Original Article

Efficacy of Aloe Vera-Loading Chitosan Nanoparticles and Submucosa on Nerve Regeneration

Hussein H. Nahi[1], Omar A. Bader[2], Farah N. Abbas[3], Moshtak A. Wtwt[4]

[1]Department of Surgery and Obstetrics, College of Veterinary Medicine, Al-Qasim Green University, Babylon, [2]Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Mosul, Mosul, [3]Department of Medical Physiology, College of Medicine, University of Babylon, Hilla, [4]Department of Medical Medicine, College of Medicine, University of Babylon, Hilla, Iraq

Abstract

Background: Many biological and synthetic materials have been tested to bridge a peripheral loss of substance, such as arteries, veins, mesothelial chambers, predegenerated or fresh skeletal muscle, and Schwann cells. **Objectives:** The objectives of this study were to evaluate nerve regeneration in an experimental model of injured sciatic nerve using various assessments. ADditionally, the study aimed to investigate the effectiveness of selenium nanoparticles (NPs) in rabbit nerve regeneration. **Materials and Methods:** The current study was conducted on 20 healthy adult rabbits. They were randomly divided into four equal groups. The rabbits were anesthetized with Ketamine and Xylazine and administered intramuscularly. Experimental induction of injury was performed on the sciatic nerve of the left side in all animals using a blade. In the treated group, the injured nerve was treated with efficacy aloe vera-loading chitosan NPs and submucosa. All rabbits were followed for 56 days, with the daily assessment of motor and sensory sciatic nerve clinical reflexes. **Results:** Results showed remarkable improvement in both gait and knuckling, reaching normal levels on day 20 and 28 postoperation (PO), respectively. Moreover, the treated group exhibited significant sensory reflexes ($P \leq 0.05$) compared to the control group on day 56 PO. The conduction velocity at 8 weeks PO did not show significant differences between the treated group and the control at $P < 0.05$. However, significant differences were observed within the same group between the left sciatic nerve (OP) and the right sciatic nerve. Histopathological examinations indicated relative convergence among all groups, including the proliferation of Schwann cells and the orientation of regenerative nerve fibers. **Conclusions:** The aloe vera-loaded chitosan NPs and submucosa played an important role in improving nerve regeneration.

Keywords: Aloe vera, chitosan nanoparticles, nerve injury, nerve regeneration, submucosa

Introduction

According to studies, traditional Chinese medicine may influence the peripheral nerve regeneration of diabetic rats by promoting the growth of Schwann cells and increasing the production of neurotrophic factors.[1] Impaired nerve fiber regeneration for various reasons is a real challenge that must be thought to solve.[2] Nanotechnology has revolutionized various fields, including public health and synthetic materials. Nanoparticles (NPs), the primary product of nanotechnology, are materials that have at least one unit within the nanometer range (1–500 nm).[3] Biological pathways have gained attention for producing NPs due to their environmental safety, minimal production of toxic byproducts, the requirement for gentle reaction conditions, and the use of natural and limited agents.[4] Consequently, NPs produced through the biological approach are relatively stable, safer, and exhibit a wider range of sizes and shapes.[5] One widely utilized biological method for creating NPs is the use of pure plant extracts, which reduces the reliance on harmful chemicals with toxic effects. Plant-assisted synthesis of NPs results in the formation of NPs with specific shapes and sizes. Researchers have also explored the use of aqueous mediums containing emulsifier solutions for

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1472_23

Address for correspondence: Dr. Farah N. Abbas, Department of Medical Physiology, College of Medicine, University of Babylon, Hilla, Iraq.
E-mail: frhnabil@yahoo.com

Submission: 26-Sep-2023 **Accepted:** 11-Dec-2023 **Published:** 28-Jun-2025

How to cite this article: Nahi HH, Bader OA, Abbas FN, Wtwt MA. Efficacy of aloe vera-loading chitosan nanoparticles and submucosa on nerve regeneration. Med J Babylon 2025;22:491-9.

the potential suspension and stabilization of materials in nanoscale sizes.[6] Aloe vera-loaded chitosan has been marked to improve the obstetrics of reactive oxygen species, mainly hydrogen peroxide.[7] Aloe vera-loaded chitosan contains different essential uses, including its stimulating, antifungal, photochemical, antibacterial, and UV-filtering properties, making it an interdisciplinary exploration subject. The present examination concentrates on the phyto-synthesis of aloe vera-loaded chitosan utilizing various kinds of Silybum marianum extracts, and several biological experiments were completed, including antimicrobial, antioxidant, and antiproliferative tests.[8] These molecules may include flavones, amides, aldehydes, polysaccharides, etc., and the synthesized green NPs demonstrate superior biomedical efficacy compared to chemically synthesized ones.

