Heliyon 10 (2024) e25414

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Chitosan nanoparticles loaded with *Foeniculum vulgare* extract regulate retrieval of sensory and motor functions in mice

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ARTICLE INFO

Keywords:
Nanoconjugate
Nerve lesions
Antioxidants
Nanocarriers
Bioavailability

ABSTRACT

In this study, chitosan nanoparticles (CSNPs) encapsulating *Foeniculum vulgare* (FV) seed extract (SE) were prepared for the controlled delivery of bioactive phytoconstituents. The prepared CSNPs encapsulating FVSE as sustain-releasing nanoconjugate (CSNPs-FVSE) was used as a potent source of functional metabolites including kaempferol and quercetin for accelerated reclamation of sensory and motor functions following peripheral nerve injury (PNI). The nanoconjugate exhibited *in vitro* a biphasic diffusion-controlled sustained release of quercetin and kaempferol ensuring prolonged therapeutic effects. The CSNPs-FVSE was administered through gavaging to albino mice daily at a dose rate of 25 mg/kg body weight from the day of induced PNI till the end of the experiment. The conjugate-treatment induced a significant acceleration in the regain of motor functioning, evaluated from the sciatic function index (SFI) and muscle grip strength studies. Further, the hotplate test confirmed a significantly faster recuperation of sensory functions in conjugate-treated group compared to control. An array of underlying biochemical pathways regulates the regeneration under well-optimized glucose and oxidant levels. Therefore, oxidant status (TOS), blood glycemic level and total antioxidant capacity (TAC) were evaluated in the conjugate-treated group and compared with the controls. The treated subjects exhibited controlled oxidative stress and regulated blood sugars compared to the non-treated control. Thus, the nanoconjugate enriched with polyphenolics significantly accelerated the regeneration and

Abbreviations: PNI, Peripheral nerve injury; HPL, Hot plate latency; WRL, Withdrawal reflex; TAC, Total antioxidant capacity; TOS, Total oxidant status; TEAC, Trolox equivalent antioxidant capacity; ABTS, 2, 2′-azinobis 3 ethylbenzothiazoline-6-sulfonate.

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https://doi.org/10.1016/j.heliyon.2024.e25414
Received 30 August 2023; Received in revised form 25 January 2024; Accepted 25 January 2024
Available online 29 January 2024

recovery of functions after nerve lesions. The biocompatible nanocarriers encapsulating the nontoxic natural bioactive constitutents have great medicinal and economic value.

1. Introduction

Over the last decade, great progress in nanotechnology has facilitated the introduction of nanoformulations of drugs and natural products [1–3]. Such sustain-releasing systems maintain a controlled delivery of bioactive ingredients over extended time periods. Generally, drugs and natural bioactive compounds experience limited bioavailability owing to their pH sensitivity, chemistry and low adsorption across biomembranes [4]. The formulations with nanocarriers improve the bioavailability of poorly soluble pharmaceutics with controlled delivery thus enhancing their bioactivity profiles [5,6]. The nanoparticles (NPs) have emerged as promising candidates to enhance the solubility and bioavailability of drugs and bioactive phytomolecules [7]. Peripheral nerve injuries (PNIs) usually cause the loss of sensory and motor functions resulting in muscular atrophy. Such abnormalities arise from any loss to the integrity of Schwann cells (SCs) or degeneration of axons. Resultantly, an array of underlying biochemical pathways and subcellular mechanisms show well-integrated coordination for axonal regeneration and recovery of functions after PNI. In general, non-neuronal SCs create growth-permissive environments for damaged axons. Accordingly, the regeneration of axons is mechanistically supported by different molecular and structural readjustments at proximal and distal stumps in damaged nerves. The mRNAs with a key role in regeneration are upregulated while those vital for neurotransmissions experience downregulation in injured neurons. The process of nerve regeneration is also supported by the overexpression of growth-inducing proteins like actin and tubulin. The damaged nerves follow a complex and slower process for regeneration. With the passage of time, a further drop is detected in activated biomachinery leading to a prolonged recovery and regeneration process. Consequently, drugs are employed for accelerated regain of sensory and motor functioning following nerve injuries. But they could not help to attain a complete regain of functions being unable to maintain a growth-permissive environment at optimum levels [8,9].

Over the years, great efforts have been made to search for nontoxic bioactive capable of supporting the regeneration of damaged nerves with complete recovery of functions. Accordingly, plant-based metabolites have emerged as alternative neurotherapeutics due to their diverse chemical nature, efficacy and safety profiles. Different herbs and dietary plants have been studied for their bioactive constituents with minimum toxicity. Several secondary metabolites of plants have established their worth as potent drugs for all types of diseases. They appear as potent leads for new drug designing owing to their multidimentional chemical nature and vunerability for further derivitization [10–12]. Various phytoconstituents including taxol, quercetin and curcumin were found highly effective as anticancer, *anti*-HCV, antimicrobial and antioxidants, thus they have become potent alternative drug candidates. Similarly, a number of phytoproducts have shown their positive role in the management of various neurodiseases including Huntington's disease, Parkinson's disease and Alzheimer's disease [13–15]. *Foeniculum vulgare* (FV) from the Apiaceae family is recognised owing to a wide range of bioactivities including anti-inflammatory, hepatoprotective and antioxidant potential [16–18]. In general, phytomolecules with antioxidant activities are effective for an accelerated regain of functions in damaged nerves. Several phytomolecules including soy lecithin, glutamic acid, vitamins, β-carotene, tocopherols and polyphenolics support peripheral nerve regeneration owing to their high antioxidant potential. A very recnt study has evaluated the phenolic composition of FVSE and validated their high antioxidative stress potential [19]. Recently, it has been observed that crude and methanolic exacts of FV were effective for accelerated recovery after induced PNI [20,21]. The applications of FV reduced neuronal toxicity by controlling oxidative stress and expressions of amyloid precursor proteins [22]. The administration of FV extracts improved memory defects in dementia and Alzheimer's disease displaying strong anti-cholinesterase and antioxidant activities [22,23]. The FV enriched with plyphenols shows promising prospects for accelerating the recovery after PNI owing to its antioxidative, analgesic and anti-inflammatory potential [16]. As described earlier, the

