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The Impact of Diabetes Mellitus on Nerve Regeneration at a Critical Level

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ABSTRACT
Randomly assigning sixteen healthy mongrel dogs (4-6 months old) to one of four groups—"control," "diabetic one," "diabetic two," or "diabetic three" they were all given intramuscular injections of ketamine and xylazine to put them to sleep. Injecting 120 mg/kg of alloxan monohydrate intraperitoneally (quarter, half, and full doses, respectively) caused diabetes mellitus in the diabetic group. Each animal had left-sided sciatic nerve induction damage prior to the experiment. For eleventh-two days, the canines were monitored using clinical reflexes of the sciatic nerve, including motor and sensory. Displayed an impressive degree of converging between the control and D1 groups as well as D2 and D3 parties. Along with the aforementioned clinical symptoms, diabetic groups also exhibit elevated blood glucose levels, depressive symptoms, polyuria, and increased water intake. The control and D1 groups had the largest ratio of relative gastrocnemius muscle means weight (0.75), and the least amount of muscle atrophy (0.70), on day 112 postoperatively (PO), compared to the D2 and D3 groups, which had the lowest ratios (0.55) (Table 4.3). The relative mean weight of the gastrocnemius muscle began to atrophy following sciatic nerve damage, according to a comparison. Based on the data shown significant differences between the left sciatic nerve (OP) and right sciatic nerve in the same group, but no significant differences between the control and D1 groups. Histopathological analyses revealed that all groups converged on a few key metrics, including the proliferation of schwann cells and the orientation of regenerative nerve fibres; however, at 16 weeks post-operatively, both the control and D1 groups showed a comparative advantage in the quantity and quality of nerve fibrous formatting. The conclusion was determined that the danger level for diabetes, which impedes and delays nerve regeneration, is 300-400 mg/dl.

Keywords: *Diabetes Mellitus, Nerve Regeneration, Critical Level*

1. INTRODUCTION

A metabolic illness known as diabetes mellitus is defined by a persistent elevation in blood glucose levels due to insulin resistance, insufficient insulin production, or both (ADA, 2003). Canine diabetes mellitus is an important public health issue on par with human diabetes mellitus. Even though diabetes mellitus is one of the most prevalent endocrine disorders in canines. There have been reports suggesting that diabetes mellitus (DM) can lead to nerve demyelination, axonal atrophy, delayed Wallerian degeneration, and nerve fibre regeneration (Zochodne et al., 2007). Possible causes of diabetic nerve regenerative failure include problems with neurotrophin and neuropeptide support, ischemia-related changes in the regenerative microenvironment, and difficulties with macrophage invasion to phagocytose myelin debris and axons after nerve damage (yasuda et al., 2003). Several neurotrophic agents, including insulin-like growth factor (IGF) and nerve growth factor (ngf) (unger et al., 1998), have demonstrated varying degrees of effectiveness in peripheral nerve regeneration in

animal models of diabetic neuropathy (lee et al., 2015). Additionally, research has demonstrated that tcm can influence diabetic nerve regeneration in rats by raising the production of several neurotrophic factors and stimulating the proliferation of schwann cells (piao et al., 2012). A prominent pathogenic aspect of human diabetic neuropathy is decreased regeneration of nerve fibres, according to the available evidence (nishida, 2013). Functional impairments with sensory loss, altered feeling, and a poor prognosis are observed in animal models of diabetes mellitus due to delayed regeneration of nerve bundles following transection and nerve functional recovery after crush injury (kennedy et al., 2005). Promoting regeneration following peripheral nerve damage in diabetes mellitus remains a significant problem to this day.This study set out to assess nerve regeneration in a diabetic dog model through a battery of tests, including functional evaluations (the walking test and electrophysiological examinations), evaluations of muscle atrophy, and histomorphometric nerve assessments. Up to this point, no studies have been published that aim to determine the critical threshold of diabetic mellitus (dm) and its effects on nerve regeneration in dogs. Understanding the crucial threshold of diabetic mellitus (DM) and its impact on canine sciatic nerve regeneration was, thus, the primary aim of this research. moreover, assess the healing process of the damaged sciatic nerve.