Materials and Methods

The current study utilized twenty healthy adult male rabbits weighing 1.5–1.25 kg. The rabbits were individually housed in separate cages and provided with unrestricted access to food and water. A 10-day acclimatization period was observed prior to the procedure. Ceftriaxone (20 mg/kg) (Sanofi/France) was administered intramuscularly once a day for 5 days. On the first day and day 14 of the acclimatization period, an antihelminthic injection of 0.2 mg/kg ivermectin (Ivomec, Pantex/Holland) and 0.4 mL/kg subcutaneously was administered.

The rabbits were randomly divided into two equal groups (n = 10). In all animals, the left side sciatic nerve was induced with an injury using a blade. Each group was further divided into two equal subgroups (n = 5). In the first group, referred to as the control group, only the injured sciatic nerves were observed. In the second group, the injured sciatic nerve was treated by applying aloe vera-loaded chitosan NPs with bovine UB submucosa around the injured site. The animals were euthanized, and the injured nerves were examined in each subgroup at 8 and 16 weeks postoperation (PO), respectively. All the techniques utilized in this study were approved by the scientific committee of the College of Veterinary Medicine at Al-Qasim Green University.

Anesthetic protocol

Prior to anesthesia, the rabbits underwent a 12-h fasting period. Anesthesia was induced by intramuscular injection of Ketamine hydrochloride (Kepro®, Holland) and Xylazine hydrochloride (Xyla®, Holland).[9,10]

Surgical protocol

The hair on the lateral and caudal side of the left hind limb, extending up to the level of the wing of the ilium dorsally, the level of the sacrum caudally, and the ventral part of the stifle joint, was trimmed off. To disinfect the skin, Hepatin (Hexatane 20®, Jordan), 70% isopropyl alcohol from Jaya Pelita Pharma SDN BHD, and tincture iodine (Lebanon) were used.

To keep the limb out of the surgical area and from the stifle joint to the distal extremity, a latex glove was placed over the distal extremity of the limb and taped securely. The limb was covered with a sterile skin towel, and the glove (Sempermed®, Austria) was fastened to the limb using towel clips. The animal was positioned in the right lateral recumbency. A fenestrated drape with an opening at the planned surgical site was placed over the left hind limb [Figure 1].

To separate the limb from the surgical area and the stifle joint, a latex glove was wrapped over the distal extremity and secured to the limb with adhesive tape. The glove (Sempermed®, Austria) was covered with a sterile skin towel and fastened to the limb using towel clips. The animal was positioned in the right lateral recumbency. The fenestrated drape with an opening at the targeted surgical site was draped over the left hind limb [Figure 1].

The stifles were used as reference points, and the greater trochanter of the femur was palpated. Using a scalpel blade #21, a 2-cm incision was made in the posterior-lateral thigh skin, caudo-lateral to the greater trochanter, at the level of the distal third of the femur. With a scalpel blade #15, the fascia lata and subcutaneous tissue were dissected along the same line. The biceps femoris muscle was split cranially, and the semitendinosus muscle was divided posteriorly through blunt dissection using Mayo scissors. This allowed for exposure of the sciatic nerve, which was carefully identified and separated from the surrounding tissues. The nerve was gently depressed with a wooden tongue depressor and then cut using a blade before applying aloe vera-loaded chitosan NPs with bovine UB submucosa [Figure 2].

Postoperative analgesia was administered to all animals. Tramadol hydrochloride (Trabar® Switzerland, 100 mg) was injected intramuscularly at a dose of 0.2 mL/kg every 12 h for 3 consecutive days.

