IBRO Neuroscience Reports 18 (2025) 512–519

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

Research paper

N-acetyl-L-cysteine mitigates diabetes-induced impairments in sciatic nerve

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ARTICLE INFO

Key words:
Diabetes
Enzymes
Lipids
N-acetylcysteine
Neuropathy
Sciatic Nerve. Membrane

ABSTRACT

Diabetic neuropathy is a consequence of long-term hyperglycemia. The emergence of neuronal condition is a result of hyperglycemia-induced oxidative stress. In the present study, streptozotocin-induced diabetes exhibited notable decrease in the levels of phospholipids, glycolipids, gangliosides, and triglycerides in the sciatic nerve. The alterations in lipids resulted in increase in cholesterol to phospholipid ratio in sciatic nerve of diabetic animals. This ratio is crucial and determines the rheological properties of membranes and resulted in substantial reduction in the activity of membrane-bound enzymes; Ca^{2+} ATPase and acetylcholinesterase. Histological examination of the cross-section of the sciatic nerve in diabetic mice revealed axonal atrophy and disarrayed myelin sheath. The potential therapeutic impact of N-acetyl Cysteine (NAC), a powerful antioxidant, on a rat model of diabetic neuropathy was evaluated. NAC was administered to rats in drinking water for a period of 8 weeks. The results indicate that administration of NAC restored lipid composition; ratio of cholesterol to phospholipids, the activity of membrane linked enzymes, and improved the structural defects in sciatic nerve. NAC plays protective role against diabetes-induced alterations in lipid composition in sciatic nerve membranes leading to improvement in structure and function of membranes. Overall, the findings suggest NAC as a potential therapeutic strategy in preventing diabetic neuropathy and other diabetic complications.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by high glucose levels in the blood due to insufficient production or effectiveness of insulin. The global population affected by diabetes currently exceeds 500 million and this number is projected to rise significantly and reach at least 1.3 billion by 2050 (Ong et al., 2023). The morbidity and mortality of diabetes is because of both macrovascular and microvascular complications (Mansour et al., 2023). Diabetic peripheral neuropathy (DPN) is one of the most common microvascular complications of diabetes affecting almost one third to one half of adults with diabetes (Feldman et al., 2019). DPN involves both somatic and autonomic peripheral nerves and is characterized as progressive axonopathy. The predominant type of diabetic neuropathy is a distal, symmetrical, sensorimotor polyneuropathy that preferentially affects sensory function in the distal regions (Itani et al., 2022). Patients may experience painful burning and tingling sensations, but all of them eventually lose normal touch and temperature sensation, leading to accidental personal injuries and impaired quality of life including paresthesia, pain, or sensory loss in the extremities (Sloan et al., 2018). The incidence of neuropathy increases with duration of diabetes and is exacerbated by poor glycemic control (Babizhayey et al., 2015)

Neuropathic damage caused by diabetes is associated with pathophysiological mechanisms involving various metabolic and signaling processes. Oxidative stress (OS) is one of the key contributing factors to the development of DPN (Pang et al., 2020; Zhu et al., 2024). Hyperglycemia induced enhanced formation of pyruvate causes increase in mitochondrial membrane potential that eventually leads to excess formation of super oxides and reactive oxygen species (ROS). The increased OS entails cascade of events culminating into apoptosis of neurons and schwann cells in the peripheral nervous system (Babizhayev et al., 2015). The DPN involves distal axonal loss and centripetal degeneration of the nerves (Malik et al., 2005). Since such degeneration is length dependent, the longest nerve fibers like sciatic and sural nerves remain at the greater risk (Smith et al., 2022)

N-acetyl Cysteine (NAC) is a known thiol antioxidant that acts as a precursor for a natural antioxidant glutathione and has been shown to scavenge hydrogen peroxide and hydroxyl radicals *in vitro* (Liu et al., 2019). It is also a powerful antioxidant and a potential treatment option for diseases characterized by the generation of free oxygen radicals

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https://doi.org/10.1016/j.ibneur.2025.02.006
Received 27 August 2024; Received in revised form 14 February 2025; Accepted 15 February 2025
Available online 21 February 2025
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(Shahin et al., 2009). NAC has been shown to be extremely effective in wide range of pathologies including neurodegenerative disorders (Martinez-Banaclocha, 2022; Monti et al., 2020), diabetic encephalopathy (Kamboj et al., 2008) and myocardial dysfunction (He et al., 2020) etc. We have previously reported that NAC is beneficial in attenuating oxidative stress mediated apoptotic changes in the sciatic nerve (Kamboj et al., 2010). The present study was designed to understand changes in sciatic nerve membrane lipid composition in Streptozotocin (STZ) induced diabetic rodent model and to evaluate the potential of a NAC in reversal of perturbations in lipid composition, activity of membrane bound enzymes and morphological alterations in sciatic nerve of diabetic animals.

2. Materials and methods

2.1. Chemicals

All the chemicals used in the present study were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, USA), Merck (Mumbai, India) and Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Streptozotocin (STZ) and NAC was purchased from Himedia Laboratories Pvt. Ltd (Mumbai, India).