Experimental Animals Design

Induction Hyperglycaemia (diabetes mellitus type I) in dogs:

Type I diabetic mellitus was induced by single intraperitoneal injection of monohydrated Alloxan (AX) in dose 120mg/kg,.The dosages of monohydrated Ax was dissolved in 5 ml sterile 0.9% saline after that animals were injected 5ml of dextrose solution 10% in order to decrease direct hypoglycemic effect of alloxan on animals. This done after checking the blood glucose of fasted dogs (Vattam et al., 2016). This induction models have been reported to exhibit pathological conditions that are similar to those of type I diabetes in humans.

Determination of Glucose

By Glucose Oxidase (GOD) catalysis the oxidation of glucose to gluconic acid according to restriction of manufacture

Anesthetic procedure

Prior to the anaesthesia, the dogs were instructed to fast for two hours. An injection of a combination of 15 mg/kg of ketamine hydrochloride (Kepro®, Holland) and 10 mg/kg of xylazine hydrochloride (Xyla®, Holland) was administered intramuscularly to induce anesthesia (Flecknell, 2003).

Surgical Protocol

The dog's hair was shaved off along its behind, from the left hind limb's lateral and caudal aspects all the way to the dorsal wing of the ilium, the sacrum, and the stifle joint. The skin was cleansed using a combination of tincture of iodine from Lebanon, isopropyl alcohol 70% from Jaya Pelita Pharma SDN. BHD, and hepatin from Hexatane 20® in Jordan.

By covering the distal extremity with a latex glove and taping it to the limb, the limb was isolated from the stifle joint and the surgery site. After covering the glove (Sempermed®, Austria) with a sterile skin towel, the limb was fastened to the glove using towel clips. The subject was positioned in a right lateral recumbent position. The fenestrated drape was placed over the left hind leg, with the opening facing the surgical target (Fig. 3.1)

Figure 1: Photograph showing the surgical operation site in dog.

It was necessary to palpate the greater trochanter of the femur in order to locate the stifles. The skin was cut on the back side of the thigh, about 2 centimetres down from the side opposite the greater trochanter, at the level of the distal 1/3 of the femur, using a blade number 21 from the scalpel.

On the same line, using scalpel blade #15, incisions were made in the subcutaneous tissue and fascia. In order to expose the

sciatic nerve and separate it from the surrounding tissues, rough dissection was performed with Mayo scissors to separate the biceps femurs muscle cranially and the semitendinosus muscle posteriorly. Ophthalmic scissors were then used for the separation. Nerve #21, which had been delicately positioned beneath a wooden tongue depressor, was sliced using scalpel blade #21.

Following surgery, all animals were given an intramuscular injection of 100 mg of Tramadol hydrochloride (Trabar® Switzerland) at a rate of 0.2 ml/kg every 12 hours for three days in a row.

Clinical Signs

Throughout the duration of the trial, which began on day 1 and ended on day 112 postoperatively, the motor and sensory sciatic nerve clinical reflexes were assessed regularly.

Motor Functions

From day one to day eleven2 post-operatively, all animals were checked daily using the grading standards. We documented the animals' onset and their capacity to walk. A regular, crouching, or crawl-on-heel gait was assigned a grade. Normal, mild, moderate, and severe knuckling were the four categories used for grading. There were three levels of muscle contraction force: mild, moderate, and powerful. There were four levels of muscle atrophy: normal, mild, moderate, and severe.

Sensory Functions

From the conclusion of the third week to the completion of the trial on day 84 PO, nerve sensory functions were recorded weekly. Toe pinch, toe prick, lateral aspect leg feeling, and toe spreading reflex were used as grading criteria to assess sensory functioning and clinical symptoms as either present (+) or absent (-). A good reaction was recorded as an indication of recovery when the foot withdrawal and vocalization tests were examined by feeling the lateral side of the leg, pinching and pricking the toes, and recording vocalizations.