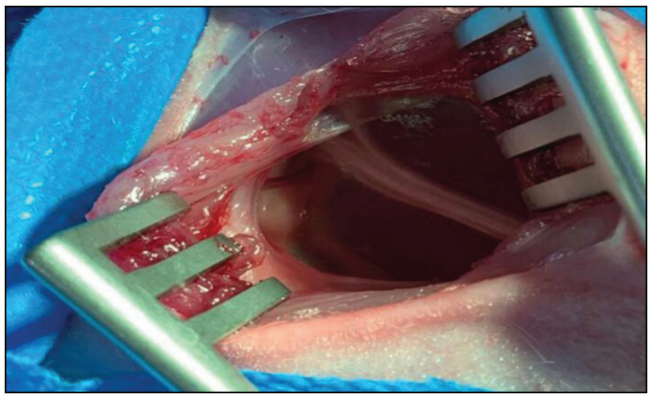

Figure 1: Photograph showing the injury site in the sciatic nerve

Loading of aloe vera on chitosan NPs

The process for preparing the aloe vera-loaded chitosan NPs was achieved according to refs.[11,12] Aloe vera is successfully loaded onto chitosan NPs, and the solution is then kept at a temperature of 4°C.

Transmission electron microscope (TEM)

TEM analysis was conducted to investigate the morphology, size properties, and shape of the NPs. This analysis also examined the extent of isolation or aggregation of the particles and their overall appearance after adsorption during the nanoloading process. To perform TEM analysis, a drop of the nanoscale solution was placed on carbon-coated copper grids and allowed to dry over a period of time. The dried grids were then examined using a TEM microscope operating at 400 kV. The particle altitude, allocation, and form were dissected and recorded. This analysis was executed at the Department of Nanotechnology of Medicine, Faculty of Advanced Technology of Medicine, University of Tehran, Iran.[13]

Particle size analysis

The particle sizes of various materials utilized in the examination, which include unloaded aloe vera extract (AE) and aloe vera-loaded chitosan NPs (CNP-AE), were estimated utilizing laser-based particle size analysis. The measurement was interested in the service of laser rays that penetrated the liquid samples, including the particles, and the particle sizes were specified as an average within a limited duration of 90 s. The data was documented in an electronic program that was particularly developed for this objective, which included a curve graph and a table displaying the average particle sizes. The particle size examination was executed at the Nanotechnology and Advanced Materials Research Center, University of Technology.[14]

Figures 3 and 4 show the outcomes of the laser-based particle size examination for AE and CNP-AE. For AE, the intermediate particle size in the aqueous explanation was chosen to be 97.1 nm, with a range of 86.5–109.1 nm. The polymerization ratio used was 1:10, and the polydispersity index was measured as 0.285. The particle size of CNP-AE was found to be 118.2 nm. The drug loading percentage was determined as 8.84%, and the encapsulation efficiency was 93.3%. The scanning electron microscopy (SEM) analysis revealed a range of 28.35–35.45 nm for the particle size.[14]

Preparation of urinary bladder matrix (UBM)

The procedure for generating a UBM as a decellularized scaffold is as follows:

a. Fresh urine bladders were obtained from slaughtered cows. The bladder's outer tissues, as well as collagenous connective and adipose tissues, were extracted utilizing scissors. All bladder layers except the submucosal layer were removed, taking into account the intraluminal water pressure that helps stretch and enlarge the bladder. The bladder was then divided into a rectangular sheet from the aperture to the apical region on one side. The serosal side of the bladder was mechanically delaminated to remove the mucosal layer, and the luminar side was delaminated to remove the tunica serosa and tunica muscularis layers. This resulted in a flattened rectangular bladder sheet with the residual submucosal layer remaining. The submucosal layer was immersed in a phosphate-buffered saline (PBS) solution containing antibiotics (such as amphotericin, streptomycin, and penicillin) to create the UBM.[15]
b. To reduce the risk of host rejection, noncollagenous components were removed from the UBM. The UBM was soaked in a solution of 0.1% peracetic acid (PAA)

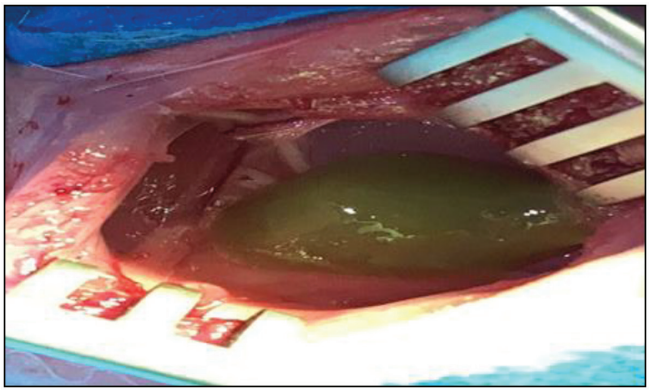

Figure 2: Applied aloe vera on chitosan nanoparticles and bovine UB submucosa on injured nerve

