Research Article

Efficacy of Chitosan Nanoparticle Transplantation on Regeneration of Acute Spinal Cord Injury in Dogs Model

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Abstract | The most common cause of persistent neurological diseases is spinal cord damage. Unfortunately, the vast majority of spinal cord injury treatments are ineffective. The current study is aimed to apply chitosan nanoparticles on spinal cord regeneration in dogs. For this objective, sixteen healthy mongrel dogs were used. They were divided into two equal groups (n=8) at random. All dogs underwent dorsal laminectomy and left lateral hemisection at the level of the second lumbar vertebra. In the control group, the hemisections were treated with 0.2 mL of phosphate buffer saline. Chitosan nanoparticles were applied to the chitosan group with hemisection stie. From the first week to the completion of the experiment 16 weeks following surgery, the open field locomotor scale, which comprised gait, proprioceptive posture, and nociception pain, was utilized to assess motor and sensory gains. After 16 weeks of postoperative testing, the motor and sensory reflexes in the treated group were substantially different ($p<0.05$) from the control group. The neurological recovery scale (normal gate to leap) in chitosan group was at 15 weeks post treatment while in control group, the animals didn't retain to that state until end of experiment. Chitosan nanoparticles group histopathological examinations revealed reduced cavitation, orientation of regenerative nerve fibers in white matter, increased number of regenerative neuron cells in grey matter, increased angiogenesis, and minimal scar tissue formation at the injured spinal cord site. In conclusion and based on clinical and histological data, chitosan nanoparticles could accelerate and promote regeneration of the injured spinal cord.

Keywords | Chitosan nanoparticles, Regeneration, Spinal cord injury, Histopathology, Nanotechnology, surgery, Dog

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INTRODUCTION

Spinal cord injuries (SCI) were classified into two categories: traumatic and non-traumatic. The contusion, compression, and stretching of the spinal cord cause traumatic spinal cord injury. The main causes of non-traumatic spinal cord injuries are congenital and inflammatory spinal cord diseases, vertebral spondylosis, tumour compression, and vascular ischemia. (Ren et al., 2023). Spinal cord injury was currently difficult to manage, and there was no definitive treatment for it, numerous studies, including experimental modeling, were being conducted to aid in the comprehension of the anatomical and biological consequences of injury and repair, as well as the evaluation of the efficacy and risk-to-benefit ratio of a proposed therapy (Weber-Levine et al., 2022). Comparing animal models to

human counterparts, there were further benefits; for example, the necessary tissue could be used and processed for histological examinations to look at the co-localization of relevant proteins, mRNA analysis to measure the expression of proteins, and protein analysis to measure protein levels. (DeRosa et al., 2019 ; Thanoon et al., 2019).

During the last decades, nanotechnology had become a widespread scientific approach in many different biological and medical fields, due to its unique benefits of improved performances and its potential toward the clinical translation, it was defined by the US National Nanotechnology Initiative as "it is concerned with materials and systems whose structures and components exhibit novel and significantly improved physical, chemical and biological properties, phenomena and processes due to their nanoscale size" (Mauri and Masi, 2020). Using nanoparticles as carriers of therapeutic agents is one of the exciting and cutting-edge approaches for treating SCI. (Zarepour et al., 2022). Nanomaterials had opened up new treatment avenues for SCI, including performance-based nanomaterials derived from a variety of materials that enhance the microenvironment of traumatic injury and, in some cases, promote neuron regeneration. (Ali and Khudair, 2019). To date, Due to their nanoscale size, a wide variety of nanoparticles (NPs) have been used in the medical profession. Based on their chemical makeup, NPs are divided into three categories: carbon-based, inorganic, and organic. To treat neurological illnesses, several materials have been created, such as liposomes, micelles, polymeric nanoparticles, carbon nanotubes, quantum dots, metallic nanoparticles, and chitosan. (Salih et al., 2015; Haleem et al., 2023).

This research is intensive on fabrication of chitosan nanoparticles and assessment their efficiency on repair of the acute injured spinal cord in dogs.

MATERIALS AND METHODS

Sixteen healthy mongrel male dogs weighing 15-20 kg and aged 8-12 months were employed in this present study. The dogs were kept in individual cages and supplied commercial food and water. The animals were housed in their separate cages for 15 days to acclimate. After that the animals were given Ceftriaxone (22 mg/kg) broad-spectrum antibiotic injection intramuscularly twice a day for five days and anthelmintic injection of 0.2 mg/kg Ivermectin (Ivomec, Holland) subcutaneously was given. From February 10, 2020, to December 14, 2022, all procedures used in this study were approved by the scientific committee of the University of Baghdad's College of Veterinary Medicine. (420/P.G.9/7/2023). The experimental animals were randomly assigned into two equal groups (n=8) for dorsal laminectomy and left lateral hemisection cordectomy at the second lumbar vertebra.

The control group (n=8) the hemisection was treated with 0.2 ml phosphate buffer saline. The chitosan group (n=8) was treated with 0.2 ml (5%) chitosan nanoparticles transplanted at the site of spinal cord hemisection. All experimental animals were followed up clinically included motor and sensory reflex weekly beginning with the first week of the study and ending with the 16th week post operation (PO). After the eighth and sixteenth weeks, the animals in each group were euthanized for histological examination.

