World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Effect Of D-Ribose-L-cysteine on associated complications in sciatic nerve crush injury model

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World Journal of Advanced Research and Reviews, 2024, 23(02), 1395–1409

Publication history: Received on 30 June 2024; revised on 10 August 2024; accepted on 12 August 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.23.2.2411

Abstract

Background: oxidative stress has been established as a significant factor in the pathogenesis of peripheral nerve injury. D-ribose-L-cysteine (DRLC) is an antioxidant agent with central neuroprotective effects. However, it is not known whether DRLC has protective effects on crush sciatic nerve injury. Twenty-five rats were divided into five groups of five rats each. In sham group (G1), the sciatic nerve was exposed but not crushed, whereas in negative control-14 group (G2), negative control-28 (G3), DRLC treated-14 group (G4) and DRLC treated-28 group (G5), the sciatic nerve was exposed and crushed with non-serrated forceps for 30 seconds. Rats in groups G2 and G3 were given normal saline orally, while DRLC (100 mg/kg, orally) was administered to rats in groups G4 and G5 for 14 and 28 straight days respectively. Fourteenth and 28th days post injury, functional recovery was analyzed using a walking track assessment and quantified using the sciatic functional index (SFI), while gastrocnemius atrophic change was evaluated using Gastrocnemius Mass Ratio (GMR), following which all rats were sacrificed, sciatic nerve tissue and gastrocnemius samples were obtained for histopathological evaluation and measurement of Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase (CAT) values.

Results: The SFI and GMR scores in groups G2, G3, G4 and G5 were significantly lower than that of G1 group ($p<0.05$). The SOD values of G2 group, the CAT value of G2 were found to be significantly lower, while MDA value of G2 group was found to be significantly higher, when compared with G1 group respectively ($p<0.05$). CAT value G5 group showed higher significant difference when compared with G2 group, while MDA value of G5 group was found to be significantly lower than those of G2 and G3 groups ($p<0.05$).

Conclusion: The results indicated that DRLC administered for 28 days, had ameliorative effect on oxidative stress markers, but no restorative effect of DRLC was seen on motor function recovery and gastrocnemius atrophy. Further studies are needed to evaluate the therapeutic effect of DRLC applied in several doses, different administration route and longer duration in crush sciatic nerve injury models.

Keywords: Peripheral Nerve Injury; D-ribose-L-cysteine; oxidative stress markers; Gastrocnemius Mass Ratio; Sciatic Functional Index

1. Introduction

Injury to peripheral nerves happen frequently in humans, leading to severe and long-term physiological and functional disabilities [1, 2]. Given that peripheral nerve injury (PNI) occurs so frequently, it has a high clinical relevance [3, 4]. Peripheral neural damage has several and different underlying causes, some of which can be traumatic events or

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iatrogenic damage that most often result from medical or surgical procedures [5, 6]. The primary associative complications of nerve injury may either be loss of motor, sensory or autonomic function in the denervated body segments, which typically causes a mild, moderate or severe functional impairment [7, 8]. The peripheral nervous system (PNS), going by its cellular characteristics, has been found to show a better self-reparative and regenerative capacity, when compared with the central nervous system (CNS). These observed regenerative difference in PNS and CNS are as follows: the functional environments in each of the systems, the age of the injured person, the type of injury and the integrity of the neural cell body of the injured nerve as factors responsible for the distinction [9, 10, 11].

Oxidative stress and inflammation processes are two major events that are evidently observed to be and associated with peripheral nerve injury, and these processes are thought to be crucial in the development of peripheral nerve injury and its consequent complications [12, 13, 14]. Recent researches have demonstrated that reducing oxidative stress following peripheral nerve injury, might hasten the healing process and enhance better functional recovery [15, 16, 17].

D-Ribose–L-cysteine (DRLC) is an exclusive ribose and cysteine derivative and a potent antioxidant molecule, that is similar or comparable to non-essential cysteine. It is developed as a prodrug, that is, a pharmacologically inactive compound that becomes pharmacologically active drug, following its metabolism. When broken down to its constituent parts, it rapidly enhances the biosynthesis of glutathione in human body Glutathione (GSH), a major endogenous antioxidant, has been found to mediates its cytoprotective effect by scavenging free radicals and detoxifying injurious cellular oxidants [18, 19, 20]. The bioprotective effects of DRLC have been demonstrated in studies done on cardiac and reproductive organs [21, 20, 22], utilized to treat chronic fatigue syndrome and as energy source to boost performance in sporting events [23], prevented mice from developing neurodegenerative diseases, oxidative stress, and the memory loss caused by lipopolysaccharide (LPS), exhibited stress reducing activity, by boosting antioxidant defense mechanisms and positively modulating the neuronal cells damaging molecules in mice exposed to Unpredictable Chronic Mild Stress [25].Based on literature search and to the best of our knowledge, research study on DRLC effect on motor function recovery and muscle atrophy restoration, following sciatic nerve crush injury is scarce. This study was therefore designed to determine the effect or potential role of DRLC in peripheral nerve regeneration, following a sciatic nerve crush injury model of PNI in Wistar rats. This was done by exploring the effect of DRLC on myelin and axonal regeneration, as well as the effect of DRLC on the activities of oxidative stress markers in sciatic nerve, motor dysfunction and gastrocnemius atrophy.

