PHYSIOLOGICAL ALTERATIONS ATTRIBUTED TO HYPERTHERMIA IN MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) INDUCED NEUROTOXICITY IN ADULT WISTAR RATS

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CERTIFICATION

This is to certify that this project work on "PHYSIOLOGICAL ALTERATIONS ATTRIBUTED TO HYPERTHERMIA IN MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) INDUCED NEUROTOXICITY" was carried out by DIVINE BEATRICE ELOGHOSA, with matriculation number; BMS2101618; in partial fulfilment for the Award of Bachelor of Science (B.Sc) Degree in the Department of physiology, School of Basic Medical Sciences, University of Benin, Benin City.

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DEDICATION

I dedicate this project study to the Lord God Almighty, whose wisdom, grace, and love have made this project successful.

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TABLE OF CONTENT

ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic (DAergic) neurons in the *substantia nigra pars compacta* (SNpc), leading to severe motor and cognitive impairments. Its complex pathophysiology involves oxidative stress, mitochondrial dysfunction, and glutamatergic excitotoxicity. Hyperthermia, or high body temperature, is also known to cause cellular damage by impairing mitochondrial function and increasing oxidative stress. This study aimed to investigate physiological alterations associated with hyperthermia in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced Neurotoxicity using the MPTP rat model. Adult Wistar rats were divided into Control, Heat only, and MPTP + heat groups, receiving intranasal administration of MPTP (0.1ml/nostril)daily for 2 days. Heat exposure was carried out with heat bulbs for 6hours/day for one week at a temperature range of 28°c - 43°c. The study assessed key biochemical parameters, including oxidative stress markers (Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), and Malondialdehyde (MDA)), and Total Protein levels. Biochemical analysis confirmed that MPTPt + HEAT treatments induced a severe state of oxidative stress. This was evidenced by a significant increase in the activities of antioxidant enzymes (SOD, CAT, GPx) alongside a decrease in the lipid peroxidation marker MDA. This pattern indicates that the cellular antioxidant defenses were overwhelmed by the toxin and heat.. Furthermore, there was a statistically significant decrease in total protein levels across the Heat only and MPTP+ heat groups, suggesting widespread cellular injury and apoptotic death consistent with severe heat exposure and MPTP neurotoxicity. This study confirms the physiological alterations caused by heat, demonstrating that MPTP administration and heat exposure promote molecular damage, oxidative stress, and widespread protein loss.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Neurotoxicity is defined as any adverse effect on the structure or function of the central nervous system (CNS) and/or peripheral nervous system (PNS) caused by biological, chemical, or physical agents, which can occur during development or at maturity (Costa *et al*., 2011). Neurotoxicity may be defined as any adverse effect on the structure or function of the central and/or peripheral nervous system by biological, chemical, or physical agents that diminish the ability of an organism to survive, reproduce, or adapt to its environment. Based on the location or the severity of neurotoxic damage, these can be accompanied by neurocognitive deficits that impact various aspects of daily life activities (Soleimani *et al*., 2016).

Neurotoxic compounds, whether artificial or natural, can disrupt normal nervous system or neuronal function through processes such as neuronal lesions (neuropathy), loss of glial cells, axonal degeneration (axonopathy), myelin cell degeneration (myelinopathy), and neurochemical alterations (Arshajyothirmayi & Gulia, 2022).

Neurotoxicity can affect the developing and adult nervous systems, although the immature nervous system is more vulnerable to permanent changes in cell migration, proliferation, and neuronal connections (Slikker Jr *et al*., 2018). Neuroanatomical abnormalities are known as

structural neurotoxic effects, whereas behavioural, neurochemical, or neurophysiological changes are known as functional (Wallig *et al*., 2017).

Common Neurotoxic Agents and Their Sources

Numerous substances, such as pharmaceuticals, industrial chemicals, and environmental pollutants, can cause neurotoxicity; it is estimated that between 3% and 28% of all commercial chemicals have the potential to cause neurotoxicity. Environmental pollution exposures may be linked to the development of neurologic impairment, according to a number of studies (Bolton *et al*., 2013).

Additional research indicates a possible link between exposure to environmental pollutants and neurodegenerative disorders like Parkinson's and Alzheimer's diseases. The same is true for neurodevelopmental diseases linked to exposure to environmental neurotoxicants(Pellacani & Costa, 2018).

Heavy metals like lead, mercury, cadmium, manganese, and nickel are known neurotoxins that can come from a variety of sources, such as industrial processes, contaminated water, occupational exposure, and environmental pollution. Damage to proteins, lipids, and DNA results from oxidative stress caused by lead, mercury, aluminium, and cadmium, which also impair mitochondrial function, lower ATP synthesis, and increase reactive oxygen species (ROS). With specific mechanisms that differ by metal, concentration, and exposure time, these metals can cause protein aggregation, neuroinflammation, disruption of the blood-brain barrier (BBB), and apoptosis (Gagnon-Chauvin *et al*., 2020).

When imbalanced, essential metals like zinc and manganese exhibit neurotoxicity, which affects enzyme functions and neurotransmitter concentrations. Manganese accumulation is associated with Parkinsonism, while zinc imbalance is associated with epilepsy and neurodegeneration (Zahoor *et al*., 2024).

The main neurotoxic agents are industrial chemicals, such as solvents and insecticides. Acetylcholinesterase is inhibited by pesticides like organophosphates and carbamates, which results in cholinergic hyperstimulation and symptoms like cognitive decline and motor disarray. Because they are lipophilic, solvents like n-hexane and toluene can enter the nervous system through ingestion, skin contact, or inhalation. Neuropathy, cognitive decline, personality disorders, and solvent-induced encephalopathy are linked to long-term solvent exposure (Gade *et al*., 2021).

Emerging neurotoxicants include nanoparticles made of cobalt, silica, silver, and zinc oxide. These particles can cause oxidative stress, inflammation, mitochondrial malfunction, apoptosis, and ferroptosis by penetrating the blood-brain barrier (BBB) or entering the brain through olfactory pathways. Proteins linked to neurodegenerative disorders, including β-amyloid and α-synuclein, may aggregate more quickly and undergo autophagy when exposed to nanoparticles. Inhalation, ingestion, skin contact, and work environments are among the exposure routes. Compared to ionic forms, metals with nanoscale shapes are more neurotoxic (Bencsik et al, 2018).

Acute or long-term neurological disorders can result from biological toxins, such as those derived from bacteria and plants, damaging neuronal tissue through interactions with ion channels and membrane proteins. Headaches, behavioural issues, sensory and cognitive

abnormalities, and limb weakness are possible symptoms. Skin contact, inhalation, or ingestion can all result in exposure (Zhang & Paule, 2010).

Alcohol, opiates, methamphetamine, and other drugs of abuse are known neurotoxicants. Methamphetamine causes myelin degradation, neuronal injury, BBB malfunction, and microglia activation by nonspecific diffusion across the BBB. Global brain shrinkage, peripheral neuropathy, and cerebellar degeneration are all brought on by alcohol. Opioids cause sleep disturbances and raise the possibility of negative neurodevelopmental effects in newborns exposed to them (Gupta & Gupta, 2019).

MECHANISMS OF NEUROTOXICITY

Neurotoxicity arises from diverse cellular and molecular mechanisms, including oxidative stress, excitotoxicity, mitochondrial dysfunction, neuroinflammation, apoptosis, autophagy, and ferroptosis. Oxidative stress is triggered by the excessive generation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, which damage proteins, lipids, and nucleic acids in neural cells (Gupta *et al.*, 2023). The buildup of ROS causes DNA damage, lipid peroxidation, and protein oxidation, all of which exacerbate brain injury. Glutamate receptor overactivation mediates excitotoxicity by causing calcium overload and activating calcium-dependent enzymes, which raise reactive nitrogen species and ROS and start cell death cascades. ROS formation is further encouraged by prolonged glutamate receptor stimulation, which results in mitochondrial malfunction and a decrease in mitochondrial membrane potential.

Energy metabolism is disturbed by mitochondrial dysfunction, which results in decreased adenosine triphosphate (ATP) synthesis and cytochrome c release, which triggers the apoptotic

pathway (Zhang & Xie, 2024). Activated microglia and astrocytes cause neuroinflammation by releasing ROS and pro-inflammatory cytokines, which worsen neuronal injury. The programmed cell death mechanism known as apoptosis is triggered when certain intracellular signals are triggered or when cells are unable to repair DNA damage. The breakdown of cellular components and organelles by autophagy, another controlled cell death mechanism, is linked to neurotoxicity (Chen & Wu, 2024). Disruption of antioxidant defenses, including glutathione depletion, heightens susceptibility to ferroptosis and oxidative damage. The interplay of these mechanisms underlies both acute and chronic neurotoxic insults, leading to neuronal injury and cell death.

