Melatonin as a Therapeutic Agent in ALS: Modulating IGF-1 and GLP-1 Pathways to Mitigate Neurotoxicity

Gurudev Singh Raina[1], Sushil Kumar[2], Milan Singh Kahlon[3], Sidharth Mehan[4], Amandeep Singh[5]*

[1]Division of Neuroscience, Department of Pharmacology, ISF College of Pharmacy, Moga, Punjab-142001, India. Phone No.: +91-7807888710; Email ID: nickykhalsa84@gmail.com

[2]Department of Regulatory Affairs, ISF College of Pharmacy, Moga, Punjab-142001, India. Phone No.: +91-7543093217; Email ID: sushilyeswant54@gmail.com

[3]Department of Pharmaceutics, ISF College of Pharmacy, Moga, Punjab-142001, India. Phone No.: +91-7831989747; Email ID: milansingh.kahlon@gmail.com

[4]Division of Neuroscience, Department of Pharmacology, ISF College of Pharmacy, Moga, Punjab-142001, India. Phone No.: +91-8059889909; Email ID: sidharthmehan@iscfcp.org

[5]Department of Pharmaceutics, ISF College of Pharmacy, Moga, Punjab-142001, India. Phone No.: +91- 9465423413; *Email ID:* ad4singh@gmail.com

With Best Regards

Corresponding Author*

Dr. Amandeep Singh, PhD

Associate Professor

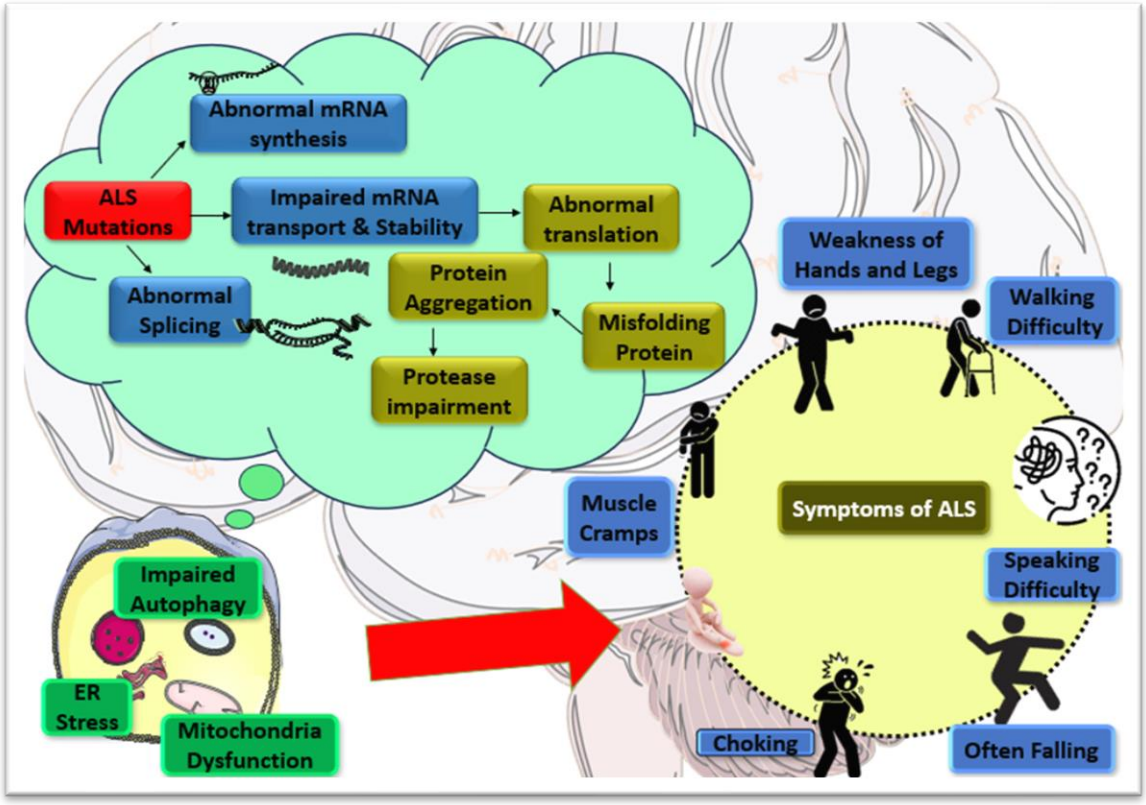
Department of Pharmaceutics

ISF College of Pharmacy Moga

Email: *sushilyeswant54@gmail.com*

Phone No.: *+91-7543093217*

Graphical Abstract



ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that progressively impairs motor neurons in the brain and spinal cord, leading to muscle weakness, paralysis, and eventual death. Globally, it affects about 4.5 individuals per 100,000, with higher incidence in the United States (19.37 Per 100,000) and Europe (10 Per 100,000), but lower rates in Asia (3.13 Per 100,000). Alarmingly, ALS cases are projected to rise by 69% from 222,801 in 2015 to 376,674 by 2040, highlighting the urgent need for effective treatments. Melatonin, a hormone with potent antioxidant and immunomodulatory properties, has emerged as a promising therapeutic candidate. By mitigating oxidative stress and inflammation key contributors to motor neuron degeneration melatonin provides neuroprotection. Additionally, it activates IGF-1 and GLP-1 signaling pathways, essential for motor neuron survival and function, offering hope for addressing the complex challenges of ALS.

Keywords: Neurodegenerative disease, Amyotrophic lateral sclerosis, Melatonin, IGF-1 (Insulin-like Growth Factor-1) and GLP-1 (Glucagon-like Peptide-1)

1. Introduction

1.1 Amyotrophic Lateral Sclerosis: Epidemiology, Environmental factors & Global prevalence

Amyotrophic Lateral Sclerosis (ALS), a progressive neurodegenerative illness characterized by the loss of motor neurons in the brain and spinal cord, causes muscle weakness, atrophy, and ultimately paralysis [1]. The global prevalence of ALS is approximately 4.5 per 100,000 people, with higher rates in Europe and the United States and lower rates in Asia [2]. The global prevalence of Amyotrophic Lateral Sclerosis (ALS) varies significantly across different countries, as illustrated in the map. Among the highlighted regions, China records the highest prevalence with 9.9 cases per 100,000 population, closely followed by the USA at 9.1 cases per 100,000. In contrast, Brazil reports the lowest prevalence at 0.9 cases per 100,000, indicating a stark difference in ALS cases across continents. India has a moderately high prevalence of 6 cases per 100,000, surpassing Japan (2.79 cases per 100,000), Australia (2 cases per 100,000), and Canada (2.5 cases per 100,000). Russia shows a relatively low prevalence at 1.25 cases per 100,000 as shown in figure (fig. 1). These variations could reflect differences in diagnostic capabilities, genetic predisposition, environmental factors, or healthcare reporting systems across regions. The data underscores the need for a deeper understanding of regional influences on ALS prevalence to guide global public health strategies. Clinical assessment, nerve conduction investigations, and electromyography (EMG) are all part of diagnosing ALS. The ALSFRS-R, or ALS Functional Rating Scale-Revised, is used to monitor the progression of the illness. Genetic mutations in over 40 genes, such as C9orf72, SOD1, TARDBP, and FUS, play a significant role in ALS risk, though risk and progression can be influenced independently by genetic and environmental factors [3]. Cognitive and behavioral changes, including frontotemporal dementia (FTD), are common in ALS, necessitating comprehensive neuropsychological assessments. Protein aggregates such as TDP-43 and SOD1 are key players in ALS's pathophysiological pathways, including altered proteostasis, cytoskeletal abnormalities, mitochondrial dysfunction, and defective RNA metabolism [4]. Environmental factors, lifestyle choices, and occupational exposures contribute to ALS risk and progression, emphasizing the importance of understanding ALS exposure. Prognosis varies, with median survival ranging from 2 to 4 years, influenced by clinical features, genetic mutations, and environmental exposures [5]. Treatment focuses on disease-modifying therapies to manage symptoms and improve quality of life. Pathological changes occur grossly and microscopically, affecting the central nervous system and peripheral muscles. Sclerosis and pallor of the corticospinal tracts, which indicate degeneration of the motor pathways, are grossly present, as is atrophy of the precentral gyrus, which represents the

motor cortex. Thinning of the spinal ventral roots and hypoglossal nerves is observed, leading to muscle atrophy, including both somatic and bulbar muscles. This results in the characteristic weakness and dysphagia seen in ALS patients. Under a microscope, ALS is characterized by the loss of more than 50% of spinal motor neurons and widespread astrocytic gliosis in the spinal gray matter, which suggests a proliferative reaction to neuronal injury [6]. In addition to atrophic and basophilic changes, motor neurons that survive have ubiquitinated inclusion bodies with a thread-like, skein-like, or compact form. The motor cortex exhibits astrocyte gliosis and a variety of big pyramidal neuron (Betz cell) losses, particularly in the gray and subcortical white matter. Microglial activity is also observed in the afflicted regions. Glial cells can also have cytoplasmic aggregation inclusions, which add to the gradual neurodegeneration seen in ALS [7].