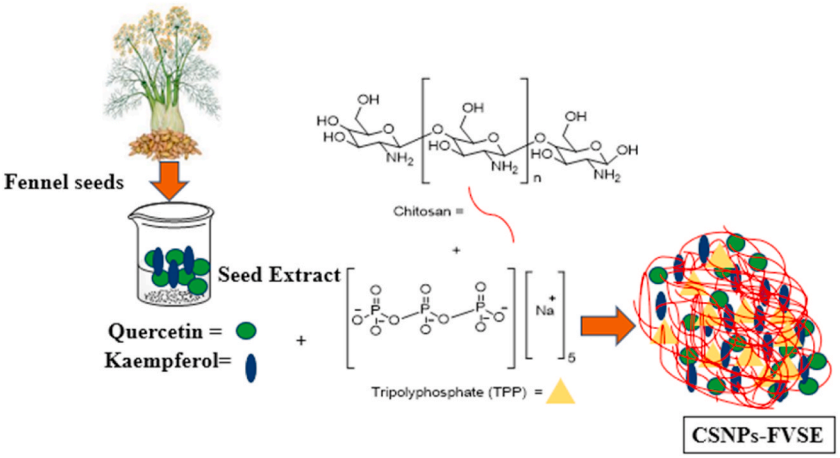

Fig. 1. Preparation of CSNPs encapsulating *Foeniculum vulgare* seed extract.

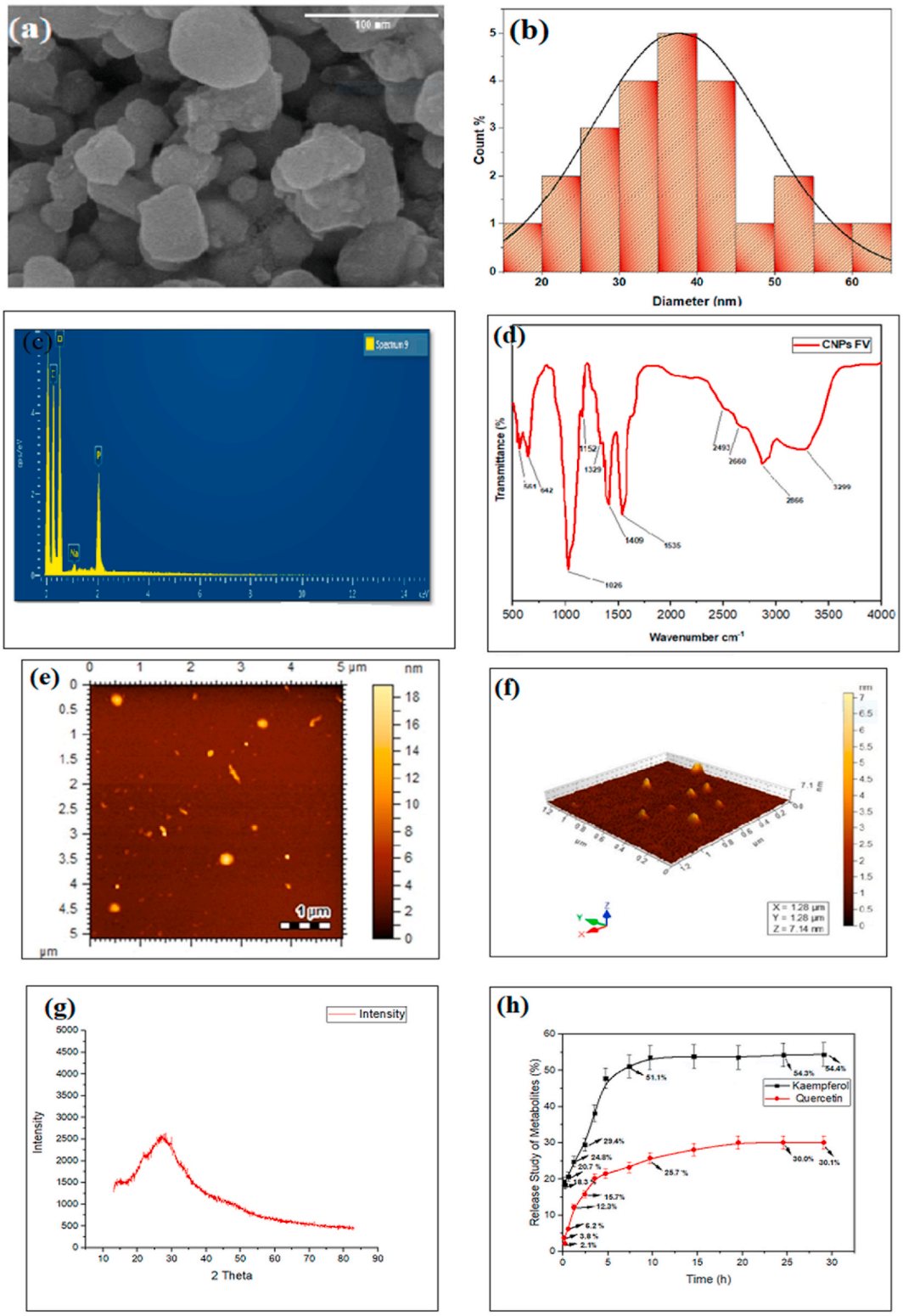

Fig. 2. Characterizations and *in vitro* release polyphenols of nanoconjugate of *F. vulgare* a) SEM analysis b) size distribution c) EDX analysis d) FTIR e) and f) AFM analysis g) XRD h) *In vitro* release study of polyphenols.

polyphenols show low solubility and limited bioavailability thus exhibit compromised efficacy profiles.