1.2 AIM OF STUDY

This study aims to investigate the physiological alteration attributed to hyperthermia in MPTP-induced neurotoxicity.

1.3 STATEMENT OF PROBLEM

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin that selectively damages dopaminergic neurons, leading to Parkinson's disease-like symptoms. This study seeks to elucidate the impact of heat stress on MPTP-induced neurotoxicity, with a focus on the underlying physiological alterations that contribute to neuronal damage. The research will investigate the effects of heat exposure on oxidative stress markers and inflammatory responses in MPTP-treated models, aiming to identify potential therapeutic targets for mitigating neurotoxicity.

1.4 RESEARCH QUESTIONS

What are the possible implications of Hyperthermia in MPTP-treated models?

1.5 OBJECTIVES OF STUDY

The objectives of this study are to :

1. To evaluate the effect of heat stress on MPTP-treated models
2. To evaluate Changes in oxidative stress markers in models.

1.6 SCOPE OF STUDY

This investigation will meticulously focus on the effects of heat stress on MPTP-treated rat models, examining physiological alterations associated with oxidative stress. It is important to delineate that the study will assess changes in oxidative stress markers, specifically within the substantia nigra pars compacta (SNpc) and striatum, key brain regions affected in Parkinson's disease pathology. The scope of the investigation will encompass a multi-faceted approach, integrating biochemical evaluations (e.g., markers of oxidative stress). The study will be conducted exclusively using an established animal model (MPTP-treated rats) and will not involve human subjects or clinical trials. The primary focus is on elucidating fundamental mechanistic insights at the cellular and molecular levels within a controlled experimental environment, contributing to the understanding of the interplay between environmental factors

and neurodegeneration, and providing insights into potential therapeutic targets for neuroprotection.

1.7 JUSTIFICATION OF STUDY

MPTP-induced neurotoxicity is a widely used model for studying Parkinson's disease, providing valuable insights into the disease's pathophysiology. However, the interplay between environmental factors, such as heat stress, and MPTP-induced neurotoxicity remains poorly understood. Elucidating the physiological alterations associated with heat stress in MPTP-treated models can provide crucial information on the disease's progression and potential therapeutic targets. This study aims to bridge this knowledge gap, contributing to the development of effective neuroprotective strategies and improving our understanding of environmental factors influencing neurodegeneration.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

The progressive loss of particular neuronal populations, which results in significant functional abnormalities, is a hallmark of neurodegenerative illnesses, which bear a significant global health burden. Studies on these conditions are increasingly pointing to shared molecular pathways as disease drivers, including excitotoxicity, proteotoxicity, mitochondrial failure, and chronic inflammation.

Parkinson's disease (PD) is the second most prevalent neurodegenerative ailment, primarily affecting those over 55. PD affects around 1 to 3 out of every 100 people who are over 60 years old worldwide. However, it can also affect younger adults and children. PD is characterised by the loss of 50-70% of dopaminergic neurones in the substantia nigra (Schober, 2004). Parkinson's disease (PD) is second to Alzheimer's disease as the most prevalent neurodegenerative disorder throughout the world (Frucht & Termsarasab, 2020; Weiner, 2008).

It is a chronic, progressive neurodegenerative disorder primarily characterized by the selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the presence of Lewy bodies, which are abnormal aggregates mainly composed of alpha-synuclein protein (Kapoor *et al*., 2024).

PD is clinically defined by motor dysfunction, which includes rigidity, bradykinesia, postural instability, and rest tremors. In addition, autonomic dysfunction symptoms like constipation and hypotension are present, along with psychological symptoms like worry and depression.

Moreover, sleep disturbances, olfactory impairment, and paraesthesia are its hallmarks (Magrinelli *et al*., 2016).

Additionally, PD puts a heavy burden on carers, frequently resulting in elevated stress and strain levels (Macchi *et al*., 2020). Nonmotor symptoms and consequences, such as autonomic dysfunction, sensory abnormalities, neuropsychiatric or neurobehavioral disorders, are acknowledged as essential components of PD in addition to motor symptoms. In people with PD, neurodegenerative processes that impact different neurotransmitter systems in different brain regions are frequently the cause of common neuropsychiatric or neurobehavioral problems, such as depression, anxiety, rapid eye movement sleep disturbance, and dementia.

There is now no known cure for Parkinson's disease, which progresses at a rate that varies greatly. Four overlapping stages can be distinguished in the condition: palliative, complex, maintenance, and diagnosis (MacMahon & Thomas, 1998). Pathologically, PD is associated with Lewy bodies (LBs), dopaminergic neuronal cytoplasmic inclusions. These are the hallmarks of PD, as they are not detectable in healthy individuals (Goedert *et al*., 2017).

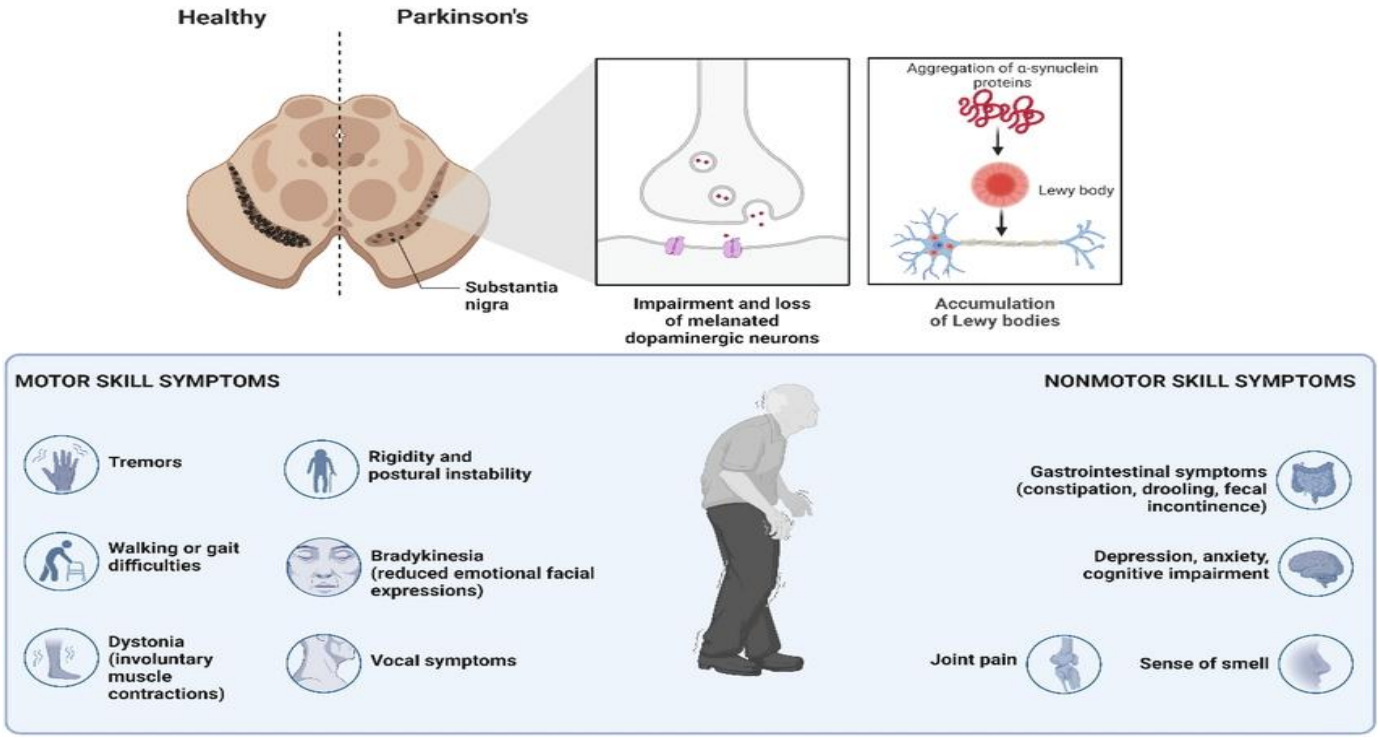

Fig 2.1 Diagram: Illustrative representation of the effects of Parkinson's disease (PD) in the brain and listing of motor and non-motor skill symptoms. Degeneration of pigmented dopaminergic neurons in the midbrain and misfolded α-synuclein and consequential accumulation of Lewis bodies are the hallmarks of PD. The neuronal loss causes dysfunction of the nigrostriatal pathway with a decrease in the levels of dopamine. Usually, this later pathway impairment results in the cardinal motor symptoms of PD.

Source: https://www.researchgate.net/figure/Illustrative-representation-of-the-effects-of-Parkinsons-disease-PD-in-the-brain-and_fig3_375663934.