2. Materials and methods

2.1. Experimental Animals

A total of 25 adult male Wistar rats (weighing 150-200g) were procured from the Animal Laboratory. The animals were housed in large cages in a room controlled for temperature and humidity, with 12:12 hour light/dark cycles. The animals were acclimatized in the environment 7 days prior to the experiment, during which they had unrestricted access to food and water.

2.2. Induction of Sciatic Nerve Crush Injury Model and Study Design

A modified procedure of sciatic nerve crush method, described in previous studies was used in this study to induce sciatic nerve crush injury [26, 15, 27, 28, 29]. Under general anesthesia, induced with ketamine (90 mg/kg), the rats were aseptically prepared by trimming the hair and using 20% iodine ethylic alcohol solution to clean the surgical area. The left sciatic nerve was bluntly exposed through a 2 cm long posterolateral longitudinal straight incision on the lateral aspect of the left thigh, after which a 3mm long sciatic nerve was uniformly clamped with straight and non-serrated heamostatic forceps by applying a steady pressure of 33N for 30 seconds, following which the crushed nerve was marked with loosely (non-constrictive) tied 2.0 suture. Thereafter, the incision was closed with sutures. The appearance of a translucent band across the clamped nerve indicated complete crush. Following induction, each rat was watched to ensure that the nerve had been completely crushed, which was demonstrated by the paralysis of the toe muscles and the absence of toes spreading. Rats with toe movement, which is a sign of an incomplete nerve crush was not used for the study. The rats were randomly grouped into five different groups: sham group (G1) represents the sham-operated group; negative control-14 group (G2) represents sciatic nerve crush + orally administered equivalent normal saline and observed for 14 days; negative control-28 (G3) represents sciatic nerve crush + orally administered equivalent normal saline and observed for 28 days; DRLC treated-14 group (G4) represents sciatic nerve crush + orally administered DRLC (100 mg/kg, orally) for 14 straight days and DRLC treated-28 group (G5) represents sciatic nerve crush + orally administered DRLC (100 mg/kg, orally) for 28 straight days.

2.3. Preparation and Dosage of DRLC

D-ribose L-cysteine was purchased from Max International (Salt Lake City, USA), it was freshly dissolved in normal saline (vehicle) and given orally at a dose of 100mg/kg per body weight The100, using the effective dose reported in studies [24].

2.4. Analyzing walking tracks

The sciatic function index (SFI) as described in previous studies was determined from walking track analysis. The rats were made to walk in the track box cardboard for five trials prior to the induction. At the conclusion of the study, one walking trial was used to evaluate the recovery of motor function. The rats were held by the chest and their hind feet was pressed against a stamp pad that has been inked with water-soluble black ink. The rats then proceeded to go through a narrow passage way (21 cm wide by 120 cm long) with a dark shelter at the end of the corridor to promote movement, leaving inked footprints on the paper.

The following measures were then made:

- the Print Length (PL), which is the distance from the heel to the third toe,
- ii the Toe Spread (TS), which is the distance from the first to fifth toe, and
- iii the Intermediate Toe Spread (ITS), which is the distance from the second to fourth toe.

The Bain formula [30] was used to determine each group's SFI value: SFI = (-38.3 x PLF) + (109.5 x TSF) + (13.3 x ITF) - 8.8 here PLF = (experimental PL - normal PL)/normal PL; TSF = (experimental TS - normal TS)/normal TS; and ITF = (experimental ITS - normal ITS)/normal ITS.

SFI values of 0 and -100 respectively, denote normal and total dysfunction [30, 31].

2.5. Biochemical procedures

Malondialdehyde (MDA) levels in serum and sciatic nerve tissue were determined using the techniques previously described. Serum MDA levels was represented as nmol/ml, and the red color produced by the interaction between thiobarbituric acid and the lipid peroxidation product MDA was quantified spectrophotometrically (Lot#:SG55431503, LOBA CHEMIE PVT. LTD. INDIA). MDA levels in sciatic nerve tissue was quantified as nmol/g of wet tissue using the VERSA max tunable microplate reader (Molecular Devices, LLC Sunnyvale, CA, USA) to calculate the absorbance of the color of the complex produced by MDA and thiobarbituric acid in an acidic environment at 532 nm.

A technique based on measuring the yellow complex generated by ammonium molybdate and hydrogen peroxide (H_2O_2) at 405 nm as previously published [32] was used to measure the levels of serum catalase (CAT) and sciatic nerve tissue. The results were expressed as U/mg protein. The method described by Sun was used to measure serum Superoxide Dismutase (SOD) activity and sciatic nerve tissue [33]. A VERSA max tunable microplate reader was used to detect the absorbance of the purple formazan molecule that results from the reduction of nitro blue tetrazolium by oxygen (O_2) and the xanthine oxidase at 560 nm. SOD activity was represented as U/mg protein and divided by the total protein concentration.

2.6. Gastrocnemius Mass Ratio

The posterior gastrocnemius muscles on the crushed and uncrushed hind limbs were harvested at the conclusion of the treatment periods, and promptly weighed to calculate the gastrocnemius wet-weight loss, is formula: Wet muscle weight of the gastrocnemius (WMW) was calculated as (WMW on the injured side / WMW on the uninjured side) x100%. [34, 35]. The group average value was used to determine the percentages.

