treatment of Paclitaxel-Induced Neuropathic Pain by TNF-α Inhibitory Action

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Received: March 25, 2020; Published: July 31, 2020

Abstract

Ginsenoside Rg1 (GRg) is a major bioactive of a ginseng plant. It is a flavonoid type of natural medicine. In this research work investigated to find the therapeutic action of GRg on paclitaxel (PT) associated neuropathic pain and tumor necrosis factor-alpha (TNF-α) regulatory action. The injection of PT (2 mg/kg, i.p.) for 5 days enhances the progression of neuropathic pain. The GRg dose 5 and 10 mg/kg; intravenous (i.v.) and pregabalin (5 mg/kg; i.v.) were administered for 10 consecutive days. The algesic responses were assessed by pinprick and plantar tests in paw dorsal surface; tail-flick test; and tail pinch test. These tests were assessed at days 0, 4, 8, 12 and 16. The biomarker changes i.e. superoxide anion, myeloperoxidase, and TNF-α protein level were estimated in sciatic nerve tissue. Further, the level of TNF-α was estimated in the serum sample and expression of TNF-α proteins studied in sciatic nerve tissue along with changes of histopathology slides. Treatment of GRg and pregabalin ameliorated the chemotherapeutic agent i.e., PT associated pain response with the control of biomarkers changes like superoxide anion, myeloperoxidase, and TNF-α protein levels and histopathological changes. GRg has potential ameliorative actions against PT induced neuropathic pain due to its free radical scavenging, anti-inflammatory; and TNF-α inhibitory actions.

Keywords: Myeloperoxidase; Neuroinflammation; Nociceptive Pain; Pregabalin, Sciatic Nerve; Tumor Necrosis Factor

Abbreviations

ANOVA: Analysis of Variance; CPCSEA; Committee for the Purpose of Control and Supervision of Experiments on Animals; CRPS: Complex Regional Pain Syndrome; ELISA: Enzyme-Linked Immunosorbent Assay; g-ratio: Ratio of Inner Axonal Diameter to the Total Outer Diameter; GRg: Ginsenoside Rg1; HRP: Horseradish Peroxidase; i.v.: Intravenous; IAEC: Institutional Animal Ethics Committee; MPO: Myeloperoxidase; NBT: Nitro Blue Tetrazolium; NIH: National Institutes of Health; PreG: Pregabalin; SD: Standard Deviation; SDS-PAGE: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis; TNF-α: Tumor Necrosis Factor-Alpha

Citation: Satbir Kaur and Arunachalam Muthuraman. "Potential Impact of Ginsenoside Rg1 on Treatment of Paclitaxel-Induced Neuropathic Pain by TNF-α Inhibitory Action". EC Pharmacology and Toxicology 8.8 (2020): 90-103.
Introduction

Neuropathic pain is a chronic progressive neuroinflammatory and neurodegenerative disorder. The major hallmark of neuropathic pain changes in nerve conduction velocity which occurs due to the neurodegeneration of central as well as the nervous system. The major behavioral symptoms are unpleasant painful sensation (called as hyperalgesia) and also lack of sensation (called as allodynia) [1]. The pathological mechanism of neuropathic pain and neurodegeneration are too complex. The well-known pathophysiological mechanism of neurodegeneration in the synthesis and expression of TNF proteins [2]. In physiological conditions, TNF proteins contribute to the cell signaling process [3]. In pathological conditions, it enhances inflammatory reactions. Various cells are synthesis into the TNF proteins like activated macrophages, CD4 T-lymphocytes, natural killer cells, mast cells, eosinophils, neutrophils including neurons. The primary action of TNF proteins regulates the immune cells leads to maintain the homeostasis condition of the body [3,4]. Paradoxically, the overproduction of TNF-α subtype contributes to the progression of various neurological disorders like Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, multiple sclerosis and neuropathic pain [4-6]. The pathological role of TNF-α protein is due to the excessive biosynthesis of TNF-α protein, overexpression of TNF receptor proteins, and lack of TNF-α degradation [7,8].