Fabrication of Chitosan Nanoparticles

Chitosan nanoparticles were modified created by dissolving 200 mg of medium molecular weight, 85% deacetylated chitosan (Sigma Chemical, St. Louis, USA) in 200 ml of deionized water and stirring the mixture at 1000 round per minute for 60 minutes at room temperature until the solution was clear (Figure 1.A). Following sonication, the solution was pH-titrated by adding Hydrochloric acid solution to reach pH 4 and stirred for an hour, then adjusted to pH 7 by adding Sodium hydroxide (Figure 1.B) and filtered through 0.2 mesh to form a semi gelly solution (Figure 1. C). (Husain et al., 2019; Amruth et al., 2022).

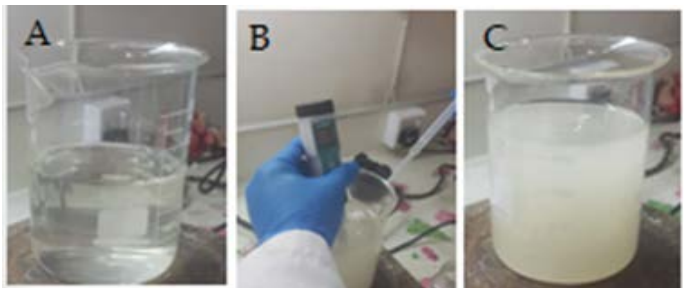

Figure 1: Photograph showing the steps of Fabrication of Chitosan nanoparticles A. Dissolving chitosan in deionized water and stirring. B. Adjusting to pH 7 by adding NaOH. C. filtering the formed semi-gel solution.

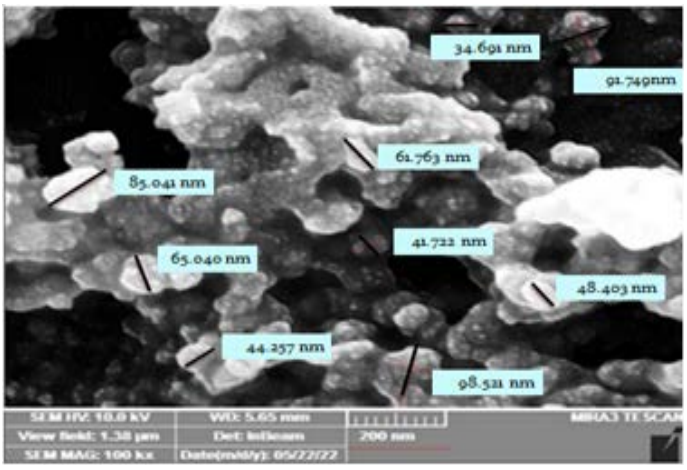

Figure 2: SEM image of synthesized nanoparticles shows spherical to elongated shape nanoparticles of about 62.065±7.632 nm.

Characterization of Nanoparticles

Scanning Electron Microscope Analysis

Scanning electron microscope (SEM) was utilized in (Center of Nanotechnology and Advance Materials, University of Technology for SEM analysis) to define the size of particles used in the experimental study. SEM (Inspect S50, FEI Company) was used to evaluate the sample. Images were captured at magnifications of 5,000 and 10,000 µm. The micrographs revealed approximately spherical nanoparticles measuring 62.065±7.632 nm in diameter (Figure 2).

Ft-Ir Spectroscopy

The Fourier-transform infrared spectroscopy (FT-IR) spectroscopy of generated chitosan nanoparticles revealed that the CH-NPs' -OH bond stretching vibrations were observed at 3344.57 cm-1, whereas the C-H bending vibrations were observed at 2121.70 cm-1. The presence of the absorption peaks at 1643.35 and 1631.78 cm-1 (N-acetylated residues, amide II band) was related to the incidence of the C=O stretching of the amide I band, which bent the vibrations of the N-H. The band was created by wagging CH3 at 1388.75 and 1253.73 cm-1. The peak at 1130.29 cm-1 was attributable to the antisymmetric stretching of the (C-OC) bridge, whereas the vibration at 1083.99 cm-1 was associated with C-O stretching (Figure 3).

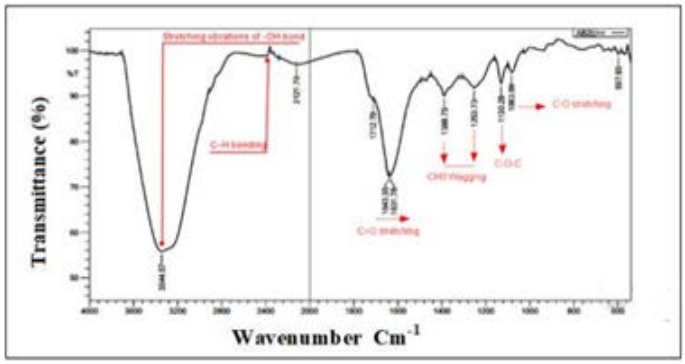

Figure 3: FT-IR spectrum of synthesized chitosan nanoparticles

Atomic Force Microscope

Atomic force microscopy (AFM) was used to examine the morphology of chitosan nanoparticles. The photos were captured with constant force. All images were assessed with Autoprobe SPM Controller software (Thermo Microscope) to prevent low-frequency noise at the scanning direction. A wide scan of the chitosan nanoparticle samples revealed several spherical-shaped structures of comparable size (Figure 4. A), suggesting that the chitosan nanoparticles form spherical structures. This is shown in a 2D AFM picture of chitosan nanoparticle morphology. The NPs tend to gather collected in the three-dimensional map of the chitosan nanoparticles (Figure 4. B) to build a stable macromolecular substance. The image showed some protruding monomeric structures (Figure 4.C).