2.7. Evaluation of the Sciatic Nerve and Gastrocnemius Histopathology

Sciatic nerve samples were taken and put in 10% buffered formalin solution. After the tissues had been fixed in solution, samples were collected for standard tissue processing. The tissues were left for 14 hours in the automated tissue processing device. The tissues were fixed in paraffin, following which 3–4 μm sections was prepared from the paraffin blocks with a microtome. Slides prepared from the sections was stained with hematoxylin-eosin for routine histopathological examination and the evaluation of axonal degeneration under 400× magnifications. The routine sections were histologically graded for axonal changes and myelin disorganization [36, 37]. The gastrocnemius muscle's histomorphometry will be evaluated since the degree of myoatrophy in target muscles is known to be a significant indicator of nerve injury and regeneration. The perfused rats' gastrocnemius mid-bellies were cut and post-fixed with

4% PFA for 24 h at C. The muscles were then transversally sliced into 10-mm slices and implanted with Tissue OCT-Freeze Medium. The myofiber areas were estimated when the sections were stained with 0.5% hematoxylin-eosin to reveal the myofiber outline and evaluated as described in previous studies [38].

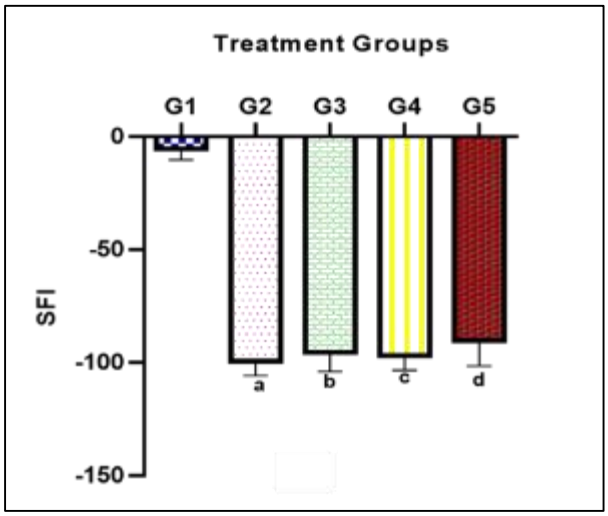
2.8. Statistical Analysis

Software from Graph Pad Software, Inc. called Graph Pad Prism 9 Biostatistics was used to analyze the data from this study. Data are presented as mean ±SEM. One-way analysis of variance (ANOVA) was used for additional analysis, followed by a post hoc test (Tukey's) for multiple comparisons. Statistically significant level was set at P< 0.05.

3. Results

3.1. Effect of DRLC on motor function recovery

The SFI scores of the groups were compared with each other. The negative control group observed for 14 days (G2) showed significantly lower SFI value when compared with sham group (G1). Significantly lower SFI were also seen in negative control group (G3) observed for 28 days, DRLC treated (14 days) group (G4) and DRLC treated (28 days) group (G5) group, when compared with G1 ($p<0.05$**), Figure 1.**

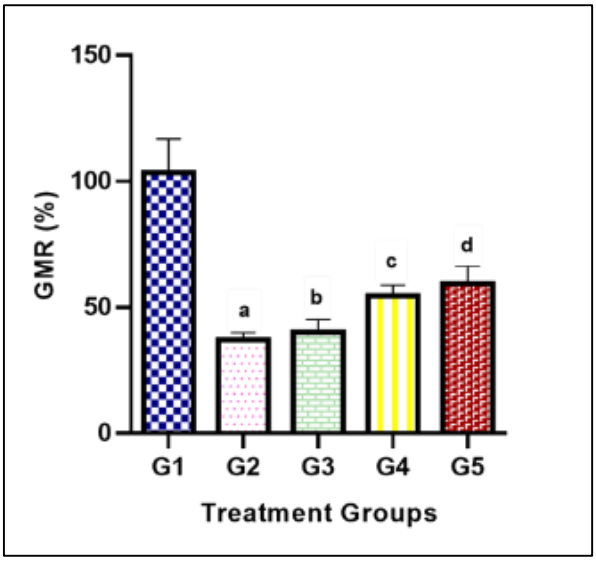


[a]P<0.05 vs. G1 group; [a]P<0.05 vs. G1 group; [b]P<0.05 vs. G1 group; [c]P<0.05 vs. G1 group; [d]P<0.05 vs. G1 group

Figure 1 Effect of DRLC on motor function recovery. Values are expressed as the mean ± standard error of the mean

3.2. Effect of DRLC on atrophic gastrocnemius

The percentage value of GMR of the groups were compared with each other. The negative control group observed for 14 days (G2) showed significantly lower GMR value when compared with sham group (G1). Significantly lower GMR values were also seen in negative control group (G3) observed for 28 days, DRLC treated (14 days) group (G4) and DRLC treated (28 days) group (G5) group, when compared with G1 ($p<0.05$). Though the GMR values of G4 and G5 appeared higher when compared with G2 and G3, but were not statistically significant, **Figure 2.**