Various TNF targeted medicines are employed for the management of TNF-α levels and inhibition of TNF-α receptor action [8]. Such agents are monoclonal antibody i.e., infliximab, adalimumab, certolizumab and golimumab (direct binding to TNFα proteins); etanercept (decoy the circulating TNF-α receptor fusion protein); thalidomide (enhancing the degradation of TNF-α mRNA); and monoclonal antibodies, etanercept, pentoxifylline, and bupropion inhibits the TNF-α receptors [9-11]. However, the major drawback of anti-TNF alpha therapy is costly, and it produces the serious complications like lupus reaction, vasculitis, worsening of congestive cardiac failure, venous thrombosis (Infliximab), severe cytopenias, and asymptomatic neutropenia [12-14]. Some of the natural medicines are also possessing the anti-TNF actions like ethanolic extract of Calyptranthes grandifolia shown inhibition of TNF-α gene expression and cytokine release [15], ethanol extract of Sargassum fulvellum reduce the synthesis of TNF-α [16], and aqueous extract of Crocus sativus regulates the levels of TNF-α [17] and ethanolic extract of Centella asiatica reduces the hippocampal TNF-α level [18]. Similarly, natural compounds have TNF-α inhibitory actions like curcumin [19], catechins [20], ferulic acid [21] and gallic acid [22,23]. The test compounds of the present study i.e. Ginsenoside Rg1 (GRg) also have TNF-α inhibitory action. Recently, Ginsenoside Rf possesses the analgesic action against the chronic constriction injury of the sciatic nerve [24,25] and incisional injury [26,27]. Pregabalin (PreG) is one of the central acting neuro-modulating agents and it has neuro-analgesic action [28]. It is also used as a reference control for neuropathic pain study. Furthermore, the repeated administration of PT enhances the TNF-α associated neurodegeneration and neuropathic pain [29]. Hence, this research work investigated to find the therapeutic action of GRg on PT associated neuropathic pain in mice model.

Materials and Methods

Animals

The male Swiss albino mice (weight 22 ± 2g) were used for the investigation of natural medicine i.e. GRg in PT associated neuropathic pain. Animals were kept at standard laboratory diet, temperature and humidity conditions and animals were allowed to access the standard laboratory diet and water ad libitum. All the animals were maintained strictly 12 hours of light-dark cycles to avoid the physiological and behavioral variability. The experimental design was duly permitted by the Institutional Animal Ethics Committee (IAEC; No: ATRC/09/14). 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 was followed for maintenance of animals in animal house and research laboratory.

Drugs and chemicals

Ginsenoside Rg1 (98% purity) was procured from Pioneer Enterprise Mumbai, India. The animal dose of GRg was dissolved in 50 mM of phosphate buffer solution. The PT drug was procured from Bristol-Mayer Squipp Pvt LTD, Mumbai, India. Sulfanilamide and 1,1,3,3-tetramethoxy propane were supplied by Sisco Research Laboratories, Mumbai, India. Thiobarbituric acid and nitro blue tetrazolium (NBT)
was obtained from Sigma Aldrich Mumbai, India. The supply of the TNF-alpha ELISA kit was made by RayBio Inc., USA. The rest of the chemicals (analytical grade) were obtained from S.D. Fine Chemicals, Mumbai, India.

**Induction of neuropathic pain**

The progression of neuropathic pain was made by the multiple (5 days) injections of PT (2 mg/kg, i.p.) in mice [13,30]. Neuroalgesic (nerve injury associated pain) responses were assessed at days 0, 4, 8, 12 and 16.

**Experimental protocol**

This experimental design consists of six groups of animals. Each group comprising eight Swiss albino mice (n = 6). Group I (Normal control): Mice were kept as naive animals without the administration of the drug. Group II (GRg per se): Mice were employed for intravenous administration of 10 mg/kg of GRg for 10 days [25]. Group III (PT control): Mice were employed for the induction of neuropathic pain by injection of paclitaxel (2 mg/kg) for 5 days. Group IV and V (GRg; 5 and 10 mg/kg): Mice were employed to the administration of 5 and 10 mg/Kg of GRg for 10 days respectively in PT treated animals. About 10 minutes time intervals were maintained between the administration of GRg and PT. The GRg administered before the injection of PT agent. Group VI (PreG; 5 mg/kg): Mice were subjected to the intravenous administration of PreG (5 mg/Kg) for 10 consecutive days. The neurobehavioral tests were assessed at different time points i.e. day 0, 4, 8, 12 and 16. On the 16th day, blood samples were collected for estimation of serum TNF-α level. Then, mice were euthanized by cervical dislocation process. The sciatic nerve and surrounding tissue of the sciatic nerve were separated for the estimation of pathological biomarkers. Each group, two animal sciatic nerve tissue was used for the analysis of histopathological changes.