2.2. Animals and induction of diabetes

Male wistar rats between 180 and 200 g (age 4–5 month) were procured from the Central Animal House of the University. The animals were given *ad libitum* access to standard laboratory diet (Ashirwad Industries, India) and drinking water. The study was approved by the Institutional Ethical Guidelines for Humane Use and Care of Laboratory Animals. Diabetes was induced in the overnight fasted rats by a single intraperitoneal injection of STZ (50 mg/kg body weight, dissolved in citrate buffer, 0.1 mol/l, pH 4.5). Control animals received equal volume of citrate buffer. STZ injected animals were given 5 % (w/v) glucose in drinking water for 24 hrs. Hyperglycemia was confirmed by elevated plasma glucose levels after 72 hours of STZ injection by using glucose oxidase peroxidase kit. The animals with plasma glucose values $>$ 15 mM were only considered diabetic and included in the study.

2.3. Experimental design

Animals were randomly divided into four groups of 6–8 animals each; control, control + NAC treated diabetic and diabetic + NAC treated group. One week after induction of diabetes, NAC was administered to the control (control + NAC) and diabetic (diabetic + NAC) in the drinking water for 7 weeks, daily. NAC concentration was kept to be 1.4–1.5 g/kg (average 1.41 ± 0.1 g/kg/day). The dose of NAC used in the study is based on preliminary studies and earlier studies (Kamboj et al., 2008; 2010). After 8 weeks, the animals were fasted overnight and were sacrificed by decapitation under light ether anesthesia (Experimental Design is shown in Fig. 1). The sciatic nerve was removed, rinsed in ice-cold isotonic saline, and stored at −80°C for further biochemical analyses.

2.4. Biochemical assays

2.4.1. Lipid Profile

Extraction of lipids: Lipids were extracted from Sciatic nerve according to the method of Folch et al. (Folch et al., 1957). Briefly, lipids from tissues were extracted with chloroform-methanol mixture (2:1, v/v). The extract was washed with 0.2 volumes of 0.88 (w/v)% KCl and was left overnight to separate into two layers. The upper aqueous layer was removed with a Pasteur pipette without disturbing the interfacial fluffy appearance. The lower layer was washed with a mixture of chloroform: methanol: water (3:48:47, v/v). The extracted lipids were dissolved in 5 ml of chloroform mixture and aliquots were used for the estimation of various lipid components.

2.4.1.1 Cholesterol: Cholesterol was estimated according to the method described by Zlatkis et al. (Zlatkis et al., 1953). Cholesterol in the presence of concentrated sulphuric acid and glacial acetic acid forms a violet-coloured complex with ferric chloride. The reaction involves initial dehydrogenation of cholesterol to 3, 5-cholestadiene or 2,4-cholestadiene which polymerises to dimer or trimer. The polymers react with $FeCl_3$-H_2SO_4 mixture to form a coloured complex, which is measured at 540 nm. The cholesterol content was expressed as mg/g tissue.

2.4.1.2 Phospholipids: Phospholipids were estimated by the method of McClare (McClare, 1971). In this method the organic phosphorus of phospholipids is converted to inorganic phosphorus by digesting with perchloric acid. The inorganic phosphate released was estimated by the method of Fiske and Subbarow (Fiske & Subbarow, 1925). The phospholipid content was expressed as mg/g tissue.

2.4.1.3 Triglycerides: Triglycerides were estimated according to the method of van Handel and Zilversmit (Van Handel & Zilversmit, 1957). Triglycerides are hydrolyzed by alcoholic potassium hydroxide and glycerol released is oxidized with periodic acid to form formaldehyde. The formaldehyde formed is the treated with chromotropic acid to form a pink coloured derivative which is measured at 570 nm. The triglyceride content was expressed as mg/g tissue.

2.4.1.1. Glycolipids. Glycolipid estimation was done according to the method of Dubois et al. (Dubois et al., 1951). Simple sugars, oligosaccharides, polysaccharides, and their derivatives including the methyl ethers with free reducing groups give furfural derivatives in presence of sulfuric acid. Furfural then condenses with phenol to form a colored complex that is measured at 490 nm. Glycolipids were calculated using the glucose as the standard expressed as mg/g tissue.

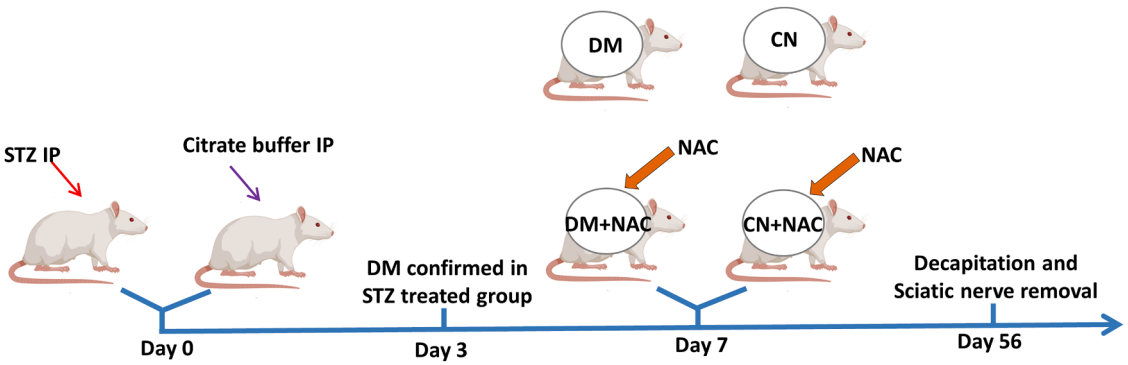

Fig. 1. Experimental Design. Streptozotocin (STZ), N-acetyl cysteine (NAC) Control group (CN), Diabetic group (DM), NAC administered control group (CN+NAC) and, NAC administered diabetic group (DM+NAC).