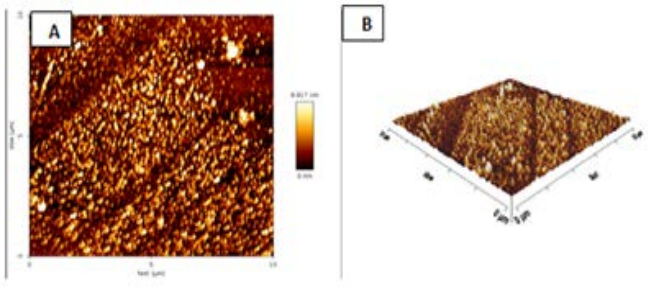

Figure 4A,B: Atomic force microscopy images of chitosan nanoparticles indicate A. 2D and B. 3D

Experimental Animals

Prior to anesthesia, the dogs were fasted for six hours and premedicated with 0.03 mg/kg atropine sulfate (Kepro®, Holland), then anaesthetized with a mixture of 5 mg/kg Xylazine hydrochloride (Xyla®, Holland) and 15mg/kg Ketamine hydrochloride (Kepro®, Holland) intramuscularly (Eesa, 2010; Ali, 2013; Lutvikadic and Maksimovic, 2022).

Surgical Protocol Of Hemicordectomy

Fossum, (2019) outlined the surgical dorsal laminectomy procedure on which the current investigation was based, as illustrated in (Figure 5 A and B).

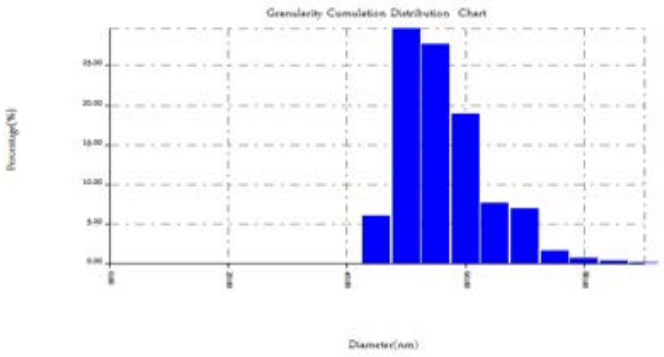

Figure 4C: Atomic force microscopy image of chitosan nanoparticles shows the average particle diameter was 65.91 nm calculated in nanoscale size.

A longitudinal incision through the meninges is made (Figure 6A), and left lateral hemisections are performed using a surgical blade No. 10 using magnifying lens (Figure 6B).

In the chitosan group is the same as control group procedure with a 200 µl solution of chitosan nanoparticles was administered locally at the site of hemisection. (Fig. 7. A). The dura mater was closed with 4/0 Vicryl a subcutaneous fat was placed over the laminectomy site. The fascia and epaxial muscles were closed with 3-0 Polydioxanone sim

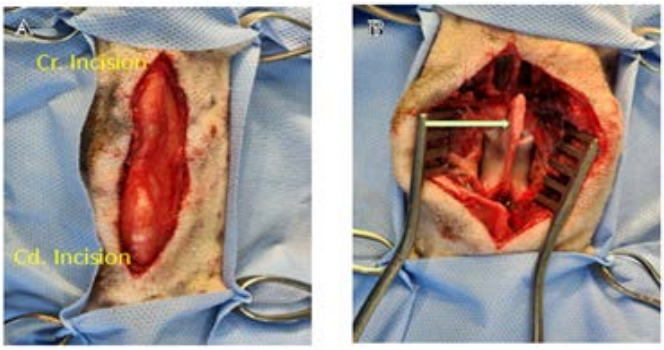

Figure 5: Photograph showing the initial steps of hemicordectomy. A. Shows the surgical incision over dorsal midline from L1 to L3. B. Elevated epaxial muscles from dorsal spinous processes, laminae, articular facets, and pedicles of L2 (arrow).

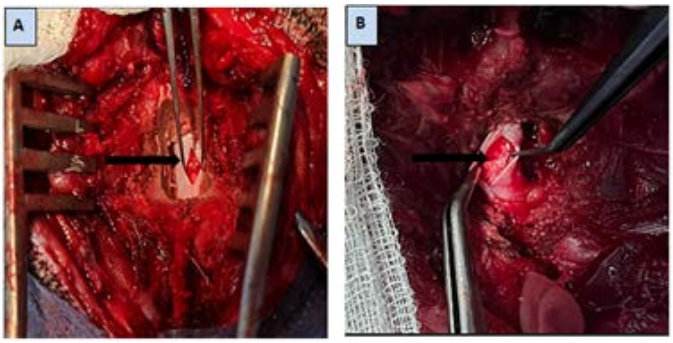

Figure 6: Photograph showing. A. A longitudinal incision is made through the meninges (arrow) B. Left lateral hemisection of the spinal cord (arrow).

ple continuous sutures. The skin was closed by 2-0 nylon in simple interrupted suturing (Figure 7 B).

