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TPGS-STABILIZED RESVERATROL NANOSUSPENSION ENHANCES FUNCTIONAL RECOVERY IN A MOUSE MODEL OF SCIATIC NERVE INJURY

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ABSTRACT

The low solubility and bioavailability issues of natural compounds challenge their applications and the wide spectrum of bioactivities. Recently, great efforts have been made to develop nanoformulations of bioactive compounds to enhance their efficacy profiles. They increase their bioavailability through a targeted, controlled and sustained release for a prolonged period. Naturally occurring resveratrol (Res), is a highly active nonflavonoid polyphenol, showing several bioactivities including anticancer, antioxidant, antidiabetic and anti-inflammatory potential. In this study, the nanosuspensions (NS) of Res were prepared with and without D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) as a stabilizing agent. Further, the prepared NS1 (Res only) and NS2 (TPGS + Res) were administered through daily gavage (20mg/mL) to accelerate functional recovery and regeneration following peripheral nerve injury (PNI) in a mouse model. A total of eighteen mice were used, equally divided (six/group) into three groups: a control and two treatment groups under a completely randomized design (CRD). The NS-mediated regain of sensory functions was evaluated by a hot plate test in NS-treated and control animals. Furthermore, the recovery of motor function was examined employing the sciatic functional index and muscle grip strength studies. Nerve regeneration highly depends on oxidative stress and blood glycemic level. Therefore, the total antioxidant capacity (TAC), total oxidant status (TOS) and blood glucose were also assessed in NS-treated groups compared to the control. The prepared NS2 induced the maximum hypoglycemic effects, resulting in a controlled glucose level and regulation of oxidants with a significantly improved TAC. These improved biochemical attributes provided a growth-permissive environment, facilitating the nerve regeneration and functional recovery after PNI owing to the enhanced bioavailability of Res.

Keywords: Drug delivery system, antioxidant, controlled release, entrapped drug

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INTRODUCTION

Peripheral nerves are delicate structures that are susceptible to traumatic injuries, which can adversely affect sensory and motor functions. Peripheral nerve injury (PNI) and the resulting impairments affect musculoskeletal sensations, mobility and cognitive processes. Sometimes such traumatic conditions result in organ failure or permanent disabilities. The functional retrieval following PNI is a complex biological process often restricted by oxidative stress-mediated slow nerve regeneration (Qiu *et al.,* 2019; André-Lévigne *et al.,* 2024). Recently, several studies have evaluated the effect of natural bioactive molecules on functional retrieval of PNI in animal models (Araújo-Filho *et al.,* 2016; Maqbool *et al.,* 2023; Mahmoud *et al.,* 2021). Over the past few decades, natural antioxidants have become a promising choice for drug development due to their diverse and tunable chemical structures. They have become vital components for drug design due to their minimal side effects and sustainable availability. However, the low solubility and bioavailability issues of natural compounds challenge their applications and the wide spectrum of bioactivities. Over the years, attention has been focused on developing nanoformulations of bioactive polyphenolic compounds to enhance their efficacy profiles (Karuppaiah *et al.,* 2024; Farhadi *et al.,* 2024). The nanoformulations increase their bioavailability through a targeted, controlled, and sustained release for a prolonged period (Yang *et al.,* 2020; Unnikrishnan Meenakshi *et al.,* 2024). Naturally occurring Res, a nonflavonoid polyphenolic antioxidant, belongs to the stilbene family and is found in berries and peanuts. and grapes. Its *trans*-isomeric form (*t*-Res) is highly stable and active, exhibiting several bioactivities including anticancer, antioxidant, antidiabetic, and anti-inflammatory potential. However, the *t*-Res shows limited applications due to poor aqueous solubility and low bioavailability, although it shows significant neuroprotective potential (Santos *et al.,* 2019). Therefore,

a suitable drug delivery system (DDS) is required to enhance its solubility and bioavailability for an improved therapeutic potential. The known DDSs include polymeric micelles, nanoparticles and liposomes, etc. Generally, the DDSs show toxicity concerns due to the use of a large proportion of surfactants and excipients. In recent years, NS has emerged as a promising DDS requiring minimal stabilizers or surfactants. The NS enhances the bioavailability of hydrophobic drugs and natural polyphenols by increasing their solubility (Du *et al.,* 2017). The TPGS is a derivative of vitamin E, exhibiting high aqueous solubility and a drug stabilization effect. Thus, highly soluble TPGS could enhance the aqueous solubility of naturally occurring *t*-Res. Recent studies have shown its drug-stabilizing potential and enhanced therapeutic effects of anti-cancer agents. The FDA has recommended it as a nontoxic excipient, and its safe use as a stabilizer, permeation enhancer and emulsifier. It exhibits higher emulsification efficiency compared to traditional emulsifiers such as PVA. It can protect against enzymatic degradation and act as a drug efflux inhibitor (Wong *et al.,* 2025). It significantly improves the stability, permeability and solubility of the entrapped drugs and bioactive natural molecules (Guo *et al.,* 2025). As a part of DDS, it enhances cellular uptake, half-life of the drug in plasma and ultimately bioavailability of bioactive molecules through controlled release and targeted delivery (Mehata *et al.,* 2023). Considering the neuroprotective nature of *t*-Res and NS as promising DDS, we envisioned preparing Res NS using TPGS as a stabilizing agent to evaluate its role in the functional recovery of nerves after induced PNI. Addressing the research gaps, this strategy presents novel insights into the neuroregenerative potential of *t*-Res, directly linking increased bioavailability to the improved physiological and biochemical attributes for the regain of functions.

MATERIALS AND METHODS

The experiment was conducted in October 2023 over a period of ten days in the Animal House, Department of Physiology, Government College University Faisalabad (GCUF). All biochemical and histopathological analyses were subsequently performed in the Neurochemical Biology and Genetics Laboratory, Department of Physiology, GCUF, under controlled experimental conditions and following established research protocols after approval from the Ethics Review Committee, Government College University Faisalabad (Ref # GCUF/ERC/313 dated 04-10-2023).