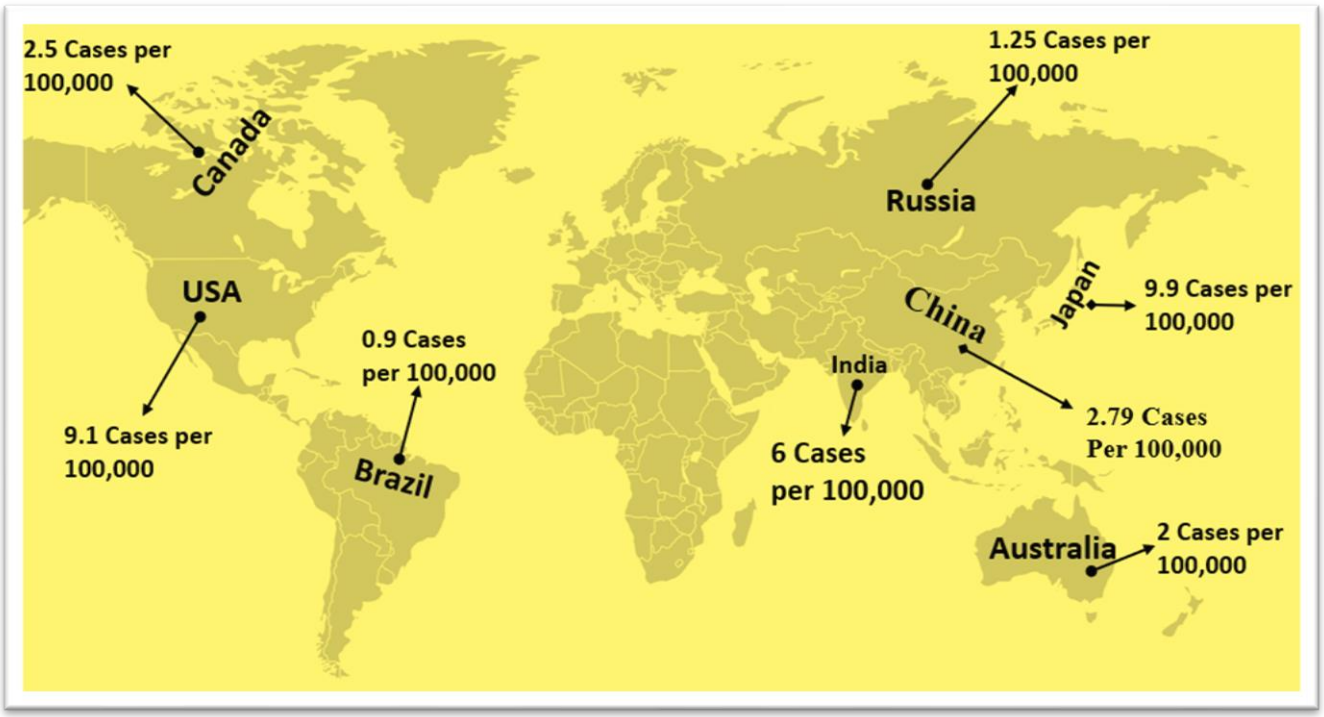


Fig. 1: Major country's prevalence rate over the globe

2. Pathogenic mechanisms involved in the progression of ALS

2.1 RNA Metabolism Impairment:

Gene mutations like C9orf72, TARDBP (encoding TDP-43), and FUS disrupt RNA metabolism in ALS. These genes play crucial roles in RNA transcription, splicing, transport, and stability. The mutations cause protein aggregates and harmful RNA species to develop, which disrupt regular cellular processes. For example, TDP-43 and FUS are RNA-binding proteins that, when mutated, aggregate and form inclusions in neurons. These aggregates sequester other vital RNA-binding proteins, disrupting their normal functions and leading to widespread RNA dysregulation. This

impairment affects numerous cellular processes, including the production of proteins essential for neuron survival and function [8-10].

2.2 Protein Homeostasis Disruption (Proteostasis):

In ALS, proteostasis, or protein homeostasis, is crucial for maintaining the balance of protein synthesis, folding, and degradation. Misfolded proteins and protein aggregates build up as a result of these processes being disrupted by mutations in genes including SOD1, TARDBP, and FUS [11]. Normally, cells use mechanisms like the ubiquitin-proteasome system and autophagy to clear damaged or misfolded proteins. However, in ALS, these systems become overwhelmed or impaired. For example, SOD1 mutations produce misfolded SOD1 proteins that cluster and accumulate inside motor neurons. These aggregates can sequester other proteins, disrupting their function and contributing to cellular toxicity. The impairment in autophagy and proteasome pathways means these toxic aggregates are not efficiently cleared, exacerbating neuronal damage. The disease can develop throughout the neurological system due to these protein aggregates' prion-like ability to move from cell to cell. This spread of aggregates further contributes to the progression of ALS and the widespread loss of motor neurons [12, 13].

2.3 Oxidative Stress

Oxidative stress drives ALS progression through several interconnected mechanisms. Gene mutations such as SOD1 cause a cycle of oxidative damage and cellular malfunction by increasing the formation of reactive oxygen species (ROS) and impairing mitochondrial function [14]. This oxidative stress initiates lipid peroxidation, damaging cell membranes and disrupting cellular integrity. ROS also oxidizes proteins, causing misfolding and aggregation of critical proteins such as TDP-43, SOD1, and FUS, accumulating and interferin with cellular functions. These protein aggregates further impair proteostasis and can spread between cells, propagating the disease [15]. Additionally, ROS-induced DNA damage results in genomic instability, contributing to neuronal degeneration. This persistent oxidative damage and cellular stress ultimately lead to motor neuron death, characteristic of ALS. The complex interplay of these molecular changes highlights oxidative stress as a crucial pathway driving ALS pathology and progression. Reactive oxygen species (ROS) production rises and antioxidant defenses are compromised, leading to cellular damage and motor neuron death [16].

2.4 Mitochondrial Dysfunction

Mitochondrial failure has a critical role in ALS pathogenesis, closely coupled with oxidative stress and other cellular pathways. Gene mutations affecting mitochondrial function, such as those affecting SOD1, TDP-43, and FUS, result in decreased ATP synthesis and elevated reactive oxygen species (ROS) production [17]. As a result, mitochondrial DNA (mtDNA) sustains oxidative damage, which hinders oxidative phosphorylation and further disrupts the electron transport chain (ETC). Reduced membrane potential ($\Delta\Psi m$) in dysfunctional mitochondria impairs energy generation and increases apoptosis through the release of cytochrome c and other pro-apoptotic proteins. Furthermore, impaired mitophagy causes damaged mitochondria to accumulate, which exacerbates cellular stress. Protein oxidation and lipid peroxidation brought on by ROS harm cellular constituents, while protein misfolding and aggregation inside mitochondria further compromise function. The degeneration of motor neurons is the result of this chain reaction between oxidative stress and mitochondrial malfunction [18]. Pharmacological interventions, such as antioxidants and compounds enhancing mitochondrial biogenesis, aim to mitigate these effects, highlighting mitochondrial dysfunction as a critical target in ALS therapy**.** Motor neuron degeneration in amyotrophic lateral sclerosis (ALS) is driven by a range of complex molecular mechanisms that involve both intrinsic neuronal dysfunction and harmful interactions with surrounding glial cells. Microglial cells initiate an inflammatory response by releasing cytokines like monocyte chemoattractant protein-1 (MCP-1), contributing to a neuroinflammatory environment. Astrocytes inhibit motor neuron damage in several ways, including by releasing inflammatory mediators such as prostaglandin E2 (PGE2) and nitric oxide (NO) and by decreasing the expression and activity of the glutamate transporter EAAT2, which causes excitotoxicity [19]. They also diminish lactate release, which deprives motor neurons of metabolic support, and activate pro-apoptotic signaling through pro-NGF binding to the p75 receptor. Protein misfolding is caused by transcriptional dysregulation, inadequate RNA processing, and excess reactive oxygen species (ROS) in motor neurons [20]. These misfolded proteins aggregate to create endoplasmic reticulum (ER) stress, which damages the proteasome by inducing autophagy and death. Additionally, it is caused by energy shortages, especially in distant axons, which are a result of impaired axonal transport. Additionally, motor neurons release complement proteins, which alert nearby cells to cellular stress and exacerbate the harm [21].

2.4 Activation of WNT/β–Catenin Signaling

The WNT/β–catenin signaling pathway is essential for regulating cell survival, proliferation, differentiation, and tissue homeostasis. At the center of the path is β-catenin, a protein that builds up in the cytoplasm when WNT ligands attach to receptors such as Frizzled and LRP6. This prevents the destruction complex from phosphorylating and breaking down β-catenin. When β-catenin stabilizes, it enters the nucleus and interacts with transcription factors to regulate the expression of genes linked to cell cycle progression, adhesion, and death [22]. Proteins like GSK3β and CK1 phosphorylate β-catenin in the absence of WNT ligands, which causes the ubiquitin-proteasome pathway to degrade. However, dysregulation of the WNT/β-catenin pathway can occur in conditions such as amyotrophic lateral sclerosis (ALS). ALS is characterized by the death of motor neurons, and research indicates that impaired WNT/β-catenin signaling may worsen this neurodegeneration. Reduced signaling in ALS may weaken the protective effects of the pathway, leading to increased oxidative stress, inflammation, and neuronal apoptosis. This impaired signaling is thought to exacerbate the disease process, accelerating neuron loss and impairing the body's natural repair mechanisms. As a result, addressing the WNT/β–catenin pathway has been proposed as a possible treatment method for decreasing ALS development and increasing neuroprotection [23].

