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Topical Dosage for Treatment of Chemotherapy-Induced Peripheral Neuropathy

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Abstract---*Peripheral neuropathy caused by chemotherapy is a general therapeutic limiting factor that greatly affects patients ' daily lives, mostly due to lack of appropriate palliative options. CIPN is a severe, often chronic condition with pain and reduced function. In addition, CIPN is its most common dose-limiting side effect, which is decreasing and could affect treatment outcomes. Interestingly, the incidence of paclitaxel (taxane) induced peripheral neuropathy is very high ranging from 53-83% with severity in 2-33% patients. The clinical symptoms of CIPN affect sensory, motor and sometimes autonomic functions. A sensory disturbance includes allodynia, hyperalgesia and spontaneous sensations such as tingling, burning, electric, stabbing, numbness, and prickling. Hence, considering the drawbacks of existing treatments, this study provides a topical gel comprising of pharmacological agents with multiple mechanisms of action and regeneration of damaged nerve fibres. The formulated gel successfully qualified the tests for semi-solid dosage form such as color, appearance, and homogeneity, measurement of pH, viscosity, spreadability, drug content and stability.*

Keywords--- *Peripheral neuropathy, chemotherapy, CIPN, pharmacological agents, allodynia, hyperalgesia, topical*

I. INTRODUCTION

Chemotherapy-induced peripheral neuropathy (CIPN) persists, according to the National Cancer Institute (NCI), one of the major shortcomings in oncology clinics that lead to poor patient adherence and disease relapse [1]. CIPN incidence depends on several factors such as patient age, dose intensity, cumulative dose, therapy duration, and diabetes or alcoholabuse pre-existingstatus[2]. Many therapeutic agents, including anticonvulsants, tricyclic antidepressants, and analgesics including NSAIDS (non-steroidal anti-inflammatory drugs) and certain opioids, have been tested for symptomatic treatment of CIPN. Symptoms slowly worsen as chemotherapy progresses, with no difference between doses [3]. The symptoms are reversible to some extent in about 80 percent of patients and fully resolve at 6 to 8 months after cessation of treatment in about 40 percent [4]. Nevertheless, for an additional 2 to 6 months post-therapy, a phenomenon known as "coasting," signs and symptoms can continue to develop and improve. Commonly reported sensory symptoms include paresthesia, dysthesia, allodynia, hyperalgesia, hypoalgesia, burning, electrical and sensation-like shock [5]. Impaired axonal transmission and decreased peripheral nutrient blood flow contribute to nerve damage and motor disturbance[3], [6].

Numerous therapeutic agents have been evaluated for symptomatic treatment of CIPN, which includes anticonvulsants (gabapentin and pregabalin), tricyclic antidepressants (amityptyline and nortyptyline), venlafexine

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hydrochloride, and analgesics include NSAIDS and some opioids. Some topical treatments like capaicin creams, ketamine and amitriptyline gel, lidocaine patches are also available[7], [8]. Due to the limited other CIPN treatment options, these agents can be presented on the basis of data supporting their effectiveness in other neuropathic pain conditions. Nevertheless, for patients undergoing cancer treatment, no well-recognized and prescribed medication is available for the prevention of CIPN. This is focused on the lack of consistent proof of high quality and a balance of benefits and harms. The treatment options available are neurotoxic and cannot be offered to cancer patients who already suffered from the side effects of cancer chemotherapy [5]. Further, these treatment options when administered orally have limited use since they exhibit intolerable side-effects and provide mild relief to only a fraction of patients.[6] The major side effects include blurred vision, peripheral edema, constipation, confusion, cardiac arrhythmia, postural hypotension, hyperhydrosis, urinary retention, weight gain, somnolence, dizziness, dry mouth, sedation, euphoria, nausea, anorexia and many more.

So, to overcome the existing therapies drawbacks, there is a need to develop a topical gel formulation containing active pharmaceutical agents with diverse mechanism of actions and a method of preparation thereof for providing relief from the symptoms of CIPN without causing intolerable side effects. Hence, the aim of this study is to provide a topical gel formulation designed comprising of US Food and Drug Administration (FDA) approved pharmacological agents (pregabalin, venlafexine hydrochloride, lidocaine, levender oil and Vit B12) with multiple mechanisms of actions for the management of CIPN [4][6]. Mechanistically, pregabalin binds strongly affinity to the $\alpha 2\pi 1$ subunit of voltage-gated calcium ($Ca2^{+}$) channels and attenuates the influx of neuronal calcium resulting in inhibition of the release of neurotransmitters like norepinephrine and glutamate proinflammatory mediators [8]. This mechanism may be responsible in all animal models for the highly significant attenuation of paclitaxel-induced pain. While venlafexine hydrochloride is a serotonin-norepinephrine reuptake inhibitor class antidepressant medication. It is used to treat major depressive disorder, common syndrome of anxiety, panic disorder, and social phobia. These neurotransmitters act in synergistic manner to decrease the transmission of pain signals from periphery to CNS.