Preparation of RES-TPGS NS: Initially, 600 mg of *t*-Res, 1500 mg of citric acid, and 900 mg of TPGS were dissolved in ethyl acetate (5 mL), and continuously stirred for one hour. The organic solvent was then evaporated using a vacuum rotary evaporator. Next, the organic acid phase was supplemented with an aqueous carbonate (1800 mg) (0.018 M $NaHCO_3$, pH ≈ 8.3 at 25 °C). As a result of the quick formation of CO_2 bubbles, the NS was produced (Wang *et al.,* 2017). The formation of nanosuspensions and their subsequent delivery for nerve regeneration is represented in **Figure 1.**

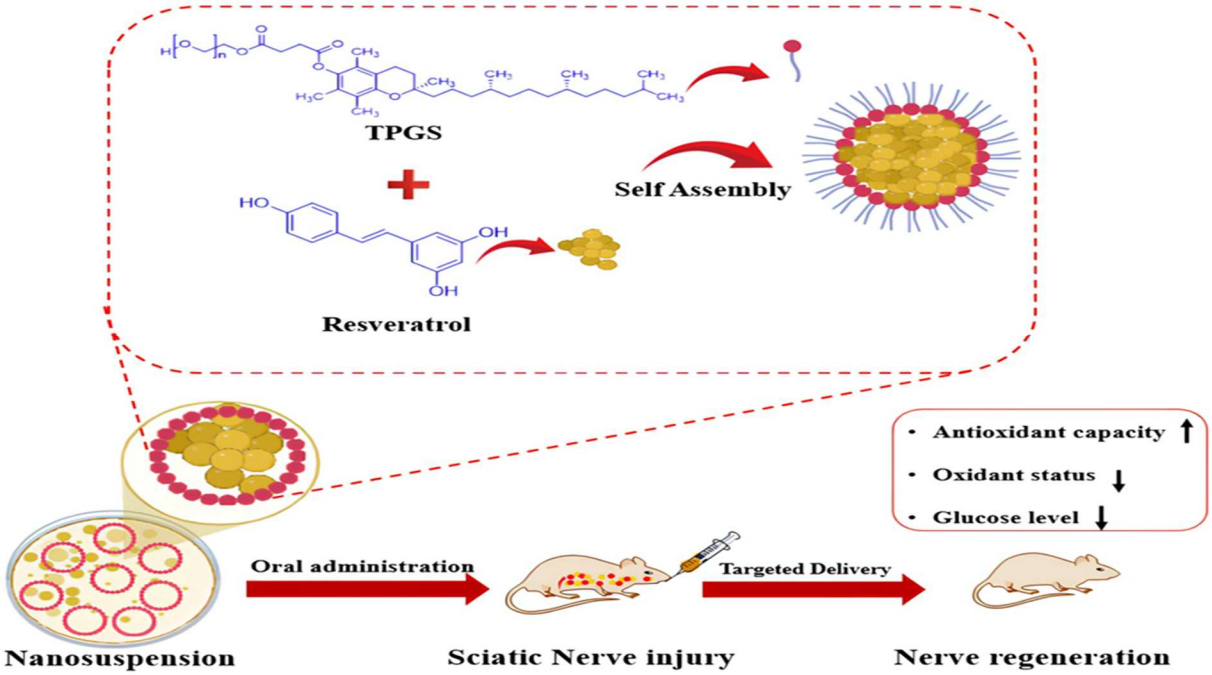

Figure 1. Preparation of NSTPGS + Res (NS2) using organic acid and aqueous carbonate

Characterization of the prepared NS TPGS + Res: The spherical surface morphology of the prepared NS2 was studied using SEM **(Figure 2A and B)**. The functional groups of compounds present in the nanosuspension were identified using FTIR spectrum. The IR spectrum of Res typically exhibits absorption bands associated with phenolic and aromatic groups. TPGS, on the other hand, shows characteristic peaks related to ester and ether functional groups. In the case of the nanosuspension, its IR spectrum reveals shifts or the appearance of new bands, which indicate interactions between resveratrol and TPGS during the encapsulation process and formulation into nanoparticles, as depicted in **(Figure 2C)**. The FTIR analysis was in agreement with those reported in prior studies, validating the presence of specific functional groups in the synthesized (Zuccari *et al.,* 2021).

Experimental Animals: Male Albino mice (BALB/C)/strain) (N=18) weighing approximately 20-22 g with an average age of 9-10 weeks were procured from the animal facility of the Government College University Faisalabad, Pakistan. They were given a continuous supply of food and water at 22+2 ℃. In this study, animals were subdivided into three groups: one treated group (n = 6, mice/cage, fed with NS Res+ normal chow), second treated group (n = 6, mice/cage, fed with RES-TPGS NS+ normal chow) and a control group (n = 6, mice/cage, fed with normal chow). Day -1, -2, or -5 denotes the pre-injury time, and the behavior-related investigations were conducted during the 12 hr light cycle at predetermined intervals.

Induction of sciatic nerve injury and NS TPGS + Res supplementation: The albino mice were acclimatized for six days before induction of compression in the sciatic nerve. The subjects were anesthetized with intraperitoneal injections of a mixture of xylazine (5mg/kg) and ketamine (70mg/kg). The skin at the mid-thigh region of the hind paw was shaved and the sciatic nerve crush was induced by compressing it for 12–15 sec with a fine pair of forceps. The skin was sutured and disinfected with pyodine. The prepared NS1 and NS2 nanosuspensions (20mg/mL) were administered via oral gavage once daily. Each mouse received a dose volume of 10 mL/kg body weight, ensuring dosing according to individual body weights measured prior to each administration. Treatments were initiated immediately after sciatic nerve crush (day 0) and continued for the entire experimental period of 10 consecutive days. Gavage was performed gently using a sterile, ball-tipped feeding needle to minimize stress and injury.

The prepared NS1 and NS2 were administered through daily gavage (20mg/mL) (El-Khadragy *et al.,* 2018). In order to harvest tissues and obtain blood samples, the mice were decapitated at the end of the trial.