2.4.1.2. Gangliosides. Ganglioside extraction was done by the method of Folch et al. (Folch et al., 1957) and estimated according to the method of Skoza and Mohos (Skoza & Mohos, 1976). Sialic acid bound to gangliosides was hydrolyzed by heating at 80ºC for 2 hours with sulphuric acid. On oxidation of sialic acid with periodate the deoxypentose sugar of sialic acid is converted to malonaldehyde, which gives a pink colored complex with thiobarbituric acid. The absorbance was measured at 549 nm. Ganglioside content was calculated using the N-acetylneuraminic acid (NANA) as standard and expressed as µmoles of NANA/g tissue.

2.4.2. Enzyme assays

2.4.2.1 Ca^{2+}ATPase activity: Ca^{2+}-ATPase was assayed in the homogenate as described by Sandhir and Gill (Sandhir & Gill, 1994). The total ATPase was assayed in a reaction mixture containing 40 mM Tris HCl buffer (pH 7.5), 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, requisite amount of crude synaptosomal fraction and 2.5 mM ATP. The reaction mixture was incubated at 37 °C for 15 min and 0.1 ml of TCA was added to stop the reaction. The contents were centrifuged at 3000 rpm for 10 minutes and the inorganic phosphorus released was estimated by the method of Fiske and Subbarrow (Fiske & Subbarow, 1925).

2.4.2.2 Acetylcholinesterase (AChE): AChE assay was performed in the homogenate according to the method of Ellman et al. (Ellman et al., 1961). Briefly, 14.9 mM acetylthiocholine iodide was added to the reaction mixture containing 0.1 M phosphate buffer, 10 mM DTNB and appropriate amount of sample to initiate the reaction. The increase in absorbance was followed at 412 nm for 3 min. AChE activity was calculated using molar extinction co-efficient of 5-mercapto-2-nitrobenzoate (13.6×10^3 M^{-1} cm^{-1}) and the results were expressed as nmoles substrate hydrolyzed/min/mg protein.

2.4.3. Estimation of protein

The protein content was estimated according to the method of Lowry et al. (Lowry et al., 1951) using bovine serum albumin as standard

2.5. Histopathological studies

Histopathological studies were performed on the transverse section of sciatic nerve to evaluate and structural changes in sciatic nerve. Sciatic nerve was dissected and kept in chilled normal saline. It was transversely cut into small pieces and fixed in Bouin's fluid. After embedding them in Paraffin wax, sections of 5 micron thickness were cut using microtome. These transverse sections were then stained with Hematoxylin and Eosin for 20 min and 30 min, respectively were observed under the light microscope (40X magnification). The internodal diameter was measured using ImageJ software (NIH, Bethesda, MD).

2.6. Statistical analysis

Values are expressed as mean ± S.D. of six animals per group. Data was analyzed using one way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple pairwise comparisons between the various treated groups. Values with $p < 0.05$ were considered as statistically significant. The statistical analyses were performed using MS Excel 2007 software.

3. Results and discussion

Eight days after induction of diabetes, increased blood glucose level, weight loss, impaired motor coordination and increased thermal nociception were observed, suggesting development of neuropathy. NAC supplementation in diet reversed these manifestations to a significant level. Lipidomic analyses of sciatic nerve from control, control + NAC, Diabetic and diabetic treated with STZ shows significant alterations in lipid composition in diabetic group viz. significant reduction in triglycerides, glycolipids and gangliosides.

3.1. Lipid profile

3.1.1 Cholesterol: As shown in Fig. 2, cholesterol levels were found to be significantly altered in diabetic animals (150.67 %). Alterations in cholesterol levels are detrimental to neuronal function (Fernández-Pérez et al., 2018; Marquer et al., 2014). NAC treatment ameliorated increase in cholesterol levels. The levels were restored close to control levels. A decrease by 75.44 % than untreated diabetic group after NAC administration was observed.

3.1.2 Triglycerides: Triglyceride levels have been shown to be increased in brain and liver of Diabetic animals (Ghebremeskel et al., 2002; Malaisse et al., 2006). A decrease of 35 % was observed in sciatic nerves of diabetic animals (Fig. 2). Lower triglyceride levels in sciatic nerve; correlate with reduction of peripheral nerve sensation in diabetic rats. Depletion of triglycerides in sciatic nerve contrasts with the accumulation of fat in most of the other organs during the development of diabetes suggesting bidirectional re-distribution of triglycerides in pathogenesis. Administration of NAC to diabetic animals causes a significant increase of 30 % in number of triglycerides in Sciatic nerve.