Currently, there are challenges for the development of nanodrugs showing longer residence time with high biocompatibility, efficacy and safety profiles. Safety is highly desirable to avoid the leakage of bioactive constituents which may cause unwanted interactions leading to side effects. The delivery process, structural attributes and composition influence the controlled release of the bioactive constituents. A controlled-releasing system, therefore, prolongs blood circulation consequently a high concentration of bioactive ingredients reaches the target site. The delivery systems with high loading capacity, appropriate pore size and large surface area enhance penetration and retention of the bioactive ingredient and furnish its controlled release at the desired site. Recently, there has been a great interest in the development of biocompatible encapsulating systems ensuring effective targeted delivery and controlled release [24–26]. Recently, the polymeric nanoparticles have emerged as promising nanocarriers of drugs and bioactive molecules. However, their applications as nanocarriers of plant extract as still very limited, which has a high economic value and broad market. In the context of nanodelivery systems, nature of the polymeric materials greatly influence the control release, initial burst and loading capacity of bioactive molecules. Accordingly, the CS has become a promising choice for the development of nanocarriers owing to its polycationic structure, biocompatibility and biodegradability [27]. It has shown potential applications in pharmaceutics, cosmetics and food industry due to its nontoxic nature and high physiochemical stability [28].

Envisioning the aforesaid facts, this study was planned to prepare CSNPs encapsultaing FVSE as a sustain-releasing conjugate for controlled delivery of active phytoconstituents (Fig. 1). The as-prepared nanoconjugate was evaluated for an accelerated regeneration and reclamation of motor and sensory functions after induced PNI in albino mice.

2. Material and methods

2.1. Preparation and characterization of CSNPs encapsulating FVSE as sustain releasing conjugate

The *FV* seeds were obtained from a local vendor, dried under shade and crushed into powder. Later, 25 g powder was mixed with water/methanol (1:1) and stirred for 24 h. Successively, the rotary evaporator was used to dry the filtrate under vacuum then heated at 50 °C for 1 h. The nanoconjugate was prepared using CS (0.4 % w/v) in 0.5 % acetic acid and pH was ajusted to 4.8 using 10 % NaOH sol. Then, the prepraed FV seed extract (FV-SE) (0.2 % w/v) was added into the aforesaid solution and stirred for 2 h. Afterward, (0.2 % w/v) TPP solution was added dropwise and further stirred for 2 h and centrifuged at 10,000 rpm for 30 min [29,30]. The pellets obtained were washed with water and lyophilized to receive CSNPs encapsulating FVSE. The prepared sample was characterized for size analysis using SEM (Fig. 2 A and B) and AFM for surface topograhoy and 3-D surface profile (Fig. 2E and F). The elemental analysis of the nanoconjugate was performed with energy dispersive X-ray (EDX) analysis (Fig. 2C). The crystallinity of the sample was studied using X-ray Diafraction (XRD) (Fig. 2G). The XRD spectroscopic result revealed the amorphous and crystalline nature of the prepared sample. A broad peak at $2\theta = 27^{o}$ confirmed the crystalline nature of the CS while the broad region from 27 to 80º validated the predominant amorphous nature of the sample [31]. The FTIR analysis detected flavonoids and polyphenolic contents in the sample and the results were in agreement with the earlier reports. It is well reported that FV seed extract majorly carries kaempferol and quercetin along with a variety of flavonoids and polyphenols [32,33]. The mean zeta potential of the prepared sample was found to be 0.7 mV.

The sustained release of phytomolecules from CSNPs was studied following the well-established method. The *in vitro* cumulative release of quercetin and kaempferol from CSNPs was administered using the phosphate buffer solution (PBS, pH = 7.4, 50 ml) containing 10 % v/v DMSO as release media. The dialysis bag containing nanoconjugate suspension (10 mg/μL) was incubated in release media at 37 °C and shaken at 100 strokes/min. After specific time intervals, 5 ml of the sample was withdrawn and replaced with an equal volume of the fresh medium. The withdrawn samples were analyzed using UV/Vis spectrophotometer at 320 and 373 nm for the determination of quercetin and kaempferol respectively [30,34,35].

2.2. Supplementation of nanoconjugate to mice with induced PNI

Male albino mice (aged around 9 weeks and weight 26 ± 1 g) were received from the local market. Under controlled environment, the subjects were allowed to acclimatize dark/light cycle at 25±1 °C. Further, they were subdivided into two groups i.e. control group and nanoconjugate-treated group. Each group included six subjects with one mice/cage. The behavior-related tests were conducted in the daylight at specified time. The as-prepared nanoconjugate was administered through gavaging by mixing the sample (25 mg/kg) with saline water [36]. They were supplemented till the end of the experiment. On daily basis, the food intake and average body mass were measured at the same time [37]. The subjects in each group were subjected to induce sciatic nerve injuries following well-estabished surgical method [38]. The surgical operation was performed to assess the efficacy of the prepared nanoconjugate on recovery of sensory and motor functioning following induced nerve injury. During the surgery, the subjects were injected intraperitonealy a mixture of Xylazine (5 mg/kg) and Ketamine (70 mg/kg) before exposing right thigh muscle. After that, sciatic nerve was compressed as a step to induce nerve injury for this comparative study. Finally, the blood and tissue samples were collected after decapitation of subjects at the end of trial.

This study was carried out by following the guideline after the approval of Institutional Animal Ethical Board GCUF with reference no. GCUF/ERC/191.

2.3. Behavior-related studies

2.3.1. Effects of nanoconjugate supplementation on SFI

The recapture of motor functioning was evaluated after injury using well-known methods [21,39]. The mice were allowed to walk and SFI measured from the paw prints as follows,

$$SFI = \left(-38.5 \times \frac{EPL - NPL}{NPL}\right) + \left(109.5 \times \frac{ETS - NTS}{NTS}\right) + \left(13.3 \times \frac{EIT - NIT}{NIT}\right) - 8.8$$

here NPL = Normal print length, EPL = Experimental print length, NTS = normal toe spread, ETS = Experimental toe spread, NIT = normal intermediate toe, EIT = Experimental intermediate toe.

2.3.2. Effects of nanoconjugate supplementation on sensory functions

The hot plate tests were done to analyze the regain of sensory functions following sciatic nerve lesion. The mice were permitted to stay on hot surface (55 ± 2 °C) and readings were documented as hot plate latency (HPL) [40].

2.3.3. Effects of nanoconjugate supplementation on muscular grip strength

The regain of muscular grip strength was evaluated using grip strength meter in accordance with established method. The injured mice put in effort against unintentional backward movements till the weakening of their grip due to withdrawing force. Accordingly, maximum pulling force was recorded for contralateral, Ipsilateral and hind limbs [41].