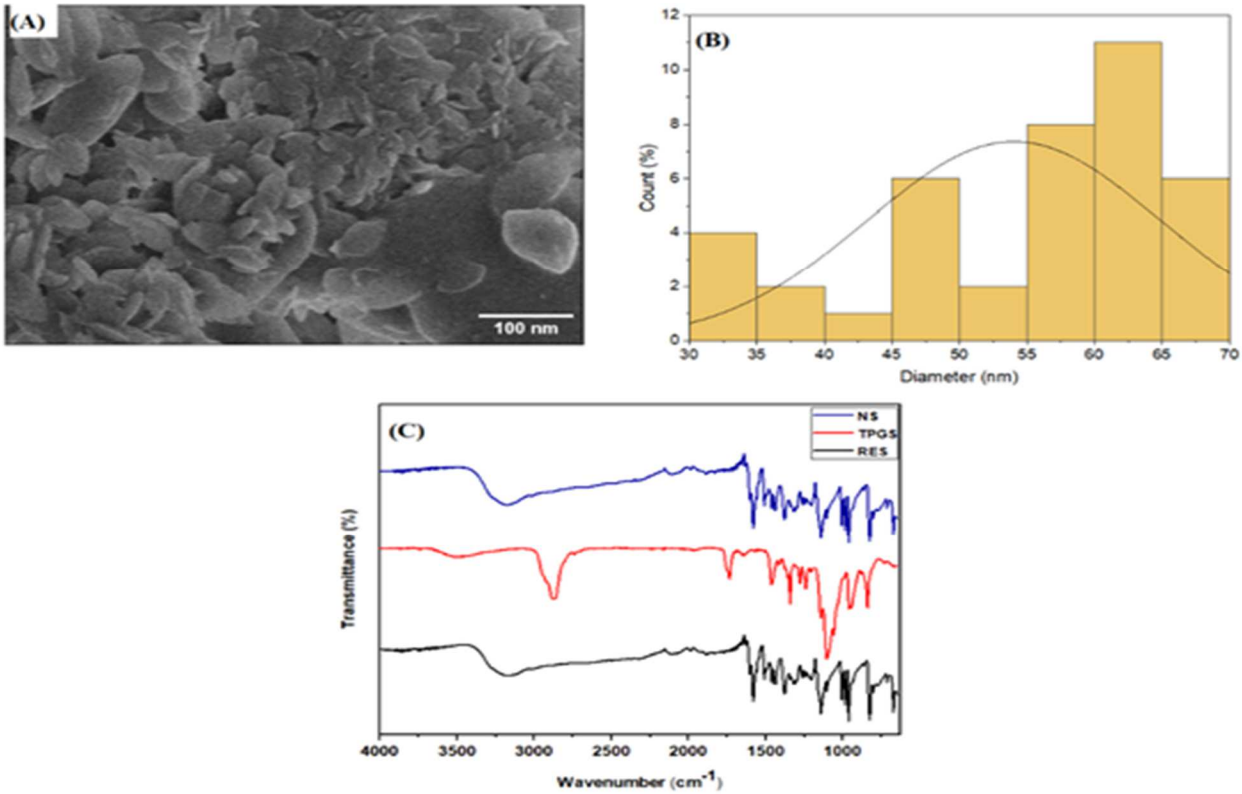

Figure 2. Characterization of NSTPGS + Res (A) SEM analysis (B) Size distribution (C) FTIR analysis

Behavioral Analyses: All assessments were conducted by observers blinded to group assignments. Mice were randomly allocated to normal chow, NS Res, or NS TPGS + Res diets before sciatic nerve crush, and cage positions were rotated to minimize environmental bias.

Diet intake and body weight assessment: The effect of treatments on subjects was assessed using body weight measurements. The daily diet was assessed for every mouse and recorded by measuring the difference between the weight of the diet provided on the preceding day and the weight of the diet remaining in the cage (Electronic balance JA5001).

Muscle grip strength analysis: This test involved putting the subjects on a grid or a metal bar to assess the grip strength of both ipsilateral and contralateral hind limbs. Each subject tried to stop the unintentional backward movements until the withdrawing force weakened their grip. For functional recovery assessment, three readings were taken for each subject with a 30 second latency and compared to the normal subject (Bajaber *et al.,* 2023).

Analysis of sciatic functional index (SFI): The SFI analysis evaluates the regain of motor function in treated subjects with PNI. The mice with inked hind paws were placed on a white-plain wooden track. The paw prints were used for calculating SFI employing the following equation (de Almeida *et al.,* 2022).

$$SFI = \left(-38.3 \times -\frac{EPL - NPL}{NPL}\right) + \left(109.5 \times \frac{ETS - NTS}{NTS}\right) \times \left(13.3 \times \frac{EIT - NIT}{NIT}\right) - 8.8$$

The experimental side (E) and normal side (N) variables were used to calculate the mean value of the SFI, with zero indicating normal functioning and -100 indicating complete impairment

PL = print length, the length from the heel to the third toe

TS = toe spread, the distance between the first toe and the fifth toe

ITS = intermediate toe spread, the distance between the second toe and the fourth toe.

Hotplate analysis: The hotplate test assessed the recovery of sensory function in subjects with induced PNI. The treated and untreated subjects were kept on a hot surface (55 ± 1°C) to study the recovery of sensory functions. The hotplate latency or reaction time was noted when the subject showed paw licking or jumped off the plate (Karandikar, Belsare, and Panditrao 2016). The final reading was determined by taking an average of three individual readings.

Pinprick Analysis: The retrieval of sensory functions was also assessed by pinpricking on five surface areas of the hind paw's plantar. The sciatic nerve's projection territory extends from the ankle to the distal end of the toes. The rapid withdrawal of the paw was recognized as a positive reaction. A subject with a positive response across all five sites scored 5, validating a complete and functional sensory response; a score below 5 indicates partial recovery and a score of 0 signifies complete functional loss (Zafar *et al.,* 2021).

Biochemical analysis:

2.6.1 Total Antioxidant Capacity: The total antioxidant capacity (TAC) means the potential of the system to counteract free radicals and to keep oxidative stress under check. The TAC was measured in treated and untreated subjects with induced PNI. Trolox solution was used as a standard for generating free radicals by reacting 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS) with H_2O_2. The serum samples were analyzed by a chemistry analyzer and TAC values were expressed in millimoles of Trolox equivalents per liter (Erel, 2004).

Total Oxidant Status (TOS): Known methodology was followed to assess the total oxidant status (TOS) in serum samples of PNI-induced subjects in units of μmoles of H_2O_2 equivalent/L. The serum sample was spiked with reagents 1 and 2 and incubated before recording the first absorbance. The final absorbance was then taken using a semi-automated chemistry analyzer (Erel, 2004).

Random blood glucose level measurement: Random glucose measurements were conducted in all subjects before and after the induction of nerve injury. The glycemic level in blood samples was analyzed using an Accu-chek glucometer.

Histology: The alterations in the tibialis anterior muscles of both treated and control subjects were evaluated by histopathology studies. In this study, the tissue was fixed in 10% formalin, transferred to a phosphate buffer (PBS) solution after 24 hrs, and dehydrated with alcohol. The xylene I and II were used as dehydrating agents at the clearing phase. Thin tissue sections (5 μm thickness) were obtained using a rotary microtome and a light microscope was used to study the stained tissue slices (Bajaber *et al.,* 2023).

Statistical analysis: The SPSS 22.0 software was used for data analysis. The data of treated and control subjects were compared using a pairwise *t*-test (Control vs treatment NS1, Control vs treatment NS2), and the results were presented as mean ± SEM. The data were subjected to statistical analysis through analysis of variance (ANOVA). For significantly different treatments ($p \leq 0.05$), post hoc Tukey test was applied ($p \leq .05$) for comparison of means. Before applying ANOVA, Normality of residuals was confirmed using the Shapiro-Wilk test, and homogeneity of variance was verified using Levene's test. Shapiro-Wilk tests indicated that

residuals for both body weight and food intake data were normally distributed (P > 0.05 for all groups). Levenes's test confirmed homogeneity of variance across groups and time points (P > 0.05).

RESULTS

Effect of NS TPGS + Res treatment on body weight and food intake: The body weight and food intake were measured daily in both control and treated subjects till the completion of the study. The non-significant difference between groups exhibited that the treatments did not affect the weight and feeding habits of the subjects. There was no significant difference in body mass and diet intake in subjects of all groups **(Figure 3A, B)**.