3.1.3 Glycolipids: Glycolipids are a broad class of molecules including glycosphingolipids. It is believed that the formation of neuromuscular junction is based on a highly specific recognition process that involves glycosphingolipids. Sulfatides have been implicated in binding of thrombospondin (Roberts, Haverstick, et al., 1985) and laminin (Roberts, Rao, et al., 1985). Therefore, it is anticipated that any change in glycolipids affects the property and function of nervous system. As shown in Fig. 2, Diabetic group showed a significant decrement of 18.7 % in glycolipids level than control group. NAC treatment to diabetic animals shows significantly improved in glycolipid levels (12 % as compared to untreated diabetic animals).

3.1.4 Gangliosides: Gangliosides have been implicated in a variety of cellular functions including neurotransmission and contribute to transduction of information across the membranes (Thomas & Brewer, 1990). Gangliosides are distributed in both the myelin and the neurons. As shown in Fig. 3, the level of gangliosides that could be from both neurons and myelin in diabetic animals was significantly lower (33.34 % of control). The low levels in STZ treated animals may affect the protein phosphorylation and consequently produce an alteration in normal structure and function of myelin sheath. NAC administration to animals showed a significant increase in the gangliosides level by 17.4 % (of untreated diabetic animals). It has been shown that structural integrity of myelin depends upon phosphorylation of myelin basic protein (Chan, 1987). In the peripheral nervous system, alterations in myelin ganglioside have been linked with pathologies like demyelination and axonal degeneration (Garbay et al., 2000). In the neurons, gangliosides are majorly the functional components of membrane lipid rafts that control critical functions in cell communication (Fantini, 2023) while the intracellular ganglioside is involved in the modulation of intra-nuclear and intracellular calcium homeostasis (Ledeen & Wu, 2008). Further, many neuronal disturbances have been shown to be associated with their metabolism such as Tay-Sachs and Lafora disease (Suzuki, 1984). Gangliosides have been shown to influence phosphorylation of these proteins (Chan, 1987). Therefore, alterations in the composition of gangliosides and cholesterol may influence signaling pathways thereby affecting membrane functions and normo-physiology of the neuron.

3.1.5 Cholesterol/Phospholipids ratio: This ratio is responsible for membrane rheology and architecture, reviewed by Oldfield and Chapman (Oldfield & Chapman, 1972). The proper distribution of axonal membrane proteins requires the formation of sphingomyelin/cholesterol-rich microdomains, lipid rafts. Studies also suggest that a proper Cholesterol/Phospholipid ratio like physiological conditions regulates function of GTP binding protein (Cumings, 1955) by inducing a change in the physical state of lipid bilayer which would favor the formation of suitable confirmation of GTP binding protein with

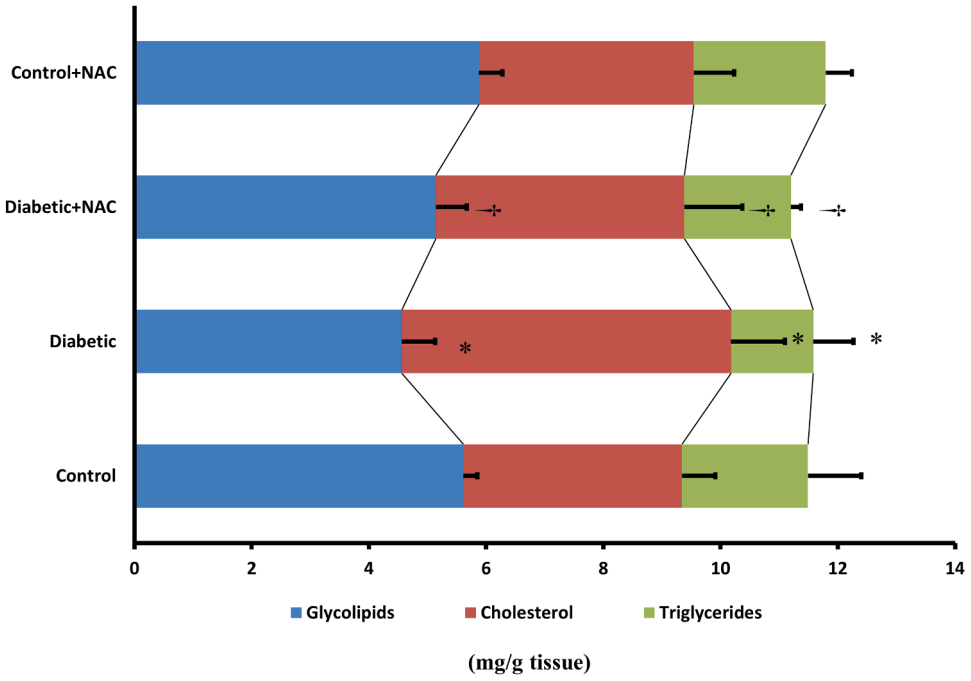

Fig. 2. Effect of NAC on lipid levels in sciatic nerve of diabetic rats. The diabetic group showed decrease in triglycerides and glycolipids accompanied by increase in cholesterol levels. The alterations in lipid profile were restored to near normal levels in NAC supplemented diabetic animals. Values are expressed as mean ± S.D. of six animals per group. *Significantly different from control group (at $p < 0.05$). †Significantly different from diabetic group (at $p < 0.05$).