2.4. Biochemical studies

2.4.1. Effects of nanoconjugate supplementation on TAC

In this study, the recovering mice were used for TAC analysis using chemistry analyzer (Biosystem, BTS-330) following known procedure. Free radicals are produced incubating 2,2′-azinobis 3 ethylbenzothiazoline-6-sulfonate (ABTS) with H_2O_2 [42]. The Trolox solution is employed as standard against serum samples and TAC expressed as mmol Trolox Equiv/L [43].

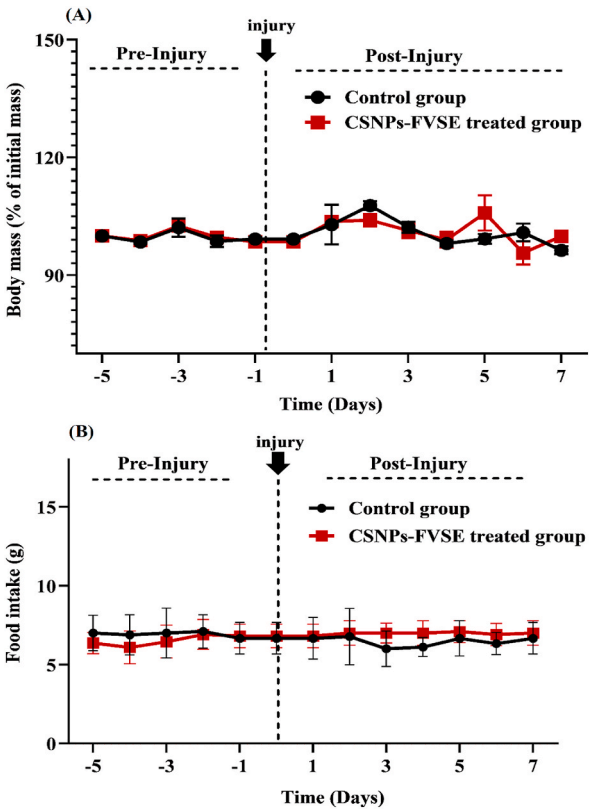

Fig. 3. Effects of nanoconjugate supplementation on mouse's a) body mass b) food intake.

2.4.2. Effects of nanoconjugate supplementation on TOS

The total oxidant status (TOS) was studied following an established procedure that involves the oxidation of Fe(II) using acid as an oxidant. The semi-automated chemistry analyzer was employed for the evaluation of oxidation in serum using xylenol orange (Bio-system, BTS-330) [44].

2.4.3. Effects of nanoconjugate supplementation on glycemic levels

The recovery of functions and regenration of nerves is mediated by an array of underlying biochemical processes at optimum glucose level. Accordingly in this study, a glucometer (Accu-chek) was employed for the monitoring of glyemic level in blood samples of conjugate-treated and control groups [21].

3. Results

3.1. Effects of nanoconjugate supplementation on eating behaviour and body mass

During this expertial study, the body mass and food intake and were evaluated in control and conjugate-treated subjects under pre- and post-injury conditions (Fig. 3A and B). There was no substantial variation detected in eating behavior due to supplementation of nanoconjugate before and after nerve lesion. It was credited to an tolerable taste, smell and physical appearance of the conjugate. More, the conjugate administration resulted in increase in body mass suggesting its positive influence on metabolic activities compared to control subjects.

3.2. Effects of nanoconjugate supplementation on muscle strength and motor functions

The retreival of motor functions in conjugate-treated and control group were examined from the improvements in SFI and muscle grip strength. Compared to control, the conjugate-treated subjects showed a significantly fast reclamation of grip strength (Fig. 4A). The conjugate-treatments induced a substantial and affirmative influence in muscle strength post-injury day 2–6. Similarly, conjugate receiving subjects exhibited a significant improvement in SFI on day 3–6 post-injury compared to controls (Fig. 4B). The nano-conjugate mediated an upgraded SFI represented an earlier retreival of motor functioning after PNI.

3.3. Effects of nanoconjugate supplementation on sensory functions

The PNIs commonly cause losing of sensory and motor functioning. In this study, the repossession of sensory functions were evaluated at different time under pre- and post injury conditions using hotplate test in conjugate-supplemented and control subjects.

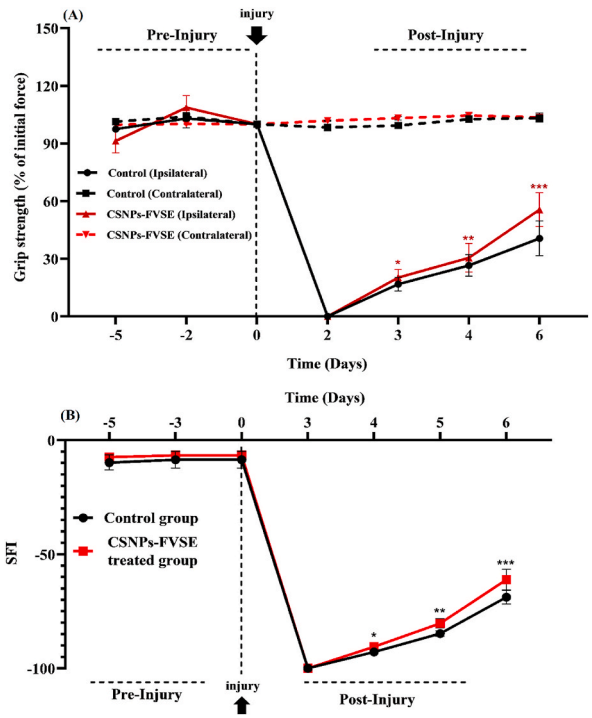

Fig. 4. Effects of nanoconjugate supplementation on a) muscle grip strength b) SFI following sciatic nerve lesion.

The conjugate-supplemented subjects presented a significantly quicker recovery of sensory function (as exhibited by paw withdrawl latencytime) on day 4 and 6 compared to controls (Fig. 5).