2.2 PATHOPHYSIOLOGY OF PARKINSON'S DISEASE

PD is a disorder that affects the extrapyramidal system, particularly the basal ganglia's motor components. It is characterised by a decline in dopaminergic activity, which lowers motor

performance and causes disease-related clinical symptoms to appear. Studies conducted in the late 1950s demonstrated that striatal dopamine depletion is the primary cause of motor symptoms in Parkinson's disease. The presence of non-motor characteristics, however, points to the participation of a number of other neurotransmitters, such as those from the glutamatergic, cholinergic, serotonergic, and adrenergic systems, as well as neuromodulators like enkephalins and adenosine. Additional study suggests that PD may first affect the anterior olfactory nucleus, as well as the dorsal motor nucleus of the vagal and glossopharyngeal neurones. The degeneration in PD is not limited to the substantia nigra pars compacta; it also involves other regions such as the basal ganglia, cortex, brainstem nuclei, and the peripheral autonomic nervous system.

Genetic research on familial PD has revealed that mutations in a single gene cause monogenic PD. The α-Syn-encoded genes, dardarin, vacuolar protein sorting-associated protein 35, parkin ligase, deglycase DJ1, and acid β-glucosidase are specifically the sites of mutations that cause Parkinson's disease. They have identified important mechanisms and molecular actors in the aetiology of Parkinson's disease (PD), even though mutations in these genes are rare and only occur in fewer than 10% of all PD patients (Deng *et al*., 2018; Ross, 2013).

This can be explained by the gene (SNCA), which has a hereditary and neuropathological connection to Parkinson's disease. Furthermore, both familial and idiopathic Parkinson's disease are known to have LBs and α-Syn. In addition to the copy number changes and SNCA mutations found in monogenic PD, common SNCA mutations are associated with a higher risk of idiopathic PD (Farrer *et al*., 2001).

Since 90% of PD patients do not exhibit a monogenic inheritance pattern, the illness is regarded as idiopathic. The aetiology of sporadic Parkinson's disease is complex, involving both genetic and environmental variables that contribute to an individual's susceptibility to the illness (Delamarre & Meissner, 2017; Nalls *et al*., 2014).

Epidemiological research has confirmed that extended exposure to chemicals found in rural areas, such as rotenone and paraquat, increases the likelihood of developing Parkinson's disease. It has been documented that dopamine structural analogues, such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and 6-hydroxydopamine (6-OHDA), cause Parkinsonism by specifically harming dopaminergic neurones (Zeng *et al*., 2018).

By directly activating dopamine receptors, which are similar to the brain's natural neurotransmitter, dopamine agonists reduce Parkinson's symptoms. Ergoline and non-ergoline are the two different kinds of dopamine agonists that target dopamine D2-type receptors. Bromocriptine, pergolide, lisuride, and cabergoline are examples of ergoline agonists, but ropinirole and pramipexole are classified as non-ergoline. By acting on both D1 and D2 receptors, apomorphine, an early dopamine agonist, reduces Parkinson's symptoms; however, it must be administered subcutaneously (Brooks, 2000).

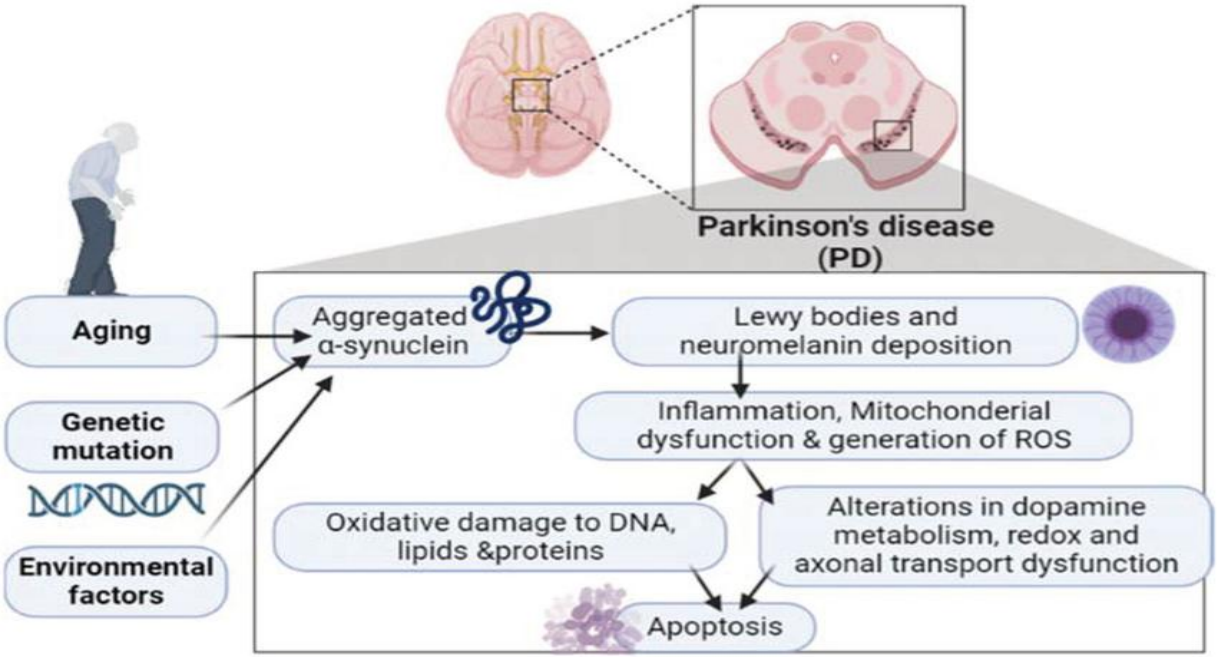

Fig 2.2 Diagram illustrating the pathogenesis of Parkinson's Disease (PD), showing how risk factors like Aging, Genetic mutation, etc, lead to the accumulation of Aggregated alpha-synuclein. This protein aggregation forms Lewy bodies and induces cellular stress (inflammation, mitochondrial dysfunction, ROS generation), ultimately causing oxidative damage and dysfunction in dopamine metabolism. This cumulative damage results in apoptosis and the death of dopaminergic neurons, leading to the clinical manifestation of PD.

Source: https://www.researchgate.net/figure/Pathophysiology-of-Parkinsons-disease-PD-Aging-genetic-mutations-and-environmental_fig1_368725665

2.3 MPTP RAT MODELS IN PARKINSON'S DISEASE

The PD model induced by MPTP is an experimental model based on the systemic treatment of MPTP, which has a high toxic affinity to dopaminergic neurons. Langston et al. have described

Parkinsonism in a group of drug abusers mediated by intravenous injection of MPTP with an illegal neurotoxin-containing drug (Langston *et al*., 1984).

MPTP was initially shown to be a Parkinsonian agent in humans, but it has also been shown to have comparable effects in a variety of other primates, cats, and rodents. It has been demonstrated that only particular mouse strains in rodents are susceptible to MPTP treatment (Smeyne & Jackson, 2005).

MPTP, a structurally similar meperidine analogue, is a by-product of 1-methyl-4-phenylpropionoxy-piperidine synthesis. When this toxin is administered to mice, its lipophilicity allows it to easily pass through the blood–brain barrier (BBB) and enter the central nervous system (CNS) (Zeng *et al*., 2018).

Glial cells (astrocytes) release the enzyme monoamine oxidase type B (MAO-B), which in the central nervous system (CNS) transforms MPTP into the intermediate metabolite 1-methyl-4-phenyl-2,3-dihydropyridine and then into the ultimate hazardous metabolite 1-methyl-4-phenylpyridinum (MPP+) (Kato *et al*.,2003). The active neurotoxin, MPP+, operates at the cellular level because it is a polar molecule and cannot pass back through the blood-brain barrier (Pasquali *et al*., 2014). MPP+ enters dopaminergic (DA) and norepinephrine (NE) neurones specifically through the DA transporter (DAT) and the NE transporter, respectively (Meredith & Rademacher, 2011).

The mouse MPTP model has been an important tool for understanding PD, despite the fact that this model does not fully recapitulate the broad spectrum of PD symptoms. While PD-toxin models have been useful in identifying important disease-related pathways and have been at the

forefront of testing new treatment strategies, they are also unable to accurately replicate symptoms that are similar to those of genuine Parkinson's disease.

A Ubiquitin-Proteasome System (UPS) failure has been suggested as the cause of Lewy body formation, one of the hallmarks of Parkinson's disease. Lewy bodies have been suggested to grow gradually, beginning as solid proteinaceous granules mixed with α-synuclein and ubiquitin-positive filament (Meredith *et al*., 2004).