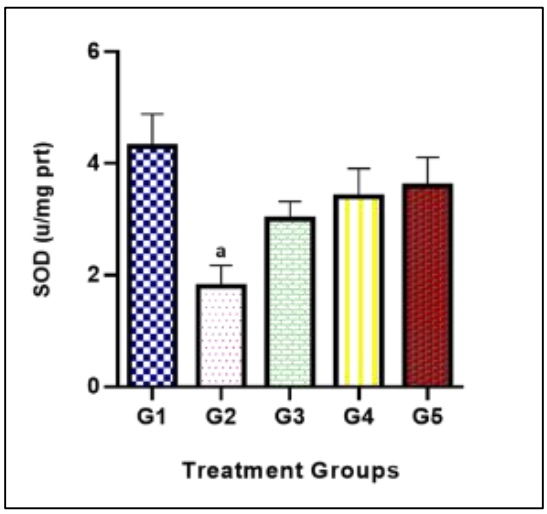
[a]P<0.05 vs. G1 group; [b]P<0.05 vs. G1 group; [c]P<0.05 vs. G1 group; [d]P<0.05 vs. G1 group

Figure 2 Effect of DRLC on atrophic changes in gastrocnemius. Values are expressed as the mean ± standard error of the mean

3.3. Biochemical analysis

3.3.1. Effect of DRLC on SOD activity

The activity level of SOD the sciatic nerve tissues of G2 group was significantly lower, when compared with G1 ($p<0.05$). when G2 and G3 groups were compared with G4 and G5 groups, higher SOD activity levels were observed, but they were not statistically significant, **Figure 3.**

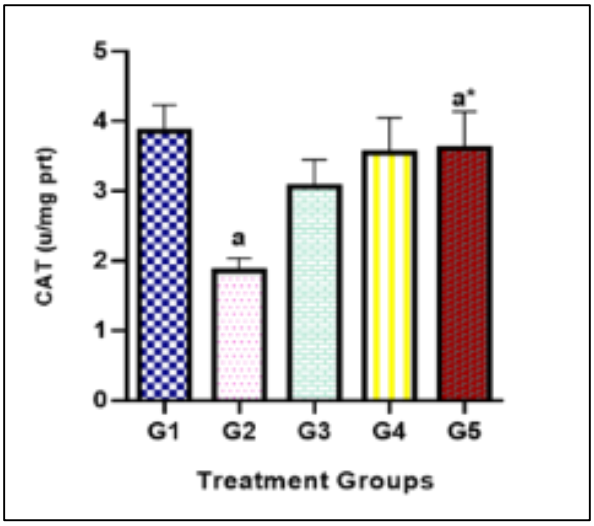


Values are expressed as the mean ± standard error of the mean. [a]P<0.05 vs. G1 group

Figure 3 Effect of DRLC on the activity level of SOD in the sciatic nerve tissue homogenate

3.3.2. Effect of DRLC on CAT activity

A significant difference was observed among the G1, G2 and G3 groups ($p<0.05$). sciatic nerve tissue CAT activity level was observed to be significantly lower in G2 group, when compared with G1 group. Also, CAT activity level was significantly higher in G5 group when compared with G2 group. No significant difference was observed among G2, G3 and G4 groups, even when the CAT activity appeared to be higher in G3 and G4 groups, **Figure 4.**

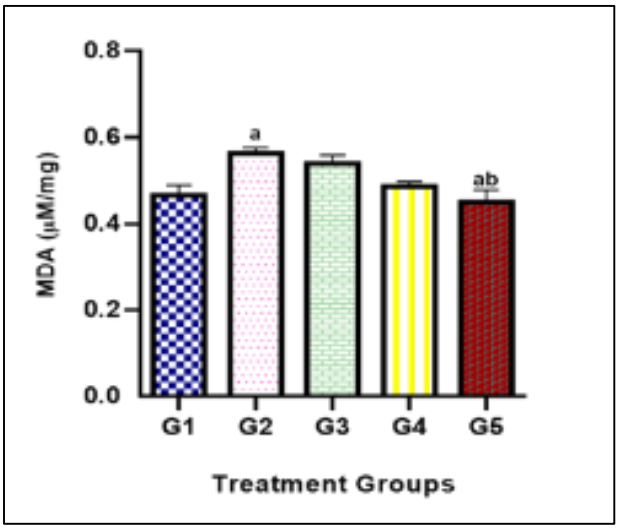


Values are expressed as the mean ± standard error of the mean. [a]P<0.05 vs. G1 group; [a*]P<0.05 vs. G2 group

Figure 4 Effect of DRLC on the activity level of CAT in the sciatic nerve tissue homogenate

3.3.3. Effect of DRLC on MDA activity

Sciatic nerve tissue MDA activity level in control and test groups were statistically significant (p<0.05). The MDA concentration was significantly higher in the G2 group when compared with the G1 group. Also, the activity level concentration of MDA was found to significantly lower in G5 group, when compared with G2 and G3 groups (p>0.05), **Figure 5.**



Values are expressed as the mean ± standard error of the mean. [a]P<0.05 vs. G1 group; [a]P<0.05 vs. G1group; [ab]P<0.05 vs. G2 group; [ab]P<0.05 vs. G3 group

Figure 5 Effect of DRLC on the activity level of MDA in the sciatic nerve tissue homogenate