3.4. Effects of nanoconjugate supplementation on glycemic level and oxidative stress

On post-injury day 5, the conjugate-supplemented subjects represented a significant controll levels of blood glucose compared to controls (Fig. 6C). The conjugate-mediated contolled glycemic levels were comparable to pre-injury day 5. The local oxidative stress greatly influence the regeneration and functional recovery process after nerve injuries. Therefore, the conjugate-mediated antioxidant effects were studied by evaluating TOS and TAC compared to controls. The conjugate-supplemented subjects exhibited significantly higher TAC values (Fig. 6A). Similarly, a regulated TOS was observed in conjugate-treated group in comparison to untreated ones (Fig. 6B). So, the supplementation of nanoconjugate controlled the oxidative stress by regulating antioxidants following induced PNI.

3.5. Effects of nanoconjugate supplementation on muscle histology

The histopathological comparison betweeen contralateral and ipsilateral sides in control and conjugate-supplented was achieved using surface area (Fig. 7A) and fiber count studies (Fig. 7B). The effects of nanoconjugate supplementation on muscular histology, the contralateral control group (Fig. 8a), Ipsilateral control group (Fig. 8b), contralateral CSNPs-FVSE treatment group (Fig. 8c), Ipsilateral CSNPs-FVSE treatment group (Fig. 8d). The injured nerves usually experience interruption in transduction of signals however, prolonged situations could lead to musclular atrophy. The conjugate administration triggered a substantial progress in the recuparation of morphology by controlling muscular dystrophy in recovering subjects.

3.6. In vitro release of quercetin and kaempferol

The interaction between polymer and bioactive ingredients, their structures and composition ratio highly influence the *in vitro* release rate and mechanism profiles. Further, the release profile from polymeric NPs is also influenced by the diffusion of adsorbed materials, degradation and swelling of the polymer. A burst release in the start followed by a sustained release of kaempferol and quercetin was recorded for 30 h. Thus, a biphasic release pattern has been observed in this case (Fig. 2H). In the first 2.5 h, the quercetin and kaempferol showed a burst release of 15.7 % and 29 % respectively. Afterward, a diffusion-controlled sustained release was observed for the subsequent 27.5 h. The loosely bound polyphenols displayed a fast diffusion during the pulsatile phase providing a relatively higher concentration to afford quick antioxidative and anti-inflammatory relief post-surgery. During the second phase, a linear trend of slow release was observed till 30 h. This release originates from the depth of the nanostructures through the diffusive transport mechanism. The small diameter and high surface area of NPs facilitates the release of the bioactive molecules due to relatively shorter diffusion pathways [45]. The diffusive release of the bioactive ingredients is controlled by the strong interactions of the polycationic structure of CS with polar groups of polyphenols. The quercetin has more polar groups so develops strong interaction leading to slow, sustained and lesser release compared to kaempferol.

Results are presented as mean ± SEM (n = 6). (A) presents time course of body weight in mice served with routine diet (Control = black line), CSNPs-FVSE dose (red line). Two-way repeated measure ANOVA followed by Tukey's multiple comparisons test along with Benjamini, Krieger and Yekutieli's correction showed a nonsignificant difference among all groups at all-time points ($\rho = 0.274$) (B) Presents time course of food intake of mice as in (A). Two-way repeated measurement ANOVA followed by Tukey's multiple comparisons test along with Benjamini, Krieger and Yekutieli's correction indicated non-significant differences among all groups at all-time points ($\rho = 0.071$).

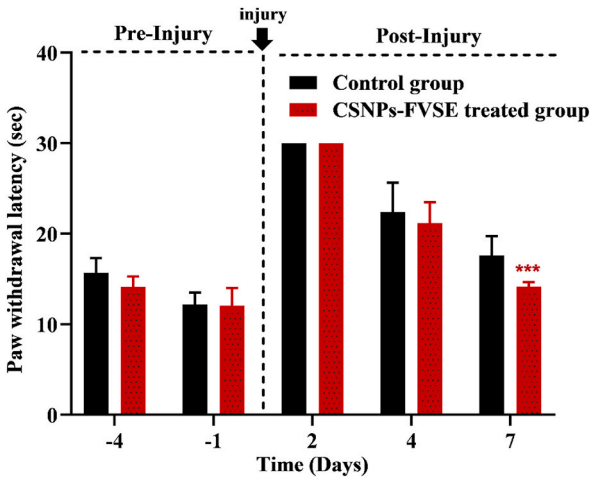

Fig. 5. Effects of nanoconjugate supplementation on recovery of sensory function following sciatic nerve lesion.