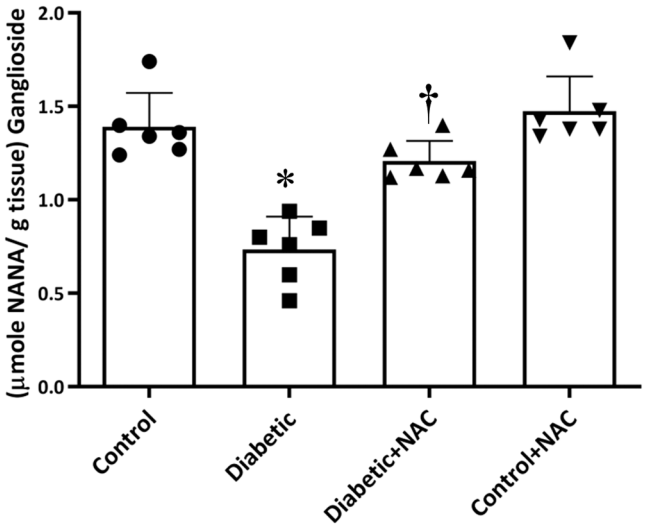

Fig. 3. Effect of NAC on ganglioside levels in sciatic nerve of diabetic rats. The amount of gangliosides was reduced in diabetic animals as compared to controls. NAC administered diabetic animals showed improvement in ganglioside levels. Values are expressed as mean ± S.D. of six animals per group. *Significantly different from control group (at $p < 0.05$). †Significantly different from diabetic group (at $p < 0.05$).

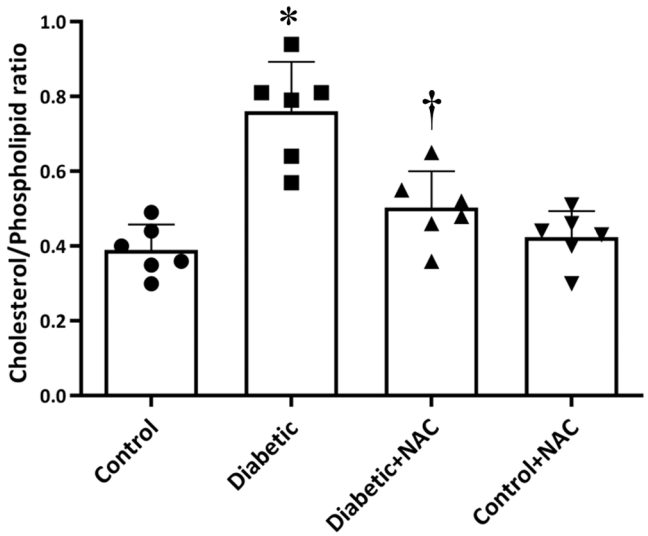

Fig. 4. Effect of NAC on cholesterol to phospholipid ratio in sciatic nerve of diabetic rats. The results show increase in cholesterol to phospholipid ratio in diabetic group. The ratio was restored in diabetic animals supplemented with NAC. Values are expressed as mean ± S.D. of six animals per group. *Significantly different from control group (at $p < 0.05$). †Significantly different from diabetic group (at $p < 0.05$).

higher activities of both binding GTP and stimulating adenylate cyclase (Bai and Youguo, 1998). Cholesterol to phospholipid ratio (shown in Fig. 4) was almost increased by 2-fold in diabetic animals as compared to control group. The previous study has also shown that higher cholesterol/phospholipid ratio inhibits GTP binding protein's function significantly by interfering to its motion in the membrane (Bai and Youguo, 1998). As shown, NAC administration rescues the alteration in cholesterol to phospholipid ratio by lowering it up to 54 % as compared to untreated diabetic group.

3.2. *Membrane bound enzyme activity*

Effect of diabetes on activity of neuronal membrane bound enzymes and effect of NAC supplementation was studied on two such enzymes viz. AchE and Ca^{2+} ATPase. A marked reduction was observed in activities of these enzymes in diabetic group compared to control. Beneficial effects of NAC were observed in the form of restoration of the activity of these enzymes. It was observed that NAC improves the activity at significant levels. Figs. 5 and 6 show the observations from