Table 1: Modified clinical signs grading and scoring system for motor abnormality (Highet, 1954; Schmidhammer et al., 2004)

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Clinical signs	Control Group	Treated Group 4ws	Treated Group 8ws
Onset	1.2±0.2 C	2.8±0.3 b	4.2±0.3 A
Walk			
Crouch	1±0.0 A	0.4±0.24 b	0.2±0.2 Bc
Crawl	2±0.0 A	0.8±0.48 b	0.4±0.4 Bc
Normal	3±0.0 A	3±0.0 a	3±0.0 A
Knuckling			
Severe	1±2.8 A	0.6±0.24 b	0.2±0.2 C
Moderate	2±0.0 A	1.2±0.48 b	0.4±0.4 Bc
Mild	3±0.0 A	1.2 ±0.73 b	0.6 ±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

Table 2: Modified Scoring evaluation of the sensory clinical signs (Highet, 1954; Schmidhammer et al., 2004)

Clinical Observations	Description	Score
Toe Spread	Lateral spearing reflex of the digits with maximum space between them when the animal is hanging (lop-eared)	
Present		1
Absent		0
Lateral aspect leg sense	Lateral aspect senses induced by pricking the leg with needle	
Present		1
Absent		0
Toe pinch	Reflex induced by pinching the most distal portion of each digit on both hind limbs with forceps	
Present		1
Absent		0
Toe Prick	Reflex induced in planter surface of foot by needle	
Present		1
Absent		0

Electrophysiological analysis

We will conduct electrophysiological studies eight weeks following nerve damage. Attached to the sciatic nerve were negative and positive clamps (recording) and stimulus electrodes; the nerve was then immersed in an AD instruments chamber filled with buffer solution. The nerve was then isolated from the body by a distance of approximately 3 cm. With a square wave of 0.2 ms length and two pulses per second delivered, the conductive velocity is calculated by multiplying the distance (in millimetres) between the recording electrodes by the time interval (in milliseconds) between the capacity proximal and distal stimulations. From the electric base line of the video display apparatus to the peak of the negative phase of the motor, the amplitude is measured in microvolts (μV), and the latency time is recorded in milliseconds from the shock artefact to the initial negative peak deflection of the response. response.

Figure 2: AD Instrument connected with nerve Kit (Isolated nerve chamber)

Electrophysiological analysis

A longitudinal incision was made on the back of the operated limb to remove the skin that covered the gastrocnemius muscle. A cut was made in the gastrocnemius muscle, and its weight was recorded right away. As a control for weight fluctuation between different dogs, the contralateral muscle was also extracted. Next, we measured the proportion of loss in muscle mass (denervated muscle weight vs. contra lateral muscle weight) by weighing each muscle independently using a 0.0001g weight. How the relative gastrocnemius muscle weight (RGMW) was determined was by comparing the weight of the muscles in

the operated limb (left) to that of the unoperated right limb (negative control). As a measure of motor function recovery, the relative group mean weight (RGMW) was calculated by comparing the experimental group's muscle weight to the negative control groups.

Histopathological Examinations:

On the eighth and sixteenth week after the procedure, nerve samples were collected. In order to prepare the tissues for examination under a light microscope, the samples were first fixed in 10% buffered formalin. Subsequently, they were embedded in paraffin and subjected to regular alcohol processing. After blocking, the tissue samples were sectioned at 5-6 micrometre intervals and stained using routine hematoxylin and eosin stain.

Statistical Analysis

Means and standard deviations (M±SD) were used to express all data after analysis. The statistical tests used for comparing groups were the Kruskal-Wallis and Mann-Witney tests, as well as the Statistical Package for the Social Sciences (SPSS) 16.0 software (non-parametric testing). For significance, a p-value of less than or equal to 0.05 was used.

2. RESULTS AND DISCUSSION

Clinical Assessment of Motor and Sensory Nerve Functions

All animals in two groups showed marked of dysfunction characterized by flaccid paralysis of the operated hind limb movement on day 1 to day 4 PO. The animals were reluctant to flex its limb from the hock joint and below, and remained crouch on the floor cage.

Control Group (non-Diabetic group)

On day 40 postoperatively (PO), the gait returned to normal, and on day 58, the knuckling did as well. Table 4.1 shows that there was a considerable decrease in muscle mass and a substantial contraction of the muscles. Recuperation of feeling in the operated left hind limb occurred on day 70 postoperatively (PO), and on days 78, 93, and 95 PO, respectively, toe pinch and prick sensations, as well as toe spread sensation, were noted (Table 4.2).