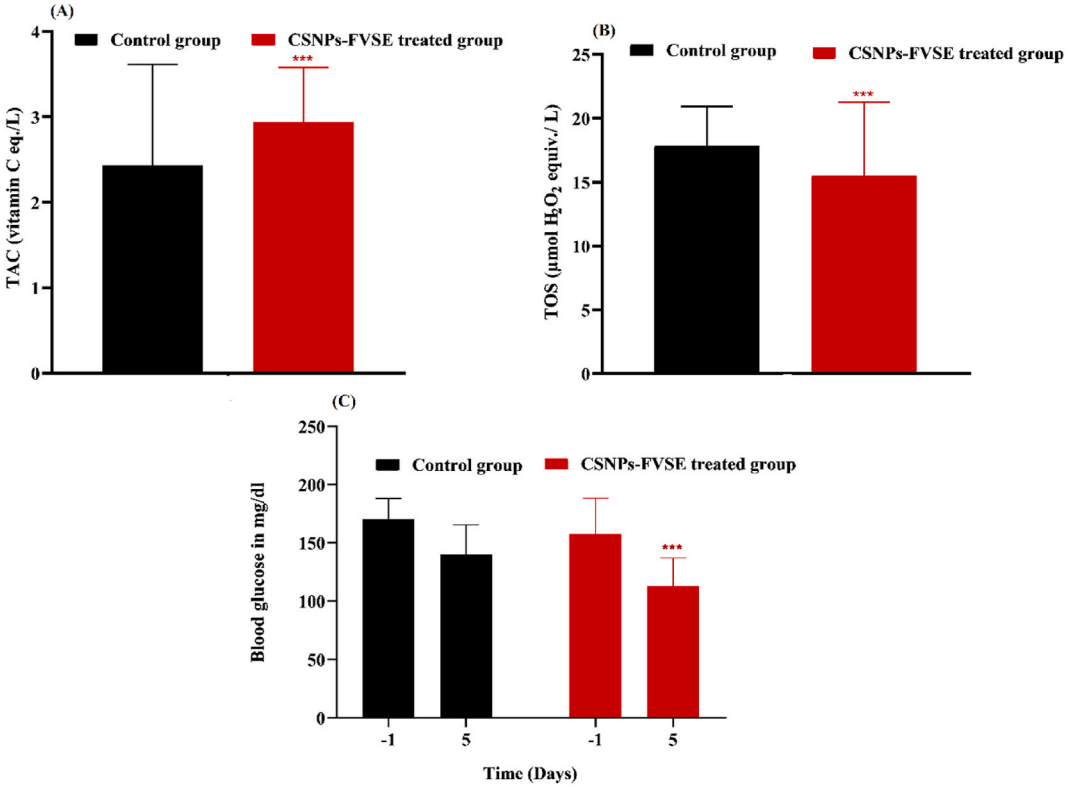

Fig. 6. Effects of nanoconjugate supplementation on a) TAC b) TOS and c) blood glucose level.

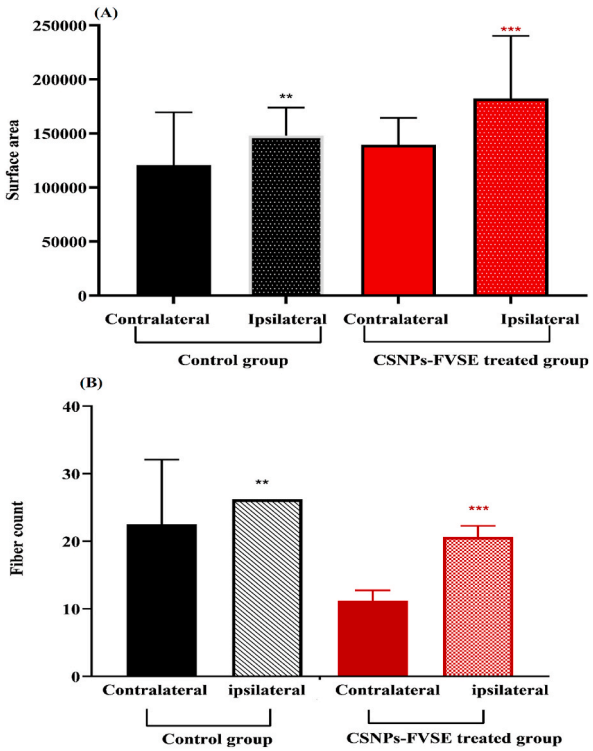

Fig. 7. Effects of nanoconjugate supplementation on a) surface area b) fiber count contralateral v/s ipsilateral in Tibialis muscles.

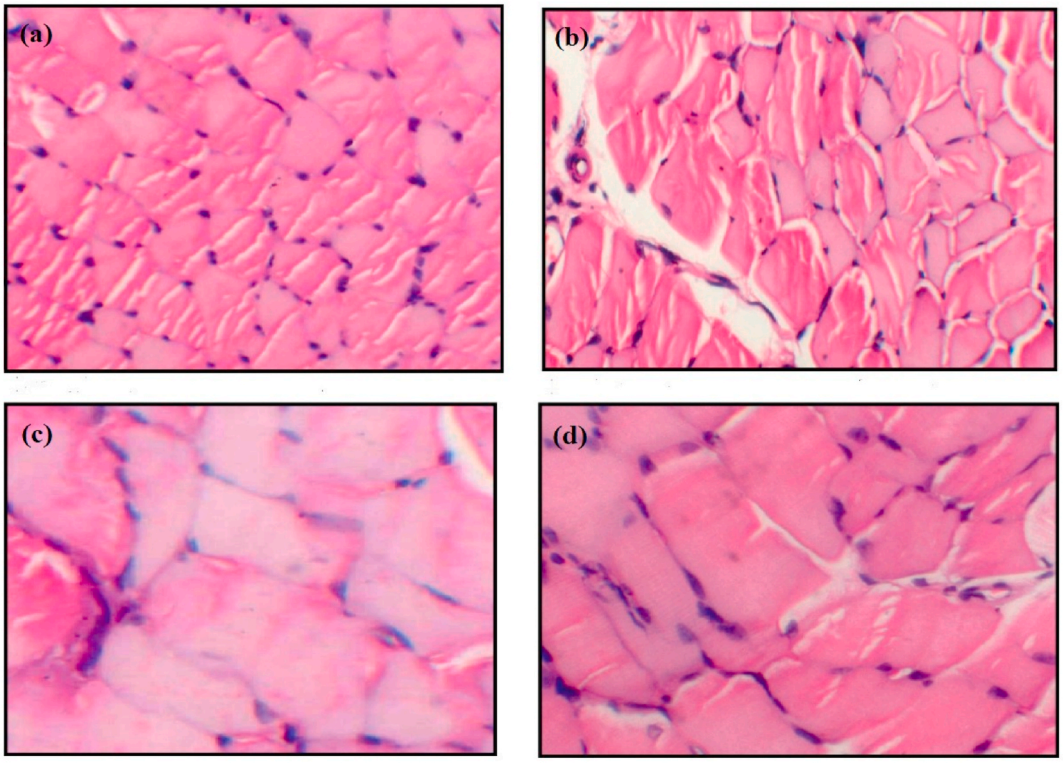

Fig. 8. Effects of nanoconjugate supplementation on muscular histology the contralateral control group (Fig. 8a), Ipsilateral control group (Fig. 8b), contralateral CSNPs-FVSE treatment group (Fig. 8c), Ipsilateral CSNPs-FVSE treatment group (Fig. 8d).

Results are presented as mean ± SEM (n = 6) (A) Presents time course of grip strength in mice served with routine diet (Control = black line), CSNPs-FVSE dose (red line). Grip strenght is expressed as percentage of mean in initial force taken at day −5 and −2 before nerve lesion.Two-way repeated measure ANOVA followed by Tukeys's multiple comparisons test along with Benjamini, Krieger and Yekutieli's correction indicated highly significant improvement after the treatment in CSNPs-FVSE group as compared to control group ($\rho < 0.001$), (B) Presents time course of Sciatic functional index (SFI) in mice as in (A).Two-way repeated measurement ANOVA followed by Tukey's multiple comparisons test along with Benjamini, Krieger and Yekutieli's correction presents significant improvement after the treatment in CSNPs-FVSE group as compared to control group ($\rho = 0.01$).