**Behavioral evaluation**

**Pinprick test**

The pinprick test (mechanical hyperalgesia) was evaluated as a described method of Erichsen and Blackburn-Munro [31]. Briefly, the blunted pinpoint needle was applied to the mid-plantar surface of the right hind paw. The pain intensity was raised as an indication of the right hind paw withdrawal reflex response. The blunted pinpoint needle was applied (six times per minute) by the uplifting process. The quick withdrawal of the right hind paw was noted as a painful response. The cut off stimuli were fixed six times per minute.

**Plantar test**

The plantar test (radiant heat hyperalgesia) was evaluated as a described method of Hargreaves., et al [32]. Briefly, the radiant heat stimuli were applied in the right hind paw. The thermal response was noted as a paw withdrawal reflex response (latency). The quick withdrawal of the right hind paw was noted as a painful response. The cut off time was retained for 20 seconds.

**Tail flick test**

The tail-flick test (radiant heat hyperalgesia) was evaluated as a described method of D’Aemour and Smith [33] with a slight modification of Hargreaves., et al [32]. Briefly, the tail terminal portion (1 cm distance) was allowed to contact the radiant heat stimuli area (lamp source). The rapid withdrawal of the tail was noted as a painful response. The cut off time was retained for 15 seconds.

**Tail Pinch test**

The tail pinch test (mechanical pressure hyperalgesia) was evaluated as a described method of Takagi., et al [34]. Briefly, Hoffmann’s clamp was applied in the base portion of the mice’s tail. The level of Hoffmann’s clamp screw was tuned to raise the mechanical pressure on the tail. The number of dislodgment attempts made on the clamp by mice was noted as a painful response. The cut off time was retained for 10 seconds.

**Biochemical estimation**

The mice serum was employed to assess the level of TNF-α protein. Besides, sciatic nerve and surrounding tissue samples were pre-
pared by homogenization (10% w/v) with 0.1M Tris-HCl buffer (pH 7.4) for total protein; with deionized water for myeloperoxidase (MPO) activity; and with phosphate buffer (pH 7.4) for TNF-α protein estimation. The level of superoxide anion generation was quantified in sciatic nerve tissue pellets.

**Estimation of serum TNF-α level**

The serum TNF-α level samples were estimated by enzyme-linked immunosorbent assay (ELISA) method as described by Muthuraman, et al. [35]; with a slight modification of Muthuraman and Ramesh [36]. The absorbance was estimated with test samples at a spectral wavelength of 450 nm using a spectrophotometer. The reference TNF-α protein plot was made with 0 to 20,000 picograms of TNF-α protein per milliliter as a standard sample. The data were expressed as pg of TNF-α protein per ml.

**Estimation of superoxide anion generation**

The concentration of tissue superoxide anion generation was quantified by Wang, et al. method [37] with a mild change of Muthuraman and Singh method [36]. The absorbance was estimated with test samples at a spectral wavelength of 540 nm using a spectrophotometer. The data of NBT reduction were expressed as picomole per minute per milligram of tissue.

**Estimation of myeloperoxidase (MPO) activity**

The concentration of tissue myeloperoxidase activity level was quantified by Patriarca, et al. method [34] with mild changes of Grisham, et al. method [39]. The absorbance was estimated with test samples at a spectral wavelength of 460 nm using by spectrophotometer. The data of myeloperoxidase activity were expressed as units per milligram of protein at one minute.