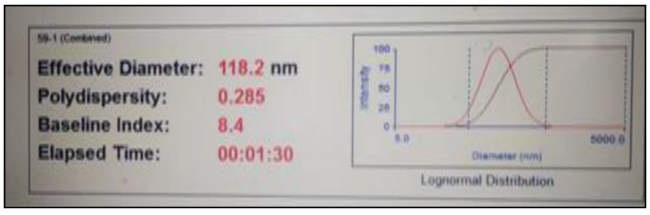

Figure 3: Refer to particle size with 118.2 nm as effective size

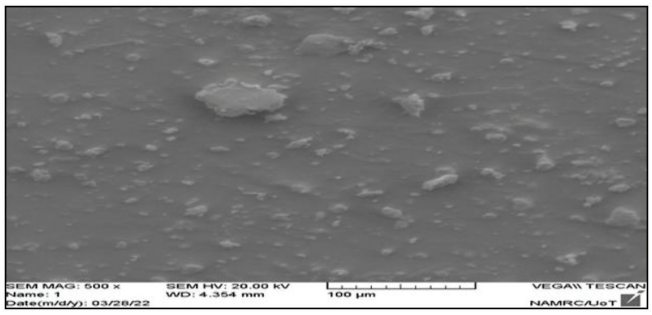

Figure 4: Refer to SEM show oval to elongated particle with different sizes

and 4% ethanol for 2 h at room temperature. Care was taken to preserve the mechanical properties and natural collagen structure of the UBM during this step. The pH level was altered by the addition of PAA, so it was necessary to thoroughly rinse the ECM with PBS to eliminate all PAA residues and restore the pH to around 7.4. This rinsing process involved shaking and rinsing the ECM with PBS and water. Each rinse lasted for 15 min at room temperature.[15]

c. The decellularized and cleaned UBM scaffold was then stored in a sterile PBS solution containing antibiotics and antifungal medications at 4°C to maintain sterility.[16]

Clinical signs evaluation

From the initial day of the investigation to day 56 PO, when the study came to a conclusion, the motor and sensory clinical reflexes of the sciatic nerve were regularly assessed.

Motor functions evaluation

From the initial day until the study's conclusion on day 56 PO, all animals were examined daily in accordance with the grading criteria. The animals' starting points and walking abilities were recorded. The different gait patterns were classified as normal, crouching, or heel-crawling. Grades for knuckling ranged from normal to mild to severe. Weak, moderate, and strong were used to classify the strength of muscle contractions. Muscle atrophy was categorized as normal, mild, moderate, or severe. According to refs.,[17,18] the modified clinical symptom grading and scoring method for motor abnormalities.

Sensory functions evaluation

Beginning at the end of the third week and continuing every week until the study's conclusion on day 42 PO, sensory functions of the nerves were monitored. The toe spreading reflex, lateral aspect leg sensation, toe pinch, and toe prick were graded as present (+) or absent (-) to assess sensory functions and clinical symptoms. Tests such as latitudinally felt leg sensation, toe pinch, and toe prick were used to evaluate foot withdrawal and vocalization. Positive results indicating recovery were recorded. The scoring evaluation of sensory clinical symptoms was performed based on refs.[17,18] as updated.

Electrophysiological analysis (EMG and CV)

Approximately 8 weeks after a nerve injury, electrophysiological tests will be conducted. The sciatic nerve will be surgically freed and separated from the body, creating a gap of around 3 cm. It will then be placed in an AD instrument chamber equipped with negative and positive clamp electrodes for recording and stimulation purposes. The conduction velocity will be calculated by multiplying the distance (in mm) between the recording electrodes by the time (in ms) between proximal and distal stimulations. Two pulses per second will be administered with a square wave duration of 0.2 ms. The amplitude of the motor response, measured in microvolts (V), will be determined by comparing the electric baseline with the peak of the negative phase. The latency time, measured in milliseconds (ms), will be calculated from the shock artifact to the initial negative peak deflection of the response observed on the video display apparatus.