Treated one group (Diabetic one)

The animals' return to normal walking abilities on day 77 postoperatively was an intriguing observation. The knuckling was not severe and persisted throughout the research. After 48 postoperative days, the force of muscular contractions became significant (Table 4.1). By the conclusion of the trial, participants' skin feeling had gradually extended to the foot, namely the fetlock joint. According to Table 4.2, there was no discernible feeling of the toe spreading reflex, lateral leg sensation, pinch, or prick.

Treated Two Group (Diabetic Two) and Treated Three

On the 56th postoperative day, it was noted that all of the animals exhibited normal gait. Day 57 post-operatively (PO) saw the disappearance of knuckling, while day 47 PO saw the onset of powerful muscular force contractions (Table 4.1). Day 85 post-operatively (PO) brought about the toe spread feeling, day 99 PO brought about the lateral aspect leg reaction, and day 105 PO brought about the toe pinch and prick sensations (Table 4.2).

Table (4.1): Motor Clinical Observations for all Groups on Day112 PO

Clinical signs	**D2+D3 Group**	**D1 group**	**Control group**
Onset	**1.2±0.2 C**	**2.8±0.3 b**	**4.2±0.3 A**
Walk			
Crouch	**1±0.0 A**	**0.4±0.24 b**	**0.2±0.2 Bc**
Crawl	**2±0.0 A**	**0.8±0.48 b**	**0.4±0.4 Bc**
Normal	**3±0.0**	**3±0.0**	**3±0.0**

	A	**a**	**A**
Knuckling			
Severe	**1±2.8** **A**	**0.6±0.24** **b**	**0.2±0.2** **C**
Moderate	**2±0.0** **A**	**1.2±0.48** **b**	**0.4±0.4** **Bc**
Mild	**3±0.0** **A**	**1.2 ±0.73** **b**	**0.6 ±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**

Sensory Clinical Observations

There were no reflexes observed in the sensory clinical symptoms, which comprised toe spread, lateral leg feeling, pinch, and prick. As shown in Table 3.4, on day 112 postoperatively, the animals in the control group exhibited significantly higher sensory reflexes compared to the control group ($p < 0.05$).

Table (4.2): Sensory Clinical Observations for all Groups on Day112 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

Relative Gastrocnemius Muscle Weight Measurement

The highest RGMWM ratio and therefore less muscle atrophy was seen in the treated group (0.75) and (0.70) at 16ws and 8ws respectively, while the smallest RGMWM ratio (0.55) was in the control group on day 112 PO (Table 4.3). A comparative on the relative gastrocnemius muscle mean weight showed that the gastrocnemius muscle started to atrophy after sciatic nerve injury. However, on day 112 PO, there was significant decreased ($p \leq 0.05$) in RGMW values in the control (45%) group compared to the control on 16ws (30%) and (25%) in 8ws group (Table 4.3).

(Airaksinen et al., 1996; Sterne et al., 1997) presented research that validated regaining muscle weight when the motor target organ and cutaneous afferents were reinnervated. According to Gillespie et al. (1987) and Bertelli et al. (1995), the maximal power of contraction is inversely proportional to the degree of reinnervation, which in turn determines the rate of mass regaining in the muscle.

The gastrocnemius muscle is the first peripheral organ that the sciatic nerve aims to reach. This muscle's relative weight change over time after nerve transection and therapy might be used to determine the sciatic nerve's regeneration state. Because muscle atrophy followed nerve damage, the injured organ lost weight (Pellegrino et al., 1963). The treated groups, the treated 8ws group, and the cntrol group all exhibited superior relative muscle weight ratios when compared to one another. (Airaksinen et al., 1996; Sterne et al., 1997) presented research that validated regaining muscle weight when the motor target organ and cutaneous afferents were reinnervated. The amount of reinnervation, which is correlated with the maximal force of contraction (Gillespie et al., 1987), determines the rate of mass regaining in the muscle (Bertelli et al., 1995).

Table 4.3: A statistical evaluation of the control, D1, D2, and D3 groups' average relative gastrocnemius muscle weight measurements.

Time	D2+D3 Gs%	D1 G %	control Group%
112 days	55.6±1.9 c	70.1±0.7 b	75.6±0.9 A

Electrophysiological analysis

Using an isolated nerve method, the electrophysiological tests were conducted. At 8 weeks post-operatively, an electromyography was performed.