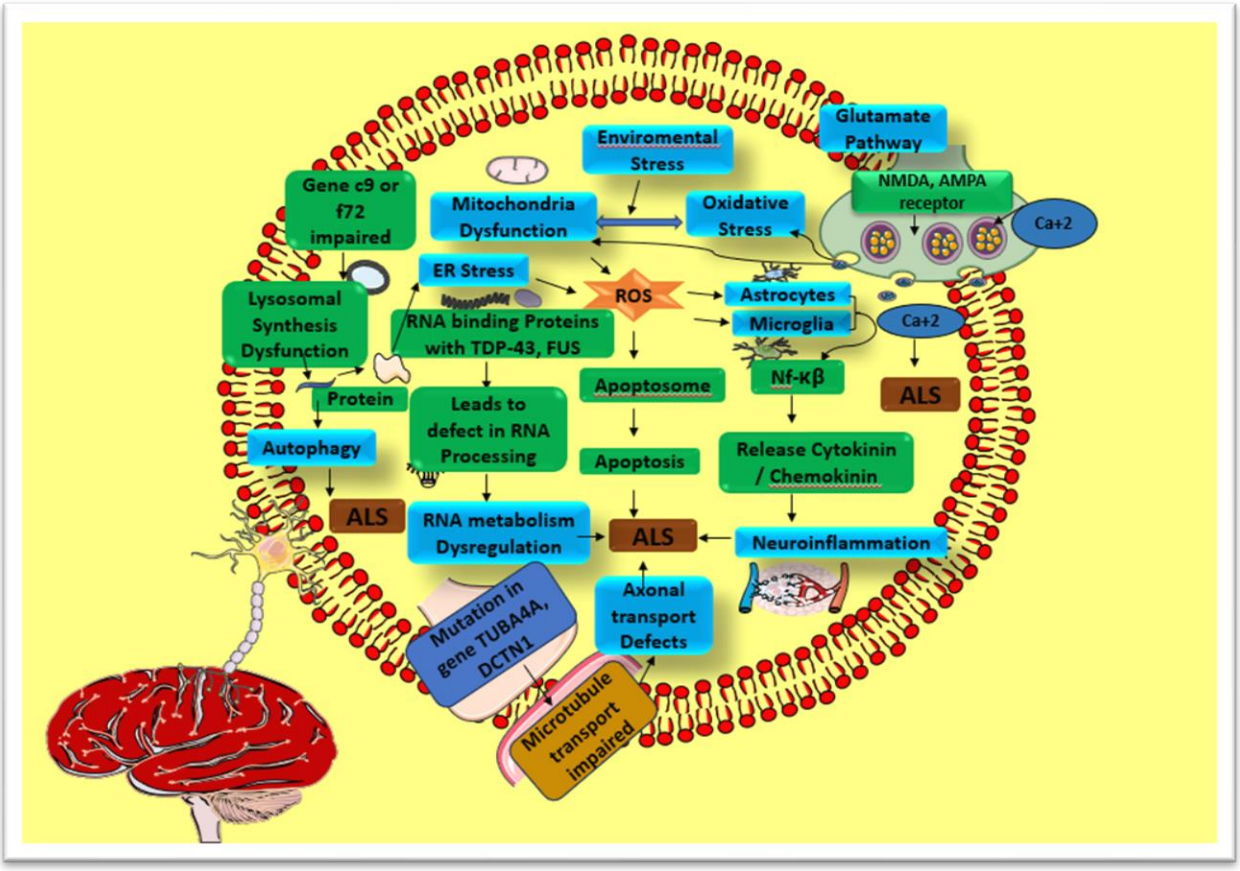


Fig. 2: Pathogenic mechanisms involved in the progression of ALS

3. Current treatments for ALS

Current treatments for ALS as depicted in Table 1 include Riluzole, which inhibits glutamate release and slows disease progression but can cause dizziness, nausea, and hepatic dysfunction. Edaravone acts as a free radical scavenger, reducing oxidative stress, with potential side effects like renal impairment and gait disturbance [24]. AMX0035, a combination of sodium phenylbutyrate and taurursodiol, helps slow neurodegeneration, though it may cause gastrointestinal discomfort and diarrhea. Tofersen, an antisense oligonucleotide targeting SOD1 mRNA, reduces SOD1 protein synthesis in mutation carriers but can lead to injection site reactions and flu-like symptoms. Stem cell therapy aims to replace damaged neurons and potentially halt disease progression, though it poses risks of immune rejection and ethical concerns [25]. Gene therapy corrects pathogenic genetic mutations and has the potential to halt disease progression, but may trigger immunogenicity and off-target effects. Melatonin emerges as a unique therapeutic option due to its natural synthesis, robust safety profile, and multifaceted approach. It creates a neuroprotective environment by acting as a strong antioxidant, neutralizing dangerous reactive oxygen species (ROS), and modifying immune responses to lower inflammation. Melatonin's regulation of circadian rhythms also supports overall physiological stability. With minimal side effects and broad neuroprotective properties, melatonin stands out as a promising remedy for ALS, complementing existing treatments and offering comprehensive protection [26]. Further research into its application could enhance our understanding and utilization of melatonin in ALS therapy**.**

4. Melatonin (Melatonin's Role in Modulating ALS Pathways)

Mostly produced by the pineal gland from the amino acid tryptophan, melatonin is released in the dark and is regulated by the light-dark cycle. G-protein-coupled receptors (MT1, MT2, MT3) affect immunological responses, sleep patterns, mood, and cardiac functions [27]. It is generated in peripheral organs such as the liver, intestines, and bone marrow. Excessive reactive oxygen species (ROS) are a sign of oxidative stress, which damages cellular constituents and plays a role in neurological conditions. First reported in 1991, melatonin's antioxidant properties involve scavenging free radicals and neutralizing ROS, offering protection against oxidative damage. Its ability to effectively lower protein oxidation and lipid peroxidation points to possible therapeutic use in neuroprotection and managing neuronal disorders [28, 29].

4.1 Regulation of Circadian Rhythm and Sleep Disturbances:

In ALS patients, sleep disturbances are common due to respiratory muscle weakness and neurodegeneration affecting sleep centers. Melatonin helps regulate the sleep-wake cycle by modulating the suprachiasmatic nucleus (SCN), ensuring better sleep quality, which is often impaired in ALS patients. ALS patients may experience less fatigue and have a higher quality of life if they get more sleep [30].

4.2 Mitochondrial Dysfunction in ALS:

In ALS, mitochondrial dysfunction is a major pathological hallmark, especially in motor neurons, where energy demands are high. Melatonin has been shown to improve mitochondrial efficiency by preserving mitochondrial membrane potential and increasing ATP production, which is often reduced in ALS. Studies have shown that melatonin localizes within mitochondria, where it acts to reduce oxidative stress and preserve mitochondrial function [31].

4.3 Regulation of Apoptosis:

One factor contributing to the loss of motor neurons in ALS is apoptosis, or programmed cell death. Melatonin can lessen the activation of pro-apoptotic proteins like Bax, but anti-apoptotic proteins like Bcl-2 can be expressed more. This balance must be maintained to protect neurons against apoptosis caused by oxidative stress and inflammation, two conditions that are frequently present in ALS [32].

4.4 Antioxidant Properties of Melatonin in ALS

The development of ALS is significantly influenced by oxidative stress, which causes neurons and glial cells to produce more reactive oxygen species (ROS) and reactive nitrogen species (RNS). ALS is commonly related to SOD1 (superoxide dismutase 1) gene abnormalities, which usually help detoxify superoxide radicals. Mutant SOD1 generates increased oxidative stress, leading to motor neuron damage. Melatonin’s antioxidant properties offer significant potential to combat this [33].

4.5 Reduction of Reactive Oxygen Species (ROS):

Melatonin is highly effective in neutralizing ROS, including hydroxyl radicals, superoxide anions, and peroxynitrite, which are involved in the oxidative damage of proteins, lipids, and DNA in ALS patients. This direct radical scavenging function is crucial for reducing oxidative injury in motor neurons [34].

Table 1: Current treatments for ALS

Treatment	Mechanism of Action	Side Effects	Unique Aspects of Melatonin	Reference
Riluzole	Inhibits glutamate release	Dizziness, nausea, weakness	Melatonin has antioxidant properties and modulates immune responses, offering broader protection	[35]
Edaravone	Scavenges free radicals	Renal dysfunction, gait disturbance	Melatonin is naturally produced by the body and regulates circadian rhythms	[36]
AMX0035	Combination of sodium phenylbutyrate and taurursodiol	Nausea, diarrhea	Melatonin has minimal side effects and is involved in multiple physiological processes	[37]
Tofersen	Targets SOD1 gene mutation	Injection site reactions	Melatonin is easily accessible and cost-effective	[38]
Stem Cell Therapy	Regeneration of damaged neurons	Immune rejection, ethical concerns	Melatonin is a natural hormone with neuroprotective effects	[39]
Gene Therapy	Corrects genetic mutations	Immune response, off-target effects	Melatonin has a well-established safety profile and is involved in immune modulation.	[40]

4.6 Stimulation of Endogenous Antioxidant Defenses:

Beyond its direct scavenging action, melatonin increases the activity of important antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). These enzymes support the maintenance of redox equilibrium in neurons and the detoxification of ROS. Melatonin treatment can help reduce oxidative stress caused by SOD1 mutations in ALS models [41].