Electrophysiological analysis (EMG and CV)

The skin covering the gastrocnemius muscle was dissected by making a longitudinal incision on the posterior aspect of the operated limb. The gastrocnemius muscles were then incised and immediately weighed. The corresponding muscle on the opposite side (contralateral muscle) was also harvested to serve as a control for any weight variations among individual rabbits.

The percentage of muscle mass loss was determined by weighing each muscle separately using a precision weight scale accurate to 0.0001 g (denervated muscle weight vs. contralateral muscle weight). To calculate the relative gastrocnemius muscle weight (RGMW), the muscle weight was expressed as a ratio of the weight of the operated (left) limb to the weight of the unoperated (right) limb. The RGMW serves as one of the parameters for assessing the recovery of motor function and is defined as the ratio of the muscle weight on the experimental side to the muscle weight on the negative control side.

Histopathological examinations

At 8 and 16 weeks after the procedure, nerve samples were obtained for analysis. After the standard processing involving alcohol and paraffin embedding, the biopsies were fixed in 10% buffered formalin. They were then stained with hematoxylin and eosin, which is a routine staining method. Finally, the stained samples were examined under a light microscope for further analysis and observation.

Statistical analysis

The data analysis, including the calculation of standard deviations (SD), was conducted for each set of data. The Statistical Package for the Social Sciences (SPSS) 23.0 software was utilized for the analysis, specifically employing non-parametric tests such as the Kruskal–Wallis and Mann–Whitney tests to compare statistics among different groups. A significance level of P-value = 0.05 or higher was considered to indicate statistical significance.

Ethical consideration

The study protocol was approved by the committee on publication ethics at College of Veterinary Medicine,

Table 1: Motor clinical observations for all groups on day 56 PO

Clinical signs	Control group	Treated group 4ws	Treated group 8ws
Onset	1.3 ± 0.2 C	2.6 ± 0.3 b	4.4 ± 0.3 A
Walk			
Crouch	2 ± 0.0 A	0.7 ± 0. 4 b	0.2 ± 0.2 BC
Crawl	1 ± 0.0 A	0.6 ± 0.5 b	0.2 ± 0.4 BC
Normal	2 ± 0.1 A	3 ± 0.1 a	3 ± 0.3 A
Knuckling			
Severe	1 ±0.3 A	0.7 ± 0. 4 b	0.2 ± 0.3 C
Moderate	2 ± 0.2 A	1.2 ± 0.6 b	0.4 ± 0.3 BC
Mild	3 ± 0.0 A	1.3 ± 0.6 b	0.5 ± 0.6 BC
Normal	0.0 ± 0.0 C	1.6 ± 0.97 b	4 ± 0.0 A
Muscle contraction force (MCF)			
Strong	3 ± 0.0 C	3 ± 0.0 b	3 ± 0.0 A

University of Mosul, under the reference No. 022 on July 20, 2023.

Results

Clinical assessment of motor and sensory nerve functions

From day 1 to day 4 PO, all animals in both groups exhibited noticeable dysfunction characterized by flaccid paralysis of hind limb movement. The animals showed resistance to flexing their limbs below the hock joint and remained in a crouched position on the cage floor.

Control group

The observation that all animals exhibited normal walking ability on day 28 PO was a significant finding. ADditionally, knuckling disappeared on day 27 PO, and muscle contractions became powerful on day 47 PO [Table 1]. Furthermore, on days 43 PO and 50 PO, the presence of toe spread sensation, lateral aspect leg response, toe pinch, and toe prick were noted [Table 2].

Treated group (4ws)

The intriguing discovery was made when the animals regained their normal walking abilities on day 38 PO. However, mild knuckling continued throughout the study period. On day 24 PO [Table 1], there was a significant increase in the force of muscular contractions. Over the course of the study, there was a gradual progression of skin sensation from the ankle to the fetlock joint. Absence of toe pinch, toe prick, lateral leg feeling, and toe spreading reflex sensation were observed by the end of the research [Table 2].

Treated group (8ws)

On days 20 and 29 PO, the gait and knuckling, respectively, returned to normal. Strong muscle force contraction and mild muscle mass atrophy can be seen in Table 1. The sensation in the surgically repaired left hind limb returned, and the toe spread, lateral aspect leg sensation, toe pinch, and toe prick all appeared on days 35, 39, 46, and 48 after the procedure [Table 2]. Values with the same superscript (MSD, $n = 5$) in the same row, denoted by letters a, b, and c, are not significant at the 0.05 level.