4.4.1 Conductive velocity on 8 weeks PO:

During 8 weeks postoperatively, there were no significant changes in conductive velocity between the control and D1 groups (50.14±4.64Aa) and D2 and D3 groups (46.42±2.87Aa) at a significance level of P:<0.05. However, there were significant differences between the same group on the left sciatic nerve (OP) and the right sciatic nerve (Table 4.5).

The study found that in the control and D1 groups, the values of latency for the left limb were 3ms, whereas for the right limb they were 4ms. At 8 weeks post-operatively, the latency values for the left leg were 3.3 ms and the right limb was 5.2 ms in the D2 and D3 groups, respectively.

In contrast, the amplitude values in the control and D1 groups were 3.5Mv and 6.9Mv, respectively, for the left and right limbs, but in the D2 and D3 groups, the corresponding values were 2.83Mv and 5.2Mv, respectively.

Table 4.5: Statistical analysis of conductive velocity of control, D1, D2 and D3 groups on 16 weeks PO.

Groups		Conductive Velocity
Control &D1	LSN (OP)	50.14 ± 4.64^{Aa}
	RSN	78.26 ± 3.54^{Ba}
D2 &D3	LSN	46.42 ± 2.87^{Aa}
	RSN	82.86 ± 7.43^{Ba}

LSN: Left sciatic nerve, RSN: Right sciatic nerve.

The similar superscript letters denote to non-significant differences at p<0.05.

The level of nerve tension at the site of healing was the determinant of peripheral nerve regeneration. One epineural suture was placed across the nerve's perimeter once the two stumps had been precisely aligned. Postoperative edoema, which causes the nerve end to enlarge, would result from failing to do so. Furthermore, during strain, the coaptation of the nerve endings should not be fixed. Endoneural pressure varies significantly after sciatic nerve transection and healing, mostly because of decreased neuronal blood and axoplasmic flow (Millesi, 1984; Hentz et al., 1993; Al-Faris et al., 2015). Nerve elongation and reduced blood flow are common symptoms of increased strain at the sciatic nerve's commissure. (Mclaren, 1989) corroborated this by finding that increasing nerve tension may cause a reduction in nerve blood flow and conduction velocity. At a stage when blood supply drops by half, peripheral nerve elongation is limited to 8-10%. Axonal degeneration starts with a 15% elongation, which causes the endoneurial lymphatic flow to stagnate, which in turn causes the endoneurial fluid pressure to rise, the oxygenation level to drop, and axonal transport to stop (Lundborg et al., 1973).

Because it increases endoneural collagenous connective tissue and decreases neural angiogenesis at the nerve suture site,

which impedes the advancement of nerve neurite sprouts regeneration, full range motion mobilisation greatly hinders functional recovery (Millesi, 1985). In contrast to previous research suggesting that the operated limb should be immobilised with a splint for two to three weeks following sciatic nerve reconstruction (Chiu et al., 1986; Hirasawa, 1996), the animals in the current study were allowed to roam freely in their cages following neurorrhaphy.