3.4. Histological Evaluation of sciatic nerve

At the end of the observation, the left sciatic nerves were removed from all the groups, were Hematoxylin-eosin (HE) stained and the microscopic images of transverse-sections were examined **(Fig. 6).** The nerve section of G1 group showed mixed axons (AX) organized in fascicle, each fascicle is covered by the endoneurium (Arrow head), Schwann cell (Arrow) and separated by the loose connective tissue. Nerve fascicles are encapsulated in a thick perineurium (PN) containing branches of blood vessels. These features are consistent with normal peripheral nerve histomorphology. G2 group section is characterized by thickening of the perineurium (TPN), zonal axonal loss (ZAL) and marked axonal degenerative changes (Circle). Features consistent with Wallerian Degeneration (stage 1). Also, the nerve section of G3 group, showed thickened perineurium (PN), degenerating nerves (DGN), marked axonal degenerative changes (Circle) and edema (Star). While the G4 group nerve section showed mixed axons (AX) organized in fascicle, sheathed by the

endoneurium (Arrow head), Schwann cell (Arrow) and separated by the loose connective tissue. Nerve fascicles are encapsulated in a thick perineurium containing branches of blood vessels. Section is free from inflammatory cell infiltration, however characterized by mild axonal thickening and myelin ballooning.

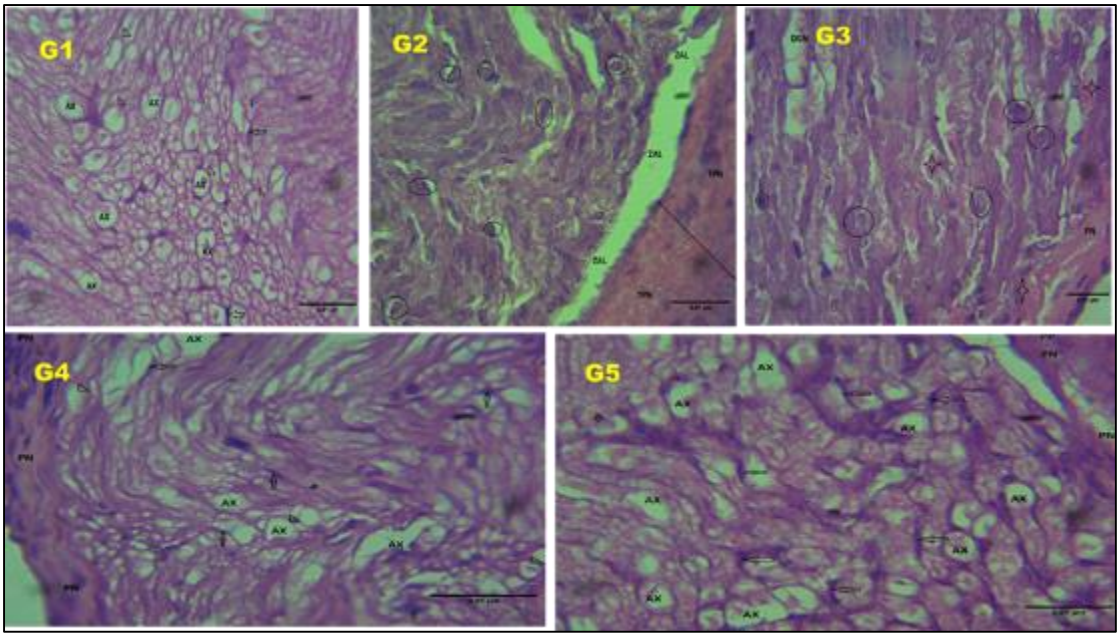


Figure 6 Representative micrographs (×400) of transverse sections of sciatic nerves of the rats in groups G1, G2, G3, G4 and G5 after hematoxylin-eosin staining. Marked axonal degeneration (Circle), vacuolization (AX) and myelin destruction (arrow heads) were found to be more severe in G2 and G3 groups, but mild in G4 and G5 groups, when compared with G1 group

3.5. Histological Evaluation of Gastrocnemius Muscle

Hematoxylin-eosin (H&E) stained and the microscopic images of section of the left hind gastrocnemius showed skeletal muscle organized in fascicles, skeletal myofibrils (SMF) are composed of large, elongated, branching and cylindrical cells with peripherally placed nuclei (N). Fibers are covered in a thin endomysium and separated by a loose connective tissue interstitium (INT) bearing networks of vessels (arrow head) and nerves **(fig 7).** Myofibrils alignments appeared essentially unremarkable, no evidence of edema and inflammatory cell infiltration in G1 group. In G2 and G3 groups, the micrographs showed skeletal muscle organized in fascicles, skeletal myofibrils (SMF) are composed of large, elongated, branching and cylindrical cells with peripherally placed nuclei (N). Fibers are covered in a thin endomysium and separated by a loose connective tissue interstitium (INT) bearing networks of vessels (arrow head) and nerves, characterized by inflammatory cell infiltration (circle), and mild vascular congestion, and these features are consistent with inflammatory response and atrophic changes. While the sections of G4 and G5 groups, showed skeletal muscle organized in fascicles, skeletal myofibrils (SMF) are composed of large, elongated, branching and cylindrical cells with peripherally placed nuclei (N). Fibers are covered in a thin endomysium and separated by a loose connective tissue interstitium (INT) bearing networks of vessels (arrow head) and nerves. Section characterized by surveilling white blood cell (arrow) while muscle showed atrophic changes, features that are consistent with cellular adaptation to injury.