4.7 Lipid Peroxidation in ALS:

Melatonin prevents the peroxidation of membrane lipids, which is an early marker of oxidative damage in neurons. Neuronal death may result from lipid peroxidation's impairment of cell membrane integrity. Research has indicated that melatonin lowers malondialdehyde (MDA) levels, which are a sign of lipid peroxidation in ALS models, thereby preserving membrane structure and function [42].

4.8 Mitochondrial Protection:

Mitochondria are significant sources of ROS generation in neurons. In ALS, mitochondrial abnormalities, such as swelling, membrane depolarization, and reduced ATP production, contribute to neurodegeneration. Melatonin is particularly effective at preserving mitochondrial function by scavenging mitochondrial ROS and maintaining membrane potential. This has been shown in ALS animal models, where melatonin therapy enhanced mitochondrial respiration and decreased oxidative stress [43].

4.9 Immunomodulatory Effects of Melatonin in ALS

One of the main causes of ALS is neuroinflammation, in which pro-inflammatory cytokines and cytotoxic substances are produced by activated microglia and astrocytes, which aid in the degeneration of motor neurons [44].

4.10 Reduction of Pro-Inflammatory Cytokines:

Melatonin inhibits pro-inflammatory cytokines that are up-regulated in ALS, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). By down-regulating these cytokines, melatonin reduces the inflammatory environment that contributes to neuronal damage in ALS. Microglial Activation Inhibition in ALS, resident immune cells called microglia in the central nervous system become activated and emit harmful substances including nitric oxide (NO) and reactive oxygen species (ROS). Melatonin inhibits microglial activation, thereby reducing the release

of these neurotoxic compounds. This action helps protect motor neurons from inflammation-induced death [45, 46].

4.11 Suppression of Nitric Oxide (NO) Production:

One of the main reasons for the neuronal death linked to ALS is excessive NO production. Activated microglia and astrocytes produce NO through the enzyme inducible nitric oxide synthase (iNOS), which melatonin inhibits to reduce nitrosative stress on motor neurons [47].

4.12 Regulation of Autophagy:

Autophagy, the process of digesting and recycling cellular components, is disrupted in ALS, resulting in the buildup of damaged proteins and organelles in neurons. Melatonin has been shown to enhance autophagy in neurodegenerative diseases, which helps in the clearance of toxic aggregates like mutant SOD1 and TDP-43 that accumulate in ALS [26].

Table 2: Studies based on the significance of melatonin in ALS treatment

Neuroactive agent	Design	Participants	Melatonin Dosage	Duration of Treatment	Key Findings	Ref.
Melatonin	Randomized Controlled Trial	50 ALS patients	3 mg/day	10 weeks	Observed improvements in muscle strength and endurance compared to the placebo group; noted positive trends in motor function.	[48]
Melatonin	Pilot Study	30 ALS patients	10 mg/day	3 months	Reduction in inflammatory cytokines (IL-6, TNF-α) levels; correlated with improved patient-reported outcomes.	[36]
Melatonin	Double-blind, Placebo-controlled Study	40 ALS patients	5 mg/day	4 weeks	Significantly improved sleep duration (avg. increase of 2 hours/night) and quality; enhanced overall quality of life.	[49]
Melatonin	Animal Study	Mice with ALS	20 mg/kg	8 weeks	Increased motor neuron survival and improved motor function were measured by grip strength tests.	[50]
Melatonin	Cohort Study	100 ALS patients	3 mg/day	6 months	Significant reduction in oxidative stress markers (malondialdehyde levels); associated with slower disease progression.	[51]
Melatonin	Randomized Controlled Trial	80 ALS patients	5 mg/day	12 weeks	Significant improvements in neuroprotective biomarkers and quality of life indicators; slower disease progression noted.	[42]

Table 3: Neuroprotective agents targeting molecular mechanisms of disease in ALS

Molecular Mechanism	Target	Disease Model	Neuroprotective Effects	Ref.
Cytochrome c	Inhibits cytochrome c release	Neurodegeneration	Prevents cytochrome c release from mitochondria	[52]
		Stroke/MCAO	Decreases cytochrome c release	[53]
		PD	Prevents cytochrome c release	[54]
Smac/Diablo	Neuroprotective	HD	Neuroprotective in HD models	[55]
AIF	Neuroprotective	Stroke	Neuroprotective in primary cortical neurons (PCN)	[56]
	Prevents mitochondrial depolarization	PD	Prevents mitochondrial depolarization	[57]
mtPTP	Inhibits mitochondrial permeability transition pore (mtPTP)	Stroke	Inhibits mtPTP in brain ischemia	[58]
		PD	Prevents mtPTP opening	[59]
Bax	Attenuates Aβ25-35-induced apoptosis	AD	Attenuates apoptosis induced by Aβ25-35	[60]
Bad	Attenuates cerebral ischemic injury	Stroke/MCAO	Reduces cerebral ischemic injury	[61]
ROS	Prevents ROS formation	PD	Prevents ROS formation	[62]
	Reduces ROS	ALS	Reduces ROS in the ALS model	[63]
PARP	Attenuates cerebral ischemic injury	Stroke/MCAO	Reduces cerebral ischemic injury	[53]
Caspase-3	Prevents caspase-3 activation	Stroke/MCAO	Inhibits caspase-3 activation	[64]
		AD	Reduces apoptosis induced by Aβ25-35	[65]
		PD	Blocks caspase-3 activation	[66]
Caspase-9	Neuroprotective	HD	Neuroprotective in HD models	[67]
Caspase-1	Neuroprotective	Stroke	Neuroprotective in primary cortical neurons (PCN)	[68]
IL-1β	Neuroprotective	Stroke	Neuroprotective in primary cortical neurons (PCN)	[68]
Rip2	Neuroprotective	HD	Neuroprotective in HD models	[69]
DNA Fragmentation	Reduces DNA fragmentation	Stroke/MCAO	Decreases DNA fragmentation	[69]
	Reduces DNA fragmentation	AD	Reduces apoptosis induced by Aβ25-35 or Aβ1-42	[70]
	Prevents DNA fragmentation	PD	Inhibits DNA fragmentation	[71]

TUNEL-positive cells	Reduces the number of DNA breaks	Neurodegeneration	Reduces DNA breaks	[72]
	Decreases TUNEL-positive cells	Stroke/MCAO	Decreases TUNEL-positive cells	[64]
INK	Inhibits cell death	PD	Inhibits cell death	[73]
Par-4	Reduces Par-4 upregulation	AD	Reduces Par-4 upregulation	[74]
NF-κB	Blocks Aβ25-35-induced apoptosis	AD	Inhibits apoptosis induced by Aβ25-35	[75]
	Anti-inflammatory effect	AD	Reduces inflammation with Aβ vaccination	[76]

Table 4: Clinical trials that involve the use of melatonin for ALS (Amyotrophic Lateral Sclerosis) treatment (www.clinicaltrails.com)

Study Title/ID	Phase	Participants	Duration	Melatonin Dosage	Key Outcomes
Study on Melatonin in ALS (NCT02987248)	Phase 2	100	12 months	10 mg/day	Reduced ALS progression, potential benefit
Efficacy of Melatonin on Oxidative Stress in ALS	Phase 1	25	6 months	20 mg/day	Reduced oxidative stress markers
Melatonin and ALS Progression (NCT02370382)	Phase 2	60	18 months	10 mg, 20 mg, placebo	No significant survival improvement
Neuroprotective Effects of Melatonin in ALS	Preclinical	N/A	N/A	Variable	Improved neuronal preservation in animals
Melatonin as an Antioxidant in ALS Patients	Phase 2	50	9 months	15 mg/day	Mild improvement in ALS symptoms
Melatonin as a Complementary Therapy for ALS	Phase 3	120	18 months	10-30 mg/day	No significant changes in the ALSFRS-R score
High-Dose Melatonin in ALS (NCT03463279)	Phase 2	80	12 months	25 mg/day	Delayed progression in early-stage ALS
Melatonin and Riluzole Combined Therapy in ALS	Phase 2	150	24 months	10 mg/day + Riluzole	Improved survival, and better outcomes than Riluzole