Following treatment with MAO-B inhibitors, such as seleginine, Cohen et al. and Heikkila et al. observed striatal MPP+ depletion, demonstrating that inhibition of this enzyme considerably inhibited the generation of this hazardous metabolite (Cohen *et al*., 1984; Heikkila *et al*., 1984).

Thus, MPTP's selective toxicity is negatively correlated with VMAT-2 levels and directly correlated with DAT levels [36].According to research by Takahashi et al. mice lacking DAT were immune to the harmful effects of MPTP. Consequently, overexpression of DAT will increase the neurotoxicity of MPTP (Takahashi *et a*l., 1997). MPP+ is transported via vesicular monoamine transporter type 2 (VMAT-2) and stored in synaptosomal vesicles after forming a compound with neuromelanin in the axoplasm of the NE/DA nerve cell. Gainetdinov et al. verified this in an experiment where VMAT-2-deficient mice displayed noticeably higher toxicity to MPTP (Gainetdinov *et al*., 1998). Consequently, MPTP's selective toxicity is negatively correlated with VMAT-2 levels and directly correlated with DAT levels (Watanabe *et al*., 2005).

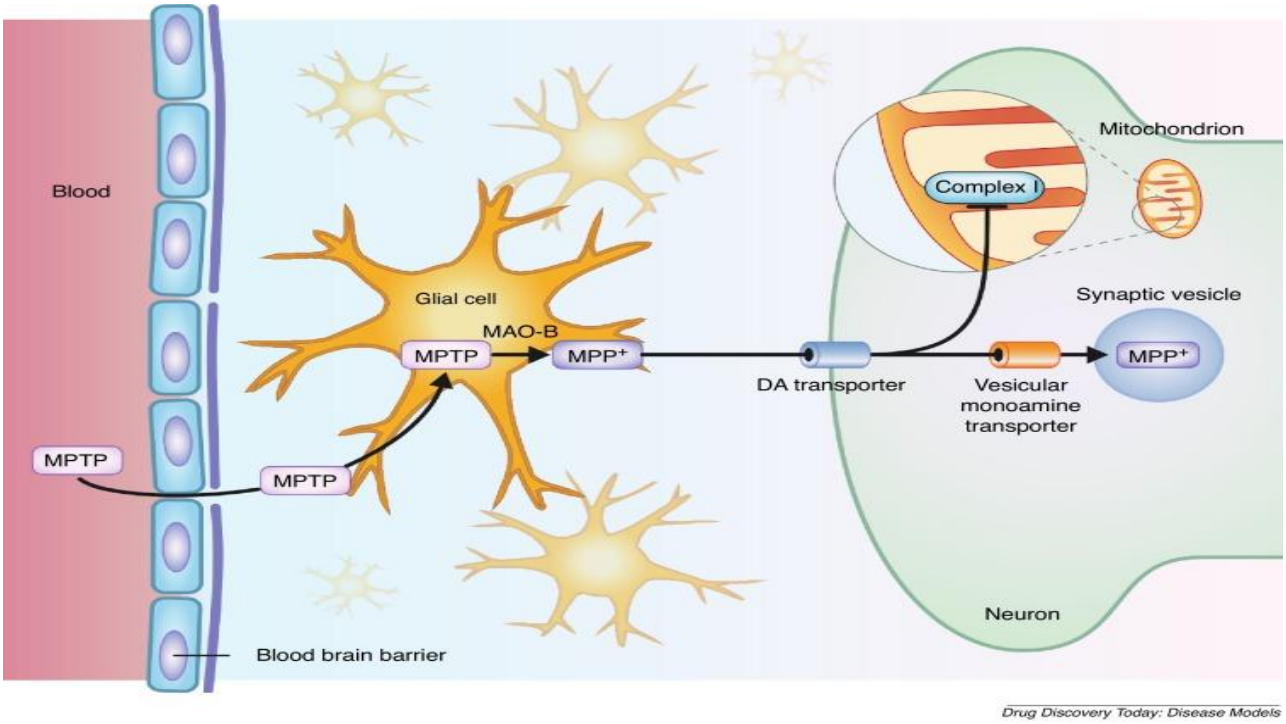

Fig 2.3 Diagram illustrating the mechanism of MPTP neurotoxicity, specifically how the compound MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) crosses the blood-brain barrier and leads to the death of dopaminergic neurons.

Source: https://support.google.com/legal/answer/3463239?hl=en-NG

2.4 MECHANISMS OF MPTP-INDUCED NEUROTOXICITY

Following its accumulation and aggregation in synaptosomal vesicles of DA neurons, the poisonous metabolite of MPTP (MPP+) gradually becomes excessive in the cytoplasm, which ultimately results in cell death in the striatum and SNpc through the following pathways:

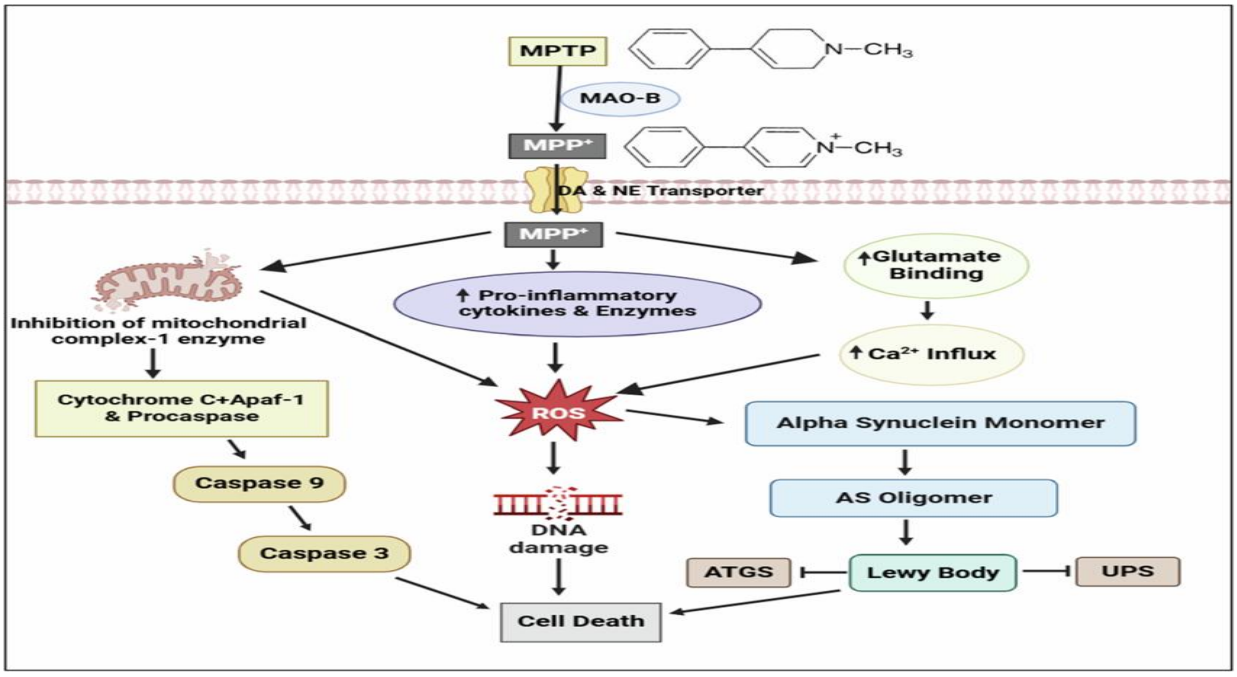

Fig. 2 .4 Overview of the neurotoxic mechanisms of MPTP. MPTP gets metabolized through MAO-B and produces MPP^+, which is taken by DAT, resulting in the prevention of complex I from entering the mitochondria. This inhibition results in the transitional pore opening and the subsequent excretion of cytochrome C, initiating a cascade of processes that culminate in the cell death process (Mitochondrial apoptotic pathway). Suppression of complex 1 results in an elevated generation of ROS, leading to cellular degradation and, ultimately, death of the cell (Oxidative stress ways). Excess generation of ROS results in the development of AS monomers, which then aggregate into toxic oligomers that impede the UPS and autophagy ATGS, ultimately culminating in cell death (Alpha-synuclein pathway). MPP^+ induces an increased level of glutamate binding within the synaptic cleft. This induces calcium influx, resulting in the overproduction of ROS, ultimately causing cellular degradation and neuronal cell death (Glutamatergic pathway). MPP^+ causes stimulation of microglia cells, and it induces the excretion of many proinflammatory mediators like cytokines/enzymes responsible for excessive

ROS generation, and after that, finally cell degeneration (Inflammatory pathway). (+): upregulates; (-): downregulates; (↑): Activates.