**Assessment of TNF-α protein expression by Western blot analysis**

The sciatic nerves TNF-α protein was estimated by western blot analysis method. The sciatic nerve specimens were homogenized with lysis buffer. Lysis buffer consists of 20 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), mM of Ethylene glycol tetraacetic acid (EGTA), 50 mM of β-glycerophosphate, 0.1% of Triton X-100, 10% of glycerol, 1 mM of dithiothreitol, 1 μg/ml of leupeptin, 5 μg/ml of aprotinin, 1 mM of phenylmethylsulfonyl fluoride and 1 mM of sodium orthovanadate. The pH-7.4 was adjusted. The homogenate of sciatic nerve suspension was centrifuged at 10,000 g-forces for 15 minutes at 4°C. The clear supernatants were electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE gel proteins were transferred to polyvinylidene difluoride membranes by the vertical electrophoretic method. TNF-α proteins were immunoblotted with corresponding to polyclonal rat anti-TNF-α antibody using Bio-Rad’s western blotting systems (Bio-Rad Laboratories, United States). Further, the membranes were stripped and reprobed with polyclonal rat anti-β-actin antibody (Bio-Rad Laboratories, United States). The incubation was made with horseradish peroxidase (HRP)-conjugated anti-rat TNF-α secondary antibody for 1 h at room temperature. The labeled proteins were developed using the chemiluminescent HRP substrate kit (ECL substrate, Bio-Rad Laboratories). To spectral signals of the membrane were digitalized after incubation of the membrane with western blot stripping buffer for 5 - 15 minutes at room temperature. The pixel density of the image was evaluated with the National Institutes of Health (NIH)-ImageJ software (NIH, Bethesda, USA). The data were as a picotogram of TNF-α protein per milligram of tissue total protein.

**Estimation of total protein content**

The tissue total protein levels were quantified as described by Lowry, et al. method [40]. 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.

**Histological evaluation**

The histological changes of the sciatic nerve were analyzed as described method of Krinke, et al. [41] with a slight modification of Muthuraman and Singh [36]. Briefly, samples of the sciatic nerve were fixed with 10% formalin (fixative solution). The cross-section of

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the sciatic nerve was made with 4 µm thickness by using a semiautomatic microtome device. Staining was done by using hematoxylin and eosin as described by Krinke, et al [41]. The histological changes of sciatic nerve sections were observed under a light microscope with 450 × magnification for detection of axonal degeneration. Furthermore, the axonal degeneration of the sciatic nerve was also analyzed quantitatively using a three-set of non-overlapping microscopic fields. The axon diameter (µm²), axon number (N), axon density (N/mm²) and g-ratio (the ratio of inner axonal diameter to total outer diameter) values were calculated with the help of NIH ImageJ software (NIH, Bethesda, USA). Scale bar indicates 35 µm.

Statistical analysis

The data in hand were revealed as mean ± standard deviation (SD). The pain response was analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni’s post-hoc method of statistical analysis via Graph pad prism (version-5.0) software. Moreover, the bio-marker changes were analyzed by one way ANOVA followed by Tukey’s multiple range test methods via Sigmastat (Version-3.5) software. The statistical probability i.e. p-value less than 0.05 (p < 0.05) was noted as a statistical significant difference between groups.

Results

Impact of GRg in pinprick test

The 5 days continuous injection of PT (2 mg/kg) shown significant (p < 0.05) rising of mechanical pain response. It is noted as an increase in the percentage withdrawal of right hind paw compared to the normal group of mice. The intravenous administration of test compound i.e. 5 and 10 mg/Kg of GRg ameliorated taxel induced paw withdrawal response in a dose-dependent manner. The response of GRg was similar to the reference compound i.e. pregabalin treated mice. The administration of vehicle and GRg (10 mg/kg; i.v.) per se treated mice didn’t show significant effect against the taxel induced mechanical hyperalgesia (Figure 1).

Figure 1: Role of GRg on PT-induced changes in pinprick 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 normal control group. #p < 0.05 Vs PT control group.
**Impact of GRg in plantar test**

The 5 days continuous injection of PT (2 mg/kg) shown significant (p < 0.05) rising of thermal pain response. It is noted as a decrease in the right hind paw withdrawal threshold compared to the normal group of mice. The intravenous administration of test compound i.e. 5 and 10 mg/Kg of GRg ameliorated taxel induced paw withdrawal response in a dose-dependent manner. The response of GRg was similar to the reference compound i.e. pregabalin treated mice. The administration of vehicle and GRg (10 mg/kg; i.v.) per se treated mice didn't show a significant effect against the taxel induced thermal hyperalgesia (Figure 2).