II. MATERIALS AND METHODOLOGY

2.1 Drugs and reagents

Paclitaxel was purchased from *Bristol-Myers*Squibb India Pvt. Ltd, Mumbai. Pregabalin, Venlafexine hydrochloride, Lidocaine and $VitB_{12}$ was obtained from All Well Pharmaceuticals Pvt. Ltd. (Chandigarh, India). “DTNB (5, 5´ -dithiobis-2-nitrobenzoic acid)”, “Folin-Ciocalteu phenol reagent”, “Bovine serum albumin”, “reduced glutathione (GSH)”, Sodium hydroxide, Hydrochloric acid, Sodium chloride, and dipotassium hydrogen phosphate were purchased from “Loba chemicals (Mumbai, India)”. Thiobarbituric acid was obtained from “Magus Chemicals”. Trichloroacetic acid was purchased from “Nice Chem. Pvt.Ltd. (Cochin India)". Carbopol utrazet, HPMC, PEG1500, glycerine, benzyl alcohol, EDTA, Propylene glycol, DMSO were purchased from Thomas Baker, India. ELISA kits were purchased from PEPROTECH.

2.2 Preparation of Topical Gel

The topical gel formulation is prepared in two parts by neutralization method. The method of preparation of the gel formulation consists of the following steps:

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1.2.1 *Preparation of Part A (50gm):* Weighed amount of carbopol ultrez as shown in Table 1 was dissolved in small quantity of water. Then PEG 1500 and HPMC were added in the mixture. Then pregabalin, Venlafexine hydrochloride, lidocaine and Vit B12 were dissolved separately in small quantities of water and then gradually mixed them in the above mixture one by one with continuous stirring. After that, camphor oil was dissolved in propylene glycol separately and put into mixture with stirring. Glycerine and benzyl alcohol were added into mixture. After mixing of all the ingredients, final pH was maintained with the addition of triethanolamine (TEA).

1.2.2 *Preparation of Part B (50gm):* Weighed amount of carbopol ultrez as mentioned in Table 1 was dissolved in small quantity of water. Then PEG 1500 and HPMC were added in the mixture. Propylene glycol was added into above mixture. Tocopheryl acetate was dissolved carefully into DMSO with continuous stirring. Then it was slowly mixed with above mixture to avoid any precipitation. Glycerine was added into mixture. After mixing of all the ingredients, final pH was maintained with the addition of triethanolamine (TEA).

1.2.3 Part A and part B were mixed with each other and allowed for mechanical stirring for 30 minutes (approx) to form a homogenous gel formulation. After mixing of both the parts, levender oil was added into gel and mixed well. The prepared gel was then packed into air tight container.

Table 1: Composition of pharmaceutical gel used to treat paclitaxel induced peripheral neuropathy

S.no	Ingredients	Quantity (g)	Quantity (%)
1.	Carbopol Ultrez	0.25	0.97
2.	HPMC	0.025	0.097
3.	PEG 1500	0.1	0.38
4.	Propylene glycol	7.5	29.16
5.	Glycerine	0.25	0.97
6.	TEA	0.75	2.91
7.	Pregabalin	0.5	1.94
8.	Venlafexine HCl	0.5	1.94
9.	Lidocaine HCl	1.25	4.86
10.	Levender Oil	1.25	4.86
11.	Cynocobalamine	0.0125	0.048
12.	Tocopheryl acetate	0.5	1.94
13.	Camphor oil	0.125	0.486
14.	Water	12.71	49.41

2.3 Characterization of topical gel

Three batches are formulated for the optimization of invented gel formulation and compared with the marketed topical gel.

2.3.1 Colour and homogeneity

The colour of invented gel and marketed gel was noticed. Moreover, the prepared gel and marketed gel were inspected visually for homogeneity, consistency and grittiness.

2.3.2 Measurement of pH

A calibrated pH meter (Eutech) was used to accurately measure the pH of the developed gel and sold gel. In 50 ml of distilled water, 1gm of gel was suspended and a homogeneous dispersion was formed. In triplicate (n=3), the pH of dispersions was calculated.

2.3.3 Measurement of rheology

"Brookfield rheometer (BrookfieldDV-3 T + Rheometer)" used a LV-4 spindle to determine the viscosity of personalized gel specimens. Gel specimens were poured into the rheometer adapter and the test samples ' viscosity was measured as a function of the shear frequency in compliance with the manufacturer's standard operating procedure. Every triplicate experiment (n=3) was performed.

2.3.4 Spreadability

Spreadability is a term that refers to the degree to which the topical gel spreads in the affected sections when applied to the body. 1 gm of gel sample was distributed between two glass slides to assess spreadability and compressed by putting 1000 gm of weight over the glass slides for 5 minutes. Then a weight of 100 gm was applied to the pan and with the aid of a string attached to the handle, the top plate was subjected to pull. The moment when the upper glass slide travels to cover a gap of 10 cm against the lower glass slide has been noted. Good spreadability is demonstrated by a shorter duration. Every triplicate experiment (n=3) was performed. The spreadability (S) was then calculated by the formula:

$$S = M.L / T$$

Where S refers to spreadability;
M denotes the weight tied to upper glass slide,
L is equal to the length covered on glass slide, and
T refers to time taken.