Source: https://www.researchgate.net/figure/Overview-of-the-neurotoxic-mechanisms-of-MPTP-MPTP-gets-metabolized-through-MAO-B-and_fig1_390799271

Mitochondrial Apoptotic Pathway

MPP+ reduces the production of anti-apoptotic proteins like Bcl2 and inhibits COMPLEX 1 in the mitochondria (Gluck *et al*., 1994; Scotcher *et al*., 1990). By impeding the electron transport chain, this inhibition prevents the synthesis of ATP and raises the formation of reactive oxygen species (ROS), which causes the mitochondrial transition pores to open (Pasquali *et al*., 2014).

After that, cytochrome c is liberated from a mitochondrion and combines with apoptosis protease activating factor-1 and pro-caspase-9 to create a complex (Schmidt & Ferger, 2001). The resultant combination triggers caspase 9 and subsequent caspases, which leads to apoptosis and ultimately DA nigrostriatal cell death in the striatum and SNpc (Basil *et al*., 2017).

Oxidative Stress Pathway

MPP+ permits the overproduction of ROS, including H2O2, NO, and hydroxyl radicals, and inhibits nicotinamide adenine dinucleotide dehydrogenase in the mitochondria (Andreassen *et al*., 2001; Maragos *et al*., 2000). Through protein cross-linking, lipid peroxidation, and DNA damage, these ROS overpower the body's antioxidant defence system and destroy DA nigrostriatal cells in the SNpc and striatum (Chun *et al*., 2001; Van Raamsdonk *et al*., 2017).

Alpha-synuclein Pathway

Increases in ROS cause production of alpha-synuclein monomers (Fornai *et al*., 2002). These monomers combine to create hazardous alpha-synuclein oligomers when their concentrations rise. This type of oligomer can also result from mutations in the alpha-synuclein gene, SNCA (Meredith *et al*., 2008; Sulzer, 2001). The oligomers block the autophagy system (ATGS) and ubiquitin proteasome system (UPS), which are in charge of preserving the neuron's metabolic equilibrium (Castino *et al*., 2008; Dehay *et al*., 2010). The development of LBs, one of the pathological characteristics of Parkinson's disease, is caused by UPS failure (Pasquali *et al*., 2014).

2.5 HYPERTHERMIA AND NEURODEGENERATION

A body temperature above 40 °C is considered hyperthermia. Sepsis, toxidromes, drug responses, and environmental factors are among the causes. Fever is the result of a dysregulated immune response in sepsis. Endogenous heat is produced by dysregulated metabolism in toxidromes, withdrawal symptoms, and drug responses. When thermoregulatory systems are unable to adjust for metabolic and ambient heat sufficiently, heat-related diseases result (Wasserman *et al*., 2017).

Given that MPTP is typically supplied peripherally, either by food or direct injection, one must also be mindful of peripheral effects that may interact with central nervous system effects. According to earlier studies, MPTP can cause renal, lung, and cardiac toxicity and alterations in muscle function (Takatsu *et al*., 2000; Wilson *et al*., 1991). It has been observed that, in addition to its capacity to harm organ systems, the peripheral injection of it seems to alter the animal's core body temperature (Freyaldenhoven *et al*., 1995; Satoh *et al*., 1987).

Heat generation and loss combine to maintain body temperature, and even slight variations in an animal's core temperature can have a major impact on its cardiovascular, endocrine, and neurological systems, all of which have been linked to MPTP and can ultimately result in death (Przedborski *et al*., 2001). The loss of body temperature correlates with a significant loss of ATP and dopamine in the striatum and ATP in the substantia nigra.

Acute hyperthermia causes the limbic system's connections in the temporal, frontal, and occipital lobes to decrease and increase (Sun *et al*., 2013). In many organs and tissues, temperature rises cause alterations in gene expression at the cellular level (Jian *et al*., 2008; Yan *et al*., 2009). Protein denaturation and the disturbance of vital cellular functions that result in apoptosis and cell death are caused by these increases (Matsuki *et al*., 2003).

2.6 EFFECT OF HYPERTHERMIA IN PARKINSON'S DISEASE

Chronic stress is associated with pathological and psychological alterations in brain function. However, it remains unclear how heat-induced stress regulates cellular response interactions (Wang et al., 2017). Ca2+, as an indispensable factor in signal transduction and cellular response regulator, seems to be significant in many aspects of heat response events. TRPV1 (Transient Receptor Potential Vanilloid1) and TRPV4 genes are members of the TRPV family, including integral membrane proteins that act as calcium-permeable channels. These channels act as thermosensors and have essential roles in the cellular regulation of heat responses. The TRPV1 and TRPV4 ion channels, as calcium-permeable cation channels, are integral membrane proteins that regulate many cell functions (Nilius & Owsianik, 2011).

By interfering with essential cellular functions, hyperthermia severely damages mitochondrial function. For instance, mild hyperthermia (around 40 °C) causes the inner membrane of the

mitochondria to become more permeable, which reduces membrane potential and impairs oxidative phosphorylation. Inefficient adenosine triphosphate (ATP) synthesis may arise from this phenomenon, especially at temperatures higher than 40 °C (Naučienė *et al*., 2012). Additionally, higher temperatures cause mitochondria to produce more reactive oxygen species (ROS), which exacerbates oxidative stress and damages cells. ROS produced in hyperthermia can initiate pathways leading to apoptosis.

Hyperthermia causes apoptosis through mitochondrial mechanisms, such as releasing cytochrome c into the cytoplasm, at extremely high temperatures (43 °C or above), which results in cell death. This process is employed therapeutically to cause tumour cells to undergo apoptosis in hyperthermia-based cancer treatments (Yuen *et al*., 2000).

In conclusion, increased membrane permeability, excessive ROS production, and compromised respiratory chain reaction all contribute to hyperthermia-induced mitochondrial dysfunction, which causes necrosis and apoptosis and ultimately various organ failure. Therefore, one of the primary causes of death in heatstroke is thermal damage to the mitochondria. (Iba *et al*., 2025).

Numerous genetic mutations, such as those in α-synuclein, parkin, UCL1, DJ-1, PINK1, and LRRK2, have been shown to exist. These mutations offer hints about the molecular processes that contribute to the loss and susceptibility of dopaminergic cells as the disease progresses (Gwinn-Hardy, 2002). Cell death in Parkinson's disease is believed to be caused by several mechanisms. Although their exact functions are yet unknown, these include aberrant protein interactions in the ubiquitin-proteasome system (UPS), oxidative stress, mitochondrial malfunction, apoptosis, inflammation, and excitotoxicity (McNaught & Olanow, 2006).

Heat shock proteins (HSPs) offer defence against oxidative stress and other harmful situations. Because of their molecular chaperone function, which promotes nascent protein folding, refolding, or the breakdown of improperly formed proteins, HSPs are thought to safeguard cells (Frydman, 2001; Wegele *et al*., 2004).

In Parkinson's disease (PD), which is characterised by protein structural abnormalities that lead to misfolding, aggregation, and intracellular Lewy body formation, there is evidence that HSPs may play a role in neuronal cell death (Klucken *et al*., 2004; McLean *et al*., 2004). In Drosophila, it has been demonstrated that overexpression of Hsp70 prevents dopaminergic neuronal loss linked to α-synuclein (Auluck et al., 2002).

A recombinant adeno-associated virus that transferred the Hsp70 gene to the dopamine neurones also shielded the mouse dopaminergic system against the loss of dopaminergic neurones caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Dong *et al*., 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Animals and Ethical Considerations

Twelve (12) adult Wistar rats, with an initial weight range of 165-270 grams, were used for this study. The rats were housed in standard polypropylene cages under controlled conditions of temperature (25 ± 2°C), humidity (55 ± 5%), and a 12-hour light/dark cycle. They were provided with standard rodent feed and water throughout the acclimatization and experimental periods

3.2 Experimental Design and Grouping

After a two-week acclimatization period, the rats were randomly divided into three experimental groups (n=4 per group):

Group A (Control): Received only standard food and water and served as the untreated control.

Group B (Heat only): exposed to heat for one week(6hours/day) using heat bulbs in enclosed boxes

Group C (MPTP + Heat): administered MPTP (0.1ml/nostril) after being exposed to heat for 7 days (6hours/day) alongside group B

3.3 Drug Preparation and Administration

MPTP Solution: MPTP was dissolved in normal saline to achieve the required concentration for intranasal administration.

Intranasal Administration: The intranasal administration was performed daily for 2 days.

3.4 Chemicals and Reagents

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was procured from Sigma-Aldrich (USA). All other chemicals and reagents used for biochemical assays, including those for the determination of oxidative stress markers, were of analytical grade.

3.5 Sample Collection and Preparation

At the end of the 2-day treatment period, the rats were anesthetized using chloroform vapor. Blood samples were collected via cardiac puncture into plain tubes.