Effect of NS TPGS + Res treatment on regain of motor functions: The retrieval of motor functions in subjects with induced PNI was evaluated using the grip strength study and SFI measurements compared to the control. The muscle grip strength and SFI were recorded at different time intervals. For grip strength studies, the readings were taken from the Ipsi-lateral (solid lines) and contralateral (dotted lines) hind limbs. On day 9, the NS2-treated group exhibited quicker recovery of motor functions than the control group **(Figure 4A)**.The NS TPGS + Res treated subjects demonstrated a significant improvement in walking patterns as indicated by the SFI values. The values restoring toward zero suggested that the retrieval of motor functions in the NS TPGS + Res treated group was more pronounced than in other groups **(Figure 4B)**. Higher variability in the control group post-injury can be observed from error bars, it is due to biological difference in nerve regeneration capacity. Here, reduced variability in NS TPGS + Res was observed which indicates the treatment stabilizes or standardizes the recovery process across the individual animals. There are no obvious extreme outlier points.

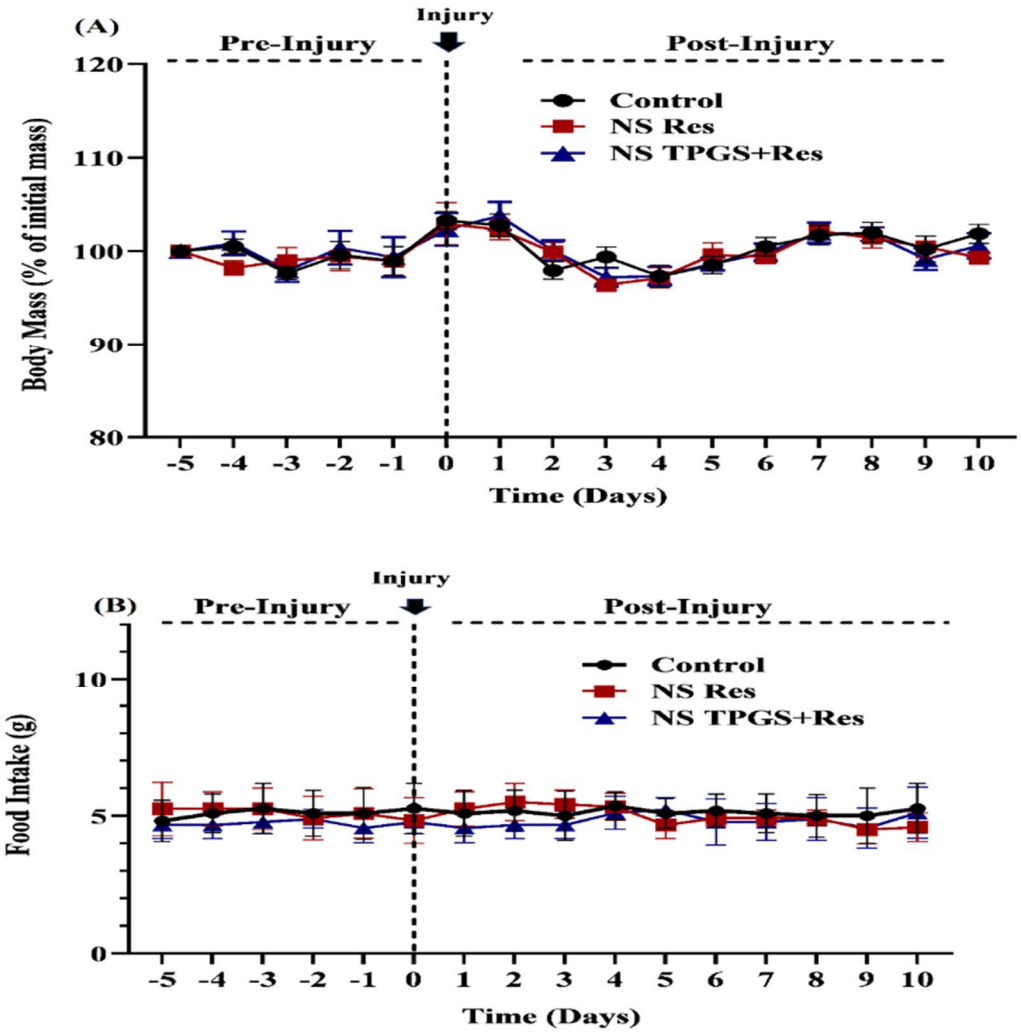

Figure 3. Impact of treatment on mice with induced PNI (A) Body mass (B) food intake. Two-way ANOVA showed a non-significant effect of NS-treatments on diet intake (ρ = 0.663) and body mass (ρ = 0.583).