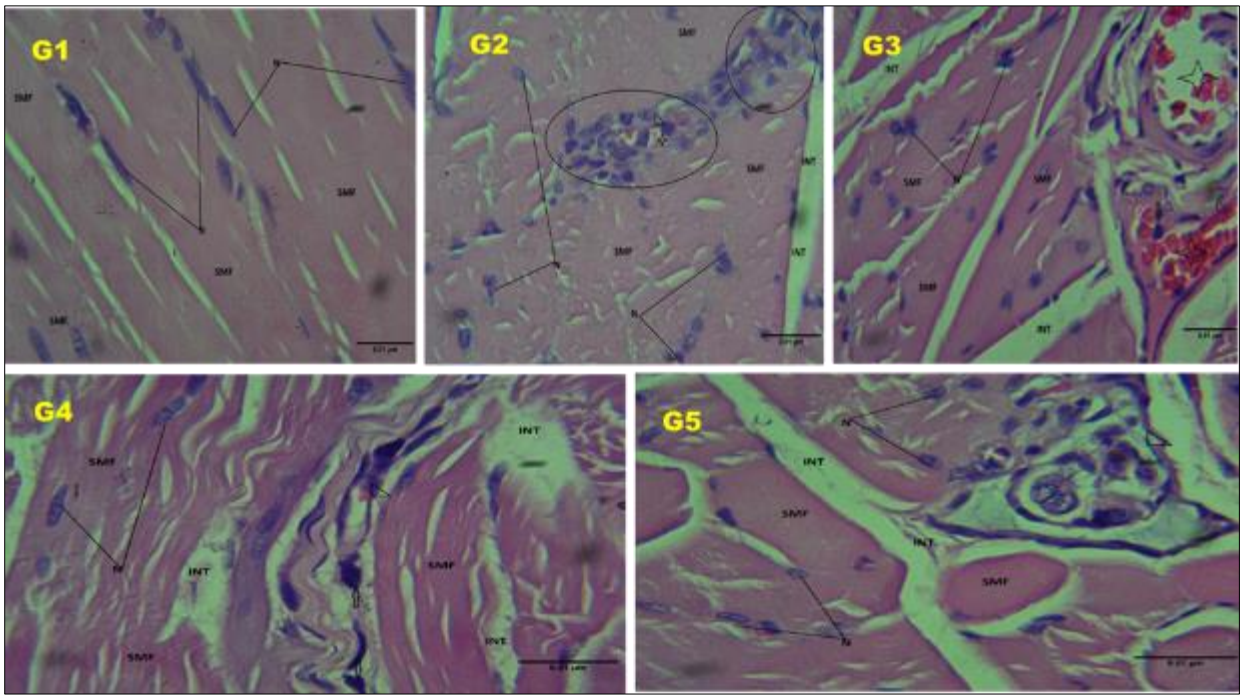


Figure 7 Representative micrographs (×400) of Hematoxylin-Eosin stained sections of gastrocnemius of the rats in G1, G2, G3, G4 and G5 groups

4. Discussion

One of the most prevalent and difficult health problems in the world is Injury (PNI) [39]. It's particularly difficult because till date, there is no standard medication or medical procedure that can cure peripheral nerve damage [40, 39]. Despite incredible progress attained in understanding the pathophysiology of PNI and medical intervention proffered so far, satisfactory optimal functional recovery is still difficult to achieve in most cases. Slow rate of axonal regeneration and muscular atrophy, among other factors, have been implicated by studies on axonotmetic model of peripheral nerve injury in Wistar rats, as key roadblocks to achieving a full recovery of motorsensory functions [41, 42]. Thus, the effort to identify effective and safe drugs that can accelerate the axonal regeneration rate, has increasingly become an area of research interest.

In crush type injury of a traumatic sciatic nerve injury, the pathological mechanism is not fully understood yet. However, two pathological mechanisms may be responsible in this crush injury: mechanical compression and ischemia [43]. It was demonstrated that both acute and chronic compression of sciatic nerve, causes disruption of Blood-Nerve Barrier and oedema within the nerve, which then causes decrease in oxygenation and morphological changes [44]. These processes cascade into formation of oxidative stress and inflammation, which are closely related pathophysiological processes, one of which can be easily induced by the other. Hence, when peripheral nerve damage induces an inflammatory response, oxidative stress will also be activated. Oxidative stress encompasses the production of Reactive Oxygen and Nitrogen Species, such as hydrogen peroxide, superoxide radicals, hydroxyl radicals, and nitric oxide radicals which imposes cellular damage [45, 46].

Although crushed sciatic nerves in experimental Wistar rats keep anatomical continuity, regenerate spontaneously and somehow reinnervate their target tissues, the longer it takes for the crushed nerve to reinnervate their target tissues, the greater the chance of permanent denervation atrophy of the target tissues [46]. Therefore, accelerated nerve regeneration is crucial to obtaining satisfactory functional outcomes. Studies on crush injury models in peripheral nerves have shown better functional recovery when therapies were directed against ischemia-reperfusion injury by using antioxidants, lipid peroxidation inhibitors and anti-inflammatory agents [47]. With this in mind, the present study evaluated the Neuro-regenerative effect of DRLC on crushed sciatic nerve, which has been previously reported to exhibit antioxidative and anti-inflammatory activity in neuro-degenerative diseases. In lipopolysaccharide and scopolamine induced amnesia, inhibition of oxidative stress and pro-inflammatory biomarkers was observed to have been influenced by DRLC [24, 48]. In mice exposed to unpredictable chronic mild stress, DRLC was found to modulate corticosterone-mediated oxido-inflammatory processes downward [25]. In a manganese-induced cognitive and motor deficit, DRLC

was reported to ameliorate the oxidative stress that was secondary to brain cells neurotoxicity [49, 50], concluded that DRLC attenuated oxidative stress-driven alterations linked with Paradoxical sleep deprivation (PSD) through its antioxidative properties.