![Figure 2](Image)

*Figure 2: Role of GRg on PT induced changes in the 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 normal control group. *p < 0.05 Vs PT control group.*

**Impact of GRg in the tail-flick test**

The 5 days continuous injection of PT (2 mg/kg) shown significant (p < 0.05) rising of thermal pain response. It is noted as a decrease in the tail withdrawal threshold compared to the normal group of mice. The intravenous administration of test compound i.e. 5 and 10 mg/Kg of GRg ameliorated PT induced tail withdrawal response in a dose-dependent manner. The response of GRg was similar to the reference compound i.e. pregabalin treated mice. The administration of vehicle and GRg (10 mg/kg; i.v.) per se treated mice didn’t show a significant effect against the PT induced thermal hyperalgesia (Figure 3).

**Impact of GRg in tail pinch test**

The 5 days continuous injection of PT (2 mg/kg) shown significant (p < 0.05) rising of mechanical pain response. It is noted as an increase in the number of dislodgement of Heffner’s clamp of mice compared to the normal group of mice. The intravenous administration of test compound i.e. 5 and 10 mg/Kg of GRg ameliorated PT induced tail withdrawal response in a dose-dependent manner. The response of GRg was similar to the reference compound i.e. pregabalin treated mice. The administration of vehicle and GRg (10 mg/kg; i.v.) per se treated mice didn’t show significant effect against the PT induced mechanical hyperalgesia (Figure 4).

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Figure 3: Role of GRg on PT 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 PT control group.

Figure 4: Role of GRg on PT 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 PT control group.
Impact of GRg in biomarker changes

The 5 days continuous injection of PT (2 mg/kg) was shown significant (p < 0.05) rising of superoxide anion, MPO and TNF-α levels compared to the normal control mice. It was indicated as PT induced the neuroinflammation, oxidative stress and neuronal damage. The intravenous administration of test compound i.e. 5 and 10 mg/Kg of GRg ameliorated PT induced above biomarker changes in a dose-dependent manner. The response of GRg was similar to the reference compound i.e. pregabalin treated mice. The administration of vehicle and GRg (10 mg/kg; i.v.) per se treated mice didn't show a significant effect against the PT induced biomarker changes (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NBT reduction (pM/Min/mg of tissue)</th>
<th>MPO (unit/Min/mg of protein)</th>
<th>TNF-α (pg/mg of protein)</th>
<th>Serum TNF-α (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.92 ± 0.98</td>
<td>13.47 ± 1.48</td>
<td>35.94 ± 1.34</td>
<td>14.91 ± 3.93</td>
</tr>
<tr>
<td>GRg (10) per se</td>
<td>3.64 ± 1.06</td>
<td>17.92 ± 2.02</td>
<td>36.82 ± 1.04</td>
<td>21.73 ± 4.24</td>
</tr>
<tr>
<td>PT (2)</td>
<td>29.27 ± 0.86*</td>
<td>106.82 ± 1.73*</td>
<td>84.93 ± 1.23*</td>
<td>204.38 ± 4.81*</td>
</tr>
<tr>
<td>PT + GRg (5)</td>
<td>9.36 ± 1.04*</td>
<td>49.58 ± 3.21*</td>
<td>49.03 ± 1.39*</td>
<td>127.29 ± 5.02*</td>
</tr>
<tr>
<td>PT + GRg (10)</td>
<td>5.26 ± 0.82*</td>
<td>37.29 ± 1.62*</td>
<td>42.83 ± 1.63*</td>
<td>94.42 ± 4.27*</td>
</tr>
<tr>
<td>PT + PreG (5)</td>
<td>4.98 ± 1.14*</td>
<td>21.83 ± 2.32*</td>
<td>38.56 ± 1.93*</td>
<td>51.64 ± 3.15*</td>
</tr>
</tbody>
</table>

Table 1: Impact of GRg in tissue biomarker changes.

Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n = 6 mice per group. *p < 0.05 Vs normal group. #p < 0.05 Vs PT control group. Abbreviation: GRg: Ginsenoside Rg1; PreG: Pregabalin; PT: Paclitaxel; NBT: Nitro Blue Tetrazolium; MPO: Myeloperoxidase; TNF-α: Tumor Necrosis Factor-Alpha.