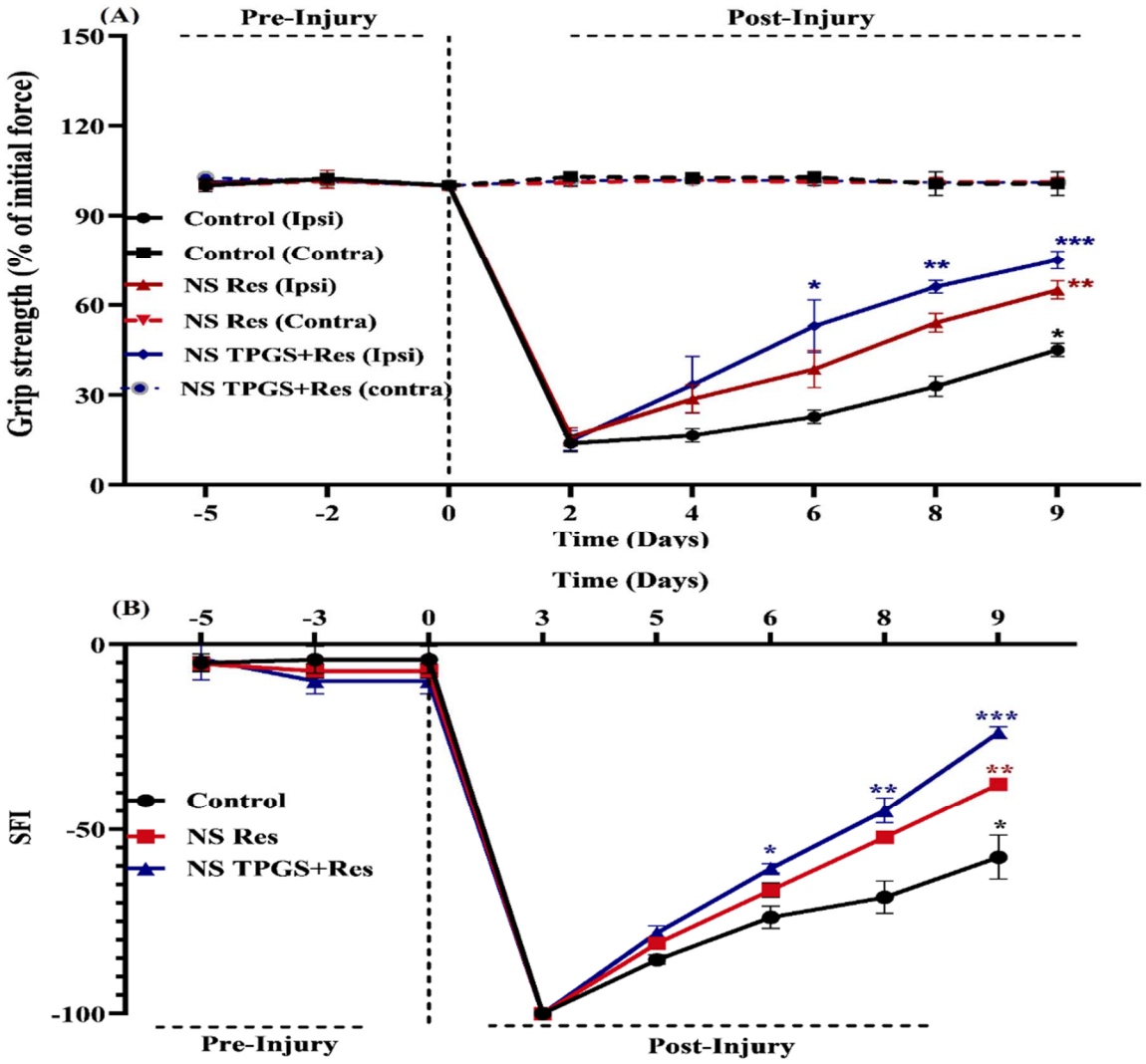

Figure 4. Effect of treatment on recovery of motor functions in mice with induced PNI, (A) Time course of muscle grip strength in mice (Two-way ANOVA), Measurements were obtained from hind limbs ipsilateral (solid lines) and contralateral (dotted lines) to the injury site. Both treated groups showed improvement at 6 day as compared to the control group. At day 8, NS TPGS + Res group showed significant (p=0.001) as compared to NS Res and control group. At day 9, NS TPGS + Res group showed significant (***p=0.001) results, and NS Res group also showed significant (**p = 0.001). (B) Time course of sciatic functional index (SFI) of mice. At day 8, both treated groups showed improvement but NS TPGS + Res group showed significant (*p=0.005) effect. At day 9, NS Res group showed significant (**p=0.002) results, NS TPGS + Res group also showed significant (***p=0.001) effects as compared to control group.**

Effect of NS TPGS + Res treatment on the restoration of sensory threshold activity: The reclamation of sensory function was examined following the pinprick and hot plate tests. A significant decrease in withdrawal latency was observed in the treated groups compared to control (**Figure 5A)**. However, a substantial regain of sensory functions was observed in NS TPGS + Res group. Further, the pinpricking test also provided insight into the ameliorative effect of the treatments on axon regeneration and functional recovery in both the treated groups **(Figure 5B).** In both paw withdrawal latency and pin prick, pre-injury variability was minimal across groups. Post-injury, the control group showed greater variability, especially at days 7 and 10, indicating inconsistent recovery among individuals. In contrast, NS TPGS + Res consistently exhibited smaller error bars, suggesting more uniform functional and sensory recovery. No clear outliers were observed, but the wide spread in control data reflect delayed recovery in some animals.

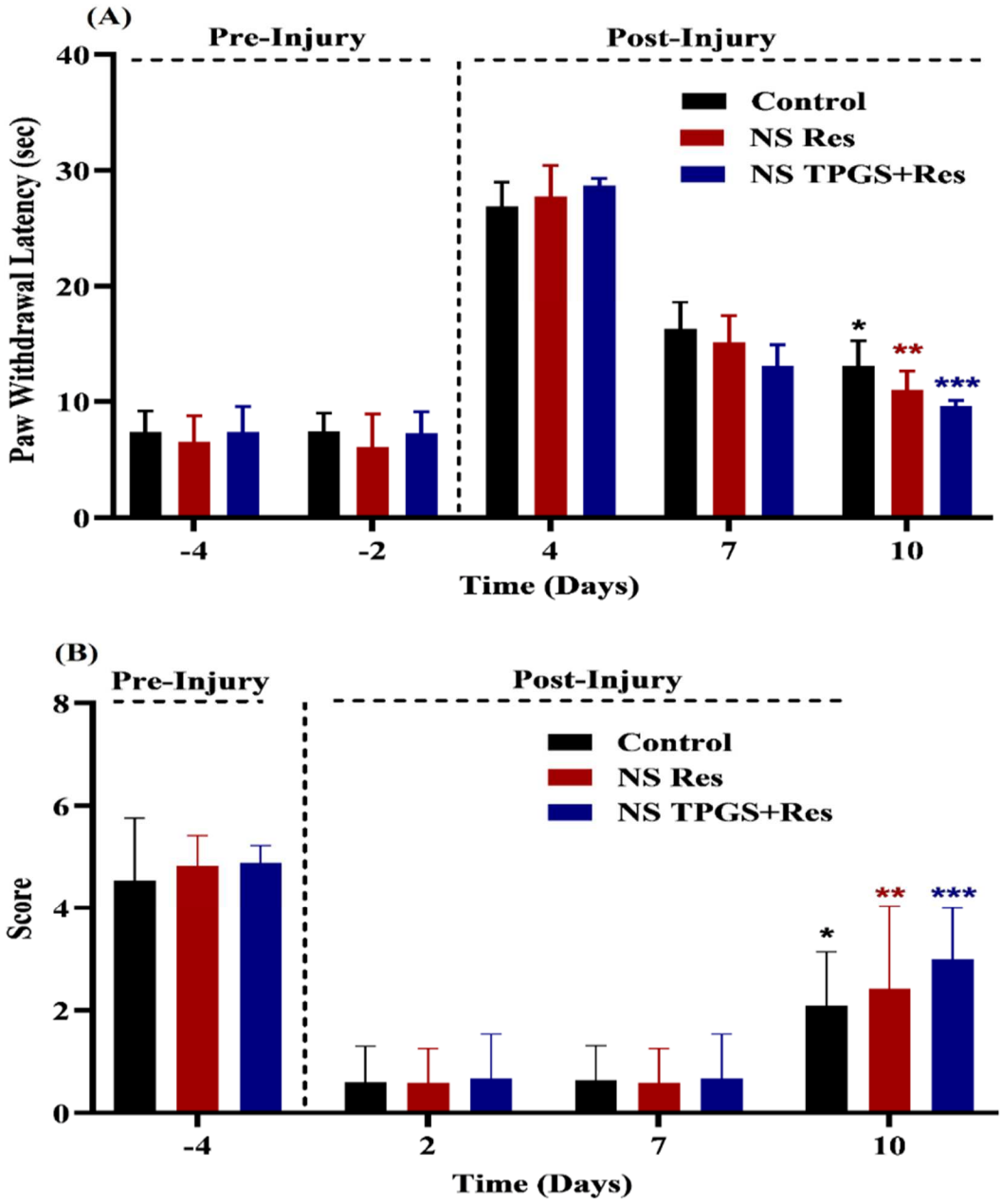

Figure 5. Effect of treatment on sensory functions recovery in mice with induced PNI, (A)

Paw withdrawal latency in response to thermal stimulation in mice, Measurements were obtained at different time points before and after injury induction (Two-way ANOVA). On day 10 Ns Res (**p=0.003) showed significant results and NS TPGS + Res group also showed significant (***p=0.001) results as compared to control group. (B) Paw withdrawal score in response to pinprick stimulation in mice, (Two-way ANOVA) at day 10 NS Res group showed significant (**p=0.002) results and Ns TPGS + Res group also showed significant (***p=0.001) results as compared to control group.