Motor Clinical Observations

This study found that compared to the D2 and D3 groups, the control and D1 groups of mice demonstrated a faster beginning of limb movement and walking. Although both groups' gaits eventually returned to normal, the animals in the control and D1 groups did so more quickly than those in the D2 and D3 groups (77 days vs. 40 and 56 days, respectively). In the control and D1 groups, knuckling disappeared after 57 and 58 days, respectively, but in the D2 and D3 groups, it remained until the end of the research, with a statistically significant difference ($p < 0.05$). Although all groups had substantial muscular contraction forces, the control group (43.6 days) and the D1 group (47 days) began contractions earlier than the D2 and D3 groups (48 days). The capacity to walk on its operated left hind foot was one of the motor clinical symptoms that were evaluated according to the degree of the pain, which was categorised as either neuropathic or inflammatory pain. The study's findings demonstrated that the gait type returned to normal in the control group of rats at a faster rate than in the D3 group. This finding provided more evidence that diabetic mellitus (DM) may have a role in neuropathic pain, damaged sciatic nerve regeneration, and other possible therapeutic effects. Possible effects of diabetes on inflammatory pain include direct interactions with all immune system cells or the inhibition of immune response activities by soluble substances, both of which shorten the duration of inflammatory pain. Supplementation with DM improved electrophysiological sensory conduction and evoked potentials in clinical settings (Tiwari et al., 2009). Research found that knuckling severity regression occurred quickly in the control group on day 57 and vanished in the D1 group on day 58, but remained in the D2 and D3 groups all the way until the study's completion on day 112 PO. It is intriguing to note that all animals in both groups (D2 and D3) showed an improvement in their walking abilities. This might be attributed to the impact of diabetes on blood flow, which in turn hinders functional recovery and innervates the damaged sciatic nerve. This study's results demonstrated that, in comparison to the other groups, the control group's animals recovered the damaged sciatic nerve's function more quickly. It is believed that diabetic complications have a dual impact on peripheral nerve damage. The prevailing belief was that DM inhibits the proliferation of Schwann cells by reducing the formation of neuronal cells and directed axonal development. As a result, endoneurial sheaths are filled to a lesser extent, and longitudinal columns, also called bands of Bungner, are prevented (Scherer et al., 1996). The chemicals that inhibit the development and proliferation of Schwann cells, including as nerve growth factor (NGF), neurotrophic factors, cytokines, and others, were reduced due to DM. The latter significantly decrease the recruitment and adherence of Schwann cells to axonal projections and promote neuronal mortality in response to reduced damage (Boyd et al., 2003). Muscle contraction force improved more quickly in the control and D1 groups than in the D2 and D3 groups, which is an intriguing finding from this study. Dystrophy and the effort required to contract muscles are associated with nerve damage and inactivity, which might lead to an increase in muscle mass as a sign that the sciatic nerve's motor function is improving. Muscle hypertrophy and force contraction are both diminished by neurotmeses of the sciatic nerve in the early stages (Burnett et al., 2004).

Sensory Clinical Observations

The present study's significant conclusion is that both the control and D1 groups had sensory clinical indicators that indicate the advancement of sensibility. When an animal walks on its dorsal foot—a common occurrence after a cruciate ligament rupture—and there is no feeling in the area, an atrophic ulcer can form. Additionally, without feeling, the foot is constantly being cleaned by licking, which just makes the problem worse. The recovery of sensory function assessment relied on the toe-spreading reaction as its primary clinical indicator. It started off nonexistent in all groups on day 1 of the experiment, but by day 112 post-intervention, it had made its way into the control and D1 groups. Upon neuron transduction, a noticeable reduction in foot height occurs; however, this dip generally fades or disappears long before the muscles are reinnervated. Observations of the gait alone are inadequate to establish the exact initiation of response because sciatic nerve damage symptoms are distinct. Restricting observations to the muscle region innervated particularly by the sciatic (peroneal) nerve provides a more reliable estimation of the beginning and course of healing. In dogs, researchers have documented reflex movements and the spreading of three toes on the back limbs following recovery from sciatic nerve injuries as good signs of the beginning of motor activity (Gutmann et al., 1942; Sarikcioglu et al., 2008). The experiment was conducted on cats by grabbing their slack backs and then swiftly lowering them into midair without allowing them to make contact with any surface. While doing so, the animals instinctively stretch their toes to increase the area of their feet, which should help them land more safely (Langley et al., 2004).

3. HISTOPATHOLOGICAL EXAMINATION

Control and Diabetic one Groups

An analysis of the longitudinal sections of damaged sciatic nerves in the control and diabetic groups by histopathologists revealed basophilic nuclei of proliferating Schwann cells with a thick myelin coating, well-oriented and myelinated nerve

fibres, and minimal fibrous tissue at the epineurium (Fig.1).

At 16 weeks postoperatively, there is a dense axon population with a thick myelin coating, a proliferation of Schwann cells, and a plethora of new blood vessels, as well as a distinct node of Ranvier (Fig.3).

According to the histopathological findings, the wounded sciatic nerve is highly vascularized because neurotrophic substances that can induce angiogenesis through impacted endothelial cells are present, leading to improved blood supply. According to earlier research, NGF can promote endothelial cell migration and proliferation, extracellular matrix remodelling, and the functional maturation of freshly generated blood arteries (Chen et al., 2004). According to Jessen et al. (1999), the main pillars that sustain peripheral nerve regeneration are Schwann cells. Previous research has shown that Schwann cells are crucial for directing neural expansion in wounded nerves, inhibiting Schwann cell proliferation, and reducing axon development; the current study's findings are in line with these prior findings (Chen et al., 2004). According to experimental evidence, the number of Schwann cells in a given area of the peripheral nervous system decreases in direct correlation with the number of nerve cells and, by extension, nerve fibres in that location. Thus, a large number of Schwann cells may suggest that there are many neurons that have survived (Jessen et al., 2019).