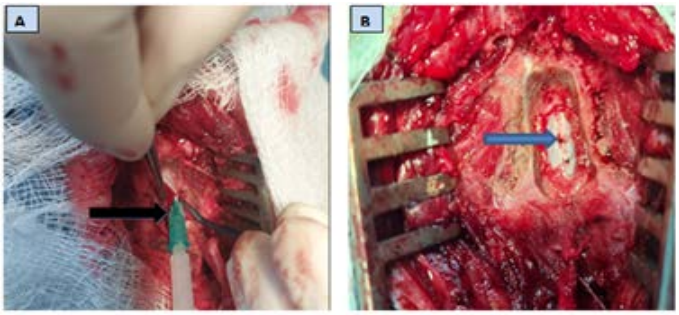

Figure 7: Photographs show. A. Implantation of chitosan nanoparticles at the injured spinal cord (arrow) B. Closed Dura matter (arrow).

Postoperativeanalgesic tramadol hydrochloride (Trabar® Switzerland, 100 mg) 0.2 ml/kg was administered to all animals intramuscularly every 12 hours for three days.

Clinical Signs Evaluation

From the first week of the study to the 16th week after treatment, clinical signs of motor and sensory reflex of injured spinal cord were examined on a weekly basis.

Evaluation of The Motor Functions

All of the animals survived surgery and were later used for analysis. Tarlov Scale Modified (Tarlov et al., 1953) and Texas Spinal Cord Injury Scale (TSCIS) (Levine et al., 2009). The locomotor recovery was evaluated using a behavioral evaluation system. From the first week through the 16th week PO, the motor functions of the spinal cord injury were documented and analyzed on a weekly basis. Knuckling function improvement was scored on a scale of normal, mild, moderate, and severe (Table 1).

Table 1: Modified clinical signs grading system for motor recovery (Tarlov, 1953 and Levine *et al.*, 2009).

Clinical observation	Grade	Description
Gate	6	complete motor activity
	5	Normal gate but inability to leap
	4	Ability to walk with minor difficulty
	3	Ability to walk with minor difficulty
	2	Ability to push upon hind leg and
	1	take few steps
	0	Ability to push upon hind leg but no take steps Total paralysis of hind leg

From the first to the sixteenth week PO, the sensory functions of SCI were assessed on a weekly basis. The Texas Spinal Cord Injury Scale (TSCIS) for dogs evaluate each limb individually was styled to reflect the typical sequence of functional loss and recovery after SCI (Levine et al., 2009). The dog was tested in lateral recumbency to determine if deep and superficial nociception was present or absent. The superficial nociception (soft tissue pain) was tested by pricking the lateral aspect of the leg and planter surface of the foot with needle, Deep nociception (bone or joint pain) was assessed by pinching the most distal region of a digit with forceps squeezing (Table 2).

Histopathological Examination

Four dogs each period was used for the neurohistopathological examination between weeks eight and sixteen after surgery. The dogs were put to sleep using xylazine and ketamine, and they were then put to death with a 10% intracardial injection of formalin. The spinal cord was imbedded in 10% neutral buffered formalin for one night in the vertebral canal before being extracted and fixed for at least one week. A 1 cm piece of the spinal cord including the lesion site was dissected. The spinal cord tissue specimens were dehydrated in a ascending graded ethanol series, cleared in xylene, embedded in paraffin, sectioned longitudinally to prepare 5µm thick sections, and stained with hematoxylin-eosin (H&E) according to standard protocols (Bancroft et al., 2008). A light microscopic analysis of the lesion site allowed for the determination of the regenerative changes at the spinal cord injury site.

Table 2: Texas Spinal Cord Injury score (TSCIS) Modified Scoring for Evaluation the Sensory Clinical Signs by (Levine *et al*., 2009).

Clinical Observation	Description	Score
1.Superficial nociception a.(lateral aspect leg sensation)	Induced by pricking the lateral aspect of leg with needle	
Absent deep and superficial Present superficial noci. Present deep noci. Present deep and superficial		0 1 2 3
b.(Toe Prick)	Reflex induced by pricking the planter surface of foot with needle	
Absent deep and superficial Present superficial noci. Present deep noci. Present deep and superficial		0 1 2 3
2.Deep nociception (Toe pinch)	Reflex induced by pinching the most distal portion of digit with forceps	
Absent deep and superficial Present superficial noci. Present deep noci. Present deep and superficial		0 1 2 3

Table 3: Neurologic Status as Measured by the Modified Tarlov Neurologic Recovery Scale in Control and Chitosan Groups (Subgroup N=4).