Effect of NS TPGS + Res on blood glucose and oxidative stress: The TOS and TAC levels were recorded to estimate oxidative stress in treated and untreated groups. The TOS represents the level of oxidants, while TAC means the antioxidant capacity of treated and untreated subjects. The TOS and TAC serum samples could interpret the underlying mechanisms for the reclamation of functions in injured nerves. The NS TPGS + Res treatment exhibited a significant impact on controlling of oxidative stress **(Figure 6A, B).**

The glucose level was noted in treated and control groups before and after induced PNI. The NS

TPGS + Res treatments induced the maximum hypoglycemic effects and a controlled glucose level compared to the control **(Figure 6C).** In TAC, TOS, and blood glucose measurements, variability within groups was low, particularly in the NS TPGS + Res group. Control groups exhibited slightly higher dispersion, especially in TAC, and blood glucose, suggesting individual differences in oxidative stress and glycemic response. No obvious extreme outliers were detected, and the reduced variability in treated groups reflects a more consistent biochemical response across the animals.

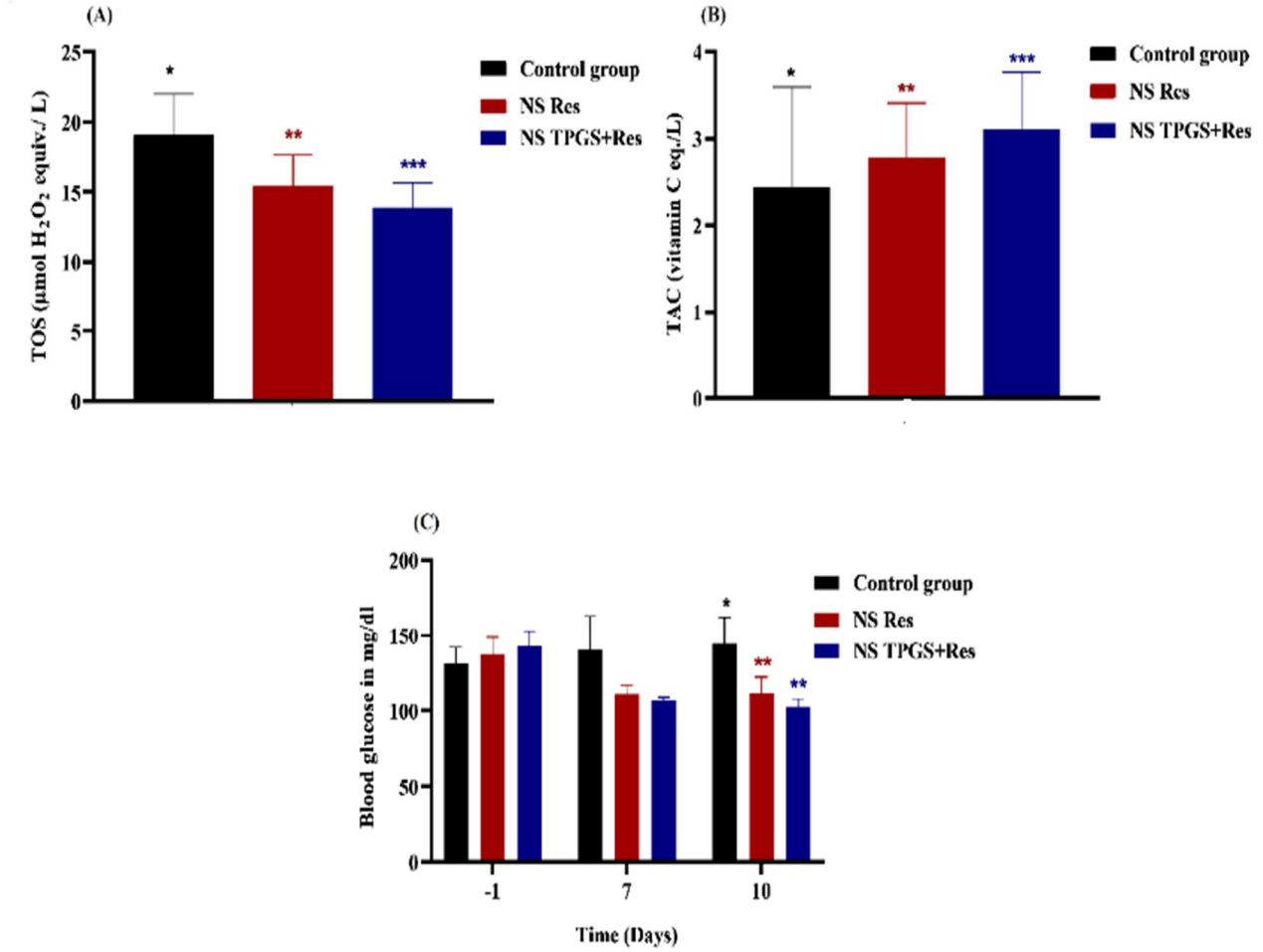

Figure 6. Effect of treatments on systemic indices. A) In total oxidant status (TOS) in mice, one-way ANOVA showed a significant (p=0.002) result in the NS Res group and the NS TPGS + Res group also showed a significant (***p=0.001) result as compared to the control group. B) Total anti-oxidant capacity (TAC) of mice, one-way ANOVA showed a significant (**p=0.006) result in the NS Res group, whereas the NS TPGS + Res group also showed a significant (***p=0.001) result as compared to the control group. C) Glycemic measurements in control and treated groups. Two-way ANOVA showed a significant (**p=0.001) result in the NS Res group and the NS TPGS + Res group also showed a significant (**p=0.001) result as compared to the control group.**

Effect of NS TPGS + Res treatment **on muscle fiber morphology**: Nerve injury causes an interruption of electrical signals in affected muscles. The prolonged interrupted signaling results in atrophy of the injured muscles. Therefore, the health status of muscle fibers is assessed from the morphology of the cross-sectional area of a small fiber. The mean surface area of ipsilateral and contralateral sections **(Figure 7A)**, and fiber count were evaluated in the NS-treated and controls **(Figure 7B)**. Dystrophy was observed in the cross-sectional area of contralateral and ipsilateral muscles of control subjects. The fiber count and surface area studies were completed on the ipsilateral and contralateral sides in the control and NS treated group (**Figure 8)**. Data represents very low variability across the groups and no obvious outliers were observed. Indicates the consistent retrieval of nerve regeneration.