Impact of GRg in the expression of TNF-α level

The 5 days continuous injection of PT (2 mg/kg) was shown significant (p < 0.05) rising the expression of TNF-α level compared to the normal control mice. The intravenous administration of test compound i.e. 5 and 10 mg/Kg of GRg ameliorated PT induced overexpression of TNF-α in a dose-dependent manner. The response of GRg was similar to the reference compound i.e. pregabalin treated mice. The administration of vehicle and GRg (10 mg/kg; i.v.) per se treated mice didn't show a significant effect against the PT induced expression of TNF-α level (Figure 5).

Impact of GRg in histopathological changes

The administration of PT (2 mg/kg, i.p. for 5 consecutive days) resulted to produce the significant histopathological changes i.e., nerve derangement, axonal swelling, number of Schwann and satellite cells. Administration of GRg (5 and 10 mg/kg, i.v.) attenuated PT induced changes of the above histopathological changes in a dose-dependent manner. This GRg effect was similar to that of pregabalin treatment. However, vehicle and GRg (10 mg/kg; i.v.) per se treated group did not show any significant changes (Figure 6). Moreover, the administration of PT was decreased the axon diameter, axon number, and axon density; and increase the g-ratio values when compared to the normal control group. The treatment of GRg was ameliorated with the PT induced above morphometric changes of sciatic nerve tissue in a dose-dependent manner: This effect was similar to that of pregabalin treatment (Table 2).

Discussion

The data of research revealed that 5 days administration of PT (2 mg/kg) was potentially (p < 0.05) produced the neuropathic pain. This action of the PT on the sciatic nerve is due to the neuroinflammation and neuronal excitation via accelerating the neuronal firings. Further, it also induced the levels of NBT reduction, MPO activity and TNF-α including TNF-α expression levels. Thus, the PT enhances the
Figure 5: Impact of GRg in the expression of TNF-α level. 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 PT control group.

Figure 6: Impact of GRg on PT induced histopathological changes. Figure 6a-6f shown a cross-section of the sciatic nerve of normal, GRg (10) per se, PT (2), GRg (5 and 10 mg/kg, i.v.) and pregabalin (5 mg/kg, i.v.) treated groups respectively. In figure 6b-6f, blue arrow showed fiber derangement, bold arrow showed swelling of nerve fiber and arrowhead showed the presence of activated satellite cells and Schwann cells. In figure 6d-6f, shown attenuation of PT induced swelling of nerve fibers by GRg (5 and 10 mg/kg) and pregabalin treatment groups respectively. Microscopic examinations were performed under 450 × light microscopy, scale bar 35 μm.
Potential Impact of Ginsenoside Rg1 on Treatment of Paclitaxel-Induced Neuropathic Pain by TNF-α Inhibitory Action

<table>
<thead>
<tr>
<th>Groups</th>
<th>Axon diameter (μm²)</th>
<th>Axon number (N)</th>
<th>Axon density (N/mm²)</th>
<th>g-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.76 ± 1.68</td>
<td>5643 ± 243</td>
<td>21029 ± 2872</td>
<td>0.93 ± 0.03</td>
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<td>GRg (10) per se</td>
<td>3.89 ± 1.73</td>
<td>5689 ± 221</td>
<td>21073 ± 2902</td>
<td>0.98 ± 0.07</td>
</tr>
<tr>
<td>PT (2)</td>
<td>1.49 ± 0.91*</td>
<td>2874 ± 532*</td>
<td>11582 ± 4121*</td>
<td>1.86 ± 0.09*</td>
</tr>
<tr>
<td>PT + GRg (5)</td>
<td>3.01 ± 0.83*</td>
<td>4956 ± 471*</td>
<td>18653 ± 3107*</td>
<td>1.12 ± 0.05*</td>
</tr>
<tr>
<td>PT + GRg (10)</td>
<td>3.31 ± 0.91*</td>
<td>5386 ± 398*</td>
<td>19849 ± 3682*</td>
<td>1.04 ± 0.04*</td>
</tr>
<tr>
<td>PT + PreG (5)</td>
<td>3.63 ± 1.03*</td>
<td>5571 ± 403*</td>
<td>20842 ± 4016*</td>
<td>0.93 ± 0.06*</td>
</tr>
</tbody>
</table>

Table 2: Impact of GRg in morphometric changes of the sciatic nerve.