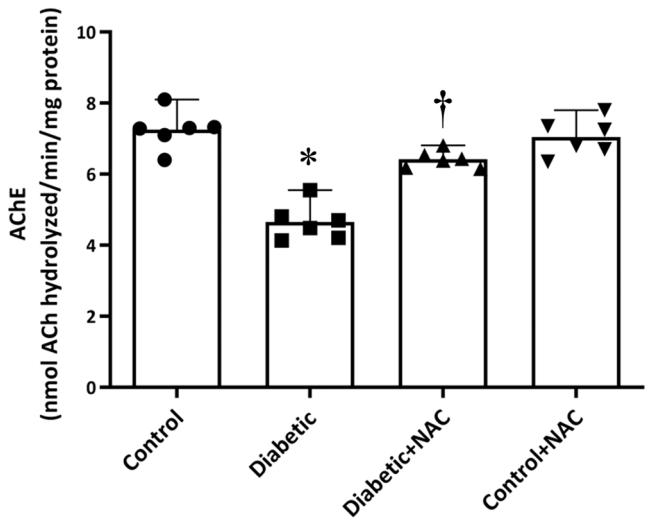

Fig. 5. Effect of NAC on acetylcholinesterase activity in sciatic nerve of diabetic rats. The activity of acetylcholinesterase was decreased in the Diabetic group. Diabetic animals supplemented with NAC showed improved acetylcholinesterase activity. *Significantly different from control group (at $p < 0.05$). †Significantly different from diabetic group (at $p < 0.05$).

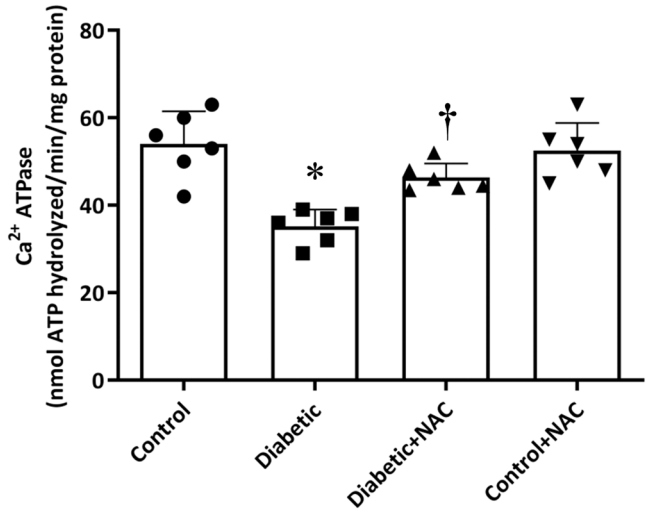

Fig. 6. Effect of NAC on Ca^{2+}- ATPase activity in sciatic nerve of diabetic rats. A significant loss in activity was observed in the diabetic animal that was partially reversed in diabetic animals supplemented with NAC. Values are expressed as mean ± S.D. of six animals per group. *Significantly different from control group (at $p < 0.05$). †Significantly different from diabetic group (at $p < 0.05$).

assays pertaining quantification of activities of these enzymes in all of the four groups.

3.2.1 Acetylcholinesterase activity: AChE activity was significantly reduced (55.29 % of control level) in diabetic animals. This phenomenon may be because of increased oxidative stress leading to formation of free radicals that may change membranous microenvironment or directly affect enzyme molecules. It has been previously reported that AChE is influenced by OS induced perturbations in membrane fluidity (Sandhir & Gill, 1994). Therefore, the reduced activity reflects alterations in membrane properties due to increased membrane lipid peroxidation, which may alter enzyme activities through lipid-protein interactions (Ashokkumar et al., 2006). Also, hydroxyl radicals produced during Fenton reaction through H_2O_2/ Fe^{2+} system have been shown to damage active sites of AChE molecules (Schallreuter et al., 2004). Treatment with NAC to diabetic animals significantly restores AChE activity 36.23 % more than that of diabetic animals, as shown in Fig. 5.

3.2.2 Ca^{+2}- ATPase activity: Ca^{+2}- ATPase is a plasmalemmal enzyme. It is important as it brings elevated intracellular calcium to resting state. As shown in Fig. 6, its decreased activity in diabetic animals (35.97 % than control) may lead to elevation in intracellular calcium levels, which may lead to calcium induced toxicity leading to neurodegenerative changes (Choi, 1992). ROS formed in diabetes attack the membranes of intracellular organelles and reported to decrease cardiac Ca^{+2}-ATPase activity (De Nicolo et al., 2023). Decrease membrane fluidity induced by oxidative stress has been linked with the abnormalities in calcium metabolism (Lehotský et al., 1999). NAC treated diabetic group showed 34.93 % increase in Ca^{+2}-ATPase as compared to untreated diabetic group.

3.3. *Histopathological Studies*

The significant deviation in lipid profile has been correlated to structural and functional integrity of sciatic nerve (Fig. 7). The nerve fibers in the transverse sections of sciatic nerve of diabetic animals showed reduced diameter, a clear implicative of axonal shrinkage. The sections reveal deformity in the axonal membrane as well as disorganised myelin sheath around nerve fibers. The NAC supplementation partially improved the morphology of fibers in their cross-sectional appearance (Fig. 7(i)). The stretched and elongated morphology suggested axonal atrophy, as compared to ovoid appearance in control animals evident with decreased internodal diameter (Fig. 7 (ii)). These fibers were seen to be restoring towards circular appearance, surrounded by myelin sheath. These findings are similar earlier finding in similar directions (Shi et al., 2013).