5. IGF-1 and GLP-1 signaling pathways and their significance in neuroprotection and ALS:

IGF-1 (insulin-like growth factor-1) signaling is necessary to maintain the health of neurons and avoid neurodegeneration. By triggering the PI3K/Akt pathway, which prevents apoptosis and supports cell survival mechanisms, IGF-1 enhances neuron survival [77]. Furthermore, IGF-1 can influence synaptic plasticity, which is the ability of synapses to change strength over time and is important for memory and learning. This pathway also encourages neurogenesis, which is the formation of new neurons in the brain and is necessary for maintaining and repairing neural networks. In ALS, where motor neuron death is a hallmark, IGF-1's ability to inhibit apoptosis and promote neural repair is especially valuable [78] [79]. GLP-1 decreases the synthesis of pro-inflammatory cytokines, which are chemicals that increase inflammation and potentially worsen brain damage, by activating GLP-1 receptors. Additionally, it increases the activity of antioxidant enzymes, which lowers oxidative stress and stops reactive oxygen species (ROS) from causing damage. This dual action helps protect neurons from the inflammatory and oxidative stress-related damage common in ALS. Furthermore, GLP-1 signaling can improve mitochondrial function, which is crucial for energy production and cell survival [80]. In ALS, where mitochondrial dysfunction and oxidative stress are prominent, the ability of GLP-1 to counteract these processes makes it a promising therapeutic target. Because of their all-encompassing neuroprotective benefits, the IGF-1 and GLP-1 pathways both hold great potential for the treatment of ALS. These pathways may be able to delay the development of the illness and enhance the quality of life for ALS patients by increasing neuron survival, decreasing apoptosis, and lowering oxidative and inflammatory stress [81]. Further investigation into these signaling pathways may result in improved treatments and results for ALS patients. The interaction between melatonin, insulin-like growth factor-1 (IGF-1), and glucagon-like peptide-1 (GLP-1) pathways is complex and interrelated, impacting oxidative stress, neuroprotection, inflammation, and metabolic regulation. These pathways work synergistically in maintaining cellular homeostasis, particularly in the brain, and their combined effects offer potential therapeutic strategies for conditions such as neurodegenerative diseases and metabolic disorders [81]. Melatonin has potent antioxidant qualities and is a crucial circadian rhythm regulator. Scavenging reactive oxygen species (ROS) and reactive nitrogen species (RNS) directly reduces oxidative damage to proteins, lipids, and DNA. In addition to its antioxidant role, melatonin protects mitochondrial function by improving electron transport chain efficiency and preventing mitochondrial permeability transition, which is crucial for maintaining cellular energy production and reducing ROS formation. This is especially crucial in neurodegenerative illnesses as neuronal damage is primarily caused by oxidative stress and mitochondrial malfunction [82]. While IGF-1 is

necessary for cell survival, growth, and neurogenesis, it also boosts the expression of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) via signaling pathways like PI3K/Akt and MAPK, which helps neurons become more resistant to oxidative stress. Moreover, IGF-1 enhances mitochondrial biogenesis, which supports energy metabolism and reduces ROS production. The neuroprotective effects of IGF-1 extend to the enhancement of synaptic plasticity, which is critical for learning and memory processes. Its ability to activate intracellular signaling cascades that inhibit apoptosis adds to its protective role against neurodegeneration. Primarily recognized for its function in glucose metabolism, GLP-1 has drawn interest due to its neuroprotective qualities. GLP-1 receptors are expressed in neurons, and their activation has been shown to promote neurogenesis, enhance synaptic plasticity, and reduce neuroinflammation. GLP-1 also significantly reduces ROS production by improving mitochondrial function and upregulating antioxidant defenses [78]. Furthermore, it lessens microglia and astrocyte activation, which in turn lessens the release of pro-inflammatory cytokines including IL-6 and TNF-α. Since prolonged neuroinflammation exacerbates neuronal damage, this anti-inflammatory action is essential in neurodegenerative disorders. The convergence of these pathways is particularly evident in their collective ability to modulate oxidative stress and mitochondrial function. Melatonin’s direct ROS scavenging complements IGF-1 and GLP-1’s ability to boost endogenous antioxidant systems, creating a robust defense mechanism against oxidative damage [83]. IGF-1 and GLP-1 enhance mitochondrial health by promoting mitochondrial biogenesis and improving mitochondrial dynamics, thus optimizing energy production and minimizing ROS output. The combined actions of these pathways help reduce apoptosis by activating pro-survival signaling, such as PI3K/Akt, which inhibits apoptotic factors like BAD and caspases. In the face of persistent oxidative stress, which is a defining feature of illnesses like Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS), this coordinated effort promotes long-term neuronal survival. In terms of metabolic regulation, the interaction between melatonin, IGF-1, and GLP-1 contributes to maintaining glucose homeostasis and insulin sensitivity. Melatonin influences glucose metabolism by enhancing insulin signaling, while GLP-1's incretin effect boosts insulin secretion and reduces glucagon levels, preventing hyperglycemia. IGF-1, which shares structural similarities with insulin, also improves glucose uptake and metabolism. This triad of pathways offers protective benefits against metabolic disorders like type 2 diabetes and obesity, where oxidative stress and inflammation play significant roles in disease progression [84] [85]. In summary, the melatonin, IGF-1, and GLP-1 pathways form a tightly interconnected network that regulates oxidative stress, mitochondrial function, inflammation, and metabolism. Their collective actions protect neurons

from oxidative damage and apoptosis and maintain metabolic balance, offering promising avenues for developing multi-targeted therapies for neurodegenerative diseases and metabolic disorders. By strengthening neuroprotection, lowering oxidative stress, and improving mitochondrial health, addressing these pathways can decrease disease progression and enhance the quality of life in afflicted patients.

6. Role of melatonin in activating IGF-1 and GLP-1 signaling pathways

Melatonin activates the IGF-1 and GLP-1 signaling pathways through several molecular mechanisms, enhancing neuroprotection and metabolic regulation. It boosts IGF-1 receptor activation, leading to increased signaling through the PI3K/Akt and MAPK pathways, which promote neuron survival, inhibit apoptosis, and support neurogenesis and synaptic plasticity. Concurrently, melatonin activates GLP-1 receptors, enhancing insulin secretion, reducing inflammation, and mitigating oxidative stress [86]. By improving mitochondrial function and antioxidant defense, melatonin reduces cellular damage caused by reactive oxygen species (ROS). Melatonin's simultaneous stimulation of these pathways highlights its potential as a treatment for metabolic and neurological illnesses, utilizing its functions in immune regulation, energy metabolism, and cell survival [87, 88]. Melatonin's interaction with IGF-1 and GLP-1 signaling pathways is critical not only for neuroprotection and metabolic regulation but also for its broader systemic effects. IGF-1 signaling through the PI3K/Akt and MAPK pathways is crucial for maintaining neuronal integrity, facilitating neuronal repair, and enhancing cognitive functions [89]. Moreover, melatonin’s activation of IGF-1 signaling contributes to the inhibition of neuroinflammation, a common underlying factor in neurodegenerative diseases. This anti-inflammatory action further helps in preserving neuronal function and preventing the progression of neurological disorders [90, 91]. In the metabolic context, melatonin’s activation of GLP-1 receptors not only enhances insulin secretion and sensitivity but also plays a role in energy balance and weight management. By promoting insulin action and glucose uptake, melatonin helps in reducing hyperglycemia and improving metabolic efficiency. The antioxidative properties of melatonin, combined with its role in enhancing mitochondrial function, ensure that cells are protected from oxidative damage and stress, which is particularly beneficial for tissues with high metabolic rates, such as the liver and muscles [92, 93]. Furthermore, melatonin’s immune-regulating functions extend to modulating cytokine production, thereby reducing chronic low-grade inflammation associated with metabolic and neurological diseases. Its ability to influence both innate and adaptive immune responses makes melatonin a versatile molecule in maintaining overall health. These combined

effects of melatonin on IGF-1 and GLP-1 signaling pathways highlight its comprehensive therapeutic potential for a range of conditions, from diabetes and obesity to neurodegenerative diseases and immune-related disorders [94-96].

7. Implications in ALS Therapy: Protective Effects Against Methyl Mercury-Induced Neurotoxicity

Methylmercury (MeHg) is a highly neurotoxic environmental pollutant known to cause oxidative stress and neurodegeneration. In the context of ALS, melatonin's protective effects against MeHg-induced neurotoxicity are particularly relevant. Melatonin is a potent endogenous antioxidant that can neutralize reactive oxygen species (ROS) generated by MeHg exposure, preventing oxidative damage to neurons a significant contributor to ALS pathology [97, 98]. By regulating immunological responses and preventing the production of pro-inflammatory cytokines, melatonin also has strong anti-inflammatory qualities, lowering MeHg-induced neuroinflammation. Additionally, melatonin activates key signaling pathways such as the IGF-1 (insulin-like growth factor-1) and GLP-1 (glucagon-like peptide-1) pathways. IGF-1 signaling enhances neuronal survival and function by activating the PI3K/Akt pathway, preventing apoptosis and supporting neurogenesis. GLP-1 signaling, on the other hand, decreases oxidative stress and inflammation. Simultaneously stimulating several pathways highlights Melatonin's potential as a treatment for ALS [99].

By mitigating oxidative and inflammatory damage caused by MeHg, melatonin may slow disease progression and improve patient outcomes. Preclinical studies provide a strong foundation, but large-scale, long-term clinical trials are essential to confirm these benefits in human patients [100]. Understanding the precise molecular mechanisms of melatonin's action in ALS will aid in developing targeted therapies and optimizing treatment strategies. Existing evidence supports melatonin's role as a neuroprotective agent, offering significant promise in ALS therapy by leveraging its antioxidant, anti-inflammatory, and neuroprotective properties, along with its ability to activate critical signaling pathways. Continued research and clinical validation are vital to unlocking its full therapeutic potential in ALS treatment [101].