Time Week	Control group	Chitosan group
1Wk PO	Total paralysis of hind leg	Total paralysis of hind leg
2Wk PO	Total paralysis of hind leg	Total paralysis of hind leg
3Wk PO	Total paralysis of hind leg	Total paralysis of hind leg
4Wk PO	Total paralysis of hind leg	Ability to push upon hind leg but don't take steps
5Wk PO	Total paralysis of hind leg	Ability to push upon hind leg but don't take steps
6Wk PO	Ability to push upon hind leg but don't take steps	Ability to push upon hind leg and take few steps
7Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with major difficulty
8Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with major difficulty
9Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with major difficulty
10Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with minor difficulty
11Wk PO	Ability to push up hind leg but don't take steps	Ability to walk with minor difficulty
12Wk PO	Ability to push up hind leg and take few steps	Ability to walk with minor difficulty
13Wk PO	Ability to push up hind leg and take few steps	Ability to walk with minor difficulty
14Wk PO	Ability to push up hind leg and take few steps	Normal gait but inability to leap
15Wk PO	Ability to push up hind leg and take few steps	Normal gait to leap
16Wk PO	Ability to push up hind leg and take few steps	Normal gait to leap

Statistical Analysis

The Statistical Analysis System- SAS (2009) program was utilized to evaluate the impact of various factors on research parameters. In this study, the least significant difference -LSD test (ANOVA) was used to compare means (Daniel, 2009).

RESULTS

Clinically, all experimental animals were maintained under observation throughout the investigation to document general health, behaviour, and alertness.

From the first to the second week after surgery, all animals displayed significant dysfunction characterised by full paralysis of pelvic limbs with dragging of the caudal half of the body during walking (Table 3) and severe knuckling (Table 4). However, there were no deep or superficial pain sensations in the hind leg recorded (Table 5).

Table 4: Statistical Analysis of Knuckling Function Tests on Weeks of the Study Period in Two Groups (Subgroup n=4)

Day	Groups	
	control	Chitosan
14	1±0Cb	1.62±0.18Ca
28	1±0Cc	1.75±0.16Cb
56	1.75±0.16Bc	2.37±0.18Bb
112	2.37±0.18Ab	3.75±0.16Aa
LSD(P<0.05)	0.413	

Table 5: The Mean Time (Weeks) of Sensory Clinical Observations in control and chitosan Groups During the Study Period (Subgroup n=4).

Time	(Sup. nociception) Lat. Aspect Leg Sense		(Sup. nociception) Toe prick		(Deep nociception) Toe Pinch	
	control	chitosan	control	chitosan	control	chitosan
2 wks	0±0Aa	0±0Ba	0±0Aa	0±0Ba	0±0Aa	0±0Ba
4 wks	0±0Aa	0±0Ba	0±0Aa	0±0Ba	0±0Aa	0±0Ba
8 wks	0±0Aa	0±0Ba	0±0Aa	0±0Ba	0±0Aa	0±0Ba
16 wks	0±0Ab	0.87±0.12Aa (96 day)	0±0Ab	1±0Aa (101day)	0±0Ab	1±0Aa (86 day)
LSD(P<0.05)	0.125		-		-	

Table 6: Statistical Analysis of Motor Clinical Observations on Weeks of the Study Period in all Groups (Subgroup n=4).

Weeks	Groups	
	Control	Chitosan
2	0±0Cc	0.37±0.18Dbc
4	0±0Cc	1.25±0.16Cb
8	0.87±0.12Bb	4.25±0.16Ba
16	1.75±0.16Ab	6±0Aa
LSD(P<0.0.5)	0.395	

*Means with different capital letters in the same column and small letters in the same row are significantly different at p ≤0.05.

Table 7: Statistical Analysis of Sensory Clinical Observations at the End of Experimental Study in All Groups (Subgroup n=4).

Signs	Control	Chitosan
Lat aspect leg sense	0±0Ab	1±0Aa
Toe Pinch	0±0Ab	1±0Aa
Toe Prick	0±0Ab	1±0Aa

Means ± SE with different capital letters in the same column and small letters in the same row are significantly different at p ≤0.05

The clinical examination revealed that total paralysis of the hind limb was obvious four weeks after surgery, and some animals suffered from skin erosion on the dorsum of the hind limbs three weeks after surgery as a result of the animals crawling on the hind limbs (Table 3). All of the animals, however, developed severe knuckling (Table 4). There were no feelings of the hind limb recorded (Table 5).

Six of the animals in this group were able to push up on their back leg but were unable to walk more than a few steps at the end of the eight-week period following surgery, while the other two had complete paralysis of their hind limbs until the end of the experiment (Table 4). Eight weeks following the procedure, knuckling was still quite bad (Table 4). The hind limb's sensations were not noted. (Table 5). Clinical assessments at the conclusion of the investigation demonstrated no improvement in normal gait after sixteen weeks (Table 3). Knuckling was moderate and

persisted throughout the trial (Table 4). Sensation was still missing (Table 5).

The clinical examination for chitosan group revealed entire paralysis of the hind limbs beginning on the first day of therapy and lasting until the end of the third week of treatment (Table 3), as well as significant knuckling at the end of the second week of treatment (Table 4). However, there were no feelings of the hind limb noted (Table 5).

At four weeks post treatment, all animals in chitosan group were able to push on the hind limb without taking a few steps (Table 3), and knuckling was severe (Table 4) but there were no sensations (Table 5).

All animals could walk with major difficulty (Table 3) and the knuckling became moderate on the 8th week post-treatment (Table 4), but the sensation was still disappeared at the completion of the eighth-week post-treatment (Table 5).