2.3.5 Drug content

To make sure the quantity of active pharmaceutical ingredients, formulated gel was assayed for the drug content by gas chromatography.

2.4 In vivo studies

The *in vivo* evaluation of novel topical gel was perfomed using Wistar rats (180-220g) of either sex. The animals were separated into 4 groups of 6 rats each. "Group 1-normal control (treated with normal saline)", "Group 2-

disease control (paclitaxel, 8mg/kg; *i.p*in four divided doses)", "Group 3- Oral group (paclitaxel, 8mg/kg; *i.p*. in four divided dosesand oral suspension)", "Group 4- Topical gel group (paclitaxel, 8mg/kg; *i.p*.in four divided doses and topical gel)". The invented topical gel was comprised of Pregabalin, Venlafexine hydrochloride, Lidocaine, Camphor oil, Vit B_{12} and Levender oil whereas oral solution consisted of Pregabalin, Venlafexine hydrochloride and Vit B12.

2.4.1 Induction of peripheral neuropathy

Paclitaxel (Taxol^{Rx}) was injected Intraperitonealy at a dosage of the 8 mg/kg in four divided doses, on days 1, 3, 5, and 7 to induce peripheral neuropathy in rats. Behavioral parameters (such as mechanical hyperalgesia, cold allodynia, heat hyperalgesia) were evaluated before paclitaxel administration and after paclitaxel administration on day 2, 7, 14, 21 and 28 to confirm induction of peripheral neuropathy. After 14 days of paclitaxel administration, drug treatment (topical gel and oral solution) was started for 2 weeks. Then after completion of drug treatment, on "28th day" all the animals were sacrificed and then the biochemical estimations (like TBARS, total protein, reduced GSH, TNF-α, and IL-6) were carried out in sciatic nerve to verify the severity of disease. TNF-α and IL-6 levels were then calculated using "enzyme-linked immunosorbent assay (ELISA)". Histopathological examination of the isolated sciatic nerve was carried out using standardized hematoxylin-eosin and toluidine blue staining techniques.

2.5 Behavioral parameters

2.5.1 Mechanical hyperalgesia: The Randall and Sellito paw stress test was used to calculate the mechanical nociceptive threshold as an indicator of mechanical hyperalgesia. The threshold of nociception has been described as the force (gm) at which the rat withdraws its paw. Treatment with the paclitaxel leads to bilateral allodynia and hyperalgesia. To determine the mechanical nociceptive threshold, removal of the hind paw was noted.

*2.5.2 Cold allodynia:*Cold allodynia was tested by immersing in cold water (10℃) the hind paw of rats. Cold sensitivity reaction was observed with regard to either paw leaking, shaking or rubbing the hind paw and reported as period of paw lifting. The cut-off was set at 20s for cold allodynia**.**

*2.5.3 Heat hyperalgesia:*The hind paw heat hyperalgesia was tested by using the hot plate from Eddy to test the reactivity to noxious thermal stimuli. The rats were placed on the top of a preheated temperature hot plate (52.5 ± 0.5 ° C). It was noted that the heat hyperalgesia was removed from the hind paw. The time limit was set at 20s.

2.6 Biochemical Estimations

Collection of samples: At the end of the experiment, all experimental animals were sacrificed by cervical dislocation. The sciatic nerve was quickly found and removed. Isolated sciatic nerve was excised into small pieces and 0.1 M Sorenson phosphate buffer (10 percent w / v, pH 7.4) was used to prepare uniform sciatic nerve homogenates. The homogeneous test tubes were held for 30 minutes in ice water and centrifuged at 4℃ (10000 rpm, 10 min). Total protein content, "thiobarbituric acid reactive species rate (TBARS)", "glutathione level (GSH)", "inflammatory mediator rates (TNF-α andIL-6)" of the sciatic nerve were determined by the supernatant of homogenates. Some of the intact sciatic nerves were conserved in the formalin for carrying out the histopathological studies.

2.6.1 Estimation of tissue total protein

Bovine serum albumin (BSA) was used as a standard to assess the total protein content in the sciatic nerve. 0.3 ml of tissue supernatant homogeneous was diluted to 1 ml with distilled water and then applied 5 ml of Lowry reagent. The contents were then thoroughly mixed and at room temperature (37℃) the mixture was held for 15 min. Diluted Folin-Ciocalteu reagent were then added to "0.5 ml of 1:1 v/v". The contents were then vigorously vortexed and 30 minutes incubated at room temperature (37℃). The protein content was spectrophotometrically measured at 750 nm against adequately prepared blank and a standard curve was plotted using the 0.2-2.4 mg/ml BSA.