3.6 Heat Exposure

Eight of the Rats were fed at least an hour before exposure. They were exposed to heat using heat bulbs in enclosed boxes for 6 hours per day for one week. The temperature was regulated and maintained within the range of 28°C - 43°C. Their body weights were recorded periodically during the exposure period.

3.7 Biochemical Analysis of Oxidative Stress Markers

3.7.1 DETERMINATION OF CATALASE (CAT)

Catalase (CAT) activity was estimated by the method described by Cohen et al. (1970).

Reagents

Hydrogen peroxidase (H_2O_2)

Sulfuric acid (6M) H_2SO_4

Preparation of reagents

0.01M $KMnO_4$ was prepared by distilling 0.158g of $KMnO_4$ in 100ml of distilled water. Phosphate buffer (pH 7.4): 0.426g of $NaHPO_4$ and 0.240g of NaH_2PO_4 were weighed and dissolved in 100ml of distilled water. 6M H_2SO_4 and 32.3ml of conc. H_2SO_4 was added to 66.7ml of distilled water.

Procedure

To an unknown volume of plasma (0.5ml), 5.0ml of H_2O_2 was added. This was mixed by inversion and allowed to stand for 30 minutes. The reaction was stopped by adding 1.5ml of 6M H_2SO_4 and 7ml of 0.01M $KMnO_4$. These were mixed by inversion and allowed to stand for 10 minutes. The absorbance was read at 480nm within 30-60 seconds against distilled water. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as μmoles of H_2O_2 decomposed/min/mg/protein.

Calculation

Activity = OD/min x V

M x V x L x Y

Where OD = Absorbance

L= Light path

V Total volume of reaction sample

M= Molar coefficient of H2O2 (40/m/cm)

V Volume of sample

Y mg protein in the sample

3.7.2 ESTIMATION OF SUPEROXIDE DISMUTASE ACTIVITY (SOD)

This was determined according to the methods of Masra and Fridorich (1972)

Principle

Adrenaline undergoes autooxidation rapidly to adrenochrome, whose concentration can be determined at 420nm with the aid of a spectrophotometer. The autooxidation of adrenaline depends on the presence of superanions.

Superoxide dismutase inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD, which is determined at 420nm.

Reagent and preparation

Carbonate buffer (0.05M) pH 10.2: This was prepared by dissolving 0.2014g of Na2CO3, 0.2604g NaHCO3, and 0.0372g of EDTA in 100ml of distilled water. The pH was adjusted to 10.2 using Sodium hydroxide.

Hydrochloric acid (0.005M): This was prepared by adding 0.044 M concentration of HCL to 99.96 mL of distilled water.

Adrenaline solution (0.3mM): This was prepared by dissolving 0.01098g of adrenaline in 100 mL of 0.005M HCL solution.

Plasma volume of 100ml was mixed with 125ml of carbonate buffer and 150ml of adrenaline solution. 100ml of distilled water was mixed with 1.25ml of carbonate buffer as a reference sample. These were mixed, and the absorbance was read at 420nm.

These were mixed and read at 420nm

% inhibition (O.D. test - ODref) x 100

OD test

Enzyme concentration can thus be calculated

unit/mg protein = % inhibition

50 x Y

Where Y = mg of protein in the volume of the sample used

3.7.3 ESTIMATION OF GLUTHATHIONE PEROXIDASE (GPx)

This was determined according to Nyman (1959)

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting in a deep brown color disposition, read at 420nm.

Reagent and preparation

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100 mL of distilled water.

Procedures

To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H2O2, 1.5ml of distilled water, and 2.5ml of pyrogallol were added.

The reaction was allowed to stand for 30 minutes at room temperature. A deep brown color was formed, which was read at 480nm.

Calculations

Activity= OD/min x vt x Df

E x Vs x Y

OD Absorbance of test

Vt: Total volume of reaction mixture

Df= Diution factor = 1

E= Molar extinction co-efficient (12/m/cm)

Vs Volume of sample

Y mg of protein used

3.7.4 DETERMINATION OF MALONDIALDEHYDE (MDA)

Malonaldehyde was determined using the thiobarbituric acid assay (Buege & Aust, 1978)

Principle

Malonaldehyde, which is a product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to give a red species.

Procedure

A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuging at 1000g for 10min. The absorbance was determined using the formula;

MDA (mol/mg protein) = A x V x 100

M x V x Y

A= Absorbance

V Total volume of reaction mixture

M Molar extinction coefficient

V volume of the sample

Y= mg protein

3.8 Protein Determination

The protein content in the serum samples was determined using the standard Bradford method to normalize all enzymatic activities and MDA levels.

3.9 ETHICAL CONSIDERATIONS

All experimental procedures were conducted in strict accordance with the guidelines of the Ethics Committee of the College of Medical Sciences, University of Benin (Approval ID:) and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

3.10 STATISTICAL ANALYSIS

All the data obtained from the experiments were expressed as mean + Standard Error of Mean (SEM): Statistical analysis was performed by one-way analysis of variance (ANOVA) to assess differences amongst multiple groups, followed by Tukey's post-hoc test using Graphpad Prism 10.0.3 statistical analysis software (Graphpad, San Diego, CA). $P < 0.05$ was considered statistically significant.

CHAPTER FOUR

RESULTS

RESULTS OF STATISTICAL ANALYSIS

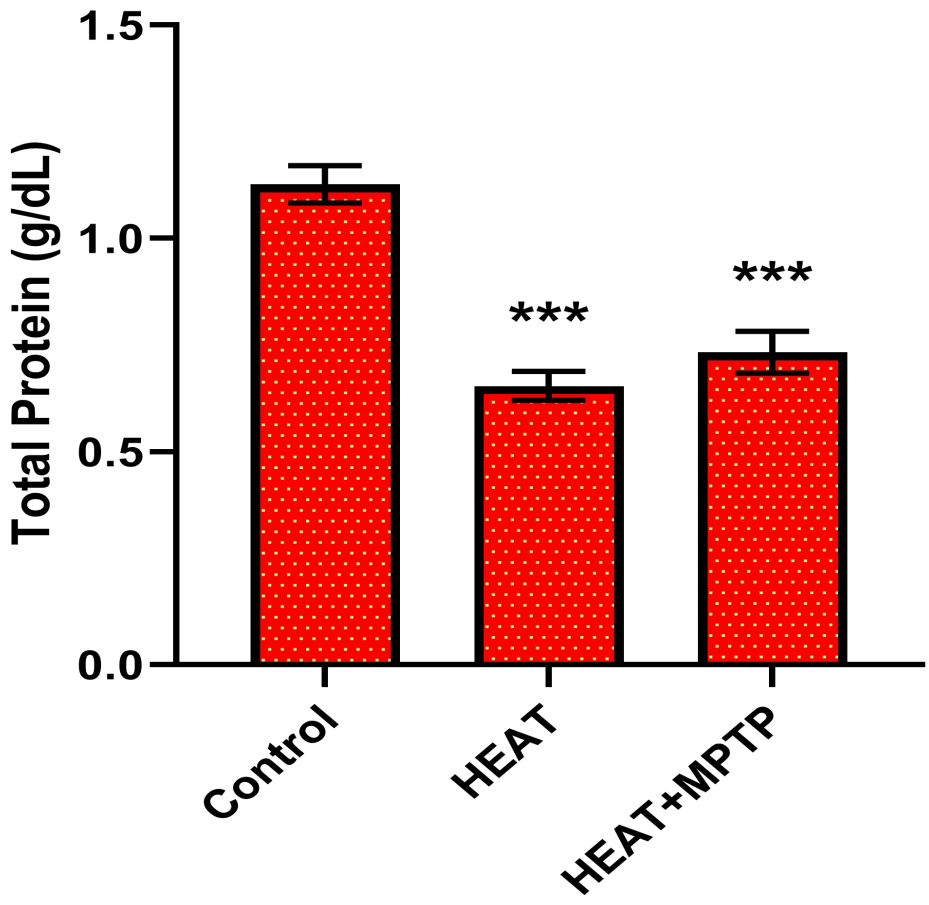

Fig 1: Effect of HEAT and HEAT+MPTP on total protein concentration

The result shows a statistically significant decrease in total protein concentration in HEAT and HEAT+MPTP-treated groups compared to the control ($p<0.05$)