Table 2: Sensory clinical observations for all groups on day 112 PO

Sensory signs	Control	Treated 4ws	Treated 8ws
Toe spread	0 ± 0 B	1 ± 0 a	1 ± 0 A
Lateral leg sensation	0 ± 0 B	1 ± 0 a	1 ± 0 A
Toe pinch	0 ± 0 B	1 ± 0 a	1 ± 0 A
Toe prick	0 ± 0 b	1 ± 0 a	1 ± 0 A

[a,b] Values with comparable superscripts in the same row (MSD, $n = 5$) are not statistically significant at the $P < 0.05$ level

Sensory clinical observations

Clinical sensory symptoms such as toe spread, lateral leg tingling, toe pinching, and toe pricking were absent, indicating a lack of sensory responses. On day 56 PO [Table 3], animals in the experimental group exhibited significantly reduced sensory reflexes ($P < 0.05$) compared to the control group.

RGMW measurement (RGMWM)

The control group showed the highest RGMWM ratio, indicating less muscle atrophy, with values of 43% and 52% at 4 weeks (4ws) and 8 weeks (8ws), respectively. In contrast, the treated groups had higher ratios of 58% and 69%, respectively [Table 3]. A comparison of relative gastrocnemius muscle mean weights revealed that muscle atrophy began after sciatic nerve injury. However, on day 56 PO, there was a significant decrease ($P \leq 0.05$) in RGMW values in the control group (45%) compared to the control at 4ws (30%) and the 8ws group (25%) [Table 3].

Table 3: Mean values of the control and treated groups for the relative gastrocnemius muscle weight measurements

Time	Control % 4ws	Control % 8ws	Treated 4ws%	Treated 8ws%
56 days	43.2 ± 3.9 D	52.1 ± 0.9 c	58.4 ± 0.3 b	69.3 ± 0.7 A

a, b, c Values with the same superscript (MSD, $n = 5$) in the same row are not statistically significant at the $P < 0.05$ level

Table 4. Statistical analysis of the conduction velocity for the control and treated groups at 8 weeks PO

Groups		Conductive velocity M/S
Treated	LSN (OP)	67.14 ± 3.64Aa
	RSN	77.26 ± 4.6Ba
Control	LSN	52.42 ± 2. 7Aa
	RSN	80.86 ± 7.43Ba

LSN: left sciatic nerve, RSN: right sciatic nerve

The similar superscript letters denote nonsignificant differences at $P < 0.05$

Electrophysiological analysis

The electrophysiological studies were conducted by performing a nerve isolation procedure. Electromyography was performed 8 weeks PO. The conduction velocity at 8 weeks PO did not show significant differences between the treated groups (67.14 ± 3.64 Aa) and the control group (52.42 ± 2.7 Aa) at $P < 0.05$. However, significant differences were observed within the same group between the left sciatic nerve (OP) and the right sciatic nerve [Table 4]. The results of the study revealed that the latency values in the left limb were 3 ms in the treated group, while in the right limb, they were 4 ms. In the control group, the latency values were 3.3 ms in the left limb and 5.2 ms in the right limb at 8 weeks PO.

Regarding the amplitude, the treated group exhibited values of 3.5 mV in the left limb and 6.9 mV in the right limb, whereas the control group showed values of 2.83 mV in the left limb and 5.2 mV in the right limb.

Discussion

The results of this study showed that animals in the medicated group regained movement and walked more quickly compared to the control group. The treatment groups (20 days) and (28 days) restored normal gait sooner than the control group (39 days), although all groups eventually achieved normal gait. In the treatment groups (28 days) and (29 days), knuckling significantly disappeared ($P < 0.05$), while it persisted in the control group until the end of the trial. All groups exhibited strong muscle contractions, but the treatment groups initiated contractions earlier than the control group (at 23 and 24 days, respectively, compared to 25 days in the control group). The study evaluated motor clinical indicators based on the degree of pain, categorized as neuropathic and inflammatory pain, and assessed the animals' ability to walk on their operated left hind foot. The findings revealed that the treatment group's animals regained normal gait at a faster rate than the control group.