Presents time course of sensory response revival as measured by earlier paw withdrawal from hot surface in mice. Two-way repeated measure ANOVA followed by Tukeys's multiple comparisons test along with Benjamini, Kerieger and Yekutieli's correction presents a significant improvement after the treatment in CSNPs-FVSE group as compare to control group ($\rho = 0.010$) on day 7 following nerve lesion.

Ordinary One-way ANOVA followed by Tukey's multiple comparisons test along with Benjamini, Krieger and Yekutieli's correction revealed a significant difference between Control vs. CsNPs-FVSE treated group for Total oxidant capacity (TAC) ($\rho < 0.001$) and for total oxidant capacity TOS ($\rho = 0.010$) as well, was significantly improved in treatment group group. Blood glucose level indicated a significant result ($\rho < .001$) in CSNPs-FVSE treated group.

Ordinary One-way ANOVA followed by Tukey's multiple comparisons test along with Benjamini, Krieger and Yekutieli's correction revealed a significant improvement in CSNPs -FVSE treatment group ($\rho = 0.012$).

4. Discussion

The plant-based polyphenols fascilitate the regeneration process due to their high antioxidant capacity and anti-inflammatory potential, a basic requirement for growth-permissive environment at the site of injury. Nerve injuries induce oxidative stress due to the overproduction of oxidants. The cellualr signaling and other coordinating biochemical pathways get disturbance with the imbalancing of ROS homeostasis. It could also damage biomolecules like DNA, proteins and lipids. The resulting prolonged inflammatory responses and cellular apoptosis cause demyelination and degeneration of axons [46]. The oxidative stress upregulates nitric oxide synthase (iNOS) and induces the overproduction of pro-inflammatory cytokines like interleukin-1 (IL-1) and IL-6. It also increases the expression of cyclooxygenase-2 (COX-2) and tumor necrosis factor alpha (TNF-α). Such alterations collectively disturb neurotransmitters, neuronal activities and functioning of mitochondria [47]. Consequently, the ROS-mediated such biochemical chnages delay regain of functions and regenration after nerve injury. Thus, polyphenolic compounds could act as diet substitutes

controlling the ROS to avoid any such changes for the maintenance of growth-permissive environment at the site of injury [48].

The functional phytomolecules usually experience bioavailability issues owing to their sensitive chemistry and poor absorption through biomembranes. In recent years, formulations with nanocarriers improve their bioavailability with controlled and targeted delivery, thus minimizing off-site interactions. The nanoformulations help to regulate sustaine releasing of molecular entities for extended timeperiods thus increasing the efficacy of potent biomolecules. The FV is a traditional nutrientive supplement and a sustainable source of potent polyphenolics having diverse chemical natures and a wide spectrum of bioactivities. The FVSE was encapsulated in nanochitosan to control the bioavailability of functional molecules for an accelerated retreival of functions and regeneration after induced PNI.

The FV-based phytoconstituents were made available from the sustain-releasing nanoconjugate to evaluate their potential for an accelerated regeneration process and regain of functions after nerve lesions. The supplementation of nanoconjugate did not induce any substantial alteration in eating behaviors of the subjects. The conjugate treatment increased body mass significantly, verifying the nutritive potential of available metabolites potent enough to regulate metabolism as a stress-mitigating step [49–51]. Due to conjugate supplementation, a maximum increment with a nonsignificant variance was noted on post-injury day 7. The FV is enriched with proteins, minerals and a variety of polyphenols, therefore, inducing a positive effect on body weight [16,52]. The polyphenolic compounds do interact with gut microbiota and influence the carbohydrate and lipid metabolism. They could control hyperglycemia and dyslipidemia and increase adipose tissue metabolism [53,54].

The grip strength evaluations confirmed the positive influence of conjugate supplementation for quick recovery of motor functioning after PNI. The conjugate-receiving mice presented a substantial regain of grip strength from post-injury day 2–6. Recovery of motor functions was at the slowest in unsupplemented subjects. The results indicated the positive role of nanoconjugate in adjudicating the recuperation of motor functions following PNI. A few preliminary reports have evaluated the potential of FV phytochemicals for the recuperation of sensory and motor functions following induced nerve lesions [55,56]. The supplementation of polyphenoles inhibits the dysfunctioning of mitochondria and avoids atrophy of muscles under extreme conditions [57]. As a stress countermeasure, they have the capacity to upregulate protein sirtuin 1 (SIRT 1) and mitochondrial energy in skeletal muscle [58].

The polyphenols could improve motor functioning and control apoptosis following nerve lesions. They regulate the expression of key proteins involved in cellular signaling for the regulation of apoptosis [59]. The polyphenols like quercetin and isoquercetin accelerated the recovery of motor functions by avoiding the muscular atrophy. They act to for the regulation of cAMP signaling and myelin function for the improvement of motor functions. They exhibit antiapoptotic activity and could control genes for the regularization of axonal growth [60–62]. In our case, the conjugate-mediated improvemed SFI signifing its key role for the recovery of motor functions. Thus, the nanoconjugate being a potent source of sustain-releasing molecules supported the recapturing of motor functioning in recovering subjects.

The phenolic compounds could interact with TRP channels and improve the regain of thermosensitivity at the site of nerve injury [63,64]. In this study, the conjugate-induced recovery of sensory functions was evaluated from the HPL time in hotplate test. Substantial progress in the retreival of sensory functioning was observed from post-injury day 4–7. The sensory function reclaimations suggested nanoconjugate as a control-delivery source of phytophenols including quercetin and kaempferol, effective for an accelerated regeneration and regain of functions.