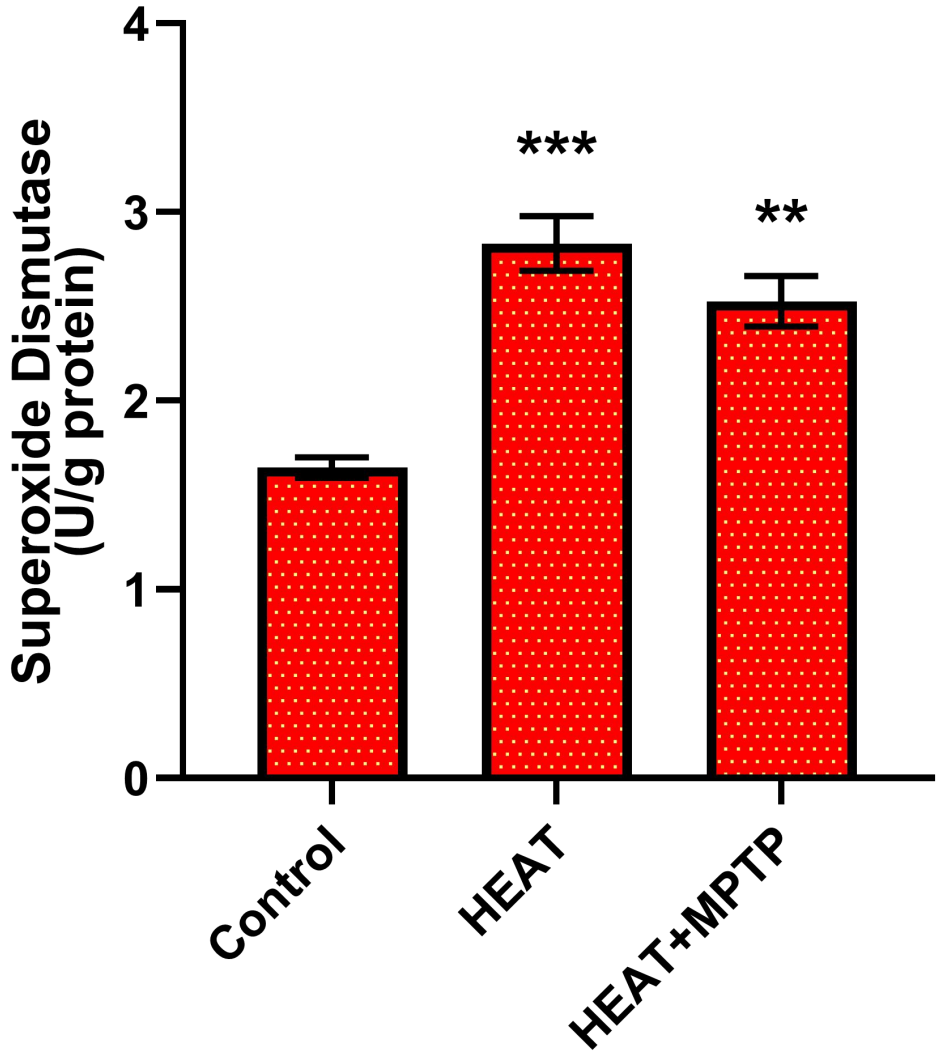

Fig 2: Effect of HEAT and HEAT+MPTP on superoxide dismutase enzyme activity

The result shows a statistically significant increase in superoxide dismutase enzyme activity in HEAT and HEAT+MPTP-treated groups compared to the control ($p<0.05$)

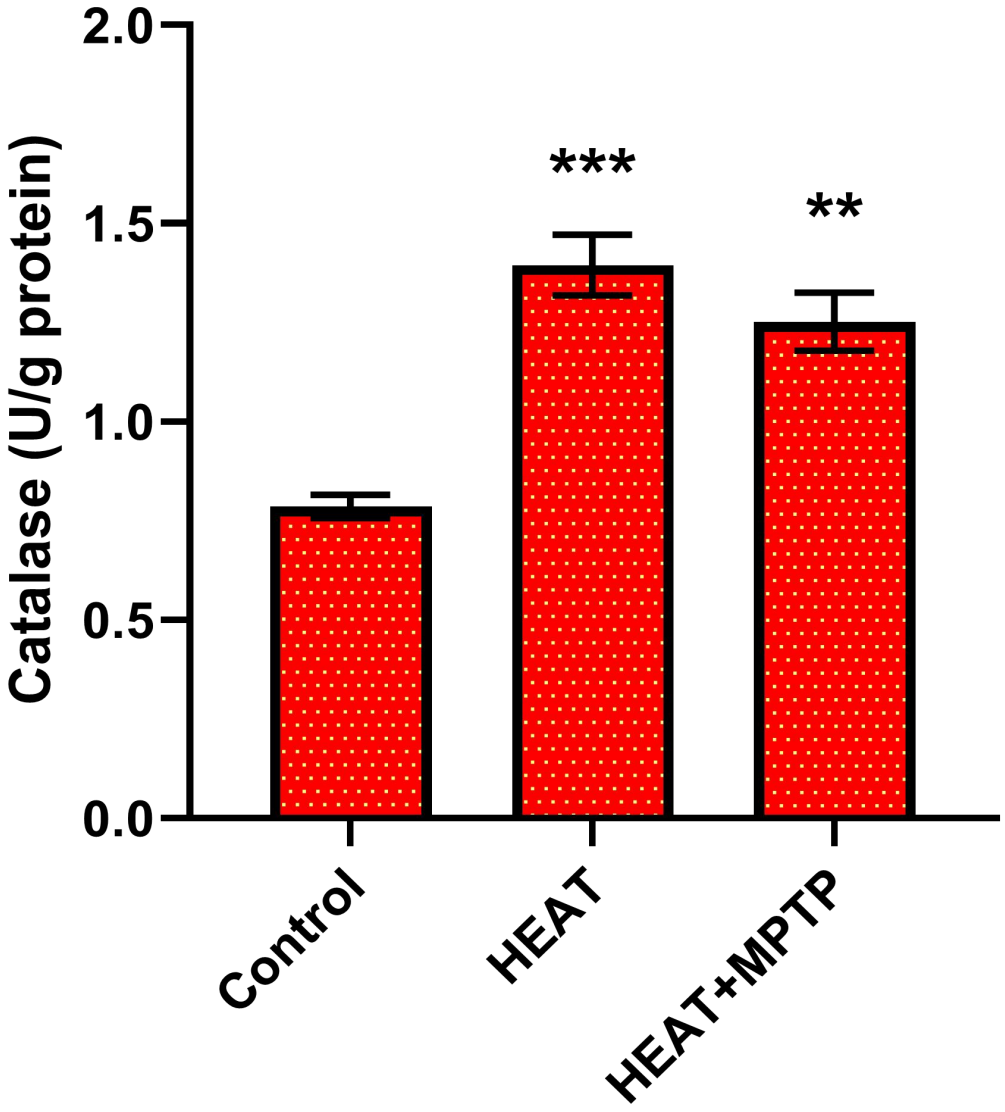

Fig 3: Effect of HEAT and HEAT+MPTP on catalase enzyme activity

The result shows a statistically significant increase in catalase enzyme activity in HEAT and HEAT+MPTP-treated groups compared to the control ($p<0.05$)