2.6.2 Estimation of thiobarbituric acid reactive substances (TBARS) level

Measurement of the thiobarbituric acid reactive substances (TBARS) has measured lipid peroxidation in the sciatic nerve. 2 ml of thiobarbituric acid-trichloroacetic acid-hydrochloric acid reagent "(1:1:1 of TBA-TCA-HCl)" was treated with 0.1 ml of homogeneous supernatant. The TBARS reagent was formed by combining the same amounts of TBA (0.37%), TCA (15%) and HCl (0.25 N) and then put in the water bath for 15 minutes, cooled and centrifuged at room temperature at 1000 rpm for 10 minutes. After centrifugation, a "spectrophotometer (UV-1800 Spectrophotometer, Shimadzu, Japan)" was used to isolate the organic layer and calculate its absorbance at 535 nm against blank reference. TBARS level "(MDA concentrations)" was then calculated from standard curve (1-10nmol) of "1, 1, 3, 3 - tetramethoxy propane (TMP)".

2.6.3 Estimation of reduced glutathione (GSH) levels in the sciatic nerve

The equal amount of sciatic nerve homogeneous supernatant was combined with trichloroacetic acid (10%) and centrifuged at 1000 rpm at 4oC for 10 min to isolate the proteins. 2 ml of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) was mixed with 0.5 ml of supernatant. Then 0.25 ml of 0.001 M freshly prepared "DTNB (5, 5'-dithiobis-2-nitrobenzoic acid)" was added to 1% w/v sodium citrate. Mixture absorption was taken within 15 minutes at 412 nm "(UV-1800 spectrophotometer, Shimadzu, Japan)". Also similar to the preparation of standard curve (10-100µm) were processed different concentrations of reduced glutathione (GSH) standard.

2.6.4 Estimation of inflammatory mediators (TNF-α and IL-6)

Levels of the IL-6 and TNF-α were measured in the sciatic nerve using the commercially available "ELISA kits" ("Rat TNF-α Mini ELISA Development Kit", "900-M73 by PEPROTECH" and "Rat IL-6 Mini ELISA Development Kit", "900-M86 by PEPROTECH") as per the manufacturer´s protocol. The colon of the dosage was monitored at 405 nm with the wavelength at 650 nm ("Micro-Plate Reader, Cyberlab").

2.7 Histopathological evaluation

Histopathological assessment of sciatic nerve was performed for estimation of nerve damage with the use of Hematoxylin and eosin dye and Toludine blue dye.

2.7.1 Hematoxylin and eosin staining method

In the fixative solution, isolated samples of distal portion of the sciatic nerve were stored (10% formalin) and fixed to preserve the integrity of the cells. Then sections of the nerves were dehydrated with the alcohol, cleaned and

finally impregnated with molten paraffin and kept for 24 hours. Sections were further cut to a thickness of 4 μm and stained with hematoxyl and eosin. Using light microscope (at magnification X 100), the micrographs of the related stained nerve sections were subsequently taken.

2.7.2 Toludine blue staining method

In 0.1 M sodium phosphate buffer, pH 7.4, isolated samples of sciatic nerves were fixed at 2.5 percent glutaraldehyde. Tissue samples were postfixed in 1.5 percent osmium tetroxide solution (pH 7.4) for 2 hours after fixation, then dehydrated with various alcohol passages (starting at 30 percent and progressing to complete ethanol) and coated in paraffin wax. Sections were subsequently cut to 5 μm thick and painted with Toludine black. Using light microscope (at magnification X 100), the micrographs of the relevant stained nerve sections were subsequently taken.

2.8 Statistical Analysis

All results have been expressed as mean ± standard means error (S.E.M.). Bonferonni's post-hoc test using Graph Pad Prism Version-5.0 software statistically analyzed the data from the behavioral results. The data from biochemical results were then analyzed statistically by using “one-way ANOVA” followed by “Tukey’s multiple range tests”. The “p-value < 0.05” was considered to be statistically significant.

III. RESULTS

3.1 Characterization of gel

The tailored topical gel successfully qualified the official and non-official tests of topical gel formulations. The color of tailored gel was observed to be peech color. In addition, the gel was homogenous in nature with absence of lumps. The pH of tailored gel and marketed gel was found in the range of 8.26 ±2.06 and 8.3 ±2.07 respectively, as recommended for the topical dermal products. The rheological analysis of prepared gel stated that the tailored gel samples followed “Non- Newtonian flow”. The spreadability of prepared gel and marketed gel was estimated to be 6.93 ±1.73 g.cm/sec and 5.49 ±1.37 g.cm/sec, respectively. In this way, the spreadability of the personalized gel samples was equivalent to the consistency of the cream on the market. Pregabalin, lidocaine and venlafexin have been reported to have a medication content of 99.3%, 98.9% and 100.3%. The customized gel should be kept at or below 20°C in a cool place.

3.2 Effect on mechanical hyperalgesia

Mechanical hyperalgesia has been assessed in the present study by the pressure stimulation procedure as described by the Randall and Sellito. The Paclitaxel administration (“8 mg/kg; *i.p.* in 4 divided doses”) resulted in the vital development of the mechanical hyperalgesia as shown by reduce in the hind paw removal threshold (Fig. 1) in comparison to normal untreated animals. Application of topical gel (twice a day for 2 weeks) to rats significantly prevented, paclitaxel-induced decrease in the hind paw withdrawal threshold. Similarly, administration of oral solution (for 2 weeks) to paclitaxel-treated rats also attenuated mechanical hyperalgesia in terms of enhance in the paw withdrawal threshold.