A significant density of Schwann cells was found in the nerve that emerged from this ganglion in the current investigation. The sensory neurons were shielded from harm by the nerve growth factors released by these cells. Previous research has suggested that injured tissue might have therapeutic advantages, thus our finding is in line with that (Liu et al., 2000).

Diabetic two and Diabetic three Groups

At 8 weeks post-operatively, the histopathology of longitudinal sections of the damaged sciatic nerve in the D2 and D3 groups showed Wallerian degeneration of nerve fibres with few Schwann cell nuclei, fibrous connective tissue, and inflammatory cells, especially macrophages and lymphocytes (Fig. 2). However, at 16 weeks post-operatively, there is disorientation of regenerative nerve fibres, vacuolar degeneration of the fibres, inflammatory cell infiltration into rounded, congested blood vessels, and differentiated staining of nerve fibre loss, with few Schwan cells and infiltrated with inflammatory cells. Deterioration of Schwann cells and proliferation of collagen fibres within the nerve fascicle (Fig. 4). Based on the histological findings, it appears that diabetic mellitus (DM) hinders the regeneration of sensory and autonomic neurons in the dorsal root ganglia and motor neurons in the ventral horn of the spinal cord. This could be because neurotrophic factors, which normally protect neurons and aid in their survival and regeneration, have been found to be reduced, even though axon regeneration has not yet been linked to target organs. But this outcome ran counter to what had been seen (Dodla et al., 2019) according to the study's authors, neurotrophic factors are lost from target organs after axonal injury, which damages the interaction between cell bodies and peripheral organs and kills as many as 35% of sensory neurons. Histopathological analysis revealed deterioration in the D2 and D3 groups, as shown by a decline in Schwann cell density and, therefore, myelin sheath thickness. Regenerated axons have reduced remyelination due to a decrease in the number of Schwann cells in the stump (Dolan et al., 2019).

Injury to the peripheral nervous system triggers an inflammatory response characterised by increased vascular permeability and intraneural edoema; tissue ischemia leads to metabolic impairment; and finally, polymorphoneuclear leucocytes infiltrating the lesion site produce the harmful oxygen metabolites superoxide anion, hydrogen peroxide, and hydroxyl radicals. When cells divide, they emit cytokines and free radicals that cause harm neutrophils (Kurtoglu et al., 2004). Because of its antioxidant properties, it can help reduce oxidative stress in a roundabout way (Aydemir et al., 2004). Research on canines with neural injuries has revealed that inducing DM thereafter increases both oxidative damage and edoema development in muscle cells (Silva et al., 2005; Al-Timmemi et al., 2012).

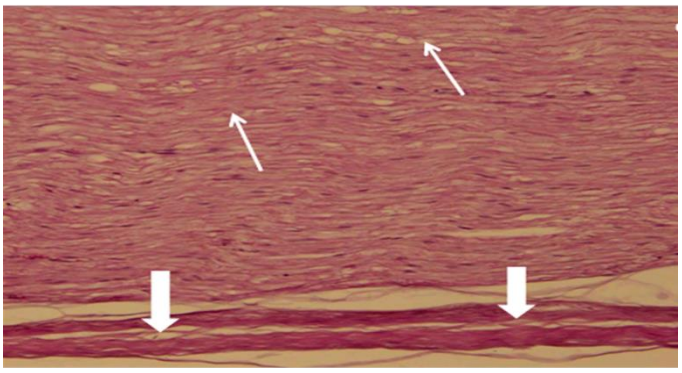

Figure 3: At 8 weeks post-operatively, light micrographs of the sciatic nerve injury site in the Control and D1 groups show that the nerve fibres are well-oriented and that myelination has occurred (thin arrows) and that the epineurium is thin (thick rows). 40. H&EX.