8. Challenges and Future Direction of Melatonin-Based ALS Treatment

Melatonin demonstrates significant potential as a therapeutic agent for ALS due to its antioxidant and immunomodulatory properties. However, several challenges must be addressed to optimize its efficacy. Determining the optimal dosage and route of administration remains a primary challenge, as current studies indicate that melatonin is safe at high doses [102]. Still, more evidence is required

to establish a standardized, effective dosage for slowing disease progression. Additionally, variability in melatonin supplement quality and the lack of long-term clinical data raise safety concerns. Although preclinical research has yielded encouraging findings, there is a dearth of solid clinical data demonstrating melatonin's effectiveness in ALS patients, and the available studies frequently have small sample sizes and brief durations [103]. Since further study is required to clarify these processes and find possible indicators for treatment response, it is also critical to comprehend the specific molecular pathways by which melatonin exerts its neuroprotective benefits in ALS. Another challenge is identifying the most effective combination therapy, as the potential synergistic effects of combining melatonin with other neuroprotective agents have been explored but not yet optimized. Investigating the most effective combinations and sequencing of therapies requires extensive preclinical and clinical studies. To determine the safety, effectiveness, and ideal dosage of melatonin in ALS patients, future research should concentrate on carrying out extensive, protracted clinical trials [104]. These trials should include diverse patient populations to ensure the generalizability of the results. Further research into the molecular mechanisms of melatonin's action in ALS will provide insights into its neuroprotective effects and help identify potential biomarkers for treatment response, guiding the development of targeted therapies. Treatment results can be improved by investigating how melatonin works in concert with other neuroprotective substances such as antioxidants, anti-inflammatory medications, and mitochondrial stabilizers. Preclinical research ought to determine the best combinations and administer them as efficiently as possible [50, 105]. Developing customized treatment strategies based on each patient's particular characteristics, such as genetic profile and stage of sickness, can improve therapy success and reduce adverse effects. Biomarker-driven studies can help tailor melatonin-based therapies to specific patient subgroups. Additionally, exploring the potential of regenerative medicine, such as stem cell therapy and gene therapy, in combination with melatonin-based treatment can offer new avenues for ALS therapy, targeting underlying disease mechanisms and promoting neural repair and regeneration [82, 106]. In conclusion, while melatonin shows promise as a therapeutic agent for ALS, addressing the challenges and exploring future directions are crucial for optimizing its efficacy. More efficient and individualized ALS treatment plans will be made possible by ongoing research and cooperation between researchers, medical professionals, and patients.

CONCLUSION

Melatonin demonstrates significant therapeutic potential for Amyotrophic Lateral Sclerosis (ALS) by addressing key pathological mechanisms, including oxidative stress, inflammation, and mitochondrial dysfunction. Its powerful antioxidant properties neutralize reactive oxygen species, prevent lipid peroxidation, and protect mitochondrial integrity, while its immunomodulatory effects reduce pro-inflammatory cytokines and suppress neuroinflammation, safeguarding motor neurons. Melatonin further enhances neuronal survival by activating the IGF-1 and GLP-1 signaling pathways, promoting neurogenesis and synaptic plasticity, and inhibiting apoptosis. Preclinical and clinical studies have shown promising results, including improvements in oxidative stress markers, motor function, and quality of life. However, challenges such as optimizing dosage, administration routes, and long-term safety remain. Future research should focus on large-scale trials to validate melatonin's efficacy, explore synergistic effects with other neuroprotective agents, and integrate it with innovative therapies like stem cell and gene therapies. With continued exploration, melatonin could play a transformative role in improving outcomes for ALS patients.

ABBREVIATIONS

ALS: Amyotrophic lateral sclerosis; IGF-1: Insulin-like Growth Factor-1; GLP-1: Glucagon-like Peptide-1; EMG: Electromyography; ALSFRS-R: ALS Functional Rating Scale–Revised; TARDBP: TAR DNA Binding Protein ; FUS: Fused in Sarcoma; FTD: frontotemporal dementia; TDP-43,RNA, ROS: Reactive oxygen species; mtDNA: Mitochondrial DNA; ETC: Electron transport chain; ΔΨm: Reduced membrane potential; MCP-1: Monocyte chemoattractant protein-1; PGE2: Prostaglandin E; NO: Nitric oxide; ER: Endoplasmic reticulum; LRP6: Low-density lipoprotein receptor-related protein 6; GSK3β: Glycogen Synthase Kinase 3β; CK1:Casein Kinase 1; AMX0035: Albrioza; MT: Melatonin; SCN: Suprachiasmatic nucleus; SOD1: Superoxide dismutase1; (GPx): Glutathione peroxidase; CAT: Catalase; SOD: Superoxide dismutase; MDA: malondialdehyde; IL-1β: interleukin-1β, IL-6: interleukin-6; TNF-α: tumor necrosis factor-alpha; INOS: inducible nitric oxide synthase; PCN: Primary cortical neurons; HD models: Huntington's Disease (HD) models; mtPTP: Mitochondrial permeability transition pore; AD: Alzheimer's Disease; PD: Parkinson's Disease; TUNEL-positive cells: Terminal deoxynucleotidyl transferase (Dutp) Nick-End Labeling; DUTP: Terminal deoxynucleotidyl transferase; INK: Inhibitors of Kinase; PAR-4: Prostate Apoptosis Response-4; NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells, GPX: Glutathione peroxidase, PI3K/Akt: Phosphoinositide 3-Kinase; Akt: Protein Kinase B; and MAPK:

Mitogen-Activated Protein Kinase; MeHg: Methylmercury; MCAO: Middle Cerebral Artery Occlusion.

AUTHORS' CONTRIBUTION

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

FUNDING

Declared none.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS Declared none.

REFERENCE

1. Morris, J., *Amyotrophic lateral sclerosis (ALS) and related motor neuron diseases: an overview.* The Neurodiagnostic Journal, 2015. **55**(3): p. 180-194.
2. Park, J., J.-E. Kim, and T.-J. Song, *The global burden of motor neuron disease: an analysis of the 2019 global burden of disease study.* Frontiers in Neurology, 2022. **13**: p. 864339.
3. Rizea, R.E., et al., *Understanding amyotrophic lateral sclerosis: pathophysiology, diagnosis, and therapeutic advances.* International Journal of Molecular Sciences, 2024. **25**(18): p. 9966.
4. Bombaci, A., *New insights in early diagnostic and prognostic clinical, instrumental, and fluids biomarkers in Amyotrophic Lateral Sclerosis.* 2024.
5. Sakowski, S.A., et al., *Role of the Exposome in Neurodegenerative Disease: Recent Insights and Future Directions.* Annals of Neurology, 2024. **95**(4): p. 635-652.
6. Nijssen, J., L.H. Comley, and E. Hedlund, *Motor neuron vulnerability and resistance in amyotrophic lateral sclerosis.* Acta neuropathologica, 2017. **133**: p. 863-885.
7. Henstridge, C.M., M. Tzioras, and R.C. Paolicelli, *Glial contribution to excitatory and inhibitory synapse loss in neurodegeneration.* Frontiers in cellular neuroscience, 2019. **13**: p. 63.
8. Buratti, E., *Trends in understanding the pathological roles of TDP-43 and FUS proteins.* Frontotemporal Dementias: Emerging Milestones of the 21st Century, 2021: p. 243-267.
9. Ratti, A. and E. Buratti, *Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins.* Journal of neurochemistry, 2016. **138**: p. 95-111.
10. Weskamp, K. and S.J. Barmada, *TDP43 and RNA instability in amyotrophic lateral sclerosis.* Brain research, 2018. **1693**: p. 67-74.
11. Ruegsegger, C. and S. Saxena, *Proteostasis impairment in ALS.* Brain research, 2016. **1648**: p. 571-579.
12. Cicardi, M.E., et al., *Proteostatic imbalance and protein spreading in amyotrophic lateral sclerosis.* The EMBO journal, 2021. **40**(10): p. e106389.