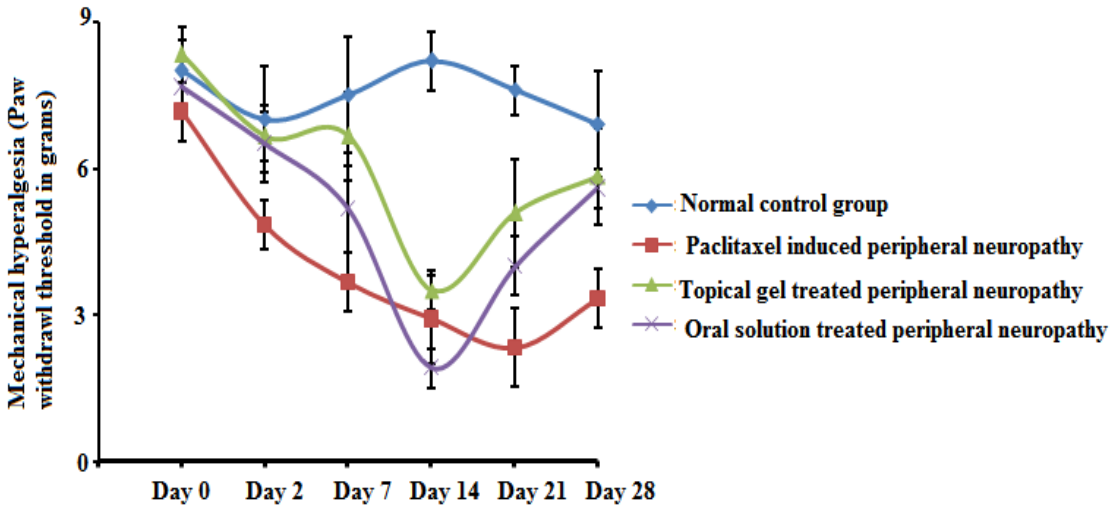

Figure 1: Effect on Mechanical hyperalgesia (paw withdrawal threshold in grams)

3.3 Effect on cold allodynia

The cold allodynia or paw lifting duration is assessed by immersing the hind paw of rats into cold water (10^{o}C). Cold sensitivity reaction with respect to shaking, paw licking or rubbing the hind paw is observed and recorded as duration of paw lifting. The administration of paclitaxel has resulted in the development of cold allodynia, which is reflected as a decrease in paw-lifting duration in water (10^{o} C) as compared to the normal group. Application of the topical gel formulation significantly attenuated paclitaxel-induced reduce in paw-lifting duration (Fig.2).

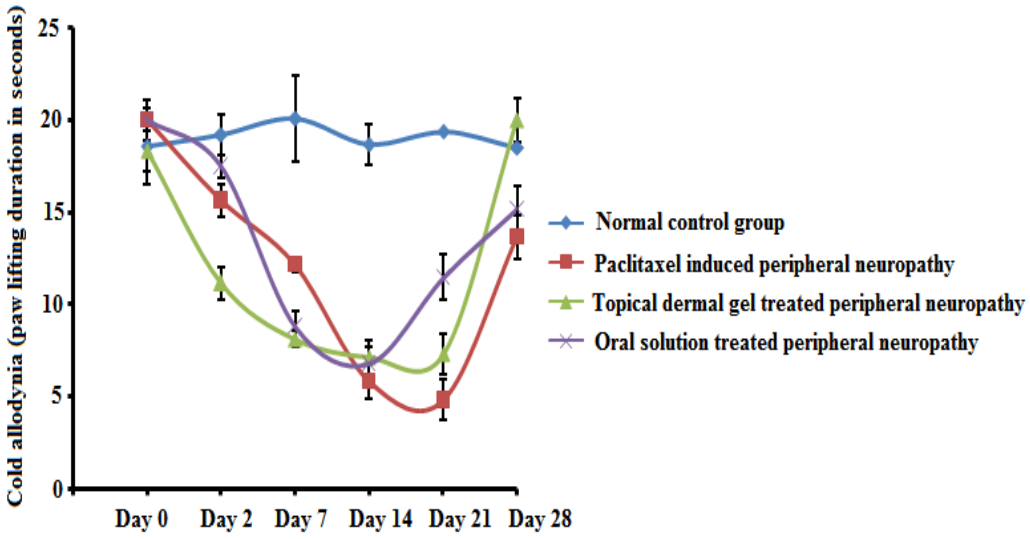

Figure 2: Effect on Cold allodynia (paw-lifting duration in seconds)

3.4 Effect on heat hyperalgesia

Using the Eddy hot plate to test the reactivity of noxious thermal stimuli, hind paw temperature hyperalgesia is evaluated. The rats are placed on top of a controlled preheated hot plate (52.5 ± 0.5°C) and it is noted that the heat hyperalgesia is eliminated from the hind paw. Pactlitaxel induces a substantial response of heat hyperalgesia in rats

as shown in the hind paw withdrawal threshold in the hot plate test of Eddy (Fig. 3) compared to normal untreated animals. Applying topical gel formulation (twice a day for 2 weeks) to rats significantly improved the reduction in the hind paw withdrawal threshold caused by paclitaxel.