Digits in parenthesis indicate dose in mg/kg. Data were expressed as mean ± SD, n = 6 mice per group. *p < 0.05 Vs normal group. **p < 0.05 Vs PT control group. Abbreviation: GRg: Ginsenoside Rg1; PreG: Pregabalin; PT: Paclitaxel; and g-ratio, the ratio of inner axonal diameter to the total outer diameter.

progression of neuropathic pain via neuroinflammation and neurodegeneration via biosynthesis of free radicals, proinflammatory cytokines and enhancing of inflammatory protein (i.e. MPO). However, administration of GRg (5 and 10 mg/kg, i.v.) attenuated the PT induced neuropathic pain with amelioration of biochemical and histopathological changes. Thus, it evidenced that, GRg possesses the potential neuroprotection via alteration of the proinflammatory cytokine pathway.

Various research reports are documented that, the cancer chemotherapeutic agent i.e., PT causing the progression of neuropathic pain at a dose of 2 mg/kg, i.p. for 5 consecutive days in the rat [42]. Clinically it mimics the polynoeuritis neuropathic pain. It is a type of complex regional pain syndrome (CRPS) in humans and in animals [43,44]. The various mechanism is involved in PT associated peripheral neuropathic pain and neurodegeneration [45]. The primary and major molecular mechanism of neuropathic pain is the synthesis and expression of TNF-α proteins and their receptor proteins [46]. Further, it enhances the degradation of neuronal myelin and axonal proteins [47]. The present study report also undergoes the same pathological mechanism. Moreover, the major action of PT in cancer cells is stabilized microtubules and inhibits tubulin polymerization [48]. Unfortunately, it also binds to microtubulin (cytoskeletal protein) proteins of host neuronal cells, which leads to cause the neurodegeneration and neuropathic pain [45]. Our preliminary research reports and other laboratory research reports are documented that, phytomedicines has neuroprotective and amelioration of neuropathic pain via TNF-α inhibitory actions such plants are Acorus calamus [35,36]; rikkunshito [49]; 7,2',3'-trimethoxy flavone, 7,2',4'-trimethoxy flavone, 7,3',4'-trimethoxy flavone and 7, 5, 4'-trimethoxy flavone [50] icariin [51] and ellagic acid [52]. Experimentally, GRg also prevents free radical formation, neuroinflammation, cytokine production and neuronal apoptosis [10,24]. And, it also possesses the TNF-α inhibitory action [53]. Another derivative of ginsenosides i.e. ginsenoside Rf showed potential analgesic action against the sciatic nerve injury [24] and incisional muscular injury [27]. Crucially, ginsenosides have anti-inflammatory and analgesic actions. However, the present research evidenced that; the GRg variety has neuro-analgesic effects against PT induced neuropathic pain.

Conclusion

Hence, GRg can is recommended for the treatment of chemotherapy (especially tubulin polymerization inhibitor: PT) induced neuropathic pain by virtue of its free radical scavenging, anti-inflammatory, inhibition of TNF-α synthesis and expression in neuronal tissue.

Acknowledgments

The authors are thankful to the Akal Toxicology Research Centre, A unit of Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur-148001, Punjab (India) for supporting this study and providing technical facilities for this work.

Citation: Satbir Kaur and Arunachalam Muthuraman. “Potential Impact of Ginsenoside Rg1 on Treatment of Paclitaxel-Induced Neuropathic Pain by TNF-α Inhibitory Action”. EC Pharmacology and Toxicology 8.8 (2020): 90-103.
Conflict of Interest
The authors declare that they have no conflicts of interest.

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Volume 8 Issue 8 August 2020
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*Citation*: Satbir Kaur and Arunachalam Muthuraman. “Potential Impact of Ginsenoside Rg1 on Treatment of Paclitaxel-Induced Neuropathic Pain by TNF-α Inhibitory Action”. *EC Pharmacology and Toxicology* 8.8 (2020): 90-103.