13. Webster, C.P., et al., *Protein homeostasis in amyotrophic lateral sclerosis: therapeutic opportunities?* Frontiers in molecular neuroscience, 2017. **10**: p. 123.
14. Cenini, G., A. Lloret, and R. Cascella, *Oxidative stress in neurodegenerative diseases: from a mitochondrial point of view.* Oxidative medicine and cellular longevity, 2019. **2019**(1): p. 2105607.
15. Obrador, E., et al., *Oxidative stress, neuroinflammation and mitochondria in the pathophysiology of amyotrophic lateral sclerosis.* Antioxidants, 2020. **9**(9): p. 901.
16. Ramalingam, M. and S.-J. Kim, *Reactive oxygen/nitrogen species and their functional correlations in neurodegenerative diseases.* Journal of Neural Transmission, 2012. **119**: p. 891-910.
17. Zhao, J., et al., *The impact of mitochondrial dysfunction in amyotrophic lateral sclerosis.* Cells, 2022. **11**(13): p. 2049.
18. Khan, T., et al., *Mitochondrial dysfunction: pathophysiology and mitochondria-targeted drug delivery approaches.* Pharmaceutics, 2022. **14**(12): p. 2657.
19. Deshmane, S.L., et al., *Monocyte chemoattractant protein-1 (MCP-1): an overview.* Journal of interferon & cytokine research, 2009. **29**(6): p. 313-326.
20. Al-Shami, A.S., A.E. Essawy, and H.T.A.E.A. Elkader, *Molecular mechanisms underlying the potential neuroprotective effects of Trifolium pratense and its phytoestrogen-isoflavones in neurodegenerative disorders.* Phytotherapy Research, 2023. **37**(6): p. 2693-2737.
21. Belosludtseva, N.V., L.A. Matveeva, and K.N. Belosludtsev, *Mitochondrial Dyshomeostasis as an Early Hallmark and a Therapeutic Target in Amyotrophic Lateral Sclerosis.* International Journal of Molecular Sciences, 2023. **24**(23): p. 16833.
22. Liu, J., et al., *Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities.* Signal transduction and targeted therapy, 2022. **7**(1): p. 3.
23. Ramakrishna, K., et al., *WNT-β catenin signaling as a potential therapeutic target for neurodegenerative diseases: current status and future perspective.* Diseases, 2023. **11**(3): p. 89.
24. Imbrici, P., et al., *Therapeutic approaches to genetic ion channelopathies and perspectives in drug discovery.* Frontiers in pharmacology, 2016. **7**: p. 121.
25. Sun, Y., X. Li, and R. Bedlack, *An evaluation of the combination of sodium phenylbutyrate and taurursodiol for the treatment of amyotrophic lateral sclerosis.* Expert review of neurotherapeutics, 2023. **23**(1): p. 1-7.
26. Loh, D. and R.J. Reiter, *Melatonin: regulation of biomolecular condensates in neurodegenerative disorders.* Antioxidants, 2021. **10**(9): p. 1483.
27. Ahmad, S.B., et al., *Melatonin and health: insights of melatonin action, biological functions, and associated disorders.* Cellular and Molecular Neurobiology, 2023. **43**(6): p. 2437-2458.
28. Reiter, R.J., *Antioxidant actions of melatonin.* Advances in pharmacology, 1996. **38**: p. 103-117.
29. Reiter, R.J., et al., *Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans.* Acta Biochimica Polonica, 2003. **50**(4): p. 1129-1146.
30. Pevet, P., E. Challet, and M.-P. Felder-Schmittbuhl, *Melatonin and the circadian system: Keys for health with a focus on sleep.* Handbook of clinical neurology, 2021. **179**: p. 331-343.
31. Cozzolino, M. and M.T. Carrì, *Mitochondrial dysfunction in ALS.* Progress in neurobiology, 2012. **97**(2): p. 54-66.
32. Bizzarri, M., et al., *Molecular mechanisms of the pro-apoptotic actions of melatonin in cancer: a review.* Expert opinion on therapeutic targets, 2013. **17**(12): p. 1483-1496.
33. Rosa, A.C., et al., *Superoxide dismutase administration: A review of proposed human uses.* Molecules, 2021. **26**(7): p. 1844.

34. Ganie, S.A., et al., *Melatonin: a potential anti-oxidant therapeutic agent for mitochondrial dysfunctions and related disorders.* Rejuvenation research, 2016. **19**(1): p. 21-40.
35. Wei, Y., et al., *Current Therapy in Amyotrophic lateral sclerosis (ALS): a review on Past and Future Therapeutic Strategies.* European Journal of Medicinal Chemistry, 2024: p. 116496.
36. Abdukadir, A., et al., *Plant-Based Extracts and Antioxidants: Implications on Multiple Sclerosis*, in *Exploring the Effects of Diet on the Development and Prognosis of Multiple Sclerosis (MS)*. 2024, Springer. p. 139-163.
37. Orsini, M., et al., *Rehabilitation in Amyotrophic Lateral Scle-rosis: Why it Matters. Current updates on Amyotrophic Lateral Sclerosis*. 2021, MedDocs Publishers.
38. Lu, L., Y. Deng, and R. Xu, *Current potential therapeutics of amyotrophic lateral sclerosis.* Frontiers in Neurology, 2024. **15**: p. 1402962.
39. Nasello, M., et al., *Stem cells and other emerging agents as innovative "drugs" in neurodegenerative diseases: benefits and limitations.* Rejuvenation Research, 2018. **21**(2): p. 123-140.
40. Hussen, B.M., et al., *Revolutionizing medicine: recent developments and future prospects in stem-cell therapy.* International Journal of Surgery, 2024. **110**(12): p. 8002-8024.
41. Monteiro, K.K.A.C., et al., *Antioxidant Actions of Melatonin: A Systematic Review of Animal Studies.* Antioxidants, 2024. **13**(4): p. 439.
42. Chen, D., T. Zhang, and T.H. Lee, *Cellular mechanisms of melatonin: insight from neurodegenerative diseases.* Biomolecules, 2020. **10**(8): p. 1158.
43. López, A., et al., *Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production.* Journal of pineal research, 2009. **46**(2): p. 188-198.
44. Rizzo, F., et al., *Cellular therapy to target neuroinflammation in amyotrophic lateral sclerosis.* Cellular and Molecular Life Sciences, 2014. **71**: p. 999-1015.
45. Stenvinkel, P., et al., *IL-10, IL-6, and TNF-α: central factors in the altered cytokine network of uremia—the good, the bad, and the ugly.* Kidney international, 2005. **67**(4): p. 1216-1233.
46. Kany, S., J.T. Vollrath, and B. Relja, *Cytokines in inflammatory disease.* International journal of molecular sciences, 2019. **20**(23): p. 6008.
47. Ikram, M., et al., *Melatonin as a potential regulator of oxidative stress, and neuroinflammation: mechanisms and implications for the management of brain injury-induced neurodegeneration.* Journal of Inflammation Research, 2021: p. 6251-6264.
48. Ienco, E.C., et al., *Oxidative stress treatment for clinical trials in neurodegenerative diseases.* Journal of Alzheimer's Disease, 2011. **24**(s2): p. 111-126.
49. GRIGG-DAMBERGER, M.M., *Sleep and Neurological Disorders.* Review of Sleep Medicine E-Book, 2017: p. 281.
50. Karaaslan, C. and S. Suzen, *Antioxidant properties of melatonin and its potential action in diseases.* Current topics in medicinal chemistry, 2015. **15**(9): p. 894-903.
51. Petrovic, S., et al., *Lipid peroxidation and antioxidant supplementation in neurodegenerative diseases: a review of human studies.* Antioxidants, 2020. **9**(11): p. 1128.
52. Brown, G.C. and V. Borutaite. *Nitric oxide, cytochrome c and mitochondria*. in *Biochemical Society Symposia*. 1999. Portland Press Limited.
53. Talley Watts, L., et al., *Stroke neuroprotection: targeting mitochondria.* Brain Sciences, 2013. **3**(2): p. 540-560.
54. Cassarino, D.S., et al., *The parkinsonian neurotoxin MPP+ opens the mitochondrial permeability transition pore and releases cytochrome c in isolated mitochondria via an oxidative mechanism.* Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1999. **1453**(1): p. 49-62.