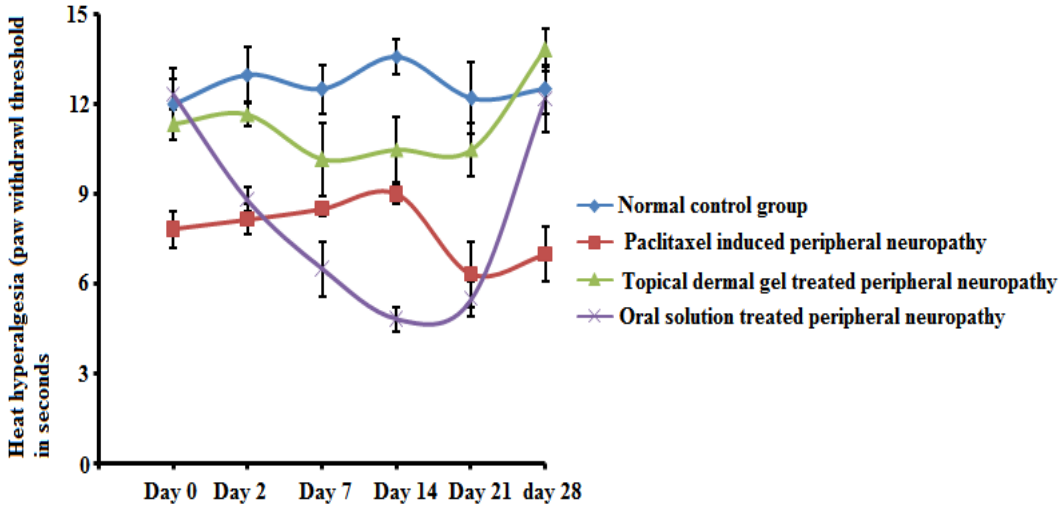

Figure 3: Effect on Heat hyperalgesia (paw withdrawal threshold in seconds)

3.5 Effect on sciatic nerve oxidative stress levels

Paclitaxel administration (8 mg / kg; i.p. in four divided doses) for 4 alternative days significantly increased the level of TBARS (Figure 4) and decreased the level of GSH (Figure 5) compared to normal untreated animals, indicating oxidative injury. The application of topical gel (twice a day for 2 weeks) to rats significantly reduced paclitaxel-induced oxidative stress in terms of decreased levels of TABRS and increased levels of GSH. Similar observations have been observed in animals treated with oral solution.

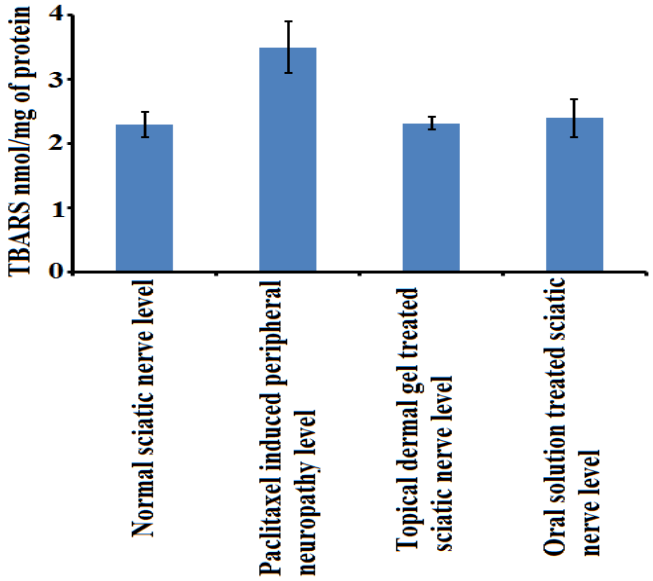

Figure 4: Effect on sciatic nerve TBARS level (nM/mg protein).