An intriguing finding was that the experimental animals resumed normal pelvic gait movements fourteen weeks after therapy (Table 3), whereas the knuckling remained mild until the end of sixteen weeks (Table 4). However, by the end of the experiment, the sensation had gradually advanced towards the foot. Lateral aspect leg and toe prick came on days 96 and 101 post-treatment, respectively, while toe pinch reaction appeared on day 86 (Table 5).

Clinical Assessment Comparison Between Groups

Neurologic testing in the control group showed no change in gait score and no hind leg posture reactions related to proprioceptive positioning. Following chitosan nanoparticle transplantation, the Texas Spinal Cord Injury Scale score and the total Modified Tarlov scores increased.. However, on the second week after treatment, the experimental animals in the chitosan group achieved a higher average Tarlov score (0.37 ± 0.18) was significant ($p < 0.05$) than control group (0.00 ± 0.00). On four weeks after treatment, the chitosan group (1.25 ± 0.16) was significantly ($p < 0.05$) higher than the control group. (0.00 ± 0.00). (Table 6).

Furthermore, eight weeks after treatment, the chitosan group (4.25 ± 0.16) were highly significant ($p < 0.05$) than the control group (0.87± 0.12). At sixteen weeks post-treatment, the chitosan group (6.0± 0.0) were significantly higher ($p< 0.05$) than the control group (1.75 ± 0.16). At the 8th and 16th weeks, there were significant differences ($p< 0.05$) between chitosan and control groups. (Table 6).

At fourteen weeks, the dogs in the chitosan group, however, had recovered their normal pelvic gait movement and showed no signs of neurological abnormalities returning.

While proprioceptive posture was detected, severe knuckling was observed in the chitosan group (1.62 ±0.18), which were significant ($P< 0.05$) as compared to the control group (1.0 ±0.0). Furthermore, on the fourth week after treatment, moderate knuckling was remained severe in the chitosan (1.75 ±0.16) group, which was significant ($p <0.05$) than the control group (1.0 ± 0.0) (Table 4).

Nevertheless, eight weeks following treatment, the knuckling was moderate in the chitosan group (2.37± 0.18), and severe in the control group (1.75± 0.16). The chitosan group was significant ($p <0.05$) when compared to the control group. There was a normal response (Planter surface of the foot facing the ground) sixteen weeks after treatment in the chitosan group (3.75± 0.16), which were highly significant ($p< 0.05$) than the control group (2.37 ± 0.18) (Table 4).

Statistical Analysis of Sensory Clinical Observations

Sensory reflexes, including superficial and deep nociception, were absent at weeks 2, 4, and 8 after therapy. On sixteen weeks after treatment, experimental animals in the chitosan group had higher significant sensory reflexes ($p \leq 0.05$) than the control group (Table 7). Nonetheless, in the group receiving therapy, the recovery of both deep and superficial pain perception in the left hind limb demonstrated lateral aspect leg on day 96, toe prick on day 101, and toe pinch reaction on day 86 following treatment. (Table 5).

Histopathological Examination

Control Group: The histopathological examination at the site of the spinal cord injury in control group at 8th week post operation revealed multiple cystic cavities containing granular cellular debris surrounded by reactive gliosis with marked vacuolation in the white matter which indicating Wallerian degeneration (Figure 8. A). While at 16th week post operation revealed large cystic cavity (thick arrows) surrounded by glial scar tissue and vacuolated nerve fibers (Figure 8. B).

Chitosan Group: At the eighth week post-op, the histological analysis at the location of the spinal cord lesion in the chitosan group revealed considerable vacuolated nerve fibre in the white matter and smaller voids surrounded by reactive glia cells. (Figure 9. A). Whereas at 16 weeks chitosan group has a small size cavity, injured area was regenerative nerve fiber and surrounded by glial cell with good remyelination regenerative nerve fibers in white matter,

and normal neuron cells in gray matter, as well as high angiogenesis. (Figure 9. B).

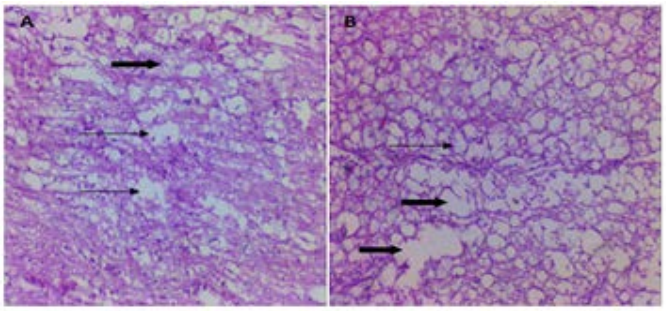

Figure 8: Micrograph of the longitudinal section of control group at the site of the spinal cord injuries **A.** 8 weeks PO shows multiple cystic cavity, containing granular cellular debris (thin arrows) surrounded by reactive gliosis and presented debris of necrotic with prominent vacuolization in white matter (thick arrow). **B.** 16 wks shows large cystic cavity (thick arrows) surrounded by glial scar tissue and vacuolated nerve fibers (thin arrow) H&EX10.