The study's results also indicated that pain may have an impact on therapeutic benefits and play a role in both neuropathic pain and the regeneration of the injured sciatic nerve. Inflammatory pain can be influenced by pain itself, which interacts with immune system cells directly or through soluble substances that slow down various immune response processes, thereby delaying the progression of inflammatory pain. It is clinically observed that supplementation to impaired degeneration leads to the electrophysiological recovery of sensory conduction and evoked potentials.[19]

The results of the current research revealed that the treated group showed a rapid improvement in knuckling severity, which disappeared thereafter, while the control group continued to experience knuckling until the end of the trial on day 56 PO. This notable improvement in walking observed in all treated animals can be attributed to the role of selenium NPs in enhancing blood supply, promoting functional recovery, and facilitating innervation of the injured sciatic nerve. In contrast to the control group, the functional state of the injured sciatic nerve in the treated group recovered at a slower pace.

Aloe vera-loading chitosan NPs were found to stimulate the differentiation and proliferation of Schwann cells, primarily through the elevation of nerve growth factors, neurotrophic factors, cytokines, and other substances. These factors play a crucial role in promoting Schwann cell migration, axonal projection, and facilitating neuronal survival in response to injury.[20]

Furthermore, it is important to note that the muscle contraction force of the animals in the treated group improved more rapidly compared to the control group. Muscle contraction force and muscle mass atrophy are indicative of the progression of the motor function of the sciatic nerve, and they are closely related to muscular denervation and muscle disuse. Early reduction in muscle mass and force contraction is associated with sciatic nerve neurometesis.[21]

One of the significant findings of the present study was the presence of sensory clinical indications in the treated groups, indicating the development of sensation. The absence of sensation, particularly in severe cases of knuckling where the animal walks on the dorsum of the foot, can contribute to the development of atrophic ulcers.

Licking the foot repeatedly without feeling anything can exacerbate the problem. The toe-spreading reflex was a key clinical indicator that aided in assessing the recovery of sensory function. Initially absent in all groups, it developed and appeared in the treated group on day 56 PO. Foot drop, which is evident immediately after nerve transection, often disappears or diminishes before muscle reinnervation occurs. Solely observing the gait is insufficient to determine the exact initiation of reflex due to the distinct symptoms of sciatic nerve injury. Focusing on the muscle group innervated by the sciatic (peroneal) nerve provides a more accurate estimation of the start and progression of healing.[22]

In the current research, the use of TPGS (d-alpha-tocopheryl polyethylene glycol 1000 succinate) with extracts of Salvia officinalis and silimarin led to a reduction in particle size. The aloe vera-loading chitosan NPs acetate organic phase was more effective in reducing particle size compared to Tween 80. TPGS is known to act as a P-gp inhibitor, solubilizer/absorption, and permeation enhancer in drug delivery formulations such as nanocrystals and nanosuspensions, which may explain its effectiveness in reducing particle size.[23]

Furthermore, bovine urinary bladder submucosa has been found to trigger various enzymes and significantly influence immune status regulation in the presence of aloe vera-loading chitosan NPs. The biosynthesized aloe vera-loading chitosan NPs have demonstrated efficacy against many bacteria.[24] The preparation of aloe vera-loading chitosan NPs involved adding the NP precursors to an aqueous solution of the herbal extract or using hydroalcoholic solutions when employing the NP precursor and seed extract in powder form. The obtained powders were washed with methanol and dried at suitable temperatures to achieve the desired level of purity.[25]

Regarding the RGMWM, the control group showed the highest RGMWM ratio, indicating less muscle atrophy, with values of 43% and 52% at 4 weeks (4ws) and 8 weeks (8 ws), respectively. In contrast, the treated groups had higher ratios of 58% and 69%, respectively [Table 4]. Comparison of relative gastrocnemius muscle mean weights revealed that muscle atrophy began after sciatic nerve injury. However, on day 56 PO, there was a significant decrease ($P \leq 0.05$) in RGMW values in the control group (45%) compared to the control at 4ws (30%) and the 8ws group (25%) [Table 3].