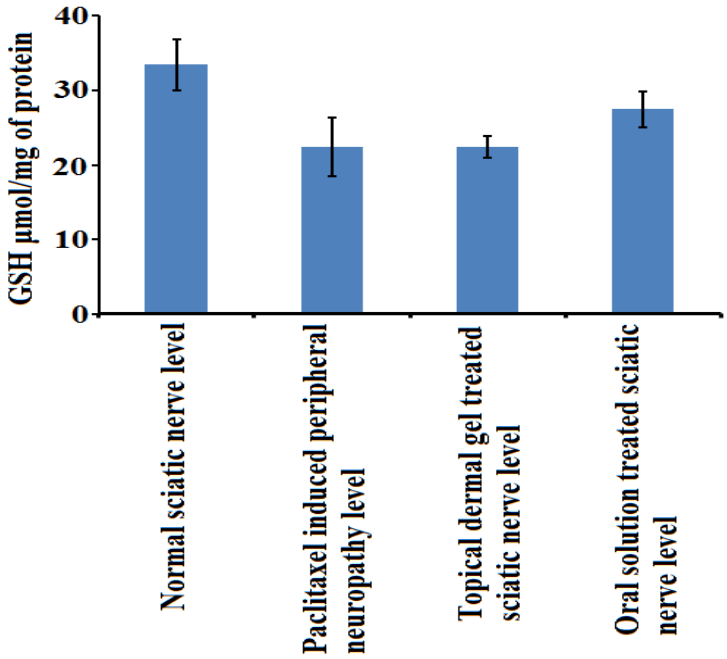

Figure 5: Effect on sciatic nerve reduced GSH level (µM/mg of protein).

3.6 Effect on sciatic nerve inflammatory mediators (TNF-α and IL-6)

There was a significant increase in TNF-α andIL-6 level in sciatic nerve homogeneous tissue on paclitaxel administration (Fig. 6 and Fig. 7) compared to normal untreated levels. Using topical gel significantly reduced enhanced levels of TNF-α andIL-6 induced by paclitaxel. Similarly, oral solution administration also considerably reduced the levels of TNF-α andIL-6 in the group treated with paclitaxel.

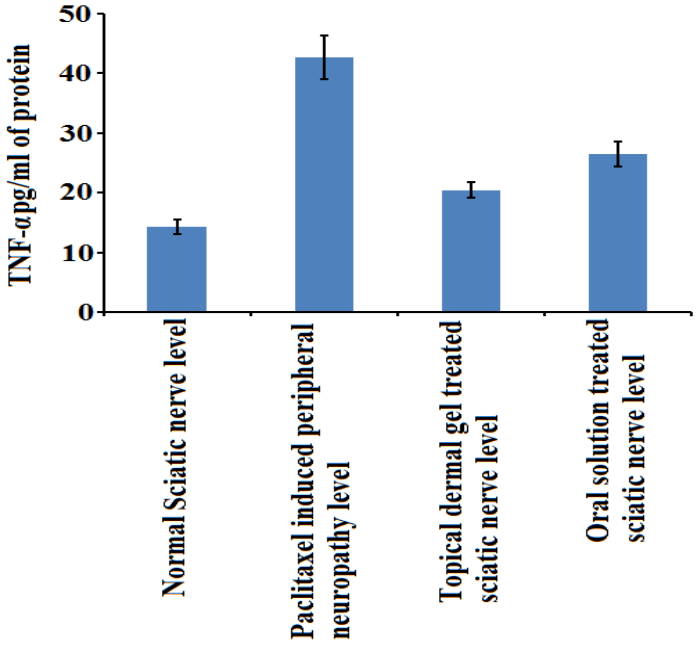

Figure 6: Effect on sciatic nerve TNF-α level (pg/ml of protein).

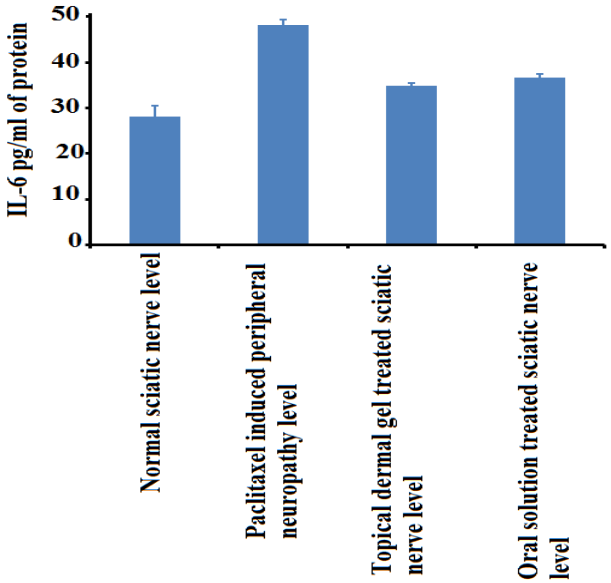

Figure 7: Effect on sciatic nerve IL-6 level (pg/ml of protein).

3.7 Effect on sciatic nerve histopathology

3.7.1 Hematoxylin and eosin stained sections: H & E stained sciatic nerve sections of paclitaxel treated animals showed marked histopathological changes such as nerve fibre derangement with areas of necrosis and reduction in number of schwan cell nuclei as compared to sections of normal animals, which bear elongated schwan cell nuclei, longitudinally oriented and arranged axons with myelin sheath. However, gel treated sections illustrated reduced areas of necrosis and derangement of nerve fibres. These sections depicted signs of improvement in terms of orderly arrangement, more schwan cells and partially disintegrated axonal myelin sheath. These changes upon gel application strongly indicated regeneration of otherwise demyelinated nerve fibres. Similarly, sections of oral treatment significantly reduced paclitaxel-induced histopathological changes in terms of uniform arrangement of nerve fibres and presence of schwan cells (Fig. 8).