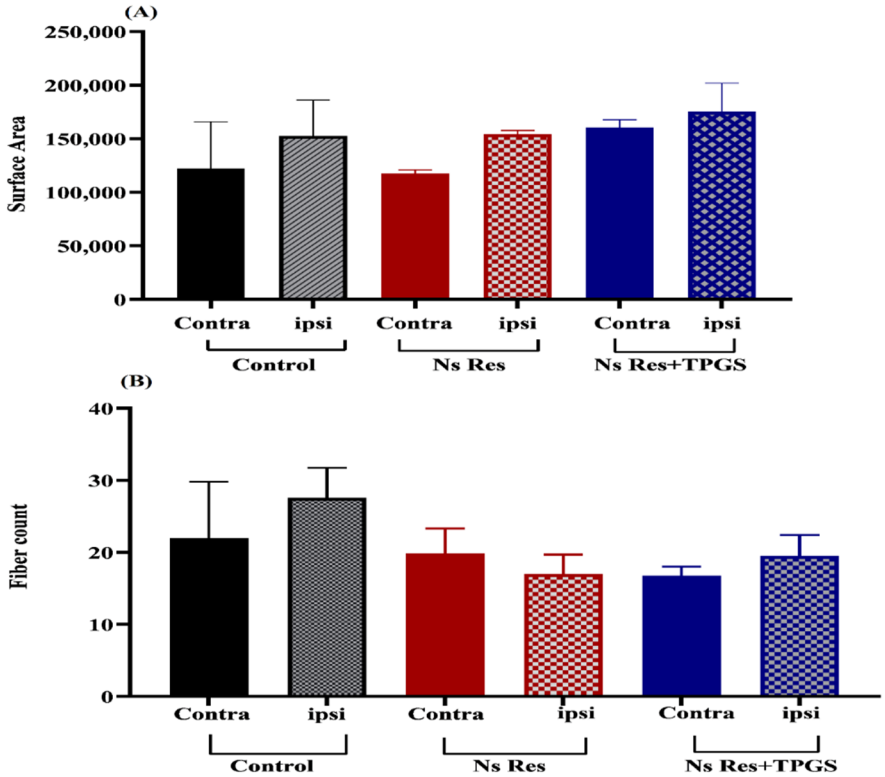

Figure 7. Histological presentation of ipsilateral and contralateral muscles of the control, NS Res and NS TPGS + Res groups. A) The comparison of muscle surface area between the ipsilateral and contralateral was found to be non-significant (p=0.413) in the NS Res group and NS TPGS + Res (one-way ANOVA) group also showing non-significant (p= 0.075) results as compared to control group. B) Whereas muscle fiber count between ipsilateral and contralateral of treated groups, the NS Res group showed non-significant (p=0.589) results, group NS TPGS + Res group also showed non-significant (p=.886) results as compared to the control group (one-way ANOVA).

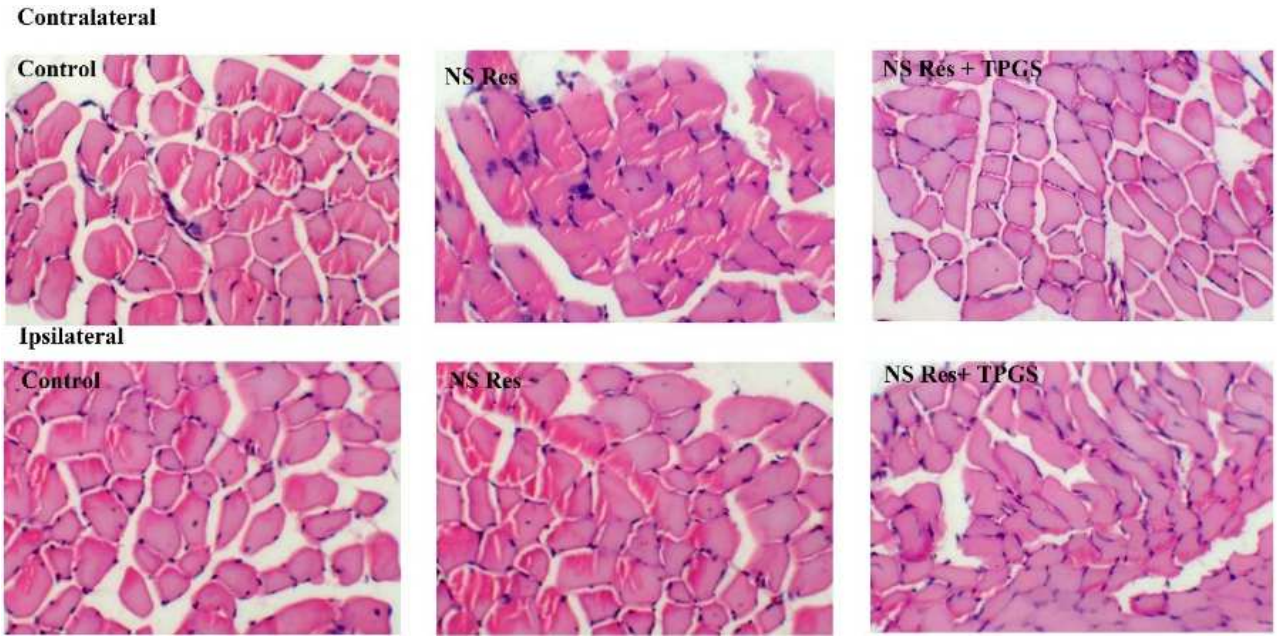

Figure 8. Histological presentation of the ipsilateral and contralateral muscles of the control, Ns Res and the NS TPGS + Res group.

DISCUSSION

Oxidative homeostasis and subcellular signaling are vital for various biological functions, including growth and regeneration. The PNI induces ROS generation, which disrupts the coordinated signaling and oxidation status in target tissues. The excessive oxidants cause damage to the cellular structures, bio-membranes and potent biomolecules. It disturbs the growth-permissive environment, neurotransmission and neuronal activities at the site of PNI. It results in inflammation and apoptosis, thus delaying the functional retrieval of nerves (Wang *et al.,* 2019). Naturally occurring polyphenolic compounds maintain oxidative homeostasis through their antioxidant activity and promote functional recovery of nerve following PNI (Muratori *et al.,* 2022). The Res is a known polyphenol with high ROS scavenging and antioxidant potential. It also has anti-inflammatory and antioxidant potential. This study assessed the potential of NS TPGS + Res (NS2) for an accelerated functional recovery following induced PNI in a mouse model. The regeneration of nerves is directly linked to the retrieval of functions; therefore, we evaluated the recovery of sensory and motor functions in the control and treated groups. The effect of NS2 on diet intake and body weight was studied in all groups. There were no significant changes in body weight in subjects with induced PNI. All subjects exhibited normal dietary behavior and even the administration of NS2 did not influence the eating behavior and metabolism. Our observation was supported by correlating previous studies, which showed the positive impact of Res on mitochondrial functions and metabolic activities in subjects with abnormal metabolism (Lagouge *et al.,* 2006). The Res supplementation modulates glucose tolerance, improves lipid and carbohydrate metabolism by controlling the AMPK pathway through SIRT1 (Chen *et al.,* 2015; Moon 2023).