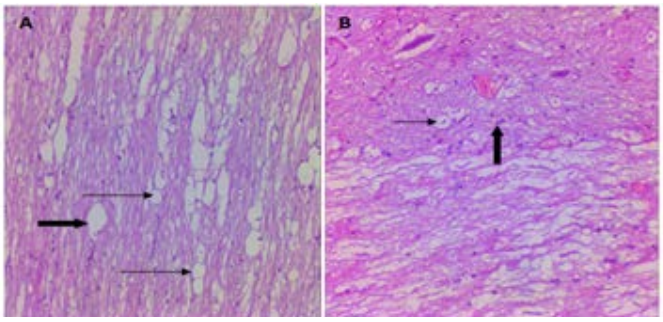

Figure 9: Micrograph of the longitudinal section of chitosan group at the site of the spinal cord injuries **A.** 8 weeks PO shows reduced cavities size and surround by glia cells (thin arrows) and moderate vacuolization (thick arrow). **B. 16** weeks chitosan group shows small size cavity, injured area was filled with dense regenerative nerve fiber (thin arrow),

DISCUSSION

One of the most challenging ailments to treat is a neurological injury, because neurons have relatively limited regeneration capacities, the majority of the deficits caused by lesions are irreversible and permanent (Cooke et al., 2022; Hussein and Al-Bayati, 2022). The Texas Spinal Cord Damage Scale and the modified Tarlov scale were utilised in the current investigation to quantitatively assess the neurological status following spinal cord damage. Increasing scores indicate less severe disability; this score was created based on the stages of motor and sensory recovery that follow spinal cord injury. (Levine et al., 2006).

The intriguing conclusion after 16 weeks PO, the motor reflex in the chitosan was significantly better (p≤0.05) compared to the control group. The dogs in the control group had paraplegia and were not expected to regain a normal gait without treatment. Tarlov, on the other hand, does not score above three until 16 weeks after SCI. These findings are consistent with Bradbury and Burnside's (2019) findings of significant spinal cord injury and the creation of cavities surrounded by scar tissue with high quantities of collagen, resulting in irreversible paraplegia after SCI. But after 105 days, the chitosan-treated groups' gaits returned to normal. The ability to walk and other motor clinical symptoms were categorised based on the type of pain, neuropathic and inflammatory pain, and its intensity. All of the experimental animals in the treatment group had no knuckling. However, it vanished in the chitosan (14-week). The control group, on the other hand, continued to knuckle until the completion of the trial. This could be due to the effectiveness of chitosan on functional recovery at the site of SCI hemisection defects via improved early innervation of the extensor and flexor muscles, which control normal limb locomotion and promote myelin sheath formation and neovascularization.

On 16 weeks PO, the sensory reflex in the chitosan nanoparticles, group was significantly better (p≤0.05) compared to the control group. However, the absence of sensation in the presence of proprioceptive positioning, particularly when severe, causes the animal to walk on the dorsum of the foot, resulting in a worse state during the healing process. The withdrawal reflex test, which involves pinching and pricking the toes, is the primary clinical indicator that supports the recovery of sensory function evaluation.However, after hemisection of the spinal cord, toe pinch and toe prick were absent in two groups at 2, 4, and 8 weeks PO, but progressed and appeared at 16 weeks PO in the chitosan nanoparticles group. Dogs in the chitosan nanoparticles group demonstrated lateral aspect leg, toe prick on days 96 and 101 PO, and toe pinch reaction on day 86 PO. The animals in the control group had lost sensation.

According to this study, functional recovery of SCI could be achieved by transplanting chitosan nanoparticles, as evidenced by increased scores on the Texas SCI scale after transplantation.

These improvements have been attributed to the ability of chitosan nanoparticles to exhibit better locomotor and sensory function recovery by increased release of neurotrophic factors, such as BDNF and NGF, in the site of injury, which are known to promote cell survival and spinal cord regeneration.

Nanoparticle treatment may prevent secondary damage in spinal cord injuries by exploiting its anti-inflammatory and antioxidant capabilities (Javdani and Barzegar, 2023). Ad-

ditionally, significantly recovered axons and neuron cells at the lesion site provided further evidence of the significant neuroprotective effects of chitosan nanoparticles against spinal cord injury; these neuroprotective effects contributed to functional recovery after SCI. (Wu et al., 2014). Chitosan application at the site of spinal cord injury was able to immediately restore compromised membrane integrity and was considered a potent neuroprotective agent that was clearly targeted the area of tissue damage, whereas uninjured spinal cord exhibited a very weak affinity for chitosan (Cho et al., 2010).

Because chitosan nanoparticles enhance the effects of extreme pressure/pulverization, they have the potential to be an important mediator in severe spinal cord damage. The functional recovery of motor, sensory, and autonomic functioning after neurotrauma is based on the physiological recovery of conduction, which is outlined here. (Darrow et al., 2019).

According to the findings of a study by Zarepour et al. (2022), chitosan nanoparticles significantly improved the recovery of sensory reflex and locomotor function. the Basso, Beattie, and Bresnahan score (BBB scores) indicated that one of the interesting and novel strategies for SCI treatment is the use of nanomaterials, which could appear as a carrier for therapeutic agents or as a platform for cells. At 8 weeks, neuro-histopathological examination of the hemisection spinal cord in the chitosan nanoparticles group revealed significant improvement and acceleration of injured spinal cord compared to the control group. The histology of the hemisection spinal cord in the control group exhibited severe vacuolation of the nerve fibres in the white matter, which was linked to pro-inflammatory cytokines which result in secondary cascades of events that occur after several hours to days of spinal cord injury, including mitochondrial dysfunction, failure of aerobic energy metabolism, and eventually the production of free oxygen radicals, which lead to lipid peroxidation and increased vascular permeability, local ischemia, intraneuronal edoema, and degenerate axons. Essa et al. (2020) and Fan et al. (2022) have found similar results.