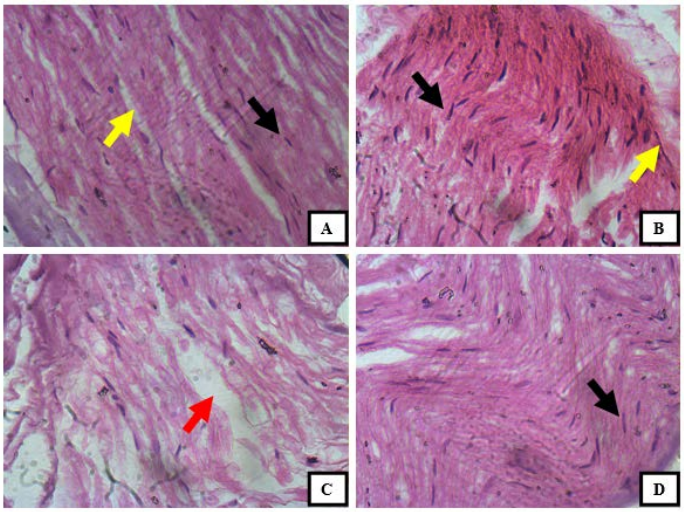

Figure 8: Effect on rat sciatic nerve histopathology (Hematoxylin and eosin staining).

As shown in **Figure 8**, A represents topical dermal gel treated paclitaxel induced Peripheral neuropathy, B represents normal sciatic nerve, C represents paclitaxel induced peripheral neuropathy and D represents oral solution treated paclitaxel induced peripheral neuropathy. The yellow, black and red arrows represent the longitudinally arranged nerve fibres, schwan cell nuclei and necrotic area.

3.7.2 Toludine blue stained sections: The untreated group showed normal histopathological features with toludine blue staining. Sciatic nerve sections of paclitaxel treated animals showed significant disorganization of nerve fibres, weaker staining and more tortuous fibre tracts along with zones of necrosis. Sections from paclitaxel treated animals also showed missing axons, vacuolation and complete demyelination of axons when compared with normal untreated sections. Application of gel significantly reversed the tortuous fibre tracts into well arranged nerve fibres. Regular, uniform and thickened myelin sheath as well as devacuolation resulted in arrest of necrosis of nerve fibres as compared to sections of paclitaxel treated animals. Oral treatment resulted in reduction of necrotic areas and restoration of few axonal myelin sheaths was also observed as compared to diseased animals (**Figure9**).

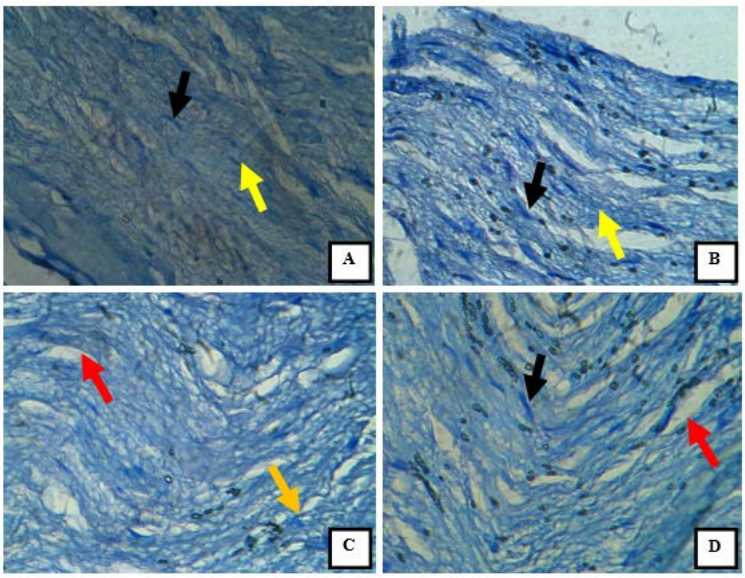

Figure 9: Effect on rat sciatic nerve histopathology (Toludine blue staining).

As shown in **Figure 9**, A represents topical dermal gel treated paclitaxel induced peripheral neuropathy, B represents normal sciatic nerve, C represents paclitaxel induced peripheral neuropathy and D represents oral solution treated paclitaxel induced peripheral neuropathy. The yellow, black, red and orange arrows represent the longitudinally arranged nerve fibres, schwan cell nuclei, necrotic area and tortous fibre tract.

IV. CONCLUSION

The results of the biochemical tests and the histopathological analysis indicates that the application of topical gel formulation effectively reverses the effects of paclitaxel induced neuropathy and is able to regenerate the myelin sheath because of inclusion of nerve tonics in the composition. The formulated topical gel is easy to apply and is readily absorbed into the skin. The formulation provides effective relief from CIPN without causing systemic side effects, thereby raises cancer patient compliance. Hence, it may be concluded that formulated gel is

pharmacologically efficacious for management of "chemotherapy-induced neuropathy" and can be excellent therapeutic option for the same.

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