Based on literature search and to the best of our knowledge, no study has been done on neuro-regenerative effect of DRLC on sciatic nerve crush injury in Wistar rats. In this present study therefore, the effect of DRLC on Oxidative Stress (CAT, SOD and MDA) biomarkers, Gastrocnemius Mass Ratio, Motor Function Assessment (motor: SFI) and histopathological changes in nerve and gastrocnemius tissues, were examined in a mechanically induced sciatic nerve crush injury in rats.

4.1. Assessment of neuro-regenerative effect of DRLC on Motor function in mechanically induced sciatic nerve dysfunctions in Wistar rats

The sciatic nerve is a mixed type of nerve that contains both sensory and motor nerve fibres. Therefore, following sciatic nerve injury, evaluation of motor function recovery is very crucial [51, 52]. Motor function distal to the point of sciatic nerve crush injury, are either partially or completely lost. This motor dysfunction result from axonal disruption and subsequent degeneration [53]. Sciatic Functional Index (SFI), is determined by walking tract analysis, which is a quantitative and non-invasive method, used in assessing motor function recovery in sciatic nerve crushed rats [54, 55]. In this present study, evaluation of SFI in G2, G3, G4 and G5 groups, showed statistically significant decrease in SFI value, when compared with G1 group respectively. No significant difference was seen between the 14- and 28-days negative control groups and the treated groups, when compared. The observed statistically significant differences in the SFI values, suggest a moderate or severe motor dysfunction, that may have resulted from the complication of denervation that is associated with induced sciatic nerve crush injury in rats. This finding agreed with *Li et al* [56], who reported significant motorsensory dysfunction in negative control rats, following experimental sciatic nerve crush injury. In an experimental study of sciatic nerve crush injury, a significant motorsensory dysfunction was also reported [57].

4.2. Assessment of Neuro-Regenerative Effect of DRLC on Gastrocnemius Mass Ratio of the Wistar Rats

The gastrocnemius muscle is supplied by the posterior tibial branch of the sciatic nerve, and its denervation can occur as a consequence of sciatic nerve injury, leading to series of histopathological and biochemical alterations that culminate in muscle atrophy, that is indicated by reduced gastrocnemius mass [58]. If reinnervation is restored, the muscle will regain its function and atrophy will be attenuated. In sciatic nerve crush injury, where the neural tube of the nerve is preserved, there is higher chance of axonal reinnervation and restoration of the neuromuscular function [56]. Atrophy level in denervated gastrocnemius is determined quantitatively by Gastrocnemius Mass Ratio (GMR) of crushed and normal limbs [7]. In the present study, statistically significant decrease in gastrocnemius Mass Ratio was observed in G2, G3, G4 and G5 groups, when compared with G1 group respectively. This observed gastrocnemius mass reduction, may have resulted from the sciatic nerve damage, caused by the crush effect of heamostatic forceps used in inducing this model of PNI. Though the G4 and G5 showed increase in GMR value when compared with the negatively controlled groups, but the different is not significant. Our results are similar to the findings of other researchers who have also used forceps to induce crush sciatic nerve injury models [28, 35]. This observation showed that DRLC did not have statistically significant effect on the gastrocnemius atrophy that resulted from the sciatic nerve crush damage in the rats. But when each group was compared with G1 group, statistically significant decrease in gastrocnemius Mass Ratio were observed. This results again established the induced sciatic nerve crush injury in the rats.

4.3. Assessment of neuro-regenerative effect of DRLC on activities of oxidative stress markers (CAT, SOD and MDA) in induced sciatic nerve crush injury in Wistar rats

The characteristic increase in production of hydrogen peroxide, superoxide, hydroxyl and nitric oxide radicals that tilts the redox homeostasis towards oxidants, following sciatic nerve crush injury, defines an oxidative stress state in the damaged nerve [42]. These increased oxidants go on impair the nerve membrane, and if sustained at this increased level of activity, increased production of MDA and negative balance level of antioxidants like CAT, SOD and GSH will prevail [59]. The antioxidative effect of DRLC has been reported in previous neuropathologic studies, with findings that suggests its protective, ameliorative and anti-inflammatory influence in pathologies that developed via oxido-inflammatory process.