Several authors confirmed the regain of muscle weight when reinnervation of the motor target organ and cutaneous afferent was re-established.[26,27] The muscle gradually regains mass depending on the degree of reinnervation,[28] which is related to the maximum force of contraction.[29]

The gastrocnemius muscle serves as the primary peripheral target organ of the sciatic nerve. The regenerative capacity of the sciatic nerve was determined by monitoring the relative weight of this muscle over time following nerve transection and therapy. The muscle weight of the target organ decreased due to atrophy that was initiated immediately after the nerve injury.[30] Comparing the relative muscle weight ratios among the different groups revealed that the treated groups exhibited better ratios, followed by the treated 8ws group and the control group with the lowest ratios.

Regarding the electrophysiological analysis, the conduction velocity at 8 weeks PO did not show significant differences between the treated groups (67.14 ± 3.64 Aa) and the control group (52.42 ± 2.7 Aa) at $P < 0.05$. However, significant differences were observed within the same group between the left sciatic nerve (OP) and the right sciatic nerve [Table 4]. The distribution, stability of the solution, loading efficiency, range of drug release, and cellular uptake are generally influenced by particle size.[31] One of the key factors affecting *in vivo* applications is the method of cellular uptake and internalization, particularly endocytosis, which facilitates the entry of particles and small molecules into cells. This process relies on the double layer of phospholipids in the cell membrane to actively transport materials through engulfment.

There are two methods of cellular uptake based on particle size: phagocytosis, which involves the ingestion of large particles with a diameter of 0.2–1 μm, and pinocytosis, which involves the cellular uptake of liquids and dissolved particles with smaller diameters. The average particle size of aloe vera-loaded chitosan was determined to be 118 nm based on the study's laser particle size test. It has been established that particles capable of entering or exiting fenestrated capillaries in tumor microenvironments and liver endothelium and other tissues are typically around 150 nm in size.[32]

Researchers highlighted the significant influence of particle size on pharmacokinetics, clearance, and distribution in therapeutic delivery systems, as particle size affects absorption, renal accumulation, and renal excretion.[33] Very small diameters (less than 10 nm) may not be significant in therapeutic delivery systems as they are filtered through renal glomerular capillary walls and are not reabsorbed. NPs with sizes ranging from 25 to 150 nm have the ability to persist for a longer duration and avoid being filtered through certain types of capillaries, thus ensuring the safe availability and delivery of drugs. However, nanoscale particles larger than 150 nm may be subject to phagocytosis by immune cells, leading to accumulation in the liver and spleen.[34] Therefore, in our research on chitosan-based nanotherapeutic delivery systems, the three materials exhibited suitable particle sizes for pharmacokinetics and passive targeting.

The degree of nerve tension at the repair site was a crucial factor influencing peripheral nerve regeneration.

Single epineural sutures were carefully applied along the circumference of the nerve to ensure proper alignment. Failure to do so could result in postoperative edema and swelling of the nerve end. It is important to note that the coaptation of nerve ends should not be fixed under tension. After sciatic nerve transection and repair, significant changes occur in endoneural pressure, primarily due to impaired neuronal blood and axoplasmic flow.[35] Increased tension at the site of sciatic nerve coaptation is typically associated with nerve elongation and reduced blood flow. The nerve blood flow and conduction velocity may decrease with increased nerve tension. The peripheral nerve's elongation limit is typically around 8%–10%, beyond which blood flow decreases by 50%. Elongation of 15% leads to stagnation of endoneurial lymphatic flow, resulting in increased endoneurial fluid pressure, decreased oxygenation, and cessation of axonal transport, ultimately leading to axonal degeneration.[33]

A study conducted by Millesi[36] demonstrated that unrestricted motion after nerve repair significantly hinders functional recovery. This is attributed to increased collagenous connective tissue formation within the endoneurium and decreased neural angiogenesis at the site of nerve suture, which inhibits the advancement of nerve neurite sprout regeneration. In the present study, the animals were placed in cages without immobilizing the operated limb, contrary to the recommendation by Chiu and Ishii,[37] who suggested immobilization for a duration of 8 days to 6 weeks following sciatic nerve reconstruction. The efficacy and the biological applications diffrent biosynthesized Chitosan Nanoparticles have been reported recently by several authors in Iraq.[38-40]

Conclusion

Based on clinical and electrophysiological findings, the study concludes that the aloe vera-loading chitosan NPs treatment facilitated faster nerve regeneration, improved motor and sensory function, and reduced muscle atrophy compared to the control group.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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