These findings suggest that NAC ameliorates the detrimental effects of diabetes induced neuropathy. It controls broad range of parameters by intervening at different levels. It seems to significantly ameliorate diabetes induced elevated cholesterol levels by down regulating the mRNA expression of malic enzyme and 3-hydroxy-3-methylglutaryl coenzyme A reductase (Lin & Yin, 2008). Similar observation has been reinstated in STZ induced diabetic rats (Kaga et al., 2018). NAC being an ROS scavenger suppresses NF Kappa B that in turn leads to inhibition of COX2 and phospholipase A2 activity which causes inhibition of hydrolysis of membrane phospholipids by activation of phospholipases (Kitatani et al., 2004; Zheng et al., 2019). NAC supplementation could restore phospholipid levels near to control by attenuating diabetes induced oxidative stress, partial restoration of arachidonic acid biosynthesis (Mîinea et al., 2002; Sztolsztener et al., 2023) and amelioration of phospholipases (Hong et al., 2006). The above two protective restoration may be the key reasons behind near normal cholesterol/phospholipid ratio in NAC treated diabetic animals as compared to untreated diabetic rats. NAC has also been found to scavenge free radicals and replenish GSH thus it blocks ceramide generation (Lavrentiadou et al., 2001; M. Liu et al., 2024) restoring gangliosides level to normal. The ability of NAC to protect AChE and Na^{+}/K^{+}-ATPase activity can be due to protective effect of NAC against hyperglycemia induced oxidative stress that might involve modulation of diabetes-induced oxidative stress keeping the membrane microenvironment unaltered and/or preventing the oxidative damage to enzyme by quenching the ROS by elevated production of thiol entities (data not shown).

4. Conclusions and future directions

Based on our results, it can be concluded that chronic hyperglycaemia perturbs lipid composition that impacts the structural and functional characteristics of the membrane via increased oxidative stress, which ultimately affects membrane bound enzymes important for proper neuronal activity. NAC supplementation not only restored levels

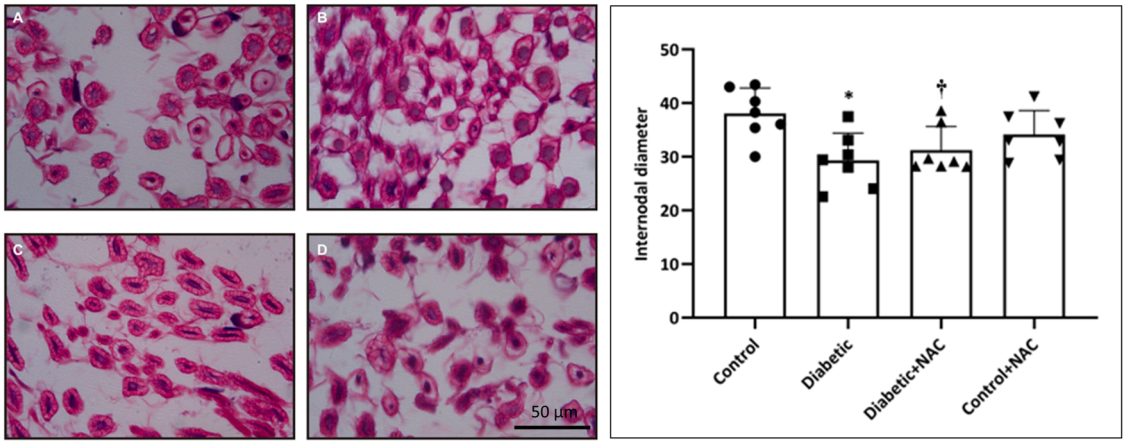

Fig. 7. Effect of NAC on histopathological changes in sciatic nerve of diabetic rats (i) Hematoxylin and eosin stained transverse sections of the sciatic nerve from Control (A), Control + NAC (B), Diabetic (C), Diabetic + NAC (D) groups. Diabetic animals showed axonal shrinkage and disorganized myelin sheath around nerve fibers as compared to control group. Administration of NAC showed partial restoration of morphology of nerve fibers and improved organization of myelin sheath. (ii) Internodal diameter was significantly decreased in the diabetic rat sciatic nerve fibers. The NAC treatment significantly ameliorated the shrinkage of nerve fibers. Values are expressed as mean ± S.D. of seven randomly observed nerve fibers. *Significantly different from control group (at $p < 0.05$). †Significantly different from diabetic group (at $p < 0.05$).

of lipids that maintain membrane fluidity and mitigated hyperglycemia-induced ROS generation. The antioxidant properties of NAC and versatility in ameliorating broad spectrum of etiological parameters responsible for diabetes induced neuropathy considered in our study suggest therapeutic potential of NAC in diabetic complications. The research shows that NAC successfully reduces the harmful impact of diabetes-induced oxidative stress on the sciatic nerve. NAC specifically restores the equilibrium of lipid composition, amplifies the functionality of essential enzymes attached to the membrane, and rectifies structural impairments in the sciatic nerve. This indicates that NAC has a defensive function in preventing the biochemical and morphological changes linked to diabetic neuropathy (Fig. 8).