A significant improvement in motor activity was recorded using SFI and grip strength analyses in NS2-treated group on the post-injury day 10 ($p \leq .0001$). The result highlighted the effective role of Res and TPGS in mediating the retrieval of motor functions in injured nerves. In a recent study, the 50-200 mg/Kg dose of Res ameliorated the motor deficits in 10 days in the animal model with sciatic nerve injury (Ding *et al.,* 2018). Motor recovery has also been observed with 30 mg/Kg dose of Res in a rat model of facial crush injury. However, the regain of motor functions took two weeks (Tanyeri *et al.,* 2015). In our case, accelerated retrieval of motor functions has been observed from day 4 to day 10 post-injury. The reclamation of motor functions is directly linked to the extent of nerve regeneration. The PNI results in axon degeneration, which triggers some underlying growth factors at the proximal stump of the injured nerve as support for regeneration. However, this intrinsic axon is generally slow, further delaying the regaining of motor functions. Motor recovery improves with the accelerated regeneration process (Tuffaha *et al.,* 2016; Zelada *et al.,* 2021).

The hotplate and pinprick tests were employed to evaluate the retrieval of senses in NS-treated and control subjects. The results from the hotplate tests indicated significant improvements in sensory functions in NS2-treated subjects compared to control on day 10 post-injury. These findings highlight the significance of Res for nerve regeneration in the context of sensory functions. The polyphenols regulate vital genes to control axonal growth, cAMP signaling and myelin functions, helping to restore motor functions (Qiu *et al.,* 2019). The Res exhibited an anti-apoptotic role by activating SIRT1 proteins for improved motor functions (Shi, Zhu, and Li 2016; Zhang *et al.,* 2019). The Res is a potent inhibitor of the TRPA1 channel responsible for pain transduction. The Res regulates its activity to improve sensory functions after nerve injury (Yu *et al.,* 2013; Zhang *et al.,* 2021). A recent study showed that Res regulated the Wnt/β-catenin signaling pathway, promoting axon regeneration and functional recovery after SCI (Xiang *et al.,* 2021). These observations were aligned with our results, validating an accelerated retrieval of functions in NS2-treated subjects.

The histopathological evaluations directly interpret the functional recovery in the target muscle on the reclamation of the nerve-muscle connection. Histopathological comparisons were conducted in both the treated and control groups. The muscle fiber characteristics and muscle mass were analyzed on both the ipsilateral and contralateral sides. The NS2-treated group exhibited a restoration of muscle mass and fiber count that closely approximated the normal levels. A significant reduction in gastrocnemius muscle mass and fiber was observed in the control group. The VEGFs and their receptors, including VEGFR1 and VEGFR2, mediate the accelerated growth and nerve generation following PNI (Guaiquil *et al.,* 2014; Calvo *et al.,* 2024). A recent study has established the positive role of Res in promoting nerve regeneration by activating the VEGF signaling (Ding *et al.,* 2018). The NF421, neurofilament, polypeptide and GAP43, an axon membrane protein, are the markers of neuronal regeneration. Their controlled expression regulates synaptic development, nerve extracellular growth and neurological functions (Liu *et al.,* 2020). Recently, Res has shown a potent role in regulating GAP3 and NF421 to improve histological damage after SCI (Xiang *et al.,* 2021). Last year, a study established the role of Res in SIRT1/FoxO1 activation, which enhanced the re-myelination process, increasing the thickness and number of myelinated axons in mice with demyelinating disease (Samy *et al.,* 2023). According to another report, it also increased the density of the myelin and the number of nerve fibers (Zhang *et*

al., 2020). Accordingly, in our case, histological changes were reversed significantly in NS2-treated subjects compared to untreated controls.

Furthermore, our study confirmed the glucose-lowering effects of NS2, attributing this impact to previously reported anti-diabetic properties of Res. The Res exhibits antidiabetic effects through various mechanisms, including increasing insulin sensitivity and glucose uptake. It could also trigger insulin secretion and promote the pancreatic β-cell population (Huang *et al.,* 2020; Fernandez-Quintela *et al.,* 2023). It modulates glucose metabolism at the cellular level and facilitates nerve regeneration because hyperglycemia impairs peripheral nerve regeneration (Pham *et al.,* 2018). Elevated blood sugar level triggers oxidative stress, which initiates various clinical issues, including nerve damage (Komirishetty *et al.,* 2016). Oxidative homeostasis is sensitive to blood glucose levels, a key factor in maintaining a growth-permissive microenvironment at the injury site. The regeneration and functional recovery process depends highly on blood glucose levels. The ROS is a key regulator and its excess delays the functional recovery after PNI (Asmat *et al*., 2016; Latini *et al.,* 2019). Given these considerations, oxidative stress mitigation may accelerate the regeneration and reclamation of functions after nerve injury. The Res have well-established ROS-scavenging and antioxidant potential for pharmacological applications (Miguel 2021). Accordingly, we measured TOS and TAC in the control and Res-treated group. The study revealed an increased TAC level and a reduced TOS level in NS2-treated subjects, confirming a favorable environment for functional recovery. The Res-mediated optimization of blood glucose levels and oxidative stress positively influenced the recovery process in our case. Therefore, the administration of NS2 significantly improved sensorimotor functions and muscle re-innervation in mice with induced PNI. The results suggested that the NS2 accelerated the functional retrieval owing to the TPGS-mediated enhanced bioavailability of Res compared to other groups. The described method is economical and easy to handle; it is recommended for scaling up NS preparation studies before potential clinical applications. Further, the effects of NS2 on nerve regeneration may be studied at the molecular level from a mechanistic perspective with a larger sample size. Moreover, the potential toxicity of long-term usage of the NS2 should be evaluated before possible clinical applications.

Conclusion: The prepared NS TPGS + Res (NS2) significantly improved the recovery of sensorimotor functions after induced PNI. Notably, the nanosuspension with TPGS demonstrated significantly higher efficacy in promoting functional recovery than the control groups. It effectively regulated glycemic levels and oxidative stress, providing a growth-permissive environment for an accelerated nerve regeneration.

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