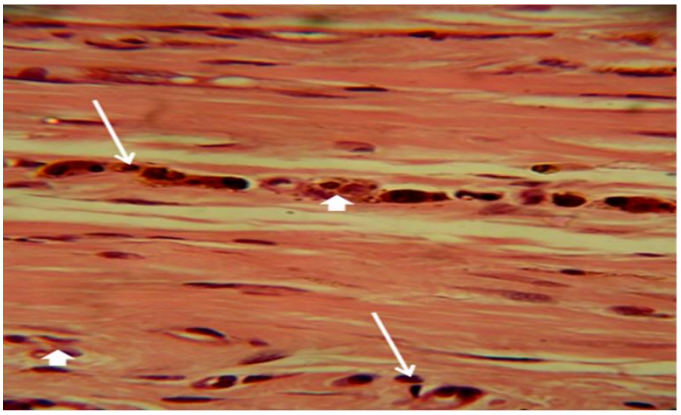

Figure 4: At 8 weeks post-operatively, light micrographs of the sciatic nerve injury location in the D2 and D3 groups reveal the presence of inflammatory cells around the blood vessels (arrows), as well as heavily vacuolated, degenerative nerve fibres (dinner chambers). (arrow heads). H&EX40.

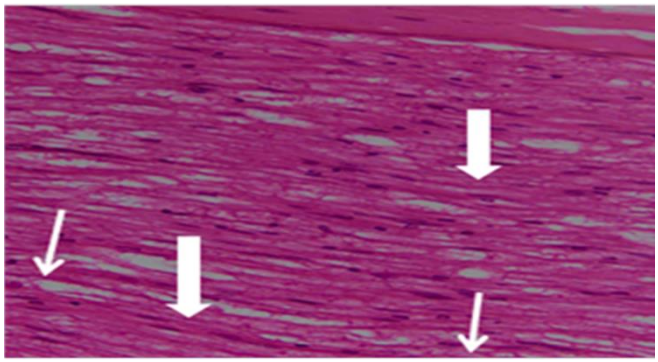

Figure 5: Light micrographs of injured site of sciatic nerve at 16 weeks PO in Control and D1 groups shows good myelinated nerve fibers with Schwan cells (thick arrow), node of Ranvier (thin arrow). H&E X40.

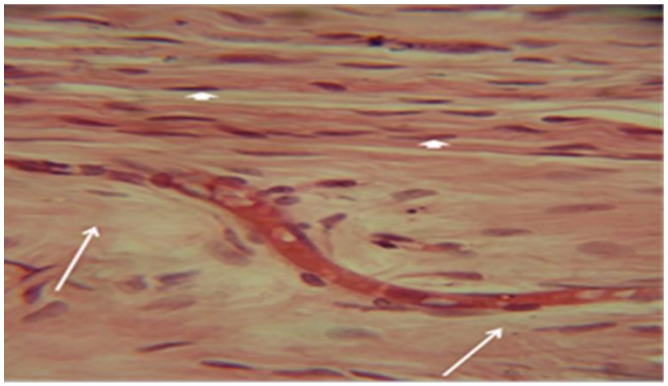

Figure 6: At 16 weeks post-operatively, light micrographs of the sciatic nerve injury site reveal a lack of Schwan cells (arrow heads), a growth of fibrous connective tissue at the epineurium (arrows), and clogged blood arteries (arrows). The H&E X40.

4. CONCLUSIONS:

The conclusions drawn from this study is based on clinical, electrophysiological examinations as follows:

1. The critical level of DM (350) that effect on nerve regeneration in dogs.
2. Clinically; the clinical signs can dis appeared early in control and D1 groups (diabetic level) compared with D2 and D3 groups (diabetic level) delayed nerve regeneration sometimes and failure at other times.
3. Motor and sensory reflexes improved earlier in the control and D1 groups compared to the D2 and D3 groups.
4. Gastrocnemius muscle atrophy was low in control and D1 groups compared with D2 and D3 groups.

5. RECOMMENDATIONS:

It is proposed that future research be focused on the following:

1. The use of other clinical experimental models such to calculate the critical level of DM effect on nerve regeneration.
2. Evaluation of the quantity of chemotactic, nerve growth factor and oxidative stress enzymes that are associated with nerve regeneration induced with DM.
3. Investigate the differentiated stem cells (Schwann cells) for the treatment of injured peripheral nerve induced with DM.