Additionally, cavitation was noted in the control group near the site of the spinal cord injury. This phenomenon consequences from the complexity of regenerative failure, A number of studies have suggested that this secondary process of cavitation is associated with ischemia (Tran et al., 2018), haemorrhage (Malomo et al., 2022), lysozyme activity (Hu et al., 2023) or macrophage infiltration (Milich et al., 2019), and inflammation (Hellenbrand et al., 2021). Inflammatory processes alone produce secondary tissue injury, increasing cavitation, and glial scarring in the CNS (Tran et al., 2018). Therefore, the anatomical structure of the spinal cord can be preserved by any therapeutic intervention that, when implemented early, can avoid secondary cascades and promote axonal regeneration. (Schmidt and Quintá, 2023).

When comparing these degenerative changes to the findings of experiments in which chitosan was transplanted into the hemisection site investigation reduced cavities size surrounded by reactive gliosis with wavy bundles of regenerate nerve fibres in the deeper part of the implanted tissues and moderately vacuolated nerve fibres in the white matter; neurons appear slightly atrophied in the grey matter and these concurred with (Yari-Ilkhchi et al., 2021; Mousa et al., 2021).

However, implantation of chitosan in a spinal cord injury site results in the elimination of cavitation and enhanced tissue repair, which improves coordinated locomotion; this functional recovery is accompanied by the preservation of myelinated white matter and motor neurons, as well as an increase in axonal re-innervation. Dynamic interactions between inflammatory cells and chitosan can promote favourable extracellular matrix remodelling, which can stimulate tissue repair after SCI injuries (Xiang et al., 2023). Following SCI, glial scars frequently form in the lesion site, impeding the regeneration of nerve fibres. Chitosan transplanted in the injured spinal cord could significantly reduce the formation of tissue cavities and glial scars, promote spinal nerve fibre regeneration, and improve locomotor function (Liu et al., 2021).

Another study by Javdani and Barzegar (2023) found that nanoparticles can control inflammation and oxidative stress in spinal cord injuries, indicating that treatment may prevent secondary damage in SCI by leveraging its anti-inflammatory and antioxidant characteristics. SCI, on the other hand, causes the generation of reactive oxygen species such as hydrogen peroxide (H2O2), superoxide (O2•–), and hydroxyl (OH•) radicals, resulting in considerable declines in antioxidant levels (Zhang et al., 2016).

When compared to the control group, histopathological analysis of hemisection spinal cord at 16 weeks PO demonstrated considerable improvement in the treatment groups. The neurons in the control group appeared atrophied in the grey matter due to reactive astrocyte proliferation, and there was still a gap between the pre and post transection area because of astrocyte hyperatrophy and proliferation, oligodentrocyte apoptosis, and microglia cell proliferation. The control group also exhibited significant vacculation in the white matter due to ongoing nerve fibre degeneration and glial scar formation. These implications on functional recovery and histology findings are consistent with clinical reports of delayed motor and sensory function progression.

These findings are congruent with those of Gaudet and Fonken (2018).

In the present study, chitosan nanoparticles transplantation on post-SCI patients reduced the size of the SCI lesion and improved long-term functional outcomes, most likely through a combination of mechanisms including white matter injury amelioration and inhibition of gliosis and microglial proinflammatory activation. Chedly et al. (2017) and AL-Ameri and Al-Timmemi (2018) found that implantation of chitosan alone into the spinal cord immediately following a bilateral dorsal hemisection enhanced spinal tissue and vasculature reconstitution and reduced fibrous glial scarring with astrocyte processes largely orientated towards the lesion.

Another study on chitosan implanted into a hemisectioned spinal cord demonstrated elicited axonal regeneration, with labeling of cortical motor neurons indicating motor axons in the corticospinal tract not only entered the injury site within the biomaterial but also grew across the lesion area and into the distal spinal cord (Rao et al., 2018). Zhang et al. (2019) used chitosan to improve healing after spinal cord injury by isolating and developing local glial scars to promote axonal regeneration and also minimize cicatrization and ensure an unobstructed space to promote cell growth compared to the control group.

CONCLUSION AND RECOMMENDATION

Transimplantation of chitosan nanoparticles at the site of spinal cord injury is able to alleviate secondary and extended inflammation to the lesion site, thereby contributing to repair and encouraging functional recovery via the early regulation of inflammatory cell recruiting with inhibition of apoptosis and secondary inflammation. In this current study, we recommend that application of nano chitosan at the site of acute spinal cord injury can improve and hasten the repairing mechanism.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

NOVELITY STATEMENT

The novelty of the study is focused on chitosan nanoparticles that can be employed as a novel treatment for acute spinal cord injury due to the major inability which resulted from this injury and lack of an approved treatment for this injury which can cause a permanent disability.

AUTHORS CONTRIBUTON

These authors each contributed equally.

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