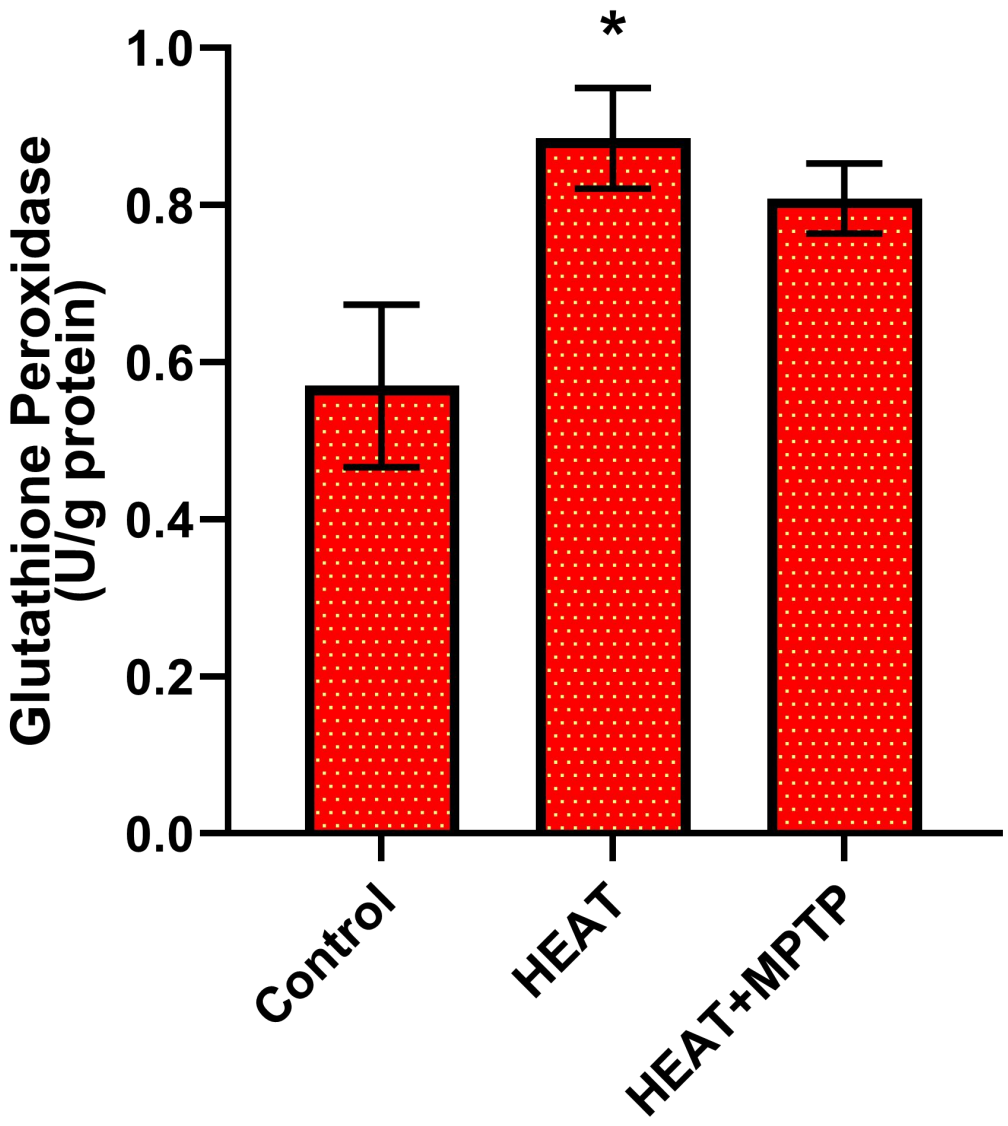

Fig 4: Effect of HEAT and HEAT+MPTP on glutathione peroxidase enzyme activity

The results show a statistically significant increase in glutathione peroxidase enzyme activity in the HEAT and HEAT+MPTP-treated groups compared to the control ($p<0.05$) compared to the control group.

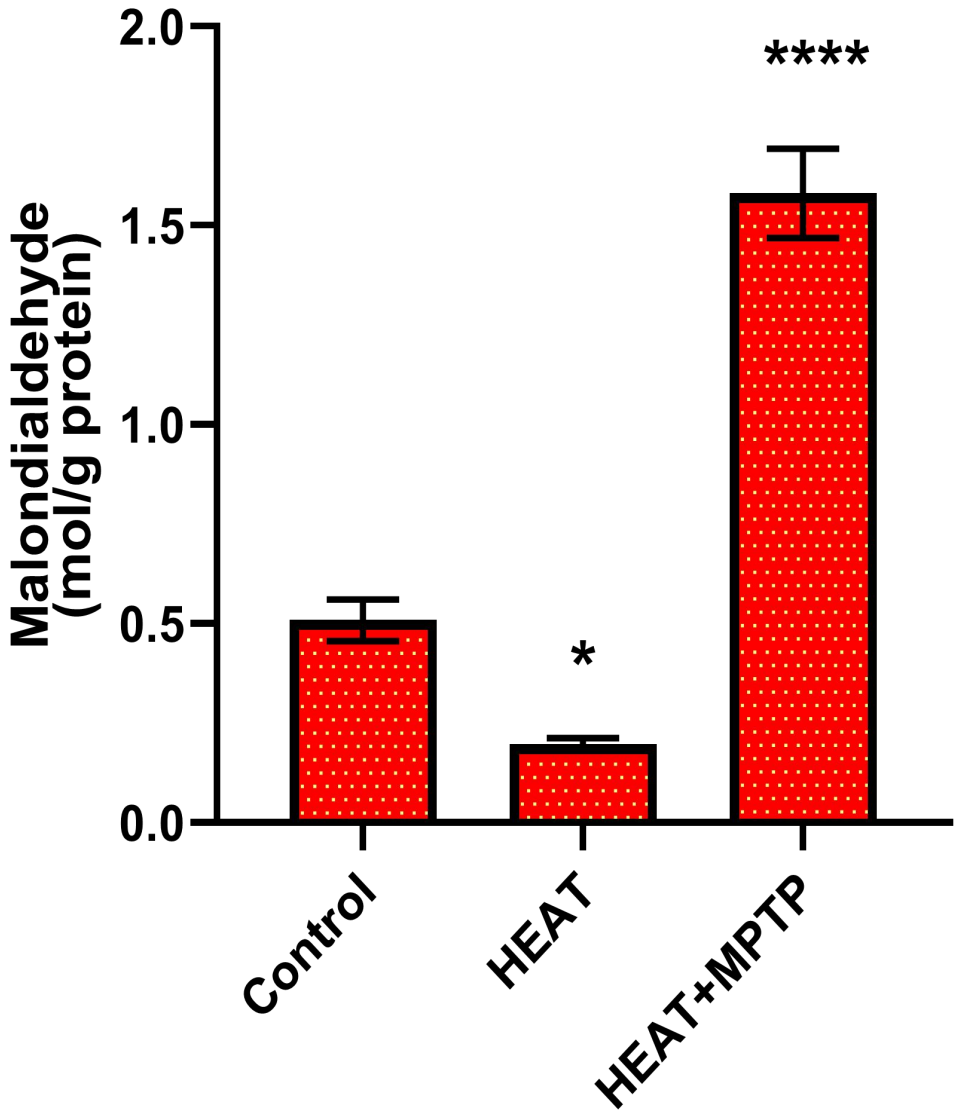

Fig 5: Effect of HEAT and HEAT+MPTP on malondialdehyde concentration

The result shows a statistically significant decrease in malondialdehyde concentration in HEAT and an increase in HEAT+MPTP-treated groups compared to the control ($p<0.05$)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

In this study, Figure 1, the result shows a statistically significant decrease in total protein concentration in HEAT and HEAT+MPTP-treated groups compared to the control ($p<0.05$). This indicates that heat stress has a profound impact on protein concentration, leading to a significant decrease. In contrast, the addition of MPTP appears to mitigate this effect, resulting in a relatively preserved protein level compared to heat stress alone.

In Figure 2, the result shows a statistically significant increase in superoxide dismutase enzyme activity in HEAT and HEAT+MPTP-treated groups compared to the control ($p<0.05$). This indicates that MPTP attenuates the heat-induced upregulation of SOD activity, as evidenced by a lesser increase in SOD activity in the HEAT+MPTP group compared to the HEAT group, suggesting that MPTP may compromise antioxidant defenses and exacerbate oxidative stress.

In Figure 3, the result shows a statistically significant increase in catalase enzyme activity in HEAT and HEAT+MPTP-treated groups compared to the control ($p<0.05$). This indicates that heat stress induces a significant increase in catalase activity, indicating an adaptive antioxidant response. However, the addition of MPTP reduces this increase, suggesting MPTP may compromise catalase-mediated antioxidant defenses, potentially exacerbating oxidative stress.

In figure 4, The result shows a statistically significant increase in glutathione peroxidase enzyme activity in the HEAT and HEAT+MPTP-treated groups compared to the control ($p<0.05$)

compared to the control group. The HEAT group shows a slightly greater increase in glutathione peroxidase activity compared to the HEAT+MPTP group, indicating that MPTP may partially attenuate the heat-induced upregulation of glutathione peroxidase, potentially compromising antioxidant defenses.

In Figure 5, the result shows a statistically significant decrease in malondialdehyde concentration in HEAT and a decrease in HEAT+MPTP-treated groups compared to the control ($p<0.05$). This indicates that heat stress leads to a significant reduction in oxidative stress, as evidenced by a huge decrease in MDA concentration. However, the addition of MPTP (HEAT+MPTP) results in a marked increase in MDA concentration, suggesting MPTP exacerbates oxidative stress and lipid peroxidation, potentially indicating increased cellular damage.

5.2 CONCLUSION

This study demonstrates that both hyperthermia and MPTP exposure significantly alter physiological and biochemical parameters associated with oxidative stress. The combined effects of heat and MPTP led to increased activities of antioxidant enzymes (SOD, CAT, and GPx) and decreased total protein levels, indicating heightened oxidative stress and cellular injury. These findings suggest that hyperthermia exacerbates MPTP-induced neurotoxicity by disrupting redox homeostasis and impairing protein stability. Overall, the results highlight the detrimental synergistic impact of environmental heat stress and neurotoxic insult on neuronal integrity, providing insight into potential mechanisms linking environmental factors to neurodegenerative processes such as Parkinson’s disease.

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