The effect of DRLC on the serum level of CAT, SOD and MDA in this present study, showed CAT nerve tissue activity level that was significantly low, when the G2 group was compared with the sham group. Also observed is the significant increase of CAT activity level in G5 group, when compared with the 14 days negative control group. Nerve tissue SOD activity level was significantly low in G2 group, when compared with G1. Though SOD activity was seen to be high in the DRLC treated G4 and G5 groups, when compared with G2, but it was not statistically significant. The nerve tissue MDA

activity level in G2 and G3 groups were observed to be high when compared with G1, but it was only statistically significant in G2 group. The comparison among G2, G3 and G5 groups, showed a significantly low activity level of MDA in G5, when compared with G2 and G3 groups respectively. This finding again corroborates the results seen in GMR and motor evaluation, which also align with previous findings by other researchers. In a study that evaluated the effect of Intraperitoneal Alpha-Lipoic Acid on sciatic nerve crush injury, the authors reported a statistically significant increase in nerve tissue MDA level in untreated rat group, which they believe to be an indication of oxidative stress [60]. Also, *Chen et al* [61], reported statistically significant increase in MDA levels in the crushed group when compared when sham group. It has been suggested that the mechanism of this significant increase in MDA, may be due to nerve compression, induced by the disruption of blood nerve-barrier and nerve oedema with a decrease in sciatic nerve oxygen supply and morphological derangement [62, 63]. The significantly low MDA activity level observed in nerve tissue of G5 group, when compared with G2 and G3 groups, may have resulted from the antioxidative effect of DRLC. The statistically significant and high activity level of CAT and SOD observed in the DRLC treated groups treated with DRLC, when compared with the negative control groups, may have occurred as a result of ameliorative effect of DRLC.

4.4. Assessment of neuro-regenerative effect of DRLC on Histopathological alterations of the sciatic nerve and gastrocnemius tissues of the Wistar rats

The histopathological micrographs of the sciatic nerve and gastrocnemius tissues of the rats used in this present study, showed significant and obvious difference in terms of the myelin sheath, axon, myofibril and vasculature of the nerve and gastrocnemius tissues, when the sham control group, the negative control groups and the treated groups were compared respectively. The myelin sheath and the axons of the negative control and treated groups, when compared with the sham control group, showed sciatic nerve degenerative changes, while same comparison showed atrophic changes in the gastrocnemius. However, sciatic nerve and gastrocnemius micrographs of the DRLC treated groups, showed features of regeneration of axons and recovery of muscle fibers. These findings aligned with the Motor Function Recovery and Gastrocnemius Mass Ratio results reported in this study. Previous studies have also reported histopathological changes, using the difference in the morphology of the myelin sheath, axons and myofibrils, following sciatic nerve injury [64, 65, 66]. *Yüce et al* [62] reported the derangement of myelin sheath, loss of myelinated fibres and absence of myelin ovoid and cytoskeleton by the end of 7 days, post sciatic nerve crush damage.

In this present study and in summary, previously used procedure for inducing axonotmetic model of sciatic nerve injury, was modified and adopted. The results obtained from the set general objectives, established an axonotmetic sciatic nerve injury in the experimental rats used for this study, as indicated by the statistically significant difference observed between the sham and negative control groups. These results aligned with findings of previous studies that used induction method we modified, that not only confirmed axonotmetic model of sciatic nerve injury, but also reported optimal and suboptimal time bound motorsensory function recovery in the untreated nerve crush injured rats [67, 68, 69, 70, 71]. A study reported optimal functional recovery of the sciatic nerve approximately 14 - 21 days after sciatic crush injury in untreated rats [72, 73]. *Hadlock et al* [74] in their study, reported that on the 34th day post-induction, functional recovery in untreated rats plateaued and the pre-crush functional level was not attained at the end of the experiment. Though we established sciatic nerve axonotmetic damage in this study, we however did not observe optimal motor function recovery in the untreated rats that had their sciatic nerve damaged at the end of this study. Therefore, the difference in the normal motor function recovery in untreated rats, observed in this study and previous studies may be associated with the experimental conditions, the pathophysiological response of sciatic nerves to different crushing force intensities and different methods used in inducing nerve injury, as no standard method of inducing axonotmetic model of sciatic nerve injury has been established [75, 3].

5. Conclusion

This study concluded that DRLC showed significant ameliorative effect on the oxidative stress markers in injured sciatic nerve of Wistar rats, but not in reversing atrophic and degenerative changes in gastrocnemius and nerve tissues or restoring motor functions. This is evidence that the use of DRLC as dietary supplement to facilitate or enhance neuro-regeneration in human subjects, may produce ameliorative effect in biochemical complication. Informed use of DRLC as dietary supplement is hereby advised. However, the potential therapeutic benefit of DRLC in the management of functional complications associated with peripheral nerve injuries, is recommended for further investigation.

Compliance with ethical standards

Acknowledgments

The authors are grateful to members of staff of Concrete and Material Testing Laboratory, Civil Engineering Department, Faculty of Engineering, Delta State University, Oleh, Nigeria and Pharmacology Department, Delta State University, Abraka, Nigeria, for their support and contribution.

Disclosure of conflict of interest

There are no conflicts of interest

Statement of ethical approval

Ethical approval (RBC/FBMC/DELSU/23/200) for this study was obtained from Research and Bio-Ethics Committee of the College of Health Sciences, Delta State University, Abraka, and was carried out at the animal facility of Faculty of Basic Medical Sciences, Department of Physiology, Delta State University, Abraka, Nigeria.

Consent for publication

All the authors have approved that this manuscript be published

Availability of data and material

Data for the Study will be provided by the corresponding author on request.

Author's contributions:

- ABT conceptualized, conducted the study and prepared the manuscript
- SOO and BCN assisted in the functional assessment procedure
- OSI prepared the methodology and supervised the study
- OLO assisted in the surgical procedure
- ACP supervised the study.

All authors approved the submission of this manuscript.

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