In biological systems, several subcellular biochemical pathways coordinate for the controlled generation of oxidants and the regulation of oxidative stress. After nerve injuries, the process of regeneration and functional regain highly depend on local oxidative stress. An optimum oxidation state with high antioxidant capacity accelerates the functional retreival after nerve injuries. So, TAC and TOS of subjects evaluates the levels of oxidative stress and its possible involvement in functional reteival and regeneration process. Compared to control, the conjugate supplementation activated a substantial rise in TAC in treated subjects. Further, the supplementation caused a substantial lessening in TOS, suggesting a well-controlled generation of oxidants. Therefore, an improved TAC and a controlled TOS followed by conjugate supplementation ensured low level of oxidative stress, favoring the functional reclamation in injured nerve.

Following nerve injuries, the growth and renegeration of axons is delayed due to several interruptions of ROS in vital intercoordinating physio-biochemical pathways. The uncontrolled oxidants cause peroxidation of fatty acids and disrupt the architectural integrity of biomembranes. The peroxidation products interfere with enzymes of metabolism including Na^{+}/K^{+}-ATPase. The FV is enriched with quercitin and kaempferols showing high ROS scavenging potential. They activate antioxidant defence machinery and control oxidative stress [48,65]. The kaempferols supprot axonal growth and regeneration by controlling oxidative stress through the regulation of proteins vital for antioxidant defense [66,67]. The quercitin could improve neuronal recovery and regulate cell proliferation. It enhances brain-derived neurotrophic factor (BDNF) by interacting with signaling pathways like JAK2/STAT3 [68]. The well-regulated provision of functional metabolites for longer period provided protection from oxidants and fascilitated the regeneration process [69–71]. Plant-based polyphenols have shown great capacity to support the regain of sensory and motor functioning after nerve injuries [48,72]. The polyphenols and flavonoids in nanoconjugate has shown a vital role in the optimization of oxidants, and improved the recapture of functions after injuries. Further, they are significantly effective for controlled glycemic levels which coordinate with underlying bioprocesses for an accelerated regeneration and recovery of senses after injury [56,73]. The FV-based functional metabolites are potent to optimize blood glucose level and for the regulation of metabolism [74,75].

The polyphenols boost the secretion and sensitivity of insulin and also increase the absorption of carbohydrates, thus help to control blood glucose level. The kaempferol improves glucose uptake by activating protein kinase C, P13K pathway and glucose transporters [76]. The quercitin also helps in the regulation of blood glucose by interferring with factors like TNFα, NRF2 and AMPK [77].The myelin-associated glycoprotein (Mag) and peripheral myelin protein 22 (Pmp 22) are the main prequisits for the growth and integral safety of myelin sheath. However, the expressions of such proteins are compromised after nerve injuries. Therefore a clear decrease in

thickness and layers-counts of myelin is observed after injuries [78,79].

The histopathological analyses of contralateral and ipsilateral sides in conjugate-supplemented and controls validated the results of behavior-related studies. The fiber count and surface area studies highlighted a substantial decrease in muscular dystrophy. Furthermore, the conjugate-induced regulation of oxidants and blood glucose controlled the process of regeneration, duly represented by the retention of normal muscular shape and area. The quercitin applications exert growth-promoting effects and fascilitates regenration by increasing the density of myelinated fibers. It inhibits apoptosis and favor remyelination of nerve fibers after nerve injuries [80]. Another report described the positive influence of quercitin on the development of myelin sheath [61]. As a mechanistic approach to avoid any peripheral nerve impairments, It can activate Nrf2 and Akt pathways and suppress caspase 3, NF-KB an ATF-6 pathways [81].

An effective drug therapy requires an optimal drug's concentration at the site of action for a prolonged period throughout the treatment cycle. The polymeric NPs increase the therapeutic index of bioactive agents by controlling their sustained release and increasing bioavailability [82]. The release study has highlighted the CSNPs as potent nanocarriers with the potential to cross biological barriers ensuring the protective and controlled delivery of bioactive molecules [83]. At the target sites, the nanoconjugate is released by enzymatic or hydrolytic cleavage. From the carrier matrix, the encapsulated phytomolecules were released through a diffusion mechanism. A biphasic release pattern has been observed in this case. In the case of injuries, the delivery of bioactive molecules through a dual-phase mechanism is highly encouraged in biological systems. A burst release at the start is effective to control microbes and the following controlled release induces a growth-permissive environment for a longer period at the site of injury [84].

The nanoconjugate supplementation produced substantial improvements in body mass, grip strength and sensory functioning after induced PNI. Further, it helped to contol oxidants by boosting TAC and reducing TOS in recovering subjects. More, the regulatory influence of FV phytoconstituents on blood glucose highlighted them vital for functional recovery and regeneration of nerves after PNI. Hence, the CS NPs encapsulating polyphenols like kaempferol and quercetin exhibited anti-inflammatory and anti-apoptotic effects and controlled oxidative stress fascilitating the functional recovery and regeneration nerve after induced injury.

5. Conclusion

The supplementation of CS NPs encapsulating FVSE accelerated the recapturing of motor and sensory functions in injured nerves. The nanocarrier regulated a controlled release of bioactive constituents like kaempferol and quercetin, enhancing their bioavailability to maintain the growth-permissive environment for a longer period. They were highly effective to maintain oxidants and glycemic at optimum level favoring the underlying mechanisms for recovery of functions. The sustained-releasing nanoconjugate presented a significantly high potential to accelerate the regeneration and reclamation of functions after nerve injury.

Funding

Partial support by MAA through Grant KKU/RGP/2/428/44.

Data availability

The datasets used and analyzed during the current study are included in this manuscript.

Institutional review board statement

Approved by the ethical review board of Government College University Faisalabad, Pakistan.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Majed A. Bajaber: Funding acquisition. **Arruje Hameed:** Resources, Methodology. **Ghulam Hussain:** Supervision, Methodology. **Razia Noreen:** Validation, Resources, Methodology. **Muhammad Ibrahim:** Validation, Resources, Funding acquisition. **Shaheera Batool:** Resources. **Muhammad Abdul Qayyum:** Resources. **Tahir Farooq:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Bushra Parveen:** Resources, Methodology, Formal analysis. **Tanzeela Khalid:** Visualization, Investigation, Formal analysis, Data curation. **Perveen Kanwal:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors express their appreciation to the deanship of scientific research at King Khalid University, Saudi Arabia for funding this work through the research group program under grant number RGP. 2/428/44.

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