55. Goffredo, D., et al., *Prevention of cytosolic IAPs degradation: a potential pharmacological target in Huntington's Disease.* Pharmacological research, 2005. **52**(2): p. 140-150.
56. Zhang, X., et al., *The MT1 receptor as the target of ramelteon neuroprotection in ischemic stroke.* Journal of Pineal Research, 2024. **76**(1): p. e12925.
57. Polster, B.M., *AIF, reactive oxygen species, and neurodegeneration: a "complex" problem.* Neurochemistry international, 2013. **62**(5): p. 695-702.
58. Li, Y., et al., *Mitochondrial MPTP: a novel target of ethnomedicine for stroke treatment by apoptosis inhibition.* Frontiers in Pharmacology, 2020. **11**: p. 352.
59. Rasheed, M.Z., H. Tabassum, and S. Parvez, *Mitochondrial permeability transition pore: a promising target for the treatment of Parkinson's disease.* Protoplasma, 2017. **254**: p. 33-42.
60. Yu, H., et al., *Neuroprotection against Aβ25–35-induced apoptosis by Salvia miltiorrhiza extract in SH-SY5Y cells.* Neurochemistry international, 2014. **75**: p. 89-95.
61. Tian, D.-S., et al., *FSAP aggravated endothelial dysfunction and neurological deficits in acute ischemic stroke due to large vessel occlusion.* Signal Transduction and Targeted Therapy, 2022. **7**(1): p. 6.
62. Kim, T.Y., et al., *Control of reactive oxygen species for the prevention of Parkinson's disease: the possible application of flavonoids.* Antioxidants, 2020. **9**(7): p. 583.
63. Rojas, F., et al., *Reactive oxygen species trigger motoneuron death in non-cell-autonomous models of ALS through activation of c-Abl signaling.* Frontiers in cellular neuroscience, 2015. **9**: p. 203.
64. Li, J., et al., *Occlusion of middle cerebral artery induces apoptosis of cerebellar cortex neural cells via caspase-3 in rats.* Turkish Neurosurgery, 2011. **21**(4).
65. Velez-Pardo, C., G.G. Ospina, and M.J. del Rio, *Aβ [25–35] peptide and iron promote apoptosis in lymphocytes by an oxidative stress mechanism: involvement of H2O2, caspase-3, NF-κB, p53 and c-Jun.* Neurotoxicology, 2002. **23**(3): p. 351-365.
66. Wang, X., et al., *Involvement of proinflammatory factors, apoptosis, caspase-3 activation and Ca2+ disturbance in microglia activation-mediated dopaminergic cell degeneration.* Mechanisms of ageing and development, 2005. **126**(12): p. 1241-1254.
67. Rigamonti, D., et al., *Huntingtin's neuroprotective activity occurs via inhibition of procaspase-9 processing.* Journal of Biological Chemistry, 2001. **276**(18): p. 14545-14548.
68. S Pandya, R., et al., *Central nervous system agents for ischemic stroke: neuroprotection mechanisms.* Central Nervous System Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Central Nervous System Agents), 2011. **11**(2): p. 81-97.
69. Wang, X., *The antiapoptotic activity of melatonin in neurodegenerative diseases.* CNS neuroscience & therapeutics, 2009. **15**(4): p. 345-357.
70. Yan, X.-Z., et al., *β-amyloid peptide fragment 31–35 induces apoptosis in cultured cortical neurons.* Neuroscience, 1999. **92**(1): p. 177-184.
71. Walker, P.R. and M. Sikorska, *New aspects of the mechanism of DNA fragmentation in apoptosis.* Biochemistry and Cell Biology, 1997. **75**(4): p. 287-299.
72. Adamec, E., J.P. Vonsattel, and R.A. Nixon, *DNA strand breaks in Alzheimer's disease.* Brain research, 1999. **849**(1-2): p. 67-77.
73. Brezniceanu, M.-L., et al., *HMGB1 inhibits cell death in yeast and mammalian cells and is abundantly expressed in human breast carcinoma.* The FASEB journal, 2003. **17**(10): p. 1295-1297.
74. Guo, Q., et al., *Par-4 is a mediator of neuronal degeneration associated with the pathogenesis of Alzheimer disease.* Nature medicine, 1998. **4**(8): p. 957-962.
75. Wang, X., Q. Chen, and D. Xing, *Focal adhesion kinase activates NF-κB via the ERK1/2 and p38MAPK pathways in amyloid-β 25-35-induced apoptosis in PC12 cells.* Journal of Alzheimer's Disease, 2012. **32**(1): p. 77-94.

76. Yang, W., et al., *Sulforaphene Ameliorates Neuroinflammation and Hyperphosphorylated Tau Protein via Regulating the PI3K/Akt/GSK-3β Pathway in Experimental Models of Alzheimer's Disease.* Oxidative medicine and cellular longevity, 2020. **2020**(1): p. 4754195.
77. Arjunan, A., et al., *Identification of the molecular mechanism of insulin-like growth factor-1 (IGF-1): a promising therapeutic target for neurodegenerative diseases associated with metabolic syndrome.* Cell & Bioscience, 2023. **13**(1): p. 16.
78. Herrera, M.L., et al., *Potentialities of IGF-1 for regulating oxidative stress in neuroinflammation and neurodegeneration: theoretical review.* Exploration of Neuroprotective Therapy, 2024. **4**(6): p. 442-458.
79. Yang, X., et al., *Neuroprotective mechanisms of glucagon-like peptide-1-based therapies in ischemic stroke: an update based on preclinical research.* Frontiers in neurology, 2022. **13**: p. 844697.
80. Rowlands, J., et al., *Pleiotropic effects of GLP-1 and analogs on cell signaling, metabolism, and function.* Frontiers in endocrinology, 2018. **9**: p. 672.
81. Cunha-Oliveira, T., et al., *Oxidative stress in amyotrophic lateral sclerosis: pathophysiology and opportunities for pharmacological intervention.* Oxidative medicine and cellular longevity, 2020. **2020**(1): p. 5021694.
82. R Ramis, M., et al., *Protective effects of melatonin and mitochondria-targeted antioxidants against oxidative stress: a review.* Current medicinal chemistry, 2015. **22**(22): p. 2690-2711.
83. Wang, S., et al., *Insulin-like growth factor 1 in heat stress-induced neuroinflammation: novel perspective about the neuroprotective role of chromium.* Stress Biology, 2023. **3**(1): p. 23.
84. Pungsrinont, T., J. Kallenbach, and A. Baniahmad, *Role of PI3K-AKT-mTOR pathway as a pro-survival signaling and resistance-mediating mechanism to therapy of prostate cancer.* International journal of molecular sciences, 2021. **22**(20): p. 11088.
85. Kalimuthu, S. and K. Se-Kwon, *Cell survival and apoptosis signaling as therapeutic target for cancer: marine bioactive compounds.* International journal of molecular sciences, 2013. **14**(2): p. 2334-2354.
86. Casado, M.E., et al., *Recent advances in the knowledge of the mechanisms of leptin physiology and actions in neurological and metabolic pathologies.* International Journal of Molecular Sciences, 2023. **24**(2): p. 1422.
87. Giri, A., et al., *Melatonin-mediated IGF-1/GLP-1 activation in experimental OCD rats: evidence from CSF, blood plasma, brain and in-silico investigations.* Biochemical pharmacology, 2023. **217**: p. 115831.
88. Anderson, G., *Tumor microenvironment and metabolism: role of the mitochondrial melatonergic pathway in determining intercellular interactions in a new dynamic homeostasis.* International Journal of Molecular Sciences, 2022. **24**(1): p. 311.
89. Nagayach, A., et al., *Advancing the understanding of diabetic encephalopathy through unravelling pathogenesis and exploring future treatment perspectives.* Ageing Research Reviews, 2024: p. 102450.
90. Shukla, M., et al., *The role of melatonin in targeting cell signaling pathways in neurodegeneration.* Annals of the New York academy of sciences, 2019. **1443**(1): p. 75-96.
91. Alamdari, A.F., et al., *Melatonin as a promising modulator of aging related neurodegenerative disorders: role of microRNAs.* Pharmacological Research, 2021. **173**: p. 105839.
92. Rajesh, P.N., *Assessment of Combination Therapy of Melatonin & GABA in Diabetic Mouse Models and Evaluation of Correlation Between Leptin & T2D Susceptibility*. 2023, Maharaja Sayajirao University of Baroda (India).
93. Ruddick-Collins, L.C., P.J. Morgan, and A.M. Johnstone, *Mealtime: A circadian disruptor and determinant of energy balance?* Journal of Neuroendocrinology, 2020. **32**(7): p. e12886.

94. Lacourt, T.E., et al., *The high costs of low-grade inflammation: persistent fatigue as a consequence of reduced cellular-energy availability and non-adaptive energy expenditure.* Frontiers in behavioral neuroscience, 2018. **12**: p. 343884.
95. Sonali, S., et al., *Mechanistic insights into the link between gut dysbiosis and major depression: an extensive review.* Cells, 2022. **11**(8): p. 1362.
96. Leisegang, K., R. Henkel, and A. Agarwal, *Obesity and metabolic syndrome associated with systemic inflammation and the impact on the male reproductive system.* American journal of reproductive immunology, 2019. **82**(5): p. e13178.
97. Islam, M.N., et al., *Superoxide dismutase: an updated review on its health benefits and industrial applications.* Critical Reviews in Food Science and Nutrition, 2022. **62**(26): p. 7282-7300.
98. Novo, J.P., et al., *Cellular and molecular mechanisms mediating methylmercury neurotoxicity and neuroinflammation.* International journal of molecular sciences, 2021. **22**(6): p. 3101.
99. Rekatsina, M., et al., *Pathophysiology and therapeutic perspectives of oxidative stress and neurodegenerative diseases: a narrative review.* Advances in therapy, 2020. **37**: p. 113-139.
100. Olufunmilayo, E.O., M.B. Gerke-Duncan, and R.D. Holsinger, *Oxidative stress and antioxidants in neurodegenerative disorders.* Antioxidants, 2023. **12**(2): p. 517.
101. Ioannidis, J.P., B.Y. Kim, and A. Trounson, *How to design preclinical studies in nanomedicine and cell therapy to maximize the prospects of clinical translation.* Nature biomedical engineering, 2018. **2**(11): p. 797-809.
102. Carrascal, L., et al., *Role of melatonin in the inflammatory process and its therapeutic potential.* Current pharmaceutical design, 2018. **24**(14): p. 1563-1588.
103. Sanchez-Barcelo, E.J., et al., *Clinical uses of melatonin in neurological diseases and mental and behavioural disorders.* Current medicinal chemistry, 2017. **24**(35): p. 3851-3878.
104. Cardinali, D.P., *Melatonin: clinical perspectives in neurodegeneration.* Frontiers in endocrinology, 2019. **10**: p. 480.
105. Chitimus, D.M., et al., *Melatonin's impact on antioxidative and anti-inflammatory reprogramming in homeostasis and disease.* Biomolecules, 2020. **10**(9): p. 1211.
106. Muñoz-Jurado, A., et al., *Melatonin and multiple sclerosis: Antioxidant, anti-inflammatory and immunomodulator mechanism of action.* Inflammopharmacology, 2022. **30**(5): p. 1569-1596.