Future studies should investigate the enduring consequences of NAC treatment on diabetic neuropathy and other problems associated with

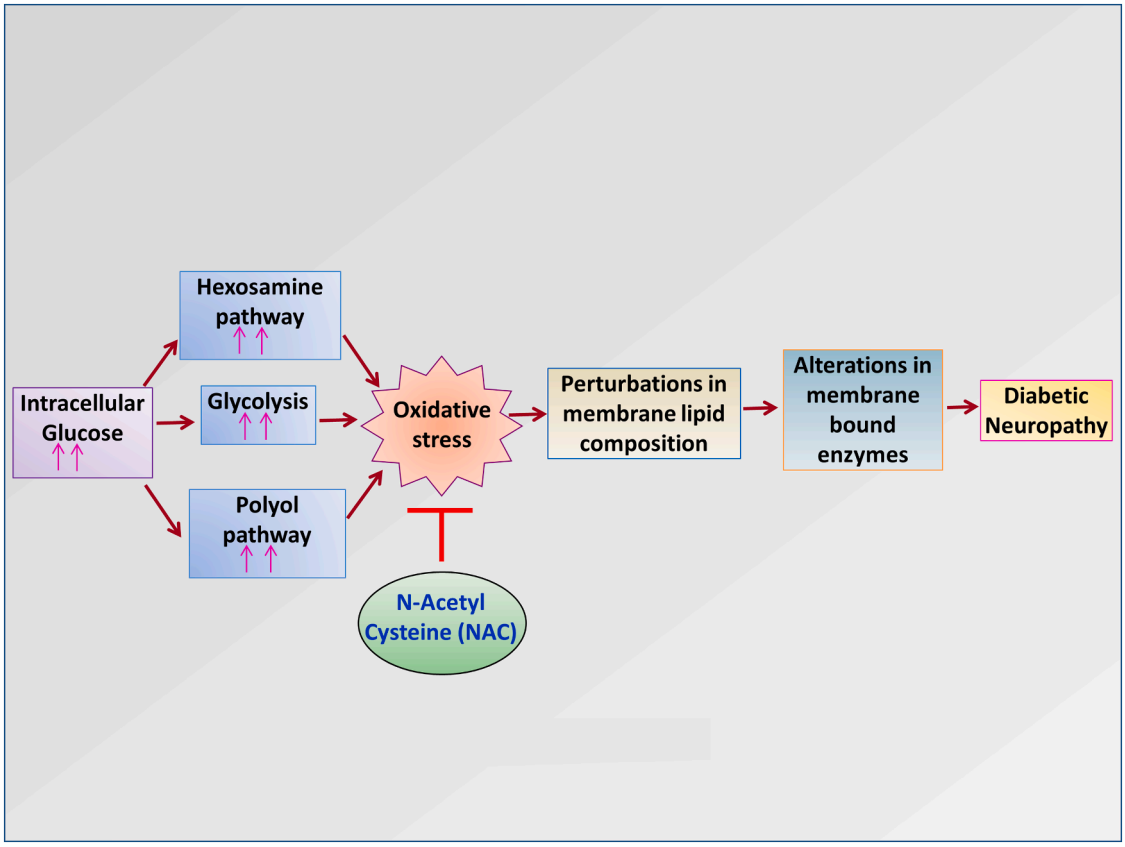

Fig. 8. Proposed Model to indicate site of action of NAC against Diabetic Neuropathy.

diabetes. Furthermore, it is necessary to conduct clinical studies in order to validate the effectiveness and safety of NAC in individuals with diabetes. Exploring the most effective dosage, timing, and integration with other therapies might also be quite beneficial. Furthermore, it is important for research to examine the influence of NAC on other tissues and organs that are impacted by diabetes in order to evaluate its wider therapeutic capacity.

Clinical Implications

The results indicate that NAC has the potential to be an effective treatment for avoiding or minimising diabetic neuropathy, a prevalent and severe consequence of diabetes. N-acetyl Cysteine (NAC) has the ability to alleviate symptoms and slow down the course of neuropathy by enhancing lipid composition and enzyme activity, hence maintaining the integrity and function of neuronal membranes. This has the potential to improve the medical results for people with diabetes, especially those who are at risk of or already experiencing neuropathy.

CRediT authorship contribution statement

Sharma Satya P.: Writing – review & editing, Writing – original draft, Visualization, Software. **Sandhir Rajat:** Validation, Supervision, Project administration, Conceptualization. **Mohamed Wael M.Y.:** Writing – review & editing, Project administration. **Kamboj Sukhdev S.:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization.

Animal model

Male wistar rats were procured from the central animal house of the Panjab University, Chandigarh (India). All the protocols were approved by the Institutional Animal Ethics Committee (IAEC) and were in accordance to the *NIH guidelines for the humane use and care of the laboratory animals*.

Financial support

Sukhdev Singh Kamboj received Senior Research Fellowship (Grant no. 3/1/2/5/07-RHN) by the Indian Council of Medical Research and DRS Phase II grant by UGC, New Delhi

Financial interests

The authors have no relevant financial or non-financial interests to disclose.

Declaration of Competing Interest

The authors declare no conflict of interest associated with this study.

Acknowledgements

The Senior Research Fellowship to Sukhdev Singh Kamboj (Grant no. 3/1/2/5/07-RHN) by the Indian Council of Medical Research and DRS Phase II grant by UGC, New Delhi are greatly acknowledged.

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